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Thèse pour obtenir le grade de :

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Discipline : Sciences et Techniques des Activités Physiques et Sportives.

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ETUDE ADIBOX

ADIPOSITE ET METABOLISME OSSEUX : EFFETS DE LA PERTE DE POIDS INDUITE PAR L'EXERCICE CHEZ LES ADOLESCENTS OBESES

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THE ADIBOX STUDY

ADIPOSIITY AND BONE METABOLISM: EFFECTS OF EXERCISE-INDUCED WEIGHT LOSS IN ADOLESCENTS WITH OBESITY

Submitted by
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Cotutelle PhD

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« Le succès n'est pas la clef du bonheur, le bonheur est la clef du succès. »

A. Schweitzer

STATEMENT OF SOURCES


This thesis contains no material published elsewhere or extracted in whole or in part from a thesis by which I have qualified for or been awarded another degree or diploma.

No parts of this thesis have been submitted towards the award of any other degree or diploma in any other tertiary institution.

No other person's work has been used without due acknowledgment in the main text of the thesis.

All research procedures reported in the thesis received the approval of the relevant Ethics Committees.

Signed: _____

A handwritten signature in blue ink, appearing to read 'Chapuis', written over a horizontal line.

Date: 03.08.2017

ABSTRACT

Introduction: This program of research targeted the impact of an 8-month weight loss intervention induced by physical activity and nutrition on bone health in adolescents with obesity. The overall aim of this thesis was to examine the impact of a lifestyle weight loss intervention on the bone parameters in adolescents with obesity.

Method: Sixty-five adolescents were recruited: 31 (6 males) adolescents with obesity in the weight loss intervention (age: 13.61 (1.27)), 23 normal weight (NW) adolescents (age: 15.90 (0.43)) and 11 (4 males) adolescents with obesity in another control group (14.02 (1.39)). Primary outcomes targeted bone densitometry (whole body, spine, hip DXA). Secondary outcomes included body composition, bone geometry and strength (hip structural analysis) and blood markers (procollagen type 1 N-terminal propeptide (P1NP), C telopeptide (CTx) estradiol, leptin). Data were collected at baseline, 4 months and 8 months.

Results: Compared with the NW controls, adolescents with obesity displayed lower unadjusted and adjusted bone density. Following successful weight loss (~ -11%) adolescents with obesity increased whole body (0.998 (0.078) $p < 0.001$) and lumbar spine (1.037 (0.128) $p = 0.014$) BMD. However, values remain lower than their NW peers after adjustment to body weight changes. After the weight loss intervention, compromised estimates of fracture risk remained especially at the narrow neck (BR 8.25 (2.00) $p = 0.005$), despite positive adaptations of some geometric properties. Also, bone accretion changes in adolescents with obesity followed an androgen-like adaptation demonstrated by periosteal expansion (% NW Δ 0.69 (3.71); Ob Δ 1.67 (9.11))

and endocortical resorption (% NW Δ -2.11 (11.79); Ob Δ 4.42 (10.56)). Among the intervention group, differences in bone markers favoured formation during the first 4 months and favoured resorption in the remaining months.

Conclusion: Bone fragility in adolescents with obesity was demonstrated by (1) baseline and post intervention lower whole body and regional BMD than NW controls, (2) post-intervention higher fracture risk index at the narrow neck, (3) blood-borne bone markers showing reduced z-scores, uncoupling indices and qualitative representations of the distribution of bone remodeling. Future investigations of links between bone and obesity during adolescence can be well informed by the results of this thesis.

SYNTHESE DU TRAVAIL DE RECHERCHE

Contexte

Définie comme une accumulation excessive de graisse corporelle pouvant engendrer des problèmes de santé, l'obésité représente un enjeu complexe pour les pays occidentaux depuis quelques décennies. Véritable pandémie ((WHO) 2000) l'obésité infantile est un facteur de complications en terme de santé (Daniels 2009) (Ebbeling et al. 2002). De nombreux travaux scientifiques se sont intéressés aux conséquences négatives de l'obésité sur les systèmes cardio-vasculaires et métaboliques.

Chez l'enfant comme chez l'adulte, le développement de l'obésité est souvent le résultat d'un déséquilibre entre les apports et les dépenses énergétiques engendrant ainsi un bilan énergétique positif et une augmentation de la masse corporelle (Rosenbaum et al. 1998). Les facteurs environnementaux, les troubles métaboliques, psychologiques et sociaux sont des éléments importants dans le développement de cette pathologie. De plus, l'augmentation de la proportion de graisses dans le régime alimentaire, la diminution de l'activité physique ainsi que l'augmentation des comportements sédentaires sont à prendre en considération.

Bien connu pour induire des troubles graves de santé (diminution / perte motrice et physique, complications psychologiques ou métaboliques, maladies cardio-vasculaires), l'obésité fut longtemps considérée comme protectrice contre l'apparition de l'ostéoporose. Effectivement,

le concept d'os plus résistants dû à une charge mécanique supérieure était considéré. Récemment, des études ont cependant mis au défi l'idée d'un effet protecteur de l'obésité sur l'os, démontrant que l'accumulation de masse grasse peut nuire à la qualité des os, particulièrement lors de la croissance.

Le squelette n'est pas seulement stimulé par une charge mécanique telle que le poids du corps, mais également via les effets métaboliques de certaines hormones (adipokines) sécrétées par le tissu adipeux. En raison de leur origine commune, les cellules osseuses et adipeuses sont intimement liées, suggérant un dialogue entre le tissu adipeux et le tissu osseux. Longtemps considéré comme inerte, dédié au stockage de l'énergie, le tissu adipeux est de nos jours reconnu comme actif d'un point de vue endocrinien. En effet, le tissu adipeux est impliqué dans le contrôle de la satiété, le contrôle homéostatique ainsi que dans le développement pubertaire (Karsenty 2006). Le tissu osseux quant à lui est reconnu pour son rôle dans la dépense énergétique et l'homéostasie du glucose (Lee et al. 2007).

Entrainant l'altération hormonale des protéines pro-inflammatoires ainsi qu'un stress oxydatif (déséquilibre cellulaire), l'accumulation de masse grasse et la perte de masse osseuse sont dès lors favorisées. Les stratégies et programmes de prise en charge de l'obésité s'orientent vers une prise en charge pluridisciplinaire (nutrition, activités physiques et soutien psychologique). Les effets positifs de la perte de poids sur la santé rencontrent cependant des effets indésirables comme entre autre la perte de masse osseuse. Il est probable que cette dégradation osseuse générée par la perte de poids lors de l'enfance et l'adolescence, soit liée aux facteurs suivants : (1) la diminution de la charge mécanique (Shapses et al. 2012), (2) l'altération des sécrétions hormonales impliquées dans le remodelage osseux (Ricci et al. 2001)

et / ou (3) la baisse de l'apport calorique (Shapses et al. 2012). De part ces observations, l'intérêt de la pratique des activités physiques semble primordial. L'activité physique induisant une contrainte mécanique (ex : activités dites à impact) est anabolisante pour le tissu osseux et ce même lors d'une perte de poids.

Contrairement à la population adulte pour laquelle les conséquences et complications de l'obésité ont été largement étudiées (Zibellini et al. 2015) (Soltani et al. 2016), peu d'informations sont disponibles sur la période de l'adolescence. En effet, la plupart des données disponibles dans les études sont limitées par la population incluse puisque celles-ci ont mélangé à la fois des adolescents en surpoids et des adolescents en situation d'obésité (Van Leeuwen et al. 2017).

Les travaux scientifiques actuels démontrent des résultats contradictoires concernant les effets de l'obésité sur le tissu osseux chez les enfants et adolescents. Cette discordance est due à plusieurs limitations.

Tout d'abord, la large hétérogénéité dans les populations recrutées (ex : le genre, l'âge, le statut pubertaire, le stade de maturation) peut expliquer l'incapacité à parvenir à un consensus concernant les effets de l'obésité sur la santé osseuse chez ces jeunes populations. Le stade de maturation revêt une importance particulière. En effet, les adolescents obèses démontrent une maturation avancée pour le même âge chronologique que les adolescents normo-pondérés. De plus, les stades de maturations et le développement osseux sont intimement liés indépendamment de la taille et de l'âge. Les effets de la masse grasse sur le pic de masse

osseuse et l'accrétion osseuse dépendent du genre et de la maturité (Shapses et al. 2012) (Wang 2002) (Dimitri et al. 2012). Cette relation est modérée en fonction des phases spécifiques de croissance (Dimitri et al. 2012).

Puis, le degré de précision des outils de mesure pourrait contribuer à la contradiction des résultats observés. Méthode de référence pour évaluer la santé osseuse des enfants et adolescents, la DXA a cependant quelques limites en raison d'une surestimation de la densité minérale osseuse lors de situation d'obésité (Crabtree et al. 2014). La normalisation des valeurs (DMO) est importante particulièrement en période de croissance lorsque plusieurs populations de tailles et poids différents sont comparés (Kröger et al. 1995) (Katzman et al. 1991). Il n'est pas possible d'obtenir des informations sur l'architecture osseuse, en effet la validité de ces mesures quant aux changements structurels liés à la croissance où à la charge mécanique sont remis en cause (Khan 2001).

En outre, afin de mieux comprendre les changements structurels osseux, les marqueurs sanguins et/ou urinaires sont nécessaires. L'analyse de marqueurs tels que P1NP, CTx, OC (unOC, COC, tOC), OPG / RANK / RANKL, sclérostine, vitamine D permettrait d'obtenir d'avantages d'informations sur le tissu osseux lors de situations d'obésité.

D'autre part, la précision des outils de mesures permettant d'évaluer la composition corporelle doit être considérée. A l'heure actuelle, l'IRM est la technique la plus précise pour différencier la graisse sous-cutanée et la graisse viscérale (Karlsson et al. 2013), son coût élevé et sa rareté

ne permettent pas, malheureusement, une utilisation fréquente. Contrairement à l'IRM, la DXA, l'ultrason et l'impédancemétrie ne mesurent pas la mesure directe de l'adiposité viscérale.

Par ailleurs, une autre limitation consiste dans le nombre d'études spécifiquement conçues de manière adéquate pour répondre à l'effet de la prise en charge par intervention pluridisciplinaire sur la santé osseuse des adolescents obèses. Seule la moitié des travaux de recherche a recruté un groupe de références (normo-pondéré et/ou obèse sans intervention). De plus, peu d'études se sont intéressées aux effets de programmes comprenant des activités physiques sur la santé osseuse. Peu de ces programmes s'intéressaient à la perte de poids.

Enfin, l'absence de report de l'adhérence des adolescents aux programmes de prise en charge affaiblit la rigueur des travaux scientifiques. Les informations relatives aux personnes délivrant l'intervention ainsi que les ressentis de cette population lors de l'intervention pourraient être utiles.

Objectifs

Le protocole ADIBOX a été pensé afin de déterminer l'influence de la perte de poids induite par une prise en charge combinant activités physiques et nutrition sur la santé osseuse des adolescents obèses.

Quatre objectifs découlent de ce projet :

(1) étudier les paramètres osseux chez les adolescents obèses et normo-pondérés ayant un même niveau de maturation ;

(2) étudier l'influence d'un programme multidisciplinaire de perte de poids combinant nutrition et activités physiques sur la santé osseuse des adolescents obèses ;

(3) étudier l'effet d'une perte de poids induite par activités physiques et nutrition sur les paramètres osseux comparé aux adolescents normo-pondérés (norme de référence) ;

(4) étudier l'influence du statut pondéral et de la perte de poids sur le remodelage osseux à l'adolescence.

Matériel et Méthodes

Conformément aux lignes directrices de l'éthique pour la recherche clinique, le protocole ADIBOX a été approuvé par le comité d'éthique (comité Hôpital Sud Est 1, France).

L'estimation du nombre de participant a été calculée sur la variabilité attendue de la masse grasse corporelle par rapport à la variabilité de la densité osseuse mesurée à la colonne vertébrale (lombaire). Un minimum de vingt et un participants (hors arrêt d'étude) par groupe est nécessaire afin de mettre en avant les potentielles différences significatives.

Soixante-cinq adolescents ont été recrutés : 31 adolescents (dont 6 garçons) atteints d'obésité inscrits dans le programme de perte de poids de TZA NOU (âge 13.61 (1.27)), 23 adolescentes de poids normaux (âge 15.90 (0.43)) et 11 adolescents (dont 4 garçons) atteints d'obésité comme groupe de référence (absence de prise en charge de perte de poids) (âge 14.02 (1.39)). Un total de vingt-quatre adolescents a complété l'intervention sur 31 initialement recrutés.

L'IMC des adolescents obèses devait se situer au-dessus du 95^e percentile alors que pour les normo-pondérés être entre le 5^e et le 85^e percentile (McCarthy et al. 2006). Afin de réduire les biais liés à la maturation, les participants invités à participer à cette étude étaient âgés entre 12 et 16 ans, avec un stade pubertaire égal ou supérieur au stade 4 de Tanner. Les adolescentes incluses dans ce travail de recherche devaient avoir atteint le stade de ménarche au moins un an avant le début de l'étude.

Les participants devaient (1) être exempts de toute histoire récente d'hospitalisation (depuis deux ans), (2) ne pas avoir d'antécédents de maladie systémique pendant plus de deux semaines au cours des douze derniers mois, (3) ne pas avoir de contre-indication à la pratique d'activité physique.

Les mesures primaires : densité minérale osseuse (DMO, g/cm²), contenu minéral osseux (CMO, g) et l'aire osseuse (cm²) ont été déterminées par DXA (DXA, QDR-4500A, Hologic, Inc., Waltham, MA). En accord avec les recommandations Internationales de l'ISCD (Crabtree et al. 2014) les mesures suivantes ont été effectuées : corps entier, corps entier moins la tête, rachis

lombaire. De plus, les mesures du score trabéculaire au rachis lombaire (TBS iNsight® version 2.1) ainsi que des mesures au niveau de la hanche ont été réalisées.

Les mesures secondaires correspondent aux mesures anthropométriques (taille, poids), au calcul de l'indice de masse corporelle (Kg.m^{-2}), aux stades de Tanner autodéterminés, à l'âge de ménarche. De plus, la composition corporelle du corps entier a été mesurée par DXA pour la masse musculaire (g), la masse grasse (g, %), la masse grasse androïde (%), la masse grasse gynoïde (%) ainsi que l'estimation de la masse grasse viscérale (% g, cm^3). La DXA fournit également la possibilité d'une analyse géométrique de la hanche au col étroit du fémur, à la zone inter trochantérienne ainsi qu'à la diaphyse fémorale. Dans chaque zone la DMO (g.cm^{-2}), le diamètre endocortical (cm), l'épaisseur corticale moyenne (cm), la largeur (cm), la coupe transversale du moment d'inertie (cm), la section transversale (cm^2), le moment de résistance (cm^3) et le rapport de masse ont été analysés. Des prélèvements sanguins à jeun ont également été effectués par une infirmière qualifiée. Le marqueur de formation osseuse P1NP (Cloud-Clone Corp, Houston, États-Unis), le marqueur de résorption osseuse CTx (Cloud-Clone Corp, Houston, États-Unis), la leptine (BioVendor, République tchèque) et l'œstradiol (BioVendor, République tchèque) ont été mesurés.

Pour répondre aux objectifs de l'étude, il est nécessaire de mieux comprendre les changements du remodelage osseux entre autre par l'utilisation de l'index de découplage. Les calculs des concentrations des marqueurs de formation et résorption sont basés sur le travail de Bieglmayer et ses collaborateurs (Bieglmayer et al. 2009) (Grimm et al. 2010). Conformément à leurs recommandations, les données ont été logarithmiquement transformées pour la

représentation graphique (Microsoft Excel et XLSTATS). Les nuages de points ont été présentés avec une ellipse de confiance de 95 %.

La prise en charge institutionnelle de l'obésité offerte par le Centre d'obésité infantile « TZA NOU » combine activités physiques, nutrition et soutien psychologique sur une période de 10 mois.

Les adolescents ont bénéficiés de quatre séances d'activités physiques supervisées par semaine. Deux de ces sessions comprenaient environ 70 minutes de travail aérobic ou de résistance. Les séances de travail en aérobic consistent en 10 minutes d'échauffement, 20 minutes de travail par intervalle, 30 minutes d'exercices continus et 10 minutes de retour au calme (étirement, relaxation). Les adolescents ont également bénéficiés de cours de natation une fois par semaine (60 min). De plus, 120 à 150 minutes basées sur la découverte de nouvelles activités physiques étaient proposées chaque semaine aux adolescents.

Concernant l'aspect nutritionnel, le centre de prise en charge de l'obésité se conforme au régime normo-calorique recommandé par rapport au niveau d'activité physique, de l'âge et du sexe (Murphy et al. 2002). La prise en charge nutritionnelle comprend également des séances d'éducation diététique toutes les deux semaines abordant des sujets tels que la perte de poids, la sensation alimentaire, les recommandations de macronutriments, les choix nutritionnels lors des repas de fêtes. Des rendez-vous avec les familles sont également organisés toutes les 10 semaines.

Le soutien psychologique a été assuré par des rencontres individuelles mensuelles, ainsi que des entretiens avec les familles toutes les 10 semaines. Des sujets comme la motivation, le retour à domicile, comment faire face aux émotions (stress, anxiété) ont été abordés lors de ces entretiens.

Les analyses statistiques ont été réalisées avec le logiciel Stata (version 13, StataCorp, College Station, US). Les données sont présentées sous forme de moyennes \pm écart-types ou médianes [interquartiles]. Les valeurs ajustées sont présentées en utilisant les moyennes [95% intervalles de confiance]. L'hypothèse de normalité a été évaluée à l'aide du test Shapiro-Wilk. L'homogénéité des échantillons a été effectuée par le test de chi-carré.

L'analyse des marqueurs sanguins concernaient les trois groupes. Par conséquent, le test ANOVA ou le test de Kruskal-Wallis (KW) ont été utilisés. Les tests de Pearson ou Spearman, selon la répartition statistique, ont servi à déterminer les coefficients de corrélation entre les paramètres de composition corporelle, les paramètres osseux ainsi que les paramètres endocriniens. Les variabilités inter et intra participants lors de l'analyse des effets fixes (groupe, temps, temps x groupe) pour les paramètres mesurés longitudinalement ont été analysées par modèles mixtes. Les analyses multivariées ajustées au poids corporel, à la masse grasseuse ou encore à la masse maigre, en fonction des résultats obtenus lors des tests univariés et de la pertinence clinique, ont été effectuées. De plus, les modélisations de régression ont également été ajustées aux modifications du poids corporel, de la masse grasse totale pendant l'intervention.

Résultats principaux

Etudier les paramètres osseux chez les adolescents obèses et normo-pondérés ayant un même niveau de maturation.

Pour cette analyse, les données de 54 adolescents ont été traitées : 31 adolescents obèses (dont 6 garçons) et 23 adolescentes normo-pondérées. Comparés aux normo-pondérées, les adolescents obèses ont un IMC (32.30 ± 4.15 vs 20.48 ± 1.32), un poids corporel (86.32 ± 15.21 vs 55.91 ± 5.90), une masse grasse totale (% 39.49 ± 3.82 vs % 20.33 ± 3.82) et spécifique (viscérale % 43.33 ± 4.22 vs % 19.37 ± 5.06) supérieurs. Les adolescentes normo-pondérées sont plus âgées que les adolescents obèses (13.61 ± 1.27 vs 15.90 ± 0.43 ans), cependant tous sont au même stade de maturation (estradiol et âge de ménarche similaire).

Concernant les paramètres osseux, les adolescents obèses ont une plus faible densité osseuse au corps entier (sans la tête) ($p < 0.001$), à la hanche ($p = 0.022$) et un plus faible contenu minéral osseux au corps entier (sans la tête) ($p = 0.048$), au rachis lombaire ($p < 0.001$) ainsi qu'à la hanche ($p = 0.008$) comparé au groupe de référence. Lorsque les données ont été ajustées au poids corporel (PC), à la masse grasse (MG) ou encore à la masse musculaire (MM), les adolescents obèses démontrent une plus grande altération des paramètres osseux à tous les sites mesurés.

L'analyse structurale de la hanche montre que les adolescents obèses ont une densité osseuse inférieure ($p = 0.008$), une plus faible épaisseur corticale ($p = 0.009$), un diamètre endocortical ($p = 0.040$) et un indice de fracture ($p = 0.028$) supérieur à la diaphyse fémorale. Après ajustement, les adolescents obèses ont une densité osseuse inférieure au niveau inter trochantérique ($p < 0.005$ ajusté PC, $p = 0.012$ ajusté MM and $p = 0.038$ ajusté MG) de même qu'à

la diaphyse fémorale ($p=0.001$ ajusté PC et MM, $p=0.022$ ajusté MG). De plus, les résultats de l'analyse géométrique mettent en avant une plus petite largeur périostéale aux trois sites de mesures (col étroit, inter trochanter, diaphyse) chez les obèses ($p=0.001$ ajusté PC, $p<0.009$ ajusté MG et $p<0.010$ ajusté MM pour le col étroit et la diaphyse, $p=0.002$ pour l'inter trochanter). A la région du col étroit, un diamètre endocortical inférieur ($p=0.002$ ajusté PC, $p=0.001$ ajusté MG) est observé chez les adolescents en situation d'obésité comparé au groupe de référence. De plus, les résultats démontrent une altération de l'épaisseur corticale dans la région inter trochantérienne ainsi qu'à la diaphyse fémorale ($p<0.008$ ajusté PC et $p<0.005$ ajusté MM).

Au début de l'étude un indice de fracture plus élevé a également été observé chez les adolescents obèses versus normo-pondérés à la diaphyse fémorale ($p=0.028$).

Etudier l'influence d'un programme multidisciplinaire de perte de poids combinant nutrition et activités physiques sur la santé osseuse des adolescents obèses.

Pour cette analyse, les données des 24 adolescents (dont 3 garçons) ayant complétés les 8 mois d'intervention de perte de poids ont été traitées. L'analyse longitudinale met en avant une diminution significative du poids corporel et de la masse grasse (totale et spécifique) lors de l'intervention ($p<0.007$). Seule la masse musculaire reste inchangée.

Lors des 8 mois de prise en charge de l'obésité, le contenu minéral osseux, la densité minérale osseuse au corps entier (sans la tête) ($p<0.001$) et le contenu minéral osseux ($p=0.003$), la densité minérale osseuse ($p=0.014$), la densité apparente osseuse ($p=0.015$) au rachis lombaire ont été améliorés.

L'analyse structurale de la hanche montre qu'à la fin des 8 mois du programme de perte de poids, les adolescents ont une densité osseuse diminuée au col étroit ($\Delta -4.74$ (6.07) % $p < 0.001$) ainsi qu'une augmentation du diamètre endocortical ($\Delta 6.20$ (6.77) % $p < 0.001$) et de la largeur périostéale ($\Delta 6.16$ (7.69) % $p < 0.001$). Des résultats similaires ont été observés au niveau inter trochantérique. L'épaisseur corticale quant à elle, augmente à la diaphyse fémorale ($\Delta 4.49$ (5.21) % $p = 0.002$).

L'analyse des paramètres de résistance osseuse met en avant une augmentation de l'index de risque de fractures principalement au col étroit du fémur ($\Delta 8.24$ (2.00) % $p = 0.005$) avec des valeurs proches du seuil de fracture.

Etudier l'effet d'une perte de poids induite par activités physiques et nutrition sur les paramètres osseux comparés aux adolescents normo-pondérés (norme de référence).

Pour cette analyse, les données de 47 adolescents ont été traitées : 24 adolescents obèses (dont 3 garçons) et 23 adolescentes normo-pondérées.

A l'achèvement du programme de perte de poids une densité osseuse plus faible au corps entier (sans la tête) ($p < 0.001$) et à la hanche ($p = 0.017$) a été observée chez les adolescents obèses versus normo-pondérés. Par ailleurs, après ajustement (changements poids corporel et masse grasse), les adolescents obèses démontrent des valeurs osseuses inférieures aux normo-pondérés : DMO corps entier (sans la tête) ($p < 0.008$), col ($p < 0.001$ ajusté PC, $p = 0.031$ ajusté MG), à la hanche ($p < 0.008$) ainsi qu'une densité minérale apparente inférieure au corps entier ($p < 0.008$).

Comparés aux changements observés chez les adolescentes normo-pondérées, les adolescents obèses ont un diamètre endocortical ($p < 0.006$), une largeur périostéale ($p < 0.017$) supérieure aux trois sites du fémur (col étroit, inter trochanter, diaphyse).

En parallèle, des valeurs plus faibles ont été observées chez les adolescents obèses versus normo-pondérés pour la densité osseuse ($p < 0.009$) et l'épaisseur corticale (IT $p = 0.031$, DF $p = 0.001$) aux sites inter trochantérien et diaphysaire. Lorsque les valeurs sont ajustées aux changements de poids ou masse grasse, l'épaisseur corticale aux deux sites reste inférieure chez les adolescents obèses. De plus, le diamètre endocortical est supérieur chez les obèses après ajustement au poids de corps (DF $p = 0.014$) et masse grasse (IT $p < 0.05$, DF $p = 0.018$). Des différences au niveau des paramètres de résistance ont été observées au col étroit ($p = 0.008$), à l'inter trochanter ($p = 0.004$) et encore à la diaphyse fémorale ($p = 0.004$) pour l'indice de fracture. Après avoir ajusté les données aux changements de poids corporel, l'indice de fracture à l'inter trochanter et à la diaphyse fémoral sont supérieur ($p < 0.004$). De même, la section transversale inter trochantérienne et diaphysaire est supérieure chez les adolescents obèses versus normo-pondérés ($p < 0.003$).

Etudier l'influence du statut pondéral et de la perte de poids sur le remodelage osseux à l'adolescence.

Pour cette analyse, les données de 38 adolescents ont été traitées : 10 adolescents obèses (groupe intervention), 11 adolescents obèses (dont 4 garçons - groupe contrôle) et 17 adolescentes normo-pondérées. Les deux groupes obèses montrent des caractéristiques corporelles (IMC, poids corporel, masse musculaire, masse grasse) supérieures au groupe normo-pondéré de référence. Les adolescents obèses ont des résultats similaires entre eux en

terme de composition corporelle à l'exception d'une masse grasse viscérale supérieure pour le groupe obèse contrôle ($p=0.034$).

Afin de comparer les valeurs dans le temps, celles-ci ont été normalisées à partir des valeurs de départ de chaque groupe.

En ce qui concerne les adolescentes normo-pondérées, l'analyse des marqueurs sanguins montre qu'à 4 mois, le remodelage osseux favorise la formation. En effet, lorsque l'on regarde la répartition au sein du nuage de point, 76% des points sont dans la zone de formation rapide, 17% en résorption rapide et 7% en résorption lente. Le calcul de l'index de découplage ($p=0.028$; 0.47 (0.78)) confirme l'observation graphique. De plus, une différence significative est mise en avant concernant la médiane de formation/résorption à 4 mois ($p=0.044$).

Le groupe contrôle d'adolescents obèses a un remodelage osseux favorisant la formation. Cependant, à 4 mois, l'ellipse de confiance se décale vers la zone de résorption rapide. Cet état de résorption a été démontré, même si non significatif, lors du calcul de l'index de découplage allant de 0.00 (1.20) à -1.32 (1.43).

Finalement, la représentation graphique du groupe ayant suivi l'intervention de perte de poids démontre un remodelage osseux à la fois en formation et en résorption rapide. Après 4 mois d'intervention, un décalage du nuage de poids en faveur de la formation osseuse apparaît. Néanmoins, cet effet positif est limité puisqu'à la fin de l'intervention les valeurs sont similaires à celle du début de la prise en charge. Ces résultats ont été confirmés par l'analyse des médianes du taux de renouvellement osseux et de l'équilibre formation/résorption. A 4 mois, la formation osseuse semble être promue par un plus faible taux de renouvellement osseux

($p=0.037$) et une activité plus forte formation/résorption en faveur de la formation osseuse ($p=0.037$). Le retour aux valeurs de base correspondrait à un équilibre formation/résorption stimulant la résorption ($p=0.007$) et un taux de renouvellement osseux supérieur ($p=0.009$).

Les valeurs des trois groupes ont été normalisées par rapport aux données de base du groupe normo-pondéré afin de comparer leur évolution. Contrairement à ce qui a pu être observé précédemment, les ellipses des deux groupes d'adolescents obèses sont représentées dans la zone de résorption osseuse.

Le remodelage osseux a également été mesuré entre les deux groupes atteints d'obésité. Pour ce faire, les valeurs ont été normalisées aux valeurs de base du groupe contrôle obèses. Le programme de perte de poids semble influencer le remodelage osseux ($p=0.037$ médiane formation/résorption, $p=0.066$ taux de renouvellement osseux).

Conclusion

Ce projet de thèse a étudié l'impact d'une intervention multidisciplinaire de perte de poids combinant nutrition et activités physiques, sur la santé osseuse des adolescents atteints d'obésité. Afin de renforcer la compréhension relative à la santé osseuse des adolescents atteints d'obésité, les données ont été recueillies lors d'un programme de perte de poids de 8 mois combinant les activités physiques et la nutrition.

Le projet ADIBOX a dans un premier temps étudié les paramètres osseux chez les adolescents ayant une même maturité. Ensuite, les effets d'un programme multidisciplinaire de perte de poids combinant nutrition et activités physiques, sur les paramètres osseux chez les adolescents obèses, ont été évalués. Une comparaison relative aux différences corporelles (obèses versus normo-pondérés) a également été effectuée sur le même intervalle de temps. Enfin, ce projet s'est intéressé à l'influence du poids et de la perte de poids sur l'activité de remodelage osseux.

Les résultats pour les variables primaires ont montré :

(1) une altération des paramètres osseux (DMO, CMO) chez les adolescents en situation d'obésité par rapport aux adolescents de poids normal, même lorsque les données ont été ajustées pour le poids corporel, la masse grasse et de la masse maigre ;

(2) une amélioration de la DMO pendant la prise en charge au corps entier et au rachis lombaire. Cependant lorsqu'ajustées au changement de poids corporel, ces valeurs sont inférieures à celles de leurs pairs de poids normal.

L'analyse structurale de la hanche (géométrie et résistance) est relativement novatrice au sein de cette population. Les principaux résultats secondaires de ce projet de recherche ont mis en avant :

(1) une altération importante des indices géométriques de la résistance de l'os au col étroit du fémur. Malgré les adaptations positives de certains paramètres géométriques suite à la prise en charge, l'index d'estimation du risque de fracture est très élevé chez cette population ;

(2) des tendances plus subtiles mais significatives concernant l'accrétion osseuse chez les adolescents obèses. Le développement osseux semble suivre une adaptation de type androgène, en stimulant l'expansion périostéale et la résorption endocorticale.

(3) une réponse positive du remodelage osseux lors des quatre premiers mois de l'intervention avant de retourner aux valeurs observées au début de la prise en charge.

Le travail présenté dans ce manuscrit présente un certain nombre de limites.

Tout d'abord, il n'était pas possible de mesurer individuellement le volume et l'intensité de la pratique sportive du programme de perte de poids. Cet effet dose-réponse de la perte de poids aux activités physiques est intéressant du fait du niveau de sédentarité des adolescents à l'entrée de ce programme. De même, plus d'informations quant à la nutrition aurait été appréciable afin de déterminer l'impact de ces deux composantes (nutrition et activités physiques) sur la perte de poids et la réponse du tissu osseux.

Ensuite, le programme d'activité physique proposé par le centre de prise en charge de l'obésité est orienté sur la remise en activité des adolescents à la pratique sportive. Il est possible que

les activités proposées n'aient pas été suffisamment ostéogéniques afin de promouvoir l'accrétion osseuse.

De plus, il n'a pas été possible de mettre en place une visite post-intervention. Le centre de prise en charge de l'obésité a un recrutement national, ce qui d'un point de vue logistique complique la mise en place d'une visite de suivi.

Par ailleurs, des difficultés telles que des données manquantes, un nombre inférieur à celui initialement souhaité ont été rencontrées lors du regroupement des groupes contrôles. De plus, le ratio garçon / fille ne permet pas une étude plus approfondie d'un point de vue des genres. L'inclusion de garçons dans l'analyse peut également être perçue comme variable de confusion, malgré la confirmation statistique du maintien de l'homogénéité dans les groupes.

Initialement le travail de recherche a été mis en place dans les deux pays. Cependant des difficultés en Australie lors du recrutement des participants ont été rencontrées.

D'autre part, les marqueurs osseux sont complexes à analyser lors de la période de croissance. En effet, ils peuvent refléter à la fois la croissance, le remodelage ou encore un statut nutritionnel.

En outre, pour raison budgétaire, l'analyse complémentaire de marqueurs sanguins (OPG/RANK/RANKL, sclérostine, ostéocalcine, PTH, GH/IGF1, adiponectine) n'a pas pu être

effectuée. Idem concernant la mesure de la testostérone et la SHBG. Ces marqueurs peuvent fournir d'importantes informations sur l'effet de croissance type androgène.

Finalement, il n'était pas possible de mesurer les différentes phases du cycle menstruel, cycle connu pour influencer les marqueurs osseux.

Ce travail présente également un certain nombre de points positifs contribuant à sa force.

Premièrement, la population recrutée était appariée d'un point de vue de la maturation. De plus, ce projet a ciblé la population adolescente en situation d'obésité et non pas un mixte entre enfants/adolescents et obésité/surpoids.

Deuxièmement, le challenge lié au recrutement des participants est bien connu, malgré l'abandon du projet Australien, en France 42 adolescents obèses ont été recrutés, ce qui représente un nombre considérable comparé à certaines études.

Troisièmement, une autre force de ce projet réside dans la durée de celui-ci. En effet, outre le fait que le temps alloué permet d'observer une réponse en termes de remodelage osseux, les mesures ont été collectées en trois temps : au début, au milieu et à la fin de l'intervention.

Quatrièmement, ce travail permet également d'obtenir d'intéressantes informations de par la présence d'un groupe contrôle sur la totalité de la prise en charge (8 mois). De plus, peu d'études ont, en plus d'un groupe normo-pondéré, recruté un groupe obèse contrôle.

Cinquièmement, bien que les données puissent être considérées comme incomplètes, ce projet :

(1) a abordé l'obésité chez les adolescents avec une intervention prolongée dans un programme résidentiel.

(2) a exploré les réponses osseuses à la hanche ; site critique et soumis aux variations de poids ; en utilisant l'analyse structurale de la hanche.

(3) a combiné les données obtenues par DXA à celles des marqueurs du remodelage osseux.

Collectivement, les résultats de ce travail de thèse contribuent à l'avancée de la compréhension des réponses osseuses à l'obésité et la perte de poids chez les adolescents.

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A PhD is more than just a scientific and intellectual challenge ... A PhD is a personal adventure that made me understand and learn a lot about myself ... about my life expectations. These 3 years made me understand what is important in my life, made me understand what I was looking for ...

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To you ...

with love

Elodie

TABLE OF CONTENTS

Introduction	57
Review of literature	61
Bone and growth	62
2.1. Bone cells	63
2.1.1. The osteoblast lineage cells	63
<i>Osteoblasts</i>	63
<i>Osteocytes</i>	64
<i>Bone-lining cells</i>	64
2.1.2. Bone resorbing cells	64
2.2. Bone remodeling cycle	65
2.3. Adolescence and gender differences	66
2.4. Bone strength	68
2.5. Bone remodeling regulation	71
2.5.1. Genetic factors	72
2.5.2. The osteoblast lineage cells' hormonal factors	72
2.5.2.1. Bone derived factors	72
<i>RANK/RANKL/OPG</i>	72
<i>Osteocalcin</i>	73
<i>Sclerostin</i>	74
2.5.2.2. Others hormonal factors arising directly or indirectly from adipose tissue ... 74	
<i>Growth Hormone/ Insulin-like Growth Factor 1</i>	74
<i>Estrogen synthase</i>	75
<i>Insulin</i>	75
<i>Leptin</i>	76
<i>Adiponectin</i>	77
<i>Ghrelin</i>	78

2.5.3. Lifestyle factors	80
2.5.3.1. Physical activity	80
2.5.3.2. Nutrition	84
<i>Calcium</i>	84
<i>Vitamin D</i>	85
2.6. Bone health assessment	86
2.6.1. Bone densitometry devices	86
2.6.1.1. Dual-energy X-ray Absorptiometry	87
<i>Classical measures</i>	87
<i>Trabecular Bone Score</i>	88
<i>Hip Structural Analysis</i>	89
2.6.1.2. Peripheral quantitative computed tomography	89
2.6.1.3. Quantitative UltraSound	90
2.6.2. Blood markers of bone turnover	95
2.6.2.1. Analysis of bone remodeling activity	96
<i>The Uncoupling Index</i>	97
<i>The bone marker plot</i>	98
2.7. Summary	100
Obesity and bone tissue: specificity of adolescence	102
2.8. Obesity	102
2.9. Bone mass in obese youth	103
2.9.1. Analysis via DXA	104
<i>Whole body analysis</i>	104
<i>Regional analysis</i>	105
<i>Meta-analysis of cross sectional data</i>	106
2.9.2. Analysis via pQCT	108
2.9.3. Analysis via biomarkers of bone formation and resorption	108
2.10. Factors influencing bone mass	109
2.10.1. Puberty and gender	109
<i>Whole body analysis</i>	109

<i>Regional analysis</i>	110
2.10.2. Fat tissue	110
2.10.3. Role of hormones?.....	111
2.10.3.1. Leptin and adiponectin: potential contributors to BMD?	111
<i>Leptin</i>	111
<i>Adiponectin</i>	113
2.10.3.2. Bone hormones: potential actor on energy metabolism?	114
<i>Osteocalcin</i>	114
<i>Sclerostin</i>	115
2.11. Effects of obesity intervention on bone parameters	115
2.11.1. Effects of physical activity intervention on bone in the absence of reporting weight loss.....	116
2.11.2. Effects of intervention directly targeting the impact of weight loss on bone... 120	
2.11.3. Meta-analysis on the effects of structured intervention on bone parameters 124	
Limitations and gaps of the current literature	129
Research questions	132
2.12.1. Do adolescents with obesity have altered bone mass compared with maturation-matched lean peers?	132
<i>Hypothesis</i>	132
<i>Aim</i>	132
2.12.2. Can the negative effects of weight loss on bone health in adolescents with obesity be attenuated with a lifestyle intervention?	133
<i>Hypothesis</i>	133
<i>Aim</i>	133
2.12.3. Does exposure to an 8-month WL intervention involving physical activity and nutrition normalise bone health in adolescents with obesity?	134
<i>Hypothesis</i>	134
<i>Aim</i>	134
2.12.4. Do weight status and weight changes influence bone markers in adolescents with obesity?	135
<i>Hypothesis</i>	135

<i>Aim</i>	135
Methodology	136
3.1. The ADIBOX study design	138
3.2. Ethics Approval and Clinical Trial registration	138
3.3. Participants	138
3.4. Sample size calculation	139
3.5. Inclusion/exclusion criteria	140
<i>Inclusion criteria</i>	140
<i>Exclusion criteria</i>	141
3.6. Data collection Overview	141
3.6.1. Primary outcomes	142
<i>Bone density assessed using DXA</i>	142
<i>Quality Assurance and Radiation dosage</i>	143
3.6.2. Secondary outcomes	144
<i>Anthropometric characteristics</i>	144
<i>Maturation</i>	144
<i>Body composition</i>	144
<i>Hip Structure Analysis</i>	145
<i>Endocrine markers</i>	146
3.7. Details of analysis involving key endocrine markers	147
<i>Uncoupling index</i>	147
<i>Bone marker plot</i>	147
3.8. Clinical intervention	148
<i>Physical activity intervention</i>	148
<i>Nutrition intervention</i>	148
<i>Psychological support</i>	149
<i>Participant compliance</i>	149
3.9. Statistical treatment of the data	149
Results	151

4.1. Does adolescents with obesity have altered bon mass compared with maturation-matched lean peers?.....	157
4.2. Does nutrition and physical activity inducing WL can reverse the negative effects of WL on bone health?	166
4.3. Does 8 months WL induced by physical activity and nutrition conduct to normal bone health?.....	183
4.4. Does weight status and weight changes influence bone markers?.....	194
Discussion and conclusion	213
5.1. Summary of major findings.....	215
5.2. Discussion of major findings	219
5.3. Limitations	235
5.4. Strengths	238
5.5. Further research considerations.....	240
5.6. Conclusion	242
References.....	244
Appendices.....	277

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LIST OF APPENDICES

<i>Appendix 1 - Confirmation of Candidature</i>	<i>278</i>
<i>Appendix 2 - Attestation letter of compulsive training module (France)</i>	<i>279</i>
<i>Appendix 3 - Ethics approval by the ANSM (National Agency for the Safety of Medicines and Health Products).....</i>	<i>280</i>
<i>Appendix 4 - Ethics approval by the University Hospital G.Montpied, Clermont-Ferrand (63)</i>	<i>281</i>
<i>Appendix 5 - Ethics amendment letter to male participants</i>	<i>282</i>
<i>Appendix 6 - Ethics application form.....</i>	<i>283</i>
<i>Appendix 7 - Clinical trial registration</i>	<i>353</i>
<i>Appendix 8 - Article 1</i>	<i>366</i>
<i>Appendix 9 - Article 2</i>	<i>377</i>
<i>Appendix 10 - Article 3</i>	<i>386</i>
<i>Appendix 11 - Article 4.....</i>	<i>428</i>
<i>Appendix 12 - Baseline data of the Ob intervention group</i>	<i>472</i>
<i>Appendix 13 - Baseline and 8 months data of the NW group.....</i>	<i>475</i>
<i>Appendix 14 - Baseline, 4 months and 8 months data of the adolescents that completed the whole WL intervention</i>	<i>481</i>
<i>Appendix 15 - Baseline and 4 months data of the adolescents with obesity in the control group</i>	<i>490</i>

<i>Appendix 16 - Excel file calculation of baseline bone turnover normalised to NW baseline median</i>	493
<i>Appendix 17 - Excel file calculation of the 4 months bone turnover normalised to NW baseline median</i>	497
<i>Appendix 18 - Excel file calculation of the 8 months bone turnover normalised to NW baseline median</i>	502
<i>Appendix 19 - Excel file calculation of baseline Ob bone turnover normalised to Ob baseline median</i>	505
<i>Appendix 20 - Excel file calculation of 4 months Ob bone turnover normalised to Ob baseline median</i>	507
<i>Appendix 21 - Excel file calculation of 8 months Ob bone turnover normalised to Ob baseline median</i>	510
<i>Appendix 22 - Excel file calculation of baseline Ob control bone turnover normalised to Ob control baseline median</i>	514
<i>Appendix 23 - Excel file calculation of 4months Ob control bone turnover normalised to Ob control baseline median</i>	517
<i>Appendix 24 - Excel file calculation of baseline Ob bone turnover normalised to Ob control baseline median</i>	520
<i>Appendix 25 - Excel file calculation of 4 months Ob bone turnover normalised to Ob control baseline median</i>	522
<i>Appendix 26 - Excel file calculation of 8 months Ob bone turnover normalised to Ob control baseline median</i>	524
<i>Appendix 27 - Bone variables of the 24 adolescents with obesity at 4 months adjusted to (A) BW changes and (B) fat mass changes</i>	526

Appendix 28 - Bone variables of the 24 adolescents with obesity at 8 months adjusted to (A) BW changes and (B) fat mass changes.....528

Appendix 29 - Baseline bone variables of the adolescents with obesity in the control group compared with the Ob intervention group530

Appendix 30 - Proposed future research question.....531

Appendice 31- Ethics approval by the ACU Human Research Ethics534

LIST OF TABLES

<i>Table 1 - Synthesis of hormonal factors influencing bone mass</i>	79
<i>Table 2 - Comparison of bone densitometry techniques (modified from Binkley et al. 2008 (Binkley et al. 2008)).</i>	93
<i>Table 3 - Meta-analysis results from cross-sectional studies</i>	107
<i>Table 4 - Aerobic based intervention described by El Hage et al. 2009 (El-Hage et al. 2009).</i>	117
<i>Table 5 - Aerobic based intervention described by McGuigan et al. 2009 (McGuigan et al. 2009)</i>	118
<i>Table 6 - Outlines of weight loss targeted interventions</i>	123
<i>Table 7 - Meta-analysis results from longitudinal studies</i>	127
<i>Table 8 - Meta-regression results from longitudinal studies</i>	128
<i>Table 9 - Outline of the intra, inter-assay coefficient of variation and sensitivity results for blood markers</i>	146
<i>Table 10 - Outline of the data collection for primary and secondary outcomes</i>	153
<i>Table 11 - Outline of the data collection and participants for the first aim</i>	158
<i>Table 12 - Descriptive statistics at baseline in the Ob group and NW control group</i>	160
<i>Table 13 - Bone variables at baseline. A. Unadjusted mean. B. Body weight adjusted. C. Fat mass adjusted. D. Lean mass adjusted.</i>	162
<i>Table 14 - Outline of the data collection and participants for the second aim</i>	167
<i>Table 15 - Unadjusted bone variables at baseline between the adolescents who dropped out of the weight loss intervention and the rest of the intervention sample</i>	171
<i>Table 16 - Biochemical characteristics of the Ob during the weight loss program</i>	177

<i>Table 17 - Bone remodeling scores and uncoupling index</i>	<i>178</i>
<i>Table 18 - Correlation analysis. A. Between hormones and bone parameters. B. Between body composition parameters and bone parameters.</i>	<i>179</i>
<i>Table 19 - Body composition parameters (A) and bone variables (B) of the 24 adolescents with obesity at baseline, 4 and 8 months.....</i>	<i>180</i>
<i>Table 20 - Outline of the data collection and participants for the third aim.....</i>	<i>184</i>
<i>Table 21 - Biochemical characteristics of the groups at baseline (T0) and 8 months (T2).....</i>	<i>188</i>
<i>Table 22 - Bone variables at 8 months. A. Unadjusted mean. B. Body weight adjusted. C. Fat mass adjusted.....</i>	<i>189</i>
<i>Table 23 - Outline of the data collection and participants for the fourth aim</i>	<i>195</i>
<i>Table 24 - Bone markers concentration</i>	<i>196</i>
<i>Table 25 - Descriptive statistics at baseline</i>	<i>197</i>
<i>Table 26 - Scores, UI and bone markers plot normalised to groups' respective median.....</i>	<i>202</i>
<i>Table 27 - Balance and turnover normalised to NW baseline median</i>	<i>205</i>
<i>Table 28 - Scores and UI normalised to NW baseline.....</i>	<i>205</i>
<i>Table 29 - Outline of the major findings within this thesis.....</i>	<i>217</i>

LIST OF FIGURES

<i>Figure 1 - Outline of chapter of this thesis</i>	60
<i>Figure 2 - Bone growth adapted from Khan et al (Khan 2001)and Seeley et al (Seeley et al. 2011)</i>	62
<i>Figure 3 – Bone remodeling cycle</i>	65
<i>Figure 4 - Peak bone mass</i>	67
<i>Figure 5 – Bone strength influencing factors (adapted from Bouxsein 2005 (Bouxsein 2005))</i>	68
<i>Figure 6 - Frost's Mechanostat theory: modeling and remodeling thresholds (adapted from Novotec Medical (Novotec Medical GmbH 2008-2013))</i>	70
<i>Figure 7 - Biomechanical communication influencing bone development (adapted from Isaacson et al. (2014) (Isaacson et al. 2014))</i>	71
<i>Figure 8 - Bone marker plot four-quadrant graph</i>	99
<i>Figure 9 - Effect size forest plot for the effects of obesity on lumbar spine bone mineral apparent density (a) and whole body bone mineral density (b).</i>	107
<i>Figure 10 - Effect size forest plot for the effects of structured physical activity intervention on bone mineral density (a) and percentage of fat mass (b)</i>	127
<i>Figure 11 - Summary of the primary and secondary outcomes measures</i>	142
<i>Figure 12 - Whole body, lumbar spine and hip positioning DXA scans</i>	143
<i>Figure 13 - Body composition analysis by Dual energy X-ray Absorptiometry (DXA)</i>	145
<i>Figure 14 - Hip Structural Analysis by Dual energy X-ray Absorptiometry (DXA)</i>	146
<i>Figure 15 - Flow charts of participants of the 3 first aims of this thesis</i>	155

Figure 16 - Body composition changes (kg) of the obese interventional group during the weight loss intervention170

Figure 17 - Unadjusted bone geometric evolution at NN, IT and FS of the obese interventional group during the weight loss program.....175

Figure 18 - Unadjusted bone strength changes at NN, IT and FS of the obese interventional group during the weight loss program.....176

Figure 19 - Schematic representation of unadjusted geometric changes at the femoral shaft at 8-months. Over the 8 months, CSA significantly increased with a similar magnitude in both group. ACT and BMD significantly increased only for the NW group.187

Figure 20 - Bone turnover changes of the three groups: normal weight (A), Ob (B) and Ob control (C), normalised to their respective baseline median201

Figure 21 - Bone turnover changes of both obese groups: Ob (A) and Ob control (B), the baseline median of NW control was used for normalisation.....206

Figure 22 - Bone turnover evolution of both obese groups: Ob (A) and Ob control (B), the baseline median of Ob control was used for normalisation.....209

Figure 23 - Bone marker plot of 3 participants (one from each group) normalised to baseline NW median (A) and normalised to group's baseline median (B).....211

Figure 24 - Schema of mechanistic hypothesis of bone metabolism in adolescents with obesity234

ABBREVIATIONS

Android Fat Mass (aFM)	Fat tissue in android region, expressed in g or %.
Average Cortical Thickness (ACT)	Estimate of mean cortical thickness calculated as follow: $(ROI\ WIDTH - ROI\ ED) / 2$ expressed in cm (Beck 2002).
Body Mass Index (BMI)	Index for assessing weight status. BMI is obtained by dividing body weight in kilograms by height in m^2 (Kuczmarski et al. 2000).
Bone Mineral Content (BMC)	The amount of bone mineral per anatomical region expressed in grams (g) (Carter et al. 1992).
Bone mineral density (BMD)	The amount of bone mineral content per projected bone scanned area expressed in $g \cdot cm^2$ calculated as follow: $BMC / projected\ area$ (Carter et al. 1992).
Bone mineral apparent density (BMAD)	The mineralised tissue mass per total tissue volume (Carter et al. 1992) WB BMAD: $(WB\ BMC / WB\ bone\ area^2) / body\ height$; LS BMAD: $LS\ BMC / LS\ bone\ area$ (Katzman et al. 1991).
Body weight (BW)	Total mass of the whole body expressed in g.
Bulking ratio (BR)	Reflect thickness and cortical instability in bulking $((ROI\ CSMI / ROI\ Z) / ROI\ ACT)$ (Beck 2002).
Center of mass position (CMP)	Distance from center of mass to medial margin $(ROI\ PCD / ROI\ WIDTH)$ (Beck 2002).

Cross-sectional area (CSA)	Estimate of BMC (exclude trabecular bone and soft tissue) calculated as follow: (Sum of pixel values in profile) * (pixel spacing along profile / 1.05) expressed in cm (Beck 2002).
Cross-sectional moment of inertia (CSMI)	Index of structural rigidity; reflect the distribution of the mass about a neutral or centroidal axis calculated as follow: (Sum of pixel mass at each point in profile times square of its distance center of mass) * (pixel spacing along profile / 1.05) expressed in cm ⁴ (Beck 2002).
C-telopeptide (CTx)	Bone resorption marker comprised of collagen molecules which are released when collagen within the bone is broken down (Szulc et al. 2007).
Endocortical diameter (ED)	Estimation of the inside diameter of the cortex calculated as follow: $(2 * ((ROI\ WIDTH / 2) * 2 - (0.6 * ROI\ CSA / \pi))) * 0.5$ expressed in cm (Beck 2002).
Dual energy x-ray absorptiometry (DXA)	A scanner that provides two-dimensional images of regional areas or the whole body, using two x-ray beams of differing energy levels to measure the absorption of each beam in order to calculate bone mineral (An et al. 1999).
Fat mass (FM)	Fat tissue in the whole body, expressed in g or %.
Femoral shaft (FS) - HSA	Region located 2 cm distally to the midpoint of the lesser trochanter (Beck 2002).
Gynoid fat mass (gFM)	Fat tissue in gynoid region, expressed in g or %.
Grams (g)	Metric unit of measure equal to 1/1000 kilogram.

Hip structural analysis (HSA)	DXA program measuring BMD and structural geometry of cross-sections traversing the proximal femur (Beck 2002).
Intertrochanteric (IT) - HSA	Region located along the bisector of the neck-shaft angle (Beck 2002).
Lean mass (LM)	Lean tissue in the whole body, expressed in g or %.
Lumbar spine (LS)	Portion of the spine comprising the lumbar vertebrae.
Narrow neck (NN) - HSA	The narrowest diameter of the femoral neck (Beck 2002).
Normal weight (NW)	Based on BMI, population classified as “normal” (Cole et al. 2005).
MoM	MoM=marker \bar{x} /median (marker) Measure of how far an individual result deviates from the median (Bieglmayer et al. 2009)
Peak bone mass (PBM)	The amount of bony tissue present at the end of the skeletal maturation (Bonjour et al. 1994).
Peripheral quantitative computed tomography (pQCT)	Scanner that provides high-resolution three-dimensional images of the peripheral skeleton and uses absorptiometry techniques to measure the attenuation of radiation passing through the scanned site in order to provide measures of volumetric bone density (An et al. 1999).
Procollagen type 1 N-terminal propeptide (P1NP)	Bone formation marker cleaved from type 1 collagen molecules during the process of incorporating collagen into the bone matrix (Szulc et al. 2007).

Profile center distance (PCD)	Distance from profile center of mass to medial margin of cortex, expressed in cm (Beck 2002).
Quantitative Ultrasounds (QUS)	Radiation free device for assessing bone mineral density by ultrasound especially to the calcaneus (An et al. 1999).
Region of interest (ROI)	Area on a digital image that circumscribes a desired anatomical location.
Section modulus (Z)	Indicator of bending strength calculated as follow: (If (ROI CMP \geq 0.5) then (ROI CSMI / ROI PCD) else (ROI CSMI / (ROI WIDTH - ROI PCD))) expressed in cm ³ (Beck 2002).
Standard deviation (SD)	Measure the dispersion in a distribution.
Tanner Stage (TS)	Scale of physical development in children, adolescents and adults (Tanner 1962).
Total area	Total surface of a two-dimensional figure or shape, expressed in m ² .
Total body less head (TBLH)	Whole body measure by DXA excluding the head region.
Trabecular area	Cross-sectional area of trabecular bone in mm ² .
Trabecular density	The amount of trabecular bone in a certain volume of bone (McGraw-Hill 2002).
Tumor necrosis factor alpha (TNF- α)	A pleiotropic cytokine synthesised widely throughout the female reproductive tract.
Uncoupling Index (UI)	Index representing the balance between bone formation and resorption during bone remodeling (Eastell et al. 1993).

Visceral fat (VFAT)	Fat tissue in largest visceral fat region, expressed in g or %.
Visceral fat mass (VFAT)	Mass of fat inside abdominal cavity, expressed in cm ³ .
Width (WIDTH)	Subperiosteal width, outer diameter of the bone. Blur-corrected width of the mass profile expressed in cm (Beck 2002).
Whole body (WB)	Relates to the entire body.

CHAPTER ONE

Introduction

The complex consequences of child and adolescent obesity represent major concerns in most developed countries ((WHO) 2000), largely contributing to metabolic complications with costly repercussions for the burden of disease (Daniels 2009) (Ebbeling et al. 2002). This burden is exemplified by high prevalence rates of overweight or obesity. Well known to lead to serious health-related disorders (compromised movement capacity, psychological or metabolic complications such as type 2 diabetes and cardiovascular disease), obesity was thought to be protective against osteoporosis. Until recently, the concept of stronger bones due to extra mechanical load had widespread acceptance. However, recent studies have challenged the concept of a protective effect of obesity on bone, indicating that fat accumulation may be detrimental to bone quality during the growing years. Even less is known about the impact of fat loss on bone quality among adolescents.

The skeletal system is not only stressed from mechanical loading such as weight bearing movements but also through the metabolic effect of some of the adipokines secreted by fat (adipose) tissue. Due to their common origin, bone cells and hormones released by fat tissue (adipocytes) are intimately connected; suggesting a cross-talk between adipose tissue and bone tissue. Adipose tissue has long been considered an inert tissue dedicated for energy storage. Recent advances have established that both adipose tissue and bone tissue are dynamic endocrine organs. Adipose tissue is involved in satiety, energy balance and pubertal development (Karsenty 2006), while bone tissue acts on energy expenditure and glucose homeostasis (Lee et al. 2007). Indeed, obesity leads to hormonal alterations associated with increased pro-inflammatory proteins released by immune cells (cytokines) and cellular imbalance known as oxidative stress. Under some conditions, inflammatory and oxidative stress can favour the accumulation of fat mass and loss of bone mass. It is possible that bone

breakdown generated by weight loss during childhood and adolescence is related to a number of factors that includes: (1) decreased mechanical loading on the skeleton (Shapses et al. 2012), (2) altered hormonal secretion involved in bone regulation (Ricci et al. 2001) and/or (3) decreased caloric intake (Shapses et al. 2012). However, weight bearing physical activity may be anabolic for bone, even during periods of weight loss during the years surrounding adolescent growth. To date, most studies have not considered adding sex-hormone status and other components of pubertal development to bone investigations among adolescents aspiring to use physical activity as part of weight loss strategies.

The overall purpose of this work was to determine the impact of weight loss induced by physical activity and nutrition on bone health among adolescents with obesity. Figure 1 provides an outline of chapters in this thesis. After a brief review on bone and growth, the existing literature on the adipocyte-osteocyte cross-talk, the impact of obesity on bone health as well as the effects of structured intervention on bone health were considered among adolescents with obesity. Finally, the methodology and results of this thesis are detailed and discussed.

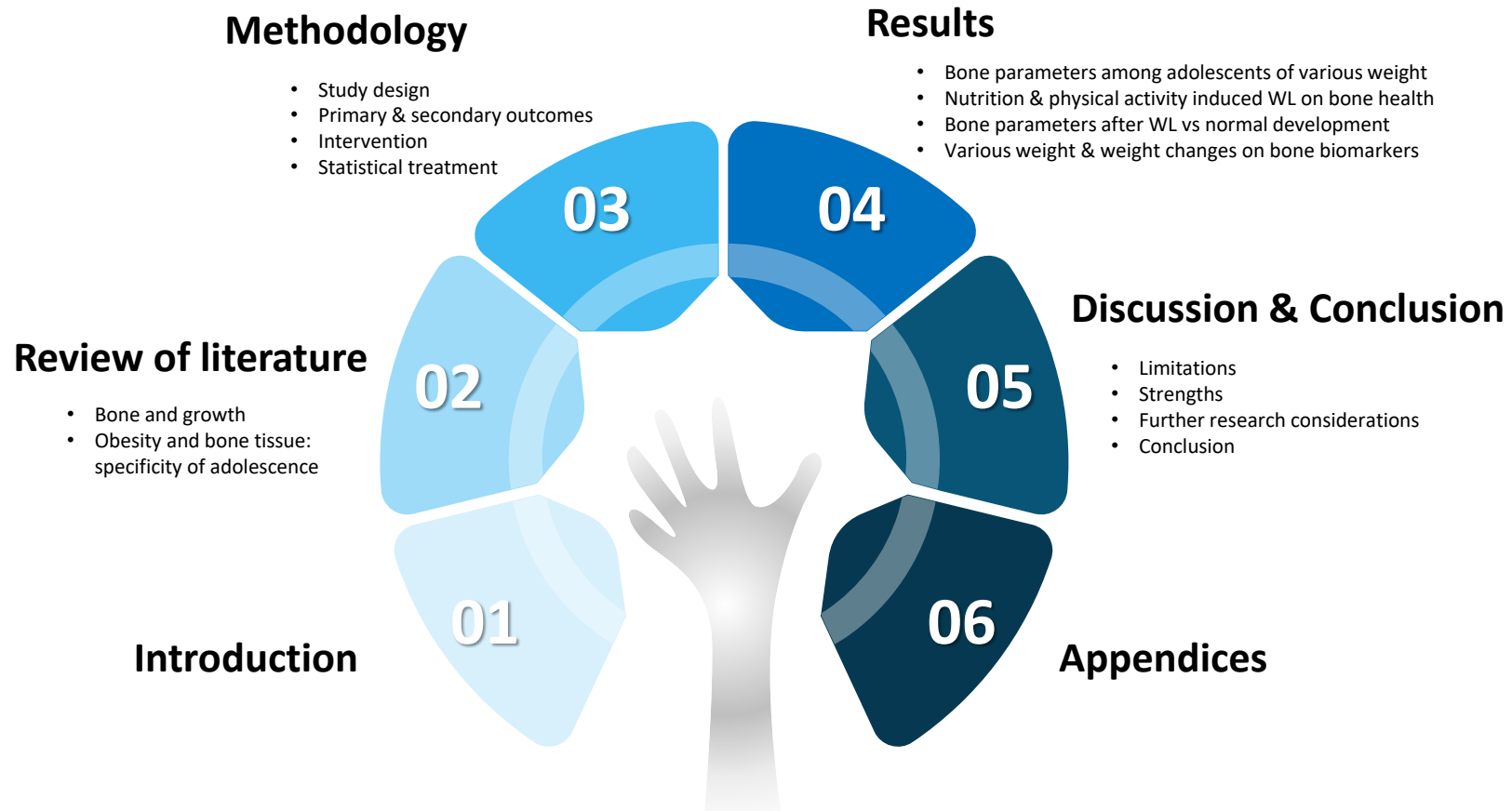


Figure 1 - Outline of chapter of this thesis

CHAPTER TWO

Review of literature

Bone and growth

Bone development (length, mass and breadth) results from complex interactions between genetics, hormonal and modifiable lifestyle factors such as nutrition and physical activity. The basic morphology of bone is genetic (50-90%), but final mass and architecture can respond to mechanical environments (10-50%) (Health et al. 2004). Investigating growth during adolescence remains critical as the skeleton, under endocrinal control, undergoes rapid changes through modelling and remodeling processes. Growth in stature during the first two decades of life is the result of multiple contribution of appendicular and axial growth.

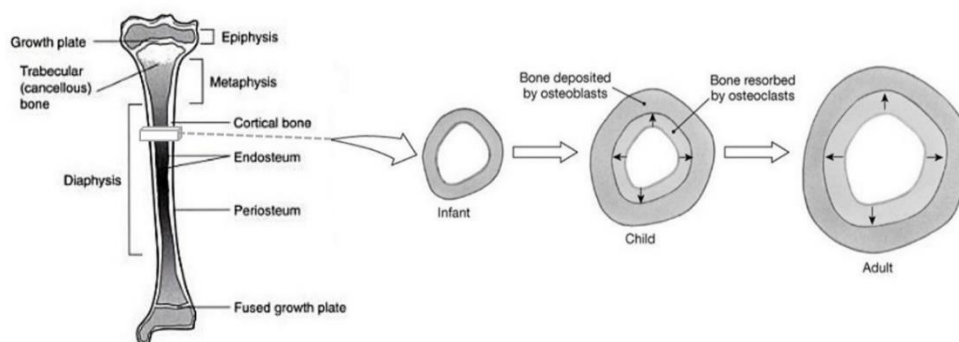


Figure 2 - Bone growth adapted from Khan et al (Khan 2001) and Seeley et al (Seeley et al. 2011)

Bones tissue in the human skeletal comprises cortical (80%) and trabecular (20%) bone (Zebaze et al. 2010). As such, the external surface of long bones comprises cortical bone, surrounding bone marrow space, while trabecular bone is principally found at the metaphysis and epiphysis of long bones (Figure 2). Trabecular and cortical bone are composed of osteons; concentric layers of compact bone tissue that are fundamental to the function of bone. Dense and solid, with less than 5% porosity (Clarke 2008), endosteum and periosteum comprise the two

surfaces of cortical bone. The endosteum surface is the inner surface of long bones and has a thin layer of cells lining the medullary cavity. With higher remodeling activity than the periosteum surface, it is postulated that endosteum experiences greater biomechanical strain or increased cytokine exposure (Clarke 2008). The periosteum forms the outside surface of the bone and consists of two shapes: an outer shape (rich in blood vessels and nerves) and an inner shape (helping to build stability in the layers of bone during growth) (Khan 2001). Therefore, the periosteum surface activity is important for growth and fracture repair.

2.1. Bone cells

Throughout life, the skeleton continuously remodels. Changes can be observed in shape, mass and intrinsic properties as well as a self-repair capacity (Frost 1987) (Macdonald et al. 2005) (Rantalainen et al. 2010). The osteoblasts, osteocytes and bone-lining cells (osteoblast lineage) and osteoclasts (bone resorbing cells) are the two bone lineage cells involved in bone remodeling.

2.1.1. The osteoblast lineage cells

Osteoblasts

Mature osteoblasts continuously add new bone between the epiphyseal plate and metaphysis (Aubin 2001). Osteoblasts are derived from chondrocytes, adipocytes, fibroblasts and bone marrow mesenchymal stem cells. They reside along the bone surface at which bone formation is active. Osteoblast differentiation has four stages: the preosteoblast, osteoblast, osteocyte

and bone-lining cells. Fifty to seventy per cent of mature osteoblasts undergo apoptosis; the remaining osteoblasts differentiate into osteocyte or quiescent lining cells (Lynch et al. 1998).

Osteocytes

Residing in newly formed osteoid and the mineralised matrix of bone, osteocytes are thought to send resorption or formation signals in response to mechanical strain (Parra-Torres et al. 2013). The fundamental roles of osteocytes are to determine and maintain bone structure. Osteocytes can undergo apoptosis when located near to the bone matrix and micro-damage when bone remodeling increases (Tatsumi et al. 2007).

Bone-lining cells

The last members of the osteoblast family are the bone-lining cells. Their suggested role is to prevent inappropriate interaction between bone surface and osteoclast precursors. Signals stimulating osteoclast formation may initiate bone-lining cells to prepare the bone for a resorption phase (Chambers et al. 1985).

2.1.2. Bone resorbing cells

Located on endosteal or periosteal surface of bone, osteoclasts are the bone cells capable of resorbing mineralised bone matrix. Osteoclast differentiation is mediated by the ratio between the expressing receptor activator (RANK) of RANKL and osteoprotegerin (OPG); an osteoclast formation paracrine inhibitor (Boyce et al. 2007).

2.2. Bone remodeling cycle

Bone remodeling phases are well established (Hadjidakis et al. 2006) and are shown in Figure 3. The activation of bone remodeling depends on acidity levels in cells (Boyle et al. 2003). Resorbing osteoclasts secrete ions that lower the pH to 4.5 within the bone-resorbing compartment. The lowered pH facilitates the mobilisation of bone mineral (Silver et al. 1988). Bone formation takes approximately 4 to 6 months (Khan 2001). Bone formation has the purpose of maintaining mineral homeostasis and bone strength by repairing micro fractured bone. During this period, about 80% of new bone is trabecular, with the remaining 20% being cortical. Indeed, less metabolically active than trabecular bone, cortical bone has a slower bone turnover. An increase in cortical remodeling causes an increase in cortical porosity and decrease cortical bone mass.

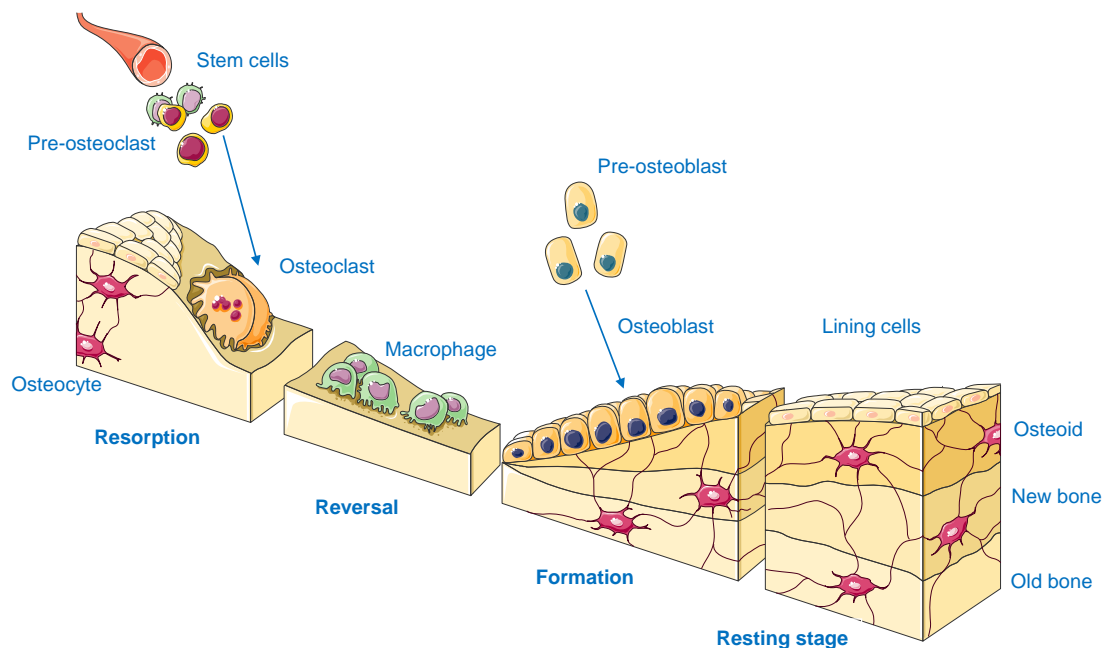


Figure 3 – Bone remodeling cycle

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2.3. Adolescence and gender differences

Unlike any other phase of development, adolescence provides optimal opportunities to boost bone mineral density. The majority of musculoskeletal mass and bone structure are accrued during childhood and peri-pubertal development (Parfitt 1994) (Baxter-Jones et al. 2011). Indeed, 26% of adult bone mass accumulates during pubertal growth (Bailey et al. 1999). Both maturation timing and sexual dimorphism have an effect on bone mass. Late maturation has been shown to have deleterious effects on whole body bone mineral content especially in female (Jackowski et al. 2011). Although the peri-pubertal period emerges as the most suitable time to boost bone structure, elongation of long bones continues into late adolescence (Figure 4). It is also during the peri-pubertal stage of development that adolescents can optimise bone strength (Parfitt 1994) (Ducher et al. 2006). Negative or positive influences during this period can subsequently modify the peak bone mass (Dimitri et al. 2012). Peak bone mass is defined as the amount of bone tissue present at the end of the skeletal maturation (Bonjour et al. 1994). However, peak bone mass, as previously mentioned is strongly influenced by dimorphism.

Wider in males than females, bones do not differ in length between sexes before puberty (Clark et al. 2007). Dimorphism in bone emerges largely during puberty (i.e. length, width, mass and strength); notably occurring two years earlier in females than males (11-13 years for females and 13-15 years for males) (Forwood et al. 2004) (Nguyen et al. 2001). Longer periods of prepubertal growth in males than females coincides with a larger increase in bone size and cortical thickness that may explain the more apparent sex differences in bone structure observed towards the end of puberty (Bonjour et al. 1994). Indeed, periosteal apposition in

females decelerates earlier than males. Although neither changes in medullary size nor contraction are observed in females, endosteal resorption enlarges the medullary cavity in males (Neu et al. 2001) (Bass et al. 1999). Similar cortical thickness for a smaller total and medullary size in females than males is attributed to greater periosteal apposition and medullary contraction (Bass et al. 1999). In contrast, trabecular thickness increases leading to greater trabecular volumetric bone mineral density (vBMD) in both sexes. However, as females enter puberty, estrogen levels increase and inhibit periosteal bone formation; promoting bone growth on the endocortical surface (Seeman 2003) (Wang et al. 2006).

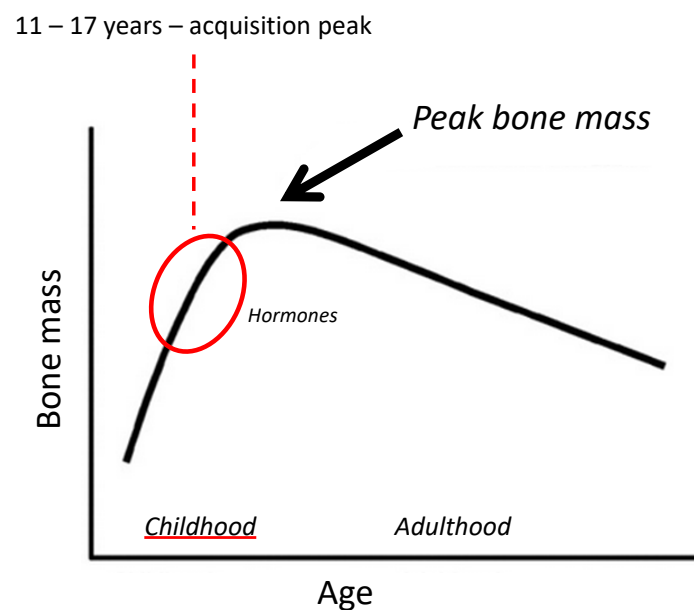


Figure 4 - Peak bone mass

2.4. Bone strength

Bone undergoes longitudinal and radial growth during childhood and adolescence. During growth, uncompromised bone health is important to potentially offset bone fragility and reduce possible risks related to exacerbated bone loss (i.e. osteoporosis) later in life. Uncompromised bone health in healthy populations can be defined by the ability of bone to resist fracture under challenging conditions such as falling, acute impact, twisting or other mechanical stress (Comité scientifique de Kino-Québec 2008).

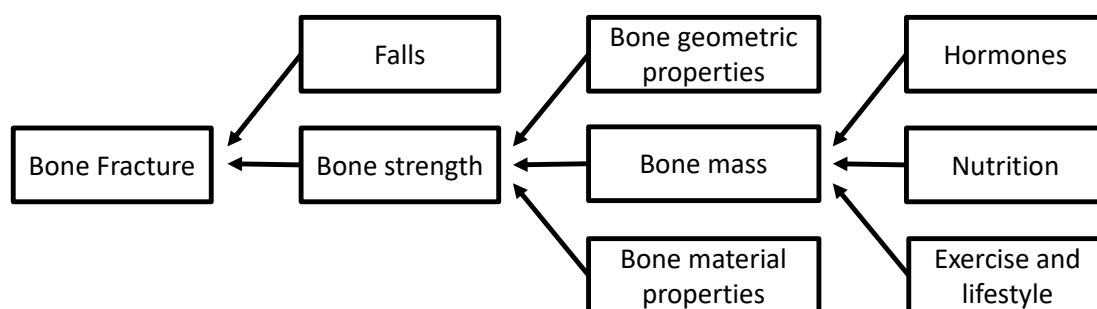


Figure 5 – Bone strength influencing factors (adapted from Boussein 2005 (Boussein 2005))

The measurement of bone strength has to account for multiple parameters including material properties (mass, density, stiffness and strength) and geometric properties (shape, cortical thickness, cross sectional area and trabecular architecture) (Khan 2001) (Figure 5). Importantly, failure to develop a strong skeleton because of factors such as insufficient mechanical loading, poor nutrition, hormonal alterations and disease may lead to bone fragility (Kontulainen et al. 2007). Material properties depend on the quality and quantity of the bone mineral mass. In contrast, bone's geometric properties depend on the resistance of bone to bending and

torsional forces. Together, bone material and geometric properties may provide useful markers of an individual's risk of fracture through an estimate of bone strength (Muehleman et al. 2000). Specifically, micro fracture risk can accumulate due to low bone turnover activity, while the main cause of micro architectural degradation is attributed to higher bone turnover activity (bone resorption activity being greater than formation) (Pocock et al. 1987).

It is well established that peak bone mass is an important determinant of future bone health and fracture risk (Clark et al. 2006) (Chevalley et al. 2011). Moreover, increasing bone strength during growth is the primary strategy for preventing osteoporosis later in life. Bone mass accounts for 50 to 70% of bone strength (Pocock et al. 1987). However, bone strength can be independently affected by the site-specific amount and proportion of trabecular and cortical bone (Pocock et al. 1987).

According to the Mechanostat theory (Figure 6) bone mass and strength depend on the peak forces caused by muscles (Frost 2003). Increasing muscle mass and muscle force during development in childhood creates the stimuli for corresponding increases in bone mass and strength (Rauch et al. 2004). Subsequently, a linear relationship exists between muscle cross sectional area and bone cross sectional area (Schoenau et al. 2002). Childhood growth and development support this muscle-bone relationship. In particular, bone strength and mass have demonstrated a linear relationship with muscle development (Schoenau 2005).

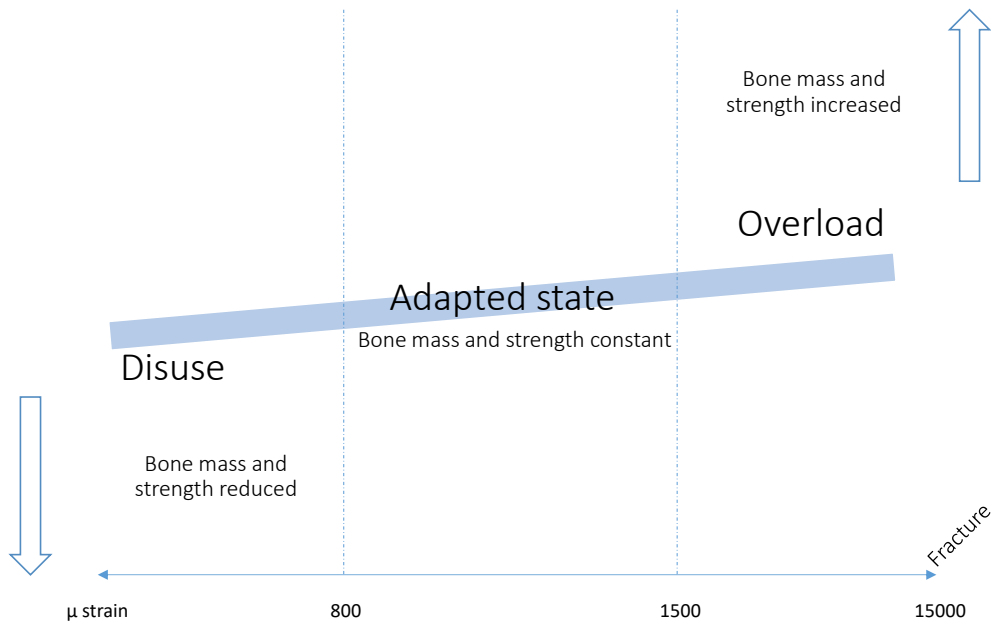


Figure 6 - Frost's Mechanostat theory: modeling and remodeling thresholds (adapted from Novotec Medical (Novotec Medical GmbH 2008-2013))

Although the muscle-bone relationship has widespread acceptance, researchers remain uncertain about the precise mechanisms of the response (Judex et al. 2009) (Kohrt et al. 2009). The Mechanostat theory is limited to the mechanical and physical interactions (Isaacson et al. 2014). Recently the literature extended the traditional mechanical view to mechanical and biochemical interactions (Isaacson et al. 2014) (Tyrovola 2017) (Figure 7). Specifically, the activation of the osteoclastogenesis function is the response of both biological (described in 2.5.) and mechanical stimuli.

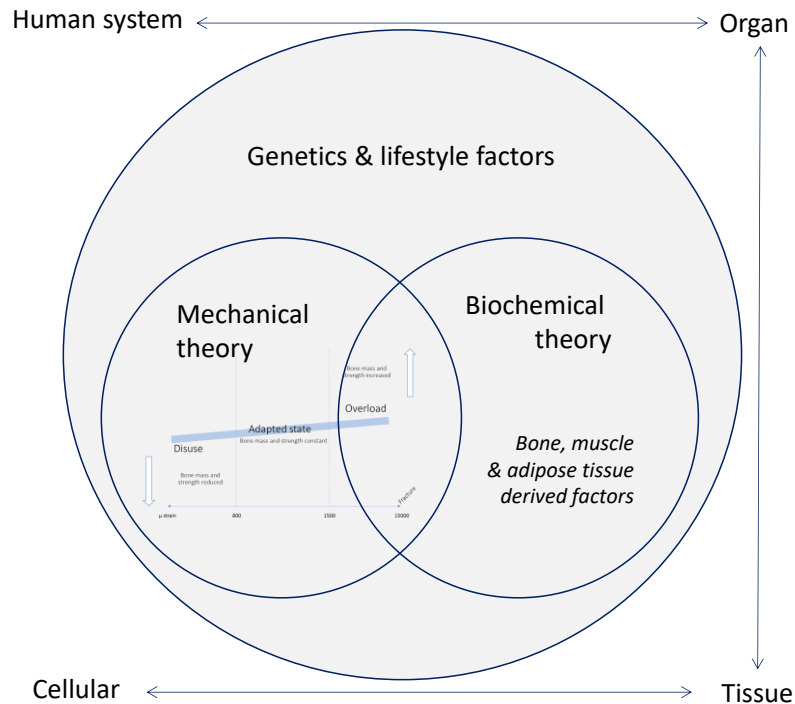


Figure 7 - Biomechanical communication influencing bone development (adapted from Isaacson et al. (2014) (Isaacson et al. 2014))

The mechanical and biochemical circles represent both the traditional Mechanostat theory and the more recent biochemical interactions, showing the possibilities of the cross talk. The largest circle represents the overall human body, influenced by genetics, nutrition and physical activity factors (detailed in section 2.5.). The interconnection between the system, organs, tissues and cells on the understanding of the biomechanical interactions are represented with double-headed arrows (Isaacson et al. 2014).

2.5. Bone remodeling regulation

Bone material properties can be influenced by genetic factors, hormones and modifiable lifestyle factors such as nutrition, or physical activity.

2.5.1. Genetic factors

Genetic factors are beyond the scope of this review. However, some important genetic determinants of bone density need to be acknowledged. The insulin growth factor I (IGF I) gene, vitamin D receptor gene polymorphisms and estrogen receptor gene polymorphisms may influence genetic variation in bone mass and density. It is recognised that genotypes may be of greater importance than environmental factors in predicting bone density early in life (Soyka et al. 2000).

2.5.2. The osteoblast lineage cells' hormonal factors

2.5.2.1. Bone derived factors

RANK/RANKL/OPG

The OPG/RANKL system has an important role in the osteoclastogenesis and is a communication mediator between osteoblasts and osteoclasts (Marie 1992). RANKL, are expressed by osteoblasts and belong to the cytokine TNF α superfamily. RANKL are critical for osteoclast differentiation. When bound to the receptor RANK, RANKL stimulate osteoclast formation (Van Wesenbeeck et al. 2002). Osteoblasts also express OPG, another member of the TNF α superfamily. OPG has the role of being a RANKL antagonist. OPG cells aim to block RANK/RANKL interactions by binding the RANK receptor and then inhibiting osteoclast differentiation (Van Wesenbeeck et al. 2002). By controlling the ratio of OPG/RANKL, osteoblasts are capable of regulating osteoclast formation (Clarke 2008).

Osteocalcin

Osteocalcin (OC) is a recognised marker of bone formation. It is secreted by osteoblasts through the OC gene, which comprises the major non-collagen protein found in the extracellular matrix of bone and directly reflects bone metabolism (Roche et al. 2011). Osteocalcin is expressed in two different forms that have two independent functions; the carboxylated (cOC) form and uncarboxylated (unOC) form. Carboxylated osteocalcin is thought to be the active form in the bone. Carboxylated osteocalcin also has a high affinity for hydroxyapatite and is mainly stored in bone matrix during osteoblast mineralisation (Capulli et al. 2014). Alternatively, uncarboxylated osteocalcin is postulated to act on energy metabolism (Bonnet 2017). Specifically, in response to decreased osteoblast proliferation via the central action of leptin, osteoblasts influence energy metabolism through expressing a product of the *Esp* gene (osteotesticular protein tyrosine phosphatase – OT-PTP). The OT-PTP inhibits the carboxylated form of osteocalcin. Consequently, the uncarboxylated form permits the β -cells' proliferation and insulin secretion in the pancreas as well as stimulating adiponectin secretion in adipocytes (Wolf 2008). In rodent studies, the uncarboxylated form of osteocalcin also contributes to glucose metabolism by increasing insulin signalling in the muscle, while in human studies total osteocalcin rather than unOC has been associated with glucose homeostasis (Bonnet 2017). Although interest in osteocalcin has increased recently, uncertainty surrounds a greater understanding of the carboxylated form of osteocalcin. This remains problematic because most of the time, the uncarboxylated and carboxylated forms have not been analysed separately. Questions about the specificity of osteocalcin to bone metabolism have been raised since the discovery of osteocalcin secretions by adipose tissue (Foresta et al. 2010) and the brain (Patterson-Buckendahl et al. 2012).

Sclerostin

Sclerostin is a protein secreted by osteocytes through the SOST gene. It acts on bone formation by means of inhibiting osteoblast activity (Morse et al. 2014) and concomitantly, osteocalcin secretion. An activation of the canonical Wnt signalling pathway enhances bone formation by stimulating osteoblastic activity, differentiation and proliferation. In addition to its action on bone, the Wnt signalling pathway acts on glucose homeostasis as is it active in pancreas, adipose tissue, liver and skeletal muscle (Bonnet 2017). The bone Wnt pathway can be antagonised by secreted inhibitors binding to lipoprotein receptor-related protein 5/6 (LRP) (Williams et al. 2009). The LRP5 protein plays important roles in the development and maintenance of bones by acting on the regulation of BMD. Sclerostin is the main inhibitor involved in mechanical loading (such as exercise) and unloading states (weight-supported environments) (Turner et al. 2009).

2.5.2.2. Others hormonal factors arising directly or indirectly from adipose tissue

Growth Hormone/ Insulin-like Growth Factor 1

The Growth Hormone (GH)/Insulin-like Growth Factor 1 (IGF1) axis is important in growth and bone remodeling (Gajewska et al. 2015). The hormone IGF1 is produced by the liver under the regulation of GH. However, GH is secreted from the anterior pituitary gland and is regulated by hypothalamic factors (E Govoni 2012). Once secreted, GH can act on bone directly or indirectly by stimulating the release of IGF1. During the peri-pubertal period, GH/IGF1 are

determinants of longitudinal bone growth (Giustina et al. 2008). The GH/IGF1 axis has an anabolic effect on bone and stimulates osteoblast proliferation (Giustina et al. 2008).

Estrogen synthase

Secreted by gonads and adipose tissue, aromatase or estrogen synthase controls the biosynthesis of androgen to estrogen. In addition to a widely accepted effect on growth (maturation and sexual development), estrogen increases fat storage and regulates bone metabolism (Riggs et al. 2002). The main function of estrogen on bone metabolism is to reduce bone resorption that subsequently increases bone formation (Alexandre 2005). The action of estrogen on osteoclasts is suggested to be indirect and mediated by products secreted from osteoblasts (i.e. OPG/RANKL) (Gruber et al. 2002). Estrogen binds to its receptor on the osteoblasts and directly increases the production of OPG and reduces the production of RANKL (Alexandre 2005).

Insulin

Insulin is produced by pancreatic β -cells and has an anabolic effect on osteoblasts. By relieving the suppression of Runx2 (Runt-related transcription factor 2 - a factor associated with osteoblast differentiation) by Twist 2 (Twist-related protein 2) via its signalling pathway, insulin stimulates osteoblast differentiation and osteocalcin expression; promoting bone formation (Fulzele et al. 2010). Insulin signalling in osteoblasts also enhances osteocalcin activity and influences glucose homeostasis (Ferron et al. 2010). The positive loop between insulin signalling in osteoblasts and osteocalcin functions implies that leptin acts as a negative regulator.

Leptin

Secreted by adipose tissue, leptin takes a major role in the regulation of energy homeostasis (Thomas et al. 2002). As a satiety hormone (ghrelin antagonist), leptin regulates food intake and increases energy expenditure. More recently leptin appears to have either a direct or indirect role on bone depending on the signal transduction pathway. By acting directly, leptin may impact and regulate bone growth through activation of the fibroblast growth factor (FGF) 23, and also osteocalcin (Upadhyay et al. 2015). The indirect role of leptin involves activation of GH and IGF1 via the hypothalamic pituitary growth hormone axis (Upadhyay et al. 2015).

Leptin also appears to have peripheral and central effects on bone. The peripheral action of leptin on bone can occur via autocrine, paracrine or endocrine mechanisms. Leptin receptors have been found in osteoblasts and chondrocytes; allowing direct action on bone (Upadhyay et al. 2015). The peripheral pathway appeared to stimulate bone growth by acting on specific receptors and stimulating cortical bone formation (Thomas 2003). Leptin increases bone mass through its interaction with bone marrow mesenchymal stem cells (BMSCs), osteoblasts and chondrocytes (Chen et al. 2015). In addition to stimulating the osteoblast cell lineage, leptin appears to moderate the OPG/RANKL ratio (Martin et al. 2005) (Thomas 2003) by stimulating OPG and inhibiting the production of RANKL.

In contrast, the central effects of leptin on bone may counterbalance the positive peripheral effects. It is postulated that when serum leptin reaches a critical threshold, the central effects of leptin override the peripheral effects (Bonnet et al. 2005). Through the central pathway, leptin acts to stimulate bone resorption (Karsenty 2006) and inhibit bone formation (Elefteriou et al. 2005). To date, researchers have identified two actions activated by the sympathetic

nervous system (SNS) after binding to the β 2 adrenergic receptor on osteoblasts (Togari 2002). On one hand, the adrenergic system suppresses osteoblasts' proliferation. On the other hand, it promotes resorptive effects of osteoclasts by increasing the production of RANKL (Chen et al. 2015). Recently, a second pathway has been identified. Leptin appears to stimulate the CART pathway (Cocaine and Amphetamine Regulated Transcript) in the arcuate nuclei of the hypothalamus. The CART expression then inhibits RANKL production via an unknown mechanism and therefore suppresses osteoclast differentiation (Chen et al. 2015).

Adiponectin

Adiponectin is another adipocyte-secreted hormone known to regulate energy homeostasis, glucose and lipid metabolism as well as inflammatory pathways (Williams et al. 2009). Adiponectin also affects insulin sensitivity (Abseyi et al. 2012). Because of the regulation of adiponectin by osteocalcin it is suggested that bone is another target of this hormone. Moreover, adiponectin receptors can be present in either osteoblasts or osteoclasts; suggesting a link with bone mass (Williams et al. 2009). However, the precise action of adiponectin on bone is complicated by multiple receptors and signalling pathways (Kajimura et al. 2013). In animal models, the existence of two opposing influences of adiponectin on bone tissue have been identified (Kajimura et al. 2013). Adiponectin appears to act via two different mechanisms, a local and a central pathway; antagonistic of each other. The local action of adiponectin inhibits osteoblast proliferation and stimulates their apoptosis. In contrast, adiponectin has the ability to decrease the activity of the SNS and favours osteoblastic proliferation. Concomitantly, for peripheral pathways adiponectin increases RANKL expression in osteoblasts, while through the brain signalling it inhibits RANKL production (Kajimura et al.

2013). Adiponectin signals in the brain inhibit the activity of the SNS, thereby increasing bone formation.

Ghrelin

Ghrelin is a gastrointestinal hormone mainly produced by the gastrointestinal track. It acts via central or peripheral pathways. Besides a major function in energy homeostasis (Delhanty et al. 2014), ghrelin demonstrates a regulatory role in multiple organs including bone. Ghrelin functions to promote bone formation and increase bone mass by increasing osteoblastic proliferation and differentiation (Pradhan et al. 2013). However, ghrelin also stimulates osteoclastogenesis when acting through the systemic pathway (van der Velde et al. 2012).

Table 1 summarises the hormonal factors influencing bone mass. The table serves to synthesise specific actions of the previously described hormones on bone.

2.5.3. Lifestyle factors

2.5.3.1. Physical activity

In past decades, the effects of physical activity on bone health have been extensively studied, with a particular focus during childhood and adolescence. Musculoskeletal gains are foremost among the well-established benefits of a physical active lifestyle in the first two decades of life. Linked to increased BMD (Bass et al. 1998) and bone strength (Ward et al. 2005), physical activity during childhood and adolescence positively influences bone parameters (Tan et al. 2014). Systematically reviewed, the mechanical component of physical activity strongly influences adaptations in the growing skeleton during childhood and adolescence and may improve BMD later in life (Tan et al. 2014) (Specker et al. 2015).

Although sexual dimorphism and maturation are associated with greater benefits during pre and peripubertal years (Ducher et al. 2006) (Vicente-Rodriguez et al. 2003) (Tan et al. 2014), the effects of physical activity are moderate by the type and intensity of exercise. From a musculoskeletal perspective, two major types of exercise are highlighted in the literature: osteogenic and non-osteogenic activities (Vico 2008). It is now well recognised that weight bearing physical activities; activities against gravity with a relatively high or intermittent impact against gravitational forces; (i.e. gymnastics, track and field) have greater osteogenic effects than non-weight bearing activities such as swimming or cycling (Greene et al. 2012) (Morel et al. 2001) (Courteix et al. 1998). Indeed, bone geometry and micro-architecture are frequently reported to be more positively influenced by high-impact sports (Grimston et al. 1993) (Proctor et al. 2002), than weight-supported sports (Courteix et al. 1998) (Duncan et al. 2002) (Ferry et

al. 2011). Moreover, osteogenic sports (i.e. soccer, runner, volleyball) seem to influence bone distribution within the cortical shell of the tibia in a different way (Specker et al. 2013).

Physical fitness levels are declining among adolescents despite an increase participation in organised physical activities (Carter et al. 2011). Participating in sports with low levels of fitness could lead to acute and chronic sports-related injuries (Carter et al. 2011). There are frequent reports of the predominance of musculoskeletal injuries in adolescent sport participants (Jacobsson et al. 2012). In addition, global concerns about sedentary behaviour in adolescents have triggered bone density comparisons between physically active adolescents and their more sedentary peers.

Although advantages in bone health parameters are frequently observed in young sporting populations compared with their less active peers, the cross sectional nature of research might be a concern. Statistical analysis is complex and involves scales that can account for the variability and impact of growth, so that bone changes can be reported relative to stages of growth and development. The benefits of exercise was confirmed in a recent systematic review and meta-analysis on exercise intervention trials (Specker et al. 2015). This revue on longitudinal studies reported higher bone mineral accretion, BMC and BMD without altering bone size in adolescents enrolled in physical activity than their less active peers. Bone mineral content benefits were up to 0.8% (95% CI; 0.3-1.3) for whole body, 1.5% (95% CI; 0.5-25) for femoral neck and 1.7% (95% CI; 0.4-3.1) greater in intervention than control groups. Greater percentage changes for bone mineral density were also seen at the femoral neck 0.6% (95% CI 0.2-3.5) and at the spine 1.2% (95% CI; 0.6-1.8), predominantly among prepubertal

adolescents. Positive bone adaptations from interventions centred on physical activity appear to be greater in adolescents who are not routinely engaged in exercise (Specker et al. 2015).

However, “conflicting” views are held on the overall health of young children participating in prolonged and relatively high impact loading exercise.

High impact loading exercise during puberty was once considered to have the potential to delay puberty and damage hormonal secretion (Theintz et al. 1994) (Courteix et al. 1998). Such findings were initially used to link exercise to a detrimental effect on normal growth and maturation (Daly et al. 2005). Effectively, regular intensive exercise is known to modify circulating steroid level so it was hypothesised that prolonged exposure to intensive exercise in children may delay the timing of the growth spurt and suppress hormonal development (Jaffre et al. 2002).

However, the notion of delayed puberty and compromised endocrine secretion in even intensity training young female gymnasts was more recently dismissed on the basis of inadequate quality of data (Malina et al. 2013). Specifically, the authors concluded that high intensity training associated particularly within the high impact sport of gymnastics was not impairing aspects of normal growth beyond those expected in genetically shorter and later maturing young people; indicating full adult height was likely to be attained. However, the review of the impact of intensive exercise on the growth of young athletes also noted that endocrine data were too limited to draw conclusions about hormonal suppression relating to growth.

From a more positive perspective, it was shown that sports-related bone benefits could be observed several years after retirement from elite sports (Bass et al. 1998) (Warden et al. 2005).

Studies frequently postulate on the specific mechanisms by which physical activity influences bone mass. Assessment of bone formation and resorption markers can support observations of the acute effects of PA on bone remodeling (Maimoun et al. 2011). However, in the absence of standardised procedures and consistently selected markers, results remain inconclusive. Some studies have observed higher values in bone formation markers without concomitant changes in resorption markers among young athletes engaged in osteogenic activities (Creighton et al. 2001) while others have reported higher values in both formation and resorption markers induced by weight bearing physical activities (Maimoun et al. 2008) (Prouteau et al. 2006). Access to multiple measures of bone formation and resorption and inconsistencies in the protocols used for analyses have compounded the currently inconclusive understanding of the blood-borne, bone-related responses to exercise in young populations.

A mechanistic understanding of how bone responds to exercise may also lie in the impact of muscle on bone strength. One recent systematic review highlighted a lack of investigations on bone related responses to exercise that have included assessments of the role of muscle in the bone strength response (Tan et al. 2014). Indeed out of the 27 studies included, 65% did not assess the role of muscle mass (Tan et al. 2014). However, when assessed, a specific influence of muscle of bone parameters has been highlighted (Tan et al. 2014). The potential of a

mediation role of muscles on bone during weight bearing physical activity requires further investigation.

2.5.3.2. Nutrition

Nutritional factors are of great importance in the development and maintenance of healthy growing bones (Bass et al. 2005) (Specker et al. 2007). Calcium and vitamin D are both key nutrients in skeletal development during growth (Schoenau et al. 2002). Furthermore, optimal bone development is promoted by adequate protein, total energy and nutritional intake (Alexy et al. 2005).

Calcium

Calcium plays a vital physiological function and comprises the structural element of bones (Rizzoli 2014) (Higdon 2003). Calcium facilitates optimal gains in bone mass (Specker et al. 2007). Some questions about recommended calcium intakes to optimise peak bone mass remain inconclusive. On one hand, some researchers advocate for the importance of sufficient calcium intake during growth to maximise bone health in adulthood (Rizzoli 2014) (Huncharek et al. 2008). On the other hand, calcium supplementation during growth may not reduce fracture risk (Winzenberg et al. 2006).

Also, international guidelines differ; with 1300 mg/day recommended dietary intake in Australia for males and females aged 12 to 18 years (Capra 2006), 800 mg and 1000 mg/day for 11 to 18 year old females and males respectively in the UK (BDA 2014) and 1200 mg/day for both sexes (9 to 19 years) in France (ANSES 2016). Nonetheless, to achieve optimal skeletal

development, adolescents must consume the recommended amounts of calcium required for bone mineral accrual (Sawyer et al. 2007). Daily recommended calcium intake may also vary depending on the amount of physical activity undertaken by children (Specker et al. 2003). In adolescents males, calcium intake has been positively associated with bone mineral density (Mouratidou et al. 2013). Coupled with physical activity and exercise, calcium supplementation among pre-pubertal children appears to promote bone health in healthy children and adolescents (Julián-Almárcegui et al. 2015) (Specker et al. 2015). However, results from a well-designed randomised controlled trial involving monozygotic twins showed the effects of calcium supplementation, at least in healthy pre-pubertal children may be transient (Greene et al. 2011).

Vitamin D

Vitamin D, especially in its active form (1,25(OH)₂D₃) has an important role in development, growth and mineralisation of the skeleton (Holick 1996). Traditionally considered for its role in calcium homeostasis (Bouillon et al. 2008), vitamin D is important in the regulation of bone formation (Saggese et al. 2015) (Bikle 2016). Similarly, osteoblasts' differentiation and the secretion of bone-specific alkaline phosphatase, osteocalcin, OPG, and other cytokines are promoted by vitamin D (Clarke 2008). However, the precise role of vitamin D on the bone-fat and glucose metabolism relationship remains controversial (Giudici et al. 2017) (Reinehr et al. 2007) (Vanlint 2013). Similarly, because of the scarcity and inconsistency of the available data, the role of vitamin D on bone growth remains unclear (Specker et al. 2007). A multi-centred cross-sectional study on 100 adolescents aged 14.81 (0.99) aimed to evaluate the influence of vitamin D on bone mineral content among adolescents (Valtuna et al. 2012). The results

demonstrated two possible interactions between vitamin D and physical activity: vitamin D might improve bone mineral content in physically active adolescents or physical activity may increase BMC only in adolescents with sufficient vitamin D levels.

2.6. Bone health assessment

2.6.1. Bone densitometry devices

Several different techniques exist to assess the material properties and geometric characteristics of bone, although no single method can adequately assess bone health (Table 2). Before deciding which device to use, advantages and disadvantages of each device available for assessing the status of overall and site specific bone should be considered. Moreover, the International Society for Clinical Densitometry (ISCD) has provided recommendations and a nomenclature in order to promote excellence in the assessment of skeletal health (Crabtree et al. 2014).

Dual-energy X-ray absorptiometry (DXA) is the most common non-invasive technique for assessing paediatric bone health. However, other methods, such as peripheral quantitative computed tomography (pQCT) or quantitative ultrasonography (QUS) may provide important insight into bone size, geometry and quality information data (Specker et al. 2005). In addition to bone, these techniques have the ability to measure soft tissue such as lean and fat mass as well as muscle and fat cross-sectional area.

2.6.1.1. Dual-energy X-ray Absorptiometry

First introduced in the late 1980's and widely available for paediatric populations since the early 1990s, DXA is currently recognised as the most extensively used two-dimensional method for measuring bone mineral density (BMD). The DXA machine is a high precision and low ionizing radiation device (i.e. less than 13 μSv for regional scans and whole body scan) with short scan times (Blake et al. 2007). However, a major limitation associated with DXA stems from the two dimensional design and the inability of the device to accurately determine and adjust results for bone depth (Ward et al. 2007). Effectively, DXA is not capable of measuring vBMD; the bone microstructure elements contributing to bone strength. Without vBMD, trabecular and cortical bone cannot be distinguished (Nishiyama et al. 2012). Often studies reporting results from DXA derived data cite limitations around the quality of bone properties compared with 3D techniques and devices with more precise imaging capacity.

Classical measures

Despite some limitations, DXA is able to reliably identify bone mineral tissue, lean tissue, and adipose tissue (El Maghraoui et al. 2008). BMD obtained from DXA is known as areal BMD (aBMD) and is obtained by dividing the bone mineral content [BMC (g)] by the projected bone area (cm^2). Areal BMD is reported in grams/cm^2 . Paediatric bone density (aBMD) and bone mineral content (BMC) results are recommended by the International Society for Clinical Densitometry to be performed at the posterior-anterior spine (PA spine) and total body less head (TBLH) sites. The ISCD also recommended reporting adjusted data (i.e. bone mineral apparent density (BMAD), height Z scores) if delays in growth or short stature have been observed in children and adolescents (Crabtree et al. 2014). Z-scores can be defined as the

standard deviation (SD) scores compiled from age and sex-specific norms. To assist in the reporting of population specific data, the availability of normative data has also evolved. Several normative data bases are now available and include data for young populations of diverse backgrounds such as Non-Hispanic whites, non-Hispanic blacks, Mexican Americans (Kelly et al. 2009).

Trabecular Bone Score

Currently, high quality DXA scans provide access to information relative to skeletal microstructure. Trabecular Bone Score (TBS) is a variable derived from lumbar spine images. Indeed, TBS is a gray level textural index that mathematically estimates 3D indices from 2D projected image (Silva et al. 2014). Access to the TBS application provides an indirect index of trabecular micro-architecture. However, some limitations are associated with TBS measurements. These limitations include the absence of recommendations from the International Society for Clinical Densitometry for the use of TBS software with children and adolescents. Also, TBS has not been validated in obese populations. However, validation in populations with obesity may be challenging because TBS measures are affected by excessive abdominal soft tissue, that can lower TBS values. In addition, as TBS data are computed from DXA images, high quality image acquisition is important for reducing image noise. Finally, in order to adjust the texture of DXA images, given that both bone tissue and soft tissue absorb X-rays, it may be necessary to adjust TBS measures for estimates of BMI (Silva et al. 2014).

Hip Structural Analysis

The Hip Structural Analysis (HSA) application within DXA devices allows estimates of geometric indices of bone strength at the proximal femur (Beck 2002). Similarly to TBS, HSA provides indices related to geometrical and mechanical properties from hip DXA images. The narrow neck (NN), the intertrochanteric (IT) and the femoral shaft (FS) are the three regions analysed for HSA (Beck 2002). Derived variables from HSA are the mineralised bone surface cross-sectional area (CSA), the subperiosteal width (WIDTH), the cross sectional moment of inertia (CSMI), the endocortical diameter (ED), the profile center distance (PCD), the center of mass position (CMP), the section modulus (Z), the bulking ratio (BR) and the average cortical thickness (ACT) (Beck 2002). Limitations of HSA are similar to TBS and include concerns about 2D images and the quality of the DXA acquisition scan (Bonnick 2007). Also there is a lack of international recommendations for the use of HSA from the International Society for Clinical Densitometry. This leading organisation does not recommend the using DXA-derived hip analysis during growth due to high variability in skeletal development. However, in some conditions such as obesity, even in young people, the hip is a site of additional external load (body mass) over a prolonged period of time and may be postulated as a site of significance.

2.6.1.2. Peripheral quantitative computed tomography

A more advanced understanding of skeletal responses in adolescents is possible using peripheral quantitative computed tomography (pQCT), a 3D scanning device. The International Society for Clinical Densitometry recommends QCT as the primary research technique to characterise bone deficits in children and adolescents (Crabtree et al. 2014). The pQCT device provides a more precise image of bone than DXA and permits investigations of bone structure

(Rauch 2012). In essence, pQCT provides 3D rather than 2D images. As such, pQCT has the advantage of measuring the true vBMD of trabecular and cortical bone separately (Kontulainen et al. 2005) (Laskey et al. 2010). Furthermore, pQCT is a relatively portable device that can determine muscle cross-sectional area and enhance distinct qualitative differences in muscle and bone of individuals (Ashe et al. 2006).

Peripheral QCT image acquisitions are based on multiple factors including the number of blocks, field of view, scan speed, and voxel size (Ashe et al. 2006). Voxel size is particularly important when scanning children as it may influence the partial volume effect (PVE). The partial volume effect occurs when more than one tissue composed of different densities is present in a voxel (Gonzalez Ballester et al. 2002). Multiple and differing tissues in the same voxel may cause inaccuracies in volumetric estimates. Because both the partial volume effects and potential artefacts attributed to even a small amount of movement during scanning, pQCT resolution is usually too low to ensure a perfect image quality (Lespessailles et al. 2006). As such, the trabecular BMD reported by pQCT is likely to contain trabeculae and bone marrow.

2.6.1.3. Quantitative UltraSound

First introduced in 1984, QUS was originally developed to assess calcaneus bone status in adults (Langton et al. 1984). Measures of QUS involves a non-ionizing radiation device. The velocity and attenuation of the ultrasound wave are provided by measures of the speed of sound (SOS) and the broadband attenuation (BUA). The SOS is defined as the ratio of the traversed distance to the transit time (m/s) and is dependent on bone stiffness. Bone stiffness is an estimate of the combined effects of the density, the micro- and macrostructure, and the

elastic modulus within the bone. Broadband attenuation is defined as the frequency dependency of the attenuation of ultrasounds signals.

Commonly used in paediatric population, data from QUS has the potential to provide information about bone architecture, which may be a determinant of fracture risks. For some researchers, the QUS results are valid predictors of bone health (Weeks et al. 2016). Emphasised for its reduced costs (Fielding et al. 2003), researchers have debated its uses in osteoporosis diagnosis (Fricke et al. 2005). Interrogations on the validity of QUS on BMD assessment were highlighted (Fricke et al. 2005). In addition, a recent systematic review assessing the accuracy of QUS in paediatric osteoporosis did not support its use due to insufficient evidence (Wang et al. 2014). A recent systematic review on diagnostic devices for osteoporosis in general populations highlighted a modest capacity of QUS to indicate BMD and predict fracture (Høiberg et al. 2016). As growth is a particular period during the lifespan, abilities of QUS to predict whole body and regional DXA bone mass were reviewed (Weeks et al. 2016). Similarly, in the previously cited systematic review, a weak to moderate relationship was found between QUS measures and DXA measurement of bone mass. Moreover, the review showed the relationships between QUS and DXA measurements of bone were strongest in the most physically mature children.

A major limitation with this device relates to the indirect measure of bone mass via broadband attenuation and the speed of sound's misclassification of children and adolescents into BMD categories based on z-scores (Williams et al. 2012). In addition, broadband attenuation and speed of sound appear to be influenced by bone density and microarchitecture (Binkley et al. 2008). The influence of SOS transmission by bone density and micro-architecture may occur because the physical distribution of trabecular and cortical bone is not homogeneous within a bone's structure. Indeed, cortical bone distribution changes significantly during growth.

Although, in children, particularly in groups with obesity, the speed of sound propagation can be reduced due to the increased thickness of the overlying fat tissue (Yao et al. 2011). The position of the foot during scanning may influence BUA because of trabecular orientation (Khan 2001), which may also be problematic for tracking bone changes during growth. Finally, large degree of measurement error was found for small and young children (Weeks et al. 2016).

Table 2 - Comparison of bone densitometry techniques (modified from Binkley et al. 2008 (Binkley et al. 2008)).

	DXA			pQCT		QUS
	Classical measures		TBS	HSA		
Bone site	Whole body, Spine Hip	Lumbar	PA spine	Proximal femur (NN, IT, FS)	Radius, Tibia	Calcaneus
Parameters	Bone area BMC aBMD		TBS	BMD Bone geometry (ED, ACT, CSA) Bone strength (CSMI, Z, BR) Shaft neck angle	Bone geometry (periosteal and endosteal circumferences, cortical thickness) BMC (total and cortical) Volumetric BMD (cortical and trabecular) Estimate of bone strength (CSMI, SSI)	SOS BUA
Advantages	Rapid scan times High precision Low cost Low radiation dose Paediatric reference data		Same as classical measures Derived from DXA images (spine or hip) Estimate skeletal micro-architecture		Measures true vBMD Low radiation dose Differentiate bone tissue Measure muscle and fat Portable scanning device	Low cost No radiation Portable scanning device
Disadvantages	No differentiation of bone tissue No bone geometry measure Bone size influence aBMD		Depend on the acquisition process (image noise) Estimation of 3D indices from 2D images		Underestimate cortical vBMD if cortical shell thickness is < 2mm Only applicable to peripheral site	Bone size (cortical thickness) will influence SOS

ISCD	<u>Preferred method:</u>	No specific guideline in adolescents' population	<u>No preferred method</u>	Yet to be published
	aBMD and BMC	Not validated in obese population	Primarily research technique	
	Total body less head	Hip not preferred measurement site in growing children	to characterise bone deficits in children and adolescents	
	PA spine			
	Data adjustment			

DXA dual energy x-ray absorptiometry, pQCT peripheral quantitative tomography, QUS quantitative ultrasounds, TBS Trabecular Bone Score score, HSA hip structural analysis, PA posterior anterior, NN narrow neck, IT intertrochanteric, FS femoral shaft, BMC bone mineral content, aBMD areal bone mineral density, ED endocortical diameter, ACT average cortical thickness, CSA cross sectional analysis, CSMI cross sectional moment of inertia, z section modulus, BR bulking ration, SSI strength stress index, SOS speed of sound, BUA broadband ultrasound attenuation, vBMD volumetric bone mineral density

2.6.2. Blood markers of bone turnover

Complementary information to BMD can be obtained via biochemical markers of bone remodeling. Moreover, in young populations those markers can also reflect physiological changes in bone growth (Vasikaran et al. 2011) (Szulc et al. 2000). Indeed, the different phases of osteoblasts and osteoclasts development and activities are more accurately represented by data that describes activity within bone turnover biomarkers (Vasikaran et al. 2011) than DXA when observations occur over relatively short periods of time, such as less than 6 to 8 months.

Due to the wide number of available bone turnover markers, international standard recommendations have been set. In 2010, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the International Osteoporosis Foundation (IOF) bone marker working group recommended procollagen type 1 N-terminal propeptide (P1NP) for markers of formation and C telopeptide (CTX) for resorption (Vasikaran et al. 2011). The use of these markers was later confirmed by the National Bone Health Alliance in 2012 (Bauer et al. 2012). The bone marker P1NP is cleaved from collagen type I during the bone matrix formation, while CTx is released when the collagen within the bone is broken down.

During growth, gender differences have been highlighted in previous sections within this review on DXA-derived variables. Increased biomarkers of bone formation and resorption have also been noted at the beginning of the pubertal growth spurt. In females, markers of bone formation were highest at Tanner Stage 3; the stage at which bone mineral accrual is the most important. During the bone mineral consolidation that occurs after the pubertal spurt, activity from both biomarkers of formation and resorption are attenuated to reach adult values (Szulc

et al. 2000). Similarly, in males after their growth spurt, markers of remodeling attenuated but remain higher than in age-matched females (Szulc et al. 2000).

Caution should be used when collecting, analysing and interpreting data from bone turnover markers. Indeed, controllable and uncontrollable factors may affect bone turnover markers. Factors include circadian rhythms, renal organs, exercise, diet, and the menstrual cycle (Delmas et al. 2000) (Vasikaran et al. 2011). Standardised protocols within and between studies appear to be important. Even with well-considered protocols, large confidence intervals are typically observed in reporting bone turnover markers; reflecting substantial within and between individual variations. Also, in younger populations, biomarkers can simultaneously reflect growth, remodeling and nutritional status (Mosca et al. 2016). Moreover, a recent study from the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the International Osteoporosis Foundation (IOF) working group (Morris et al. 2016) highlighted another limitation regarding bone markers. Indeed, standardising clinical analytic methods appears to be of importance and without streamlined analysis, reports using results for between studies comparisons can lack validity, especially for CTx markers.

2.6.2.1. Analysis of bone remodeling activity

As outlined in the section above, bone turnover can be assessed by measurements of biochemical markers that reflect bone remodeling activity (Eastell et al. 1993). Despite standardised recommendations of the most suitable markers to use (IOF-IFCC 2010), different markers and methods are found in the literature for describing bone remodeling activity.

Diversity in analyses is exemplified by estimates of balance (uncoupling index) or turnover rates (bone marker plot). One of the major advantages in bone markers is the insight of a possible response within a few months while BMD responses can take longer to occur. The other major advantage lies in the capacity to understand more about the balance between bone formation and bone resorption markers. In addition, an association between P1NP and CTx markers and bone fracture prediction was shown in a meta-analysis (Johansson et al. 2014). Thus, adding measures of bone markers into DXA based studies deepen the richness of the data and provide additional options to describe bone metabolism beyond the slower modelling of bone properties that are detectable through scanning devices. Although there are multiple ways of using bone marker data, the following two approaches have been selected as relevant and innovative in this thesis.

The Uncoupling Index

The uncoupling index (UI) developed by Eastell (Eastell et al. 1993) provides insight into the relative formation and resorption balance during bone remodeling process. The UI is derived from individual z-scores calculations for formation and resorption markers. The index is calculated as the z-score formation marker minus the z-score resorption marker. A positive UI indicates an imbalance of the bone remodeling activity in favour of formation, while a negative UI indicates a remodeling activity favouring bone resorption (Lane et al. 2000). Calculations of UI were developed to compare individual values with the mean value from a reference group (Faulkner 2005). Results of the UI are expressed in standard deviations. The uncoupling index might provide complementary information helping in the understanding of DXA measurements. Previously used among elite adolescents practicing rhythmic gymnasts

the observed positive uncoupling index was associated with a greater rate of bone remodeling provided complementary information on the higher observed bone mass and density compared with control (Courteix et al. 2007).

An example of a Z-scores calculation is as follows: Person A's bone formation marker z-scores = (person A's bone formation marker value - mean of the reference group bone formation marker) / standard deviation of the reference group bone formation marker. Person A's bone resorption marker z-scores = (person A's bone resorption marker value - mean of the reference group bone resorption marker) / standard deviation of the reference group bone resorption marker.

Thus the status of bone activity is established for an individual compared with normative values for bone formation and resorption.

The bone marker plot

A bone marker plot was developed by Bieglmayer et al. (2009) with the purpose of innovatively visualising changes of in the bone resorption and formation balance as well as the bone turnover rate (Bieglmayer et al. 2009). Balance and rate of bone turnover are presented graphically with a 95% confidence ellipse which circumscribes the groups' characteristics. As shown in Figure 8 dominant resorption with a high turnover (i.e. fast bone resorption) is represented in the upper left quadrant on the graph. The left bottom quadrant symbolises slow resorption. The right side of the central vertical axis represents fast bone formation (upper right) and slow formation (bottom right). The left side of the central vertical axis represents

resorption activity. Values above the central horizontal axis represent rapid bone marker activity and values below it denote slower responses. To prepare for graphical presentation, transformation of bone formation and resorption markers concentrations require three calculations: (1) calculation of the multiple of median (MoM), (2) calculation of the balance ratio (MOM_F/MOM_R) and the turnover rate ($\sqrt{MOM_F^2+MOM_R^2}$), and (3) logarithmic transformation.

Interpretation of the marker plot relies on the distributions of participants among the features of the four-quadrant graph. For example, based on Figure 8, the distribution among participants was 6% in slow formation, 18% in fast resorption and 76% in fast formation.

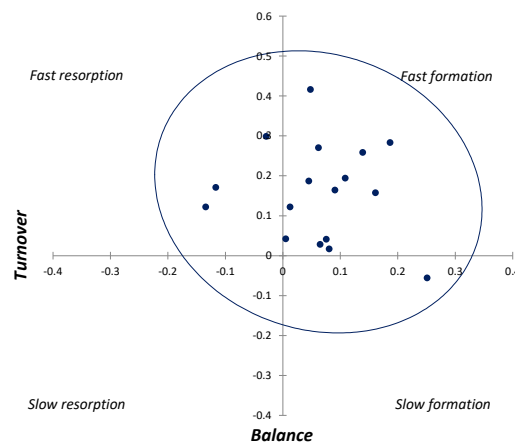


Figure 8 - Bone marker plot four-quadrant graph

Bone marker plot cross-sectional analysis with 95% confidence ellipse from 17 females' adolescent (data from the normal weight control group of the ADIBOX study) calculated from CTX-P1NP.

2.7. Summary

Bone tissue undergoes rapid changes during growth. In childhood and adolescence bone tissue continuously adapts to meet functional needs of young populations through synergies between non-modifiable and modifiable factors. Childhood and adolescence are recognised as critical periods for maximising bone mass and strength.

During growth, bone strength and muscle mass follow a linear relationship, supporting the theory of mechanical stressors that can strongly explain bone modelling and remodeling. However, traditional mechanical discussions of bone mass and strength accrual have been deepened by including biochemical interactions. The biomechanical communication extends the possible explanations of mechanisms within bone responses. Indeed, bone tissue is subject to multiple hormone-released products derived from bone and directly or indirectly derived from adipose tissue.

This complex cross talk between bone and adipose tissue highlights bone as an active organ reciprocally influencing and influenced by other tissues/organs. In addition to mechanistic cross talk, other factors such as physical activity and nutrition influence the human body and bone development. Physical activity and nutrition are both important lifestyle factors to optimise bone mineral acquisition. Sedentary behaviour, lower physical fitness and poor nutrition intake (i.e. lower levels of calcium and vitamin D) constitute global health concerns that might decrease metabolic health through suboptimal fitness, as well as potentially increasing sports-related injuries and fracture risks.

Currently, various diagnostic devices are available for the assessment of bone health. However, the addition of conditions such as obesity to adolescent growth can confound the reported accuracy of these devices. Densitometry devices weakness should be considered as none can

adequately assess bone health. However, coupled with bone turnover markers, complementary information reflecting physiological changes in bone growth can be obtained.

Common child and adolescent conditions such as obesity can exacerbate or attenuate some typical hormonal functions in bone regulation. Greater focus on the impact of obesity on bone during adolescent growth using a combination of densitometry and innovative reporting in blood-borne growth and bone marker measures may strengthen the evidence to include bone health in future health goals for adolescents with obesity.

Obesity and bone tissue: specificity of adolescence

2.8. Obesity

According to the World Health Organisation (WHO), obesity is defined as excessive fat accumulation that may impair health (WHO 2000). The rising prevalence of overweight and obesity in paediatric populations has become a major concern (WHO 2000). Globally, the estimated 155 million school-aged children who are overweight or obese represent a 47% increase between 1980 and 2013 (Ng et al. 2014). National health promotion strategies have been devised to prevent health risks associated with obesity and where appropriate, to manage existing overweight obesity in most developed nations. These strategies include the “Plan National Nutrition Santé” in France or the NH&MRC’s “Clinical practice guidelines for the management of overweight and obesity in adults, adolescents and children in Australia” in Australia. However, prevalence rates of overweight or obesity in young people aged less than 20 years in Australia are 24% in males and 23% in females while in France the prevalence rates are 20% for males and 16% for females (Ng et al. 2014).

Obesity is an economic burden and a health care national priority in developed countries, once again including France and Australia. Indeed, in France, in 2006, it represented between 2€ to 6€ /\$3 to \$7 billion and between 1.5 and 4.6% of the current health expenditure (Emery et al. 2007). In Australia, the costs of obesity were estimated at \$58 billion (Colagiuri et al. 2010). Beyond the global economic concern, is the fact that undesirable consequences of unhealthy lifestyles during the two first decades of life can lead to long-term serious health problems; which can commence even in childhood. More than just fitness, motor and psychological issues

(i.e. depression, body image, self-esteem) associated with obesity can lead to metabolic complications such as hypertension, dyslipidaemia, insulin resistance and diabetes. Also obesity can alter an individuals' bone health and potentially exacerbate the onset of osteopenia (Daniels 2009) (Ebbeling et al. 2002).

2.9. Bone mass in obese youth

Currently, the complex relationship between fat mass and bone mass is well established (Shapses et al. 2012). Obesity effectively leads to hormonal alterations associated with increasing pro-inflammatory cytokines and oxidative stress; favouring the accumulation of fat mass and loss of bone mass (Shapses et al. 2012). In fact, excess body mass plays an important role in the mechanical response of the skeleton (Frost 2003) via dysregulation attributed to adipocyte production that leads to metabolic dysfunction (Karsenty 2006) (Lee et al. 2007). Moreover, the distribution (subcutaneous, central or visceral) of adipose tissue could be a relevant confounder in this complex process that links obesity to osteoporosis (Júnior et al. 2013). A recent systematic review and meta-analysis (Van Leeuwen et al. 2017) included 27 studies (only 1 longitudinal study) with population ranging from 2 to 18 years. Results highlighted greater unadjusted total body bone mineral content and density in children who were overweight or obesity than their lean peers. Authors also noticed an overall trend for higher unadjusted bone density at specific site (i.e. spine, femoral neck) in children who were overweight or obese compared with their leaner peers. Due to inconsistently reported data within the 27 studies, analysis of adjusted data was not described. It is possible that knowledges of the impact of obesity on bone, in adolescents would have been more advanced

if data could have been reported using adjustments for key factors such as body weight, fat mass, lean mass, and/or bone size.

Subsequently, contrary to adults where obesity has been extensively studied (Zibellini et al. 2015) (Soltani et al. 2016), the obesity relationship with bone during adolescence lacks analysis. As shown in the recently published systematic review and meta-analysis (Van Leeuwen et al. 2017), most of the available evidence is limited by a combination of overweight plus obesity rather than obesity only.

This review targets only obesity (not obesity plus overweight) during adolescence. However, due to the paucity of available literature, the review of studies relating to obesity and bone within the remainder of this chapter has included children and adolescents with obesity. Despite ISCD recommendations, with the exception of one study (Dimitri et al. 2010), none of the studies reviewed for this thesis did not reported total body less head BMD. The following reviewed literature first synthesises evidence from cross sectional studies (age between 10 to 17 years) and then extends into current information about obesity and bone from interventional designs (age between 9 to 17 years).

2.9.1. Analysis via DXA

Whole body analysis

Systematically reviewed, the available literature did not allow a consensus relating to bone mass and obesity using the whole body analysis. On the available evidence, some studies reported similar (El Hage et al. 2012) (Fintini et al. 2011) (Russell et al. 2010), or higher (Maggio

et al. 2011) (Rocheffort et al. 2011) (Rocher et al. 2013) or lower (Dimitri et al. 2010) unadjusted BMD in children and adolescents with obesity compared with their normal weight peers. When BMD data were adjusted for confounders, in order to reduce the risk of measurements variance due to body weight and body size, all results showed lower values for WB BMD in adolescents with obesity (El Hage et al. 2012) (Ellis et al. 2003) (Rocher et al. 2008) (Rocher et al. 2013). Even if results were inconsistent in raw WB BMD findings (El Hage et al. 2012) (Maggio et al. 2011), all studies demonstrated higher values for whole body BMC in adolescents with obesity (El Hage et al. 2012) (Ellis et al. 2003) (Haroun et al. 2005) (Maggio et al. 2011) (Rocher et al. 2008).

Volumetric whole body mineral density was assessed in two studies: adolescents with obesity had lower values while there was no significant difference reported in the WB BMD between normal weight and adolescents with obesity (El Hage et al. 2012) (Rocher et al. 2008).

Regional analysis

Similar inconsistency was observed in regional analysis. Indeed, at the lumbar spine higher BMD or BMC values were observed in some (Fintini et al. 2011) (Hasanoğlu et al. 2000) (Rocher et al. 2008) (Rocher et al. 2013) but not all studies (Dimitri et al. 2010) (Fintini et al. 2011). For volumetric data, studies showed higher LS BMAD results in population with obesity than their leaner peers (Dimitri et al. 2010) (Rocher et al. 2008) (Fintini et al. 2011).

At the femoral neck and total hip, studies also showed higher BMD in adolescents with obesity (El Hage et al. 2012) (Rocher et al. 2013). To have further investigate structural geometry of

cross-sections traversing the proximal femur, one study performed hip structural analysis (HSA) at the femoral neck and the shaft (Rocher et al. 2013). Results highlighted a higher cross-sectional area (CSA) at the femoral neck only. However, no differences between adolescents with obesity and the control participants were found for section modulus at both sites (Rocher et al. 2013). After adjustment for body weight, all HSA values became significantly lower in adolescents with obesity.

Meta-analysis of cross sectional data

As the effect of excess body weight on bone health remains inconclusive, we aimed to determine by meta-analysis the effect of obesity on bone health in children and adolescents (Chaplais E 2017). Twelve cross sectional studies assessing bone health by DXA were included in this meta-analysis. Participants had a mean age ranging between 10 to 17 years. The meta-analysis methodology and details from included studies can be found in the appendix (Appendix 9). Results from the meta-analysis for unadjusted whole body BMC, unadjusted whole body BMD, unadjusted lumbar spine BMD and LS BMAD are reported in Table 3.

The meta-analysis confirmed that children and adolescents with obesity had significantly higher raw bone content and density than their normal weight peers (Figure 9). In the present analyses, data were not adjusted for confounders since most of the studies included in the meta-analysis present unadjusted data only.

Table 3 - Meta-analysis results from cross-sectional studies

	<i>n</i>	<i>Effect size</i>			<i>95% CI</i>		<i>p-value</i>	<i>Heterogeneity</i>			
		Min	Max	Mean	Min	Max		<i>I</i> ²	<i>Q</i>	<i>df</i>	<i>p-value</i>
Whole body BMC	8	0.155	1.750	1.019	0.629	1.409	<0.001	81.75	38.36	7	<0.001
Whole body BMD	10	-0.131	1.154	0.568	0.273	0.863	<0.001	67.14	27.39	9	<0.001
Lumbar spine BMD	13	-0.169	1.121	0.529	0.260	0.798	<0.001	72.87	44.23	12	<0.001
Lumbar spine BMAD	5	0.000	1.100	0.653	0.292	1.013	<0.001	58.29	9.59	4	0.048

BMC bone mineral content, BMD bone mineral density, BMAD bone mineral apparent density

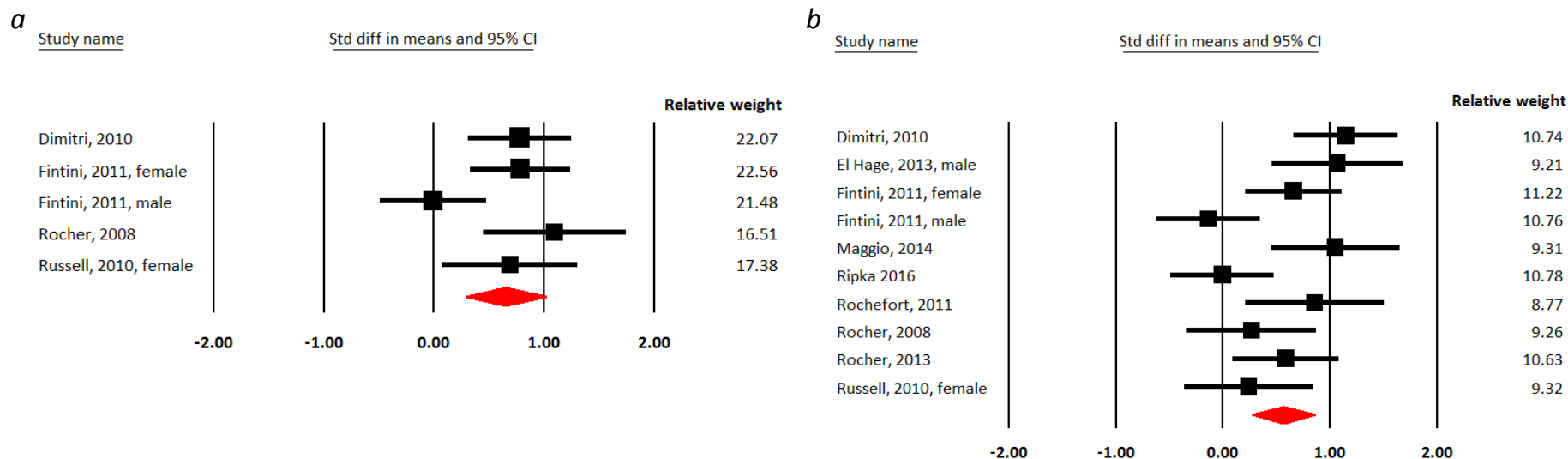


Figure 9 - Effect size forest plot for the effects of obesity on lumbar spine bone mineral apparent density (a) and whole body bone mineral density (b).

2.9.2. Analysis via pQCT

There is a paucity of literature relating to three-dimensional analysis as only 2 of the 7 studies used pQCT on adolescents with obesity. Indeed, most of the studies included both overweight and obese adolescents (Laddu et al. 2013) (Farr et al. 2011) (Ehehalt et al. 2011) or late adolescents (Viljakainen et al. 2015) (Pollock et al. 2011).

Bone geometry and volumetric density assessed by pQCT revealed that at the tibial site, similar results were observed between an obese and non-obese groups for trabecular and cortical volumetric density (Dimitri et al. 2015) (Leonard et al. 2015). Adolescents with obesity had a greater cortical section modulus, and a greater cortical periosteal circumference than non-obese participants at the tibia (Leonard et al. 2015). Also, one study found lower trabecular thickness and cortical pore diameter in adolescents with obesity (Dimitri et al. 2015). Similar results were found for trabecular and cortical volumetric density between groups at the radius (Dimitri et al. 2015) (Leonard et al. 2015). Cortical periosteal circumference at the radial site was higher among adolescents with obesity than their normal weight peers (Leonard et al. 2015) and cortical porosity and cortical pore diameter were lower in obese than non-obese groups (Dimitri et al. 2015).

2.9.3. Analysis via biomarkers of bone formation and resorption

Bone biomarkers were analysed in one cross sectional study recruiting 391 adolescents (105 obese and 46 extremely obese) aged between 10 to 19 years (Mosca et al. 2016). When classified according to age group, higher levels of osteocalcin (at the age of 14 to 15 and 16 to 19 years) and CTx (16 to 19 years) were observed in females with excess weight compared with

their normal weight peers. In males with excess weight, lower levels of both osteocalcin and CTx were observed between the ages of 14 and 15 years. In addition, results highlighted a correlation between fat mass and bone markers in females but not males. Indeed, levels of bone markers were lower when fat mass or fat percentage was higher.

2.10. Factors influencing bone mass

2.10.1. Puberty and gender

Whole body analysis

Puberty and gender effects on bone density are well established (Nagasaki et al. 2004) (Fintini et al. 2011). Indeed, females (Rogol et al. 2002) and adolescents with obesity (Wang 2002) experience an earlier maturation than males. Unlike normal weight adolescents for whom the gain in BMD begins to increase from about 12 years of age, children and adolescents with obesity display higher BMD values for bone age compared with reference values before puberty and lower BMD values after puberty. Males with obesity could be predicted to have higher (Tanner Stage 3-4) or lower (Tanner Stage 5) whole body BMD; depending on maturation (Fintini et al. 2011) (Nagasaki et al. 2004). Alternatively, in females with obesity, BMD is likely to increase with advancing puberty (Tanner Stages 3-4) (Fintini et al. 2011) (Nagasaki et al. 2004). Further gender differences were identified by BMD values in females being higher than males with similar levels of obesity (Fintini et al. 2011) (Maggio et al. 2011) (Nagasaki et al. 2004).

Only one study questioned the impact of maturation status on bone over time in adolescents with obesity. Results showed similar sex-hormones status (oestradiol) among adolescents with

obesity compared with normal-weight adolescents; however, the control group was of a younger chronological age (Klein et al. 1998).

Regional analysis

Similar to whole body BMD, maturation stages appear to influence lumbar spine BMD. Young males with obesity had higher lumbar spine BMD values at Tanner Stages 3 to 4 than Tanner Stage 5 (Fintini et al. 2011). While the overall results showed higher LS BMAD for females with obesity, when only the participants from Tanner Stages 3 to 4 were considered similar LS BMAD was observed between adolescents with obesity and their normal weight peers (Fintini et al. 2011) (Russell et al. 2010). Therefore, regional analysis in adolescents with obesity may require strong consideration for maturational status for data interpretation.

2.10.2. Fat tissue

Despite an emerging interest in the relationship between fat mass and bone mass among adolescents with obesity, results remain contentious. Studies assessing total body fat mass suggest a negative association between the percentage of fat and bone measures (Pollock et al. 2007) (Rhie et al. 2010) (Ripka et al. 2016). One exception was reported with a positive relationship between fat mass and bone mass. However, when adjusted for height and lean body mass, no differences between fat mass and bone mass were evident (Shaikh et al. 2014). In order to better understand the role of fat mass on bone mass interactions, recent studies have more specifically investigated the effects of fat mass distribution on bone health. Using magnetic resonance imaging (MRI), a reciprocal association was observed between

subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT) and bone density (Russell et al. 2010). Specifically, the investigation involved the relative proportion of VAT and SAT which determined concentrations of adipokines in the circulation and the subsequent effect of BMD. These results are confirmed by others who have reported a negative association between abdominal obesity (Júnior et al. 2013), VAT (Campos et al. 2012) and bone density as well as a positive association between SAT and bone density (Campos et al. 2012). In addition, a systematic review (Sioen et al. 2016) recently demonstrated that the association between fat mass and bone parameters was contradictory depending on participants' age and sex. However, the site of fat mass may also be salient factor.

2.10.3. Role of hormones?

To better understand interrelations, it seems necessary to focus on the potential interactions between adipokines and the physiological factors involved in bone metabolism. Indeed, the skeletal system is not only stressed from mechanical loading, but also through the metabolic effect of some of the proteins (adipokines) secreted by the adipose tissue (Klein et al. 1998). Adipokines play important roles in the modulation of biological functions and could potentially impair skeletal acquisition in children and adolescents with obesity (Dimitri et al. 2011).

2.10.3.1. Leptin and adiponectin: potential contributors to BMD?

Leptin

More frequently described in cross sectional than longitudinal studies, higher serum leptin and

lower adiponectin levels (Cao 2011) (Giudici et al. 2017) are associated with obesity. As stated previously, the actions of leptin appear to be complex depending on the activated pathway, with the potential for both positive (peripheral pathway) and negative effects (central pathway) and may depend on the mode of activation (Bonnet et al. 2005). In addition, a gender effect, independent of pubertal status, might be observed in relation to the influence of leptin on bone.

Current evidence offers little consistency in discussions around leptin levels or its implication on bone in young populations with obesity. Some studies report no difference in leptin levels between children and adolescents with obesity and their normal weight peers (Russell et al. 2010) (Klein et al. 1998). In contrast, others report higher leptin levels in children and adolescents with obesity (Vandewalle et al. 2013) (Rhie et al. 2010) (Dimitri et al. 2011) (Giudici et al. 2017). However, independent of the age, female with obesity have higher leptin levels than males with obesity (Klein et al. 1998) (Campos et al. 2012) (Do Prado et al. 2009). Differences in leptin levels might be also mediated and moderated by the maturation process. A pubertal stage effect can be observed on leptin levels and be explained by an acceleration of the maturation process in the presence of secretions of steroid hormones. Indeed through the action of estrogen and progesterone stimulating adipose tissue acquisition, leptin will be secreted in higher levels in females than males, whereas in males, testosterone will foremostly stimulate muscle mass. Leptin secretion is positively correlated with body fat mass and can lead to leptin “resistance”. Leptin resistance corresponds to a state of hyperleptinemia, when elevated leptin levels fail to suppress feeding sensations (Myers et al. 2008) (Crujeiras et al. 2015). As leptin is a major regulator of bone mass (Karsenty 2006) a leptin deficiency may alter BMD (Ducy et al. 2000). Leptin is also increased by emotional stress which is one of the multiple

environmental factors potentially leading to obesity (Kohlboeck et al. 2014) (Sominsky et al. 2014). Leptin's action on bone metabolism remains controversial. There is some support for leptin as a positive predictor for BMD in both pubertal and prepubertal females with obesity (Russell et al. 2010) (Rhie et al. 2010). However, there is also discussion of a contrasting inverse association between leptin and BMD in males with obesity (Do Prado et al. 2009). Moreover, others find a negative correlation in females with obesity (Campos et al. 2012).

Adiponectin

There is a paucity of data on the role of adiponectin in obesity on bone parameters. Obesity tends to reduce adiponectin levels independent of the well-established contribution of fat mass, gender, ethnicity, and dietary status.

In contrast, some studies report no relationship between adiponectin levels and weight status (Russell et al. 2010) (Rhie et al. 2010) (Abseyi et al. 2012). Moreover, one study reported an absence of adiponectin differences between genders in individuals with obesity (Campos et al. 2012). Only two of the included bone related studies reported lower adiponectin levels in adolescents with obesity than their leaner peers (Dimitri et al. 2011) (Giudici et al. 2017). To sustain its potential action on bones, an inverse relationship has been suggested between adiponectin levels and bone accrual in children, with adiponectin levels that were negatively correlated with BMD (Russell et al. 2010) (Rhie et al. 2010). Recently, a study assessing 198 adolescents aged between 14 to 18 years highlighted a positive association ($r^2=0.035$, $p=0.005$) between adiponectin and the uncarboxylated form of osteocalcin (Giudici et al. 2017). The positive association suggests a role of circulating adiponectin in the osteocalcin and glucose homeostasis relationship among adolescents with obesity (Giudici et al. 2017). Also,

adiponectin levels were found in lower concentrations in young patients with metabolic syndrome (Abseyi et al. 2012).

2.10.3.2. Bone hormones: potential actor on energy metabolism?

Osteocalcin

The level of circulating osteocalcin remains poorly documented. Some studies observed no differences (Rhie et al. 2010), while others reported lower levels of osteocalcin in children and adolescents with obesity (Abseyi et al. 2012) (Garanty-Bogacka et al. 2013) (Giudici et al. 2017). Independent of its expression (unOC, OC, total), pubertal status does not appear to interact with osteocalcin levels (Abseyi et al. 2012) (Garanty-Bogacka et al. 2013). Moreover, no association between osteocalcin levels and the presence of metabolic syndrome has been reported (Abseyi et al. 2012), which contrasts with other reports of a negative correlation with an insulin resistance index ($r=-0.33$, $p<0.001$) (Garanty-Bogacka et al. 2013). A favourable function of circulating osteocalcin on glucose homeostasis among children and adolescents with obesity is then suggested (Garanty-Bogacka et al. 2013). Although this was not in adolescents with obesity, a potential role of ucOC was confirmed in the skeletal regulation of energy metabolism in non-obese postmenopausal women (Schafer et al. 2011). The increased unOC was associated with decreased body fat, and increased adiponectin levels. Further analyses are needed for adolescents with obesity to better understand the implication of osteocalcin in the adipocyte/osteocyte interaction, more specifically, the uncarboxylated form that regulates glucose homeostasis (Yang et al. 2011).

Sclerostin

Sclerostin is yet to be investigated in adolescents with obesity even in cross sectional studies. However, its effects on older adults with obesity has been reported (Armamento-Villareal et al. 2012). In older adults, exercise prevented an increase of sclerostin, in a similar way that it prevents bone loss and increases bone marker turnover. Because sclerostin is released with mechanical unloading, sclerostin should be considered as part of the outcome variables in intervention involving exercise for young people with obesity.

2.11. Effects of obesity intervention on bone parameters

Strategies and guidelines to address the high prevalence of obesity have been published and are frequently updated. Because obesity is mainly due to an imbalance between energy intake and energy expenditure, obesity programs based on caloric restriction and/or physical activity training have been proposed.

The effects of exercise training versus caloric restriction on body weight (overweight plus obesity) were compared in a systematic review and meta-analysis (Verheggen et al. 2016). Results showed that even in the absence of weight loss, exercise training was related to decreased-fat tissue, in particular visceral adipose tissue (Verheggen et al. 2016). Also, the effectiveness of lifestyle interventions on specific criteria such as weight loss, comorbidities, health behaviour, side effects and quality of life was assessed in overweight children (Reinehr 2011). Their review highlighted a lack of efficacy within lifestyle interventions. Indeed, most of the studies targeted weight loss only, ignoring the sustainability of weight loss, comorbidities

(Martin et al. 2014) (Oude Luttikhuis et al. 2009) (Mead et al. 2016). A third of the included studies were short-term interventions (from 6 to 12 weeks). However, it is well known that long-term interventions (6 to 12 months) rather than short-term interventions are more efficient to sustain weight loss (Reinehr 2011). In addition, in the context of bone health, short term interventional studies provide some bias as the bone remodeling cycle takes 4 to 6 months (Shapses et al. 2012).

As previously stated, little is known about the effectiveness of structured intervention with a physical activity component on bone density in children and adolescents with obesity (overweight is not considered here). First, intervention programs focusing only on the physical activity effects on bone will be reviewed. In those programs, weight loss was not the primary outcome. Specifically, researchers were interested in positive associations between physical activity on bone health among adolescents with obesity. Second, obesity programs inducing weight loss on bone will be discussed.

2.11.1. Effects of physical activity intervention on bone in the absence of reporting weight loss

Programs only based on physical activity appear counterproductive to bone density accretion in adolescents with obesity. Based on the available literature, five studies described the effects of exercise training on bone health among adolescents with obesity. Interventions were a combination of supervised physical activity performed two (Rocheffort et al. 2011) or three times a week (El-Hage et al. 2009) (Lau et al. 2010) (McGuigan et al. 2009) (Tsang et al. 2009). All studies included children and adolescents with obesity who were part of single sex groups or combined sex groups who were exposed to a physical activity intervention.

A large heterogeneity between studies in the age of children and adolescents can be observed. Indeed, age ranged from 15.8±0.8 (El-Hage et al. 2009), 13.4±2.1 (Tsang et al. 2009), 12.4±1.8 (Lau et al. 2010), 9 to 12 years (Rocheffort et al. 2011) and 7 to 12 years (McGuigan et al. 2009). The duration of intervention also varied from 6 weeks (Lau et al. 2010), 8 weeks (McGuigan et al. 2009), 12 weeks (El-Hage et al. 2009) and 26 weeks (Tsang et al. 2009) (Rocheffort et al. 2011). Details of the studies can be found in appendix (Appendices 8 & 9).

Endurance training programs were conducted and assessed by El Hage et al. (2009) and Rocheffort et al. (2011). One endurance based approach involved a physical activity program three times per week. The researchers reevaluated the intensity at mid intervention. Details of the incremental aerobic training intervention are outlined in Table 4 (El-Hage et al. 2009).

Table 4 - Aerobic based intervention described by El Hage et al. 2009 (El-Hage et al. 2009)

Weeks	Session 1	Session 2	Session 3
1 to 6	90 minutes predominantly aerobic + strength, proprioceptive exercise and stretching	60 minutes aerobic @ 70% max aerobic speed	90 minutes sports based
7 to 12	90 minutes individual sports (badminton, tennis)	30 minutes high intensity interval training: 15s at ~100% max exercise 15s rest	90 minutes modified sports (larger area or few players)

In the other endurance based study the impact of aerobic activities on skeletal changes was tested in three groups: obese trained, obese untrained, and for baseline only, an age matched non-obese group (Rocheffort et al. 2011). The intervention involved 6 months of individualised

aerobic program running for 90 minutes, twice a week (cycling, rowing, jumping, games, hip hop). Few details were specified within the exercise intervention.

Some interventions have involved resistance training for adolescents with obesity (Lau et al. 2010) (McGuigan et al. 2009). Specifically, a weekly load for resistance training over the 6 weeks comprised of three sets of 5 to 8 repetitions at 75/85% 1RM. Exercises for the upper body included shoulder press and biceps curls and for the lower body, leg press and extensions. Then participants also performed 3 sets of a custom designed circuit based training. Adolescents were allowed 3 to 5 minutes of rests between sets (Lau et al. 2010). An alternative resistance training regime centred on a combination of different body weight and power exercises varying training loads and increasing intensity (McGuigan et al. 2009). An outline of this program is presented in Table 5.

Table 5 - Aerobic based intervention described by McGuigan et al. 2009 (McGuigan et al. 2009)

Weeks	Session 1	Session 2	Session 3
1 to 8	3 sets - 8 to 10 repetitions, with 90s rest e.g. squats, bench press, sit-ups	<u>high volume moderate intensity</u> 3 sets - 10 to 12 repetitions, with 60s rest e.g. squats, biceps curls, heel raises	<u>moderate to high explosive exercise</u> 3 sets - 5 to 8 repetitions, with 1800s rest e.g. squats jumps, rows, hang pulls

Innovatively, a sport related intervention was also conducted for adolescents with obesity by comparing the effects of Kung Fu participation with Tai Chi over 26 weeks (Tsang et al. 2009).

Three 60 minute sessions comprising approximately 40 minutes of active exercise used basic

non-contact Kung Fu technique focusing on mitts and kicking with these adolescents (Tsang et al. 2009).

At the end of each of this exercise intervention, major finding emerged. The exact impact of physical activity interventions (aerobic, resistance or sports related activity) on bone health remains unclear. Indeed, 3 of the 5 physical activity studies recorded higher (El-Hage et al. 2009) (Lau et al. 2010) or similar (McGuigan et al. 2009) whole body BMC following the intervention, however results with comparison groups were not reported. Despite some reports of higher values of BMD in the targeted groups after the physical activity (El-Hage et al. 2009) (Tsang et al. 2009) (Rochefort et al. 2011), the two studies with comparison groups lacked differences between their trained and untrained groups. Thus an intervention effect was not supported in these two studies for which the duration of the intervention exceeded 6 months (Tsang et al. 2009) (Rochefort et al. 2011). It is possible that higher BMD values in young populations over prolonged period of time can be attributed to growth, independent of physical activity interventions. The absence of comparison groups in some studies precludes any strong trends for the impact of physical activity intervention on whole body bone parameters in adolescents with obesity.

With equivocal results from WB BMD analysis, the site-specific nature of weight bearing physical activity may show greater sensitivity in regional rather than WB analysis. Similar to results from WB analysis significance regional changes in the exercise group failed to reach significance in comparison with untrained groups (Tsang et al. 2009). The absence of differences in regional bone density between both obese and comparative groups may once again be attributed to skeletal growth.

Finally, a dearth of literature currently describes the bone marker responses to training in adolescents with obesity. Only one endurance based study reported that training increased insulin, osteocalcin and uncarboxylated osteocalcin levels and decreased adiponectin levels compared with normal-weight controls and obese baseline measures (Rocheffort et al. 2011). However, only the uncarboxylated form of osteocalcin was significantly higher in the trained than untrained group at program completion (Rocheffort et al. 2011). The lack of significant difference in total osteocalcin levels between trained and untrained adolescents with obesity after 6 months, may be attributed to elevated uncarboxylated osteocalcin while the carboxylated form stored in the bone matrix decreased; unfavourably up-regulating glucose homeostasis. These results highlight the necessity of additional analysis to distinguish between carboxylated and uncarboxylated osteocalcin for outcomes relating to metabolic profiles.

Only one study could report that leptin levels remained unchanged even when the relative leptin (leptin/FM) value decreased to improve leptin sensitivity following the intervention (Lau et al. 2010).

2.11.2. Effects of intervention directly targeting the impact of weight loss on bone

The positive effects of weight loss interventions combining nutrition and physical activity on BMI and fat mass are well established (Gajewska et al. 2013) (Campos et al. 2012) (Campos et al. 2013) (Reinehr et al. 2010) (Stettler et al. 2008) (Campos et al. 2014). However, the effects of such interventions on the dialogue between adipokines and bone density remain uncertain. Beyond the primary outcome of weight loss via decreases in BMI and fat mass, secondary

markers of overall health most frequently disregard bone.

Protocols include combination of nutrition education and restriction, physical activity and psychological support (Campos et al. 2012) (Campos et al. 2013) (Reinehr et al. 2010) (Stettler et al. 2008), nutrition education and restriction and physical activity (Campos et al. 2014) (Gajewska et al. 2013), or physical activity, diet counselling and psychological support (Blüher et al. 2014). The duration of interventions ranged between 12 weeks (Gajewska et al. 2013), and 52 weeks (Campos et al. 2013) (Campos et al. 2014) (Campos et al. 2012) (Stettler et al. 2008) (Blüher et al. 2014) (Reinehr et al. 2010). Details of the studies can be found in appendix (Appendices 8 & 9).

Nutritional advice largely focused on recommended dietary intakes for adolescents with low levels of physical activity, based on age and gender. Targeted ranges for balanced macronutrients described approximately 30 to 38% fat, 13 to 20% protein and the remaining intake in carbohydrate (Campos et al. 2012) (Campos et al. 2013) (Reinehr et al. 2010) (Stettler et al. 2008) (Campos et al. 2014) (Gajewska et al. 2013). Often a nutrition education approach was adopted. Only one study offered generic diet counselling (Blüher et al. 2014).

More diversity were seen in the prescription of physical activity. Some intervention used supervised aerobic and/or resistance training (Campos et al. 2012) (Campos et al. 2013) (Campos et al. 2014) (Reinehr et al. 2010) (Blüher et al. 2014), while others provided unsupervised exercise sessions (Stettler et al. 2008) (Gajewska et al. 2013) (Blüher et al. 2014).

Descriptions of weight loss targeted interventions using aerobic and/or resistance training are summarised in Table 6. More detailed exercise prescription is largely observed in the most recent studies compared with the first reports of exercise training for weight loss in

adolescents with obesity. Moreover, the absence of considerations about weight bearing activities to facilitate bone health, prescribed intensity and progression within the duration of the program to assist in increasing metabolic activity may be major limitations to the existing literature.

Densitometry investigation showed higher baseline bone mineral content in adolescents with obesity than normal weight controls. However, after 12 months of weight loss programs no changes were observed in the BMC of the intervention groups (Campos et al. 2013) (Stettler et al. 2008). Total bone density decreased concurrently (Campos et al. 2013) or a decrease in upper and lower limbs BMD was observed. Also, results included increases in whole body and lumbar spine bone density (Stettler et al. 2008). In contrast, increases in BMC were reported without changes in BMD (Campos et al. 2012). The absence of changes in BMD may be related to some additional investigations involving bone markers. For example, after 3 months of weight loss intervention, there were lower levels of bone alkaline phosphatase, which is a sensitive and reliable indicator of bone formation (Gajewska et al. 2013).

At the end of weight loss interventions involving exercise prescription, controversial results were observed for blood markers. Indeed, some researchers reported that a substantial weight loss was associated with lower insulin (Campos et al. 2012), higher osteocalcin levels (Reinehr et al. 2010), lower leptin levels (Reinehr et al. 2010) (Gajewska et al. 2013) (Blüher et al. 2014) (Campos et al. 2014) and higher adiponectin levels (Reinehr et al. 2010) (Campos et al. 2013). In contrast, others report no within group changes in adiponectin (Blüher et al. 2014) (Campos et al. 2012) and leptin levels (Campos et al. 2012) (Campos et al. 2013) following the

intervention. Only one study has investigated the relationship between ghrelin and bone metabolism in response to weight loss in adolescents with obesity.

Table 6 - Outlines of weight loss targeted interventions

Authors	Years	Training descriptions	
Campos et al.	2012	30 minutes moderate intensity aerobic training	+ 30 minutes resistance training (ACSM guidelines)
	2013	e.g. treadmill and cycle ergometer	e.g. chess press, leg press
Campos	2014	60 minutes moderate intensity aerobic training e.g. treadmill and cycle ergometer	<u>versus</u> 30 minutes moderate intensity aerobic training as described in Campos et al. 2012 and Campos et al. 2013
		+ 30 minutes resistance training	
Reinehr et al.	2010	aerobic exercise once weekly with sports related activity	
Blüher et al.	2014	120 minutes /week: 90 minutes	+ 60 minutes
		supervised aerobic + resistance training 120 minutes (weekly goals setting)	unsupervised activity
Stettler et al.	2008	unsupervised exercise e.g. walking or other aerobic activity	
Gajewska et al.	2013	No details provided	

Although modifications to ghrelin levels remained unaltered between groups, the potential role of ghrelin was hypothesised to be a predictor of reduced total BMD. However, without

correlation analysis between these factors. However, this hypothesis remains untested. Also, cautious interpretation of results is required due to unequal baseline values between groups with the combined aerobic and resistance training group showing lower baseline ghrelin concentrations than the aerobic group (Campos et al. 2014).

Conclusions on the most efficacious exercise and dietary prescriptions for promoting weight loss and bone health in adolescents with obesity remain elusive. Results from a systematic review on weight loss in young people with obesity recently highlighted the need for structured exercise with strategic prescription planning that centred on the intensity, exposure time and frequency of physical activity in combination with dietary restriction/education (Hernandez et al. 2015). However, increased metabolic activity required for weight loss may or may not simultaneously promote bone health. For example, high intensity interval training can increase metabolic activity and facilitate weight loss. However, the impact on bone may depend on whether the prescribed medium of exercise is weight bearing or weight supported (Vico 2008). If high intensity interval training is performed on a weight supported cycle ergometer, exercise may be more sustainable than weight bearing activity on a treadmill under the same prescribed intensity. Future challenges may lie in investigations optimising exercise prescriptions to increase sustainable metabolic activity that also promotes both weight loss and bone health.

2.11.3. Meta-analysis on the effects of structured intervention on bone parameters

A meta-analysis was performed in this thesis to assess the effect of interventions that included physical activity on bone health in children and adolescents with obesity (Chaplais E 2017). The

meta-analysis search strategy, method and details from included studies are located in appendix (Appendix 9). In the present analyses, data were not adjusted for confounders such as body weight, fat mass and lean mass, since most of the included studies present unadjusted data only. Including only bone and physical activity related studies inducing weight loss would have been ideal the scarcity of studies would have limited the capacity for a meta-analysis and meta-regression. Therefore, the following results include some physical activity intervention affecting bone health without reporting weight changes.

Contrary to the studies reviewed previously, the analysis was limited to studies with participants having a mean age between 9.7 to 17 years. This limitation related to a goal of describing peri and post pubertal populations. Within this thesis selected variables for the meta-analysis involved only whole body BMC and BMD. Table 7 summarises a number of key data comparisons. Figure 10 shows the effects size plot for the structured physical activity interventions on the whole body BMD and percentage of FM loss in adolescents with obesity. In addition, a meta-regression was performed (Table 8) on the influence of the length of the intervention on BMC and BMD, the influence of BMI variation on BMC, fat mass variation on BMC and BMD as well as fat free mass variation on BMC and BMD.

The meta-analysis revealed that structured physical activity interventions did not influence BMC and BMD among adolescents with obesity. Of interest, 4 of the 8 interventions combined structured physical activity and nutrition while the other 4 exclusively involved physical activity. The diversity of the proposed interventions may contribute to the difficulties encountered in understanding the real effects of physical activity. Moreover, no information regarding the intensity and progression of physical activity was provided, which might be of particular

importance given that exercise performed at light to moderate intensities do not influence bone mineral parameters (Ivuškāns et al. 2015). The meta-regression results were non-significant. However, it provides indicative information on interventions length on WB BMC outcomes, on greater loss of fat mass on WB BMC and BMD outcomes and increased muscle mass on variability of WB BMC and BMD.

These inconclusive results can be influenced by significant weight loss being observed in only 50% of the included studies. Yet, weight loss and its magnitude might be suggested as key factors to induce bone changes in response to physical activity. At the end of the structured weight loss interventions, three out of the four studies with significant weight loss found that children and adolescents with obesity increased their whole body BMC (Campos et al. 2014) (Campos et al. 2012) (Stettler et al. 2008), while in one study, participants lost BMD (Campos et al. 2013).

Table 8 - Meta-regression results from longitudinal studies

	<i>n</i>	<i>Coef.</i>		<i>95% CI</i>		<i>t</i>	<i>p-value</i>	<i>Heterogeneity</i>	
		Mean	SD	Min	Max			I ²	Q
Intervention's length on BMC	8	0.001	0.005	-0.012	0.014	0.27	0.79	18.35	7.35
Interv.'s length on BMD	9	-0.015	0.018	-0.057	0.028	-0.83	0.44	0.00	6.76
BMI on BMC	4	-0.218	0.345	-1.704	1.267	-0.63	0.59	0.00	0.01
FM on BMC	7	-0.118	0.182	-0.585	0.349	-0.65	0.54	26.67	6.82
FM on BMD	6	-1.543	1.245	-4.999	1.914	-1.24	0.28	0.00	3.83
FFM on BMC	6	1.297	0.653	-0.515	3.109	1.99	0.12	0.00	3.25
FFM on BMD	6	1.430	0.932	-1.156	4.015	1.53	0.20	0.00	3.12

BMC bone mineral content, BMD bone mineral density, BMI body mass index, FM fat mass, FFM fat free mass

Limitations and gaps of the current literature

The available literature proposes contradictory results for the effects of obesity on bone health in children and adolescents.

First, the large heterogeneity between study designs (including gender, age, pubertal status, maturation stage) may explain the inability to reach a consensus on the effects of obesity on bone health in young populations. Diversity in populations' gender, age, pubertal status and maturational stage as well as inconsistencies in methodologies best describes the heterogeneity in this review.

Maturation status is of particular importance in young populations. Indeed, adolescents with obesity usually demonstrate an advanced biological maturation for the same chronological age as their normal weight peers. Also, there are complex and strong associations between maturation phases and bone accrual that body size and age cannot explain. Indeed, the effects of fat mass on peak bone mass and bone mass accrual are gender and maturation dependent (Shapses et al. 2012) (Wang 2002) (Dimitri et al. 2012). In addition, this relationship may be moderated by specific growth phases (Dimitri et al. 2012). Unfortunately, most of studies did not take into consideration sex-hormone status and pubertal stages which are important factors as adolescence is a critical period of bone development, fat distribution (VAT and SAT) (Karlsson et al. 2013) and hormones secretion. Study age ranges often crossed puberty, which may have masked growth-stimulated responses.

Second, the accuracy of the techniques used to measure parameters might contribute to conflicting findings. Even if DXA is an acceptable standardised method to evaluate bone health,

limitations can apply due to an overestimation of BMD in adolescents with obesity (Crabtree et al. 2014). Moreover, DXA does not provide information on bone architecture and subsequently has questionable validity for reflecting structural changes due to growth or mechanical loading (Khan 2001). Normalisation of BMD values is important especially when comparing growing populations of different sizes and shapes (Kröger et al. 1995) (Katzman et al. 1991). Also, when assessing bone strength, whole body scans should be combined with regional data from weight bearing sites such as the spine and hip. Both these sites have interest for sensitivity to bone change including indices of fracture risk.

Third, to better understand structural changes in bones, blood and/or urine markers of bone metabolism are required. These markers include such as P1NP, CTx, OC (unOC, COC, tOC), the OPG/RANK/RANKL, sclerostin, vitamin D. Yet, few studies of adolescents with obesity have analysed such bone markers.

Fourth, the accuracy of techniques known to assess body composition have to be considered. Currently, MRI is the most accurate technique used to differentiate VAT and SAT (Karlsson et al. 2013), yet, high costs and poor availability preclude frequent use. Body composition changes detected from DXA, ultrasound (US) and bioelectrical impedance do not provide direct measures of visceral adiposity despite lower cost and improved accessibility.

Fifth, another limitation may lie in the number of studies that have been specifically designed and adequately powered to question the effect of structured lifestyle interventions on bone health of adolescents with obesity. Only half of the intervention studies had a control group, and comparison with obese population were less frequent. Also, few studies considered the

effect of structured physical activity programs alone on bone health as a primary outcome measure and fewer of these studies included reports of weight loss. Although, the central role of weight bearing physical activity to enhance bone parameters in children and adolescents is well known (Tan et al. 2014), little is known regarding bone specific responses in adolescents with obesity.

Finally, the absence of reported compliance within the intervention studies weakened the rigour of the available literature. Also the consistency of reporting who delivers the intervention and the type of experience in this population might be useful. Understanding how adolescents responded to the intervention would also have improved the quality of reporting within these studies and helped inform future research plans/ clinical practice.

Research questions

2.12.1. Do adolescents with obesity have altered bone mass compared with maturation-matched lean peers?

Hypothesis

- i.* Adolescents with obesity will display lower bone density at the whole body and specific weight bearing sites.
- ii.* Adolescents with obesity will display altered bone geometry and strength.

Aim

To profile bone parameters among maturation-matched adolescents of various weight.

2.12.2. Can the negative effects of weight loss on bone health in adolescents with obesity be attenuated with a lifestyle intervention?

Hypothesis

- i. The WL induced by physical activity and nutrition will prevent the loss of bone mass caused by weight loss in adolescents with obesity.
- ii. The WL induced by physical activity and nutrition can prevent estimates of fracture risk.

Aim

To investigate the impact of a multidisciplinary weight loss program combining nutrition and physical activity on the bone health of adolescents with obesity, including estimate of fracture risk

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2.12.3. Does exposure to an 8-month WL intervention involving physical activity and nutrition normalise bone health in adolescents with obesity?

Hypothesis

- i. The WL intervention will support positive adaptations in bone parameters reaching the bone parameters values in normal weight adolescents.
- ii. Bone parameters at weight bearing sites will be more responsive than whole body measures following weight loss induced by a lifestyle intervention in adolescents with obesity.

Aim

To investigate the impact of body weight changes induced by a structured weight loss intervention on bone parameters in adolescents with obesity compared with normal weight maturation-matched peers.

2.12.4. Do weight status and weight changes influence bone markers in adolescents with obesity?

Hypothesis

- i.* Obesity in adolescents is associated with altered bone remodeling markers.
- ii.* The 8-month weight loss intervention will stimulate the remodeling activity in favour of bone formation in adolescents with obesity.
- iii.* The weight loss intervention experienced by adolescents with obesity will induce a shift of bone turnover towards positive bone formation compared with an obese control group; trending towards bone formations values similar to a lean control group.

Aim

To investigate the influence of body weight status and weight loss intervention on bone remodeling in adolescents with obesity and normal weight controls.

CHAPTER THREE

Methodology

The review of literature (Chapter 2) supported the alternative hypothesis that limited data currently exist in understanding more about body composition and changes in body composition can alter bone in adolescents with obesity. The chapter specifically highlighted deficits in:

- i.* longitudinal research;
- ii.* in obese rather than obese plus overweight recruitment;
- iii.* problems when maturation differed within groups in the same age;
- iv.* scarcity of data on site-specific bone parameters that body weight changes could alter;
- v.* the need to combine both bone parameters assessed with DXA with biomarkers of bone remodeling;
- vi.* the need to incorporate key adipokines and bone related hormones that may or may not be altered in adolescents with obesity.

To address these gaps, this methodology chapter begins with an outline of the intervention design, ethical approval and trial registration. The next section describes participants' selection and recruitment, sample size calculations. Primary and secondary outcomes measures are next to be detailed. Data analytical techniques that may be considered as innovative within this thesis are defined. Then the intervention is described. The chapter ends with details of the statistical treatment of the data.

3.1. The ADIBOX study design

The ADIBOX study was developed as a 8-month longitudinal study with repeated measures on three occasions (baseline, 4 months and 8 months). This protocol has been set in order to understand the effect of physical activity-induced weight loss on the bone adipocyte cross-talk in adolescents with obesity (Chaplais et al. 2016).

3.2. Ethics Approval and Clinical Trial registration

Approval was obtained from the Hospital Sud Est 1 committee (2015-33) (Appendices 3, 4, 5 & 6). In accordance with Ethical considerations, the chief investigator is responsible of ensuring that participants understand potential risks and benefits of taking part in the study. Moreover, the chief investigator is responsible for obtaining writing consents from both the adolescents and their legal guardians/parents.

The study was registered as a Clinical Trial (registration number: NCT02626273 - <https://clinicaltrials.gov/ct2/show/NCT02626273>) (Appendix 7).

3.3. Participants

A total of 65 adolescents (42 with obesity including 10 males) were enrolled in the study. Participants were aged between 12 to 16 years with a self-reported pubertal status equal to or above Tanner Stage 4. The adolescent stage of development was selected to understand more about maturation processes, growth changes and the possible exploratory aspects of weight

changes on bone parameters. Although all participants were French nationals, race and ethnicity of participants were mixed.

This intervention primarily targeted adolescents with obesity; a population that can be problematic to recruit. To address this issue, recruitment was strategically aligned to a single centred tertiary referral clinic treating adolescents with obesity either as residential or non-residential patients.

For the intervention group, a paediatrician first checked the suitability of adolescents to complete the intervention. This process partially involved reviewing medical records for key information such as medical history of the family, early childhood development and the history of obesity.

Adolescents with obesity were recruited to the obesity intervention clinic from the “Tza Nou” Children Obesity Center (SSR Tza Nou, UGECAM, La Bourboule, France) (n=31, 6 males, Intervention group) and the Local Ambulatory Nutrition-Obesity Hospital (SSR Nutrition Obésité UGECAM, Clermont-Ferrand, France) (n=11, 4 males, Control group with obesity). In addition, a normal weight control group was enrolled a related ongoing project in the paediatric department of the University Hospital G.Montpied (Clermont-Ferrand, France).

3.4. Sample size calculation

Sample size estimation centred on the expected variability within participants’ body fat mass relative to variability in a key marker of bone mass (BMD) measured at the lumbar spine.

Indeed, lumbar spine is a representative site of fracture risk for young population (Silva et al. 2014). The index difference between the two most extreme groups was estimated to be 1.3 (standard deviations) based on existing research (Campos et al. 2014). To highlight statistically significant differences with a statistical power of 90% and two-sided type I-error less of 5%, a minimum of 21 participants (without drop-out) per group were required for recruitment.

3.5. Inclusion/exclusion criteria

Inclusion criteria

Obese participants were required to have a BMI above the 95th percentile (McCarthy et al. 2006), while the normal weight control group had a BMI between the 5th and 85th percentiles (McCarthy et al. 2006). To reduce bias, participants who were invited to take part in this study were aged between 12 and 16 years, with a self-reported pubertal status equal to or above Tanner Stage 4. Females were required to have reached menarche at least one year prior to the study. Of note, adolescents in the intervention group had already consented to take part in a residential program described later in this chapter (section 3.8.).

Participants had to be free of any recent history of hospitalisation (past two years) and without history of systemic illness lasting more than two weeks in the past 12 months. In addition, the recruited adolescents had to be free of any contraindications for physical activity or extreme dietary allergies.

The non-inclusion criteria related to a known history of bone or muscle disease, metabolic diseases such as diabetes, insulin-resistance, and or, hypo- or hyper- thyroid activity. Additional

non-inclusion criteria were congenital cardiovascular disease, alcohol use, smoking, and the use of drugs known to alter bone metabolism, hormones or calcium supplements (vitamin D, calcium, protein).

Exclusion criteria

Exclusion criteria applied only to participants from the interventional weight loss program. Adolescents were excluded if educators at the residential site of the intervention observed major treatment and/or protocol deviations (i.e. non-adherence to the Obesity Center rules, to physical activity or nutritional programs). Educators were asked to complete a “daily journal” reporting adolescents’ involvement (attendance and perceived compliance) during physical activity sessions. This journal allowed us to assess adolescents’ compliance to the physical activity program.

3.6. Data collection Overview

Data collection was undertaken three times between September 2015 and 2016: (1) at baseline; (2) four months; (3) and eight months. Measures were performed at the “Tza Nou” Obesity Center (La Bourboule (63), France) and at the University Hospital G.Montpied (Clermont-Ferrand (63), France). Figure 11 summarises the primary and secondary outcomes selected to meet the study aims.

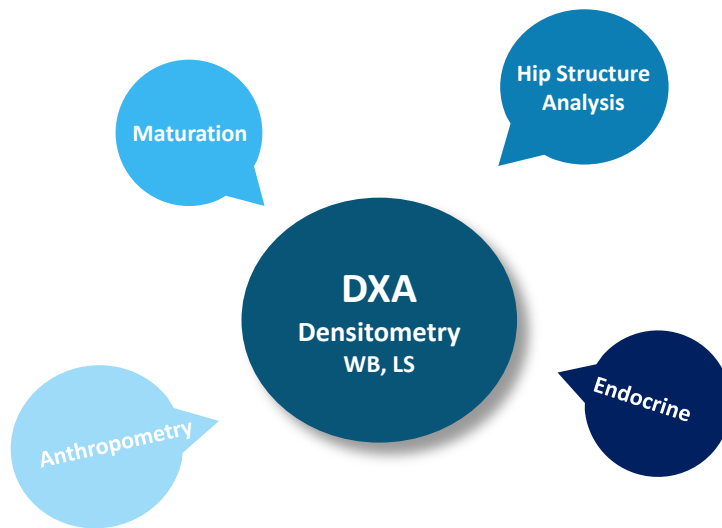


Figure 11 - Summary of the primary and secondary outcomes measures

3.6.1. Primary outcomes

Bone density assessed using DXA

Bone mineral density (BMD, g/cm²), bone mineral content (BMC, g) and bone area (cm²) were determined using DXA device (DXA, QDR-4500A, Hologic, Inc., Waltham, MA). In agreement with the ISCD recommendations (Crabtree et al. 2014) measurements were: whole body (WB), total body less head (TBLH) and lumbar spine (LS). Recommended bone data also included the trabecular bone score (TBS), derived from bone texture analysis of the spine (TBS iNsite[®] version 2.1). Also, due to the likely excessive loading on the hip of adolescents with obesity investigations included the non-dominant hip. Specifically, hip bone density provided data on parameters at the femoral neck, as well as the trochanteric and intertrochanteric regions (Figure 12).

The in vivo coefficients of variability of the DXA were 0.35 and 0.9% after repositioning for BMD, and 0.57 and 1.2% for BMC at the lumbar spine and total body, respectively. Bone mineral apparent density (BMAD, g.cm⁻³) was calculated using the following equation: WB BMAD = BMC/(WB bone area²/body height) and LS BMAD = BMC (LS)/spine bone area (Katzman et al. 1991).

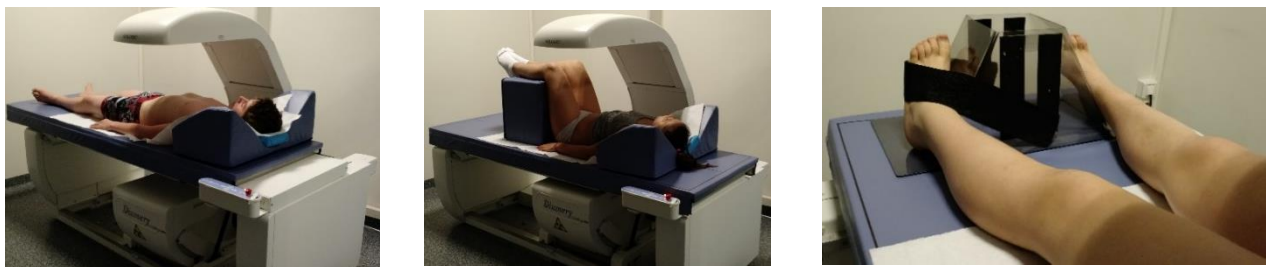


Figure 12 - Whole body, lumbar spine and hip positioning DXA scans

Quality Assurance and Radiation dosage

All DXA scans were conducted by the same investigator and quality assurance checks were performed routinely. The DXA scans were analysed by the same experienced investigator using the APEX software (APEX version 5.5.3., Hologic, Inc., Waltham, MA). Measures of body composition and bone properties provided by DXA exposed participants to low level radiation: 0.0056 mSv from DXA scans (whole body, lumbar and hip) (Damilakis et al. 2010). Parents were informed about the radiation dosage involved in the research project as well as the risks associated with this low level of exposure (Appendix 6).

3.6.2. Secondary outcomes

Anthropometric characteristics

Assessment of anthropometric measures for stature (m) and body mass (kg) occurred in accordance with the International Society for the Advancement of Kinanthropometry (Marfell-Jones et al. 2012) and were conducted by a researcher accredited in anthropometry. Body mass was measured with participants wearing light clothing on a digital electronic scale (SECA 813, Hamburg, Germany, ± 0.1 kg) and stature on a stadiometer (Seca 240, UK, ± 0.2 cm). Body mass index was calculated by dividing the body mass by the stature squared (Kg.m^{-2}). We converted BMI into BMI z-score relative to age using the references recommended by the Center for Disease Control and Prevention (Ogden et al. 2002).

Maturation

Maturation was estimated using self-reported Tanner Stages (TS) at baseline. Although, self-assessment can lack clinical precision it has some acceptance in the literature (Morris et al. 1980) (Taylor et al. 2001). Also, the age of menarche (defined as the onset of menstruation in females) was self-reported for female.

Body composition

Whole body composition was measured using the same DXA device with the capacity to assess lean mass (LM, g), fat mass (FM, % and g), android fat mass (aFM, %), gynoïd fat mass (gFM, %), as well as estimating visceral fat (VFAT %, g and cm^3) (Figure 13).

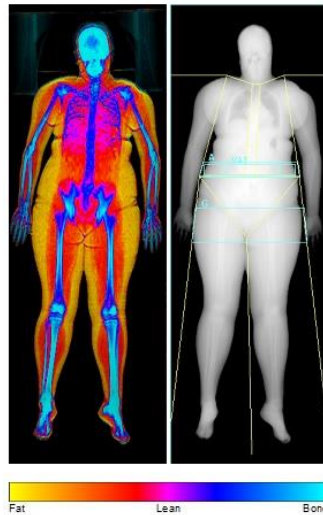


Figure 13 - Body composition analysis by Dual energy X-ray Absorptiometry (DXA)

Hip Structure Analysis

The DXA provided the opportunity for additional geometric and strength analyses including regional analysis of the narrow neck (NN), the femoral shaft (FS) and the intertrochanteric area (IT) (Figure 14). At each region, we analysed multiple variables: the BMD ($\text{g}\cdot\text{cm}^{-2}$), the endocortical diameter (ED, cm), the average cortical thickness (ACT, cm), the width (WIDTH, cm), the cross-sectional moment of inertia (CSMI, cm^4), the cross-sectional area (CSA, cm^2), the section modulus (Z, cm^3) and the Bulking Ratio (BR). The intra-observer CV following repositioning for hip structure analysis in our laboratory was 1.35%.

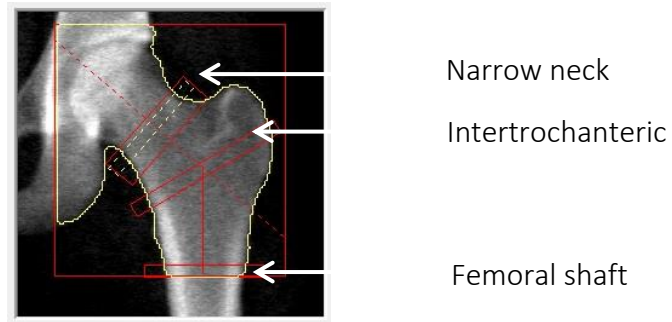


Figure 14 - Hip Structural Analysis by Dual energy X-ray Absorptiometry (DXA)

Endocrine markers

Blood samples were collected by a qualified paediatric nurse after participants had fasted overnight. The blood was centrifuged at a rotor speed of 4000 RPM for 10 minutes and aliquots were frozen for subsequent analyses. The bone formation marker P1NP (Cloud-Clone Corp, Houston, US), the bone resorption marker CTx (Cloud-Clone Corp, Houston, US), leptin (BioVendor, Czech Republic) and estradiol (BioVendor, Czech Republic) were measured at the University Hospital's accredited laboratory following manufacturers' recommendations. Table 9 shows intra, inter-assay coefficient of variation and sensitivity results for blood markers used in this thesis.

Table 9 - Outline of the intra, inter-assay coefficient of variation and sensitivity results for blood markers

Hormones	Coefficient of variations		Sensitivity
	Intra-assay	Inter-assay	
Oestradiol	<10%	<12%	10pg/ml
Leptin	<8%	<7%	0.2 ng/ml
P1NP	<10%	<12%	<12.3 pg/ml
CTx	<10%	<12%	<44.3pg/ml

3.7. Details of analysis involving key endocrine markers

Uncoupling index

To address the study aims it was necessary to understand more about changes in bone remodeling markers such as the uncoupling index. To do this a series of t-score and z-score calculation were used.

Within-study reference data were compiled using baseline values from each group as reference data. For example, baseline data for normal weight controls could be compared with changes in the normal weight group (t-score) or changes in either of the groups with obesity (z-score). Specifically, an uncoupling index (UI) (Eastell et al. 1993) favouring formation was denoted by a positive value, while a negative UI suggested an imbalance in favour of resorption (Lane et al. 2000).

Bone marker plot

Calculations of markers concentrations were based on the work of Bieglmayer and collaborators (Bieglmayer et al. 2009) (Grimm et al. 2010). As described in Chapter 2, (section 2.6.2.1), markers of bone concentration can be converted to visually displayed balance vectors based on the following equation: $\text{Balance} = \text{MoM}_F / \text{MoM}_R$ (Bieglmayer et al. 2009). A balance vector is a surrogate for formation and resorption forces representing the ratio between both factors. Then, the calculation of the rate of bone turnover follows ($\text{Turnover rate} = \sqrt{(\text{MoM}_F^2 + \text{MoM}_R^2)}$) (Bieglmayer et al. 2009). Data from obese groups were normalised to control group(s), as well as their own baseline data.

In line with the bone marker plot recommendations, data were log transformed for graphic representation in order to demonstrate symmetrical distribution patterns. All calculations and

scatter plots were derived using Microsoft Excel and XLSTATS. Scatter plots were presented with a 95% confidence ellipse, based on Fisher.

3.8. Clinical intervention

Adolescents from the intervention group joined the residential program provided by “Tza Nou” Children’s Obesity Center for the whole school year. The Obesity Center program is a French National initiative, combining physical activity, nutrition education and psychological support.

Physical activity intervention

Four physical activity sessions were planned and supervised per week. Two of these sessions comprised approximately 70 minutes of aerobic or resistance training. Aerobic training sessions had the following structure: 10 minutes of warm-up, 20 minutes of interval training, 30 minutes of continuous exercise and 10 minutes for cooling-down (stretching, relaxation). Also, adolescents in the intervention group had swimming lessons once a week (60 min). The final session provided 120 to 150 minutes of various opportunities for sports and recreational activities such as ball and racquet sports or trekking and snowshoeing for recreation. The physical activity intervention focused more on physical practice than on specific osteogenic and metabolically challenging modalities.

Nutrition intervention

The nutrition of adolescents in the obesity center conformed to a normo-caloric diet. Food and drink consumption was strategically planned to comply with recommended levels of dietary

intake relative to predicted physical activity level, age and gender (Murphy et al. 2002). Adolescents also received fortnightly nutrition education sessions including topics such as weight loss, food sensation, macronutrient recommendations, home-based nutrition choices, nutrition choices during festivities, cooking skills and interpreting food labels. Also, approximately every 10 weeks adolescent's and their family met with the clinic's dietician.

Psychological support

Similarly, psychological support consisted of meetings with the adolescent's and their family approximately every 10 weeks during the intervention. Also monthly individual meetings with a clinical psychologist addressed the following topics: motivation, how to prepare for holidays and/or going home, how to cope with emotions such as stress and anxiety and mindfulness around food.

Participant compliance

Participants' daily engagement and adherence to the weight loss lifestyle intervention were monitored by educators working at the Obesity Center. Educators were asked to complete an individualised daily journal on the compliance of each participant.

3.9. Statistical treatment of the data

Statistical analyses were performed using Stata software (version 13, StataCorp, College Station, US). The tests were two-sided with a type I error set at $\alpha = 0.05$. Data were presented

as the mean \pm standard deviation or median and interquartile range, as appropriate. When data were adjusted for key explanatory variables, data were presented as mean and 95% confidence intervals. Assumption of normality was assessed using the Shapiro-Wilk test. A cross tabs chi-square test was performed to test the homogeneity of the sample.

For blood markers only, at baseline comparisons involved all three groups. Therefore baseline comparisons used ANOVA or Kruskal-Wallis (KW) test if two key assumptions for ANOVA were not met. These assumptions were normality and homoscedasticity using the Bartlett test.

To initially explore the relationships among body composition, bone and endocrine parameters, correlation coefficients (Pearson or Spearman, according to statistical distribution) were determined. Repeated correlated data (parameters measured longitudinally) were investigated using mixed models to take into account between and within participant variability (as random effect) while studying the impact of fixed effects for group, time point evaluations and their interactions. In multivariate analysis, these regression models adjusted for baseline body weight, fat mass or lean mass depending on univariate results and clinical relevance. Additional regression modelling included adjustment for changes in body weight and whole body fat mass during the intervention.

CHAPTER FOUR




Results

This chapter presents the results of the methods described in the previous chapter, dealing first with a review of aims and then results from each separate aim are described and summarised. Primary outcomes of bone parameters are addressed first but secondary outcomes are often blended into the results through adjustments using variables for key “explanatory” data such as body weight, fat mass and lean mass.

The first 3 aims share the same population and methods. The fourth aim involved separate method, analysis and a slightly different comparison group. Therefore, to minimise repetition in the first section of this results chapter, the first 3 aims and their hypothesis are presented together and the fourth aim is presented separately.

Also of note was the “imperfect” data collection. In addition to some incomplete numbers within groups, not all groups were available for all primary and secondary measures during the intervention period (Table 10).

Table 10 - Outline of the data collection for primary and secondary outcomes

Groups	Anthropometry, body composition, maturation	Baseline				Anthropometry, body composition	4-month				Anthropometry, body composition	8-month			
		Densitometry		Blood collection			Densitometry		Blood collection			Densitometry		Blood collection	
		DXA	HSA	Other	Bone		DXA	HSA	Other	Bone		DXA	HSA	Other	Bone
Intervention  Adolescents with obesity residential WL program (physical activity & nutrition)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	n = 31 (6 ♂)				n=29 (4 ♂)				n = 24 (3 ♂)						
	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Control  Normal weight adolescents		✓	✓	✓		✓	✓	✓	✓		✓	✓	✓	✓	✓
	n = 23 (♀)				n = 23 (♀)				n = 23 (♀)						
	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Control  Adolescents with obesity no-residential program	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	n = 11 (4 ♂)				n = 11 (4 ♂)										

To address the hypothesis that:

- i.* Adolescents with obesity will display lower bone density at the whole body and specific weight bearing sites.
- ii.* Adolescents with obesity will display altered bone geometry and strength.
- iii.* The weight-loss induced by physical activity and nutrition will prevent the loss of bone mass caused by weight loss in adolescents with obesity.
- iv.* The weight-loss induced by physical activity and nutrition can prevent estimates of fracture risk.
- v.* The weight loss intervention will support positive adaptations in bone parameters reaching the bone parameters values in normal weight adolescents.
- vi.* Bone parameters at weight bearing sites will be more responsive than whole body measures following weight loss induced by a lifestyle intervention in adolescents with obesity.

The following aims were addressed:

- i.* To profile bone parameters among maturation-matched adolescents of various weight.
- ii.* To investigate the impact of a multidisciplinary weight loss program combining nutrition and physical activity on the bone health of adolescents with obesity, including estimate of fracture risk
- iii.* To investigate the impact of body weight changes induced by a structured weight loss

intervention on bone parameters in adolescents with obesity compared with normal weight maturation-matched peers.

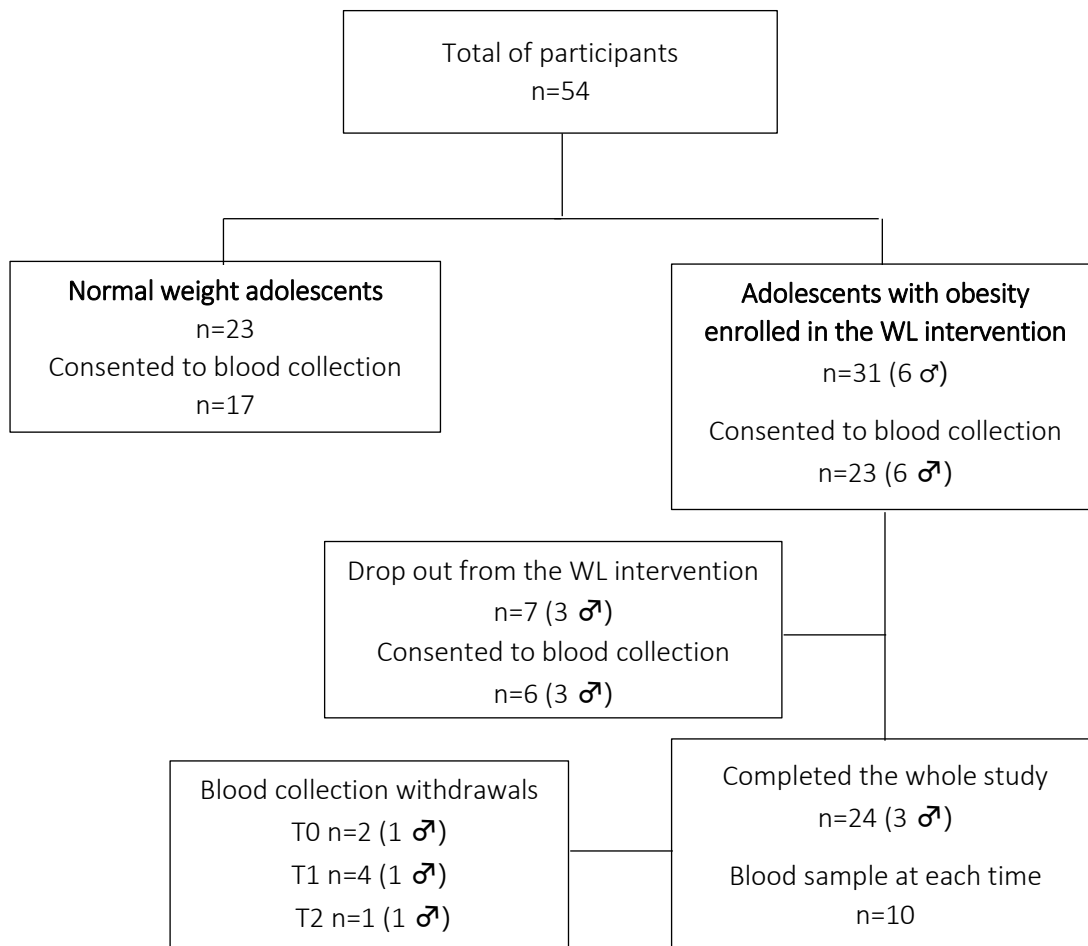


Figure 15 - Flow charts of participants of the 3 first aims of this thesis

WL weight loss

To answer the first 3 aims of this thesis, a total of 54 adolescents: 31 obese (Ob) (89% of female) and 23 normal-weight (NW) adolescents females were recruited. Adolescents with obesity were enrolled in a structured weight loss intervention that combined physical activity

and nutrition. However, the participant flow chart shows that not all participants consented to the blood collection at each of the three scheduled periods of data collection. Figure 15 highlights the complexities of recruiting even from clinical populations. The low number of adolescents with obesity who dropped out of the intervention ($n = 7$) was noted and less than planned numbers in the blood collection periods weakened the statistical power within the analyses.

The inclusion of males within the results of the WL weight loss intervention.

A chi-square test was performed to demonstrate if the presence of 6 males had an influence on the distribution of the data in the group. Despite lower estradiol levels in males than females results from other variables showed no influence of the inclusion of males; with results showing acceptable homogenous data ($p=0.08$).

4.1. Does adolescents with obesity have altered bon mass compared with maturation-matched lean peers?

Aim

To profile bone parameters among maturation-matched adolescents of various weight.

Table 11 - Outline of the data collection and participants for the first aim












Groups		Baseline	4-month	8-month
Intervention	 Adolescents with obesity residential WL program (physical activity & nutrition)	 n = 31 (6 ♂)	 n=29 (4 ♂)	 n = 24 (3 ♂)
	 Normal weight adolescents	 n = 23 (♀)	 n = 23 (♀)	 n = 23 (♀)
Control	 Adolescents with obesity no-residential program	 n = 11 (4 ♂)	 n = 11 (4 ♂)	

Table 11 indicates that participants targeted in meeting the first aim of the research were the adolescents with obesity who underwent the intervention and the normal weight control group. The shading indicates that only baseline comparisons were analysed.

Body composition comparison between adolescents with obesity and their leaner peers

Descriptive characteristics relating to body composition are first presented. Table 12 presents the results from anthropometric characteristics of the obese and lean. Body Mass Index and body weight were both higher in the Ob group than their normal weight (NW) peers ($p < 0.001$ for all). Similarly, estradiol levels did not differ between the two groups (median [IQR], Ob 56 [97]; NW 49 [46]). Height and years since menarche of the females was similar in both groups, despite the older chronological age of the NW group ($p < 0.001$). Using data derived from DXA, at baseline, compared with the NW group, the Ob group had 19% higher lean mass, 67% more whole body FM, 56% more android FM and 36% more gynoid FM ($p < 0.001$ for all). Measures of visceral tissue (% , g and cm^3) were also higher in Ob than NW ($p < 0.001$ for all).

Table 12 - Descriptive statistics at baseline in the Ob group and NW control group

	Ob (n= 31)		NW (n= 23)	
	Mean	SD	Mean	SD
Age (years)	13.61	1.27 *	15.90	0.43 *
Menarche age (years)	12.50	0.76	13.21	1.31
BMI	32.30	4.15 *	20.48	1.32 *
zBMI	2.26	0.30 *	-0.12	0.48 *
Height (cm)	161.38	8.62	164.48	5.48
Body weight (Kg)	86.32	15.21 *	55.91	5.90 *
WB Lean Mass (g)	51995.47	8380.66 *	42194.69	4200.64 *
WB FM (%)	39.49	3.82 *	20.33	3.82 *
WB FM (g)	34330.76	7942.64 *	11436.73	2807.79 *
Android (%)	42.31	4.56 *	18.53	4.80 *
Gynoid (%)	41.17	3.78 *	26.19	4.15 *
V FAT (%)	43.33	4.22 *	19.37	5.06 *
V FAT (g)	315.71	97.67 *	128.25	54.35 *
V FAT (cm3)	341.31	105.59 *	138.64	58.76 *

* p<0.05 in comparison between Ob and NW

Ob obese group, NW normal weight control group, SD standard deviation, BMI body mass index, WB LM whole body lean mass, WB FM whole body fat mass, V FAT visceral fat

Baseline results from bone parameters between adolescents with obesity and lean

Results from group comparisons of the primary outcomes of bone measurements expressed as unadjusted and adjusted values (body weight (BW) or fat mass (FM) or lean mass (LM)) are presented in Table 13.

Adolescents with obesity had lower total body less head bone density (TBLH BMD) (p<0.001), lower hip BMD (p=0.022), lower whole body bone mineral content (p=0.048) lower spine BMC (p<0.001) and lower hip BMC (p=0.008) than the NW group. In addition, Ob had a lower bone mineral apparent density at the whole body (p<0.001). Even when raw scores for bone

parameters were adjusted for body weight (BW) or fat mass (FM), or lean mass (LM) the Ob group, still displayed lower quantitative bone at all sites than the NW group. However, one exception to the trend of lower BMD values in the Ob than NW group were noted with LS BMAD ($p>0.05$) when adjusted to FM.

Similarly, unadjusted bone parameters from the hip structure analyses showed the Ob group had lower femoral shaft (FS) density ($p=0.008$), lower FS cortical thickness ($p=0.009$) and higher FS endocortical diameter ($p=0.040$) and bulking ratio ($p=0.028$) than NW. When adjusted for body weight, or fat mass, or lean mass, Ob displayed lower bone density at the intertrochanteric (IT) ($p<0.005$ adjusted BW, $p=0.012$ adjusted LM and $p=0.038$ adjusted FM) and femoral shaft ($p=0.001$ adjusted BW or LM, $p=0.022$ adjusted FM).

In addition, results showed lower width at all sites (NN, IT FS) in Ob than the NW group ($p=0.001$ adjusted BW, $p<0.009$ adjusted FM and $p<0.010$ adjusted FM at NN and FS, $p=0.002$ at IT). At the narrow neck, lower endocortical diameter was observed in the Ob than NW group after adjustment for BW ($p=0.002$) and FM ($p=0.001$). Finally, results showed lower cortical thickness at the intertrochanteric and the femoral shaft in Ob than NW after adjusting for body weight or lean mass ($p<0.008$ adjusted BW and $p<0.005$ adjusted LM).

At baseline, Ob had a unfavourable higher raw bulking ratio at the shaft ($p=0.028$) than the NW group. Otherwise, all data were similar between both groups. Once differences in body weight, fat mass, or lean mass were accounted for, adolescents with obesity compared with NW peers, displayed lower cross sectional area, cross sectional moment of inertia and section modulus at all site (NN, IT, FS) ($p<0.001$), mainly adjusted for BW adjustment (Table 13).

Table 13 - Bone variables at baseline. A. Unadjusted mean. B. Body weight adjusted. C. Fat mass adjusted. D. Lean mass adjusted.

A	WB (TBLH BMD)				Lumbar Spine				Hip				Neck						
	Ob <i>n</i> =31		NW <i>n</i> =23		Ob <i>n</i> =31		NW <i>n</i> =23		Ob <i>n</i> =31		NW <i>n</i> =23		Ob <i>n</i> =31		NW <i>n</i> =23				
	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>			
BMD (g/cm ²)	0.941	0.088	1.054	0.071	*	0.964	0.150	1.030	0.107	1.021	0.137	1.102	0.100	*	0.953	0.139	1.005	0.102	
BMC (g)	2082.98	379.55	2275.94	285.84	*	49.62	11.69	63.55	10.79	*	33.42	5.83	38.00	5.41	*	4.71	0.85	5.05	0.60
BMAD (g/cm ³)	0.09	0.01	0.1	0.01	*	0.97	0.15	1.03	0.11										

	Narrow Neck				Intertrochanteric				Femoral Shaft				
	Ob <i>n</i> =31		NW <i>n</i> =23		Ob <i>n</i> =31		NW <i>n</i> =23		Ob <i>n</i> =31		NW <i>n</i> =23		
	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	
BMD (g/cm ²)	1.179	0.187	1.183	0.137	1.104	0.177	1.178	0.133	1.515	0.171	1.651	0.152	*
ED (cm)	2.75	0.22	2.75	0.3	4.50	0.39	4.46	0.4	1.81	0.30	1.65	0.23	*
ACT (cm)	0.23	0.05	0.23	0.03	0.48	0.09	0.52	0.06	0.58	0.08	0.65	0.09	*
WIDTH (cm)	3.20	0.25	3.21	0.27	5.45	0.39	5.51	0.38	2.99	0.25	2.91	0.2	
CSA (cm ²)	3.60	0.68	3.6	0.42	5.75	1.06	6.17	0.69	4.27	0.67	4.58	0.56	
CSMI (cm ⁴)	2.90	0.84	2.81	0.72	14.94	4.04	15.16	3.12	3.49	1.12	3.51	0.80	
Z (cm ³)	1.69	0.42	1.67	0.31	4.93	1.07	4.96	0.76	2.27	0.56	2.31	0.38	
BR	7.70	1.56	7.41	1.4	6.48	1.22	5.87	0.81	2.70	0.51	2.36	0.39	*

B	WB (TBLH BMD)		Lumbar Spine		Hip		Neck					
	Ob <i>n</i> =31		Ob <i>n</i> =31		Ob <i>n</i> =31		Ob <i>n</i> =31					
	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>				
BMD (g/cm ²)	0.891	(0.858 - 0.925)	*	0.890	(0.834 - 0.946)	*	0.966	(0.914 - 1.018)	*	0.908	(0.855 - 0.960)	*
BMC (g)	1847.56	(1727.28 - 1967.83)	*	44.73	(40.04 - 49.41)	*	30.57	(28.33 - 32.82)	*	4.32	(4.03 - 4.61)	*
BMAD (g/cm ³)	0.09	(0.08 - 0.09)	*	0.93	(0.86 - 0.99)	*						

	Narrow Neck		Intertrochanteric		Femoral Shaft	
	Ob n=31		Ob n=31		Ob n=31	
	mean	95% CI	mean	95% CI	mean	95% CI
BMD (g/cm ²)	1.130	(1.056 - 1.204)	1.066	(0.995 - 1.137) *	1.467	(1.392 - 1.541) *
ED (cm)	2.59	(2.48 - 2.70) *	4.32	(4.14 - 4.51)	1.65	(1.52 - 1.78)
ACT (cm)	0.22	(0.21 - 0.24)	0.46	(0.42 - 0.49) *	0.57	(0.52 - 0.61) *
WIDTH (cm)	3.02	(2.92 - 3.12) *	5.24	(5.08 - 5.40) *	2.77	(2.67 - 2.86) *
CSA (cm ²)	3.27	(3.06 - 3.50) *	5.17	(4.684 - 5.51) *	3.87	(3.62 - 4.12) *
CSMI (cm ⁴)	2.34	(2.07 - 2.61) *	12.50	(11.17 - 13.82)) *	2.78	(2.41 - 3.15) *
Z (cm ³)	1.47	(1.32 - 1.61) *	4.19	(3.84 - 4.52) *	1.91	(1.73 - 2.10) *
BR	7.49	(6.77 - 8.20)	6.32	(5.78 - 6.85)	2.60	(2.38 - 2.81)

C

	WB (TBLH BMD)		Lumbar Spine		Hip		Neck	
	Ob n=31		Ob n=31		Ob n=31		Ob n=31	
	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI
BMD (g/cm ²)	0.901	(0.857- 0.945) *	0.894	(0.823 - 0.964) *	0.974	(0.909 - 1.039) *	0.914	(0.849 - 0.980) *
BMC (g)	1863.54	(1695.02 - 2032.05) *	45.59	(39.63- 51.53) *	31.25	(28.30 - 34.21) *	4.37	(3.99 - 4.75) *
BMAD (g/cm ³)	0.09	(0.09 - 0.10) *	0.95	(0.87 - 1.02)				

	Narrow Neck		Intertrochanteric		Femoral Shaft	
	Ob n=31		Ob n=31		Ob n=31	
	mean	95% CI	mean	95% CI	mean	95% CI
BMD (g/cm ²)	1.144	(1.053 - 1.235)	1.070	(0.984 - 1.156) *	1.485	(1.395 - 1.576) *
ED (cm)	2.55	(2.42 - 2.67) *	4.32	(4.10 - 4.60)	1.63	(1.48 - 1.78)
ACT (cm)	0.23	(0.20 - 0.25)	0.47	(0.42 - 0.51)	0.58	(0.53 - 0.62)
WIDTH (cm)	2.97	(2.85 - 3.09) *	5.24	(5.04 - 5.44) *	2.76	(2.64 - 2.88) *
CSA (cm ²)	3.28	(2.99 - 3.57) *	5.21	(4.75 - 5.66) *	3.91	(3.59 - 4.24) *
CSMI (cm ⁴)	2.29	(1.93 - 2.66) *	12.62	(10.82 - 14.42) *	2.80	(2.30 - 3.30) *
Z (cm ³)	1.48	(1.29 - 1.66) *	4.21	(3.73 - 4.69) *	1.93	(1.68 - 2.18) *
BR	7.16	(6.34 - 7.98)	6.28	(5.65 - 6.91)	2.54	(2.28 - 2.80)

D

	WB (TBLH BMD)		Lumbar Spine		Hip		Neck	
	Ob		Ob		Ob		Ob	
	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI
BMD (g/cm ²)	0.910	(0.8862 - 0.934) *	0.922	(0.877 - 0.967) *	0.991	(0.950 - 1.032) *	0.934	(0.893 - 0.976) *
BMC (g)	1958.88	(1872.87 - 2044.89) *	47.20	(43.54 - 50.86) *	31.90	(30.25 - 33.56) *	4.50	(4.28 - 4.73) *
BMAD (g/cm ³)	0.09	(0.08 - 0.09) *	0.95	(0.89 - 0.99) *				

	Narrow Neck		Intertrochanteric		Femoral Shaft	
	Ob		Ob		Ob	
	mean	95% CI	mean	95% CI	mean	95% CI
BMD (g/cm ²)	1.159	(1.099 - 1.218)	1.096	(1.038 - 1.154) *	1.490	(1.430 - 1.549) *
ED (cm)	2.67	(2.57 - 2.76)	4.40	(4.25 - 4.55)	1.71	(1.60 - 1.82)
ACT (cm)	0.23	(0.21 - 0.24)	0.47	(0.44 - 0.50) *	0.57	(0.53 - 0.60) *
WIDTH (cm)	3.11	(3.02 - 3.20) *	5.33	(5.20 - 5.45) *	2.84	(2.77 - 2.92) *
CSA (cm ²)	3.44	(3.27 - 3.61) *	5.45	(5.21 - 5.70) *	4.05	(3.86 - 4.23) *
CSMI (cm ⁴)	2.61	(2.40 - 2.83) *	13.63	(12.66 - 14.59) *	3.09	(2.82 - 3.37) *
Z (cm ³)	1.57	(1.46 - 1.68) *	4.51	(4.27 - 4.75) *	2.07	(1.94 - 2.20) *
BR	7.61	(7.02 - 8.20)	6.30	(5.85 - 6.75)	2.64	(2.45 - 2.82)

* p<0.05 Ob different from NW.

Ob obese intervention group, NW normal weight control group, SD standard deviation, WB whole body, TBLH total body less head, LM lean mass, FM fat mass, BMD bone mineral density, BMC bone mineral content, BMAD bone mineral apparent density, ED endocortical diameter, ACT average cortical thickness, WIDTH width, CSA cross sectional area, CSMI cross sectional moment of inertia, Z section modulus, BR bulking ratio

To summarise, despite maturational similarities, size and indices of body composition differed between adolescents with obesity and normal weight controls. With the exception of the neck and the spine, adolescents with obesity had lower unadjusted primary outcomes in bone mineral density and bone mineral content than their leaner peers. Those differences exacerbated after adjustment to body weight, fat mass or lean mass. In addition, bone geometric and strength indices were lower in adolescents with obesity after body composition adjustments.

4.2. Does nutrition and physical activity inducing WL can reverse the negative effects of WL on bone health?

Aim

To investigate the impact of a multidisciplinary weight loss program combining nutrition and physical activity on the bone health of adolescents with obesity, including estimate of fracture risk.

Table 14 - Outline of the data collection and participants for the second aim












Groups		Baseline	4-month	8-month
Intervention	 <p>Adolescents with obesity residential WL program (physical activity & nutrition)</p>	 n = 31 (6 ♂)	 n=29 (4 ♂)	 n = 24 (3 ♂)
	 <p>Normal weight adolescents</p>	 n = 23 (♀)	 n = 23 (♀)	 n = 23 (♀)
Control	 <p>Adolescents with obesity no-residential program</p>	 n = 11 (4 ♂)	 n = 11 (4 ♂)	

Table 14 indicates that participants targeted in meeting the second aim of the research were only the adolescents with obesity who underwent the intervention. The shading indicates that baseline, 4-month and 8-month comparisons were analysed.

Analysis addressing this aim involve the 24 participants that completed the 8 months intervention. Seventy-seven percent of the recruited adolescents with obesity completed the whole study including three males (Figure 15). As explained previously, groups were homogenous and the presence of males did not influence the distribution of the data.

Statistical comparisons performed using mixt models occurred between participants who remained in the study and those who dropped out. However, the low numbers of adolescents not completing study may require cautious interpretation of the following bivariate analyses. Statistical analyses of this aim included only the adolescents with obesity that completed the whole study (n=24). Data are presented in Table 19.

Baseline descriptive characteristics (body composition) did not differ between the adolescents who dropped out of the weight loss intervention (n=7) and the rest of the intervention sample (n=24). However, significant differences were observed in bone parameters (Table 15).

Indeed, lower TBLH BMD ($p=0.005$), WB BMC ($p=0.006$), WB BMAD ($p=0.002$), LS BMD ($p=0.02$), LS BMC ($p=0.01$), LS TBS ($p=0.004$), hip BMC ($p=0.046$), neck BMC ($p=0.029$) were observed in the adolescents with obesity who dropped out the study compared with those who completed it.

Results in geometry parameters also showed lower BMD ($p=0.007$) and cortical thickness ($p=0.029$) at the shaft.

In addition, strength parameters were lower for the cross section area at the IT ($p=0.043$) and FS in the adolescents with obesity who dropped out and those who did not. Results were

similarly lower for the cross sectional moment of inertia ($p=0.018$) at the shaft and for section modulus at the narrow neck ($p=0.020$), the intertrochanteric ($p=0.023$) and the femoral shaft ($p=0.008$) sites in the adolescents with obesity who dropped out the study than those who completed it.

Body composition changes over the 8-month weight loss program in adolescents with obesity

Longitudinal analysis revealed that adolescents with obesity reduced their body weight and fat mass (total (g, %) over the time of the intervention ($p<0.007$). (Figure 16). Although the data are not presented in this figure, similar changes were also observe for BMI, android, gynoid and visceral fat (g, %, cm^3) during the 8-month weight loss program. Lean mass remained unchanged (Figure 15).

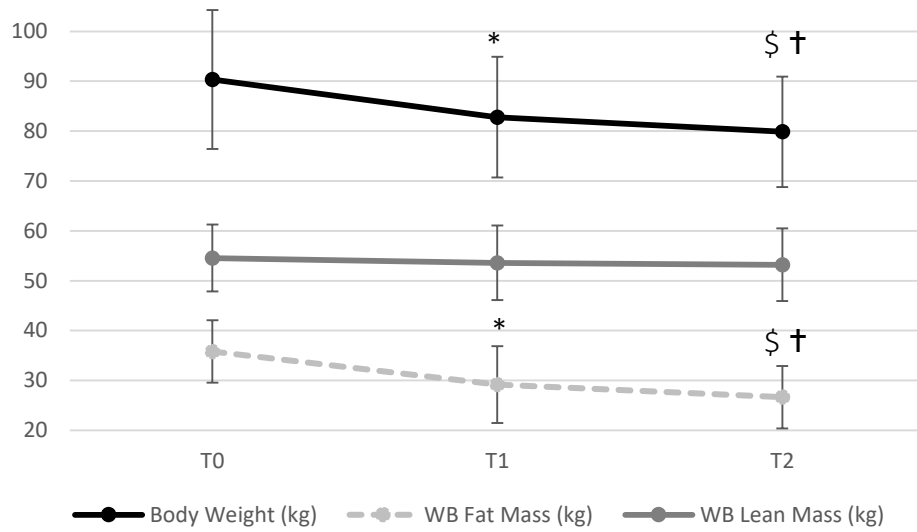


Figure 16 - Body composition changes (kg) of the obese interventional group during the weight loss intervention

* Significant difference between T0 and T1; \$ significant differences between T1 and T2; † significant differences between T0 and T2; *WB whole body*

Table 15 - Unadjusted bone variables at baseline between the adolescents who dropped out of the weight loss intervention and the rest of the intervention sample

<u>A</u>	WB (TBLH BMD)				Lumbar Spine				Hip				Neck			
	Ob n=24		Ob_out n=7		Ob n=24		Ob_out n=7		Ob n=24		Ob_out n=7		Ob n=24		Ob_out n=7	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
BMD (g/cm ²)	0.943	0.087	0.854	0.588 *	0.985	0.146	0.834	0.121 *	1.038	0.141	0.937	0.069	0.978	0.145	0.889	0.081
BMC (g)	2148.33	363.67	1734.51	264.03 *	52.25	10.87	40.16	7.35 *	34.51	5.80	29.61	3.80 *	4.88	0.82	4.11	0.60
BMAD (g/cm ³)	0.092	0.005	0.084	0.005 *	0.953	0.147	0.977	0.188								
TBS					1.306	0.109	1.15	0.79 *								

	Narrow Neck				InterTrochanteric				Femoral Shaft			
	Ob n=24		Ob_out n=7		Ob n=24		Ob_out n=7		Ob n=24		Ob_out n=7	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
BMD (g/cm ²)	1.212	0.198	1.090	0.102	1.129	0.188	1.082	0.148	1.557	0.163	1.364	0.115 *
ED (cm)	2.75	0.19	2.72	0.34	4.56	0.39	4.32	0.38	1.81	0.29	1.72	0.43
ACT (cm)	0.24	0.05	0.21	0.02	0.49	0.1	0.44	0.04	0.59	0.08	0.515	0.07 *
WIDTH (cm)	3.2	0.23	3.15	0.35	5.53	0.36	5.20	0.43	2.99	0.25	2.75	0.33 *
CSA (cm ²)	3.72	0.69	3.23	0.48	5.96	1.05	5.03	0.82	4.45	0.66	3.56	0.38 *
CSMI (cm ⁴)	3.04	1.16	2.40	0.76	15.81	0.72	12.35	3.77	3.72	0.21	2.55	0.73 *
Z (cm ³)	1.79	0.41	1.37	0.30	5.14	1.04	4.02	1.19	2.4	0.57	1.74	0.32 *
BR	7.43	1.49	8.40	1.74	6.48	1.36	5.98	1.23	2.63	0.47	2.86	0.63

* p<0.05 Ob significantly different than Ob_out.

Ob obese intervention group, Ob_out obese adolescents that dropped out the study, SD standard deviation, WB whole body, TBLH total body less head, LM lean mass, FM fat mass, BMD bone mineral density, BMC bone mineral content, BMAD bone mineral apparent density, NN narrow neck, IT intertrochanteric, FS femoral shaft, ED endocortical diameter, ACT average cortical thickness, WIDTH width, CSA cross sectional area, CSMI cross sectional moment of inertia, Z section modulus, BR bulking ratio

Bone parameters changes over the 8-month weight loss intervention in adolescents with obesity

Bone mineral density increased within the first four months of the weight loss intervention (Δ mean variation (SD)) LS BMD Δ 2.66 (2.94) % $p < 0.001$) as well as at the end of the 8 months compared with baseline (TBLH BMD (Δ 3.22 (3.58) % $p < 0.001$, WB BMD (Δ 3.82 (3.06) % $p < 0.001$, LS BMD (Δ 3.67 (4.04) % $p < 0.001$, LS TBS (Δ 3.41 (4.11) % $p = 0.001$).

Additional analysis showed at 4 months an increase in TBLH BMC ($p = 0.003$), WB BMC ($p < 0.001$), WB BMD ($p = 0.027$), LS BMC ($p < 0.001$), LS BMD ($p < 0.001$) and neck BMD ($p = 0.042$) after adjusted to body weight changes and fat mass changes. Between 4 and 8 months, bone mineral density continued to increase for TBLH, WB ($p < 0.001$) and the neck ($p = 0.038$) as well as for WB BMAD ($p = 0.028$) and BMC for TBLH and WB ($p < 0.001$).

In conclusion, between baseline to the end of the program, adolescents with obesity significantly increased TBLH / WB BMC, TBLH / WB BMD ($p < 0.001$), LS BMAD ($p = 0.015$), LS BMC ($p = 0.003$), LS BMD ($p = 0.014$) after adjustment for body weight and fat mass changes. Results from data adjusted can be found in appendix (Appendices 28 & 29).

At 4 months, reduced NN BMD (Δ -4.35 (6.19) % $p < 0.001$) and NN cortical thickness (ACT) (Δ -7.19 (8.79) % $p < 0.001$) were observed. Also, the NN endocortical diameter (ED) (Δ 2.85(0.26) % $p = 0.009$) and width (WIDTH) (Δ 5.48(10.84) % $p = 0.016$) increased. At the IT reduced BMD was observed (Δ -1.64 (3.24) % $p = 0.02$) along with increased ED (Δ 4.24 (5.24) % $p = 0.010$) and width (Δ 3.49 (4.40) % $p = 0.01$). Also, the FS endocortical diameter (Δ 5.15 (8.09) % $p = 0.010$) and WIDTH increased (Δ 3.14 (2.68) % $p < 0.001$).

From mid-intervention to the end of the 8-month intervention, adolescents with obesity significantly increased cortical thickness at both the intertrochanteric and femoral shaft. However, compared with baseline value, at program completion adolescents with obesity demonstrated a reduction in NN BMD (Δ -4.74 (6.07) % $p < 0.001$) and an increase in their endocortical diameter (Δ 6.20 (6.77) % $p < 0.001$) and width (Δ 6.16 (7.69) % $p < 0.001$) compared with their baseline scores. Moreover, IT BMD reduced (Δ -3.43 (4.62) % $p < 0.001$) while intertrochanteric ED (Δ 4.00 (4.31) % $p < 0.001$) and width (Δ 3.06 (2.79) % $p < 0.001$) continued to increase. Finally, the FS cortical thickness (ACT) (Δ 4.49 (5.21) % $p = 0.002$) increased (Figure 17).

The observed changes in unadjusted data at 4 and 8 months were confirmed after adjustment for each body composition changes. In addition, at the end of the weight loss program, increased in femoral shaft endocortical diameter and lower average cortical thickness at the narrow neck were observed after adjustment.

At 4 months, bone strength variables showed increases in FS bulking ratio (BR) (Δ 4.95 (9.28) % $p = 0.020$), IT BR (Δ 3.66 (7.09) % $p = 0.020$) and femoral shaft cross sectional moment of inertia (CSMI) (Δ 3.88 (1.27) $p = 0.030$). NN section modulus (Z) (Δ -1.70 (0.39) $p = 0.039$) significantly decreased.

No changes were observed between 4 months to 8 months. However, at the end of the intervention compared with baseline values, narrow neck BR (Δ 8.24 (2.00) % $p = 0.005$), intertrochanteric BR (Δ 9.25 (6.43) % $p < 0.001$), femoral shaft cross-sectional area (Δ 4.67 (5.29) % $p < 0.001$) and FS cross sectional moment of inertia (CSMI) (Δ 7.09 (15.99) % $p = 0.030$) also increased (Figure 18).

When adjusted for Δ BW or Δ FM similar results were observed at 4 months and 8 months. In addition, at 4 months, after adjustment for body weight changes, cross sectional area at the narrow neck significantly decreased ($p=0.044$).

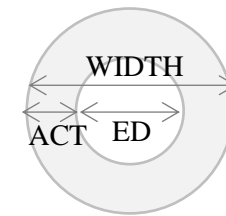
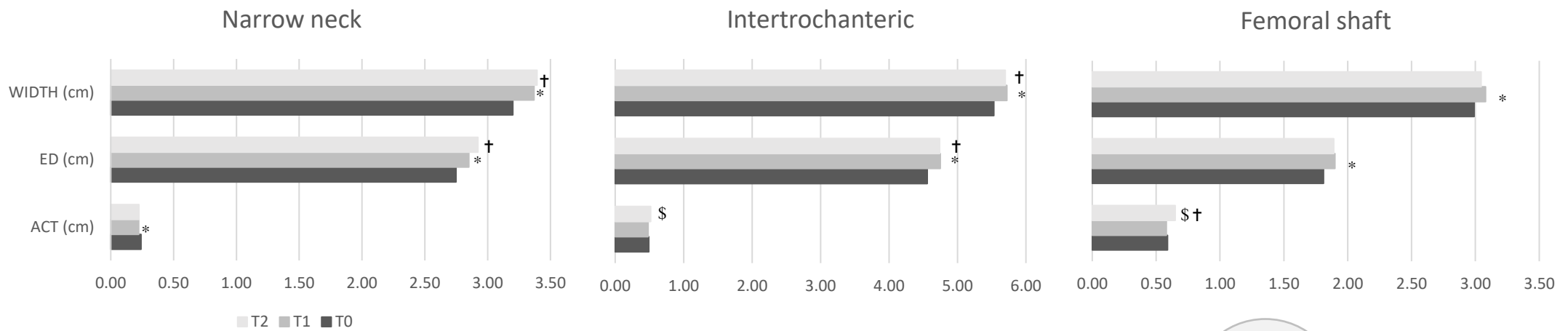


Figure 17 - Unadjusted bone geometric evolution at NN, IT and FS of the obese interventional group during the weight loss program

* Significant difference between T0 and T1; \$ significant differences between T1 and T2; † significant differences between T0 and T2

ACT average cortical thickness, ED endocortical diameter, WIDTH width

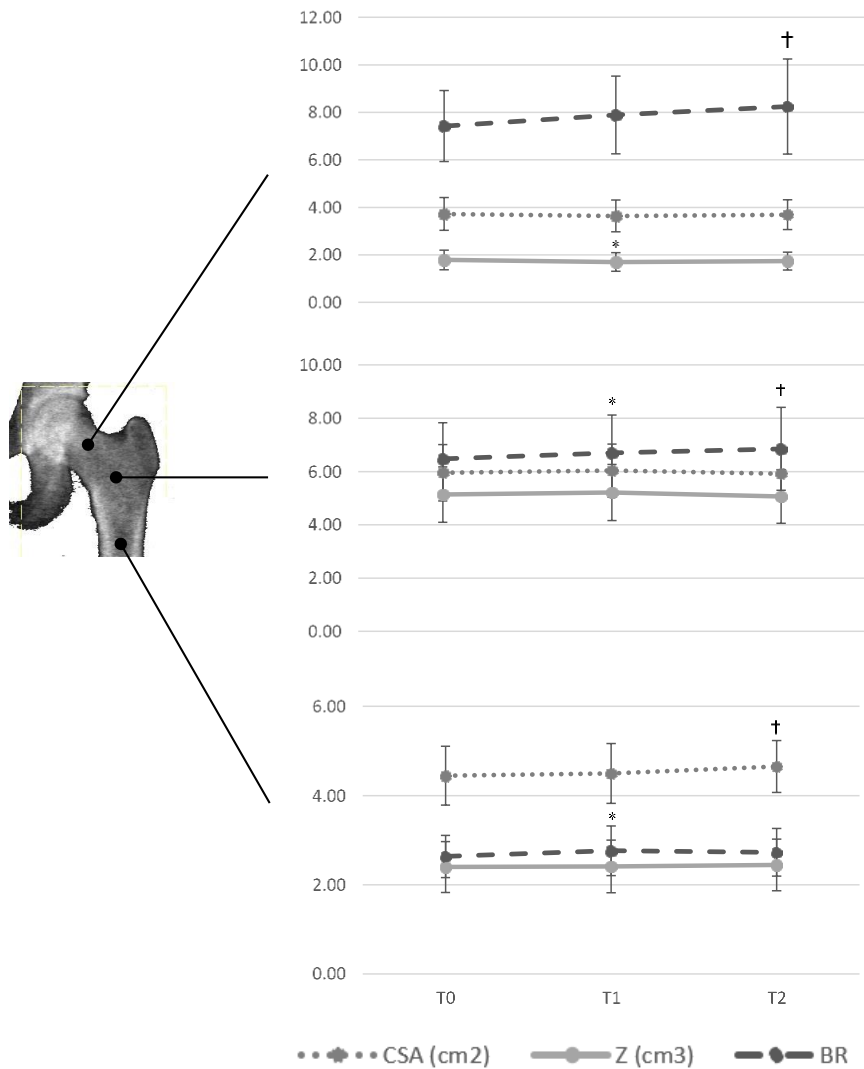


Figure 18 - Unadjusted bone strength changes at NN, IT and FS of the obese interventional group during the weight loss program

* Significant difference between T0 and T1; † significant differences between T1 and T2; ‡ significant differences between T0 and T2

CSA cross sectional area, Z section modulus, BR bulking ratio

Biomarkers changes over the 8-month weight loss program in adolescents with obesity

Results from non-parametric analysis of leptin, estradiol and P1NP showed similar concentration throughout the weight loss program (Table 16). However, a lower CTx concentration at 4 months were observed ($p=0.037$), while an increase was shown at 8 months ($p=0.013$).

The uncoupling index and z- scores were calculated from the Ob baseline data (Table 17). A significant reduction in marker CTx was observed at 4 months ($p=0.037$) before returning to its baseline secretion level ($p=0.013$). Despite an acute formation phase observed in the UI at 4 months (0.98 (2.19)) before declining at 8 months (0.32 (1.80)) no changes were observed over the weight loss intervention in the uncoupling index of bone remodeling.

Table 16 - Biochemical characteristics of the Ob during the weight loss program

	Ob <i>n</i> =10					
	T0		T1		T2	
	<i>median</i>	<i>IQR</i>	<i>median</i>	<i>IQR</i>	<i>median</i>	<i>IQR</i>
Leptin ng.ml ⁻¹	29.83	19.63	19.01	29.85	25.14	18.04
Estradiol pg.ml ⁻¹	56.67	97.49	80.77	104.59 *	80.08	72.47 †
P1NP ng.ml ⁻¹	41.30	4.09	38.55	9.46	41.22	7.74
CTx ng.ml ⁻¹	4.42	1.50	3.47	0.94 *	4.34	1.31 †

* Significant difference between T0 and T1; † significant differences between T1 and T2; ‡ significant differences between T0 and T2

Ob obese intervention group, IQR interquartile range, P1NP Procollagen type 1 N-terminal propeptide, CTx collagen type 1 cross-linked C-telopeptide

Table 17 - Bone remodeling scores and uncoupling index

	T0		Ob n=10 T1		T2	
	mean	SD	mean	SD	mean	SD
score F	0.00	1.00	-0.03	2.05	-0.36	1.90
score R	0.00	1.00	-1.01	1.00 *	0.05	0.68 †
UI	0.00	1.74	0.98	2.19	0.32	1.80

* Significant difference between T0 and T1; † significant differences between T1 and T2; ‡ significant differences between T0 and T2

Ob obese intervention group, SD standard deviation, F formation, R resorption, UI uncoupling index

Additional exploration of bone parameters using correlations

At the end of the intervention, changes in leptin was positively associated with changes in bone density (LS) (p=0.02) and cortical thickness (narrow neck, intertrochanteric, femoral shaft) (p=0.002, p=0.046, p=0.049 respectively).

In contrast, the bone resorption marker of CTx displayed an inverse correlation with bone density (WB p=0.002; LS p<0.001; neck p<0.001, hip p=0.012, NN p<0.001, IT p=0.046), cortical thickness (NN p<0.001, IT p=0.026), cross sectional area (NN p<0.001, IT p=0.013) and section modulus (NN p=0.004, IT p=0.016) and was positively correlated with the bulking ratio (NN p=0.016) (data and coefficients are detailed in Table 18). Correlational analysis at the completion of the weight loss intervention showed no association of VFAT (% or g) with bone parameters. However, body weight (g) and fat mass (g) were moderately correlated with all bone parameters, except for NN BR (BW p=0.006) (data and coefficients are detailed in Table 18).

Table 18 - Correlation analysis. A. Between hormones and bone parameters. B. Between body composition parameters and bone parameters.

<u>A</u>	Bone Mineral Density								CTx	
	TBLH		LS		neck		hip		r	p
	r	p	r	p	r	p	r	p		
Leptin ng.ml ⁻¹			0.397	0.02					-0.419	0.02
CTx ng.ml ⁻¹	-0.552	0.002	-0.625	<0.001	-0.565	<0.001	-0.462	0.012		

	Bone Mineral Density				Average Cortical Thickness				Cross Sectional Area				Section Modulus				BR			
	NN		IT		NN		IT		FS		NN		IT		NN		NN			
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p		
Leptin ng.ml ⁻¹					0.502	0.002	0.345	0.046	0.339	0.049										
CTx ng.ml ⁻¹	-0.614	<0.001	-0.374	0.046	-0.579	<0.001	-0.414	0.026			-0.594	<0.001	-0.457	0.013	-0.517	0.004	-0.443	0.016	0.443	0.016

<u>B</u>	TBLH		LS		Hip		Neck													
	BW		LM (g)		BW		FM (g)		LM (g)		BW		FM (g)		LM (g)					
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p				
BMD (g/cm ²)	0.511	<0.001	0.735	<0.001	0.455	0.001	0.533	<0.001	0.568	<0.001	0.334	0.025	0.695	<0.001	0.562	<0.001	0.330	0.027	0.687	<0.001
BMAD					0.305	0.035	0.450	<0.001												

	Narrow Neck				Intertrochanteric				Femoral Shaft									
	BW		FM (g)		LM (g)		BW		FM (g)		LM (g)		BW		FM (g)		LM (g)	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
BMD (g/cm ²)	0.553	<0.001	0.349	0.017	0.648	<0.001	0.505	<0.001			0.625	<0.001	0.562	<0.001	0.319	0.033	0.705	<0.001
ACT (cm)	0.341	0.021			0.324	0.028											0.316	0.033
WIDTH (cm)					0.349	0.017	0.342	0.020			0.498	<0.001	0.389	0.008			0.503	<0.001
CSA (cm ²)	0.635	<0.001	0.401	0.006	0.743	<0.001	0.587	<0.001	0.314	0.034	0.754	<0.001	0.623	<0.001	0.347	0.019	0.791	<0.001
Z (cm ³)	0.678	<0.001	0.462	<0.001	0.753	<0.001	0.606	<0.001	0.325	0.027	0.778	<0.001	0.585	<0.001	0.355	0.015	0.702	<0.001
BR	-0.398	0.006			-0.480	<0.001												

BW body weight, FM fat mass, LM lean mass, BMD bone mineral density, BMAD bone mineral apparent density, TBLH total body less head, LS lumbar spine, CTx collagen type 1 cross-linked C-telopeptide, NN narrow neck, IT intertrochanteric, FS femoral shaft, CSA cross sectional area, ACT average cortical thickness, WIDTH width, Z section modulus, BR bulking ratio

Table 19 - Body composition parameters (A) and bone variables (B) of the 24 adolescents with obesity at baseline, 4 and 8 months.

<u>A</u>	Ob <i>n</i> =24					
	T0		T1		T2	
	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>
Age (years)	14.05	1.08	14.39	1.09	14.74	1.09
BMI	33.02	4.02	29.89	2.38	28.19	2.89
Height (cm)	163.83	7.11	164.49	6.57	165.41	6.42
Body weight (kg)	90.37	13.93	82.80	12.09	79.86	11.06
Fat mass (g)	35813.94	7695.14	29204.10	6247.12	26666.43	6274.81
FM (%)	39.35	3.85	35.03	4.07	33.07	5.01
Lean mass (g)	54559.85	7451.10	53597.40	7285.22	53198.28	6722.13
a FM (%)	42.50	4.81	37.72	4.39	35.30	5.53
g FM (%)	41.25	3.83	36.94	4.24	35.47	5.14
VFAT (g)	310.63	102.46	233.14	63.72	197.02	61.51
VFAT (%)	43.40	4.39	38.52	4.69	36.70	5.78
VFAT (cm ³)	335.81	110.77	252.05	68.89	213.00	66.49

<u>B</u>	WB						TBLH						LS					
	T0		T1		T2		T0		T1		T2		T0		T1		T2	
	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>
BMD (g/cm ²)	1.102	0.091	1.111	0.091	1.143	0.085	0.943	0.087	0.969	0.084	0.988	0.078	0.985	0.146	1.011	0.133	1.037	0.128
BMC (g)	2148.33	363.67	2228.51	365.18	2305.14	362.92	1667.65	283.80	1712.30	287.78	1788.79	285.26	52.25	10.87	55.89	10.79	58.56	10.43
BMAD (g/cm ³)	0.092	0.01	0.092	0.005	0.093	0.005	0.092	0.005	0.091	0.005	0.091	0.005	0.953	0.147	0.992	0.138	1.037	0.128
TBS													1.306	0.109	1.324	0.111	1.345	0.113

	Hip						Neck					
	T0		T1		T2		T0		T1		T2	
	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>	<i>mean</i>	<i>SD</i>
BMD (g/cm ²)	1.038	0.141	1.035	0.135	1.039	0.140	0.978	0.145	0.962	0.131	0.969	0.132
BMC (g)	34.51	5.80	34.61	4.85	35.10	5.88	4.88	0.82	4.82	0.84	4.92	0.79

	NN						IT						FS					
	T0		T1		T2		T0		T1		T2		T0		T1		T2	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
BMD (g/cm ²)	1.212	0.198	1.158	0.197	1.152	0.189	1.129	0.188	1.107	0.168	1.091	0.174	1.557	0.163	1.532	0.168	1.572	0.162
ED (cm)	2.75	0.19	2.85	0.26	2.92	0.31	4.56	0.39	4.75	0.42	4.74	0.44	1.81	0.29	1.90	0.34	1.89	0.34
ACT (cm)	0.24	0.01	0.22	0.01	0.23	0.01	0.49	0.02	0.48	0.02	0.52	0.01	0.59	0.02	0.58	0.01	0.65	0.02
WIDTH (cm)	3.2	0.23	3.37	0.30	3.40	0.28	5.53	0.36	5.72	0.38	5.70	0.35	2.99	0.25	3.08	0.27	3.04	0.37
CSA (cm ²)	3.72	0.69	3.64	0.67	3.69	0.63	5.96	1.05	6.03	1.00	5.92	0.97	4.45	0.66	4.50	0.67	4.65	0.58
CSMI (cm ⁴)	3.04	1.16	2.95	0.88	3.10	0.80	15.81	0.72	16.50	4.06	16.10	3.77	3.72	0.21	3.89	1.27	3.94	1.16
Z (cm ³)	1.79	0.41	1.70	0.39	1.73	0.38	5.14	1.04	5.21	1.05	5.07	1.00	2.40	0.57	2.41	0.59	2.45	0.57
BR	7.43	1.49	7.89	1.64	8.25	2.00	6.48	1.36	6.70	1.43	6.85	1.56	2.63	0.47	2.76	0.56	2.73	0.54

Ob adolescents with obesity, SD standard deviation, BMI body mass index, FM fat mass, a FM android fat mass, g FM gynoid fat mass, VFAT visceral fat, WB whole body, TBLH total body less head, BMD bone mineral density, BMC bone mineral content, BMAD bone mineral apparent density, TBS Trabecular Bone Score, NN narrow neck, IT intertrochanteric, FS femoral shaft, ED endocortical diameter, WIDTH width, CSA cross sectional area, ACT average cortical thickness, Z section modulus, BR bulking ratio

To summarise, an overall increase in bone mineral density was observed at whole body and site-specific lumbar spine in adolescents with obesity over the weight loss intervention. However, bone geometry and strength appeared to be weakened during the weight loss program, particularly at the narrow neck with a score moving closer to the fracture prediction threshold. Furthermore, uncoupling index observations suggested bone remodeling activity in favour of formation only during the first 4 months of the intervention, but without reaching significance and returning to baseline at 8 months.

4.3. Does 8 months WL induced by physical activity and nutrition conduct to normal bone health?

Aim

To investigate the impact of body weight changes induced by a structured weight loss intervention on bone parameters in adolescents with obesity compared with normal weight maturation-matched peers.

Table 20 - Outline of the data collection and participants for the third aim




Groups		Baseline	4-month	8-month
Intervention	 Adolescents with obesity residential WL program (physical activity & nutrition)	● n = 31 (6 ♂)	● n=29 (4 ♂)	● n = 24 (3 ♂)
	 Normal weight adolescents	● n = 23 (♀)	● n = 23 (♀)	● n = 23 (♀)
Control	 Adolescents with obesity no-residential program	● n = 11 (4 ♂)	● n = 11 (4 ♂)	

Table 20 indicates that participants targeted in meeting the third aim of the research were the adolescents with obesity who underwent the intervention and the normal weight control group. The shading indicates that only baseline and 8-month comparisons were analysed.

Out of the 54 participants, 47 (87%) of them completed the whole study including three males (Figure 15). As explained previously, baseline body composition descriptive characteristics were similar but bone variables differ between the adolescents who dropped out and the other. Only the adolescents with obesity that completed the whole study (n=24) were considered for statistical analysis using mixt-model analysis and multivariate analysis. In addition, groups are homogenous and the presence of males do not influence the distribution of the data.

Bone parameters time-related differences

Bone measurements expressed as unadjusted and adjusted values are presented Table 22.

Differences in lower bone density unadjusted values were observed for TBLH BMD, BMAD ($p < 0.001$) and hip BMD ($p = 0.017$) between Ob and NW. However, once the longitudinal changes in body weight (BW), or fat mass (FM) were accounted for results changed. Compared with their NW peers, adjusted data from adolescents with obesity showed lower density at TBLH ($p < 0.008$ for all adjustment), neck ($p < 0.001$ adjusted BW, $p = 0.031$ adjusted FM), hip ($p < 0.008$ for all) and lower bone mineral apparent density ($p < 0.008$ for BW and FM) at the whole body. Differences in the changes of bone parameters (Δ LS BMAD ($p = 0.02$), Δ neck BMD ($p = 0.03$), Δ hip BMD ($p < 0.001$)) between both groups were explained by variations in BW and Δ FM. Only Δ hip BMD remained significantly different after adjustment Δ BW.

Compared with changes over time in NW, at the end of the weight loss intervention Ob showed greater values in narrow neck endocortical diameter (ED) ($p = 0.005$), NN width ($p = 0.003$),

intertrochanteric endocortical diameter ($p=0.005$), IT width ($p=0.017$) and femoral shaft ED ($p=0.006$). In contrast, lower values were observed in adolescents with obesity than their NW peers for IT BMD ($p=0.009$), IT cortical thickness (ACT) ($p=0.031$), FS BMD ($p=0.004$) and FS ACT ($p=0.001$) (Figure 19). When data were corrected for longitudinal changes in body weight (BW), or fat mass (FM) adolescents with obesity had continued to show lower bone density and cortical thickness at IT ($p<0.010$) and FS ($p<0.010$). Lower cortical thickness remained at NN ($p=0.05$) in the Ob than NW groups after adjustment for body weight changes. In addition, higher endocortical diameter after adjustments for BW (FS $p=0.014$) and FM (IT $p<0.05$, FS $p=0.018$) changes were seen in adolescents with obesity than their NW peers. Time-related changes in bone parameters between adolescents with obesity and NW were at least partially explained by changes in body weight or Δ fat mass. Only the Δ IT BMD maintained significance when adjusted for Δ BW.

Differences in raw strength indices were observed for NN bulking ratio (BR) ($p=0.008$), IT BR ($p=0.004$) and FS BR ($p=0.004$). When adjusted for Δ BW, cross-sectional area (IT and FS) was higher than NW ($p<0.003$ for all). Cross-sectional area at IT ($p=0.020$) was also higher when corrected for Δ FM. Higher bulking ratio at IT and FS for adolescents with obesity than NW were observed once the adjusted for changes in BW or FM ($p<0.004$). Time-related changes of bone parameters between groups for Δ IT BR ($p<0.001$), Δ FS BR ($p=0.022$), were explained by variations in BW or Δ FM.

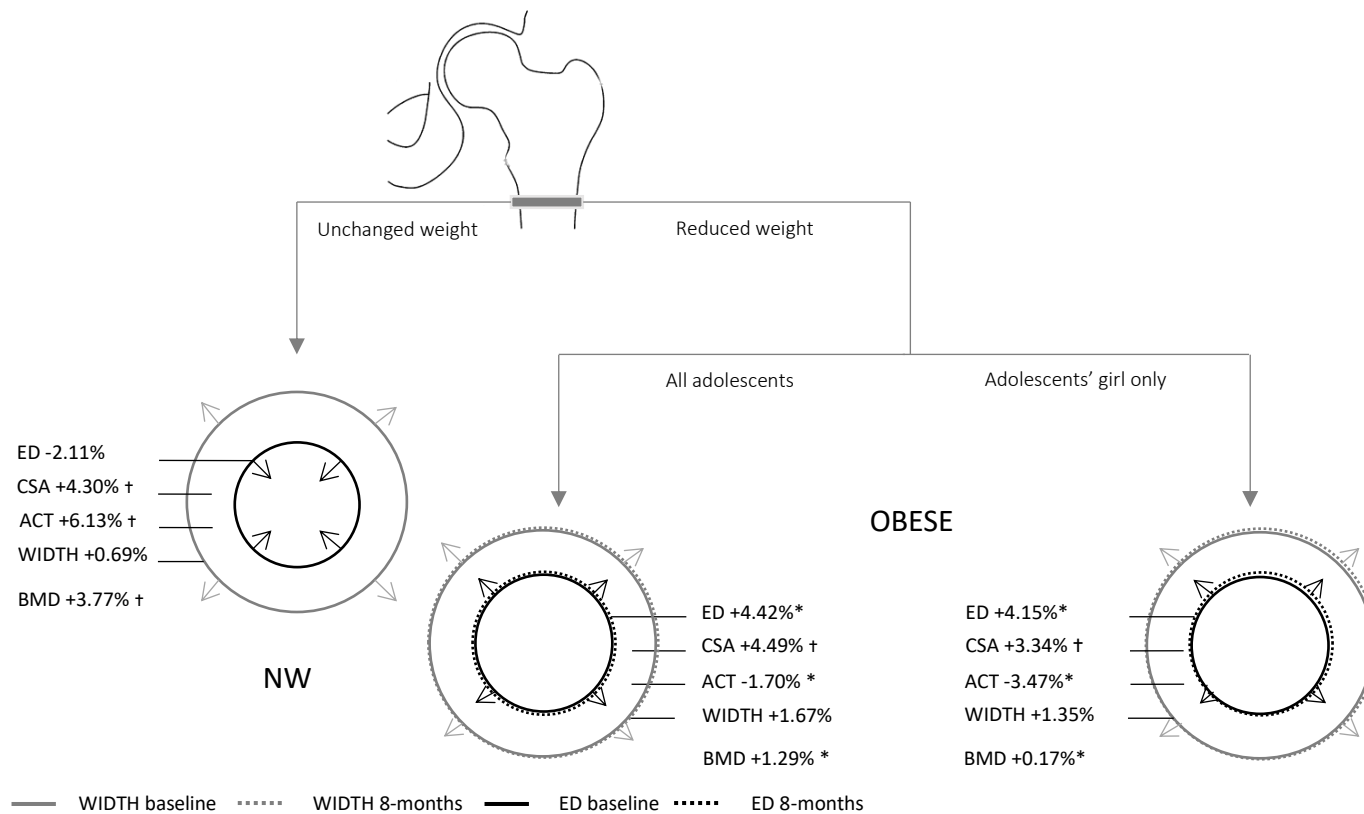


Figure 19 - Schematic representation of unadjusted geometric changes at the femoral shaft at 8-months. Over the 8 months, CSA significantly increased with a similar magnitude in both group. ACT and BMD significantly increased only for the NW group.

† p<0.05 between baseline and 8 months; * p<0.05 at 8-months between Ob and NW when adjusted to fat mass changes

NW normal weight control group, Ob obese group, ED endocortical diameter, CSA cross-sectional area, ACT average cortical thickness, WIDTH subperiosteal width, BMD bone mineral density

Biomarkers time-related differences

At the end of the weight loss intervention, no difference was observed in estradiol levels between groups. (Table 12).

Table 21 - Biochemical characteristics of the groups at baseline (T0) and 8 months (T2)

	Ob		NW	
	<i>median</i>	<i>IQR</i>	<i>median</i>	<i>IQR</i>
Leptin ng.ml ⁻¹ T0	29.83	19.63		
Leptin ng.ml ⁻¹ T2	25.14	18.04		
Estradiol pg.ml ⁻¹ T0	56	97	49	46
Estradiol pg.ml ⁻¹ T2	80	72.47	42	30

* p<0.05 in comparison between OB and NW

OB obese intervention group, NW normal weight control group, SD standard deviation

Table 22 - Bone variables at 8 months. A. Unadjusted mean. B. Body weight adjusted. C. Fat mass adjusted.

<u>A</u>	WB (TBLH BMD)				Lumbar Spine				Hip				Neck			
	Ob		NW		Ob		NW		Ob		NW		Ob		NW	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
BMD (g/cm ²)	0.988	0.078	1.092	0.065 *	1.037	0.128	1.048	0.099	1.039	0.140	1.130	0.101 *	0.969	0.132	1.021	0.093
BMC (g)	2305.14	362.92	2363.88	276.79	58.56	10.43	64.95	10.72 *	35.10	5.88	38.10	5.21	4.92	0.79	5.09	0.49
BMAD (g/cm ³)	0.093	0.005	0.102	0.005 *	1.037	0.128	1.037	0.086								

	Narrow Neck				Intertrochanteric				Femoral Shaft			
	Ob		NW		Ob		NW		Ob		NW	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
BMD (g/cm ²)	1.152	0.189	1.214	0.120	1.091	0.174	1.219	0.144 *	1.572	0.162	1.711	0.144 *
ED (cm)	2.92	0.31	2.67	0.27 *	4.74	0.44	4.36	0.43 *	1.89	0.34	1.61	0.30 *
ACT (cm)	0.22	0.03	0.24	0.03	0.48	0.09	0.53	0.06 *	0.58	0.10	0.69	0.11 *
WIDTH (cm)	3.40	0.28	3.15	0.24 *	5.70	0.35	5.42	0.39 *	3.04	0.37	2.93	0.25
CSA (cm ²)	3.69	0.63	3.63	0.35	5.92	0.97	6.28	0.71	4.65	0.58	4.76	0.53
CSMI (cm ⁴)	3.10	0.80	2.78	0.60	16.10	3.77	15.20	3.28	3.94	1.16	3.77	0.99
Z (cm ³)	1.73	0.37	1.69	0.26	5.06	1.00	5.03	0.79	2.44	0.57	2.44	0.41
BR	8.25	2.00	6.92	1.08 *	6.85	1.56	5.72	0.91 *	2.73	0.54	2.27	0.47 *

<u>B</u>	WB (TBLH BMD)		Lumbar Spine		Hip		Neck	
	Ob		Ob		Ob		Ob	
	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI
BMD (g/cm ²)	0.966	(0.926 - 1.006) *	0.969	(0.917 - 1.021) *	0.972	(0.910 - 1.035) *	0.910	(0.850 - 0.969) *
BMC (g)	2024.60	(1915.29 - 2133.90) *	51.38	(46.87 - 55.88) *	31.43	(28.73 - 34.13) *	4.47	(4.15 - 4.78) *
BMAD (g/cm ³)	0.094	(0.091 - 0.097) *	0.975	(0.924 - 1.025) *				

	Narrow Neck		Intertrochanteric		Femoral Shaft	
	Ob		Ob		Ob	
	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>
BMD (g/cm ²)	1.084	(1.001 - 1.167)	1.047	(0.953 - 1.141) *	1.504	(1.414 - 1.593) *
ED (cm)	2.86	(2.69 - 3.03)	4.76	(4.50 - 5.02)	1.95	(1.77 - 2.15) *
ACT (cm)	0.21	(0.19 - 0.23) *	0.44	(0.39 - 0.49) *	0.55	(0.49 - 0.62) *
WIDTH (cm)	3.33	(3.17 - 3.48)	5.64	(5.42 - 5.87)	3.08	(2.89 - 3.28)
CSA (cm ²)	3.47	(3.17 - 3.76)	5.61	(5.12 - 6.11) *	4.42	(4.09 - 4.75) *
CSMI (cm ⁴)	2.78	(2.38 - 3.18)	15.10	(13.03 - 17.18)	3.78	(3.15 - 4.44)
Z (cm ³)	1.59	(1.41 - 1.78)	4.78	(4.25 - 5.31)	2.34	(2.04 - 2.64)
BR	8.30	(7.33 - 9.26)	7.40	(6.68 - 8.13) *	2.92	(2.63 - 3.21) *

<u>C</u>	WB (TBLH BMD)		Lumbar Spine		Hip		Neck	
	Ob		Ob		Ob		Ob	
	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>
BMD (g/cm ²)	0.966	(0.926 - 1.006)	1.019	(0.955 - 1.083)	1.003	(0.932 - 1.073) *	0.933	(0.867 - 0.999) *
BMC (g)	2240.34	(2061.47 - 2419.20)	57.90	(51.98 - 63.82)	33.08	(29.89 - 36.27) *	4.82	(4.42 - 5.21)
BMAD (g/cm ³)	0.092	(0.089 - 0.095) *	1.023	(0.961 - 1.084)				

	Narrow Neck		Intertrochanteric		Femoral Shaft	
	Ob		Ob		Ob	
	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>
BMD (g/cm ²)	1.128	(1.036 - 1.220)	1.043	(0.953 - 1.134) *	1.523	(1.434 - 1.611) *
ED (cm)	2.84	(2.68 - 3.00)	4.76	(4.51 - 5.01) *	1.94	(1.75 - 2.12) *
ACT (cm)	0.22	(0.20 - 0.23)	0.44	(0.40 - 0.49) *	0.56	(0.50 - 0.62) *
WIDTH (cm)	3.31	(3.17 - 3.46)	5.66	(5.44 - 5.88)	3.07	(2.88 - 3.25)
CSA (cm ²)	3.52	(3.23 - 3.81)	5.61	(5.13 - 6.08) *	4.49	(4.16 - 4.81)
CSMI (cm ⁴)	2.83	(2.44 - 3.23)	15.19	(13.18 - 17.20)	3.85	(3.22 - 4.46)
Z (cm ³)	1.63	(1.44 - 1.81)	4.80	(4.29 - 5.31)	2.37	(2.07 - 2.66)
BR	8.10	(7.16 - 9.03)	7.34	(6.63 - 8.04) *	2.86	(2.58 - 3.15) *

* p<0.05 OB significantly different than NW.

Ob obese intervention group, NW normal weight control group, SD standard deviation, WB whole body, TBLH total body less head, LM lean mass, FM fat mass, BMD bone mineral density, BMC bone mineral content, BMAD bone mineral apparent density, NN narrow neck, IT intertrochanteric, FS femoral shaft, ED endocortical diameter, WIDTH width, CSA cross sectional area, CSMI cross sectional moment of inertia, ACT average cortical thickness, Z section modulus, BR bulking ratio

To summarise, despite favoured bone density adaptation over the 8 months, adolescents with obesity demonstrated lower bone parameters than their normal weight peers after adjustment for body weight. Yet, some positive adaptations were seen for hip geometry most specifically at the narrow neck. However, as stated in the previous chapter, the index of fracture prediction at the narrow neck remained of concern. Finally, bone accretion among adolescents with obesity appeared to follow androgen like adaptations, which was not apparent in the normal weight control group.

The final aim addresses the hypothesis that:

- i. Obesity in adolescents is associated with altered bone remodeling markers.
- ii. The 8-month weight loss intervention will stimulate the remodeling activity in favour of bone formation in adolescents with obesity.
- iii. The weight loss intervention experienced by adolescents with obesity will induce a shift of bone turnover towards positive bone formation compared with an obese control group; trending towards bone formations values similar to a lean control group.

Aim 4:

- i. To investigate the influence of body weight status and weight loss intervention on bone remodeling in adolescents with obesity and normal weight controls.

To answer the final aim of this thesis, we used the data of 38 adolescents recruited for the program of research. Data in this section were collected from 10 adolescents with obesity (Ob) enrolled in a weight loss intervention, 17 normal-weight (NW) adolescent females and 11 Ob controls (4 ♂).

4.4. Does weight status and weight changes influence bone markers?

Aim

To investigate the influence of body weight status and weight loss intervention on bone remodeling in adolescents with obesity and normal weight controls.

Table 23 - Outline of the data collection and participants for the fourth aim




Groups		Baseline	4-month	8-month
Intervention	 Adolescents with obesity residential WL program (physical activity & nutrition)	●	●	●
		n = 31 (6 ♂)	n=29 (4 ♂)	n = 24 (3 ♂)
Control	 Normal weight adolescents	●	●	●
		n = 23 (♀)	n = 23 (♀)	n = 23 (♀)
	 Adolescents with obesity no-residential program	●	●	
		n = 11 (4 ♂)	n = 11 (4 ♂)	

Table 23 indicates that participants targeted in meeting the final aim of the research were the adolescents with obesity who underwent the intervention, the normal weight control group and the adolescents with obesity who did not receive the intervention and therefore acted as a control group. The shading indicates that only baseline and 4-month data were used and the 8-month data were only available for the adolescents with obesity enrolled in the weight loss intervention.

Table 24 - Bone markers concentration

	Baseline				4 months				8 months					
	P1NP ^{*,§}		CTx ^{*,§}		P1NP ^{*,§}		CTx ^{*,†}		P1NP		CTx			
	median	IQR	median	IQR	median	IQR	median	IQR	median	IQR	median	IQR		
Ob	41.30	4.09	4.42	1.50	38.55	9.46	3.47	0.94	£	41.22	7.74	4.34	1.31	¤
NW	120.00	77.50	7.04	2.27	136.00	69.50	6.76	2.68						
Ob control	41.76	7.28	5.60	1.97	39.67	4.75	6.30	2.07	£					

* Significant difference between Ob and NW; § significant differences between Ob control and NW; † significant differences between Ob and Ob control, £ significant differences between baseline and 4 months, ¤ significant differences between 4 months and 8 months, µ significant differences between baseline and 8 months

IQR interquartile range, Ob obese group, NW normal weight control group, Ob control obese control, CTx collagen type 1 cross-linked C-telopeptide, P1NP Procollagen type 1 N-terminal propeptide

Analysis of biomarkers used the non-parametric Wilcoxon test. Bone marker plot analyses occurred on data from baseline and 4 months for both control groups and at baseline, 4 and 8 months for the Ob group. Table 24 described bone markers concentration of each group.

Table 25 - Descriptive statistics at baseline

	Ob <i>n</i> = 10		NW <i>n</i> = 17		Ob control <i>n</i> = 11 (4 σ)		
	Mean	SD	Mean	SD	Mean	SD	
Age (years)	14.12	1.39	16.06	0.39	14.02	1.39	*, £
Menarche age	12.33	0.86	13.00	1.15	12.29	0.95	
BMI	33.70	4.85	20.71	1.42	30.80	4.80	*, £
Height (cm)	161.60	8.26	164.38	5.08	165.82	7.25	
Body weight (Kg)	88.17	15.65	57.19	5.93	87.71	16.83	*, £
WB Lean Mass (g)	52393.42	8970.61	45454.15	4648.97	52912.17	9669.91	*, £
WB FM (%)	40.35	4.08	20.46	3.12	37.75	3.43	*, £
WB FM (g)	35783.42	8067.86	11734.42	2380.31	33414.61	8127.23	*, £
Android (%)	43.39	5.62	18.06	4.03	40.87	4.84	*, £
Gynoid (%)	42.70	3.87	26.00	3.12	40.12	3.28	*, £
V FAT (%)	44.01	5.32	19.08	4.37	39.51	5.57	*, £
V FAT (g)	274.79	71.47	140.33	54.86	407.64	185.04	*, £, \$
V FAT (cm ³)	297.07	71.47	151.70	59.31	398.25	163.45	*, £,

* $p < 0.05$ comparisons between Ob and NW, ^{\$} $p < 0.05$ comparisons between Ob and Ob control, [£] $p < 0.05$ comparisons between Ob control and NW

Ob obese group, NW normal weight control group, SD standard deviation, BMI body mass index, WB LM whole body lean mass, WB FM whole body fat mass, V FAT visceral fat

Mean and Standard Deviation (SD) at the baseline of adolescents with obesity in the intervention group, adolescents with obesity who formed a control group and normal-weight control groups are summarised in Table 25. As expected, compared with normal weight

participants, both of the groups with obesity were greater in term of body mass index ($\text{kg}\cdot\text{m}^{-2}$) ($p<0.001$), body weight (kg) ($p<0.001$) but were younger in age (years) ($p<0.001$). However, no differences in height (m) were observed.

At baseline, both groups with obesity had higher values for lean mass (g) than their NW peers (Ob $p=0.032$, Ob control $p=0.017$). Similarly, compared with the NW group, both groups with obesity had higher values for whole body FM (% and g), as well as for android FM (%), gynoid FM (%) ($p<0.001$) and visceral fat (% , g and cm^3) (% $p<0.001$; g: Ob $p=0.021$, Ob control $p<0.001$; cm^3 : Ob $p=0.006$, Ob control $p<0.001$). However, a difference for VFAT (g) was observed between both obese group ($p=0.034$), with higher value in the control group.

Bone markers changes among adolescents of various weight normalised to their respective baseline median

Data were normalised to group's respective baseline median to observe each group's changes over time (Figure 20). Table 26 details bone remodeling scores and uncoupling index.

For the normal weight adolescents (NW), confidence ellipses of bone turnover overlapped at baseline and 4 months. At baseline, the distribution of adolescents relating to bone remodeling activity was 53% in fast formation, 35% in fast resorption and 12% in slow resorption. With the exception of one adolescent, all data were close to the balance central axis, in the upper quadrant of the turnover axis, which defines the separation between fast/slow activities. At 4 months, the ellipse appeared to shift towards dominant formation. Indeed, the distribution

among the features of resorption and formation were 76% for fast formation, 17% for fast resorption and 7% for slow formation.

To confirm the observed graphical representation and the uncoupling index favouring bone formation ($p=0.028$; 0.47 (0.78)), statistical differences were reported for the median of the balance between baseline and 4 months ($p=0.044$). The shift towards bone formation might be explained by an increase of the balance in favour of formation, while bone turnover did not change.

At baseline, bone markers of the adolescents with obesity who did not receive the intervention favoured bone formation. Surprisingly, confidence ellipse of this Ob control group appeared to move backward to a fast bone resorption state after 4 months. The accelerated bone resorption was visualised by a shift of the ellipse towards the left upper quadrant. Also at baseline, data were more scattered than at 4 months. The calculated distributions of data from adolescents for fast formation and fast resorption at baseline and 4 months for CTx-P1NP were 56% to 44% and 27% to 73%, respectively.

The resorption state of the adolescents with obesity enrolled in the control group was confirmed by differences over time in the median value for balance ($p=0.010$) and turnover ($p=0.050$); indicating accelerated bone resorption. However, changes in the UI (from 0.00 (1.20) to -1.32 (1.43)) were not observed between baseline to 4 months in this group ($p=0.075$).

Finally, for the adolescents enrolled in the WL intervention, the three ellipses overlapped. At baseline, adolescents with obesity in the intervention demonstrated bone remodeling activity favouring fast resorption/fast formation. The distribution of the adolescents among the bone marker plot was approximately 50% in fast resorption and 50% in fast formation. At the mid-point of the intervention, a shift occurred in bone turnover. Indeed, during this period of weight loss (Δ weight loss %, mean (SD) -9.04 (4.57)) bone remodeling activity appeared to induce bone formation, with 90% of the adolescents in the “formation” quadrant of the bone plot. Data from the uncoupling index supports a formation bias. Indeed, at 4 months adolescents enrolled in the weight loss intervention demonstrated a positive UI (0.98 (2.19)). However, the UI was not significantly different from baseline. At the end of the weight loss intervention bone turnover in adolescents with obesity returned towards baseline distribution with 40% of the population in fast resorption and 60% in fast formation (Δ weight loss %, mean (SD) -3.28 (4.20)). Although the uncoupling index remained positive (0.32 (1.80)) bone formation had slowed down.

To more quantitatively support the observed shifts in bone marker plots, differences in median values were assessed using non-parametric statistics. Between baseline and 4 months, the bone formation process was promoted by a lower turnover rate ($p=0.037$), and higher balance between formation and resorption ($p=0.037$). In addition, differences in median values were also observed between 4 and 8 months. The return of the bone remodeling activity to baseline values (fast resorption/fast formation) is explained by a lower median for balance suggesting higher resorption ($p=0.007$) and a higher median for turnover ($p=0.009$).

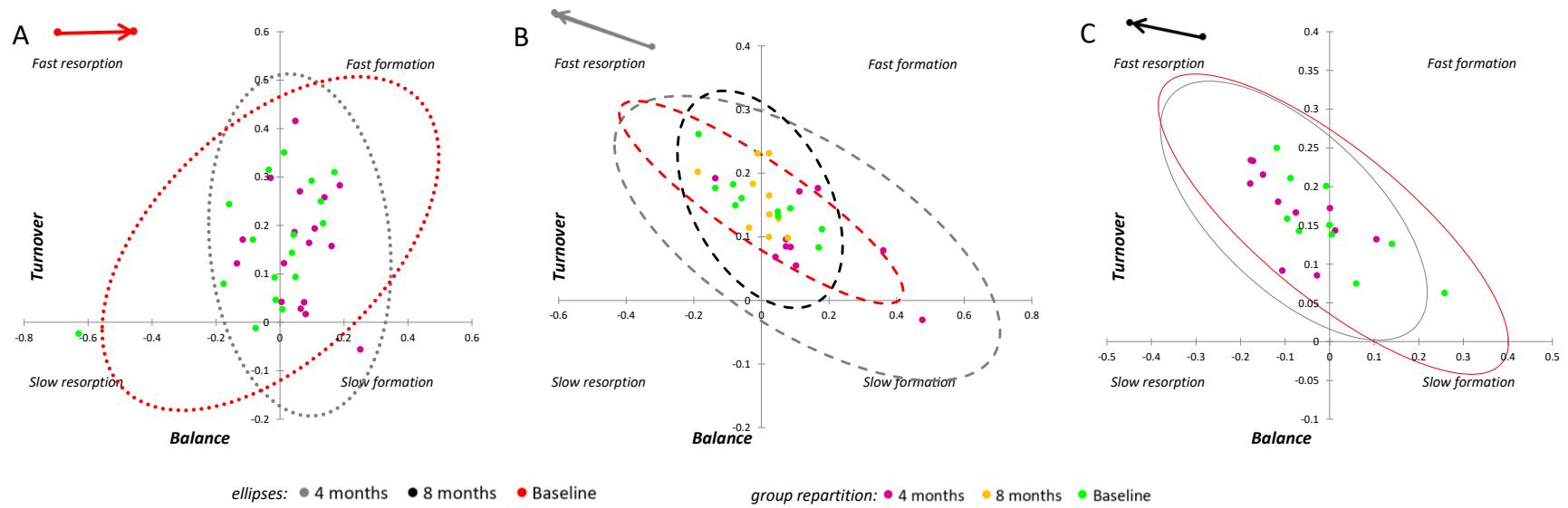


Figure 20 - Bone turnover changes of the three groups: normal weight (A), Ob (B) and Ob control (C), normalised to their respective baseline median

NW normal weight control group, Ob obese intervention group, Ob control obese control

Table 26 - Scores, UI and bone markers plot normalised to groups' respective median

	Ob						NW				Ob control						
	Baseline		4 months		8 months		Baseline		4 months		Baseline		4 months				
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD			
score F	0.00	1.00	-0.03	2.06	0.36	1.90	0.00	1.00	0.18	0.87	0.00	1.00	-0.69	1.13			
score R	0.00	1.00	-1.01	1.00	0.05	0.68	£, ✕	0.00	1.00	-0.29	1.22	0.00	1.00	0.63	0.86	£	
UI	0.00	1.75	0.98	2.20	0.32	1.80		0.00	0.87	0.47	0.78	£	0.00	1.20	-1.32	1.43	
Balance	0.00	0.12	0.13	0.17	0.00	0.07	£, ✕	-0.02	0.18	0.06	0.09	£	0.01	0.11	-0.08	0.09	£
Turnover	0.15	0.04	0.09	0.06	0.15	0.05	£, ✕	0.16	0.11	0.15	0.12		0.14	0.05	0.16	0.05	£

£ significant differences between baseline and 4 months, ✕ significant differences between 4 months and 8 months, µ significant differences between baseline and 8 months

Ob obese group, NW normal weight control group, Ob control obese control, F formation, R resorption, UI uncoupling index,

Bone markers comparison over time normalised to baseline NW median

Bone remodeling markers were also assessed in the two groups with obesity relative to baseline median values from the normal weight peers. In contrast to previous observation, when normalised to NW baseline medians, ellipses of both obese groups shifted towards bone resorption area (Figure 21). However, the shape of the ellipses mostly remained unchanged.

The confidence ellipse of the Ob control group were subsequently located in the lower left quadrant, suggesting altered remodeling activity compared with normal development. The distributions of adolescents with obesity in the control group indicated fast resorption and slow resorption at baseline and 4 months for CTx-P1NP were 18% to 82% and 45% to 55%, respectively. Also, the resorption state of the adolescents with obesity enrolled in the control group was confirmed by the UI ($p=0.004$; from $-0.71 (0.76)$ at baseline to $-1.25 (0.68)$ at 4 months) (Table 28). Moreover, observation of resorption were confirmed by the difference between baseline and 4 months in the median values for balance ($p=0.010$) and turnover ($p=0.004$); accelerating bone resorption (Table 27).

Similar observations were noted in data from the adolescents with obesity enrolled in the WL intervention. Indeed, at baseline, with the exception of one participant (fast resorption), all data were in the slow resorption quadrant of the graph. The UI confirmed the graph plot ($-0.16 (0.65)$). When analysing the distribution of those data, approximately half of the participants were located in the upper quadrant near to the fast resorption line, while the other half were closer to the slow formation area.

At 4 months, the bone marker plot showed that the weight loss intervention may potentially have influenced the adolescents' bone turnover by a shift towards slow formation ($p=0.037$ differences between baseline and 4 months for both balance and turnover median). Closer examination of the distribution within the graph showed 80% were in slow resorption and 20% in slow formation. However, with the exception of one participant, all adolescents in the intervention group moved closer to the slow formation quadrant ($n=7$), or were in the slow formation quadrant ($n=2$). The positive score of the UI ($p=0.022$; 0.46 (0.62)) supported this information (Table 28).

Finally, at 8 months, the bone marker plot returned to slow resorption with a tighter scatter of data than previously observed. A similar pattern was calculated in the UI at 8 months ($p=0.007$; -0.17 (0.40)).

The lower turnover rate previously observed in this group using bone marker plots was supported quantitatively by a tendency for a lower turnover rate between baseline and 4 months ($p=0.069$). The return to baseline between 4 and 8 months was associated with a lower median for balance, suggesting higher resorption ($p=0.007$) and a higher bone turnover ($p=0.005$) (Table 27).

Table 27 - Balance and turnover normalised to NW baseline median

	Baseline				4 months				8 months							
	Balance		Turnover		Balance		Turnover		Balance		Turnover					
	median	IQR	median	IQR	median	IQR	median	IQR	median	IQR	median	IQR				
Ob	-0.27	0.20	-0.15	0.10	-0.17	0.15	£	-0.23	0.11	£	-0.24	0.05	¤	-0.15	0.11	¤
NW	0.01	0.15	0.17	0.20	0.06	0.11	£	0.16	0.22							
Ob control	-0.36	0.18	-0.06	0.15	-0.47	0.17	£	-0.02	0.13	£						

£ significant differences between baseline and 4 months, ¤ significant differences between 4 months and 8 months

Ob obese group, NW normal weight control group, Ob control obese control, IQR interquartile range

Table 28 - Scores and UI normalised to NW baseline

	Ob						NW						Ob control					
	Baseline		4 months		8 months		Baseline		4 months		Baseline		4 months		Baseline		4 months	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
z-score F	-1.78	0.06	-1.79	0.11	-1.76	0.11	0.00	1.00	*,§	0.18	0.87	*,§	-1.77	0.07	-1.82	0.08		
z-score R	-1.62	0.62	-2.25	0.62	-1.59	0.42	£,¤	0.00	1.00	*,§	-0.29	1.22	*,†	-1.06	0.78	-0.57	0.67	
UI	-0.16	0.65	0.46	0.62	-0.17	0.40	£,¤	0.00	0.87	§	0.47	0.78	§,†	-0.71	0.76	-1.25	0.68	

* Significant difference between Ob and NW; § significant differences between Ob control and NW; † significant differences between Ob and Ob control, £ significant differences between baseline and 4 months, ¤ significant differences between 4 months and 8 months, μ significant differences between baseline and 8 months

Ob obese group, NW normal weight control group, Ob control obese control, F formation, R resorption, UI uncoupling index, Procollagen type 1 N-terminal propeptide

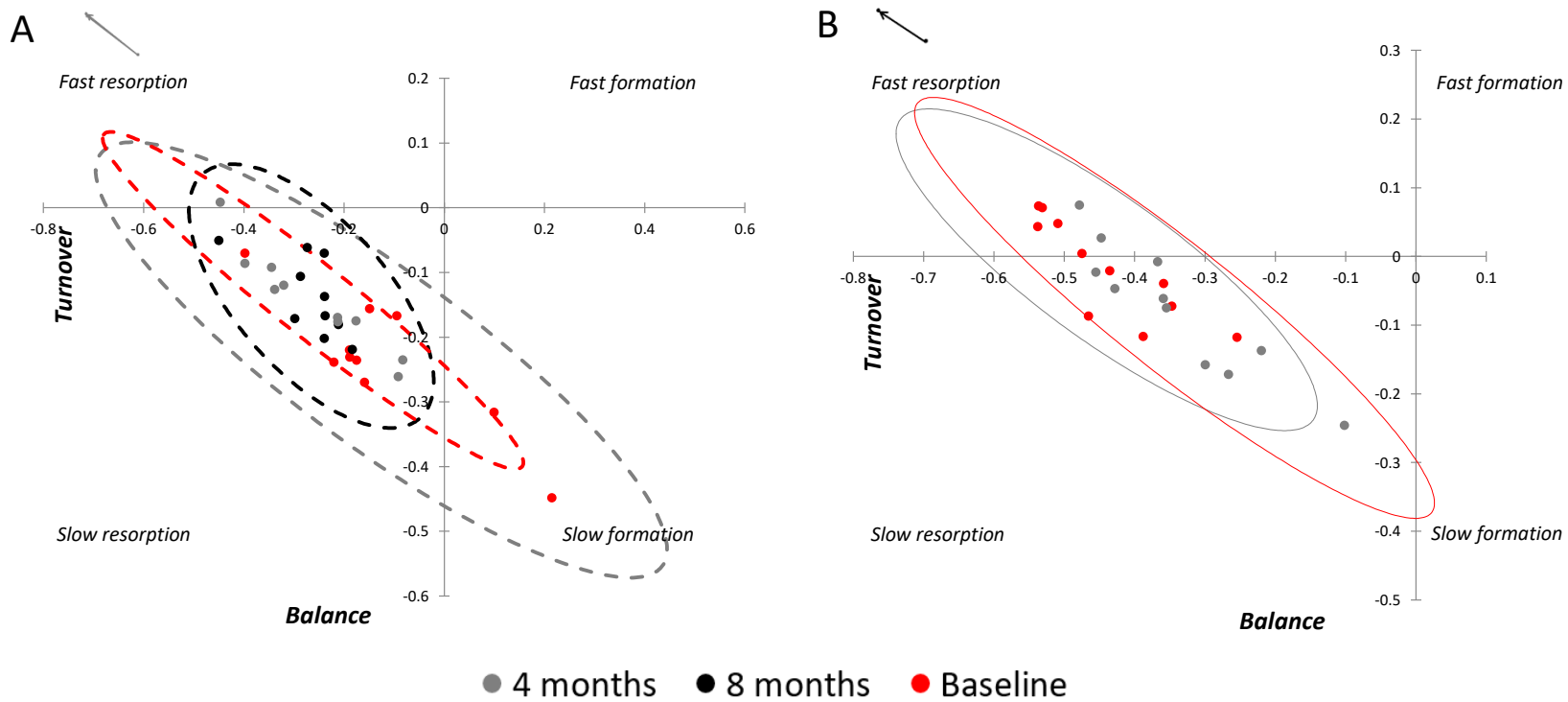


Figure 21 - Bone turnover changes of both obese groups: Ob (A) and Ob control (B), the baseline median of NW control was used for normalisation
Ob obese intervention group, Ob control obese control

Bone markers comparison over time normalised to baseline Ob control median

To compare bone turnover changes of adolescents with obesity undertaking a weight loss intervention with a control group of adolescents with obesity, the baseline median value for the control group with obesity was used for normalisation (Figure 22). As stated previously, despite the same characteristics for sedentary behaviour, body composition and bone parameters, adolescents enrolled in the control group with obesity had higher VFAT (g) at baseline.

Although, no significant differences in bone parameters were observed for unadjusted and adjusted values (VFAT,g) at baseline between both obese groups (Appendix 30), adolescents with obesity enrolled in the weight loss program had bone turnover values mostly favouring fast formation compared with the “control” adolescents with obesity. The UI confirmed the graph plot (0.57 (1.40)).

Moreover, similarly to previous observations of the intervention group at 4 months, the bone marker plot showed that the weight loss intervention program may potentially have influenced bone turnover. More specifically, normalising data against the control group with obesity showed a shift towards bone formation after 4 months of the intervention in these adolescents (p=0.037 differences between baseline and 4 months for balance median; p=0.066 for turnover median). The positive score of the UI (1.35 (1.77)) supported the graphical trends, however, data did not differ from baseline.

Finally, at 8 months, the bone marker plot returned to baseline characteristics, with three adolescents with obesity in the fast resorption quadrant, while others were in the fast formation quadrant but closer to the central axis delimiting the resorption area. Similarly, this observation was confirmed by the UI at 8 months ($p=0.037$; -0.32 (1.80)). The shift back from 4 to 8 months was associated with a lower median for balance, suggesting higher resorption ($p=0.005$) and a higher bone turnover ($p=0.005$).

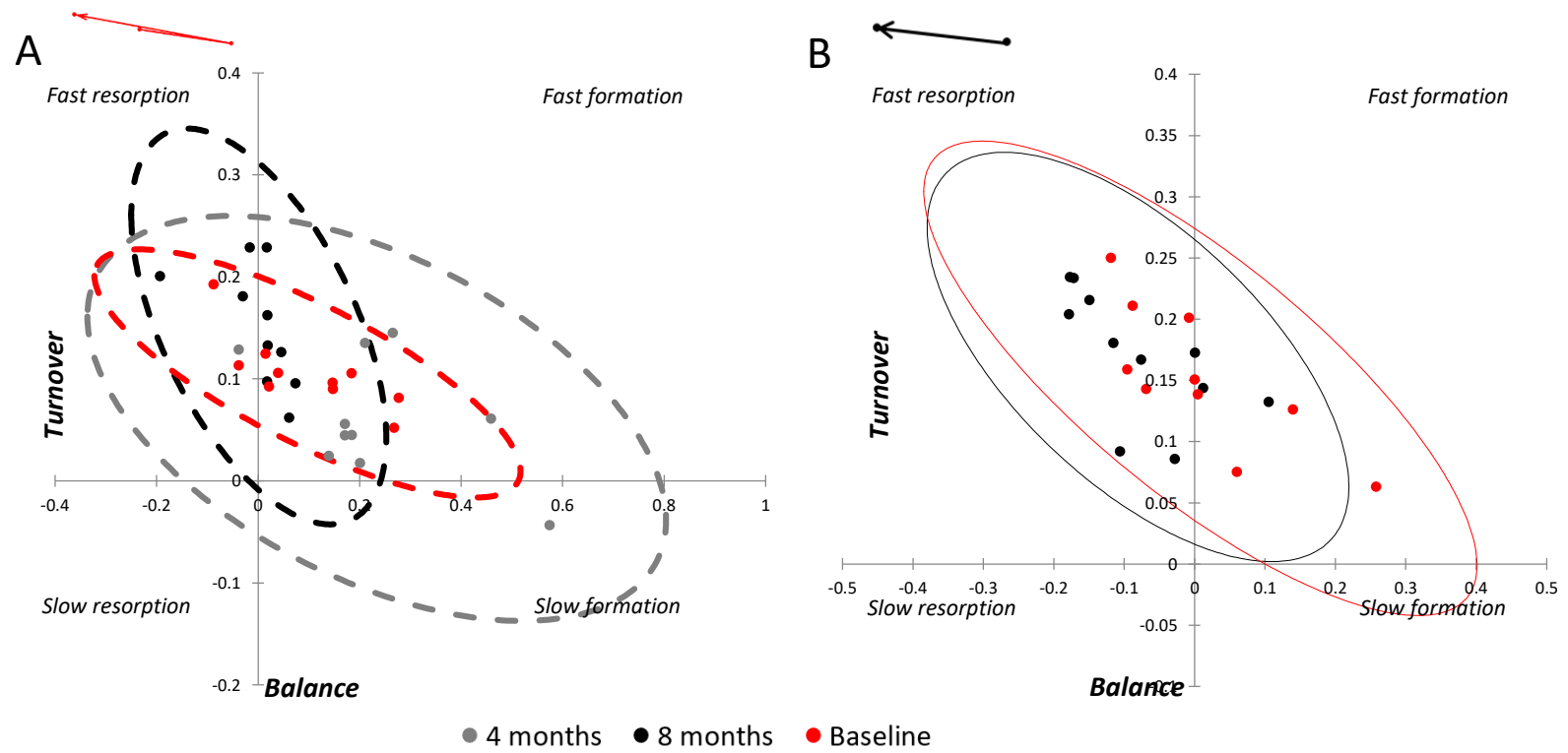


Figure 22 - Bone turnover evolution of both obese groups: Ob (A) and Ob control (B), the baseline median of Ob control was used for normalisation

Bone turnover of one randomly selected adolescent from each group

To confirm the observed results one adolescent from each group was randomly selected for additional analysis (Figure 23). When normalised to their respective group baseline median values (Figure 23.B), the NW adolescent appeared to benefit from perhaps growth related activity via a shift towards accelerated bone formation. The NW adolescent had a bone turnover favouring fast formation. However, both adolescents with obesity had bone turnover predominantly located in the fast resorption quadrant, possibly due to their excess body weight and fat. Interestingly, at 4 months, the turnover activity of the two adolescents with obesity followed different trajectories. Indeed, the bone turnover activity of the adolescent with obesity from the control group deteriorated, while the adolescent enrolled in the WL intervention showed remodeling activity favouring formation, despite a slowdown of its activity at 8 months.

When normalising to the baseline NW median value (Figure 23.A), similar to the results from the previously presented groups' ellipses, the turnover pattern of each adolescent with obesity remained similar. However, both adolescents with obesity showed that their remodeling activity had shifted towards a slower resorption phase. Nonetheless, results for these two adolescents with obesity showed an overall dominance of resorptive bone remodeling activity.

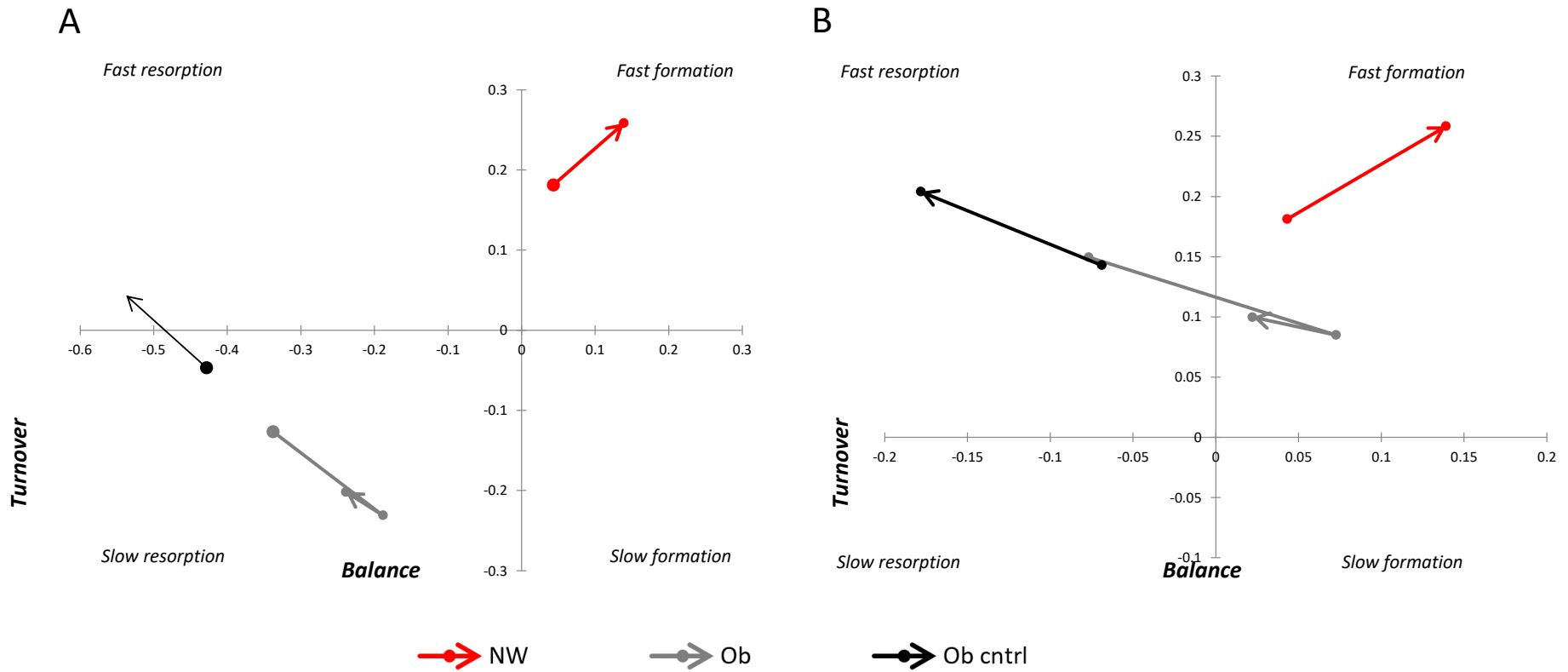


Figure 23 - Bone marker plot of 3 participants (one from each group) normalised to baseline NW median (A) and normalised to group's baseline median (B)

NW normal weight control group, Ob obese intervention group, Ob control obese control

To summarise, after 4 months, with the exception of the adolescents with obesity in the control group, normal weight adolescents as well as adolescents with obesity in the weight loss intervention showed both quantitative and qualitative markers of bone formation. However, bone formation had slowed by 8 months in the obese state. Despite a positive remodeling activity, when compared with normal weight controls, adolescents with obesity generally displayed altered bone remodeling favouring resorption.

CHAPTER FIVE

Discussion and conclusion

This thesis comprised four inter-related investigations sharing the overarching purpose of determining the impact of weight loss induced by physical activity and nutrition on the bone health of adolescents with obesity. Critical appraisal of current literature highlighted the equivocal understanding of how obesity and then weight loss might impact the typically anabolic status of adolescent bone growth. Few studies have isolated overweight and obese individuals so existing results for adolescents with obesity may lack strong external validity. Maturation always complicates research-targeting adolescents. Maturation is an issue that few studies into obesity address in recruitment and subsequent data analyses. A paucity of research into adolescent obesity has extended metabolic investigations into the area of bone health. Arguably, bigger bones can be healthier. However, when bigger bones are subjected to perhaps growth-related stimuli, unusual hormonal activity related to nutrition and sedentary behaviour with prolonged exposure to excessive load bearing on the skeleton, bone health may be compromised.

The methods selected for the program of research therefore focussed on the identified gaps in the literature; choosing methods that would advance the understanding of investigation into the status of bone health in adolescents with obesity compared with maturation-matched peers, who had a normal weight status. Bone health measurements extended from whole body and regional bone scans using a DXA device, to new thinking around relationships among blood-borne markers of bone remodeling, maturation and adiposity. Then, more innovatively, responses to a residential intervention for adolescents with obesity were investigated and compared with normal weight peers. Also, using blood-borne variables, comparisons of the impact of the intervention on bone markers were compared with a group of adolescents with obesity who did not receive the intervention and a normal weight group of adolescents.

5.1. Summary of major findings

The ADIBOX study first investigated bone parameters among maturation-matched adolescents. Then, the effects of a multidisciplinary weight loss program combining nutrition and physical activity on bone parameters in adolescents with obesity were assessed. A weight status comparison was also performed over the same timeframe. Finally, the influences of body weight status and weight loss on the bone remodeling activity were analysed.

Summary of the primary outcomes major findings

Results for the primary outcome variables showed (1) lower bone parameters of key BMD and BMC measures, among adolescents with obesity compared with normal weight controls even when data were adjusted for significant variables of body weight, fat mass and lean mass. (2) Some improvements in BMD for the whole body and lumbar spine were observed in adolescents with obesity during the intervention. However, BMD adjusted values to body weight remained lower than their normal weight peers.

Summary of the secondary outcomes major findings

Results from site-specific bone changes (geometry and strength) were novel among the adolescents with obesity. Specifically, additional major findings from secondary outcomes were (3) that geometric indices of bone strength at a significant weight bearing site (NN) for individuals with obesity remained altered. Specifically, despite positive adaptations of some geometric properties, the weight loss intervention in adolescents with obesity remained

associated with a compromised estimate of fracture risk. (4) Also, the results highlighted more subtle but significant trends by finding that bone accretion in adolescents with obesity followed an androgen-like growth adaptation through a stimulation of periosteal expansion and endocortical resorption associated with weight loss. (5) Furthermore, bone remodeling activity positively responded during the first four months of the weight loss intervention before returning to baseline the bone resorption activity. Table 29 summarise the major findings from the present thesis.

Table 29 - Outline of the major findings within this thesis

Aims	Experimental hypothesis	Majors findings
To profile bone parameters among maturation-matched adolescents of various weight.	<p>Compared with their lean peers, adolescents with obesity will display:</p> <ul style="list-style-type: none"> - lower bone density at whole body and specific weight bearing sites - altered bone geometry and strength 	<ul style="list-style-type: none"> - Adolescents with obesity displayed lower unadjusted and adjusted BMD at whole body and most site-specific regions than their normal weight peers. - Geometric indices of bone strength were compromised when adjusted for body composition variables of body weight, fat mass and lean mass.
To investigate the impact of a multidisciplinary weight loss program combining nutrition and physical activity on the bone health of adolescents with obesity, including estimate of fracture risk.	<p>The structured weight loss intervention combining physical activity and nutrition will:</p> <ul style="list-style-type: none"> - prevent the loss of bone mass caused by weight loss in adolescents with obesity. - prevent estimates of fracture risks. 	<ul style="list-style-type: none"> - Induced WL favoured positive adaptations in bone parameters at whole body and lumbar spine. - Geometric indices of bone strength at the weight-bearing site of the narrow neck remained compromised with scores nearing the fracture prediction threshold. - Bone remodeling activity in favour of formation was observed during the WL intervention with higher values during the first 4 months of WL intervention but values from the UI did not reach significance.
To investigate the impact of body weight changes induced by a structured	<p>The 8 months WL intervention will:</p>	<ul style="list-style-type: none"> - Despite positive adaptation, bone density of adolescents with obesity participating in a weight loss intervention remained compromised when adjusted for body weight.

weight loss intervention on bone parameters in adolescents with obesity compared with normal weight maturation-matched peers.

- support positive adaptations in bone parameters reaching the bone parameters values in NW adolescents.
- induce a higher responsiveness in bone parameters at weight bearing sites than whole body.

- Positive geometric adaptations were observed with weight loss, with better responses at the narrow neck than the shaft. However, strength indices remained compromised (i.e. aim 2)
- Bone accretion in adolescents with obesity participating in a weight loss intervention appeared to follow an “androgen like” growth adaptation through a stimulation of periosteal expansion and endocortical resorption.

To investigate the influence of body weight status and weight loss intervention on bone remodeling in adolescents with obesity and normal weight controls.

- Obesity is associated with altered bone remodeling markers during adolescence.
- The 8 months WL program will stimulate the remodeling activity in favour of bone formation in adolescents with obesity.
- The WL program will induce a shift of bone turnover towards positive bone formation compared with Ob control; trending towards bone formation values similar to a lean control group.

- Normalised to their baseline value, adolescents with obesity from the WL intervention demonstrated an acute remodeling activity in favour of bone formation after 4 months.
- Normalised to NW, both obese groups demonstrated remodeling activity favouring bone resorption.
- Weight loss may benefit bone health in adolescents with obesity during the initial phase of an intervention.

5.2. Discussion of major findings

Bone parameters among maturation-matched adolescents

The current literature shows inconsistency in the status of bone mineral density in adolescents with obesity (Dimitri et al. 2010) (Rocher et al. 2013) (Goulding et al. 2000) (Rocher et al. 2008) (El Hage et al. 2012) (Russell et al. 2010) (El Hage et al. 2012) (Leonard et al. 2004). In accordance with some studies (Dimitri et al. 2010) (Rocher et al. 2013) (Goulding et al. 2000) (Rocher et al. 2008) (El Hage et al. 2012), but not all studies (Russell et al. 2010) (El Hage et al. 2012) (Leonard et al. 2004), the results from baseline comparisons showed lower unadjusted data for bone mineral density measures involving WB (less head) BMD, WB BMAD, LS BMAD and no differences at LS BMD, neck BMD, hip BMD in adolescents with obesity compared with NW. In contrast, the current findings agree with all previous studies of lower BMD when data from adolescents with obesity were adjusted for the key body composition variables of body weight (kg), or fat mass (g) and compared with their normal weight peers. Although the influence of lean mass (g) on bone is well demonstrated (Courteix et al. 1998), lean mass did not change through the weight loss program; justifying only adjusting for lean mass at baseline.

The discordances (BMD) observed in the literature might be related to in-between study inconsistencies in pubertal (Garabédian et al. 2009) (Dimitri et al. 2012) and maturation status (Wang 2002), single or mixed sex among participants (Shapses et al. 2012) or, the degree of obesity (Pollock et al. 2007) (Laddu et al. 2013) (Campos et al. 2012) (Ripka et al. 2016). In the present study, adolescents shared a similar maturation status despite adolescents in the obesity groups being younger than their normal weight peers. Indeed, both groups had similar

oestradiol levels, age at menarche and all participants from this study were at least at Tanner Stage 4. In addition, only adolescents above the 95th percentile for BMI were included (BMI percentile mean and SD of the adolescents in the weight-loss program group was 98.25 (1.89)). Moreover, all the adolescents with obesity enrolled in the present research had a history of early onset obesity; with a BMI above the 97th percentile at a mean age of 3.76 (1.54) years. The incidence of obesity in early childhood among children aged between 2 to 7 years is a critical time for developing bone parameter weaknesses (Garabédian et al. 2009). The reviewed literature in this thesis showed that hormones related to excessive obesity can compromise bone health. It is postulated that any mineralisation delay potentially occurring in the prepubertal years due to the early onset of obesity was too substantial to be corrected during pubertal years.

Influence of the WL program on bone parameters

During the weight loss intervention, trabecular and cortical bone appeared to change in association with body weight changes. This change was not always consistent with previous observations of negative associations between weight loss and BMD (Whipple et al. 2004). In the current thesis, adolescents with obesity increased their BMD (WB and LS). However, when compared with changes in NW, adolescents with obesity had similar unadjusted bone density values at WB, LS and neck but lower values at the hip. With adjustments for *body weight* changes, BMD became lower in adolescents with obesity than NW at all sites except the LS. When adjusted for *fat mass* changes, similar LS BMD was identified in both groups but adolescents with obesity showed lower BMD at WB, neck and hip than their NW peers. Despite the well-known effects of excessive body weight-stimulating mechanical loading (Shapses et

al. 2015), adolescents with obesity seemed to have less WB and site-specific bone density than their normal weight peers to cope with their relative body weight.

Even if fat-bone mechanisms remain uncertain, fat mass plays an important role during bone growth depending of the degree of obesity (Pollock et al. 2007) (Laddu et al. 2013) (Campos et al. 2012) (Ripka et al. 2016). Growing bones are fashioned into a structure adapted to support the skeleton. During adolescence, changes in modular volume depend on endocortical resorption. This reportion enlarges the marrow cavity and is stimulated by bone growth and endocortical apposition of bone mineral, often in response to physical activity (Parfitt 1994). Moreover, most increases of cortical thickness are attributed to gains in bone width (Seeman 2001). Impairment of periosteal apposition might result in smaller bones and increased fracture risks in response to bending loads (Seeman 2001). In this thesis, adolescents with obesity demonstrated lower quantitative bone parameters. Reduced weight appeared to cause higher fragility at the narrow neck than the shaft. This could be explained by modifications in compressive and tensile stresses attributed to weight loss. Results from investigations with peripheral Quantitative Computed Tomography (pQCT) (Laddu et al. 2013) (Farr et al. 2010) support the finding that skeletal adaptations may be compromised during growth relative to body mass and location of the fat mass. It is possible that regardless of the weight bearing effects of excess loading through obesity, the location of fat mass may independently act to compromise bone health during growth.

In addition, when focusing on the bone geometric findings from the shaft of the hip, the results highlighted a significant increase in cortical area with a similar magnitude of change in

adolescents with obesity and their normal weight peers. However, only the normal weight group increased cortical thickness and bone density. Typically, during normal bone growth during late puberty bone growth is stimulated by periosteal apposition and medullary contraction (endocortical apposition); increasing cortical thickness (Seeman 2003) (Bass 2003) (Forwood et al. 2004). In the adolescents with obesity who received the intervention, reduced weight was associated with periosteal expansion and endocortical resorption, a typical male rather than female growth response. Yet, during pubertal growth, estrogen in young females is known to inhibit periosteal expansion and stimulate endocortical acquisition (Bass 2003). The finding of periosteal expansion in the predominantly female group of adolescents with obesity occurred despite demonstrating similar oestradiol values as their normal weight peers. Because of the observation of androgen-like growth within the bone, it could be speculated that the presence of males (three boys) influenced geometric development. As such, additional analyses involved withdrawing the males from the data set. As shown in Figure 19, similar results were observed between groups with or without the inclusion of males in the analysis. Despite low numbers of males, less compromised cortical thickness within a female only adolescent group with obesity was hypothesised. Surprisingly, the decline observed in cortical thickness worsened with the female group. Strengthening the observation of androgynous-like adaptations in bone distribution in female with obesity who experienced weight loss. It is possible that an 8-month weight loss intervention would produce a similar or even more exaggerated pattern in pubertal growth in boys who experience a typical dimorphic stimulation of periosteal expansion and endocortical resorption.

The investigation into hip bone strength and geometry could be regarded as controversial given it lies outside the ISCD recommendations. Hip investigations were included as it is a site of considerable and prolonged loading for adolescents with obesity. Results from HSA analysis reflect a bone's ability to withstand forces generated during walking or during a fall. The impact of weight loss on local modifications at specific bone sites is of interest at least in this population. Section modulus (Z) represents an endpoint in mechanical homeostasis in long bones, able to show adaptation through bone geometry, depending on the demand of environmental load (Beck et al. 2001). Conflicting findings about bone strength have been reported in adolescents with obesity. Cross-sectional pQCT studies reported higher bone strength in adolescents with obesity, which was mainly explained by higher maturational levels (Vandewalle et al. 2013) (Leonard et al. 2015), while HSA studies have reported similar (Gong et al. 2012) or lower (Rocher et al. 2013) (El Hage 2012) bone strength at the hip.

In the current thesis, baseline data showed that adolescents with obesity had lower resistance to bending, torsional and axial stresses at the narrow neck, the intertrochanteric site and femoral shaft after adjustment to body weight or fat mass. Changes in hip loading are associated with mechanical alteration of section modulus and are likely to weaken femoral neck (cortical and trabecular bone) than shaft (cortical bone) (Beck et al. 2001). The 8-month WL program unfavourably reduced these values for adolescents with obesity. This was exemplified by results for the bone buckling ratio at narrow neck, with scores trending closer to the fracture prediction threshold at the end of the intervention. Indeed, results showed estimates of fracture risk worsened in adolescents with obesity when changes were adjusted for weight loss. Higher fracture risk in adolescents who lose weight is a major concern and must be addressed in the exercise prescriptions used in future interventions. It is postulated that

progressive load bearing exercise can better support bone strengthening than the sport-related experiences offered to adolescents with obesity in the present intervention.

Correlation analyses confirmed previous associations between body composition and bone mass parameters (Lanyon et al. 1984) (Forwood et al. 1995) (Frost 1997). In the current study, lean mass and bone parameters (density, geometry and strength) correlated strongly, while body weight and fat mass were moderately associated with geometry and strength parameters. In a pQCT study (Farr et al. 2010) assessing the relationship between WB fat mass and bone geometry on pre-pubertal females it was highlighted that females with higher WB FM had attenuated volumetric bone mineral density, geometry and bone strength at femur and tibia than females with lower WB FM. In addition, others (Campos et al. 2012) (Russell et al. 2010) have demonstrated links between fat mass location and bone mineral density, suggesting that visceral fat or visceral fat/subcutaneous fat ratio were negative predictors of BMD in adolescents with obesity after pubertal growth. Whole body fat mass was inversely correlated to lean mass, making it difficult to determine whether bone parameters were influenced by either the fat mass or the lean mass percentages (Specker et al. 2001). However, in this thesis, during the weight loss intervention, adolescents with obesity did not significantly change their lean mass and significantly decreased their fat mass. Maintenance of lean mass suggested that the observed effect after adjustment for weight change might be linked to the improvement of the fat mass/lean mass ratio. The absence of variation of lean mass during the weight loss intervention was unexpected. Indeed, most of the studies report an increase in lean mass during WL interventions (Campos et al. 2014) (Campos et al. 2012) (Stettler et al. 2008). Although this study did not aim specifically for lean mass changes, a possible explanation

for the lack of increased lean mass might be that the physical activity program was more focused on sports-related experiences than on specific osteogenic modalities.

Influence of body weight status and weight loss on the bone remodeling activity

Finally, the assessment of the bone remodeling activity assists in the understanding of skeletal growth and allows a rapid response by providing complementary information on slower changes in bone density (Vasikaran et al. 2011). The relationship between weight status and markers of bone turnover among maturation-matched adolescents was assessed. The threefold purpose of the graphical representation of the bone remodeling activity was: (1) To understand the balance of bone turnover in the adolescents with obesity who received the intervention and bone turnover changes over time. (2) To observe the changes pattern of the bone turnover in the group of adolescents with obesity who received the intervention by comparing them with an obese control group. (3) To observe and set the balance of the bone turnover in adolescents with obesity who received the intervention compared with a normal weight control group.

Lower baseline concentrations of the bone markers (P1NP and CTx) were observed for both obese groups compared with their normal weight peers. The results from these bone markers concentrations are in accordance with some (Viljakainen et al. 2014) but not all (Dimitri et al. 2011) previous studies. Bone markers in adolescents and young adults with obesity (15-25 years of age) have shown lower bone turnover activity when compared with their maturation-matched lean peers (Viljakainen et al. 2014). Other researchers have assessed the bone formation marker P1NP and bone resorption marker CTx in younger populations; 103 children

and adolescents aged 5 to 16 years (Dimitri et al. 2011). Contrary to the results from the current thesis, circulating CTx level were found to be higher in children with obesity while no difference in P1NP was reported compared with normal weight peers (Dimitri et al. 2011). Also, the results from the study with younger children suggested an increased rate of the bone remodeling in favour of resorption.

In order to observe the changes of bone turnover activities of each groups data were normalised to their respective baseline values. In a general observation, at baseline, the three groups has a bone remodeling activity favouring bone formation.

Surprisingly, over the 4 months, bone remodeling biomarkers of the adolescents with obesity from the control group were in favour of bone resorption. This observation was not expected. Indeed, both the normal weight control and the adolescents with obesity enrolled in the WL program showed bone remodeling activity in favour of formation during the first four months. An association between fat mass and bone resorption has been highlighted among children with obesity (Dimitri et al. 2011). Higher fat mass was linked with circulating leptin levels, which induces OPG to reduce. Reduced OPG leads to osteoclastogenesis due to higher bone resorption and lower formation, or a reduction in osteoblast numbers, inducing CTx and P1NP secretion (Dimitri et al. 2011). Moreover, others have speculated that under obese conditions, bone turnover is regulated by leptin secretion (Viljakainen et al. 2014). However, in the current thesis, no differences in baseline circulating leptin levels, were observed between both obese groups. This finding does not support the notion that the higher visceral fat profile of the control group with obesity than the intervention group would lead to increased circulating leptin levels. One explanation might be because of higher level of visceral adipose tissue in the adolescents with obesity than the intervention group. Visceral adipose tissue has been shown

to be negatively associated with bone parameters in population with obesity (Schorr et al. 2016) (Zhang et al. 2015) (Donner et al. 2015) (Gilsanz et al. 2009) (Russell et al. 2010).

Interestingly, in the first four months, the period during which adolescents lost the most weight a marked response of the bone remodeling activity in favour of formation was observed. However, at the end of the weight loss program, bone turnover values slowed down and reduced bone formation. Because of the bone marker plot pattern during this active period of bone growth, those results lets us hypothesis a possible effect of the WL intervention in addition to growth pattern. As WL induced by diet only is well known to induce bone loss (Zibellini et al. 2015), physical activity might induce a positive effect during the acute phase of weight loss (% -9.04 (4.57)) on bone turnover by inhibiting resorption. However, the osteogenic stimuli from the physical activity component of the intervention may have lacked similar or sufficient intensity of weight bearing activity between the 4 to 8 months of the intervention. As such markers of positive effects on bone were not maintained and would have possibly required a stronger mid-intervention review to elicit a more progressive, bone enhancing exercise regime. In adult studies, it has been well established that losing weight via caloric restriction alone can induce bone loss, but when weight loss is combined with weight-bearing exercise, BMD loss is attenuated (Schafer 2016) (Martyn-St James et al. 2008) (Zibellini et al. 2015). Bone loss induced by weight loss might be explained by a reduction in mechanical loading (Shapses et al. 2012), which stimulates sclerostin secretion by osteocytes (Turner et al. 2009). Because sclerostin is inhibited by mechanical loading, future consideration should be given to the inclusion of measures of sclerostin level to refute or support this hypothesis in effective physical activity impact on bone.

When bone marker plots were normalised to NW baseline median values to perform a group comparisons, interesting results emerged. Despite both obese groups sharing similar P1NP and CTx baseline levels, the UI of the obese control group was lower than their NW peers. When reviewing results from the 4-month data normalised to NW baseline median values, it might be suggested that osteoblasts under obese conditions are less functional and produce less collagen. However, osteoclasts in the control group with obesity appeared to be as active as in the normal weight state. Because of this observation, it is speculated that the weight loss intervention combining nutrition and physical activity potentially inhibited osteoclast activity. Indeed, compared with leaner individuals, both obese groups showed lower bone formation activity, while similar CTx levels were observed between both control groups (NW and Ob). Also, adolescents enrolled in the WL intervention displayed lower circulating CTx than both control groups. The results of less bone formation activity are supported by previous work in young adults with a history of early onset of obesity (Viljakainen et al. 2014). Specifically, the young adults with obesity had lower bone formation markers without any differences in the formation/resorption calculated index than their lean peers (Viljakainen et al. 2014).

Mechanistic hypothesis

Obesity leads to complex alterations of hormonal secretions. Selected secretions potentially affect bone metabolism, with stronger alterations observed during critical periods of developmental change such as puberty (Dimitri et al. 2012) (Douchi et al. 2000). Major hormonal alterations with maturation, bone and obesity have been previously described in this thesis (Chapter 2 - Review of literature).

The results demonstrated lower bone mass, potential alteration of osteoblasts activities, as well as an “androgen-like” bone growth adaptation in adolescents with obesity. In the absence of extensive additional hormonal analyses, which could have helped in elucidating bone responses under conditions of obesity, the following questions and hypotheses are proposed:

(1) Can obesity alter osteoblastic activity?

Hypotheses: Fewer osteoblast numbers and/or more limited activity of osteoblasts could be the result of diminished mesenchymal stem cells differentiation and/or diminished control of the disruptive hormones.

This mechanistically based theory hypothesis extends previous discussions within the literature (Shapses et al. 2012).

Due to a shared origin, osteocytes and adipocytes are intimately connected. Indeed, mesenchymal stem cells differentiate at least into osteoblasts, chondrocytes and adipocytes lineages (Bruder et al. 1994). The Mitogen-Activated Kinase pathway (MAPK) was suggested as one of the transduction signalling pathway regulating the adipogenesis and osteogenesis differentiation. Cross-talk in the MAPK pathway is essential as it is composed of three enzymes; the extracellular signal-regulated kinase (ERK), p38, and c-Jun amino N terminal kinase (JNK). More precisely, these three enzymes are active within MAPK pathway and are known to be involved in the adipogenesis/osteogenesis differentiation process (Rodríguez-Carballo et al. 2016). The various activators to this pathway include growth factors, insulin (Boulton et al. 1990), inflammatory cytokines (i.e. TNF- α , IL-6) (Cuadrado et al. 2010), estrogen (Migliaccio et

al. 1996), glucocorticoids (Lukert et al. 1990), leptin (Takahashi et al. 1997), and 1.25 vitamin D (Zhang et al. 2012).

Agreement on the precise action of JNK on osteogenesis remains controversial. However, reports of strong evidence of the ERK and p38 enzymes promoting osteogenesis (Rodríguez-Carballo et al. 2016). Also, it is highlighted that the p38 enzyme is involved in adipocyte differentiation (PPAR γ phosphorylation) (Engelman et al. 1999) (Cargnello et al. 2011) (Jaiswal et al. 2000). Overexpression of the protein kinase MKK6 may be responsible for the p38 activation that favours adipocyte differentiation over osteogenesis (Engelman et al. 1999). Moreover, in rodent studies, inhibition of the p38 has been linked with decreased weight loss, increased fat deposits as well as increased adipocyte size (Maekawa et al. 2010). As obesity can disrupt hormonal factors that activate the MAPK pathways, it might affect the mesenchymal stem cell differentiation with an imbalance in typical osteogenesis/adipogenesis differentiation to the detriment of osteoblastic lineage; due to an overexpression or activity of the PPAR γ , specifically through an upregulation of adipocyte differentiation.

In addition to a postulated reduced number of osteoblasts, their activity could be limited by a negative loop of dysregulated hormones. Under conditions of obesity, inflammatory cytokines (Fain 2006), insulin (Reid 2010), aromatase (Rosen et al. 2009), and leptin (Bell et al. 2006) are known to have higher circulating levels. However, under the same obesogenic conditions, circulating levels of GH/IGF1 axis, 1.25 vitamin D (Konradsen et al. 2008), SHBG (Hautanen 2000), and adiponectin (Balistreri et al. 2010) are lower. The relationship of the cited hormones with bone health has been previously demonstrated. Some hormones act in favour of formation (i.e. estrogen, SHBG), others of resorption (i.e. GH/IGF1, insulin, 1.25 vitamin D,

inflammatory cytokines) and sometimes both formation and resorption are possible (i.e. leptin, adiponectin); depending on whether the pathway is central or peripheral (Shapses et al. 2012) (Shapses et al. 2017).

As previously stated in the review of literature, bone secreted hormones can also influence fat metabolism. The skeletal system is not only responsive to altered hormonal secretions but also may respond to other metabolic responses altered by obesity. Higher mechanical loading on bone induced by obesity (“static loading” due to higher fat tissue) does not appear sufficient to compensate hormonal effects. Mechanical loading is known to inhibit PPAR γ (David et al. 2007) and to stimulate osteoblasts (Ehrlich et al. 2002). It is postulated that disruptions to hormonal levels directly associated by obesity have stronger negative than positive influences on bone formation.

(2) Is the “androgen-like” bone growth adaptation a specific response to obesity?

Hypotheses: The “androgen-like” bone growth could result from a loss of sensitivity in estrogen (aromatase conversion) coupled with higher androgen levels and/or a mechanical bone growth response to excessive body weight under conditions of obesity.

As previously stated, the following considerations are hypothetical due to the absence of complementary hormonal analyses that would have helped explain bone responses. However, the possibility that lower osteoblast activity due to mesenchymal stem cells differentiation and hormonal dysregulation lacks a sound rationale for the observed “androgen-like” bone growth

within the two groups with obesity recruited for this thesis. Thus, other potential mechanisms require consideration.

First, during typical growth, estrogen is known to stimulate endocortical apposition and inhibit periosteal expansion. Also, SHBG levels, which are inversely proportional to bone growth are triggered by multiple stimuli including estrogen, but inhibited by testosterone and visceral fat accumulation (Peter et al. 2010). Interestingly, under conditions of obesity, the literature reports higher levels of estrogen (aromatase conversion) and lower levels of SHBG (Peter et al. 2010). Theoretically, as lower SHBG levels are observed in obesity (SHBG inhibits free estrogen action by binding it), free estrogen levels are supposed to increase; leading to positive effects on bone geometric and densitometric development (Wang et al. 2004). However, our results did not demonstrate positive bone development. Based on these observations, a potential importance of body fat distribution in the crosstalk between androgens and obesity might be postulated (Pasquali 2006). In the recruited adolescents with obesity, visceral fat represented 47.14 (5.07) %. Similar to leptin which under obese conditions fails to return the body's adiposity to a normal range (Myers et al. 2008), a "loss of sensitivity" to estrogen from aromatase conversion might be compromised by obesity. On one hand, estrogen plays an important role in lipid homeostasis and adipose tissue (Mauvais-Jarvis 2011). On the other hand, estrogen deficiency or resistance, is associated with an absence of pubertal growth spurt (MacGillivray et al. 1998). The pubertal growth spurt had already occurred in the recruited adolescents so decreased growth was not apparent. However, it is known that lipid homeostasis and adipose tissue are altered with obesity, which may link to the hypothesis that bone growth can be enhanced and compensated by high levels of androgens (Öz et al. 2000)

(Fisher et al. 1998). Indeed elevated androgen levels are found under certain conditions (i.e. central adiposity, polycystic ovary syndrome) (Pasquali 2006) (Coviello et al. 2006). This hypothetical explanation, reinforces the speculation of a potential “loss of sensitivity” from estrogen (aromatase conversion).

Second, postural stability is the major component of children and adolescents development (Shumway-Cook et al. 1985). Growth but also obesity is connected to morphological changes that interfere with postural stability (Nantel et al. 2010). The negative effects of obesity on gait are well known and reported in the literature (Jegede et al. 2017). Excessive body weight leads to compensatory body movement strategies in adolescents with obesity compared with normal weight adolescents (Strutzenberger et al. 2011). Compensatory movements during gait due to excess weight may alter the hip (Nantel et al. 2006), the knee (Gushue et al. 2005) and the ankle (Shultz et al. 2009) loading; instigating a reorganisation of walking patterns. Moreover, adolescents with obesity have higher absolute ground reaction forces with greater muscle activity during walking and stairs climbing (Strutzenberger et al. 2011) (Browning et al. 2007). The observed bone growth might simply result from mechanical adaptations to excess weight. Indeed, higher loads generated by muscle forces have been shown to increase periosteal apposition and to increase cortical thickness in order to improve bending resistance (Turner et al. 2003).

Figure 24 summarise the mechanistic hypothesis of bone metabolism in adolescents with obesity.

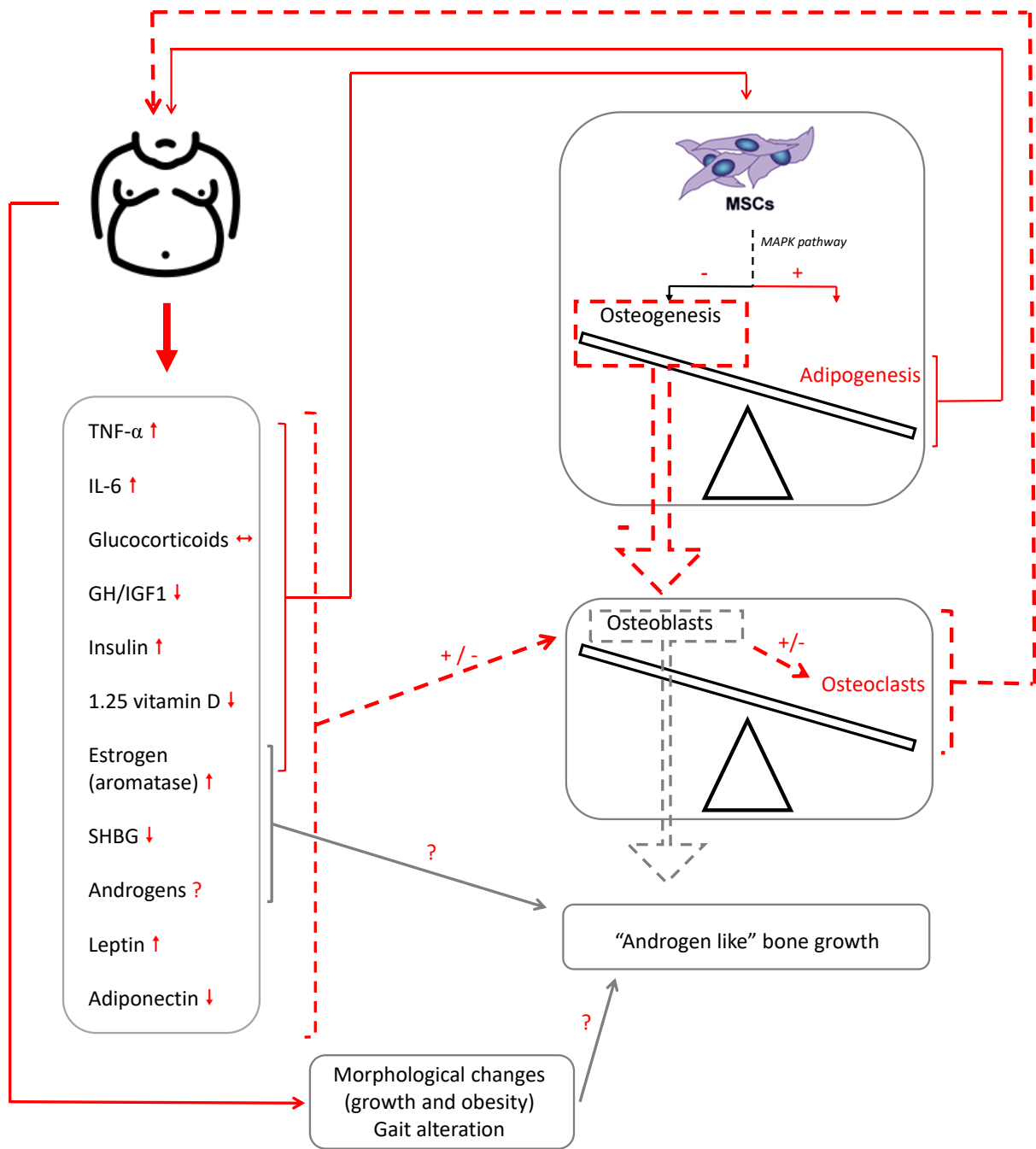


Figure 24 - Schema of mechanistic hypothesis of bone metabolism in adolescents with obesity
 ↔ similar, ↓ lower, ↑ higher, + stimulate, - inhibit

To summarise, obesity is postulated to disrupt the mesenchymal stem cell differentiation between osteoblasts and adipocytes via the MAPK pathway. Alternatively, the reduced number

of osteoblasts and/or a reduction of osteoblasts' sensitivity might explain the osteoblastic dysfunction. Also, a strong influence generated by an imbalance between osteoblast and osteoclast activity is observed in obesity states. This imbalance appears to be mediated by key hormones. Each, some or all of these hormones can inhibit/stimulate, down/up regulate osteoblast and/or osteoclasts. There is also the possibility that the androgen-like adaptations in adolescents with obesity might be the results of a loss of sensitivity to estrogen, or the results of mechanical body adaptations strategies to adapt the skeletal system.

5.3. Limitations

There were several limitations to the implementation and assessment of the planned protocol within this thesis.

- Although the adolescents took part in a structured physical activity intervention, it has not been possible to individually track the volume and intensity of the intervention. Because of the established links between weight bearing physical activity and bone, access to what occurred and how the adolescents responded to this component of the intervention could have been helpful in explaining a dose-response effect. This would have been particularly interesting because all adolescents with obesity were inactive before enrolling the WL intervention. However, the health providers delivering the program also could not supply sufficient data on nutrition, which again would have been helpful in understanding bone responses of the adolescents with obesity who were participating in the intervention. Similarly, the physical activity and nutrition data

of the control groups used within the study were not available; again leaving some uncertainty around the explanation of results relating to bone.

ii. Because of the “sports-related” nature of the physical activity program individuals may not have been subjected to sufficient osteogenic activities than a more bone promotion focused program that should be possible in future reiteration of this intervention. Despite the initiative to repeat baseline assessments of primary and secondary outcomes at 4 months, these results were not used to strengthen osteogenic possibilities from exercise within the intervention. However, the intervention focused on re-introducing positive experiences in physical activity, with the practice and discovery of various activities, it is possible that adolescents were supported to sustain some form of physical activity when they returned home.

iii. We were not able to assess the sustainability of this WL intervention for the whole group as the recruitment of the obesity center is national and participants could live anywhere in France, with some participants having limited possibilities to return to the center. However, the obesity center offered adolescents the opportunity to re-enter the intervention for one week, 3 months after they returned home, which would have provided at least some interesting follow-up data. However, time and resource limitations within the present thesis precluded follow-up data collection.

iv. It would have been interesting to have a complete set of data on all three groups involved in this research. However, restricted access to adolescents with obesity suitable for the control group for the whole period of the weight loss intervention (8 months) was not

possible. Moreover, the number of participants recruited in the obese control group was lower than originally anticipated. Low response rates in this population presented a major challenge during this research. Also, the inclusion of a few males to the larger number of females within the two groups with obesity could be seen as confounding the results. However, statistical support for the inclusion of males was confirmed via unaltered homogeneity testing.

- u.* The original design of this project was set in both countries. However, because of difficulties in recruitment in Australia, only the French-designed project have been performed and can be presented within this thesis.

- ui.* Although DXA-derived techniques have already shown acceptable reliability (Gordon et al. 2008), the use of 2D technologies to assess bone strength and bone trabecular microarchitecture may also comprise a limitation. Nonetheless, the available data was comprehensively explored, particularly presenting data for the first time using hip geometry as a region of particular interest in adolescents with obesity.

- iii.* Markers of bone turnover are not easy to interpret during growth as they reflect growth, remodeling as well as nutritional status (Mosca et al. 2016). However, some interesting responses were monitored, particularly in the first four months of the intervention.

- iii.* The study did not measure specific hormones known to influence osteoblast and osteoclasts activity such as OPG/ RANK/ RANKL, sclerostin, osteocalcin (unOC, oC, tOC) but also parathyroid hormone, GH/IGH1, adiponectin.
- iv.* Also due to budgetary considerations, testosterone and SHBG hormone (Sex hormone-binding globulin), were not measured, but these data could have provided some high quality information relating to the observations of androgen-like bone growth.
- x.* Among the adolescent females, it was not possible to control for the menstrual cycle phases, which may have also influence bone markers.

5.4. Strengths

This study has several strength that contribute to the existing literature.

- i.* The population of adolescents were maturation-matched. The absence of matched-maturation was previously identified as problematic in the reviewed literature. In addition, the targeting of only adolescents rather than children and adolescents and of obese rather than overweight and obese individuals can also be presented as a strength within the existing thesis.
- ii.* Difficulties recruiting adolescents with obesity are well known. The challenges encountered in recruiting adolescents with obesity in Australia were significant and this planned component of the research had to be abandoned. Nonetheless, in France with strong clinical support, a total of 42 adolescents with obesity were recruited. This

number is similar to one previous study on adolescents with obesity (Campos et al. 2014) and substantially more than others (Campos et al. 2013) (Rocheftort et al. 2011) (El-Hage et al. 2009).

- iii.* Another strength may lie in the duration of the intervention, which importantly allowed time for potential bone remodeling. Additionally, within the 8-month intervention, mid-intervention data were collected, to detect any acute changes in body composition and bone parameters. Without this scheduled data collection, some key adaptations would have been missed.
- iv.* Although not all three groups were involved with complete sets of data, having three groups enhanced investigative opportunities. Having a normal weight control group beyond baseline to 8 months is not typically observed in the literature. Also, few studies are design to recruit an additional control group of adolescents with obesity, who do not receive the intervention.
- v.* It is acknowledged that limiting bone remodeling to only one bone formation marker (P1NP) and one bone resorption marker (CTX) may not provide an incomplete description of blood-borne responses to growth and the intervention. However, data were used innovatively in quantitative (median values for the uncoupling index) and qualitative representation of the distribution and elliptical patterns of bone remodeling via bone marker plots.

vi. Finally, although the data could be seen as incomplete, few previous studies have addressed adolescent obesity with a prolonged intervention within a residential program. Nor have they explored bone responses at the critical weight-bearing site of the hip using the hip structural analysis. Also, longitudinal studies on bone rarely combine DXA derived-data with blood-borne markers of bone turnover and maturation. Collectively, the results advance the existing understanding on bone responses to obesity and weight loss in adolescents and strengthen the directions for future research.

5.5. Further research considerations

This thesis opens several perspectives for future research.

First, future research designs for interventions targeting adolescents with obesity will require strong considerations to improve rigour in scientific investigations. For example, compliance can not be assumed. Protocols for planning monitoring and assessing physical activity and nutrition would strongly support potential dose-responses among participants in both intervention and comparative groups.

Second, the protocols in this thesis could be expanded to include additional blood markers of bone activity to investigate potential mechanistic explanations for the observed results. As stated in the limitations, additional blood markers may more comprehensively explain the effects of weight loss on bone health following physical activity and nutrition intervention in adolescents with obesity. Specifically, measurements of RANKL/RANK/OPG, osteocalcin (unOC,

OC, tOC) and sclerostin would provide additional information on bone remodeling activity. Also, the assessment of vitamin D could be of interest as vitamin D insufficiency has been linked with the uncarboxylated form of osteocalcin (Giudici et al. 2017). Indeed, vitamin D deficiency or insufficiency often occurs under obese conditions.

Third, to improve the understanding of maturational status and gender specific hormones, additional measures of FSH/LH could be assessed. This type of information could be used to add quality to data collection by (1) profiling additional measures of maturation, (2) coinciding blood sampling within similar phases of the menstrual cycle. Also, because of the observed androgen-like adaptation growth it would be of interest to measure both testosterone and the SHBG hormone as its secretion is influenced by physical activity (van Gemert et al. 2015). Indeed, it is possible that females with obesity have a substantially higher risk of developing polycystic ovary syndrome (hyperandrogenism) which may potentially explain the observed androgen-like bone growth. Therefore, future research could include additional bone, maturation and sex-specific hormones to help explain dose-related responses to lifestyle intervention in adolescents with obesity.

The thesis also generated specific research question to be address in future research (Appendix 31).

5.6. Conclusion

This thesis investigated the impact of a multidisciplinary weight loss intervention combining nutrition and physical activity, on the bone health of adolescents with obesity.

The research first identified contextual information and literatures gaps through rigorously reviewing the literature relating to the implication of the bone-adiposity cross-talk in paediatric obesity and the effects of interventions with a physical activity component on bone health in children and adolescents with obesity.

To strengthen the understanding of adolescents with obesity bone-related parameters, using a longitudinal intervention design, evidence were collected across an 8-month weight loss program combining physical activity and nutrition in a residential program. This study identified compromised bone mineral density through whole body and regional analysis. Even when adjusted for body weight and fat mass, lower bone mineral density was observed in adolescents with obesity than their normal weight peers. Despite some positive regional adaptations of bone geometry associated with the 8-month multidisciplinary weight loss intervention, fracture risk remained high in the adolescents with obesity, especially at the narrow neck. Moreover, bone accretion in adolescents with obesity undergoing a weight loss intervention appeared to follow androgen-like growth adaptations. These adaptations were shown through changes in periosteal expansion and endocortical resorption. An acute response at the midpoint of the intervention favoured bone formation. Yet, bone fragility in adolescents with obesity observed via DXA was supported by results from (1) bone marker z-scores, (2) the uncoupling index as well as by (3) the graphical representation. Future

investigations of links between bone and obesity during adolescence can be well informed by the results of this thesis.

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CHAPTER SIX

Appendices

Appendix 1 - Confirmation of Candidature



7/07/2015

Student ID: S00141116
Elodie Chaplais
Email: eechap002@myacu.edu.au

Dear Ms Chaplais,

Successful completion of probationary candidature

This is to inform you that your successful completion of the Probationary stage of your Doctor of Philosophy Candidature has been approved and your candidature in this program is confirmed as follows:

Thesis topic: *ADIBOX & SMART Health: outcomes and process measures of lifestyle intervention in paediatric obesity.*

Principal Supervisor: Assoc Prof David Greene

Co-Supervisor: Prof Geraldine Naughton

Assoc. Supervisor: NA

Date of Confirmation: 25/11/2014

Should you at any stage have any questions about your candidature, please do not hesitate to contact me. Please accept our best wishes for a successful and enjoyable continuation of your candidature.

Yours sincerely,

A handwritten signature in cursive script, appearing to read 'C. Condotta'.

Chiara Condotta
Graduate Research

Copies to: Associate Dean (Research): Prof Maree Johnson
National Head of School/Institute: Prof Justin Kemp
Principal Supervisor: A/Prof David Greene
Co-supervisor: Prof Geraldine Naughton

Graduate Research

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North Sydney New South Wales 2059
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Appendix 2 - Attestation letter of compulsive training module (France)



*Ecole Doctorale 65
Sciences de la Vie, Santé, Agronomie, Environnement*



**ATTESTATION DE VALIDATION FINALE DES MODULES
ANNEE 2016/2017**

NOM : CHAPLAIS Elodie

	VALIDATION EN 2016/2017	VALIDATION ANNEES ANTERIEURES		VALIDATION EN 2016/2017	VALIDATION ANNEES ANTERIEURES		VALIDATION EN 2016/2017	VALIDATION ANNEES ANTERIEURES	
MODULES OSP			MODULES OSP			MODULES OSP			
OSP 1 COMMUNICATION			OSP 10 GESTION DE PROJET		X	OSP 21 BILAN DE COMPETENCES	X		TOTAL
OSP 2-1 CREATION D'ENTREPRISES			OSP 11 EXPRESSION CORPORELLE			OSP 22 SCIENCES ET SOCIETE			
OSP 2-2 POLE ENTREPREUNARIAT			OSP 12 LES DEFIS DE L'EVALUATION			OSP 23 MANAGEMENT			
OSP 3 CULTURE D'ENTREPRISE			OSP 13 OPTIMISATION DE DOCUMENTS			OSP 24 MAITRISER L'INFORMATION			
OSP 4 ENVRONNEMENT TERRITORIAL			OSP 14 E-LEARNING			DISPENSES		2	
OSP 5 DEMARCHE QUALITE			OSP 15 DOCTORIALES (validation de 2 modules)			AUTRES			
OSP 6 PROPRIETE INTELLECTUELLE			OSP 16 SEMINAIRE ENSEIGNEMENT (validation de 2 modules)						
OSP 7 INSERTION PROFESSIONNELLE			OSP 17 FLE (validation de 4 modules)						
OSP 8 LA THESE			OSP 18 MARKETING						
OSP 9-1 ENSEIGNEMENT			OSP 19 COMMERCE INTERNATIONAL						
OSP 9-2 ENSEIGNEMENT			OSP 20 COMMUNIQUER LES SCIENCES						4/4
MODULES BIOLOGIE	0	4							4/4

Je soussignée, Monique ALRIC, Directrice de l'ED SVSAE, atteste que tous les modules sont validés.



Nous ne vous délivrerons pas de duplicata



AUTORISATION D'ESSAI CLINIQUE NE PORTANT PAS SUR UN PRODUIT DE SANTE (ESSAI-HPS)

Nombre de pages : 1
(Incluant la page de garde)

Envoi par Télécopie

Date : 11 AOUT 2015

Identifiants de l'essai clinique			
Titre : Effet de l'exercice physique sur la relation tissu osseux - tissu adipeux chez l'enfant obèse : projet ADIBOX.			
Promoteur	CHU de Clermont-Ferrand	Réf. CPP	Non disponible
Réf. Promoteur	AdiBoX	N° ID RCB	2015-A01024-45
		Réf. ANSM	150903B-21
Expéditeur		Destinataire (demandeur : nom / société / tél.)	
ANSM / Direction Produit Médicaments en cardiologie, endocrinologie, gynécologie, urologie / Equipe VASC		Patrick Lacarin CHU de Clermont-Ferrand 58 rue Montalembert BP 69 63003 Clermont-Ferrand cedex 04 73 75 11 95	
Dossier suivi par : Louise TOURNOYS Tél : 33 (0) 1 55 87 38 53 / Fax : 33 (0) 1 55 87 30 53 Mel : louise.tournoys@ansm.sante.fr		Fax : 04 73 75 47 30	
CPP destinataire en copie		Fax	Code 22
Sud-Est VI (Clermont-Ferrand)		04.73.75.10.69	

Vu le code de la santé publique et notamment ses articles L. 1123-8, R. 1123-32 et vu le dossier de demande d'autorisation d'essai clinique adressé à l'Agence nationale de sécurité du médicament et des produits de santé (ANSM)

L'autorisation mentionnée à l'article L. 1123-8 du code de la santé publique est accordée pour l'essai clinique cité en objet. Cette autorisation est valable pour toute la durée de l'essai à compter de la date de la présente décision.

Toutefois, conformément à l'article R. 1123-33 du code de la santé publique, la présente autorisation devient caduque si la recherche n'a pas débuté dans un délai d'un an.

Cette autorisation est délivrée, considérant que les examens complémentaires mis en œuvre pour les besoins de la recherche doivent être effectués avec les mêmes mesures de sécurité que celles habituellement recommandées en pratique clinique. Il revient donc aux investigateurs et intervenants de se conformer aux usages en vigueur.

En outre, je vous rappelle notamment que pendant le déroulement de la recherche et pour ce qui concerne l'ANSM :

- toute modification substantielle du dossier initialement soumis doit faire l'objet d'une demande d'autorisation en vertu des articles L. 1123-9 et R. 1123-35 du code de la santé publique ;
- les effets indésirables graves inattendus ainsi que les faits nouveaux susceptibles de porter atteinte à la sécurité des personnes doivent être déclarés conformément aux articles L. 1123-10 et R. 1123-46 du code de la santé publique.

Direction nationale de cardiologie, endocrinologie, gynécologie, urologie
Pôle « cardiovasculaire, thrombose, métabolisme »

Docteur Lotfi BOUDALI
Chef Produit

Je vous demande de transmettre toute demande d'informations complémentaires concernant ce dossier par courriel adressé à la boîte : ams-essaiscliniques@ansm.sante.fr. Je vous précise qu'il vous est possible d'utiliser à cet effet le système de messagerie électronique sécurisée Eudralink. Lors de l'envoi de ces dossiers, je vous demande de veiller à reporter dans l'objet du message les mentions suivantes :

- pour les MS transmises à l'Ansm pour information : **MSI/ Réf ANSM du dossier**
- pour les MS soumises pour autorisation ou pour les dossiers mixtes (comportant des modifications soumises pour autorisation et d'autres pour information) : **MSA/ Réf ANSM du dossier**

Si vous ne recevez pas toutes les pages de cette télécopie, veuillez contacter le secrétariat de la Direction Produit Médicaments en cardiologie, endocrinologie, gynécologie, urologie / Equipe VASC
au : 33 (0) 1 55 87 38 05

Confidentialité
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COMITE DE PROTECTION DES PERSONNES SUD-EST I

Président : M. Philippe RUSCH - Vice-Président : Dr Joël JUGE - Secrétaire : Mme Anne GROSSELIN - Trésorier : Pr Frédéric ROCHE

Date du comité	07/09/2015	Référence CPP	2015-33
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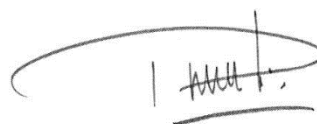
Projet relatif à	Recherche biomédicale ne portant pas sur un produit mentionné à l'article L.5311-1 du Code de la Santé Publique
Réception datée du	04/09/2015
Demande d'avis concernant un	Projet initial
Dans le cadre d'une	Nouvelle soumission d'un projet modifié
Documents concernés	Les documents soumis à l'approbation du Comité dans le cadre de la soumission du projet modifié figurent en annexe de cette délibération

Numéro EudraCT ou ID RCB	2015-A01024-45	Réf. Promoteur	RBHP 2015 DUTHEIL
Titre du projet	Effet de l'exercice physique sur la relation tissu osseux - tissu adipeux chez l'enfant obèse : projet ADIBOX		
Promoteur	CHU de CLERMONT-FERRAND		
Investigateur	Dr Frédéric DUTHEIL - Service de Médecine Préventive		

Avis du comité après examen, réexamen ou prise en compte des réserves mineures émises lors de la délibération initiale	Conformément à l'Article L. 1123-7, le comité a adopté la délibération suivante :		
	AVIS FAVORABLE		
Ont participé à la délibération	Membres du comité	Titulaires	Suppléants
1er collègue	Qualifiés en recherche biomédicale	Pr D. GUYOTAT	
		Pr F. ROCHE	
	Compétents en biostatistique ou épidémiologie		Dr C. BERGER
		M. P. RUSCH	
		Dr I. CARRIERE	
Pharmaciens	M. X. SIMOENS		
	Infirmiers		
2ème collègue	Compétents en questions éthiques	Mme C. SOLER	
	Juristes		
	Psychologues	Mme A. GROSSELIN	Mme I. RAMOS
	Représentants d'associations agréées de malades et usagers du système de santé	M. F. FAISAN	
M. G. BERNE		Mme C. MARTIGNAGO	

A Saint-Etienne, le 08/09/2015

Le Président de séance - M. Philippe RUSCH



Appendix 5 - Ethics amendment letter to male participants



Occupational Medicine
– CHU G. MONTPIED
– AUVERGNE UNIVERSITY
63000 Clermont-Ferrand
France



Laboratory of Metabolic Adaptations to
Exercise in Physiological and Pathological
conditions (AME2P, EA3533)
– BLAISE PASCAL UNIVERSITY
63000 Clermont-Ferrand, France



Faculty of Health
– AUSTRALIAN CATHOLIC UNIVERSITY
Locked Bag 4115 Fitzroy MDC
Melbourne, VIC 3065
Australia

Dr Frédéric DUTHEIL
Praticien Hospitalo-Universitaire
Tel: +33 4 73 17 82 60
Mobile: +33 6 88 22 48 48
Mail: fdutheil@chu-clermontferrand.fr

28 septembre 2015

Objet: Amendement au protocole de recherche AdiBoX (accord éthique du CPP Sud-Est VI du 08 septembre 2015)

Promoteur: CHU Clermont-Ferrand

Réf. CPP: 2015-33

Réf. ID-RCB: 2015-A01024-45

Réf. Promoteur: RBHP 2014 DUTHEIL 3

Monsieur le Président,
Mesdames et Messieurs les membres du Comité

Le centre Tza Nou a étendu son programme de prise en charge de l'obésité aux adolescents de sexe masculin depuis cette année.

Nous souhaitons donc **étendre l'inclusion aux adolescents de sexe masculin** afin d'être en adéquation au potentiel de recrutement et à la réalité du terrain.

Le genre n'a a priori qu'une influence mineure sur les relations ostéo-adipocytaire. Cette éventuelle influence sera toutefois étudiée lors de l'analyse statistique.

Nous vous remercions d'avance pour votre réponse que nous espérons positive, et vous prions d'agréer, Monsieur le Président, Mesdames et Messieurs les Membres du Comité, l'expression de nos sentiments respectueux.

Frédéric Dutheil



Effet de l'exercice physique sur la relation tissu osseux – tissu adipeux chez l'enfant obèse : projet ADIBOX

Titre abrégé : AdiBoX

Version : 3

en date du : 28.09.2015

Code Promoteur	N° d'enregistrement ANSM
RBHP 2015 DUTHEIL	2015-A01024-45

Promoteur

C.H.U de Clermont-Ferrand

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Investigateur principal

Dr Frédéric DUTHEIL

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CHU de Clermont-Ferrand - Villa annexe IFSI
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63003 Clermont-Ferrand cedex
bpereira@chu-clermontferrand.fr

Lieu de réalisation de l'étude

Tza Nou - Maison médicale pour enfants et adolescents – 230, rue Vercingétorix – B.P. 77 – 63150 La Bourboule

CHU Gabriel Montpied – Service Médecine du sport – 58 rue de Montalembert – 63003 Clermont-Ferrand Cedex 1

CHU Gabriel Montpied – Cardiologie – 58 rue de Montalembert – 63003 Clermont-Ferrand Cedex 1

CHU Gabriel Montpied – Médecine Préventive et des risques professionnels – 58 rue de Montalembert – 63003 Clermont-Ferrand Cedex 1

SSR Nutrition-Obésité – 33-35 rue Maréchal Leclerc – 63000 Clermont-Ferrand

RESUME

Contexte / Justification de l'étude :

Problème sanitaire mondial de par la gravité de ses maladies collatérales (hypertension artérielle, diabète de type 2, maladies cardiovasculaires, ...) l'obésité constitue l'un des plus grand défis du 21^e siècle. La prévalence du surpoids et de l'obésité infantiles augmente de façon préoccupante. L'accès à cette population bien spécifique nous est de plus favorisé. En effet, notre principal partenaire, la maison médicale pour enfants et adolescents « Tza Nou » accueille un public exclusivement d'enfants ou adolescents obèses.

L'adolescence est une période clef dans le développement de l'enfant (changements physiologiques, psychologiques, culturels et émotionnels) pouvant influencer sur une prise de poids. D'un point de vue osseux, l'adolescence, plus précisément la période péri-pubertaire est le meilleur moment pour stimuler la structure osseuse. Il est donc important de s'intéresser à cette tranche d'âge afin de réduire et limiter au maximum les risques de répercussions de l'obésité sur le métabolisme, y compris sur le tissu osseux.

Comprendre les interactions du tissu adipeux et du tissu osseux durant la croissance est un élément important. En effet, contrairement aux études faites chez l'adulte obèse¹, les données chez l'enfant suggèrent un risque de fracture important, particulièrement lors de la période péri-pubertaire². Plusieurs équipes ont analysé la santé osseuse d'enfants et d'adolescents obèse utilisant divers techniques (densitométrie, ultra-sonore ou encore micro-architecturale). Toutes ont obtenu des résultats discordants³⁻¹². Toutefois, il faut noter que les mesures micro-architecturales mettent en avant un déficit de minéralisation osseuse chez l'enfant obèse compris entre -1.5 et -2.5 de déviation standard (DS) comparé à une population témoin de même âge, même sexe¹³. Ce déficit est considéré par de nombreux investigateurs comme risque d'ostéoporose précoce.

Afin de comprendre cette altération osseuse, il est nécessaire de s'intéresser à l'influence de la masse grasse sur la masse osseuse, d'autant plus que la littérature a mis en exergue l'existence d'un axe physiologique os-adiposité¹⁴.

Une relation complexe entre le tissu adipeux et le tissu osseux est clairement établie¹⁵. En effet, l'obésité entraîne une altération hormonale associée à une augmentation des facteurs inflammatoires et du stress oxydatif stimulant les facteurs contribuant à l'augmentation des graisses, et de la perte osseuse¹⁵. L'augmentation de la production de facteur inflammatoire (e.g. TNF- α) et la réduction de sécrétion d'hormone produite par le tissu adipeux (e.g. adiponectine) aboutissent à la suppression du développement des adipocytes, favorisant le stockage des lipides et augmentant l'insulino-résistance^{16,17}.

Le tissu adipeux sécrète divers peptides, parmi lesquels la leptine dont la production est proportionnelle à la masse grasse périphérique. Les taux de leptine sont donc plus élevés chez l'adolescent obèse comparativement à l'adolescent normo-pondéré avec un phénomène induit de résistance à la leptine. Cette hormone intervient dans le contrôle de la masse corporelle en agissant au niveau de récepteurs hypothalamiques impliqués dans le contrôle de la prise alimentaire et de la dépense énergétique, en augmentant l'oxydation des lipides dans le muscle^{18,19}. Il est démontré que la masse grasse et une plus grande sécrétion de leptine observée chez l'enfant obèse sont associées à une réduction de marqueur sanguin et urinaire du remodelage osseux. D'où l'intérêt de contrôler l'action de régulation locale du remodelage lors de la prise en charge de ces adolescents.

D'autre part, chez les adolescents obèses comparativement aux normo-pondérées, les taux d'hormones de régulation de l'appétit (ghréline, PYY) sont significativement inférieurs²⁰. Ces taux plus faibles pourraient être expliqués par une suralimentation des adolescents obèses pouvant refléter

un mécanisme de rétroaction pour réduire l'appétit et être en partie le résultat de la résistance à l'insuline associée à un excès de poids²¹. Il est établi que ces hormones de régulation de l'appétit sont négativement impliquées dans la régulation de la densité osseuse²². De plus, la fragilisation du tissu osseux est accentuée par la perte de poids induite par restriction énergétique²³.

En conclusion, dans ce contexte, il est aisé de concevoir que la compréhension du rôle des sécrétions hormonales du tissu adipeux lié à la prise/perte de poids est fondamentale pour comprendre leurs relations sur la structure osseuse (fragilité osseuse de l'adolescent et ostéoporose précoce) et la prévention de l'obésité à l'âge adulte. A notre connaissance aucune étude ne s'est intéressée aux rôles des facteurs hormonaux interagissant sur les métabolismes énergétiques et osseux lors d'une perte de masse corporelle chez l'adolescent obèse. L'objectif de ce projet est donc d'explorer les relations entre le métabolisme du tissu adipeux et le métabolisme du tissu osseux chez l'adolescent obèse, ainsi que les effets d'une perte de poids induite par l'activité physique sur ces relations.

Objectifs :

- Objectif principal :

Etudier les effets d'une prise en charge de 10 mois combinant activité physique et nutrition en comparaison avec un groupe contrôle sur l'interaction 'tissu adipeux – tissu osseux' chez des adolescents obèses,

- Objectifs secondaires :

Etudier l'effet d'une perte de poids induite par une prise en charge de 10 mois combinant activité physique et nutrition sur la variation de masse adipeuse et masse osseuse.

Etudiez les effets du statut pondéral de l'adolescent sur la relation métabolisme osseux – métabolisme adipeux et la prise énergétique.

Etudier les effets d'une prise en charge de 10 mois par l'activité physique et la nutrition sur le contrôle de la prise énergétique d'adolescents obèses.

Etudier les effets d'une prise en charge de 10 mois par l'activité physique et la nutrition sur les paramètres cardiaques (dont le tissu adipeux épicaudique) et leurs relation avec le tissu osseux et adipeux général, sous cutané et viscéral.

Type d'étude : interventionnelle comparant deux cohortes d'adolescents obèses (prise en charge de 10 mois combinant activité physique et nutrition en comparaison avec un groupe contrôle).

Nombre de centres :

Les adolescents obèses (groupe intervention) seront recrutés parmi les adolescents de la maison d'enfants de prise en charge de l'obésité de Tza Nou, La Bourboule.

Les adolescents obèses « contrôle » seront recrutés par l'intermédiaire de consultations pédiatriques externes ainsi que lors de consultation au SSR Nutrition-Obésité de Clermont-Ferrand.

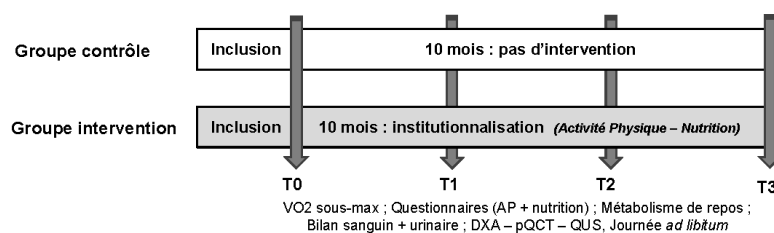
Lieux de réalisation des mesures:

- Maison médicale pour enfants et adolescents Tza Nou, La Bourboule
- Service de Médecine du Sport, CHU Clermont-Ferrand
- SSR Nutrition-Obésité – 33-35 rue Maréchal Leclerc, Clermont-Ferrand

Description de l'étude / Plan expérimental :

Après une visite d'inclusion pour assurer l'aptitude des adolescents à compléter l'ensemble de l'étude, chacune devra réaliser une épreuve d'effort sous-maximale sur bicyclette ergométrique, des mesures densitométriques (composition corporelle et structure osseuse), un prélèvement sanguin, un test d'évaluation de leur métabolisme de repos, une journée *ad libitum*, une échocardiographie et

compléter des questionnaires afin d'évaluer leur prise énergétique et leur activité physique. Ces mesures seront à T0, T1, T2 et T3 pour toutes les participantes, soit tous les deux mois et demi. Ces temps de mesure permettront de comparer les effets d'une perte de poids induite par l'activité physique sur les interactions tissu adipeux – tissu osseux. L'inclusion d'un groupe contrôle est primordiale afin de nous permettre d'obtenir des informations relatives à l'effet propre de l'intervention (AP + nutrition) sur les paramètres tissu osseux, tissu adipeux et apport alimentaire.



Critère de jugement principal :

Le critère d'évaluation principal est la variation de la masse adipeuse rapportée à la variation d'un marqueur de la masse osseuse (densité minérale osseuse) mesuré au rachis lombaire. C'est donc un ratio considéré comme une donnée quantitative et traitée comme telle.

Nombre d'adolescents :

50 adolescents obèses minimum (25 pour le groupe intervention, 25 pour le groupe contrôle)

Critères d'inclusion :

Pour être inclus dans l'étude, les adolescents (interventions et contrôles) devront respecter les critères communs de sélection. De sexe indifférent, les participantes devront être âgées entre 12 et 16 ans, être mature (ménarchée), avoir un IMC supérieur au 97th percentile des courbes nationales²⁴ et être apte à la pratique d'activité physique. Les adolescents ne devront pas présenter de carences alimentaires (vitamine D, calcium, protéine) ainsi que de maladie métabolique susceptible de perturber les mécanismes étudiés (obésité, nutrition, remodelage osseux) telle que du diabète / pré-diabète, de l'insulino-résistance, de l'hyper/hypo-thyroïdie. De plus les adolescents et deux titulaires de l'autorité parentale devront avoir signé la fiche d'information et de consentement.

En complément des critères énumérés ci-dessus, les adolescents du groupe intervention devront intégrer la cure de 10 mois du centre Tza Nou, quant aux adolescents du groupe « contrôle », elles ne devront pas pratiquer une activité physique qui excède 150 min par semaine en dehors du temps scolaire.

Critères d'exclusion :

Les principaux critères d'exclusions sont la déviation majeure au protocole, ainsi que toutes causes ne permettant pas au sujet de participer complètement au programme et de respecter les consignes concernant la diète et l'activité physique.

Déroulement de l'étude

Maison médicale pour enfants et adolescents
Tza Nou
230, rue Vercingétorix – B.P. 77

63150 La Bourboule
SIRET : 423 977 792 00088
FINESS : 63 078 0559

Centre Hospitalo-Universitaire
Service de Médecine du Sport et des
Explorations Fonctionnelles
SSR Nutrition-Obésité
33-35 rue Maréchal Leclerc
63000 Clermont-Ferrand
SIRET : 423 977 792 00088
FINESS : 63 001 1823

Hôpital Gabriel Montpied
58, rue Montalembert BP 69
63003 Clermont-Ferrand cedex
Autorisation de lieu n°03022S

Centre Hospitalo-Universitaire
Médecine Préventive et des risques
professionnels
Hôpital Gabriel Montpied
58, rue Montalembert BP 69
63003 Clermont-Ferrand cedex

Centre Hospitalo-Universitaire
Cardiologie
Hôpital Gabriel Montpied
58, rue Montalembert BP 69
63003 Clermont-Ferrand cedex

Bénéfices et risques de cette étude :

Les adolescents obèses (du groupe intervention) bénéficieront d'une prise en charge institutionnelle combinant activité physique – éducation thérapeutique et suivi nutritionnel visant une perte de poids et améliorant leur condition physique. Concernant les adolescents du groupe contrôle, leur participation n'aura aucun effet bénéfique direct sur leur santé mais celle-ci fera progresser leurs connaissances visant à une meilleure compréhension du métabolisme énergétique de l'enfant. En effet, à l'issue du deuxième temps de mesure, nous leur offrirons une prise en charge sous forme d'éducation thérapeutique. Elles bénéficieront de conseils concernant les comportements nutritionnels à adopter ainsi que les recommandations liées à la pratique d'activités physiques.

Pour l'ensemble des adolescents, les risques liés aux mesures sont minimes dans la mesure où l'étude est réalisée dans un cadre clinique et encadrée par des professionnels de santé et d'activité physique adaptée (prélèvement biologique, tests capacités physique et musculaire, évaluation nutritionnelle, activité physique). En ce qui concerne les mesures densitométriques le taux d'irradiation est également très faible ce qui limite les risques encourus.

SOMMAIRE

1. INFORMATIONS GENERALES.....	9
1.1. TITRE DE LA RECHERCHE.....	9
1.2. CODE PROMOTEUR.....	9
1.3. PROMOTEUR.....	9
1.4. COORDINATION ET SUIVI DE L'ETUDE	9
1.5. INVESTIGATEURS	9
1.5.1. <i>Investigateur coordonnateur</i>	9
1.5.2. <i>Responsable scientifique</i>	9
1.6. PARTENAIRES ASSOCIES	10
1.7. LIEUX DE REALISATION D'ETUDE	10
1.8. TRAITEMENT DES DONNEES.....	10
1.9. COMITE DE PROTECTION DES PERSONNES	11
1.10. CALENDRIER PREVISIONNEL DE L'ETUDE	11
2. RATIONNEL DE L'ETUDE / JUSTIFICATION SCIENTIFIQUE.....	12
2.1. EPIDEMIOLOGIE DE L'OBESITE INFANTILE	12
2.2. QUE DIT LA LITTERATURE ?	12
2.3. RELATIONS TISSU ADIPEUX/TISSU OSSEUX	12
2.4. DERNIER ETAT DES CONNAISSANCES SUR L'EXPERIMENTATION PRE CLINIQUE	13
2.5. RESUME DES BENEFICES ET DES RISQUES PREVISIBLES ET CONNUS POUR LES PERSONNES SE PRETANT A LA RECHERCHE.....	13
2.6. REFERENCES A LA LITTERATURE SCIENTIFIQUE ET AUX DONNEES PERTINENTES SERVANT DE REFERENCE POUR LA RECHERCHE.....	14
3. OBJECTIFS DE L'ETUDE.....	15
3.1. OBJECTIF PRINCIPAL	15
3.2. OBJECTIFS SECONDAIRES	15
4. DESCRIPTION DE L'ETUDE	15
4.1. CRITERES D'EVALUATION	15
4.1.1. <i>Critère d'évaluation principal</i>	15
4.1.2. <i>Critères d'évaluation secondaires</i>	15
4.2. DESCRIPTION DE LA METHODOLOGIE DE LA RECHERCHE.....	15
4.3. DESCRIPTION DES MESURES PRISES POUR REDUIRE ET EVITER LES BIAIS	16
5. REALISATION PRATIQUE DU PROTOCOLE	17
5.1. DESCRIPTION DES ACTES PRATIQUES SUR LES PERSONNES (DESCRIPTION DE CHACUNE DES VISITES)/ DES PRODUITS UTILISES AU COURS DE LA RECHERCHE.....	17
5.1.2. <i>Temps de mesure</i>	17
5.1.3. <i>Mesures au cours de l'intervention (T0, T1, T2, T3)</i>	17
5.1.4. <i>Prise en charge au centre Tza Nou</i>	18
5.2. DESCRIPTION DE L'ORGANISATION LOGISTIQUE GENERALE DE L'ESSAI	18
5.3. PARAMETRES MESURES.....	18
5.3.1. <i>Mesures cliniques</i>	18
5.3.2. <i>Prise alimentaire</i>	18
5.3.3. <i>Activité Physique</i>	19
5.3.4. <i>Capacité aérobie</i>	19
5.3.5. <i>Composition corporelle et structure osseuse</i>	19
5.3.6. <i>Prélèvements / analyses biologiques et urinaire de l'étude</i>	20

5.3.7. Echocardiographie.....	22
5.4. DESCRIPTION DE L'ORGANISATION LOGISTIQUE GENERALE DE L'ESSAI	22
5.5. DUREE PREVUE DE PARTICIPATION DES PERSONNES ET DESCRIPTION DE LA CHRONOLOGIE DE L'ESSAI.....	23
6. POPULATION ETUDIEE.....	23
6.1. CRITERES D'INCLUSION.....	23
6.2. CRITERES DE NON INCLUSION.....	23
6.3. PROCEDURE D'ARRET PREMATURE DE LA RECHERCHE	24
6.4. PERIODE D'EXCLUSION ET PARTICIPATION A UNE AUTRE RECHERCHE.....	24
6.5. INDEMNISATION DES VOLONTAIRES	24
6.6. MODALITES DE RECRUTEMENT	24
7. TRAITEMENT ET PRODUITS ADMINISTRES AUX PERSONNES QUI SE PRETENT A LA RECHERCHE	24
7.1. DESCRIPTION DU TRAITEMENT	24
7.2. POSOLOGIE, MODALITES D'ADMINISTRATION ET DUREE DU TRAITEMENT (LE CAS ECHEANT)	24
7.3. PRESENTATION DES PRODUITS.....	25
7.4. MEDICAMENTS ET TRAITEMENTS AUTORISES ET INTERDITS PENDANT L'ESSAI.....	25
8. DONNEES RECUEILLIES.....	25
9. CONSIDERATIONS STATISTIQUES.....	25
9.1. NOMBRE D'ADOLESCENTS A INCLURE	25
9.2. ANALYSE DE DONNEES : GENERALITES	25
9.3. DESCRIPTION DES ECHANTILLONS A L'INCLUSION.....	26
9.4. ANALYSE PRINCIPALE	26
9.5. ANALYSES SECONDAIRES.....	26
9.6. METHODE DE PRISE EN COMPTE DES DONNEES MANQUANTES, INUTILISEES OU INVALIDES	26
9.7. RESPONSABLE DE L'ANALYSE	26
10. EVALUATION DE LA SECURITE – GESTION DES EVENEMENTS INDESIRABLES.....	27
10.1. DEFINITIONS	27
10.2. DESCRIPTION DES PARAMETRES D'EVALUATION DE LA SECURITE	27
10.3. DECLARATION DES EVENEMENTS INDESIRABLES GRAVES	28
10.4. EFFETS INDESIRABLES.....	28
10.5. SUIVI DES ADOLESCENTS AYANT PRESENTE UN EVENEMENT INDESIRABLE	28
11. DROIT D'ACCES AUX DONNEES ET DOCUMENTS SOURCE.....	29
11.1. ACCES AUX DONNEES.....	29
11.2. DONNEES SOURCE	29
11.3. CONFIDENTIALITE DES DONNEES.....	29
11.4. INSCRIPTION AU FICHIER NATIONAL DES PERSONNES SE PRETANT A UNE RECHERCHE BIOMEDICALE.....	30
12. CONTROLE ET ASSURANCE DE LA QUALITE	30
12.1. ENGAGEMENT DES INVESTIGATEURS ET DU PROMOTEUR	30
12.2. ASSURANCE DE QUALITE	30
12.3. CONTROLE DE QUALITE	30
12.4. CAHIER D'OBSERVATION.....	30
13. CONSIDERATIONS ETHIQUES.....	31

13.1.	COMITE DE PROTECTION DES PERSONNES ET AUTORITE COMPETENTE	31
13.2.	INFORMATION AUX ADOLESCENTS ET FORMULAIRE DE CONSENTEMENT ECLAIRE ECRIT.....	31
13.3.	AMENDEMENTS AU PROTOCOLE.....	31
13.4.	PRISE EN CHARGE RELATIVE A LA RECHERCHE.....	31
14.	TRAITEMENT DES DONNEES ET CONSERVATION DES DOCUMENTS ET DONNEES RELATIVES A LA RECHERCHE.....	32
14.1.	SAISIE ET TRAITEMENT DES DONNEES	32
14.2.	CNIL	32
14.3.	ARCHIVAGE	32
15.	FINANCEMENT ET ASSURANCE.....	32
15.1.	BUDGET DE L'ETUDE.....	32
15.2.	ASSURANCE	33
16.	COMMUNICATION - REGLES DE PUBLICATION	33
17.	FAISABILITE DE L'ETUDE.....	33
18.	BIBLIOGRAPHIE.....	34
	LISTE DES ANNEXES.....	38

1. Informations générales

1.1. Titre de la recherche

Titre : « **Effets de l'exercice physique sur la relation tissu osseux – tissu adipeux chez l'adolescent obèse : projet ADIBOX** »

Titre abrégé : *AdiBoX*

n° d'enregistrement ANSM : 2015-A01024-45

1.2. Code Promoteur

RBHP 2015 DUTHEIL

1.3. Promoteur

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58 Rue de Montalembert
63003 Clermont-Ferrand Cedex 1

1.4. Coordination et suivi de l'étude

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Campus Universitaire des Cézeaux
63170 Aubière Cedex

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1.5.2. Responsable scientifique

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1.6. Co-investigateurs

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1.7. Lieux de réalisation d'étude

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Montpied - 58, rue Montalembert – BP 69 63003 Clermont-Ferrand cedex
- Centre Hospitalo-Universitaire, Médecine Préventive et des risques professionnels,
Hôpital Gabriel Montpied, 58, rue Montalembert BP 69 63003 Clermont-Ferrand
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- Maison médicale pour enfants et adolescents Tza Nou - 230, rue Vercingétorix – B.P.
77 - 63150 La Bourboule - SIRET : 423 977 792 00088 FINESS : 63 0780559
- SSR Nutrition-Obésité – 33-35 rue Maréchal Leclerc – 63000 Clermont-Ferrand -
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1.8. Traitement des données

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Theix
63122 St Genès Champanelle

1.9. Comité de Protection des Personnes

Comité de Protection des Personnes Auvergne (CPP) Sud est 6

1.10. Calendrier prévisionnel de l'étude

Soumission CPP : Juin 2015

Autorisation de l'autorité compétente : Juin 2015

Début d'étude : Août 2015

Période d'inclusion : 3 mois

Fin d'étude prévisionnelle : Juillet 2016

Rapport de fin d'étude : Octobre 2016

2. Rationnel de l'étude / Justification scientifique

2.1. Epidémiologie de l'obésité infantile

Problème sanitaire mondial de par la gravité de ses maladies collatérales (hypertension artérielle, diabète de type 2, maladies cardiovasculaires, ...) l'obésité constitue l'un des plus grand défis du 21^e siècle. La prévalence du surpoids et de l'obésité infantiles augmente de façon préoccupante.

L'adolescence est une période clef dans le développement de l'enfant (changements physiologiques, psychologiques, culturels et émotionnels) pouvant influencer sur une prise de poids. D'un point de vue osseux, l'adolescence, plus précisément la période péri-pubertaire est le meilleur moment pour stimuler la structure osseuse. Il est donc important de s'intéresser à cette tranche d'âge afin de réduire et limiter au maximum les risques de répercussions de l'obésité sur le métabolisme, y compris sur le tissu osseux.

2.2. Que dit la littérature ?

Comprendre les interactions du tissu adipeux et du tissu osseux durant la croissance est un élément important. En effet, contrairement aux études faites chez l'adulte obèse¹, les données chez l'enfant suggèrent un risque de fracture important, particulièrement lors de la période péri-pubertaire². Plusieurs équipes ont analysé la santé osseuse d'enfants et d'adolescents obèses utilisant divers techniques (densitométrie, ultra-sonore ou encore micro-architecturale). Toutes ont obtenu des résultats discordants³⁻¹². Toutefois, il faut noter que les mesures micro-architecturales mettent en avant un déficit de minéralisation osseuse chez l'enfant obèse compris entre -1.5 et -2.5 de déviation standard (DS) comparé à une population témoin de même âge, même sexe¹³. Ce déficit est considéré par de nombreux investigateurs comme risque d'ostéoporose précoce.

Afin de comprendre cette altération osseuse, il est nécessaire de s'intéresser à l'influence de la masse grasse sur la masse osseuse, d'autant plus que la littérature a mis en exergue l'existence d'un axe physiologique os-adiposité¹⁴.

2.3. Relations tissu adipeux/tissu osseux

Une relation complexe entre le tissu adipeux et le tissu osseux est clairement établie¹⁵. En effet, l'obésité entraîne une altération hormonale associée à une augmentation des facteurs inflammatoires et du stress oxydatif stimulant les facteurs contribuant à l'augmentation des graisses, et de la perte osseuse¹⁵. L'augmentation de la production de facteur inflammatoire (e.g. TNF- α) et la réduction de sécrétion d'hormone produite par le tissu adipeux (e.g. adiponectine) aboutissent à la suppression du développement des adipocytes, favorisant le stockage des lipides et augmentant l'insulino-résistance^{16,17}.

Le tissu adipeux sécrète divers peptides, parmi lesquels la leptine dont la production est proportionnelle à la masse grasse périphérique. Les taux de leptine sont donc plus élevés chez l'adolescent obèse comparativement à l'adolescent normo-pondérée avec un phénomène induit de résistance à la leptine. Cette hormone intervient dans le contrôle de la masse corporelle en agissant au niveau de récepteurs hypothalamiques impliqués dans le contrôle de la prise alimentaire et de la dépense énergétique, en augmentant l'oxydation des lipides dans le muscle^{18,19}. Il est démontré que la masse grasse et une plus grande sécrétion de leptine observée chez l'enfant obèse sont associées à une réduction de marqueur sanguin et urinaire du remodelage osseux. D'où l'intérêt de contrôler l'action de régulation locale du remodelage lors de la prise en charge de ces adolescents.

D'autre part, chez les adolescents obèses comparativement aux normo-pondérées, les taux d'hormones de régulation de l'appétit (ghréline, PYY) sont significativement inférieurs²⁰. Ces taux plus faibles pourraient être expliqués par une suralimentation des adolescents obèses pouvant refléter un mécanisme de rétroaction pour réduire l'appétit et être en partie le résultat de la résistance à l'insuline associée à un excès de poids²¹. Il est établi que ces hormones de régulation de l'appétit sont négativement impliquées dans la régulation de la densité osseuse²². De plus, la fragilisation du tissu osseux est accentuée par la perte de poids induite par restriction énergétique²³.

En conclusion, dans ce contexte, il est aisé de concevoir que la compréhension du rôle des sécrétions hormonales du tissu adipeux lié à la prise/perte de poids est fondamentale pour comprendre leurs relations sur la structure osseuse (fragilité osseuse de l'adolescent et ostéoporose précoce) et la prévention de l'obésité à l'âge adulte. A notre connaissance aucune étude ne s'est intéressée aux rôles des facteurs hormonaux interagissant sur les métabolismes énergétiques et osseux lors d'une perte de masse corporelle chez l'adolescent obèse. L'objectif de ce projet est donc d'explorer les relations entre le métabolisme du tissu adipeux et le métabolisme du tissu osseux chez l'adolescent obèse, ainsi que les effets d'une perte de poids induite par l'activité physique sur ces relations.

2.4. Dernier état des connaissances sur l'expérimentation pré clinique

Au regard de la nature et de la thématique considérée ici, les données issues d'évaluation précliniques sont rares. A ce jour, une étude publiée semble pouvoir servir de base à ce travail, aussi bien méthodologiquement que scientifiquement. Cette étude conduite par Campos et collaborateurs 2013 au Brésil nous montre chez l'adolescent obèse que la ghréline, la graisse viscérale et le ration leptine/adiponectine sont des facteurs influençant négativement la DMO et CMO²⁵. Les hypothèses formulées dans la discussion de ce travail concernant les relations entre le métabolisme adipeux et le métabolisme du tissu osseux chez l'adolescent obèse, sont en accord avec les objectifs et la méthodologie de ce projet.

2.5. Résumé des bénéfices et des risques prévisibles et connus pour les personnes se prêtant à la recherche

Bénéfices : Par leur participation, les adolescents obèses (du groupe intervention) bénéficieront d'une prise en charge institutionnelle combinant activité physique – éducation thérapeutique et suivi nutritionnel visant une perte de poids et améliorant leur condition physique. Concernant les adolescents « contrôle », leur participation n'aura aucun effet bénéfique direct sur leur santé, toutefois, à l'issue du deuxième temps de mesure, nous leur offrirons une prise en charge sous forme d'éducation thérapeutique. Elles bénéficieront de conseils concernant les comportements nutritionnels à adopter ainsi que les recommandations liées à la pratique d'activités physiques.

Risques :*· Liés à l'exercice physique*

L'exercice physique peut être nuisible à la santé de l'enfant dans certaines conditions. Les effets préjudiciables des exercices physiques comprennent : les lésions dues au sport, les syndromes d'hyper-utilisation, et les réactions physiologiques anormales. Bien que la plupart des lésions résultent le plus souvent d'un traumatisme au cours de sports de contact comme le football, le rugby, le hockey sur glace, elles peuvent survenir en tous sports, et chez tous sportifs sans rapport avec ses compétences ou ses aspirations.

Les syndromes d'hyper-utilisation peuvent résulter d'un mouvement répété, souvent à une intensité élevée, pendant des mois et des années entraînant des lésions mécaniques, osseuses, cartilagineuses, tendineuses ou musculaires. Ces lésions sont souvent observées chez de jeunes athlètes s'entraînant de façon intensive.

Chez de jeunes adolescents saines un exercice au long cours peut induire une déshydratation, un épuisement, un coup de chaleur ou une protéinurie. Ces effets négatifs sont souvent dus à une accumulation de facteurs défavorables.

Les risques encourus lors des exercices de ce protocole sont minimes notamment en raison du très faible effet traumatisant des épreuves physiques (pédalage lors de l'épreuve sous-maximale). Plus spécifiquement pour les adolescents du groupe « intervention », les activités physiques proposées sont adaptées et encadrées par des éducateurs spécialisés.

· Liés à l'analyse corporelle et structure osseuse

L'irradiation des outils de mesure est le principal risque lié à l'analyse corporelle et de la structure osseuse, cependant ce risque est minime du fait de la faible émission provoqué par les outils de mesures soit entre 0,0042 – 0,0048mSv pour une DXA du corps entier chez l'enfant de 10 à 15 ans et moins de 0,01mSv par scan pour la pQCT²⁶.

· Liés aux analyses biologiques

Les risques liés aux prises de sang (allergies, hématomes, malaises, piqûres dans le nerf, contamination, syncopes) seront réduits du fait de la réalisation de ces prélèvements par des professionnels de la santé aguerris.

2.6. Références à la littérature scientifique et aux données pertinentes servant de référence pour la recherche

De la même manière que pour ce qui concerne les données précliniques disponibles aujourd'hui, l'étude de Campos et collaborateurs de 2013²⁵ comparant les interactions entre la densité minérale osseuse, les adipokines et les hormones chez l'adolescent obèse, sert de référence principale (méthodologie et scientifique) à ce travail.

3. Objectifs de l'étude

3.1. Objectif principal

Etudier les effets d'une prise en charge de 10 mois combinant activité physique et nutrition en comparaison avec un groupe contrôle sur l'interaction 'tissu adipeux – tissu osseux' chez des adolescents obèses,

3.2. Objectifs secondaires

- Etudier l'effet d'une perte de poids induite par une prise en charge de 10 mois combinant activité physique et nutrition sur la variation de masse adipeuse et masse osseuse.
- Etudiez les effets du statut pondéral de l'adolescent sur la relation métabolisme osseux – métabolisme adipeux et la prise énergétique.
- Etudier les effets d'une prise en charge de 10 mois par l'activité physique et la nutrition sur le contrôle de la prise énergétique d'adolescents obèses.
- Etudier les effets d'une prise en charge de 10 mois par l'activité physique et la nutrition sur les paramètres cardiaques (dont le tissu adipeux épicaudique) et leurs relation avec le tissu osseux et adipeux général, sous cutané et viscéral.

4. Description de l'étude

4.1. Critères d'évaluation

4.1.1. Critère d'évaluation principal

Le critère d'évaluation principal est la variation de la masse adipeuse rapportée à la variation d'un marqueur de la masse osseuse (densité minérale osseuse) mesuré au rachis lombaire. C'est donc un ratio considéré comme une donnée quantitative et traitée comme telle.

4.1.2. Critères d'évaluation secondaires

Les critères secondaires d'évaluation sont :

- 1) La variation d'un marqueur adipocytaire (adiponectine) et la variation d'un marqueur quantitatif de la masse osseuse (densité minérale osseuse) ainsi qu'un marqueur biologique du remodelage, l'index de découpage par mesure des activités de formation et résorption de l'os.
- 2) La prise alimentaire et la sensation de faim. La prise alimentaire sera évaluée par le biais d'une journée *ad libitum* (petit déjeuner, déjeuner, dîner). Les aliments seront alors pesés par l'équipe investigatrice. La sensation de faim sera quant à elle renseignée à intervalles réguliers lors de chaque temps de mesure par questionnaires (échelles visuelles analogues).
- 3) Les paramètres de remodelages cardiaques précoces et classiques (volumes, épaisseurs des ventricules droit et gauche, fraction d'éjection ventriculaire gauche, Strain 2D et 3D), ainsi que le tissu adipeux épicaudique.

4.2. Description de la méthodologie de la recherche

Etude interventionnelle comparant deux cohortes d'adolescents obèses (prise en charge de 10 mois combinant activité physique et nutrition en comparaison avec un groupe contrôle).

Après une visite d'inclusion pour assurer l'aptitude des adolescents à compléter l'ensemble de l'étude, chacune d'entre elles devra réaliser une épreuve d'effort sous-maximale sur bicyclette ergométrique, des mesures densitométriques, un prélèvement sanguin, un test d'évaluation de leur métabolisme de repos, une journée *ad libitum*, une échocardiographie trans-thoracique et répondre à des questionnaires sur leur activité physique et nutrition. Ces mesures seront réalisées à T0, T1, T2 et T3 pour toutes les adolescents. Seul le groupe intervention bénéficiera du programme d'activités physiques (4 fois par semaine) intégré dans la maquette d'enseignement de la Maison Médicale pour enfants et adolescents Tza Nou (La Bourboule, 63). L'inclusion d'un groupe contrôle est primordiale afin de nous permettre d'obtenir des informations relatives à l'effet propre de l'intervention (AP + nutrition) sur les paramètres tissu osseux, tissu adipeux et apport alimentaire.

Ces temps de mesure permettront de comparer les effets combinés de la pratique d'activités physiques et de la perte de poids sur les interactions tissu adipeux – tissu osseux. Ces trois visites seront séparées par un minimum de 15 semaines et l'intervention durera au total 10 mois. La figure ci-dessous illustre l'organisation de cette étude et chaque session et méthode utilisée sont décrites ci-après.

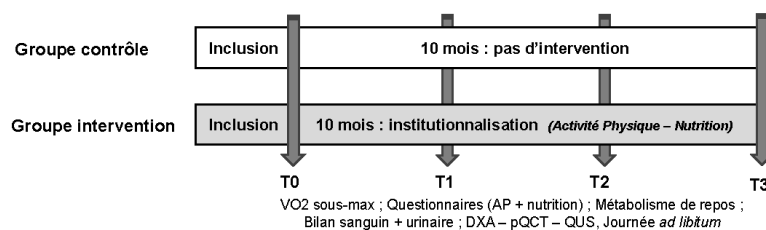


Illustration schématique du déroulement du protocole.

4.3. Description des mesures prises pour réduire et éviter les biais

- De manière à éviter les biais liés à la connaissance par les adolescents du critère d'évaluation secondaire : de la même manière pour éviter tous les biais liés à prise alimentaire *ad libitum*, il ne leur sera pas précisé que la quantité d'aliments ingéré sera pesée. Il leur sera indiqué que l'objectif est d'évaluer leur métabolisme énergétique. De la même manière pour éviter tous biais liés à une potentielle surconsommation alimentaire (majoritairement induite par les phénomènes de palatabilité), un questionnaire de préférences alimentaires sera rempli par chaque participante avant l'étude, et les aliments indiqués comme préférés ne seront pas proposés lors des buffets repas (comme détaillé méthodologiquement par notre équipe²⁷).
- Afin de limiter les biais liés aux mesures biologiques, les analyses seront réalisées en triplicate et prélevées avec 3 jours d'intervalles. Les biais liés aux impacts de la maturation sur les adaptations nutritionnelles sont pris en compte par le choix de ne recruter que des adolescents aux stades 3 et 5 de maturation (Tanner), et de n'inclure que des filles.
- Appariement entre groupes selon âge et IMC (30-35, 35-40, >40)
- Bien qu'improbable, l'éventuelle prise de contraception orale sera également contrôlé par appariement.
- L'échographie cardiaque sera réalisée par le même examinateur qualifié et identique pour chaque patient.

5. Réalisation pratique du protocole

5.1. Description des actes pratiqués sur les personnes (description de chacune des visites) / des produits utilisés au cours de la recherche

5.1.1. Visite d'inclusion

L'inclusion des adolescents obèses sera réalisée à la Maison Médicale pour les enfants et adolescents Tza Nou par le Docteur DUTHEIL ou le Dr FOUROT, les adolescents obèses du groupe contrôle seront recrutées lors des consultations externes ainsi que lors de consultation au SSR Nutrition-Obésité. Les inclusions pourront être effectuées soit au CHU Gabriel Montpied, Service de Médecine du Sport et Exploration Fonctionnelle et Respiratoire, soit au SSR Nutrition-Obésité ou encore à la maison médicale Tza Nou. Les adolescents seront sélectionnés après un interrogatoire médical, un examen clinique complet effectué par le médecin investigateur ou co-investigateur. Les adolescents sélectionnés seront inclus après information complète des objectifs, de la nature et des risques encourus, vérification de la conformité aux critères d'inclusion/exclusion et obtention d'un accord écrit de leur part ainsi que de deux représentants légaux. Lors de cette visite, les adolescents devront aussi remplir un questionnaire de préférences et d'habitudes alimentaires qui permettra de composer les repas proposés lors des temps de mesure.

5.1.2. Temps de mesure

Les temps de mesure seront réalisés en début d'étude, ainsi que toutes les 15 semaines pour une durée de 10 mois pour toutes les adolescents (T0, T1, T2, T3). Les adolescents devront se rendre disponibles sur trois demi-journées. Au cours de ces demi-journées, elles devront réaliser les mesures décrites dans la partie 5.3.

5.1.3. Mesures au cours de l'intervention (T0, T1, T2, T3)

Il sera demandé aux adolescents de ne pas s'engager dans des activités physiques et sportives lors des deux journées précédents chaque temps de mesure, ainsi que de garder une alimentation équilibrée (un relevé sera effectué avant la première session et il leur sera demandé de respecter ce dernier du mieux que possible avant les autres sessions). Un intervalle de 3 jours devra être respecté entre les visites.

	Demi-journée 1		Demi-journée 2		Demi-journée 3	
	Contr.	Inter.	Contr.	Inter.	Contr.	Inter.
Mesures cliniques (5.3.1)	SSR	Tza Nou				
Journée <i>ad libitum</i> (5.3.2)					SSR	Tza Nou
Questionnaires (5.3.2 ; 5.3.3)	SSR	Tza Nou				
Capacité aérobie (5.3.4)			CHU	Tza Nou		
Compo corporelle str. osseuse (5.3.5)			CHU	CHU		
Bilan biologique (5.3.6)	SSR	Tza Nou				
Echocardiographie (5.3.7)			CHU	CHU		

Contr. : Obèses - groupe contrôle / Inter. : Obèses - groupe interventions

5.1.4. Prise en charge au centre Tza Nou

Dans le cadre de la prise en charge par l'activité physique et la nutrition mise en place depuis des années au sein de la Maison Médicale pour enfants et adolescents Tza Nou (La Bourboule, 63), des éducateurs sportifs spécialisés (Professeurs et cadres APAS) dispensent aux adolescents 4 séances d'activité physique par semaine.

En plus du programme d'activité physique, les adolescents suivent un programme d'éducation nutritionnelle dispensé par des diététiciens diplômés. Les participantes suivront également un régime alimentaire normo-calorique basé sur les recommandations nutritionnelles adaptées.

5.2. Description de l'organisation logistique générale de l'essai

Les adolescents obèses (groupe intervention) seront recrutés parmi les adolescents de la maison d'enfants de prise en charge de l'obésité de Tza Nou, La Bourboule.

Les adolescents obèses contrôle seront recrutés par l'intermédiaire de consultation pédiatriques externes ainsi que lors de consultation au SSR Nutrition-Obésité.

Lieux de réalisation des mesures T0 à T3:

- Maison médicale pour enfants et adolescents Tza Nou, La Bourboule (adolescents obèses)
- Service de Médecine du Sport, CHU Clermont-Ferrand (adolescents obèses « intervention + contrôle »)
- SSR Nutrition-Obésité, 33-35 rue Maréchal Leclerc, 63000 Clermont-Ferrand (adolescents obèses « contrôle »)

5.3. Paramètres mesurés

5.3.1. Mesures cliniques

- poids, taille et calcul de l'IMC²⁸
- périmètre abdominal (Tour de Taille)²⁹
- pression artérielle et fréquence cardiaque : mesurée après 15 minutes de repos utilisant un tensiomètre électronique automatique de bras (SunTech Medical, Model 222)
- longueur des membres : mesurée pour le radius de l'olécrâne à la styloïde ulnaire et pour le tibia de la malléole médiale à la partie médiale du plateau tibial³⁰
- stade de Tanner

5.3.2. Prise alimentaire

Les adolescents devront compléter un journal alimentaire de 3 jours (2 jours de semaines et 1 jour de week-end). Pour les aider, le carnet de type SUVIMAX³¹ sera détaillé et expliqué lors de la première demi-journée.

Lors de la journée complète, la prise énergétique des adolescents sera mesurée *ad libitum* sous forme d'un buffet dont la composition sera basée sur le questionnaire de préférences alimentaires rempli lors de la visite d'inclusion (de manière à éviter les aliments préférés et non consommés). La prise énergétique des adolescents lors de ce buffet sera pesée et analysée par l'équipe investigatrice à l'aide

du logiciel Bilnut. Tout au long de la matinée et sur deux heures après les repas, des questionnaires de sensations de faim (Echelles Visuelles Analogues³²) seront complétés par les adolescents. Cette méthode d'évaluation de la prise énergétique a été utilisée à de nombreuses reprises par notre équipe auprès d'adolescents minces et obèses^{33,34}.

5.3.3. Activité Physique

Les adolescents devront compléter un questionnaire de manière à évaluer leur engagement dans de l'activité physique régulière³⁵. Leur activité physique sera aussi appréhendée à travers les mesures sur 24 heures d'actimétrie, de variabilité sinusale et d'activité électrodermale grâce à des outils non invasifs et indolores (Cardiscop[®], Movisens[®]).

5.3.4. Capacité aérobie

La capacité sous-maximale aérobie sera réalisée sur bicyclette ergométrique et consiste en un effort composé de 4 paliers de 6 minutes et de difficulté croissante. Au cours de ce test, les adolescents seront reliés à un système portable de calorimétrie indirecte pour la mesure de la consommation d'oxygène et de la production de CO₂ (figure ci-dessous. K4b²). Le K4b² répond aux normes MDD (93/42ECC) & FDA R10(k) de la Communauté Européenne. Ce dispositif d'usage courant est validé chez l'adulte comme chez l'enfant³⁶.



K4b2

5.3.5. Composition corporelle et structure osseuse

La composition corporelle et la structure osseuse des adolescents seront évaluées par les techniques suivantes :

- Dual energy X-ray Absorptiometry (DXA, QDR-4500A, Hologic, Inc., Waltham, MA).

Le balayage du corps par un faisceau de rayon X à 2 niveaux d'énergie différents permet la mesure de la composition corporelle (masse minérale, la masse tissulaire active et la masse grasse) et la géométrie osseuse³⁷ chez l'adulte comme chez l'enfant. Les mesures seront effectuées au corps entier, au rachis lombaire et à la hanche, nécessitant environ 15 minutes d'examen. Cette méthode est également peu irradiante (moins qu'une radiographie thoracique classique).



DXA

- Peripheral Quantitative Computed Tomography (XCT 2000; Stratec Medizintechnik, Pforzheim, Germany).

Le balayage en rotation des membres par un faisceau de rayon X permet une reconstruction en trois dimensions mesurant la géométrie et distinguant l'os trabéculaire et l'os cortical (volume, aire, densité, résistance osseuse). Pour chaque adolescent, le tibia et radius non dominant seront mesurés distalement, proximatement et au niveau de l'os sous-chondral^{30,38} nécessitant le maintien d'une position stable durant 15 minutes. L'irradiation est de moins de 0,01mSv²⁶.



pQCT

- Quantitative Ultra-Sound (QUS ; Insight ; Achilles Lunar ; Belgium). L'atténuation du faisceau selon la vitesse et la fréquence de propagation des ultrasons permet d'évaluer la densité du tissu osseux au niveau du calcaneum^{39,40}. Le recueil de cette mesure non irradiante nécessite environ cinq minutes.



QUS

Les risques associés à l'exposition aux radiations de la DXA et pQCT sont considérés comme très faible : soit pour une DXA (corps entier, lombaire et hanche) chez l'enfant de 10 à 15 ans 0.0056mSv et pour la pQCT (tibia, radius) 0.0016mSv.

Le taux de radiation pour toute la durée de l'étude est de 0.03mSv (0.28µSv).

Sachant que dans la vie de tous les jours, les rayonnements « naturels » sont chaque année d'environ 2 à 3 mSv, le taux de radiation total de l'étude par enfant (0.03mSv) peut être considéré comme « négligeable ».

5.3.6. Prélèvements / analyses biologiques et urinaire de l'étude

La veille de chaque visite les enfants devront consommer un repas standardisé. Le repas sera constitué de 2 tranches de jambon cuit découpé, dégraissé, d'environ 100g (poids cru) de pâtes type spaghetti, d'un yaourt nature avec environ 20 g de sucre blanc en poudre. Le repas devra être pris entre 19h et 21h et sera accompagné uniquement d'eau du robinet.

Les enfants devront vider complètement la vessie à l'issue du repas. Ils devront également s'abstenir de consommer, dans la mesure du possible, tous produits médicamenteux ayant des effets reconnus sur le profil métabolomique, tel que l'aspirine ou l'ibuprofène.

Les prélèvements sanguins se réaliseront à jeun.

· Etiquetage des tubes

Afin de permettre l'anonymat, chaque tube sera étiqueté avec les trois premières lettres du nom de famille puis les deux premières du prénom ainsi que le temps du prélèvement (Exemple pour DUPONT Pierre : DUPPI_t0).

- Pour l'analyse biologique (marqueurs adipocytaires/ostéocytaires) : après centrifugation (10 minutes à 4°, 4000 rpm), les prélèvements seront conditionnés et stockés par type de prélèvements à -80°C, au laboratoire AME2P.
- Pour l'analyse métabolomique : le plasma sera aliquoté et stocké à -80°C, à l'UNH. De même pour l'urine.
- Pour l'analyse mécanistique *ex vivo* : après aliquotage le sérum sanguin sera stocké à -80° à l'UNH.

a) Analyses biologiques (marqueurs adipocytaires/ostéocytaires)

Variables	Références	Tubes
Marqueurs osseux :		
Ostéocalcine décarboxylée	41-44	EDTA
Ostéocalcine totale	41-44	EDTA
Sclérostine	45-47	EDTA
RANK-L	8,42	EDTA
Vitamine D	48,49	SEC
Marqueurs d'inflammation		
TNF- α	50	SEC
IL-6	43	SEC
Marqueurs de croissance		
IGF1	5,12,42	SEC
IGFBP3	5	SEC
Parathyroïdienne /calcitonine	43,51,52	SEC
Œstradiol	5,12	SEC
FSH/LH		SEC
Régulation de l'appétit :		
Ghréline AC	22	EDTA
Leptine	8,53-55	SEC
Adiponectine	8,41,43,55,56	SEC
PYY ₃₋₃₆	22	SEC
GLP ₁	57	SEC
Biologie de routine:		
TG	41,53,55	SEC
Cholestérol	41,55	SEC
LDL	53	SEC
HDLC	41,55	SEC
Glycémie	5,41,55	SEC
Insuline	25,53,58,64	SEC
CRP ultra sensible	58,59	SEC

Compte tenu de la quantité d'analyse, ces dernières seront réalisées par le biais de la méthode MULTIPLEX. Pour ce faire, deux tubes de 5ml seront prélevés : un tube sec de 5ml et un tube EDTA de 5ml.

b) Analyse métabolomique, suivi des trajectoires métaboliques

Une étude métabolomique sera conduite chez les enfants. Cette technique non invasive permet l'analyse quantitative, exhaustive et non biaisée du contenu en métabolites (plusieurs centaines voire milliers de molécules) d'un système biologique dans un biofluide (plasma et urine), le plus souvent par chromatographie liquide couplée à un spectromètre de masse (LC-MS), et donc de prendre en compte la complexité du vivant.

Le métabolome représente effectivement l'ultime réponse d'un organisme à un patrimoine génétique, une pathologie, une exposition nutritionnelle ou toute cause environnementale. Dans la mesure où il inclut non seulement des biomarqueurs d'exposition, mais également des molécules issues du fonctionnement de l'organisme, une analyse multivariée permet de comprendre les voies mécanistiques mise en jeu et éventuellement de suivre les trajectoires métaboliques.

L'analyse des plasmas et des urines du matin prélevés à chaque point d'étude et après consommation la veille d'un repas standardisé nous permettra de suivre l'apparition de métabolites liés à la prise en charge des enfants et de mieux comprendre l'impact de cette intervention.

Pour ce faire, deux tubes héparinate de lithium de 5ml seront prélevés pour le sang. Pour l'urine, recueil classique (deuxième miction du matin) sera réalisé.

c) Etude mécanistique ex vivo

Une étude mécanistique sera également conduite pour compléter l'approche métabolomique, afin d'investiguer l'impact de l'intervention sur le fonctionnement des cellules osseuses. Dans ce but, 10 ml de sang seront prélevés à chaque stade sur tubes secs. Le sérum ainsi récolté sera ensuite incubé in vitro soit en présence d'ostéoblastes (cellules ostéoformatrices), soit d'ostéoclastes (responsables de la résorption osseuse). Une étude de leur prolifération, de leur activité et des différentes voies de signalisation sera pratiquée, afin de mieux comprendre l'influence de la cure d'amaigrissement sur le métabolisme et le capital osseux.

Pour ce faire, trois tubes secs de 5 ml seront prélevés.

5.3.7. *Echocardiographie trans-thoracique*

Une échocardiographie permettra d'évaluer le tissu adipeux épique et ses possibles relations avec le tissu osseux et adipeux général, sous cutané et viscéral, ainsi qu'avec les paramètres de remodelages cardiaques précoces et classiques (volumes, épaisseurs des ventricules droit et gauche, fraction d'éjection ventriculaire gauche, Strain 2D et 3D).

5.4. Description de l'organisation logistique générale de l'essai

Cette étude est sous la responsabilité du Dr DUTHEIL (investigateur principal), en collaboration avec le Pr Daniel COURTEIX (laboratoire AME2P), qui assureront la coordination et le bon déroulement des étapes de cette étude, du recrutement jusqu'au traitement des données. Les mesures et sessions expérimentales seront également conduites avec la participation des Dr David THIVEL (AME2P), Romain ESCHALIER, Guillaume CLERFOND et Charles VORILHON (Cardiologie CHU), aidés par Mlle Elodie CHAPLAIS, doctorante sur le projet (Laboratoire AME2P), Mme Monique ETIENNE, technicienne de recherche (Laboratoire AME2P)

5.5. Durée prévue de participation des personnes et description de la chronologie de l'essai

Durée de l'étude estimée : 10 mois pour les adolescents

Date de début de l'étude (premier adolescent inclus) : 1^{er} août 2015 (en fonction de l'accord éthique)

Date de fin (fin de suivi du dernier adolescent de l'étude) : Juillet 2016

Durée totale de participation à l'étude pour les adolescents : 10 mois pour les adolescents

La date de fin d'étude sera transmise à l'autorité compétente et au CPP dans un délai de 90 jours.

En cas d'arrêt prématuré de l'étude, l'information sera transmise dans un délai de 15 jours à l'autorité compétente et au CPP.

6. Population étudiée

6.1. Critères d'inclusion

- Critères d'inclusion communs aux 2 groupes :
 - Age : 12 à 16 ans
 - Sexe indifférent, stade de Tanner 3-5
 - IMC > 97th percentile
 - Signature de la fiche d'information et de consentement par l'adolescent et le titulaire de l'exercice de l'autorité parentale
 - Etre assujettie à un régime de Sécurité Sociale
- Critère d'inclusion des adolescents obèses « groupe intervention » :
 - Intégrer la cure de 10 mois du centre de traitement de l'obésité de Tza Nou
- Critères d'inclusion du « groupe contrôle » :
 - < 150min de pratique d'activité physique par semaine en dehors de l'école
 - Etre considéré comme sain sur l'examen clinique et l'interrogatoire médical

6.2. Critères de non inclusion

- Antécédents médicaux ou chirurgicaux jugés par l'investigateur comme étant non compatibles avec l'étude
- Prise de médicaments pouvant interférer avec les résultats de l'étude
- Maladies métaboliques (pré-diabète, diabète, insulino-résistance, hyper/hypo-thyroïdie)
- Carences alimentaires (vitamine D, calcium, protéine)
- Adolescents à problèmes cardio-vasculaires
- Intervention chirurgicale dans les 3 mois précédents
- Personne sous tutelle ou non assujettie à un régime de sécurité sociale
- Refus de signature de la fiche d'information et de consentement
- Refus d'être inscrit sur le Fichier National des Volontaires
- Personne en période d'exclusion d'une autre étude
- Consommation régulière de tabac ou alcool.
- Participation à des activités sportives régulières et intenses.

6.3. Procédure d'arrêt prématuré de la recherche

L'essai peut être arrêté prématurément pour les raisons suivantes :

- ★ Déviation majeure au protocole
- ★ Maladie intercurrente interférant avec le déroulement normal du protocole
- ★ Décès
- ★ Décision du volontaire
- ★ Perdu de vue (un adolescent perdu de vue est un adolescent qui ne s'est pas présenté à la visite prévue au protocole et pour lequel on reste sans nouvelle au moment de statuer. Tout doit être mis en œuvre pour obtenir de ses nouvelles et connaître la raison d'arrêt de l'essai. La raison "perdu de vue" ne sera invoquée que si l'enquête effectuée reste infructueuse).

6.4. Période d'exclusion et participation à une autre recherche

Au regard de la nature de cette étude, aucune période d'exclusion n'est prévue.

6.5. Indemnisation des volontaires

Cette étude ne concernant que des personnes mineures, aucune indemnisation n'est prévue..

Le laboratoire s'engage à payer les frais de déplacement engagés par les parents / enfants pour participer au protocole.

6.6. Modalités de recrutement

Les adolescents seront recrutés de manière à garantir au moins 25 adolescents par groupe. Les volontaires seront répartis en 2 groupes d'adolescents âgés de 12 à 16 ans (obèse intervention et obèse contrôle). Les adolescents devront présenter un IMC supérieur au 97th percentile des courbes nationales²⁴ et seront recrutées, pour le groupe intervention en collaboration avec la Maison Médicale pour enfants et adolescents Tza Nou (63 – Puy-de-Dôme). Les adolescents du groupe contrôle seront recrutées par le biais de consultations pédiatriques externes ainsi que lors de consultation au SSR Nutrition-Obésité. Conformément aux exigences formulées par le Comité de Protection de la Personne, un formulaire d'informations sera distribué et un formulaire de consentement complété par les adolescents volontaires, ainsi que par deux titulaires de l'exercice de l'autorité parentale.

7. Traitement et produits administrés aux personnes qui se prêtent à la recherche

7.1. Description du traitement

Aucun traitement médical n'est prévu, seulement une prise en charge institutionnelle (intégrée au fonctionnement habituel du centre Tza Nou) par activité physique et nutrition.

7.2. Posologie, modalités d'administration et durée du traitement (le cas échéant)

Aucun traitement ne sera administré

7.3. Présentation des produits

Aucun traitement ne sera administré

7.4. Médicaments et traitements autorisés et interdits pendant l'essai

Les adolescents ne devront pas être sous traitement médicamenteux impactant le tissu osseux.

8. Données recueillies

· Cahier d'observation

Les résultats des évaluations des paramètres anthropométriques, de la composition corporelle et structure osseuse, de l'épreuve sous-maximale, ainsi que les bilans sanguins seront consignés dans le cahier d'information. Les résultats du journal alimentaire de 3 jours seront également inscrits dans le cahier.

9. Considérations statistiques

9.1. Nombre d'adolescents à inclure

L'objectif principal de ce projet étant d'étudier les effets d'une prise en charge de 10 mois combinant activité physique et nutrition en comparaison avec un groupe contrôle sur l'interaction 'tissu adipeux – tissu osseux' chez des adolescents obèses, la justification du nombre de sujets nécessaire repose sur un test de comparaison entre groupes à l'étude de l'index variation de la masse adipeuse rapportée à la variation d'un marqueur de la masse osseuse mesuré au rachis lombaire.

A ce jour, peu d'éléments concernant ces paramètres sont référencés dans la littérature. Néanmoins, au vu des travaux publiés par Campos et al. ⁶⁰, une différence d'index de l'ordre de 1.2 à 1.5 peut être attendue entre groupes et il semble raisonnable de fixer une variabilité de l'index de l'ordre de 1.3 (écart-type). Considérant ces éléments, n=21 sujets par groupe permettront de mettre en évidence une différence minimale de 1.35 concernant l'index I, pour un risque d'erreur de 1^{ère} espèce de 5% (bilatéral) et une puissance de 90%. Afin de pallier aux pertes de vue inhérent à ce type de protocole, il est *in fine* convenu d'inclure 25 adolescents par groupe.

9.2. Analyse de données : généralités

L'ensemble du traitement statistique sera réalisé en intention de traitée (en première intention) à l'aide des logiciels Stata (StataCorp, College Station, USA) et SPSS version 19.0 pour Windows (SPSS, Chicago, IL, USA). Tous les tests statistiques seront effectués au risque d'erreur de première espèce $\alpha=5\%$.

Les variables continues seront présentées sous forme de moyenne et écart-type, sous réserve de la normalité de leur répartition (test de Shapiro-Wilk au besoin). En cas de non normalité, elles seront présentées sous forme de médiane, quartiles et valeurs extrêmes. Les variables qualitatives seront exprimées en effectifs et pourcentages associés. Des représentations graphiques seront, autant que possible, associées à ces analyses.

Les comparaisons entre groupes se feront (1) sans ajustement (2) en ajustant sur des facteurs dont la répartition pourrait être déséquilibrée entre groupes malgré l'appariement.

9.3. Description des échantillons à l'inclusion

Les sujets seront décrits et comparés entre groupes à l'inclusion selon leurs caractéristiques cliniques et épidémiologiques. Une description des déviations du protocole, des patients répartis selon ces déviations et des causes d'abandon sera également réalisée.

Le nombre d'adolescents inclus et la courbe des inclusions (par groupe de randomisation), le nombre de visites théoriques correspondant au nombre d'adolescents inclus, le nombre de visites réellement effectuées et le rapport des deux seront présentés par groupe.

9.4. Analyse principale

La comparaison entre groupes concernant le critère d'évaluation principal, à savoir l'index I variation de la masse adipeuse rapportée à la variation d'un marqueur de la masse osseuse mesuré au rachis lombaire sera réalisée par test t de Student ou test non paramétrique de Mann-Whitney si les conditions du test de Student ne sont pas respectées (normalité vérifiée par le test de Shapiro-Wilk et égalité des variances par le test de Fisher-Snedecor).

9.5. Analyses secondaires

Les comparaisons entre les 2 groupes seront réalisées (1) de manière analogue à ce qui a été présenté précédemment pour les critères de nature quantitative (par exemple prise énergétique) et (2) par test du chi-deux ou test exact de Fisher pour les variables catégorielles.

Afin d'étudier l'effet d'une perte de poids induite par une prise en charge de 10 mois combinant activité physique et nutrition sur la variation de masse adipeuse et masse osseuse, des coefficients de corrélation seront calculés (Pearson ou Spearman au regard de la distribution statistique) et comparées par test de Fisher (commande *corcor* Stata). Ces analyses seront complétées par une ancova considérant les effets variation de poids et groupe à l'étude.

L'évolution des différents paramètres recueillis de manière longitudinale sera étudiée par modèles mixtes permettant d'étudier les effets fixes groupe, temps et interaction groupe x temps tout en prenant en compte la variabilité inter et intra sujet (effets aléatoires « *random intercept* » et « *slope* »). L'impact de covariables (par exemple observance, statut pondéral (IMC) et hormonal) pourra être exploré.

9.6. Méthode de prise en compte des données manquantes, inutilisées ou invalides

Concernant l'analyse des données longitudinales, le recours à une méthode d'imputation des données pourra être envisagé selon la quantité (niveau d'attrition) et la nature (indépendance vis-à-vis du groupe de randomisation) des données manquantes. Une analyse de sensibilité sera effectuée notamment pour assurer la pertinence des données longitudinales (MAR ou MCAR).

9.7. Responsable de l'analyse

Bruno Pereira (PhD Biostatistique)
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CHU de Clermont-Ferrand - Villa annexe IFSI
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10. Evaluation de la sécurité – Gestion des événements indésirables

L'investigateur a la responsabilité de rapporter tous les événements indésirables dans le cahier d'observation.

10.1. Définitions

Événement indésirable : on définit comme événement indésirable toute manifestation nocive survenant chez une personne qui se prête à une recherche biomédicale, que cette manifestation soit liée ou non à la recherche ou au produit sur lequel porte cette recherche

Effet indésirable : l'effet indésirable d'une recherche correspond à tout événement indésirable dû à la recherche.

On classe les effets indésirables graves en sous-classes que voici :

- *Effet indésirable grave attendu* : lorsqu'il est déjà mentionné dans la version la plus récente de la brochure pour l'investigateur, ou dans le Résumé des Caractéristiques du Produit pour les médicaments ayant une Autorisation de Mise sur le Marché, ou dans la notice d'instruction lorsque la recherche porte sur un dispositif médical qui fait l'objet d'un marquage CE.

- *Effet indésirable grave inattendu* : si sa nature, sa sévérité ou son évolution ne concorde pas avec les informations relatives aux produits, actes pratiqués et méthodes utilisées au cours de la recherche.

Événement ou effet indésirable grave : tout événement ou effet indésirable qui entraîne la mort, met en danger la vie de la personne qui se prête à la recherche, nécessite une hospitalisation ou la prolongation d'une hospitalisation, provoque une incapacité ou un handicap importants ou durables, ou bien se traduit par une anomalie ou une malformation congénitale.

Les décès, quelle que soit leur cause, y compris lorsqu'ils correspondent à une progression de la maladie traitée, sont considérés comme des événements graves.

D'autres événements ne répondant pas aux qualifications ci-dessus énumérées, peuvent être considérées comme « *potentiellement graves* », notamment certaines anomalies biologiques. Le jugement médical de l'investigateur ou du promoteur pourra conduire à la déclaration de tels événements de la même façon que les événements « graves ».

Fait nouveau : événement concernant le déroulement de la recherche ou le développement du médicament ou du produit assimilé faisant l'objet de la recherche, lorsque ce fait nouveau est susceptible de porter atteinte à la sécurité des personnes qui se prêtent à la recherche.

Tout fait nouveau intéressant la recherche (ou le produit utilisé) et susceptible de porter atteinte à la sécurité des personnes qui se prêtent à la recherche fera l'objet de mesures de sécurité urgentes appropriées et d'une information sans délai par le Promoteur auprès de l'autorité compétente et du Comité de Protection des Personnes.

10.2. Description des paramètres d'évaluation de la sécurité

La nature du présent protocole ne laisse pas envisager la survenue d'un effet indésirable en lien avec la recherche en question. Tout événement indésirable pouvant survenir au cours de la durée d'inclusion sera renseigné par un suivi téléphonique (avec les parents) dans les jours précédents chaque session expérimentale ainsi que la veille.

10.3. Déclaration des événements indésirables graves

L'investigateur a obligation de déclarer dans les 24h au promoteur tout événement indésirable grave survenu chez toute adolescent inclus dans l'étude,

- lors de la phase active de l'étude,
- dans les semaines suivant l'arrêt du traitement (délai précisé par le promoteur en fonction du profil du produit à l'étude),
- dans les délais établis de suivi de tolérance sans traitement, avant (phase de wash out ou sevrage) ou après la phase active,
- Après l'arrêt de l'essai, quel que soit le délai, dès lors qu'aucune autre cause que la recherche ne peut raisonnablement être incriminée,

sur un formulaire " Evénement indésirable grave", figurant dans les cahiers d'observation.

L'investigateur doit se prononcer sur la relation de causalité entre l'événement indésirable grave avec la recherche.

Un rapport narratif devra être complété et transmis au promoteur dès l'obtention de nouvelles informations pertinentes. Suivant la nature et la gravité de l'événement, des copies du dossier médical anonymisé de l'adolescent peuvent être jointes, ainsi que les résultats des analyses de laboratoire.

A réception du formulaire " Evénement indésirable grave", le promoteur analyse cet événement et se prononce sur son imputabilité par rapport à l'étude et sur son caractère inattendu au moyen d'une analyse conjointe avec le Centre Régional de Pharmacovigilance.

Conformément au décret d'application n° 2006-477 du 26/04/2006 modifiant le chapitre I^{er} du titre II du livre 1^{er} de la première partie du code de la santé publique relatif aux recherches biomédicales, toutes les suspicions d'effets indésirables graves inattendus, feront l'objet d'une déclaration du Promoteur à l'autorité compétente, au CPP, dès qu'il en a connaissance et au plus tard 7 jours après la survenue de l'événement.

La survenue et la déclaration des événements indésirables graves seront systématiquement vérifiées lors des visites de monitoring.

Le promoteur tiendra des registres détaillés de tous les événements indésirables qui lui sont notifiés par le ou les investigateurs.

Une fois par an ou sur demande, le promoteur transmettra à l'autorité compétente et au CPP un rapport annuel de sécurité tenant compte de toutes des informations de sécurité disponibles.

Le promoteur transmettra également aux investigateurs de l'étude toute information susceptible d'affecter la sécurité des personnes.

10.4. Effets indésirables

Aucun effet indésirable grave n'est attendu.

10.5. Suivi des adolescents ayant présenté un événement indésirable

Dans le cas de l'apparition d'événements indésirables, une prise en charge et un suivi médical seront mis en place automatiquement.

11. Droit d'accès aux données et documents source

11.1. Accès aux données

Le promoteur est chargé d'obtenir l'accord de l'ensemble des parties impliquées dans la recherche afin de garantir l'accès direct à tous les lieux de déroulement de la recherche, aux données source, aux documents source et aux rapports dans un but de contrôle de qualité et d'audit par le promoteur. Les investigateurs mettront à disposition les documents et données individuelles strictement nécessaires au suivi, au contrôle de qualité et à l'audit de la recherche biomédicale, à la disposition des personnes ayant un accès à ces documents conformément aux dispositions législatives et réglementaires en vigueur (articles L.1121-3 et R.5121-13 du code de la santé publique).

11.2. Données source

Les documents source étant définis comme tout document ou objet original permettant de prouver l'existence ou l'exactitude d'une donnée ou d'un fait enregistrés au cours de l'étude clinique seront conservés pendant 15 ans par l'investigateur ou par l'hôpital s'il s'agit d'un dossier médical hospitalier. Il sera plus particulièrement question des observations établies lors des visites d'inclusions, des résultats des explorations anthropométriques et de la composition corporelle ainsi que des résultats de l'épreuve aérobie sous-maximale.

11.3. Confidentialité des données

Conformément aux dispositions concernant la confidentialité des données auxquelles ont accès les personnes chargées du contrôle de qualité d'une recherche biomédicale (article L.1121-3 du code de la santé publique), conformément aux dispositions relatives à la confidentialité des informations concernant notamment la nature des médicaments expérimentaux, les essais, les personnes qui s'y prêtent et les résultats obtenus (article R. 5121-13 du code de la santé publique), les personnes ayant un accès direct prendront toutes les précautions nécessaires en vue d'assurer la confidentialité des informations relatives aux médicaments expérimentaux, aux essais, aux personnes qui s'y prêtent et notamment en ce qui concerne leur identité ainsi qu'aux résultats obtenus.

Ces personnes, au même titre que les investigateurs eux-mêmes, sont soumises au secret professionnel (selon les conditions définies par les articles 226-13 et 226-14 du code pénal).

Pendant la recherche biomédicale ou à son issue, les données recueillies sur les personnes qui s'y prêtent et transmises au promoteur par les investigateurs (ou tous autres intervenants spécialisés) seront rendues anonymes.

Elles ne doivent en aucun cas faire apparaître en clair les noms des personnes concernées ni leur adresse.

Seules les trois premières lettres du nom de l'adolescent et les deux premières lettres de son prénom seront enregistrées, accompagnées d'un numéro codé propre à l'étude indiquant l'ordre d'inclusion des adolescents.

Le promoteur s'assurera que chaque personne qui se prête à la recherche a donné son accord par écrit pour l'accès aux données individuelles la concernant et strictement nécessaires au contrôle de qualité de la recherche.

11.4. Inscription au fichier national des personnes se prêtant à une recherche biomédicale

Les adolescents volontaires sains participant à l'étude seront inscrits sur le Fichier National des volontaires qui se prêtent à des recherches biomédicales (fichier VRB = Volontaires pour la Recherche Biomédicale), conformément à la réglementation en vigueur.

12. Contrôle et assurance de la qualité

12.1. Engagement des investigateurs et du promoteur

L'investigateur s'engage à ce que cette étude soit réalisée en conformité avec les Bonnes Pratiques Cliniques et la loi de santé publique n°2004-806 du 9 août 2004 concernant les recherches biomédicales, le décret d'application n° 2006-477 du 26/04/2006 modifiant le chapitre I^{er} du titre II du livre 1^{er} de la première partie du code de la santé publique relatif aux recherches biomédicales ainsi que les arrêtés en vigueur.

L'investigateur s'engage également à travailler en accord avec la Déclaration d'Helsinki de l'Association Médicale Mondiale (Tokyo 2004, révisée).

12.2. Assurance de Qualité

Un Attaché de Recherche Clinique (ARC) mandaté par le promoteur s'assurera de la bonne réalisation de l'étude, du recueil des données générées par écrit, de leur documentation, enregistrement et rapport, en accord avec les Procédures Opératoires Standards mises en application au sein du CHU de Clermont-Ferrand et conformément aux Bonnes Pratiques Cliniques ainsi qu'aux dispositions législatives et réglementaires en vigueur.

12.3. Contrôle de Qualité

L'investigateur se porte garant de l'authenticité des données recueillies dans le cadre de l'étude et accepte les dispositions légales autorisant le promoteur de l'étude à mettre en place un contrôle de qualité.

L'investigateur coordinateur et les investigateurs associés acceptent donc de se rendre disponibles lors des visites de Contrôle de Qualité effectuées à intervalles réguliers par l'Attaché de Recherche Clinique. Lors de ces visites, les éléments suivant seront revus :

- consentement éclairé
- respect du protocole de l'étude et des procédures qui y sont définies
- qualité des données recueillies dans le cahier d'observation : exactitude, données manquantes, cohérence des données avec les documents "source" (dossiers médicaux, carnets de rendez-vous, originaux des résultats de laboratoire, etc....)
- gestion des produits éventuels.
-

12.4. Cahier d'observation

Toutes les informations requises par le protocole doivent être consignées sur les cahiers d'observation et une explication doit être apportée pour chaque donnée manquante. Les données devront être

recueillies au fur et à mesure qu'elles sont obtenues, et transcrites dans ces cahiers de façon nette et lisible.

Les données erronées relevées sur les cahiers d'observation seront clairement barrées et les nouvelles données seront copiées, à côté de l'information barrée, accompagnées des initiales, de la date et éventuellement d'une justification par l'investigateur ou la personne autorisée qui aura fait la correction.

13. Considérations éthiques

13.1. Comité de Protection des Personnes et Autorité compétente

Le protocole, le formulaire d'information et de consentement ainsi que le cahier d'observation de l'étude seront soumis pour avis au Comité de Protection des Personnes *Sud Est 5*

La notification de l'avis favorable du CPP sera transmise au promoteur de l'étude et à l'autorité compétente. Une demande d'autorisation sera adressée par le Promoteur à l'autorité compétente avant le début de l'étude.

13.2. Information aux adolescents et formulaire de consentement éclairé écrit

Les adolescents seront informés de façon complète et loyale, en des termes compréhensibles, des objectifs et des contraintes de l'étude, des risques éventuels encourus, des mesures de surveillance et de sécurité nécessaires, de leurs droits de refuser de participer à l'étude ou de la possibilité de se rétracter à tout moment. L'investigateur doit également informer les adolescents de l'avis rendu par le CPP.

Toutes ces informations figurent sur un formulaire d'information et de consentement remis aux adolescents. Le consentement libre, éclairé et écrit de l'adolescent sera recueilli par l'investigateur. Ces documents (*Cf. Annexes 1 et 2*) sont approuvés par le CPP compétent et sont à utiliser pour l'essai concerné, à l'exclusion de tout autre document.

Deux exemplaires originaux seront co-signés par le médecin investigateur et l'adolescent. Un exemplaire sera remis à l'adolescent, le second exemplaire conservé dans le dossier médical de l'adolescent.

13.3. Amendements au protocole

Les modifications apportées au protocole devront être qualifiées de substantielles ou non.

Elles feront, selon leur nature, l'objet d'un nouvel avis du Comité de Protection des Personnes et/ou d'une autorisation de l'autorité compétente.

13.4. Prise en charge relative à la recherche

Aucune prise en charge au regard de la nature de l'étude.

14. Traitement des données et conservation des documents et données relatives à la recherche

14.1. Saisie et traitement des données

La saisie des données sera effectuée par CHAPLAIS Elodie (AME2P) étudiante en doctorat ainsi que par le Pr COURTEIX et Mme ETIENNE. Mlle CHAPLAIS assurera aussi l'analyse statistique en collaboration avec Mr Bruno Pereira de la DRCI.

14.2. CNIL

Cette étude entre dans le cadre de la « Méthodologie de Référence » (MR-001) en application des dispositions de la loi du 6 août 2004 relative à la protection des personnes physiques à l'égard des traitements de données à caractère personnel et modifiant la loi du 6 janvier 1978 relative à l'informatique, aux fichiers et aux libertés. Ce changement a été homologué par décision du 5 janvier 2006. Le CHU de Clermont-Ferrand, promoteur de l'étude, a signé un engagement de conformité à cette « Méthodologie de Référence » en date du 15/03/2007.

14.3. Archivage

Les documents suivants seront archivés par le nom de l'étude dans les locaux du laboratoire AME2P de Clermont-Ferrand jusqu'à la fin de la période d'utilité pratique. Durée de l'étude : 18 mois } 12 mois d'analyse des données.

Ces documents sont :

- Protocole et annexes, amendements éventuels,
- Formulaire d'information et consentements originaux signés
- Données individuelles (copies authentifiées de données brutes)
- Documents de suivi
- Analyses statistiques
- Rapport final de l'étude

A l'issue de la période d'utilité pratique, l'ensemble des documents à archiver, tels que définis dans la procédure PG.06.005 « Gestion de la documentation relative aux protocoles » du CHU de Clermont-Ferrand sera transféré aux archives centrales et sera placé sous la responsabilité du Promoteur pendant 15 ans après la fin de l'étude conformément aux pratiques institutionnelles.

Aucun déplacement ou destruction ne pourra être effectué sans l'accord du Promoteur. Au terme des 15 ans, le promoteur sera consulté pour destruction. Toutes les données, tous les documents et rapports pourront faire l'objet d'audit ou d'inspection.

15. Financement et assurance

15.1. Budget de l'étude

Dépenses	Montants (euros)	Financements
Matériel		
pQCT	70 000€	Cœur et Artères
Kit analyses biologiques	31 550€	Cœur et Artères + fonds propre
Actes		

DXA + QUS	15 000€	Prix Européen Tanita (acquise)
Infirmière	15 000€	Cœur et Artères
Divers		
Calibration calorimétrie	1 800€	AME2P (fonds propre)
Administratif / bureautique	2 500€	AME2P (fonds propre)
Congrès / publications	3 000€	Appel d'offre soumis
Doctorant	53 000€	Bourse Australienne (acquise)
Sous-total fonds propres AME2P	5 850€	
Sous-total Cœur et Artères	115 000€	
Sous-total Bourse Australienne	53 000€	
Sous-total Prix Européen Tanita	15 000€	
Sous-total appel d'offre soumis	6 000€	
TOTAL	194 850€	

Le prix européen Tanita 2014 (financier + matériel) obtenu par le Dr Thivel, partenaire associé à cette étude, sera utilisé en partie afin de réaliser les explorations de la composition corporelle des adolescents.

Si nous n'obtenons pas le financement de la Fondation Cœur et Artères, les partenaires franco-australiens de ce projet s'engagent à le financer totalement.

Le service de Cardiologie du CHU de Clermont-Ferrand s'engage à réaliser gratuitement ces échocardiographies.

15.2. Assurance

Conformément aux dispositions réglementaires, le CHU de Clermont-Ferrand en sa qualité de promoteur a souscrit une assurance responsabilité civile destinée à garantir les éventuels dommages résultant de la recherche auprès de la Société Hospitalière d'Assurances Mutuelles (SHAM). Le numéro de contrat est 147161.

Il est à noter que le non-respect des conditions légales de la recherche (absence d'avis du CPP, absence d'autorisation de l'autorité compétente, non consentement de la personne, poursuite d'une recherche suspendue ou interdite) est une clause d'exclusion de la garantie.

16. Communication - Règles de publication

Les données ne seront divulguées qu'après accord conjoint préalable de l'investigateur et du promoteur. Les résultats feront l'objet de communications et de publications.

Ce protocole sera enregistré sur ClinicalTrials.gov.

L'investigateur principal sera a minima 2^e ou avant dernier auteur sur au moins la publication principale de l'étude.

17. Faisabilité de l'étude

Ce travail s'inscrit dans la continuité de plusieurs études menées par les équipes concernées. Depuis 15 ans les équipes en question (AME2P, LNH) travaillent en collaboration avec le Service de Médecine du Sport du CHU de Clermont-Ferrand, sur la physiologie de l'enfant à l'exercice et plus

particulièrement de l'enfant obèse. Depuis 2008, plusieurs travaux centrés sur les adaptations nutritionnelles des adolescents minces et obèses ont été menés par ces équipes et ont apportés des résultats importants dans ce domaine, qui ont donné lieu à plusieurs publications internationales (plus de 20 en 4 ans).

Toutes ces études se sont déroulées sans avoir rencontré de difficultés de réalisation.

Le critère non-invasif et ludique des épreuves expérimentales est également un gage de sa faisabilité.

La maîtrise des techniques employées par les équipes en question est une caution de la réussite pratique du protocole.

La durée (13 mois) prévue pour la réalisation de ce travail semble également largement suffisante et permettra son déroulement dans de bonnes conditions.

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Liste des annexes

- Annexe 1 : Fiche d'information
- Annexe 2 : Formulaire de consentement
- Annexe 3 : Schéma général de l'étude
- Annexe 4 : Tableau de suivi de l'étude
- Annexe 5 : Carnet alimentaire
- Annexe 6 : Questionnaire de sensations de faim : échelles visuelles analogiques
- Annexe 7 : Questionnaire d'activité physique IPAQ
- Annexe 8 : *Curriculum Vitae*
- Annexe 9 : Fiche de déclaration d'événement indésirable grave

ANNEXE 1



FORMULAIRE D'INFORMATION PARTICIPANTE

Etude de l'effet de l'exercice physique sur la relation tissu osseux – tissu adipeux lors d'une prise en charge institutionnelle combinant activité physique et nutrition

Promoteur de l'étude : C.H.U. Gabriel Montpied – Clermont-Ferrand (63)

Investigateur principal : Dr Frédéric DUTHEIL – C.H.U. Gabriel Montpied
06 88 22 48 48 - fred_dutheil@yahoo.fr

Chère participante,

Tu es invitée à participer au projet de recherche décrit ci-dessous.

Quel est le projet de recherche ?

L'adolescence est une période importante dans le processus de développement entraînant de nombreux changements (changements physiologiques, psychologiques, culturels et émotionnels) pouvant influencer sur la prise de poids. A l'adolescence, la jeune fille a une sensibilité toute particulière, contrairement aux garçons, au développement de la masse grasse. De plus, cette période est le meilleur moment pour stimuler l'os. La pratique d'activité physique régulière est un facteur permettant de modifier la composition corporelle, c'est-à-dire à la fois la masse grasse (tissu adipeux) et le capital osseux.

La survenue de surpoids ou d'obésité à cette période clef de développement entraîne de nombreuses dysfonctions au niveau des hormones.

Nous sommes très intéressés par l'évolution et les interactions de ces deux paramètres (tissu adipeux et tissu osseux) et souhaitons analyser les éventuelles connexions entre la masse grasse et la qualité du squelette.

Pour cela, nous sommes à la recherche de volontaire âgée entre 12 et 16 ans.

Qui s'occupe de la réalisation de ce projet ?

Le projet sera conduit par Mlle Elodie CHAPLAIS sous la supervision du Professeur Daniel COURTEIX.

Qui peut participer à ce projet ?

Nous sommes à la recherche de participants âgés entre 12 et 16 ans. Cependant, il y a certaines conditions qui peuvent limiter ta participation à cette étude. Si tu souhaites intégrer ce projet, tu ne dois pas avoir de problème cardiovasculaire, d'ostéopénie, ou encore être enceinte. Tu ne dois également pas être une consommatrice régulière d'alcool ou de cigarette et ne pas prendre des médicaments pouvant altérer le métabolisme osseux (hormones – calcium – Vitamine D si elle est associée à une supplémentation calcique).

La contraception n'est pas un critère de non inclusion.

Si tu souhaites participer à cette étude, le docteur DUTHEIL ou le docteur FOUROT te poseront des questions afin de vérifier ton aptitude à participer à cette étude.

Quelles sont les risques associés à ta participation à cette étude ?

Les risques associés à ce projet sont minimes et détaillés ci-après.

- L'analyse de la composition corporelle :

Afin de connaître ta composition corporelle (muscle, os, masse grasse), nous utilisons deux outils de mesure qui sont actuellement des outils de référence. Ces outils de mesures sont complètement indolores. Cependant l'utilisation de ces outils nécessite l'exposition à de très faible dose de radiation.



Les risques associés à cette dose de radiation sont considérés comme « négligeables » sachant que dans la vie de tous les jours, tu es naturellement exposée à des rayonnements « naturels » et reçois une dose bien supérieure à celle provenant de l'appareil.



- L'analyse de la capacité aérobie :

Afin de connaître ta capacité aérobie, tu devras réaliser un test de mesure de consommation d'oxygène (il permet d'évaluer tes possibilités d'endurance). Les risques associés à ce test sont minimes. En effet, tu le réaliseras sous la supervision de professionnels de l'activité physique. Etant donné qu'il s'agit d'un test analysant ta capacité physique, il est possible que tu sois un peu fatiguée à la fin de l'exercice.

- L'analyse sanguine et urinaire :

Afin d'analyser certains marqueurs sanguin, nous te prélèverons un peu de sang. Les risques liés aux prises de sang (allergies, hématomes, malaises, piqûres dans le nerf, contamination, syncopes) seront réduits du fait de la réalisation de ces prélèvements par des professionnels de la santé aguerris.

Un prélèvement urinaire sera également réalisé le matin, cela nous permettra d'obtenir d'avantages d'informations sur les métabolites (vitamines, hormones, molécules énergétiques comme le glucose – lipides ...).

Que va-t-il m'être demandé ?

Il te sera demandé de participer à 4 temps de mesure que nous appelons T0, T1, T2 et T3. Pour chacune de ces périodes :

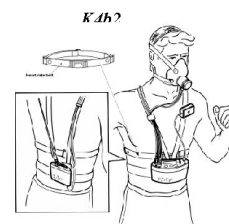
- Si tu n'as pas intégré la maison médicale Tza Nou, tu devras venir 3 fois, soit au CHU Gabriel Montpied, soit au laboratoire AME2P (campus des Cézeaux) ou encore au SSR Nutrition-Obésité.
- Si tu fais partie de la cure de la maison médicale Tza Nou, c'est nous qui nous déplacerons pour les temps de mesure à l'exception d'une demi-journée où tu devras te rendre au CHU Gabriel Montpied.

En dehors des exercices physiques qu'il te sera demandé de réaliser, tu ne devras pas faire d'activité intense avant de venir nous voir.

Tu devras réaliser les tests suivants :

★ Mesures anthropométriques - (poids, taille, tour de taille, pression artérielle, ...)

★ Mesures de métabolisme énergétique - (VO_2 sous maximal, métabolisme de repos) Le test de VO_2 sera composé de quatre paliers de six minutes chacun avec une difficulté croissante. Pendant que tu pédaleras, tu auras un masque sur le nez et la bouche qui nous permettra de connaître l'évolution de ta respiration pendant l'exercice. Ce masque ne te gênera pas et tu pourras respirer tout à fait normalement.



★ Mesure de ta nutrition et de ton activité physique - (par questionnaires et journée à volonté petit déjeuné – déjeuné - diner)

★ Mesure de ta composition corporelle - (DXA, pQCT, QUS) Ces tests sont complètement indolores.



★ Echocardiographie. Ce test est complètement indolore.

★ Mesure sanguine et urinaire – Le jour de la visite la prise de sang se fera à jeun - un petit déjeuné te sera bien évidemment offert une fois le prélèvement réalisé. Tu devras également recueillir ton urine (nous te donnerons le nécessaire) la deuxième fois que tu iras aux toilettes le matin. La veille de chaque prélèvement sanguin et urinaire, tu devras avoir un diner « standardisé ». C'est-à-dire que ton repas sera pris dans des conditions particulières (exemple : 2 tranches de jambon cuit découpé, dégraissé, environ 100g de pâtes, un yaourt nature, eau plate uniquement). A la fin du repas, tu devras également vider complètement ta vessie.

Quelles sont les bénéfices liés à ta participation à ce projet ?

En tant que participante tu bénéficieras d'un retour sur ta résistance et densité osseuse ainsi que d'information sur ta composition corporelle.

Puis-je me retirer librement de l'étude ?

Ta participation est complètement volontaire, tu es donc libre de participer ou non à cette étude et libre d'arrêter à tout moment sans qu'aucune explication ne te soit demandée.

Qui dois-je contacter si je souhaite obtenir davantage d'information ?

Si tu souhaites, avec tes parents, avoir plus d'informations ou de précisions tu peux me poser tes questions à n'importe quel moment en me contactant par email à l'adresse suivante : fred_dutheil@yahoo.fr

Merci et à bientôt

Dr Frédéric DUTHEIL – C.H.U. Gabriel Montpied
06 88 22 48 48 - fred_dutheil@yahoo.fr

Paraphe de l'adolescent

Version N.1 – 14-06-2015

42



FORMULAIRE D'INFORMATION PARENTS

Etude de l'effet de l'exercice physique sur la relation tissu osseux – tissu adipeux lors d'une prise en charge institutionnelle combinant activité physique et nutrition

Promoteur de l'étude : C.H.U. Gabriel Montpied – Clermont-Ferrand (63)

Investigateur principal : Dr Frédéric DUTHEIL – C.H.U. Gabriel Montpied
06 88 22 48 48 - fred_dutheil@yahoo.fr

Madame, Monsieur,

Nous vous proposons de faire participer votre enfant à un travail de recherche médicale.

Il est important que vous preniez connaissance de toutes les informations données dans ce formulaire avant d'accepter ou non sa participation à ce travail de recherche.

Nous restons à votre entière disposition pour répondre à toutes les questions que vous pourriez vous poser.

Si vous acceptez que votre enfant participe à cette étude, nous vous demandons de bien vouloir signer les deux exemplaires de l'avis d'acceptation ci-joint :

- un exemplaire vous étant destiné, à conserver avec le formulaire d'information,
- un exemplaire à remettre au médecin.

But de l'étude

Problème sanitaire mondial de par la gravité de ces maladies collatérales (hypertension artérielle, diabète de type 2, maladies cardiovasculaires...), la lutte contre l'obésité constitue l'un des plus grands défis du 21^e siècle. L'obésité infantile a dramatiquement augmenté au cours de ces quatre dernières décades et a été montré comme étant un facteur de risque d'obésité à l'âge adulte.

L'adolescence est une période clef dans le développement de l'enfant (changements physiologiques, psychologiques, culturels et émotionnels) pouvant influencer sa prise de poids et toutes influences au cours de cette période, qu'elles soient positives ou négatives, influenceront l'acquisition osseuse et modifieront le pic de masse osseuse.

L'objectif de ce projet est d'explorer et comparer entre l'adolescent obèse et l'adolescent normo-pondéré les relations entre le métabolisme adipeux et le métabolisme du tissu osseux, ainsi que les effets d'une perte de poids sur ces relations.

Déroulement de l'étude

Cette étude respecte les normes de Bonne Pratique Clinique définies par le Ministère de la Santé. Elle a été conçue dans l'esprit des assemblées d'Helsinki et de Tokyo. Elle a été soumise à l'approbation du Comité de Protection des Personnes Sud Est VI (comité réuni spécialement pour juger de la possibilité de réaliser ce travail sans danger pour les adolescents concernés) qui a donné son approbation en date du [REDACTED].

Visite d'inclusion

La visite d'inclusion se déroulera soit au Service de médecine du sport du CHU de Clermont-Ferrand, soit à la maison médicale Tza Nou, ou encore au SSR Nutrition-Obésité et sera constituée d'un examen médical. Si vous êtes disposé à la participation de votre enfant à l'étude, vous devrez signer cette fiche d'information ainsi que votre accord de participation.

Lorsque votre enfant sera définitivement inclus, il devra compléter un questionnaire de préférence alimentaire.

1^{ère} demi-journée : (C.H.U Gabriel Montpied ou Tza Nou ou SSR Nutrition-Obésité)

Si sa candidature est retenue, votre fille devra être à jeun afin que l'on puisse réaliser un prélèvement sanguin et recueil d'urine. Ce prélèvement aura lieu soit à la maison médicale Tza Nou si votre fille a intégré la cure, soit au SSR Nutrition-Obésité ou CHU Gabriel Montpied en fonction du lieu par lequel elle a été recrutée pour l'étude. Un petit déjeuner lui sera ensuite bien évidemment offert.

La veille de cette visite votre enfant devra avoir un diner « standardisé », c'est-à-dire que nous vous demanderons de bien vouloir respecter un menu et les quantités que nous vous donnerons. De plus, à la fin du repas votre enfant devra vider entièrement sa vessie.

Lors de cette matinée nous évaluerons également son métabolisme de repos et procéderons à des mesures dites cliniques (*poids, taille, tour de taille, pression artérielle, fréquence cardiaque*).

2^{ème} demi-journée : (C.H.U Gabriel Montpied)

Votre enfant devra réaliser un test de VO₂ sous-max. Cet exercice sera composé de quatre paliers de six minutes chacun avec une difficulté croissante. Elle sera équipée d'un système portable d'analyse de ses échanges respiratoires. Cette technique est indolore. Comme l'illustre la figure, un harnais lui sera passé autour des épaules. Sur ce harnais seront disposés une batterie et un boîtier d'analyse, les deux étant très légers. Enfin un masque lui sera apposé sur nez et la bouche. Ce dernier ne gênera en rien sa respiration.



Des évaluations de sa composition corporelle par absorptiométrie (DXA) et de sa densité minérale osseuse par tomographie (pQCT) et ultrason seront aussi effectuées. Ces techniques sont totalement indolores et non ou très peu irradiantes (0.03mSv pour la durée totale de l'étude alors que les radiations « naturelle » dans la vie de tous les jours sont de 2 à 3mSv pour une année).



DX



pQCT



Ultrason

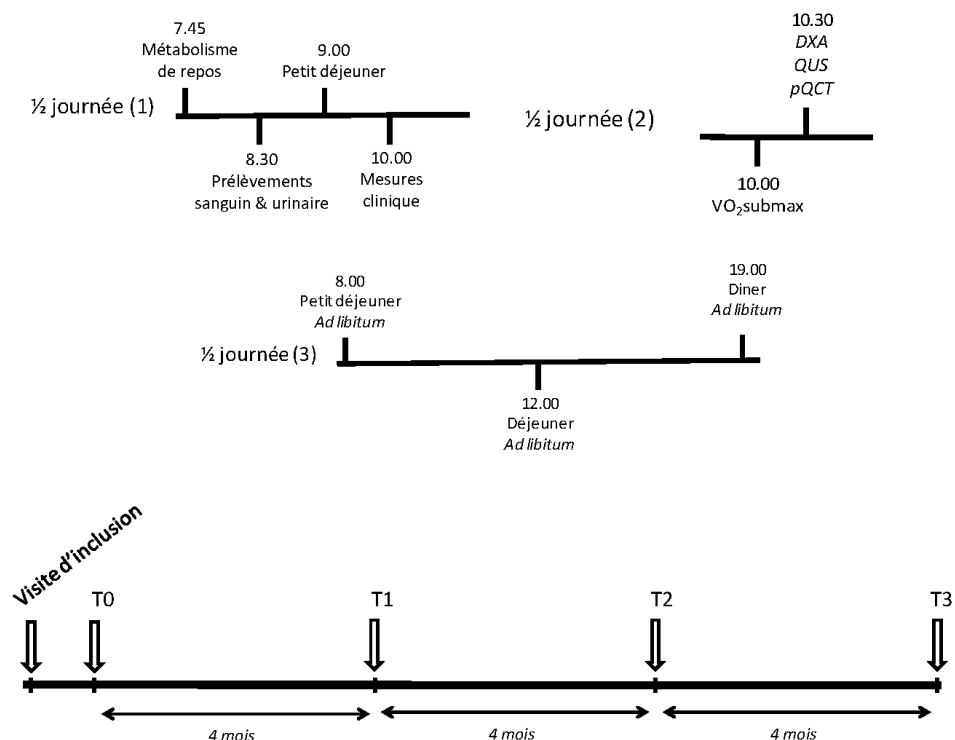
Une échocardiographie sera également réalisée afin d'évaluer le retentissement de l'obésité sur le cœur de votre enfant, ainsi que d'évaluer le tissu adipeux épicaudique (celui qui entoure le cœur). L'échocardiographie est complètement indolore.

3^{ème} demi-journée : (Tza Nou ou SSR Nutrition-Obésité)

Si votre enfant a intégré la maison médicale Tza Nou nous nous rendrons sur place afin de réaliser une évaluation de la prise alimentaire par repas *Ad libitum* (petit déjeuner – déjeuner – diner). Si votre enfant n'a pas intégré la maison médicale Tza Nou, il devra se rendre au SSR Nutrition-Obésité (33-

35 rue Maréchal Leclerc à Clermont-Ferrand) afin de réaliser la journée nécessaire à l'évaluation de la prise alimentaire.

Votre enfant devra réaliser à quatre reprises ces 3 demi-journées. Les schémas ci-dessous illustrent le déroulement prévisionnel des demi-journées ainsi que le déroulement général de l'étude.



Nous lui demanderons également de remplir divers questionnaires de nutrition et d'activité physique. En dehors des exercices physiques imposés par le protocole, votre enfant ne devra pas effectuer d'exercice intense dans les 12h avant les visites.

Effets indésirables / Rapport bénéfice-risque

Aucun risque ou effet indésirable n'est identifié quant à ce protocole.

La participation volontaire à cette étude de votre enfant peut faire progresser le corps médical dans la compréhension des relations entre les hormones sécrétées par le tissu adipeux et le métabolisme osseux ; cependant, vous ne devez en attendre aucun bénéfice pour son propre état de santé.

Modalités pratiques

La durée totale de l'étude sera de 15 mois. **La durée de la participation de votre fille sera de 10 mois.** Compte tenu de la nature de cette étude, aucune durée d'exécution n'est prévue.

Aspects éthiques et réglementaires

Les données recueillies seront traitées confidentiellement et identifiées par un numéro de code. Conformément aux dispositions de loi relative à l'informatique aux fichiers et aux libertés, vous disposez d'un droit d'accès et de rectification et d'un droit d'opposition à la transmission des données couvertes par le secret professionnel.

Vous acceptez :

- que les données puissent faire l'objet d'un traitement informatisé anonyme.
- que les données médicales concernant votre enfant et les données relatives à ses habitudes de vie soient transmises au Promoteur de la recherche (CHU Clermont-Ferrand), aux collaborateurs adolescents à la recherche et éventuellement à un représentant des autorités de santé.
- son inscription dans le fichier national des personnes qui se prêtent à des recherches biomédicales (article L.1121-16 du Code de la santé publique)

Vous pouvez également accéder directement ou par l'intermédiaire d'un médecin de votre choix à l'ensemble des données médicales de votre enfant (article L 1111-7 du Code de la Santé Publique) et les résultats globaux du projet vous seront communiqués à l'issue de la recherche.

Le promoteur a souscrit une assurance responsabilité civile contractée auprès de la compagnie SHAM (N° de police : 147161), afin de pouvoir vous dédommager si l'état de santé de votre enfant s'altérait suite à sa participation à cette étude et dans la mesure où il pourra être établi que ces dommages sont la conséquence directe de l'étude.

Cette étude a reçu un accord du Comité de Protection des Personnes Sud Est VI de la Région Auvergne (CPP), en date du _____ et elle est couverte par une assurance.

Vous ne signerez votre consentement écrit que si, après lecture de cette note et discussion avec le médecin, vous vous sentez, à priori, disposé à accepter la participation de votre enfant à l'étude. Si vous décidez qu'il participe, vous signerez le consentement écrit indiquant que vous avez lu et compris cette information et que sa participation a été librement décidée. Toutefois si vous changez d'avis pour quelques raisons que ce soit, vous restez libre de retirer votre consentement à tout moment, sans préjudice légal ou médical.

Merci de votre aide.

Fait à _____, le _____,

Enfant NOM _____, Prénom _____

Signature des deux titulaires de l'exercice de l'autorité parentale suivi de la mention manuscrite « lu et compris »

Père Mère Tuteur

Nom _____

Prénom _____

Signature

Père Mère Tuteur

Nom _____

Prénom _____

Signature

ANNEXE 2



**FORMULAIRE DE CONSENTEMENT DE PARTICIPATION
A UNE RECHERCHE BIOMEDICALE PARTICIPANTE**



**Etude de l'effet de l'exercice physique sur la relation tissu osseux – tissu adipeux
lors d'une prise en charge institutionnelle combinant activité physique et nutrition**

Promoteur de l'étude : C.H.U. Gabriel Montpied – Clermont-Ferrand (63)

Investigateur principal : Dr Frédéric DUTHEIL – C.H.U. Gabriel Montpied
06 88 22 48 48 - fred_dutheil@yahoo.fr

Je déclare :

- que le Dr _____ m'a expliqué en détail l'étude et qu'il m'a présentée :
 - o l'objectif, la méthode et la durée de l'étude
 - o les contraintes et les risques
 - o l'avis du Comité Consultatif de Protection des Personnes
 - o ma possibilité de refuser de participer et d'arrêter à tout moment sans aucun problème
 - o que je ne pourrai pas participer à d'autres études en même temps

- que j'ai répondu sincèrement aux questions concernant mon état de santé et ma participation à d'autres études.

Les informations de l'étude recueillies par les organisateurs sont traitées confidentiellement. J'accepte :

que toutes les données me concernant soient utilisées de façon anonyme (Loi informatique et liberté, Art. 40).

mon inscription dans le fichier national des personnes qui participent à des recherches biomédicales (Art. L 1121-16 du Code de la Santé Publique).

Après avoir discuté et obtenu des réponses à toutes mes questions, j'accepte, en toute connaissance de participer à cette étude.

Fait à _____, le ____/____/____

Signature de l'adolescent

Consentement recueilli par le Dr _____



**FORMULAIRE DE CONSENTEMENT DE PARTICIPATION
A UNE RECHERCHE BIOMEDICALE PARENTS**



Etude de l'effet de l'exercice physique sur la relation tissu osseux – tissu adipeux lors d'une prise en charge institutionnelle combinant activité physique et nutrition

Promoteur de l'étude : C.H.U. Gabriel Montpied – Clermont-Ferrand (63)

Investigateur principal : Dr Frédéric DUTHEIL – C.H.U. Gabriel Montpied
06 88 22 48 48 - fred_dutheil@yahoo.fr

Je soussigné(e) _____ autorise ma fille
_____ né le ____/____/____
domiciliée à _____ à participer à l'étude
susnommée.

Je déclare :

- que le Dr _____ m'a expliqué en détail le protocole et qu'il m'a notamment fait connaître :
 - o l'objectif, la méthode et la durée de l'étude
 - o les contraintes et les risques potentiels encourus
 - o l'avis du Comité de Protection des Personnes
 - o mon droit de refuser sa participation et en cas de désaccord de retirer notre consentement à tout moment sans encourir aucune responsabilité
 - o que ma fille ne sera pas autorisé(e) à participer à d'autres études cliniques au cours de ce protocole.
- que j'ai répondu en toute bonne foi aux questions concernant son état de santé et sa participation à d'autres études.

Les informations relatives à l'étude recueillies par l'investigateur sont traitées confidentiellement.

J'accepte :

que ces données puissent faire l'objet d'un traitement informatisé anonyme (Loi informatique et liberté, Art. 40), y compris, compte tenu des nécessités de la recherche, mes origines ethniques. J'ai bien noté que le droit d'accès prévu par la loi « Informatique et liberté (Article 40) » s'exerce à tout moment auprès du Dr _____ ou de son représentant. Je pourrai exercer un droit de rectification auprès de lui. Ma participation entre dans le cadre des articles L1121-1 et suivants du Code de Santé Publique.

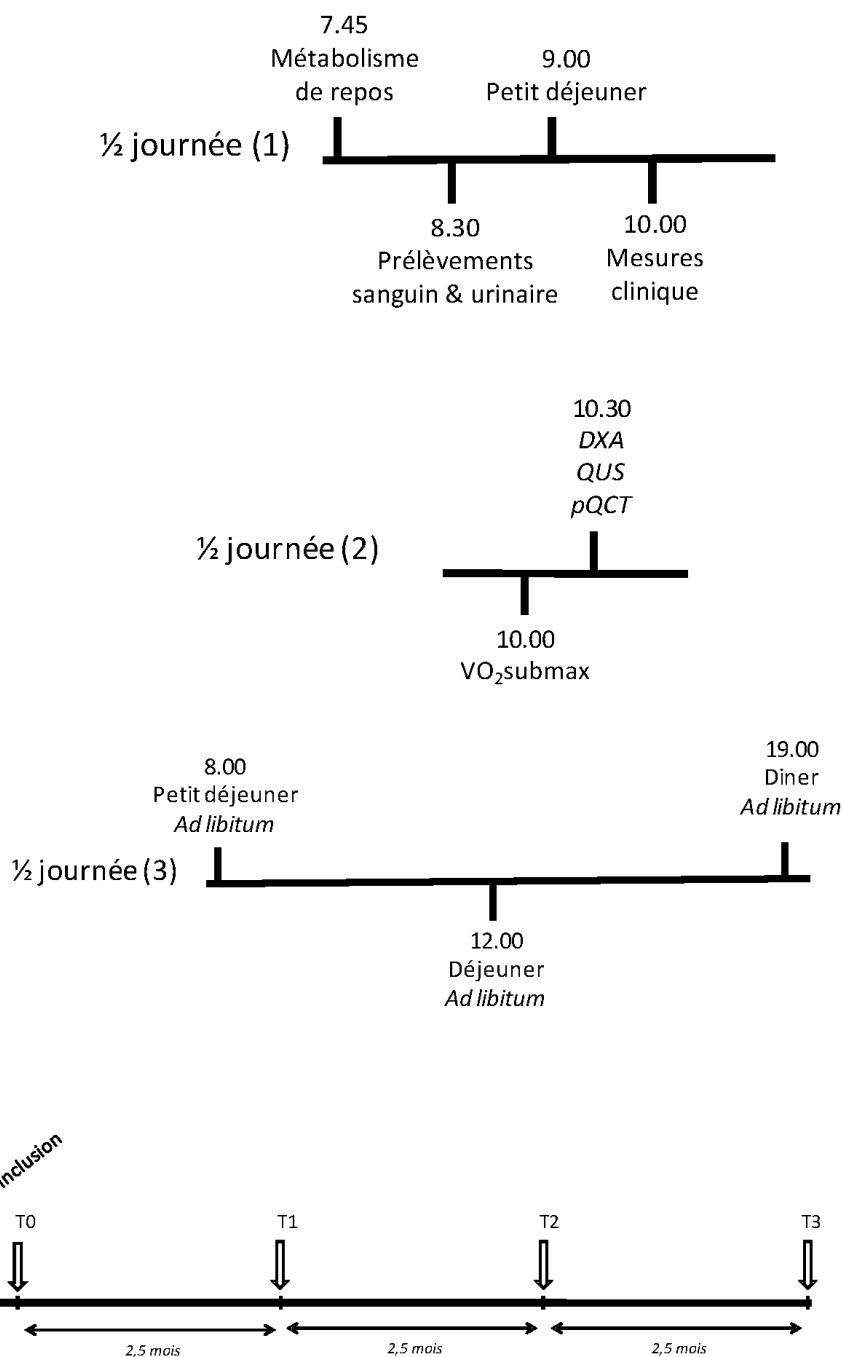
mon inscription dans le fichier national des personnes qui se prêtent à des recherches biomédicales (Art. L 1121-16 du Code de la Santé Publique).

Après avoir discuté librement et obtenu réponse à toutes mes questions, j'accepte, en toute connaissance la participation de ma fille à cette étude.

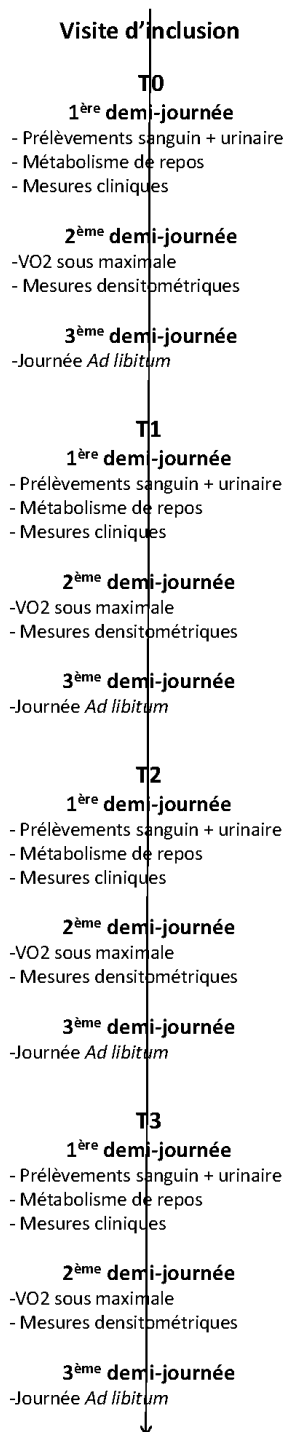
Fait à _____, le ____/____/____
Signature des deux titulaires de l'exercice de l'autorité parentale _____

Consentement recueilli par le Dr _____

ANNEXE 3



ANNEXE 4



ANNEXE 5

CARNET ALIMENTAIRE

(A tenir pendant au moins 3 jours de semaine consécutifs)

Renvoyer à :

Vos coordonnées :

Que faut-il faire pour tenir un carnet alimentaire ?

- Pratiquer un relevé complet de toute la nourriture que l'on consomme, sans oublier les boissons, les en-cas et les condiments.
- Noter immédiatement dans un carnet, après chaque repas ou en-cas, ce qui vient d'être consommé (boisson ou aliment solide) afin de ne pas oublier.
- Dans la première colonne, indiquez : le type de repas, l'heure et le lieu (domicile, restaurant, cantine, stade...) de celui-ci.
- Dans la deuxième colonne, notez le nom des aliments (solides et liquides) consommés, sans oublier les condiments qui sont ajoutés aux aliments tels que beurre, margarine, huile, crème fraîche, mayonnaise, sauce, vinaigrette, moutarde ou ketchup.
- Dans la troisième colonne, décrivez les aliments consommés :
 - Origine (industrielle – marque - ou maison)
 - Type (écrémé, ½ écrémé, fromage blanc 10%, 20% de MG),
 - Caractéristique (pour le fromage : gruyère, bleu,...., pour le céréales : Muesli, All Bran,...
 - Type de préparation des aliments : grillés, sautés, frits, cuits au four (avec ou sans matière grasse) ou à la vapeur (ou bouilli).
- Ne pas oublier de noter également tous les suppléments alimentaires consommés tels que les vitamines ou minéraux, boissons énergétiques pour sportifs ou protéinées, aliments de régime ou laxatifs.
- Dans la quatrième colonne, notez la quantité (ou le volume pour les liquides) des aliments consommés :
 - Pour les liquides : un bol ou une grande tasse (250ml), une petite tasse de café (50ml), une cuillère à soupe (cs), une cuillère à café (cc), un grand verre (200ml), un petit verre (120ml), une louche (125g) ou une assiette à soupe (250g) ;
 - Pour les solides : une tranche (tr) fine, moyenne ou grosse pour la viande et la charcuterie, en cm pour le pain (mais aussi ¼ ou 1/3 ou ½ baguette), en portion (1=30g) pour le fromage ;
 - Pour le sucre : nombre de morceaux (et le n° : 3 ou 4,...) ou le nombre de cuillerées (à soupe ou à café) quand il est en poudre ;
 - Pour le beurre ou la margarine : une grosse, moyenne (=1cc) ou petite noix ;
 - Pour le rations : nombre de cs (pour purée, pâtes ou légumes) ou nombre d'assiettes (1 de riz ou pâtes = 250g) ;
 - Pour le sel, le poivre et les épices, estimez leur quantité par : + (peu), ++ (moyen) et +++ (beaucoup).

Exemple

NOM	Sexe	Taille (cm)	Poids (kg)	Date de naissance
DURAND F.	M	175	75	30.12.57

Activité quotidienne : travail de bureau + 2h bricolage

Activité sportive : 45 mn footing + 15 mn fractionné en nature

Repas /en-cas Horaire, lieu	Aliment ou boisson	Description (composition et/ou marque)	Quantité consommée
Petit déjeuner Heure : 7h15 (domicile)	-thé +sucre -céréales +sucre +lait -Jus d'orange	Blanc Muesli sans sucre Blanc ½ écrémé 100% Pur jus	1 gde t 1 cc 1 bol 1 cc 3 cs 1 gd v
En-Cas Heure : 10h00 (bureau)	-eau - barre chocolatée	Evian Mars	1 gd v 1
Déjeuner Heure : 12h30 (restaurant)	-apéritif -eau -olives vertes -vin rouge -carottes -vinaigrette -haricots verts -pommes de terre -poulet -pain -pomme -café -sucre	Ricard Robinet Natures 12° crués (râpées) classique sautés vapeur rôti blanc (baguette) cru noir blanc	1 dose 1 pt v 5 2 pt v 4 cs 2 cc ½ assiette 1 moyenne 1 cuisse 3 tr de 3 cm 1 1pte t 1
En-Cas Heure : 16h	-thé sucré -biscuits secs	Distributeur Petits beurre (LU)	1 gde t 3 (soit 25g)
Dîner Heure : 20h (domicile)	-potage de légumes -pâtes -sauce tomate au basilic -fromage râpé -yaourt	Maison (pommes de terre, carottes, poireaux) Coquillettes Heinz Gruyère Entier aux fruits	1 assiette 1 assiette 3 cs 1 cs 1
En-Cas Heure : 22h (domicile)	-cidre	Doux	1 pt v

Jour 1

NOM **Sexe** **Taille (cm)** **Poids (kg)** **Date de naissance**

Activité physique :

Repas /en-cas Horaire, lieu	Aliment ou boisson	Description (composition et/ou marque)	Quantité consommée
Petit déjeuner Heure : 7h15 (domicile)			
En-Cas Heure : 10h00 (bureau)			
Déjeuner Heure : 12h30 (restaurant)			
En-Cas Heure : 16h			
Dîner Heure : 20h (domicile)			
En-Cas Heure : 22h (domicile)			

Jour 2

NOM **Sexe** **Taille (cm)** **Poids (kg)** **Date de naissance**

Activité physique :

Repas /en-cas Horaire, lieu	Aliment ou boisson	Description (composition et/ou marque)	Quantité consommée
Petit déjeuner Heure : 7h15 (domicile)			
En-Cas Heure : 10h00 (bureau)			
Déjeuner Heure : 12h30 (restaurant)			
En-Cas Heure : 16h			
Dîner Heure : 20h (domicile)			
En-Cas Heure : 22h (domicile)			

Jour 3

NOM **Sexe** **Taille (cm)** **Poids (kg)** **Date de naissance**

Activité physique :

Repas /en-cas Horaire, lieu	Aliment ou boisson	Description (composition et/ou marque)	Quantité consommée
Petit déjeuner Heure : 7h15 (domicile)			
En-Cas Heure : 10h00 (bureau)			
Déjeuner Heure : 12h30 (restaurant)			
En-Cas Heure : 16h			
Diner Heure : 20h (domicile)			
En-Cas Heure : 22h (domicile)			

Jour 4

NOM **Sexe** **Taille (cm)** **Poids (kg)** **Date de naissance**

Activité physique :

Repas /en-cas Horaire, lieu	Aliment ou boisson	Description (composition et/ou marque)	Quantité consommée
Petit déjeuner Heure : 7h15 (domicile)			
En-Cas Heure : 10h00 (bureau)			
Déjeuner Heure : 12h30 (restaurant)			
En-Cas Heure : 16h			
Dîner Heure : 20h (domicile)			
En-Cas Heure : 22h (domicile)			

ANNEXE 6

Questionnaire de sensations de faim :
Echelles Visuelles Analogiques

As-tu faim ?
Pas du tout ----- Très

Te sens tu repus ?
Pas du tout ----- Très

Comment pourais tu encore manger ?
Rien du tout ----- énormément

Désires tu manger ?
Pas du tout ----- énormément

Ces échelles visuelles analogiques sont réalisées à plusieurs reprises tout au long de la journée.

ANNEXE 7

Questionnaire d'activité physique IPAQ

IPAQ

International Physical Activity Questionnaire

(Version française juillet 2003)

Nous nous intéressons aux différents types d'activités physiques que vous faites dans votre vie quotidienne. Les questions suivantes portent sur le temps que vous avez passé à être actif physiquement au cours des **7 derniers jours**. Répondez à chacune de ces questions même si vous ne vous considérez pas comme une personne active. Les questions concernent les activités physiques que vous faites au lycée, lorsque vous êtes chez vous, pour vos déplacements, et pendant votre temps libre.

Bloc 1 : Activités intenses des 7 derniers jours

1. Pensez à toutes les **activités intenses** que vous avez faites au cours des **7 derniers jours**. Les activités physiques intenses font référence aux activités qui vous demandent un effort physique important et vous font respirer beaucoup plus difficilement que normalement. Pensez seulement aux activités que vous avez effectuées pendant **au moins 10 minutes d'affilée**.

1-a. Au cours des **7 derniers jours**, combien y a-t-il eu de jours au cours desquels vous avez fait des **activités physiques intenses** comme porter des charges lourdes, bêcher, faire du VTT ou jouer au football ?

__ jour(s)

Je n'ai pas eu d'activité physique intense

➔ **Passez au bloc 2**

1-b. Au total, combien de **temps** avez-vous passé à faire des **activités intenses au cours des 7 derniers jours** ?

__ heure(s) __ minutes

Je ne sais pas

Bloc 2 : Activités modérées des 7 derniers jours

2. Pensez à toutes les **activités modérées** que vous avez faites au cours des **7 derniers jours**. Les activités physiques modérées font référence aux activités qui vous demandent un effort physique modéré et vous font respirer un peu plus difficilement que normalement. Pensez seulement aux activités que vous avez effectuées pendant **au moins 10 minutes d'affilée**.

2-a. Au cours des **7 derniers jours**, combien y a-t-il eu de jours au cours desquels vous avez fait des **activités physiques modérées** comme porter des charges légères, passer l'aspirateur, faire du vélo tranquillement ou jouer au volley-ball ? Ne pas inclure la marche.

__ jour(s)

Je n'ai pas eu d'activité physique modérée

➔ **Passez au bloc 3**

2-b. Au total, combien de **temps** avez-vous passé à faire des **activités modérées au cours des 7 derniers jours** ?

__ heure(s) __ minutes

Je ne sais pas

Bloc 3 : La marche des 7 derniers jours

3. Pensez au temps que vous avez passé à **marcher au moins 10 minutes d'affilée** au cours des **7 derniers jours**.

Cela comprend la marche au lycée et à la maison, la marche pour vous rendre d'un lieu à un autre, et tout autre type de marche que vous auriez pu faire pendant votre temps libre pour la détente, le sport ou les loisirs.

3-a. Au cours des **7 derniers jours**, **combien y a-t-il eu de jours** au cours desquels vous avez marché pendant **au moins 10 minutes d'affilée**.

__ __ jour(s)

Je n'ai pas fait de marche

➡ **Passez au bloc 4**

3.b. Au total, combien d'épisodes de marche d'au **moins 10 minutes d'affilée**, avez-vous effectué au cours des **7 derniers jours** ?

__ __ __ __ nombre d'épisodes de 10 minutes d'affilée

Exemples :

Lundi :	1 marche de 60 minutes		6 épisodes
Mardi :	1 marche de 20 minutes et 3 marches de 5 minutes		2 épisodes
Mercredi :	1 marche de 35 minutes		3 épisodes
Jeudi :	1 marche de 8 minutes		0 épisode
Vendredi :	1 marche de 6 minutes puis 3 marches de 4 minutes	→	0 épisode
Samedi :	1 marche de 18 minutes		1 épisode
Dimanche :	1 marche de 10 minutes et 3 marches de 5 minutes		1 épisode
		Total	13 épisodes

Je ne sais pas

Bloc 4 : Temps passé assis au cours des 7 derniers jours

4. La dernière question porte sur **le temps que vous avez passé assis** pendant les jours de semaine, au cours des **7 derniers jours**. Cela comprend le temps passé assis au lycée, à la maison, lorsque vous étudiez et pendant votre temps libre. Il peut s'agir par exemple du temps passé assis à un bureau, chez des amis, à lire, à être assis ou allongé pour regarder la télévision, devant un écran.

4-a. Au cours des **7 derniers jours**, pendant les jours de semaine, **combien de temps**, en moyenne, avez vous passé **assis** ?

__ __ heure(s) __ __ minutes

Je ne sais pas

ANNEXE 8

CURRICULUM VITAE (*) abrégé des investigateurs

Nom : Frédéric DUTHEIL	
Fonctions : Praticien Hospitalo-Universitaire	
Titres : MD, PhD, HDR	
Établissement :	
- Médecine Préventive, CHU de Clermont-Ferrand, Hôpital Gabriel Montpied, 58 rue Montalembert BP 69, 63000 Clermont-Ferrand	
Affiliation éventuelle à un organisme de recherche :	
INSERM	<input type="checkbox"/>
CNRS	<input type="checkbox"/>
Autres (préciser) x	
Adresse :	
- Médecine Préventive, CHU de Clermont-Ferrand, Hôpital Gabriel Montpied, 58 rue Montalembert BP 69, 63000 Clermont-Ferrand	
- EA 3533 Laboratoire AME2P – Université Blaise Pascal Bâtiment de Biologie B - Campus Universitaire des Cézeaux - 63170 Aubière Cedex	
Téléphone : 06 88 22 48 48	Télécopie : 04 73 27 46 49
e-mail : fred_dutheil@yahoo.fr	
N° d'inscription au conseil de l'ordre : 63/5926	n° ADELI 63 10 5926 8
n°RPPS 10 100 161438	

Principales publications :

Ollier M, Garcier JM, Naughton G, Chamoux A, Pereira B, Dutheil F. CT scan procedure for lung cancer screening in asbestos-exposed workers. *Chest*. 2014 Aug 1;146(2):e76-7. doi: 10.1378/chest.14-0831. Impact factor 2014: **7.13**

Ollier M, Chamoux A, Naughton G, Pereira B, Dutheil F. Chest computed tomography screening for lung cancer in asbestos occupational exposure: a systematic review and meta-analysis. *Chest*. 2014 Jan 30. doi: 10.1378/chest.13-2181. Impact factor 2014: **7.13**

Dutheil F, Trousselard M, Perrier C, Lac G, Chamoux A, Duclos M, Naughton G, Mhazaganian G, Schmidt J. Urinary Interleukin-8 Is a Biomarker of Stress in Emergency Physicians, Especially with Advancing Age - The JOBSTRESS* Randomized Trial. *PLoS One*. 2013 Aug 19;8(8):e71658. doi: 10.1371/journal.pone.0071658. Impact factor 2013: **4.09**

Dutheil F, Lac G, Lesourd B, Chapier R, Walther G, Vinet A, Sapin V, Verney J, Ouchchane L, Duclos M, Obert P, Courteix D. Different modalities of exercise to reduce visceral fat mass and cardiovascular risk in metabolic syndrome: the RESOLVE* randomized trial. *International Journal of Cardiology*. 2013. doi:10.1016/j.ijcard.2013.05.012. Impact factor 2013: **7.21**

Dutheil F, Boudet G, Perrier C, Lac G, Ouchchane L, Chamoux A, Duclos M, Schmidt J. JOBSTRESS study: Comparison of heart rate variability in emergency physicians working a 24-hour shift or a 14-hour night shift - A randomized trial. *Int J Cardiol*. 2012 Jul;158(2):322-325. Impact factor 2013: **7.21**.

February 28th, 2015:



Nom : DUCLOS Martine

Fonctions : Praticien Hospitalier - Professeur des Universités, PU-PH

N° ADELI: 63/5449 **N° d'inscription à l'ordre** : 63/5449 **N° RPSS**: 10002773744

Titres : Docteur en médecine (DES Endocrinologie), PhD, HDR

Organisme : CHU de Clermont-Ferrand et CRNH Auvergne, Université d'Auvergne 1

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5 Principales publications sur les 4 dernières années :

Duclos M, Ouerdani A, Mormède P, Konsman JP. Food-restriction-induced hyperactivity: addiction or fleeing famine? *Psychoneuroendocrinology* 2012 Oct 8. Impact factor 5,809

Duclos M, Oppert JM, Verges B, Coliche V, Gautier JF, Guezennec Y, Reach G, Strauch G; SFD diabetes and physical activity working group. Physical activity and type 2 diabetes. Recommendations of the SFD (Francophone Diabetes Society) diabetes and physical activity working group. *Diabetes & Metabolism* 2013;39:205–216. Impact factor 2,72

Guidoux R, **Duclos M**, Fleury G, Lacomme P, Lamaudiere N, Manenq PH, Paris L, Ren L, Rousset S. A smartphone-driven methodology for estimating physical activities and energy expenditure in free living conditions. *Journal of Biomedical Informatics* 2014;52:271-278 Impact factor 2.131

Buffiere C, Mariotti F, Savary-Auzeloux I, Migné C, Meunier N, Hercberg S, Cano N, Rémond D, **Martine Duclos**, Dardevet D. Slight chronic elevation of C reactive protein is associated with lower aerobic fitness but does not impair meal-induced stimulation of muscle protein metabolism in healthy old men. *J Physiol.* 2015 Mar 1;593(5):1259-72. Impact factor 4.544

Chaput JP, Genin P, LeMoel B, Pereira B, Boirie Y, **Duclos M**, Thivel D. Lean adolescents achieve higher intensities but not higher energy expenditure while playing active video games compared with obese ones. *Pediatric Obesity*, In Press. Impact factor 3,025.

le 2 juin 2015



<p>Nom : ESCHALIER Romain</p> <p>Fonctions : Investigateur</p> <p>N° d'inscription à l'ordre : 63 / 5927</p> <p>Titres : Cardiologue, Praticien Hospitalier (MD, PhD)</p> <p>Etablissement : pôle de cardiologie, CHU Clermont-Ferrand</p> <p>Affiliation éventuelle à un organisme de recherche :</p> <p>INSERM <input type="checkbox"/> CNRS <input checked="" type="checkbox"/> Autres (préciser) <input type="checkbox"/></p> <p>Adresse : service de cardiologie, CHU Gabriel Montpied, 58 rue montalembert, 63100 Clermont-Ferrand</p> <p>Téléphone : 04.73.75.14.12 Télécopie : 04.73.75.19.33</p> <p>Courriel : reschali@chu-clermontferrand.fr</p>
--

Principales publications :

- Eschali R, Chenaf C, Mulliez A, Yalioua A, Clerfond G, Authier N, Vorilhon C, Citron B, Pereira B, Jean F, Souteyrand G, Motreff P, Eschali A, Lusson JR. Impact of clinical characteristics and management on the prognosis of unselected heart failure patients. Cardiovasc Drugs Ther. 2015 Feb;29(1):89-98
- Vorilhon C, Chenaf C, Mulliez A, Pereira B, Clerfond G, Authier N, Jean F, Motreff P, Citron B, Eschali A, Lusson JR, Eschali R. Heart failure prognosis and management in over-80-year-old patients: data from a French national observational retrospective cohort. Eur J Clin Pharmacol. 2015 Feb;71(2):251-60
- Eschali R, Jean F, Pereira B, Monzy S, Vorilhon C, Mactoux V, Citron B, Sapin V, Motreff P, Lusson JR. Is there benefit in optimising heart failure treatment in over-80 year-old patients? (HF-80 study): study protocol for a randomized controlled trial. Trials. 2012 Mar 6;13:25.
- Eschali R, Girerd N, Rossignol P. Do not analyze too quickly a result: how spironolactone is always point at! Am J Cardiol. 2015 Jan 1;115(1):155-6.
- Eschali R, Ploux S, Ritter P, Haïssaguerre M, Ellenbogen KA, Bordachar P. Nonspecific intraventricular conduction delay: Definitions, prognosis, and implications for cardiac resynchronization therapy. Heart Rhythm. 2015 May;12(5):1071-9.

Nom : CLERFOND Guillaume	
Fonctions : médecin	
N° d'inscription à l'ordre : 63/6306	
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Établissement : pôle de cardiologie, CHU Clermont-Ferrand	
Affiliation éventuelle à un organisme de recherche :	
INSERM	<input type="checkbox"/>
CNRS	<input type="checkbox"/>
Autres (préciser)	<input checked="" type="checkbox"/>
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Téléphone : 0473751414	Télécopie : 0473751933
Courriel : gclerfond@chu-clermontferrand.fr	

Principales publications :

- Clerfond G, Pereira B, Innorta A, Motreff P, Gilard M, Laskar M, Eltchaninoff H, Jung B, Leprince P, Teiger E, Chevreur K, Prat A, Lievre M, Leguerrier A, Donzeau-Gouge P, Fajadet J, Souteyrand G. Comparison of Outcomes After One-Versus-Two Transcatheter Aortic Valve Implantation During a Same Procedure (from the FRANCE2 Registry). *Am J Cardiol*. 2015 May 1;115(9):1273-80
- Clerfond G, Bière L, Mateus V, Grall S, Willoteaux S, Prunier F, Furber A. End-systolic wall stress predicts post-discharge heart failure after acute myocardial infarction. *Arch Cardiovasc Dis*. 2015 May;108(5):310-20
- Eschalier R, Chenaf C, Mulliez A, Yalioua A, Clerfond G, Authier N, Vorilhon C, Citron B, Pereira B, Jean F, Souteyrand G, Motreff P, Eschalier A, Lusson JR. Impact of clinical characteristics and management on the prognosis of unselected heart failure patients. *Cardiovasc Drugs Ther*. 2015 Feb;29(1):89-98
- Vorilhon C, Chenaf C, Mulliez A, Pereira B, Clerfond G, Authier N, Jean F, Motreff P, Citron B, Eschalier A, Lusson JR, Eschalier R. Heart failure prognosis and management in over-80-year-old patients: data from a French national observational retrospective cohort. *Eur J Clin Pharmacol*. 2015 Feb;71(2):251-60
- Grall S, Biere L, Clerfond G, Mateus V, Prunier F, Furber A. ECG characteristics according to the presence of late gadolinium enhancement on cardiac MRI in hypertrophic cardiomyopathy. *Open Heart*. 2014 Aug 5;1(1):e000101

Nom : VORILHON Charles		
Fonctions :		
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Organisme : Pôle de Cardiologie, CHU Clermont-Ferrand		
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Email : c_vorilhon@chu-clermontferrand.fr		

Principales publications :

- Eschalier R, Jean F, Pereira B, Monzy S, **Vorilhon C**, Mactoux V, Citron B, Sapin V, Motreff P, Lusson JR. Is there benefit in optimising heart failure treatment in over-80 year-old patients? (HF-80 study): study protocol for a randomized controlled trial. *Trials*. 2012 Mar 6;13:25.
- Nana A, **Vorilhon C**, Adjtoutah D, Azarnoush K, Kissel V, Chabin X, Chailloux A, Belhakem A, Tixier V, Ferrier N, Croisille P, Long JL, Marcaggi X. Contribution of magnetic resonance imaging in diagnosis of pericardial mesothelioma: a case report. *Ann Cardiol Angeiol (Paris)*. 2012 Nov;61(5):370-4.
- **Vorilhon C**, Chenaf C, Mulliez A, Pereira B, Clerfond G, Authier N, Jean F, Motreff P, Citron B, Eschalier A, Lusson JR, Eschalier R. Heart failure prognosis and management in over-80-year-old patients: data from a French national observational retrospective cohort. *Eur J Clin Pharmacol*. 2015 Feb;71(2):251-60.
- Eschalier R, Chenaf C, Mulliez A, Yalioua A, Clerfond G, Authier N, **Vorilhon C**, Citron B, Pereira B, Jean F, Souteyrand G, Motreff P, Eschalier A, Lusson JR. Impact of clinical characteristics and management on the prognosis of unselected heart failure patients. *Cardiovasc Drugs Ther*. 2015 Feb;29(1):89-98.

<p><u>Nom</u> : Anne-Véronique FOUROT</p> <p><u>Fonctions</u> : Médecin</p> <p><u>Titres</u> : MD</p> <p><u>Etablissement</u> : - Maison médicale pour enfants et adolescents, 230 rue Vercingétorix BP 77 63150 La Bourboule</p> <p><u>Affiliation éventuelle à un organisme de recherche</u> : INSERM <input type="checkbox"/> CNRS <input type="checkbox"/> Autres (préciser) <input type="checkbox"/></p> <p>Adresse :</p> <p><u>Téléphone</u> : 04 73 81 31 36 <u>Télécopie</u> :</p> <p><u>e-mail</u> : avfb@wanadoo.fr</p> <p><u>N° d'inscription au conseil de l'ordre</u> : 63/4733 <u>n° ADELI</u> 63 10 47 33 9</p> <p><u>n°RPPS</u></p>
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Principales publications :

Evolution : Amélioration Stabilité Aggravation Survie avec séquelles
 Décès (cause : lié à l'événement Oui Non) Evolution inconnue

Description (joindre les comptes-rendus anonymisés d'hospitalisation d'examens et/ou résultats de laboratoire) :

Un ou des produits ont-ils été réintroduits ? Réapparition de l'événement après réintroduction ?
 Oui N° N° N° Non Oui N° N° N° Non

Si oui, date : |_|_|_|_|_|_|_|_| heure : |_|_|_|_|_|_|

DIAGNOSTIC DIFFERENTIEL

Autres étiologies envisagées:

Examens complémentaires réalisés et résultats :

7. INFORMATIONS SUR LE DECLARANT

Nom et adresse du centre investigateur :

Centre n° : _____ Investigateur : _____
 Tél. : _____ Email : _____ @ _____
 Service : _____

Nom et qualité du déclarant : _____ Signature : _____

8. INFORMATIONS SUR LE PROMOTEUR *(cadre réservé au promoteur, ne pas remplir)*

Nom et adresse du promoteur :

Date de réception par le promoteur : |_|_|_|_|_|_|_|_| Type de rapport : initial
 suivi n° _____
 Date de déclaration aux autorités : |_|_|_|_|_|_|_|_|

N° d'identification de l'événement par le promoteur : _____

Identification de l'autorisation de recherche : _____

Nom et qualité du représentant du promoteur: _____

Tél. : _____ Email : _____ @ _____
 Fax : _____ Signature : _____

Appendix 7 - Clinical trial registration



Title IND Sponsor Summary Status Design Interventions Conditions Eligibility Locations Citations Links

Title: ADIBOX – ADIposity and Bone metabolism: effects of eXercise-induced weight loss in obese adolescents

Unique Protocol ID:	2015-A01024-45
Brief Title:	ADIBOX
Official Title:	ADIposity and Bone metabolism: effects of eXercise-induced weight loss in obese adolescents
Secondary ID's: <i>(One ID per line)</i> Definition: Other identification numbers assigned to the protocol, including any applicable NIH grant numbers. Provide up to 5 Secondary ID Numbers. Example: NCI-793-0115D	Référence CPP 2015-33

Title IND **Sponsor** Summary Status Design Interventions Conditions Eligibility Locations Citations Links

Sponsor: Definition: Name of sponsoring organization that takes responsibility for and initiates a clinical investigation	University Hospital of Clermont-Ferrand (CHU), Clermont-Ferrand, France
Collaborators: <i>(Enter up to 10 agencies, one agency per line)</i> Definition : Full names of all organizations co-sponsoring and/or providing financial support for the protocol. The data provider is responsible for confirming all collaborators before listing them. Provide up to 10 full names of collaborating organizations	<ul style="list-style-type: none"> - AME2P Laboratory (EA 3533), Laboratory of Metabolic Adaptations to Exercise in Physiological and Pathological conditions – University Clermont Auvergne 63000 Clermont-Ferrand, France - Faculty of Health Science, Australian Catholic University, Locked bag 2002, Strathfield NSW 2135, Australia - Tza Nou Medical House for children and adolescents, 230, rue Vercingétorix - B.P. 77 63150 La Bourboule, France

REVIEW BOARD

Board Approval Number: *	2015-A01024-45 N° de référence attribué par le CPP au 1 ^{er} avis favorable
Board Name: *	cpp sudest1 Nom du CPP
Board Chair: * (Not made public)	Name: M. Philippe Rusch Business Phone: 04.77.12.70.09 Extension: <input type="text"/> Business Email: cpp.sudest1@chu-st-etienne.fr Business Address: CHU de Saint Etienne Direction des Affaires Médicales et de la Recherche Hopital Bellevue – Pavillon 31 42055 Saint Etienne Cedex , France

Title IND Sponsor **Summary** Status Design Interventions Conditions Eligibility Locations Citations Links

Brief Summary:

Definition: Short description of the primary purpose of the protocol intended for the lay public. Include a brief statement of the study hypothesis.

The present protocol is mainly involved in the understanding of the local interaction between the released products by fat tissue and hormones production of bone tissue. These complex interactions between adipocyte and osteocyte activities could explain the mechanisms of the body responses to the strategies of weight loss that include diet and/or physical activity program, as well as the side effects encountered by these interventions.

Adolescence is a period of development characterized by many metabolic and somatic changes that may influence weight. Weights bearing physical activities are a key factor allowing body composition changes (i.e. fat and bone tissue). The difficulties of managing weight and the onset of overweight and obesity during this very important growth spurt lead to various hormonal dysregulation. The specific mechanisms of the evolution and interactions between these two parameters (fat and bone tissue) are not yet elucidated; therefore our aim is to analyze the possible connections between fat tissue and the quality of the skeleton in order to reduce related risks of the consequence of weight loss in obese individuals.

<p>Detailed Description: Definition: Extended description of the protocol, including information not already contained in other fields, such as comparison(s) studied.</p>	<p>The complex consequences of childhood obesity represent major concerns in most developed countries, largely contributing to metabolic complications with costly repercussions for the burden of disease. The burden is exemplified by high prevalence rates of overweight or obesity.</p> <p>The ADIBOX protocol was designed to provide a better understanding of the bone-adipocyte cross-talk in adolescents with obesity and the effects of physical activity-induced weight loss on this cross-talk.</p> <p>Obesity effectively leads to hormonal alterations favoring the accumulation of fat mass and loss of bone mass. Advancing the knowledge of the complex interactions between adipocyte and osteocyte activities may contribute to the mechanistic understanding of the body's responses to weight loss during adolescence and prevent cardiovascular risk. Indeed, the adipose-bone tissue cross-talk has been recently linked with cardiovascular diseases. Similarly as adipose tissue, released-products from bone tissue may act directly or indirectly on cardiovascular risk and diseases.</p> <p>The ADIBOX study, a 40 weeks longitudinal study (LS) with repeated measures on four occasions (baseline and every fourteen weeks), will allow us to understand the effects of physical activity-induced weight loss on this cross-talk in obese adolescents.</p> <p>Data will be analyzed using Stata (StataCorp, College Station, USA) and IBM Statistics SPSS version 22 (IBM Corp, 2013, Chicago, IL, USA) and significance will be accepted at a two-sided alpha level of $p < 0.05$. After testing for normal distribution (Shapiro-Wilk test), data will be treated either by parametric or non-parametric analyses according to statistical assumptions. Student t tests or Mann-Whitney U test will be performed to compare adipose tissue (total, subcutaneous, visceral) variation reported to bone mass variation at lumbar spine between groups at baseline. Pearson (or Spearman when appropriate) correlation coefficient will be used and compared with Fisher test (command <i>corcor</i> Stata) to measure the link between exercise-inducing weight loss on adipose tissue and bone mass variations. Longitudinal data will be treated using a mixed model analyses in order to treat fixed effects group, time and group x time interaction taking into account between and within participant variability.</p>
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Study Phase:	
Study Type:	Interventional
Overall Study Status: Definition: Overall protocol accrual activity for the protocol	Recruiting
Record Verification Date: Definition: Date the protocol information, including recruiting status, was last verified, whether changes were made or not.	
Key Trial Dates	
Study Start Date: Definition: Date that enrollment to the protocol begins	September 2015
Last Follow-Up Date: Definition: Date that follow-up with all study participants is complete	June 2016
Data Entry Closure Date: Definition: Date that data submission for the study is complete.	October 2016
Study Completion Date: Definition: Expected or actual date that analysis is concluded for the protocol.	October 2016

Study Type Interventional

Title IND Sponsor Summary Status **Design** Interventions Conditions Eligibility Locations Citations Links

Purpose: Reason for the protocol	Improve our understanding about the effects of a weight loss management program combining physical activity and restrictive diet on the bone-adipocyte cross-talk in obese adolescents.
Allocation: Participant selection <ul style="list-style-type: none">Nonrandomized Trial: participants are expressly assigned to intervention groups	Non Randomized Trial
Masking: knowledge of intervention assignments <ul style="list-style-type: none">Open: no masking is used. All involved know the identity of the intervention assignment.	Open

<p>Control: Nature of the intervention control</p> <ul style="list-style-type: none"> • Uncontrolled: no controls are used 	<p>Controls will be obese adolescents who will not undergo the management program combining physical activity and restrictive diet</p>
<p>Assignment:</p> <p>- intervention assignments</p> <ul style="list-style-type: none"> • Single Group: all participants receive the same intervention throughout the protocol 	<p>Two groups of adolescents will be recruited:</p> <ul style="list-style-type: none"> - an intervention group who will undergo the management program combining physical activity and restrictive diet at Tza Nou Medical House for 10 months - a control group who will not undergo any intervention
<p>Endpoints: overall outcome that the protocol is designed to evaluate. Select one.</p> <ul style="list-style-type: none"> • Safety/Efficacy 	<p>Safety/Efficacy</p>
<p>Primary Outcomes: Definition: The specific measure that will be used to determine the effect of the intervention(s). The description should include the time at which the measure will be taken. Examples: all cause mortality at one year; score on a depression rating scale at 6 weeks</p>	<p>Title: change from baseline fat mass (total, subcutaneous, visceral) measured by <u>Dual energy X-ray Absorptiometry (DXA)</u> at 10 months Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p> <p>Title: change from baseline bone mass (measured at the L2-L4 lumbar spine level) measured by <u>Dual energy X-ray Absorptiometry (DXA)</u> at 10 months Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p>
<p>Secondary Outcomes: Definition: Other measures that will be used to evaluate the intervention(s), and that are specified in the protocol. The description should include the time at which the measures will be taken. Examples: cardiovascular mortality at 6 months; functional status at 4 weeks</p>	<p>Title: Whole body, and non-dominant hip (including the femoral neck, trochanteric and intertrochanteric regions) measured by <u>Dual energy X-ray Absorptiometry (DXA)</u> Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months.</p>

	<p>Safety issue : no</p> <p>Title: height. Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p> <p>Title: weight. Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p> <p>Title: waist circumference. Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p> <p>Title: lower limb bone lengths/breadths. Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p> <p>Title: <u>Physical activity measured with International Physical Activity Questionnaire</u> and by educators Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p> <p>Title: Maturation assessed by Tanner's stages model for pubertal maturation as well as laboratory blood analyses Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p> <p>Title: <u>Energy metabolism assessed by</u> cycle-ergometer submaximal aerobic fitness Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p> <p>Title: <u>bone mineral density</u> measured by Peripheral Quantitative Computed Tomography (pQCT) at the distal (4%), proximal (66%) site of the non-dominant tibia and radius Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p>
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	<p>Title: bone mineral status measured by Quantitative Ultra-Sound (QUS) on the non-dominant calcaneus. Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p> <p>Title: <u>Endocrine assays</u> (TG, cholesterol, LDL, HDLC, Glycaemia, Insulin, CRP ultra-sensible, serum osteoprotegerin (OPG), receptor activator of nuclear κB ligand (RANKL), sclerostin, bone alkaline phosphatase, type I collagen C-telopeptides (CTX), PINP uncarboxylated, osteocalcin total and vitamin D osteocalcin uncarboxylated (unOc), leptin, adiponectin, TNF-alpha, IL-6, GH, IGF1, IGFBP-3, estradiol, FSH/LH and PTH/calcitonin) Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p> <p>Title: <i>Ad libitum</i> meal and hunger feelings questionnaire measured with a SUVIMAX book Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p> <p>Title: Adolescents' observance to the weight loss lifestyle program measured by Tza Nou educators. Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p> <p>Title: Adolescents' observance to the weight loss lifestyle program measured by self-reported questionnaires. Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p> <p>Title: <u>Metabolomics analysis in blood plasma</u> Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p> <p>Title: <u>Ex-vivo mechanistic analysis</u> in blood plasma</p>
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	<p>Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p>
	<p>Title: left ventricular end diastolic diameter measured by echocardiography Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p>
	<p>Title: left ventricular end systolic diameter measured by echocardiography Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p>
	<p>Title: posterior wall thickness measured by echocardiography Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p>
	<p>Title: interventricular septum thickness measured by echocardiography Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p>
	<p>Title: left ventricular mass indexed measured by echocardiography Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p>
	<p>Title: left ventricular ejection fraction measured by echocardiography Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p>
	<p>Title: valves velocity measured by echocardiography Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p>
	<p>Title: isovolumic relaxation time measured by echocardiography</p>

	<p>Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p>
	<p>Title: strain rate measured by echocardiography Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p>
	<p>Title: myocardial dyssynchrony measured by echocardiography Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p>
	<p>Title: epicardiac fat measured by chest echocardiography Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p>
	<p>Title: carotid-intima-media thickness measured by echography Time frame: Measures will be recorded three times: at the beginning of the study, at 5 months and at 10 months. Safety issue : no</p>

Study Type Observational

Title IND Sponsor Summary Status **Design** Interventions Conditions Eligibility Locations Citations Links

<p>Purpose: Reason for the protocol</p> <ul style="list-style-type: none"> • Natural History: protocol designed to investigate a disease or condition through observation under natural conditions (i.e., without intervention) • Screening: protocol designed to assess or examine persons or groups in a systematic way to identify specific markers or characteristics (e.g., for eligibility for further evaluation) • Psychosocial: protocol designed to observe the psychosocial impact of natural events 	
<p>Duration * length of protocol</p> <ul style="list-style-type: none"> • Longitudinal: studies in which participants are evaluated over long 	

<p>periods of time, typically months or years</p> <ul style="list-style-type: none"> • Cross-sectional: studies in which participants are evaluated over short periods of time, typically up to 10 weeks 	
<p>Selection * sample selection</p> <ul style="list-style-type: none"> • Convenience Sample: participants or populations are selected due to ease of recruitment • Defined Population: participants or populations are selected based on predefined criteria • Random Sample: participants or populations are selected by chance • Case Control: participants or populations are selected to match the control participants or populations in all relevant factors except for the disease; only the case participants or populations have the disease 	
<p>Timing * - time of protocol</p> <ul style="list-style-type: none"> • Retrospective: a protocol that observes events in the past • Prospective: a protocol that observes events in real time (may occur in the future) • Both: a protocol that combines retrospective and prospective observation 	
<p>Primary Outcomes: Definition: The specific measure that will be used to determine the effect of the intervention(s). The description should include the time at which the measure will be taken. Examples: all cause mortality at one year; score on a depression rating scale at 6 weeks</p>	
<p>Secondary Outcomes: Definition: Other measures that will be used to evaluate the intervention(s), and that are specified in the protocol. The description should include the time at which the measures will be taken. Examples: cardiovascular mortality at 6 months; functional status at 4 weeks</p>	

Title IND Sponsor Summary Status Design **Interventions** Conditions Eligibility Locations Citations Links

Provide a type and specific name for each intervention. For drugs, please use the generic name if known.

<u>Intervention Type:</u>	<p><i>Select one</i> per intervention</p> <ul style="list-style-type: none"> • Radiation/Biological
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<u>Intervention Name:</u>	Weight loss induced by a program combining physical activity and restrictive diet in obese adolescents
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generic name of the precise intervention being studied Examples: Zidovudine (drug) Self-hypnotic relaxation (behavior)	
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Specify the primary condition or disease being studied.

<u>Conditions:</u> (Enter 1 to 5 conditions, one per line) <small>Definition: Primary diseases or conditions being studied, using the National Library of Medicine's Medical Subject Headings (MeSH) controlled vocabulary. The conditions are used to index studies in ClinicalTrials.gov. Select up to five disease or condition terms.</small>	Obesity
<u>Keywords:</u> (One per line) <small>Definition: Words or phrases that best describe the protocol. Keywords help users find studies in the database. Use NLM's Medical Subject Heading (MeSH) controlled vocabulary terms where appropriate. Be as specific and precise as possible. Avoid acronyms and abbreviations.</small>	obesity, adolescents, physical activity, adipocytes, osteocytes, weight loss, bone, fat,

<u>Eligibility Criteria:</u>	<p>All adolescent girls will have to be free of any recent history of hospitalization (past two years) or of systemic illness lasting more than two weeks in the past 12 months. Moreover, adolescents and their parents have to sign assent and consent forms, respectively.</p> <p>Also, adolescents recruited shouldn't have any known history of metabolic bone or muscle disease, nor metabolic syndrome such as diabetes, insulin-resistance, hypo- or hyper- thyroid. Adolescents will need to be free from a diagnosis of congenital cardiovascular disease, not be regularly consuming alcohol, non-smokers and not taking medication known to alter bone metabolism, nor hormones or calcium preparations (vitamin D, calcium, protein).</p>
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	<p>Moreover, girls will be excluded if they are pregnant. Obese girls (BMI > 97th percentile) that will be recruited will be at Tanner stage 3-5.</p> <p>Adolescents with obesity enrolled in this study will have to be covered by a social health insurance. Moreover, adolescents will need to have no limitations to being physical active.</p>
<p>Gender: Definition: Physical gender of individuals who may participate in the protocol. Select one.</p> <ul style="list-style-type: none"> • Both: both female and male participants are being studied 	Both
Age Limits:	12 and 16 years
Participants:	<p>Adolescents girls from:</p> <ul style="list-style-type: none"> - Tza Nou obesity center, La Bourboule, France (intervention group) - SRR Nutrition-Obesity, Clermont-Ferrand, France (control group)
<p>Expected Total Enrollment: Definition: Estimated number of participants to be studied</p>	<p>50 adolescents:</p> <ul style="list-style-type: none"> - 25 adolescents within the intervention group - and 25 adolescents within the control group

Title IND Sponsor Summary Status Design Interventions Conditions Eligibility **Locations** Citations Links

<p>Facility: Name: Full name of the organization where the protocol is being conducted. Examples: UCLA Eye Institute; Springfield Memorial Hospital</p> <ul style="list-style-type: none"> • City • State/Province • Postal Code • Country 	<p>Tza Nou Medical House for children and adolescents, 230, rue Vercingétorix - B.P. 77 63150 La Bourboule, France</p>
Recruitment Status:	Recruiting
<p>Facility Contact:</p> <ul style="list-style-type: none"> • First Name • Middle Initial • Last Name • Degree • Phone: office phone of the facility contact person. Use the format 123-456-7890 within 	<p>Frederic Dutheil, MD, PhD, HDR, Preventive and Occupational Medicine, University Hospital of Clermont-Ferrand (CHU), Clermont-Ferrand, France, +33688224848, fred_dutheil@yahoo.fr</p>

the United States and Canada. Otherwise, provide the country code. <ul style="list-style-type: none"> • Ext: phone extension, if needed • Email: electronic mail address of the facility contact person 	
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Bone-adiposity cross-talk: implications for pediatric obesity

A narrative review of literature

Elodie Chaplais · David Thivel · David Greene ·
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Geraldine Naughton · Daniel Courteix

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Abstract The rising prevalence of overweight and obesity among pediatric populations has become a major global concern. The objective of this review is to demonstrate potential interactions between the products released by fat tissue and the hormonal production of bone tissue in obese children and adolescents. Advancing the understanding of the complex interactions between adipocyte and osteocyte activities may contribute to the mechanistic understanding of the body's responses to weight loss during adolescence. This knowledge could also reveal any side effects encountered with these interventions. Currently, the concept of bone-adiposity crosstalk has not been fully elucidated, and the mechanisms remain controversial. Understanding the local interactions between the released

products by fat tissue and hormones produced in bone tissue requires further investigations.

Keywords Obesity · Adolescents · Adipocyte · Osteocyte

Introduction

Obesity is a world health concern with high prevalence among European and Australasian nations, including pediatric populations [1]. Childhood obesity prevalence has dramatically increased over the past 4 decades [2]. For example, in 2013, respectively, 20 and 24 % of boys and 16 and 23 % of girls were overweight or obese in France and Australia [3]. Childhood obesity is known to be a risk factor for obesity later in life [4], leading to various repercussions on the metabolism, including the bone remodeling process. Indeed, bone mass is acquired by a complex and dynamic process involving resorption of bone by osteoclasts and formation of bone by osteoblasts [5]. As the peripubertal period is the most opportune time to boost bone structure, any negative influence during this period can adversely alter peak bone mass and contribute to increased risk of skeletal fractures in children and adolescents [6, 7].

Due to the important skeletal generation of adipocyte hormonal products, the impact of obesity on bone metabolism is gaining the attention of researchers. Currently, the complex relationship between fat mass and bone mass is well established [8]. Obesity effectively leads to hormonal alterations associated with increasing proinflammatory cytokines and oxidative stress, favoring the accumulation of fat mass and loss of bone mass [8]. These physical and biological relations must be considered in obesity investigations and interventions. In fact, excess body mass plays an important role in the mechanical response of the skeleton

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[9] via dysregulation attributed to adipocyte production, which leads to metabolic dysfunction [10, 11]. Moreover, the distribution (subcutaneous, central or visceral) of adipose tissue could be a relevant confounder in this complex process that links obesity to osteoporosis [12].

Contrary to existing knowledge of adiposity-bone relationships of adults, the understanding of the impact of obesity on bone mass in adolescents remains unclear and conflicted. The present review focuses on evidence regarding potential relationships between adiposity and bone mass in children and adolescents relative to their body weight status. This is followed by an overview of the physiological factors implicated in this adiposity-bone metabolism crosstalk, and the impact of weight loss programs on potential interactions of adiposity and bone will also be considered. A search of online databases (PubMed and MEDLINE) was limited to studies published in French and English languages including the following key words and mesh terms where appropriate: obesity, bone, fat mass, child*, adolescents, adipocyte and osteocyte. Where possible, only studies including adolescents were extracted. However, the age ranges of some of the listed studies also included prepubertal children.

Is there any relationship between fat and bone mass in obese youth?

In contrast to investigations with obese adults, results in obese adolescents regarding bone quality show little consistency. Areal bone mineral density (BMD) and apparent bone mineral density (BMAD) may not differ between obese and normal weight children and adolescents for the whole body, spine or hip [13, 14]. However, lower whole body [6, 15, 16] or regional [15, 16] BMD and bone area [6] in obese children and adolescents has also been demonstrated, and yet others reported higher bone mass for the whole body [17, 18] or the peripheral skeleton [19].

Extending previous results, Nagasaki et al. [20] found an effect of puberty as well as a gender effect on bone density. In their study, obese children had higher BMD values between the ages of 7–9 years for girls and 7–11 years for boys, as well as lower BMDs in 11-year-old girls and 12-year-old boys, respectively. The study also found that obese girls had higher BMDs than obese boys. Unlike nonobese adolescents for whom the gain in BMD begins to increase from about 12 years of age, obese children had higher BMD values for bone age compared with the reference value before puberty and lower BMD values after puberty. Results suggest that discrepancies between studies cannot be explained by different body fat percentages. Several studies effectively found different bone mass in 9–11-year-old children while showing relatively

similar body fat values [14, 15, 17, 18]. Differences might be explained by the sex hormone status. Indeed, females [21] and obese adolescents [22] share an earlier maturation than boys. Only one study has analyzed the impact of maturation status on bone over time in obese adolescents. Results of this study showed a similar sex hormones status compared with normal-weight adolescents, but the normal weight adolescents were of a younger chronological age [14]. However, a correlation between sex hormone and adipokine concentrations in obesity was not apparent.

Despite emerging interest in the relationship between fat mass and bone mass among obese adolescents, the results remain contentious. Studies assessing total body fat mass, whether using dual-energy X-ray absorptiometry (DXA) [23] or bioelectrical impedance [24], suggest a negative association between the percentage of fat and bone measures. One exception was reported in a positive relationship between fat mass and bone mass. However, when this positive relationship between fat mass and bone mass was adjusted for height and lean body mass, no differences between fat mass and bone mass were evident [25].

In order to better understand the role of fat mass on bone mass interactions, recent studies have more specifically investigated the effects of the distribution of fat mass on bone health. Using magnetic resonance imaging (MRI), a reciprocal association was observed among subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT) and bone density [13]. Specifically, the investigation involved the relative proportion of VAT and SAT, which determined the concentrations of adipokines in the circulation and the subsequent effect of BMD. Their results have been confirmed by others who have reported a negative association between abdominal obesity and bone density [12] as well as a positive association between SAT and bone density and a negative association between VAT and bone density [26].

When interpreting the data, the technique used to assess body composition has to be considered. Although MRI is the most acceptable technique used to differentiate VAT and SAT [27], the more accessible DXA, ultrasound (US) and bioelectrical impedance do not provide direct measures of visceral adiposity. Also, with the exception of studies enrolling girls only [13, 24], other studies did not discriminate between gender in their analyses. This lack of discrimination masks the fact that the VAT and SAT distribution varies considerably depending on sex, with boys presenting more VAT and less SAT than girls [27]. The absence of gender differentiation certainly imposes an important limitation potentially leading to misinterpretation of the results.

Study in menopausal women [28] highlights an age effect with the strengthening of the negative association between abdominal obesity and bone mineral content (BMC) with aging. Unlike adult studies, developmental processes appear not to impact these relations in children

and adolescents with obesity. Of note, participants' pubertal status did not seem to impact these relations since similar results were observed among prepubertal [12, 24] or pubertal participants [13, 23, 26].

Relationships between adiposity and bone development were also assessed in a longitudinal study via DXA and peripheral quantitative computed tomography (pQCT) [29]. The total body fat mass and android fat mass in young girls were both positive predictors of bone strength and bone density at the proximal and distal site of the femur and tibia when data were monitored over 2 years [29]. However, it was argued that if the android fat mass reached a certain level, it might impair growth in cortical volumetric BMD.

Relationships have been observed between fat mass and bone mass, and distribution-specific adiposity is a significant determinant of bone development during bone mineral accrual. Subsequently, a cross-talk between the physiological and metabolic activity of bone and adipose tissue is postulated. Details from the previously mentioned studies are reported in Table 1.

Adiposity-bone metabolism cross-talk

To better understand interrelations, it seems necessary to focus on the potential interactions between adipokines and the physiological factors involved in bone metabolism (Table 1). Indeed, the skeletal system is not only stressed from mechanical loading, but also through the metabolic effect of some of the proteins (adipokines) secreted by the adipose tissue [14]. Adipokines play important roles in the modulation of biological functions and could potentially impair skeletal acquisition in obese children and adolescents [30]. More specifically, leptin and adiponectin, depending on the *Ob* gene activity, are potential contributors to BMD. Obesity is associated with significantly higher serum leptin and lower adiponectin levels [31]. Although leptin is mainly implicated in satiety control, it is also involved in the regulation of energy expenditure and bone metabolism [32]. Current evidence offers little consistency in the discussion of leptin levels or their implication for bones in younger populations with obesity. Some studies have reported no difference in leptin levels between obese children and adolescents and their normal weight peers [13, 14], whereas others have reported higher leptin levels in obese children and adolescents [19, 24, 30]. However, independent of age, all agree that obese girls have higher leptin levels than obese boys [14, 26, 33].

Differences in leptin levels might also be explained by the maturation process. A pubertal stage effect can be observed on leptin levels and be explained by an acceleration of the maturation process in the presence of secretions of steroid hormones. Indeed, through the action of estrogen

and progesterone stimulating adipose tissue acquisition, leptin will be secreted in higher levels in girls than boys, whereas in boys, testosterone will primarily stimulate muscle mass. Leptin secretion is positively correlated with body fat mass and can lead to leptin "resistance." As leptin is a major regulator of bone mass [10], a leptin deficiency may alter the BMD [34]. Leptin is also increased by emotional stress, which is also one of the many environmental factors potentially leading to obesity [35, 36].

Leptin's action in bone metabolism is also controversial. Studies emphasize leptin as a positive predictor for BMD in both pubertal and prepubertal girls [13, 24], with a contrasting inverse association between leptin and BMD in obese boys [33]. Moreover, others find a negative correlation in obese girls only [26]. Subsequently, the actions of leptin appear to be complex depending on the activated pathway, with the potential for both positive (peripheral pathway) and negative effects (central pathway), which may depend on the mode of activation [37]. In addition, a gender effect, independent of pubertal status, might be observed in relation to the influence of leptin on bone.

Other adipocytokines have also been shown to interact with bone metabolism. The association between the skeletal system and insulin resistance has started to attract the attention of researchers. The role of adiponectin on increased insulin sensitivity has generated some interest. Specifically, adiponectin has been shown to regulate bone mass through a central regulation that stimulates osteoblasts and a local regulation that inhibits osteoblasts. Similarly, it increases RANKL expression (a stimulus for osteoclast formation, function and survival) in osteoblasts, but inhibits them via the sympathetic nervous system [38]. The influence of this adipocytokine on bone mass via different pathways has been supported in adult study [39]. However, its action remains controversial.

According to some studies, no differences have been reported in adiponectin levels between obese and normal weight children and adolescents [13, 24, 40] as well as between obese girls and boys [26]. Only one study reported lower adiponectin levels in obese adolescents than in their leaner peers [30]. However, adiponectin levels were found in lower concentrations in young patients with metabolic syndrome [40].

To sustain its potential action on bones, an inverse relationship has been suggested between adiponectin levels and bone accrual in children, with adiponectin levels that were negatively correlated with BMD [13, 24]. Independent of fat mass, gender, ethnicity and dietary status, which may also be factors contributing to the observed differences, obesity tends to reduce adiponectin levels.

Osteocalcin is a known marker of bone formation secreted by osteoblasts through the *Ocn* gene, which comprises the major noncollagen protein found in the

Table 1 Studies analyzing the effect of weight status on adipocyte/osteocyte interrelations

References	Sample (age ^a)	BMI	Blood sample	Densitometry measures	Other measures	Adipocytes/osteocytes interactions
Goulding et al. [6]	♀ 9.7 ± 4.2 ♂ 11.0 ± 4.2			DXA (whole body)		BMD, bone area ↓
Júnior et al. [12]	Ob 11.1 ± 2.6	45.4 ± 5.2 % fat		DXA (whole body, trunk fat mass) US (abdominal region)		Abdominal obesity—BMD
Russell et al. [13]	Ob♀ 14.0 ± 1.9 Cntr ♀ 15.9 ± 1.7	34.4 ± 7.1 kg/m ² 21.7 ± 1.9 kg/m ²	Leptin, IL-6, adiponectin	DXA (whole body, spine, hip) MRI (VAT, SAT) Bone age		BMD, BMAD ↔ SAT + BMD VAT – BMD Leptin ↔ ♀ leptin + BMD Adiponectin ↔ Adiponectin – BMD
Klein et al. [14]	Ob 8.9 ± 1.8 Cntr 10.0 ± 1.9	3.9 ± 2.2 SD –0.2 ± 0.8 SD	Leptin, insulin, glucose, estradiol, GH, IGF-1	DXA (whole body) Bone age (radiograph of the left hand)		BMD ↔ Leptin ↔ Ob ♀ vs Ob ♂ leptin ↑ BMD ↓
Rocher et al. [15]	Ob 10.7 ± 1.2 Cntr 10.9 ± 1.1	8.0 ± 4.5 kg/m ² 16.7 ± 1.8 kg/m ²		DXA (whole body, LS) QUS (n.d. calcaneus)		BMD ↓ Regional BMD ↓
Dimitri et al. [16]	Ob 12.1 ± 2.9 Cntr 10.6 ± 3.2	3.3 ± 0.6 SD 0.2 ± 1.0 SD		DXA (whole body, LS, ultra distal radius, 33 % radius) DXA (whole body)	PA questionnaire	BMD ↓ Regional BMD ↓ BMD ↑
Ellis et al. [17]	Ob, Ov, Cntr 11–12 years	Ob ♀ 36.5 ± 5.0 % Ob ♂ 36.1 ± 4.2 % Cntr ♀ 19.9 ± 3.6 % Cntr ♂ 14.9 ± 4.8 %				
Leonard et al. [18]	Ob 9.8 ± 2.9 Cntr 9.9 ± 3.9	28.1 ± 4.9 kg/m ² 16.9 ± 2.3 kg/m ²		DXA (whole body, LS)		BMD ↑
Vandewalle et al. [19]	Ob ♂ 14.4 ± 2.3 Age matched cntr ♂ 14.4 ± 2.3 Bone age matched cntr ♂ 15.0 ± 2.0	2.5 ± 0.4 SD –0.2 ± 0.9 SD –0.4 ± 1.0 SD	Leptin, IGF-1, SHBG, E1, E2, testosterone	pQCT [n.d. radius (4; 66 %) n.d. tibia (4; 38 %)] Bone age: X-ray left hand and wrist DIP (2nd metatarsal left hand) Biological impedance (% fat) Bone age	Ground reaction force platform	BMD ↑ Leptin ↑
Nagasaki et al. [20]	Ob ♀ 10.1 ± 2.3 Ob ♂ 10.2 ± 2.0	33.4 ± 4.0 % fat 32.4 ± 4.3 % fat	Insulin, glucose			Prepubertal stage ↑ BMD (♀ 7–9 yo, ♂ 7–11 yo.) Postpubertal stage ↓ BMD (♀ up to 11 ♂ up to 12) Ob ♀ BMD ↑ Ob ♂ % fat mass – bone
Pollock et al. [23]	Ow ♀ 18.4 ± 0.5 Cntr ♀ 18.2 ± 0.4	25.0 ± 2.8 kg/m ² 21.7 ± 1.9 kg/m ²		DXA (whole body) pQCT [n.d. tibia (4; 20 %), n.d. radius (4; 20 %)]	7-day recall PA questionnaire + 3-day diet records	

Table 1 continued

References	Sample (age ^a)	BMI	Blood sample	Densitometry measures	Other measures	Adipocytes/osteocytes interactions
Rhie et al. [24]	Ob ♀ 8.3 ± 1.2 Ctr ♀ 8.8 ± 1.3	21.6 ± 2.5 kg/m ² 16.8 ± 1.6 kg/m ²	Insulin, glucose, leptin, adiponectin, osteocalcin	DXA (L2-L4, femoral neck) Bioelectrical impedance		% fat mass – bone Leptin ↑ ♀ leptin + BMD Adiponectin ↔ Adiponectin – BMD Osteocalcin ↔
Shaikh et al. [25]	Hypothalamic Ob 6–18 Congenital hypopituitarism 6–17 Simple Ob 6–14 Ob ♀ 16.6 ± 1.6 Ob ♂ 16.0 ± 1.9	2.0–4.9 SD –0.2 to 5.2 SD 2.2–4.7 Sd 36.6 ± 4.8 kg/m ² 36.3 ± 4.4 kg/m ²	Leptin, adiponectin Leptin, adiponectin, IR	DXA (whole body) Calcium FMI and FFM	Triaxial accelerometers 7 days	% fat mass + bone
Campos et al. [26]	Ob ♀ 16.6 ± 1.6 Ob ♂ 16.0 ± 1.9	36.6 ± 4.8 kg/m ² 36.3 ± 4.4 kg/m ²	Leptin, adiponectin, IR	DXA (whole body) US (VAT, SAT)		SAT + BMD VAT – BMD Ob ♀ vs Ob ♂ leptin ↑ Ob ♀ leptin – BMD Adiponectin ↔
Laddu et al. [28]	B 10.6 ± 1.1 E 12.7 ± 1.1	18.34 ± 3.2 kg/m ² 20.2 ± 3.7 kg/m ²		DXA (whole body) pQCT (n.d. femur (4;20 %), n.d. tibia (4;66 %))	2 years measure PA questionnaire Diet questionnaire	TBFM + bone strength and BMD AFM + bone strength and BMD ↑ AFM – cortical vBMD
Dimitri et al. [29]	Ob 12.9 ± 2.9 Ctr 10.6 ± 3.2	3.3 ± 0.6 SD 0.2 ± 1.1 Sd	Free leptin, adiponectin, RANK-L, OPG, DKK1, CTx, PINP	DXA (total body fat, truncal fat mass) Bone age (radiography of the left wrist)		Leptin ↑ Adiponectin ↓ OPG ↓ (↓ when prior fracture)
Do Prado et al. [32]	Ob ♀ 16.7 ± 1.7 Ob ♂ 17.1 ± 1.6	35.1 ± 4.1 kg/m ² 36.0 ± 3.7 kg/m ²	Leptin, IR, insulin	DXA (whole body)		Ob ♀ vs Ob ♂ leptin ↑ Ob ♂ leptin – BMD
Abseyi et al. [39]	Ob ♀ 11.8 ± 3.2 Ob ♂ 12.3 ± 3.7	29.2 ± 4.8 kg/m ² 27.9 ± 4.4 kg/m ²	Insulin, IR, glucose, osteocalcin, adiponectin			Ob ♀ leptin ↑ Adiponectin ↔ MES adiponectin ↓ Pubertal stage ∅ osteocalcin
Giranty-Bogacka et al. [43]	Ob 13.8 ± 2.6	30.1 ± 4.4 kg/m ²	Insulin, IR, glucose, total osteocalcin	Biomechanics Tanita (% body fat mass, fat mass)		Ob ♀ osteocalcin ↓ Osteocalcin ∅ MES Pubertal stage ∅ osteocalcin Ob ♀ osteocalcin ↓ Osteocalcin – IR

♀ female, ♂ male, B baseline, E end, Ob obese, ctr control, IR insulin resistance, MES metabolic syndrome, DXA dual-energy X-ray absorptiometry, US ultrasound, QUS quantitative ultrasound, DIP digital image processing, n.d. non-dominant, TBFM total body fat mass, AFM android FM, ↔ no differences, ↑ higher, ↓ lower, + positive, – negative, ∅ no interaction

^a Data for ages are presented as mean ± SD

extracellular matrix of bone and directly reflects bone metabolism [41]. Osteocalcin is expressed in two different forms that have two different functions: the carboxylated (cOC) and uncarboxylated (unOC) forms. Carboxylated osteocalcin is thought to be the active form in the bone. Carboxylated osteocalcin also has a high affinity for hydroxyapatite and is mainly stocked in the bone matrix during osteoblast mineralization [42]. Alternatively, uncarboxylated osteocalcin is suggested to act on energy metabolism. Specifically, in response to decreased osteoblast proliferation via the central action of leptin, osteoblasts influence energy metabolism by expressing a product of the *Esp* gene (osteostecticular protein tyrosine phosphatase, OT-PTP). OT-PTP inhibits the carboxylated form of osteocalcin. Consequently, the uncarboxylated form permits the β cell proliferation and insulin secretion in the pancreas and also stimulates adiponectin secretion in adipocytes [43]. Although interest in osteocalcin has increased recently, uncertainty surrounds a greater understanding of the carboxylated form of osteocalcin. This remains problematic because most of the time, the uncarboxylated and carboxylated forms have not been analyzed separately.

Independent of form, pubertal status does not appear to interact with osteocalcin levels [40, 44]. However, the level of circulating osteocalcin remains poorly documented. For example, no differences have been observed in osteocalcin levels between obese and nonobese children and adolescents [24], and in contrast [40, 44], lower levels of osteocalcin have been reported in 5–18-year-old obese girls. Moreover, no association between osteocalcin levels and the presence of metabolic syndrome has been reported in a population aged between 5 and 18 years [40], which contrasts with other reports of a negative correlation with an insulin resistance index [44]. Although this was not in obese youth, Rosen's research group [45] confirmed the potential role of ucOC in the skeletal regulation of energy metabolism in nonobese postmenopausal women. They effectively found that an increase in unOC was associated with decreased body fat and increased adiponectin levels. Further analyses are needed in the obese adolescent population to better understand the implication of osteocalcin for adipocyte/osteocyte interaction, more specifically, the uncarboxylated form, which regulates glucose homeostasis [46].

Sclerostin, a protein secreted by osteocytes through the *SOST* gene, acts on bone formation by means of inhibiting osteoblast activity [47] and concomitantly osteocalcin secretion. Mechanical stress (such as exercise) influences circulating sclerostin levels. An activation of the canonical Wnt signaling pathway leads to osteoblastic differentiation, proliferation and activity, resulting in enhanced bone formation. However, this pathway can be antagonized by secreted inhibitors binding to lipoprotein receptor-related

protein 5/6 (LRP) [48]. LRP5 plays an important role in the development and maintenance of bones by acting on the regulation of BMD. Sclerostin appears to be the main inhibitor involved in states of mechanical loading and unloading [49]. Sclerostin is yet to be investigated in obese adolescents; however, its effects on obese older adults have been reported [50]. In these older adults, exercise prevented the increase of sclerostin in a similar way to how exercise prevents bone loss and increases bone marker turnover.

Effects of weight loss on bone-adipocyte cross talk

Observations on the effectiveness of weight loss therapy improving overall health in obese individuals remain limited by the concomitant side effects (i.e., loss of bone mass). An understanding of the effects of weight loss programs on the bone-adipocyte cross-talk in adolescents is required in order to prescribe effective and safe weight loss programs. Protocols combining nutritional, physical activity and psychological approaches [51–54], nutritional and physical activity [55, 56] or physical intervention alone [41] have been proposed. However, the exact mechanisms for weight loss induced-bone loss remain unknown, and hypotheses have been proposed involving alterations of some bone-regulating hormones. Longitudinal studies are reported in Table 2.

First, programs only based on physical activity appear counterproductive to bone density accretion in obese adolescents. Basically, the impact of physical activities (aerobic activities) on skeletal changes was questioned, and three groups were involved: obese trained, obese untrained and for baseline only, an age-matched nonobese group [41]. The two obese groups undertook a 6-month physical activity program. Three findings emerged. First, normal-weight and obese adolescents had similar whole-body BMDs, leptin levels, adiponectin levels and osteocalcin concentrations at baseline. Second, after 6 months of physical activity both the trained and untrained obese groups improved their whole-body BMDs compared with the baseline values of the normal weight control group. However, only the trained group increased insulin, osteocalcin and uncarboxylated osteocalcin levels and decreased adiponectin levels compared with normal-weight control and obese baseline measures. Third, the uncarboxylated form of osteocalcin for the obese trained group was significantly higher than in the obese untrained group after the 6-month program. The lack of a significant difference in total osteocalcin levels between trained and untrained obese adolescents after 6 months may have masked the observed increase in the level of uncarboxylated osteocalcin while the level of the carboxylated form decreased, up-regulating glucose homeostasis.

Table 2 Effects of weight loss on the bone-adipocyte cross-talk

References	Sample (age ^b)	BMI (kg/m ²)	Blood sample	Densitometry	Other measures	Period	PA	Nutr	Psy	Results
Rocheffort et al. [40]	Ob 10.27 ± 0.92 Cnr 10.59 ± 0.96	25.56 ± 2.91 16.48 ± 1.89	B Insulin adiponectin Osteocalcin (total and unOC)	DXA (WB)	PA: self reported Q Diet: self Q	6 months	2/w supervised AT (90 min): cycling, rowing, jumping, games, hip-hop			B Ob ↑ BMD 6 months Trained Ob and untrained Ob ↔ BMD Trained Ob vs enr ↑ osteocalcin (↑unOC) Trained Ob vs enr ↓ adiponectin ↑ BMC, lean mass ↓ BMD, FM
Campos et al. [50]	Ob 17 ± 1.7	B 35.8 ± 5.3 Y 32.2 ± 5.0	IR Insulin Leptin adiponectin	DXA (WB) US (VAT, SAT)	Nutr: 3-day record (start + end) Psy: BES Q (during therapy)	1 year	3/w supervised AT (30 min): treadmill RT (30 min)	Hypo caloric diet + diet lesson 1/w	1/week	
Campos et al. [51]	Ob 16 ± 1.5	37.2 ± 4.8	Leptin Ghrelin Adiponectin	DXA US (VAT, SAT)	Nutr: 3-day record (start + end)	1 year	3/w supervised AT (30 min): treadmill RT (30 min)	Food diet + diet lesson 1/w		↓ BMD, BMD, FM ↑ adiponectin B ∅ Y ghrelin levels Ghrelin – BMD B ↓ adiponectin, osteocalcin B ↑ leptin, IR Y ↑ adiponectin, osteocalcin Y ↓ leptin, IR ↑ BMC ↓ BMD, FM
Reinehr and Roth [52]	Ob 10.9 ± 0.3 Cnr 11.6 ± 0.4	28.3 ± 0.5 17.0 ± 0.5	Leptin Insulin Adiponectin Glucose Osteocalcin			1 year	1/w: ballgames, jogging, trampolene jumping	Fat and sugar reduced diet	Yes	
Stettler et al. [53]	Ob 14.5 ± 1.1 Cnr 13.3 ± 2.7	36.8 ± 3.7 21.1 ± 5.4		DXA (WB, L,S)		1 year	120 min aerobic/w	1200–1500 kcal/day	Group sessions	↑ BMC ↓ BMD, FM
Campos et al. [54]	Ob AT B 16.1 ± 1.27 Ob AT Y 16.9 ± 1.8 Ob AT + RT B 16.3 ± 1.5 Ob AT + RT Y 17.1 ± 1.5	35.82 ± 4.52 32.06 ± 4.92 37.6 ± 5.44 32.6 ± 4.56	Leptin Ghrelin Adiponectin IR	DXA (WB) US (VAT, SAT)	Nutr: 3-day record (start + end) Psy: BES Q (during therapy)	1 year	3/w supervised AT (60 min): treadmill AT (30 min) RT (30 min): training treadmill, cycle, strength	Food restrict	Body image, self esteem, family problems, eating disorders	↑ BMC ↓ BMD, FM AT + RT ↓ VAT/ SAT ratio AT + RT ↑ lean mass B AT + RT ↓ ghrelin Y AT + RT ↓ ghrelin

Table 2 continued

References	Sample (age ^a)	BMI (kg/m ²)	Blood sample	Densitometry	Other measures	Period	PA	Nutr	Psy	Results
Gajewska et al. [55]	Ob 8.3 Ctr 8.3	B 24.6 M 22.9 B 15.6	Leptin BLAP Adiponectin	DXA (WB)	Nutr: 3-day record PA, parents Q	3 months		Low energy diet		↑ BLAP (decreased compared to baseline) ↓ leptin ↑ adiponectin ↓ leptin ∅ adiponectin
Bliher et al. [56]	Ob 12.2 ± 0.2	B 30.6 Y 30.6	Insulin Glucose Leptin adiponectin			1 year	150 min/w 90 min supervised AT + RT			

♀ female, ♂ male, Ob obese, ctr control, B baseline, Y year, M months, AT aerobic training, RT resistance training, PA physical activity, Nutr nutrition, Psy psychology, Q questionnaire, WB whole body, LS lumbar spine, IR insulin resistance, DXA dual-energy X-ray absorptiometry, US ultrasound, n.d. non-dominant, unOC uncarboxylated osteocalcin, ↔ no differences, ↑ higher, ↓ lower, + positive, - negative, ∅ no interaction

^a Data for ages are presented as mean ± SD

It is possible that the nature of the physical activity program may have been perceived as easy for the trained group and challenging for the untrained group. Also, the absence of details of nutritional care may further explain the lack of significant differences between both obese groups after the 6-month program. However, the absence of measures of body composition and fat distribution before and after the 6-month program was a limitation of the study. In addition, differences in BMD between both obese groups after 6 months and the control group (baseline measure) might be attributed to body growth and/or advanced pubertal status among the obese group.

Second, BMI and fat mass significantly decreased during the weight loss programs combining nutrition and physical activities in interventions ranging in time from 3 [56] to 12 [51–54] months' duration. However, the effects of such programs on the dialogue between adipokine hormones and bone density remain uncertain. Physical activities [aerobic training (AT) only or a combination program including aerobic and resistance training (RT)] were performed once, twice or three times a week with exercise sessions ranging in duration from 60 to 150 min. Consistently, nutritional interventions for obese groups have comprised balanced but somewhat restricted dietary recommendations. When comparing blood samples, controversial results are observed. Indeed, some researchers have reported a substantial weight loss was associated with higher osteocalcin levels [53], lower leptin levels [53, 56, 57] and higher adiponectin levels [22, 51, 53], while others found no changes in adiponectin [51, 57] and leptin levels [51, 52] compared with baseline measures. Moreover, densitometry results have shown that the BMC appeared to be higher in obese than normal-weight children at baseline. However, after a 12-month weight-loss program, studies showed no change in BMC [52, 54] with a decrease in total bone density [52] or a decrease in the upper and lower limbs and increases in whole-body and lumbar spine bone density [54]. However, increases in BMC without changes in BMD have also been reported [51]. The absence of changes in BMD may be related to other results reporting that after 3 months of weight loss, there were lower levels of bone alkaline phosphatase, which is a sensitive and reliable indicator of bone formation [56].

All studies agreed on outcomes of decreases in BMI and fat mass after combined physical activity and nutrition weight loss programs. However, results have highlighted discrepancies in associations between adipokines and bone density that may be partially explained by gender differences. Indeed, all of the aforementioned studies were mixed gender [51, 53, 54, 56], with the exception of one girls-only study [52].

Third, a more prescriptive combination of AT/RT plus nutrition appears to be the most reliable program for

sustaining weight loss and reducing bone loss. Weight loss and associated changes in the VAT/SAT ratio, lean mass and bone quality were made through comparisons of programs involving nutrition plus AT vs. nutrition and AT + RT in obese adolescents [55]. The AT + RT group had higher BMC values than the AT group, who showed a decreased BMC. However, BMD remained unchanged in both subgroups. Although both groups decreased their VAT, stronger results were observed in the AT + RT group. SAT also decreased significantly in both groups, as did insulin, but increased adiponectin and decreased leptin concentrations were only observed in AT + RT. This study was the first to investigate the relationship between ghrelin and bone metabolism in response to weight loss in obese adolescents. Although modification of the ghrelin levels (in both groups) was not observed, the potential role of ghrelin was discussed as a predictor of reduced total BMD. Specifically, a potentially negative correlation with bone density was postulated in obese girls. However, cautious interpretation of results is required because of the unequal baseline values between groups (with the AT + RT group showing lower baseline ghrelin concentrations) [55]. Moreover, in their study total ghrelin was analyzed without subanalysis of the acylated form, which has a major role in appetite regulation [58]. Despite the importance of combining exercise, results remain uncertain on the role of adipokines on bone after weight loss. Responses to this dimorphism need further analysis to better understand the effects of puberty and gender on the adipocyte-bone cross-talk after substantial weight loss.

Limitations

Currently, the literature provides contradictory results regarding the impact of bone-adipocyte interactions on obese adolescents, confounded by the limited number of studies in this population (12 studies involved 3 to 20 year olds, 4 studies 9–13-year-old adolescents, 8 studies 14–19-year-old adolescents and 4 unknown age range).

The large heterogeneity in study designs (including gender, age, pubertal status, maturation stage) may explain the inability to find a consensus and highlights the inconsistencies in methodologies. Indeed, most studies did not take into consideration sex-hormone status and pubertal stage, which are important factors as adolescence is a critical period of development and hormone secretion that may or may not alter obesity. As previously stated, the study age ranges often crossed puberty, which may have masked growth-stimulated responses. Also, the accuracy of the techniques used to measure parameters might contribute to conflicting findings. For example, DXA, US and bioimpedance techniques do not provide direct measures

of visceral fat, nor can they differentiate between visceral and subcutaneous fat. Moreover, most of the bone density measurements were conducted on whole-body DXA scans. Site-specific DXA measurements such as the lumbar spine or hip give more precise information about bone fragility. However, DXA does not provide the bone architecture and subsequently does not reflect structural changes due to growth or mechanical loading [59]. In order to better understand structural changes in the bones, blood and/or urine markers of bone metabolism are required. Few studies of adolescents with obesity have analyzed bone markers such as osteocalcin; further, when the osteocalcin level is reported, the osteocalcin carboxylated and uncarboxylated forms have not been differentiated. The lack of differentiation results in questioning of the precision and reliability that clearly limit the interpretation of data. Also, to our knowledge, no study in obese adolescents has assessed sclerostin, a protein suggested to have deleterious effects on bones. Moreover, as the fundamental cause of obesity is an energy imbalance between calories consumed and calories expended, a ghrelin analysis, more precisely the acylated form and total ghrelin, seems to be a useful additional analysis.

In conclusion, the review emphasized many possible interactions between adiposity and bone for adolescents, but the low volume and inconsistent methodologies of existing studies prevent strong conclusions about the presence of definitive relationships. Understanding the local interactions between the released products by fat tissue and hormones produced in bone tissue requires further investigations. Assessing the bone-adipocyte cross-talk in large cohort studies and measuring participants on a regular basis (i.e., every 2 years, following a longitudinal design) may be helpful in understanding the maturation effect, gender effects, adipocyte effects, osteocyte effects, bone-adipocyte cross-talk, etc. Large cohorts of prepubertal adolescents allow researchers to provide important health-related information with a reduced impact of dropout. This kind of study is well developed in North America, for example, the "Quality study" [60].

Moreover, longitudinal studies focusing on adipocyte-osteocyte interactions after a weight loss program combining physical activity and diet could explain the mechanisms of the body's responses to the strategies of weight loss. Longitudinal studies could also report any side effects encountered by these interventions. To our knowledge, no studies to date have investigated the effects of exercise and weight loss programs with an advanced understanding of adipokine markers, quantitative measure of bone mass, biologic markers of bones, appetite regulator markers and growth markers.

Conflict of interest All authors have no conflicts of interest.

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BMJ Open Cross-sectional and longitudinal study protocols of the 'ADiPosity and BOne metabolism: effects of eXercise-induced weight loss in obese adolescents' (ADIBOX) project

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ABSTRACT

Introduction: A need exists for sustainable and clinically effective weight management interventions, suitable for preventing well-linked chronic disease such as diabetes and cardiovascular disease and some less investigated secondary conditions such as bone alteration. The ADiPosity and BOne metabolism: effects of eXercise-induced weight loss in obese adolescents (ADIBOX) protocol was designed to provide a better understanding of the interaction between adipokines and bone hormones in adolescents with obesity and how a 10-month physical activity programme may affect these interactions.

Methods and analysis: The ADIBOX protocol combines 2 studies. The first study involves a total of 68 adolescents aged 12–16 years. This cross-sectional study will include both males and females (1:1 ratio), either living with obesity/overweight (n=34; body mass index (BMI) \leq 97th centile and \geq 85th centile) or normal weight (n=34; BMI <85th centile). The second study is a longitudinal study that will include 50 obese adolescent girls and track them over a period of 42 weeks. Weight loss programme will consist of a combination of physical activity and a normocaloric diet. Bone and adiposity-related measurements will be performed every 14 weeks. Both studies will assess participants' anthropometric profile, nutrition and physical activity, body composition, bone densitometry and blood markers of bone, growth and adiposity.

Ethics and dissemination: The ADIBOX protocol complies with the ethics guidelines for clinical research and has been approved by their respective ethics committee (Australian Catholic University Committee Ethic, Australia and Hospital Sud Est 1 committee, France). Findings from this protocol are expected to clarify the possible interactions between adiposity and bone in childhood obesity and will be disseminated at several research conferences and published articles in peer-reviewed journals.

Trial registration number: NCT02626273; Pre-results.

Strengths and limitations of this study

- Advancing the understanding of the bone adipocyte cross-talk in obese adolescents.
- Investigating the effects of physical activity-induced weight loss on bone, growth and adiposity markers.
- Longitudinal study only assessing female adolescents.
- Unable to accurately account for the different phases of the female menstrual cycle.

INTRODUCTION

The complex consequences of childhood obesity represent major concerns in most developed countries,¹ largely contributing to metabolic complications with costly repercussions for the burden of disease.^{2–5} This burden is exemplified by high prevalence rates of overweight or obesity. For instance, in Australia the prevalence of overweight and obesity is 24% in boys and 23% in girls, while in France the prevalence is 20% of boys and 16% of girls.⁴

Understanding the effects of obesity-induced fat mass accumulation on bone during growth is of particular interest given that obesity is a risk factor for fracture during the peripubertal period.⁵ Childhood and adolescence are characterised by significant bone accrual. The achievement of optimal skeletal gains throughout the maturation process is crucial in order to optimise bone mass before adulthood. However, the levels of some circulating hormones, either dependent or independent of fat mass, vary widely from childhood to puberty and adulthood and may subsequently strongly affect bone density.

Owing to their common origin, bone cells and adipocytes are intimately associated, suggesting a cross-talk between adipose tissue and bone tissue. Adipose tissue has long been considered an inert tissue dedicated for energy storage. Recent advances have established that both adipose tissue and bone tissue are endocrine organs. Adipose tissue is involved in satiety, energy balance and pubertal development,⁶ while bone tissue acts on energy expenditure and glucose homeostasis.⁷

The impact of obesity on bone metabolism is gaining the attention of researchers. The skeletal system is stressed from mechanical loading and also through the metabolic effect of some of the adipokines secreted by the adipose tissue. Indeed, obesity leads to hormonal alterations associated with increasing proinflammatory cytokines and oxidative stress. These events favour the accumulation of fat mass and loss of bone mass. Moreover, weight loss induced by dietary restriction can lead to weakening bones.⁸

Decreased mechanical loading on the skeleton,⁹ altered hormonal secretion involved in bone regulation¹⁰ or decrease of caloric intake⁹ may contribute to the bone breakdown generated by weight loss strategies during childhood. However, weight bearing physical activity may be anabolic for bone, even during periods of weight loss.

A better understanding of the effects of weight loss programmes on the bone adipocyte cross-talk in adolescents is required in order to prescribe effective and safe weight loss programmes. As previously shown, the effectiveness of weight loss programmes for improving the overall health in obese youth may be compromised by potential side effects (ie, loss of bone mass).

Our team recently justified the need of further investigations exploring the bone adiposity cross-talk in adolescents with obesity.¹¹ Fat and bone are linked by multiple possible interactions but the low volume and inconsistent methodologies of existing studies prevent strong conclusions about the presence of definitive relationship. A specific project is required to advance the understanding of the complex relationship between fat mass and bone mass in adolescents with obesity.

The overall purpose of this work is to investigate the effects of body mass and its variations (taking into account body composition) on the bone adipocyte cross-talk in adolescents with obesity. To do so, a cross-sectional and a longitudinal study will be conducted. The ADIposity and BOne metabolism: effects of eXercise-induced weight loss in obese adolescents (ADIBOX) protocol will contribute to the evidence required to promote the holistic significance of sustainable weight management in obese youth.

METHODS AND ANALYSIS

Cross-sectional study—study I

Protocol design

This study will investigate whether the interaction between adipokines and bone hormones differ between obese and their normal weight peers (figure 1).

Selection criteria

Adolescents aged between 12 and 16 years, over Tanner stage 3, free of any recent history of hospitalisation (past 2 years) or of systemic illness lasting more than 2 weeks in the past 12 months will be recruited. In compliance with Human Ethics guidelines, adolescents and their legal representatives will sign assent and consent forms, respectively. Also, the recruited adolescents will not have any known history of metabolic bone or muscle disease, nor metabolic diseases such as diabetes, insulin resistance, or hypothyroid or hyperthyroid activity.

Additional inclusion criteria relate to being free from a diagnosis of congenital cardiovascular disease, not regularly consuming alcohol, being a non-smoker and not taking medication known to alter bone metabolism, nor hormones or calcium preparations (vitamin D, calcium, protein). Owing to the low exposure to radiation, women will be excluded if they are pregnant and will need to have a regular menstrual cycle. Obese men and women recruited for this study will have a body mass index (BMI) ≥ 95 th centile.¹² Furthermore, adolescents who are overweight or with obesity will be ineligible if they were enrolled in a weight management programme during the past 2 years.

Age-matched and gender-matched normal weight participants (BMI < 85th centile)¹² will also be recruited. If necessary, in order to include a sufficient number of participants, we will consider recruiting both obese and overweight (BMI ≥ 85 th centile) adolescents. Normal weight, overweight and obese adolescents will be excluded if they participate in more than 250 min of physical activity outside of school per week. The 250 min was derived by excluding the physical education and school sport from guidelines recommending 60 min per day or 420 min per week.¹³ This ensured that none of the participants exceeded the recommended guidelines for physical activity. Minutes of weekly physical activity will be monitored during the screening visit using the International Physical Activity Questionnaire (IPAQ).

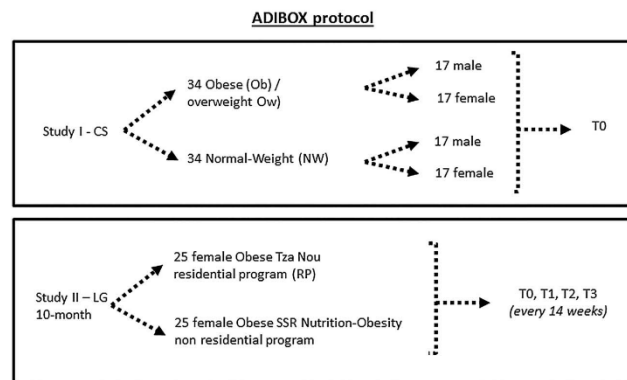
Power analysis

Owing to a lack of data and reported discrepancies in the literature it seemed difficult to propose an a priori accurate sample size estimation. The number of participants to be included in the study has been extrapolated from the data obtained on previous works.¹⁴ For a two-side type I error at 5% and a statistical power equivalent to 80%, 17 participants of each sex per group of obese and lean body composition status (a total of 68 participants) will allow us to highlight a clinical and realistic effects size of 1 SD between groups in key obesity outcomes.¹⁵

Participants

As previously stated participants engaged in this protocol will be adolescents aged between 12 and 16 years, with a self-reported pubertal development status equal to or exceeds Tanner stage 3. Differences in race and

Figure 1 ADIBOX protocol. ADIBOX, ADIposity and BOne metabolism: effects of eXercise-induced weight loss in obese adolescents; CS, cross-sectional study; LG, longitudinal study.



ethnicity of participants will be accepted. The adolescent stage of development was selected to advance the understanding of maturation processes, growth changes and the possible exploratory aspects of weight changes. For this study, we will recruit both male and female adolescents who are obese or overweight (1:1 ratio) as well as their age-matched and sex-matched lean peers. The non-obese group is necessary for a better understanding of the hormonal changes induced by obesity.

Participant recruitment

Following approval from ethic committees, and based on our calculation, a total of 68 adolescents, men and women will be enrolled in the first study of the ADIBOX protocol. All participants as well as their legal representatives will be given written information regarding the project and be asked to sign consent and assent forms. Adolescents will be recruited from medical clinics in Melbourne and Sydney, Australia, and from the Australian Catholic University (Victoria or New South Wales—Australia) using the 'snowballing' principle. The 68 adolescents will be split into two groups of 34 each; a non-obese group and an obese/overweight group. Groups will comprise 17 men and 17 women, in order to understand more about sex-based comparisons.

Measurements

After a screening visit to ensure the suitability of adolescents to complete the study, each adolescent will perform a battery of tests. Data collection will be performed only once (figure 2).

Ability to complete the study

The researcher will make a phone call prior to the beginning of the study to ensure the suitability of adolescents to complete it. If the participant is willing to participate, general information such as medical history of the family, early childhood development and history of obesity will be gathered during this phone call.

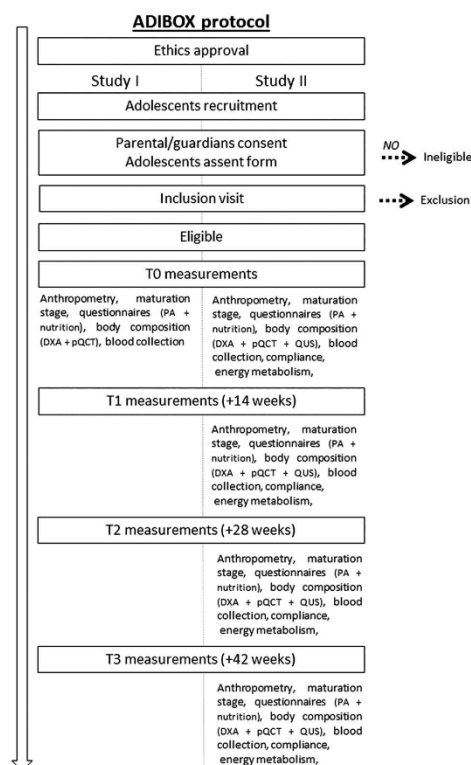


Figure 2 ADIBOX protocol overview. ADIBOX, ADIposity and BOne metabolism: effects of eXercise-induced weight loss in obese adolescents; DXA, dual energy X ray absorptiometry; PA, physical activity; pQCT, peripheral quantitative CT; QUS, quantitative ultrasound.

Maturation

Pubertal status will be assessed using the Tanner's Stage of Pubertal Development model for biological

maturation. An experienced researcher will be provided with a series of pictures showing the five stages of puberty for breast or genitalia and pubic hair development.¹⁶ Despite the fact that self-reported maturation stages is less precise than a paediatric assessment it still has a good validity and reliability.¹⁷

Anthropometry

Anthropometric measurements will be taken according to the anthropometrics recommendations of the International Society for the Advancement of Kinanthropometry for standing height (m) and body mass (kg), waist circumference (cm), and lower limb bone lengths/breadths (cm).¹⁸

Physical activity and nutrition questionnaires

Among participants, validated sedentary activity¹⁹ and physical activity questionnaires (IPAQ)²⁰ will be distributed to assess physical activity. In addition, a food frequency questionnaire,²¹ current eating habits questionnaire²² and a food preferences questionnaire²³ will be distributed.

Body composition

Body composition will be measured by dual energy X ray absorptiometry (DXA) (DXA, iDXA, GE healthcare, Lunar Corporation, Madison, Wisconsin, USA). Bone mineral density (BMD, g/cm^2), bone mineral content (BMC, g), bone area (cm^2), and lean and fat mass (subcutaneous and visceral) will be determined for each adolescent. The DXA measurements will be taken for the whole body, lumbar spine (L2–L4) and the non-dominant hip (including the femoral neck, trochanteric and intertrochanteric regions). All DXA scans will be conducted by the same technician and quality assurance checks will be performed routinely. This scanning protocol and offline analyses have been validated among comparable population.²⁴

Peripheral Quantitative CT

Musculoskeletal parameters for bone geometry including bone strength will be obtained using a peripheral quantitative CT (pQCT) XCT 2000 in study I (XCT 2000, Stratec Medizintechnik, Pforzheim, Germany). BMC (g/cm), volumetric cortical and trabecular BMC (mg/cm^3), total area (mm^2), cortical and trabecular area (mm^2) and density (g/cm^2), bone strength (mm^3) will be assessed at the distal (4%), proximal (66%) site of the non-dominant tibia and radius. A planar scout scan was first conducted to determine the anatomical reference line for the radius and tibia. Tomographic slices of 1 mm thickness were obtained at the 4% and 66% sites measured distally. Scan speed and voxel size were 30 mm/s and 0.5 mm, respectively. To assure quality of measurement, calibration checks will be performed by scanning a standard phantom with known densities, prior to each scan.

In order to calculate BMC, volumetric cortical BMC, volumetric trabecular BMC, cortical area (CoA), cortical density (CoD), trabecular area (TrA), trabecular density (TrD) and stress-strain index (SSI) will be analysed with Stratec pQCT software. Contour mode 1 with a threshold of $180 \text{ mg}/\text{cm}^3$ was used to separate soft tissue and bone in order to analyse trabecular bone. Cortical bone was identified and removed using a constant default threshold of $710 \text{ mg}/\text{cm}^3$. A contour mode 3 with peel mode 1 at a threshold of $40 \text{ mg}/\text{cm}^3$ was used to assess muscle and fat cross-sectional area. Radial distribution and polar density will be estimated with an open source bone image analysis tool, known as Image J (rsbweb.nih.gov/ij). To differentiate the cortical bone a threshold of $710 \text{ mg}/\text{cm}^3$ and a 3x3 median filtering of the image was used. For mean cortical polar bone mass distribution and polar distribution the cortex is divided into equal sectors, while the 66% slice radial distribution will be estimated by subdividing the cortex into concentric rings. For more information, this open source software was validated and described in two studies of Rantalainen *et al.*^{25 26}

Endocrine assays

Blood samples will be collected by a qualified paediatric nurse after the participants have fasted overnight. Blood will be collected by a venipuncture at the brachial vein. After collection, blood will be centrifuged and aliquots will be stored (-80°) for subsequent analysis.

Basic biology (triglycerides (TG), cholesterol, low-density lipoprotein (LDL), high-density lipoprotein cholesterol (HDL), glycaemia, insulin, ultrasensible C reactive protein (CRP)) will be assessed in the biochemistry laboratory of the Australian Catholic University (Sydney, Australia). Bone markers will be assayed in the biochemistry laboratory of the Sydney University (Sydney, Australia): serum osteoprotegerin (OPG), receptor activator of nuclear κB ligand (RANKL), sclerostin, bone alkaline phosphatase and undercarboxylated osteocalcin (uOc) will be assayed using ELISA kits while type I collagen C-telopeptides (CTX), PINP, osteocalcin total and vitamin D by Cobas 6000 (Roche Diagnostics).

All other biochemical determination (leptin, adiponectin, tumour necrosis factor (TNF)- α , interleukin (IL)-6, growth hormone (GH), insulin-like growth factor-1 (IGF1), insulin-like growth factor-binding protein 3 (IGFBP-3), oestradiol, follicle stimulating hormone/luteinizing hormone (FSH/LH) and parathyroid hormone (PTH)/calcitonin) will be made using commercial kits, following manufacturers' recommendations, including sampling steps, allowing the best performances of coefficient of variation and sensitivity. All analyses will be conducted in duplicate by the same technician.

Statistical analysis

Data will be analysed using Stata (StataCorp, College Station, Texas, USA) and significance will be accepted at

a two-sided α level of $p < 0.05$. After testing for normal distribution (Shapiro-Wilk test), data will be treated either by parametric or non-parametric analyses according to statistical assumptions. Means and SDs will be reported for descriptive statistics and used to summarise the data. In case of non-Gaussian distribution, median and IQR will be reported. For general linear model (GLM) or multilevel model, the data will be log transformed or Box-Cox transformed, where appropriate. Group comparisons will be assessed using analysis of covariance (ANCOVA) to compare quantitative parameters between obese and lean adolescents, as well as for gender comparison after controlling for pubertal status.

Ethical considerations and dissemination

The cross-sectional study has been approved by the Australian Catholic University Ethic Committee (2014 320N). In accordance with ethical considerations, the principal investigator is responsible of ensuring that participants understand the potential risks and benefits of taking part in the study. Moreover, the principal investigator is responsible for obtaining a written consent from adolescents and their legal guardians/parents.

Ten-month longitudinal study—study II

Protocol design

This 42-week longitudinal study with repeated measures on four occasions (baseline and thereafter every 14 weeks) will allow us to understand the effect of physical activity-induced weight loss on the bone adipocyte cross-talk in obese adolescent females (figure 1). For this study, we will compare two groups of obese adolescents: one following a residential weight loss programme and compare their results to a group not receiving any intervention or advice for weight loss.

Selection criteria

Similarly to the cross-sectional study, obese females (BMI > 95th centile)¹² who are invited to take part in this study will be aged between 12 and 16 years, with a self-reported pubertal status equal to or exceeding Tanner stage 3, free of any recent history of hospitalisation (past 2 years) or of systemic illness lasting more than 2 weeks in the past 12 months. In compliance with Human Ethics guidelines, adolescents and their legal representatives will sign assent and consent forms, respectively, and will have to be covered by social health insurance.

Also, the recruited adolescents will need to have no limitations to being physically active and will not have a known history of metabolic bone or muscle disease, nor metabolic diseases such as diabetes, insulin resistance or hypothyroid or hyperthyroid activity. Additional inclusion criteria relate to being free from a diagnosis of congenital cardiovascular disease, not regularly consuming alcohol, being a non-smoker and not taking medication known to alter bone metabolism, nor hormones or calcium preparations (vitamin D, calcium, protein). Owing to the low exposure to radiation, women will be

excluded if they are pregnant and will need to have a regular menstrual cycle.

Exclusion criteria

Adolescents will be excluded if major treatment and/or protocol deviations are observed by the obesity centre team. In addition, they will be excluded and not considered in the analysis if their compliance to the programme is < 80%. This threshold has been chosen based on our previous experience²⁷ and the fact that adolescents are integrated as a specific institution.

Power analysis

Sample size estimation has been set within the context of the variation index of body fat relative to the variation of a marker of bone mass measured at the lumbar spine. Currently, few articles provide information about the combined effects of body fat and lumbar spine parameters in the literature. However, based on limited previous research²⁸ the index difference of the variation index of body fat, relative to the variation of a marker of bone mass at lumbar spine between groups, of 1.2–1.5 (SDs) can be expected. It seemed reasonable to set the variation index at 1.3 (SDs). Based on this, a sample size of 21 female participants was predicted to highlight statistically significant differences with a statistical power of 90% and a two-sided type I error less of 5%. Anticipating a potential 20% drop-out among participants, a minimum of 25 volunteers per body composition group will be invited to take part in the study.

Participants

As previously stated participants in this second study will be adolescents aged between 12 and 16 years, with a paediatrician-assessed maturation stage equal to or exceeding Tanner stage 3. The adolescent stage of development was selected to advance the understanding of maturation processes, growth changes and the possible exploratory aspects of weight changes. Race and ethnicity of participant is mixed.

As the obesity centre that our study group is associated with is hosting only women, we will restrict recruitment to adolescent women. The community dwelling control group with obesity is necessary in this study to provide useful information on setting the effects of exercise-inducing weight loss on the bone adipocyte cross-talk.

Participant recruitment

Following approval from ethic committees, and based on our calculations, a total of 50 adolescents' girls will be enrolled for the second study of the ADIBOX protocol. All participants as well as their legal representatives will be given written information regarding the project. Both the adolescents and their legal guardians will have to sign consent forms before enrolment of the adolescent. Adolescents will be recruited from the 'Tza Nou' Medical Center for Children and the SSR (ambulatory care and rehabilitation department) Nutrition-Obesity

in Auvergne (France). The participants will be divided into two groups on a convenient basis: an intervention group (25 obese women from 'Tza Nou'—France) who will undertake the residential programme and an obese control group (25 obese women from the SSR Nutrition-Obesity—France) who will remain with their family and in a community setting.

Institution programme

Adolescents from the intervention group will be enrolled at the obesity centre for the whole school year (ie, 10 months). The obesity centre employs a multidisciplinary team to provide the best weight management care to adolescents during their stay. The weight loss programme is an integral part of the obesity centre programme and fundamentally combines physical activity with a normocaloric diet monitored by a dietician. The physical activity programme consists of two training sessions (aerobic and resistance training) per week. Moreover, adolescents will be engaged in two additional sessions per week, consisting in recreational activities such as ball and racquet games, trekking, snowshoeing or swimming.

Measurements

After a screening visit to ensure the suitability of adolescents to complete the group in the study to which they have been assigned, each adolescent will perform a battery of tests (described below). Data collection will be performed four times for the 42-week longitudinal study. Adolescents will be screened at baseline (T0) and thereafter every 14 weeks. This period of testing coincides with school holidays and includes the bone remodelling cycle period of 3 months (figure 2).

Ability to complete the study

A paediatrician will meet participants assigned to the weight management group prior to the beginning of the study to ensure the suitability of adolescent to complete the weight loss protocol. If the participant is willing to participate, general information such as medical history of the family, early childhood development and history of obesity will be gathered by the paediatrician.

Maturation

Pubertal status will be assessed by a paediatrician using Tanner's Stage of Pubertal Development model for biological maturation¹⁶ as well as laboratory blood analyses (oestradiol, testosterone, FSH/LH). Information about the age at menarche and regularity of menstruation will be gathered by the paediatrician.

Anthropometry

Anthropometric measurements will be taken according to the recommendations of the International Society for the Advancement of Kinanthropometry for the following: standing height (m) and body mass (kg), waist

circumference (cm), and lower limb bone length/breadth (cm).¹⁸

Physical activity and nutrition questionnaires

Among participants, validated sedentary activity¹⁹ and physical activity questionnaires (IPAQ)²⁰ will be distributed to assess physical activity. In addition, a food frequency questionnaire,²¹ current eating habits²² and food preferences questionnaire²³ will be distributed.

Body composition

Body composition will be measured by DXA (DXA, QDR-4500A, Hologic, Waltham, Massachusetts, USA). BMD (g/cm²), BMC (g), bone area (cm²), and lean and fat mass (subcutaneous and visceral) will be determined for each adolescent. The DXA measurements will be taken for whole the body, lumbar spine (L2–L4) and the non-dominant hip (including the femoral neck, trochanteric and intertrochanteric regions). All DXA scans will be conducted by the same technician and quality assurance checks will be performed routinely.

Peripheral quantitative CT

Similarly to the cross-sectional study, musculoskeletal parameters for bone geometry including bone strength will be obtained using the pQCT XCT 3000L (XCT 3000L, Stratec Medizintechnik, Pforzheim, Germany). BMC (g/cm), volumetric cortical and trabecular BMC (mg/cm³), total area (mm²), cortical and trabecular area (mm²) and density (g/cm²), and bone strength (mm³), will be assessed at the distal (4%) and the proximal (66%) site of the non-dominant tibia and radius. Pictures will be analysed with Stratec pQCT software and Image J, an open source bone image analysis tool (rsbweb.nih.gov/ij).^{25 26}

Quantitative ultrasound

This method may provide additional information on bone quality and architecture. To predict fracture risk, quantitative ultrasound (QUS) measurements will be made with Achilles Insight+ (Achilles Insight, GE, Lunar Corporation, Madison, Wisconsin, USA) on the non-dominant calcaneus. The QUS results will be expressed in terms of broadband ultrasound attenuation (BUA, dB/MHz) which is postulated to reflect bone mass and architecture, and speed of sound (SOS, m/s) which is estimated to reflect the mass and elasticity of bone. This technique has been shown to be useful in child and adolescent bone investigations.²⁹ The in vivo coefficient of variation assessed in our laboratory for paediatric use is 1.4% for BUA and 0.16% for SOS measurements. All analyses will be conducted in duplicate by the same observer.

Endocrine assays

Similarly to the cross-sectional study, blood samples will be collected by a qualified paediatric nurse after the participants have fasted overnight. Blood will be collected

by a venipuncture at the brachial vein. After collection, blood will be centrifuged and aliquots will be stored (-80°) for subsequent analysis.

Basic biology (TG, cholesterol, LDL, HDLC, glycaemia, insulin, ultrasensible CRP) will be assessed in the biochemistry laboratory of Clermont-Ferrand University Hospital, while bone markers will be assayed in the biochemistry laboratory of Montpellier University Hospital (OPG, RANKL, sclerostin, bone alkaline phosphatase, unOc, CTX, PINP, osteocalcin total and vitamin D).

All other biochemical determination (leptin, adiponectin, TNF- α , IL-6, GH, IGF1, IGFBP-3, oestradiol, FSH/LH and PTH/calcitonin) will be made using commercial kits, following manufacturers' recommendations, including sampling steps, allowing the best performances of coefficient of variation and sensitivity. All analyses will be conducted in duplicate by the same technician.

Energy metabolism

Routinely performed in our laboratory, resting metabolism rate (RMR) is a reliable measure that reflects muscle mass. RMR will be measured in the morning using indirect calorimetry (K4b², Cosmed, Rome, Italy). Before each test, gas analysis is calibrated in accordance with the manufacturer's recommendations. Participants will be asked to lie in a supine position in a thermoneutral environment (22–25°C room temperature) for 45 min before starting the measurements. After achieving a steady state, O₂ consumption and CO₂ production standardised for temperature, barometric pressure and humidity will be recorded at 1 min intervals for 20–45 min and averaged over the whole measurement period.

Compliance

Volunteers engaged in the residential arm of the longitudinal study will be monitored by educators working at the obesity centre in order to control for their daily engagement and adherence to the weight loss lifestyle intervention combining physical activity and nutrition (normocaloric diet). Educators will complete an individualised daily journal on the compliance of each participant.

Statistical analysis

Data will be analysed using Stata and IBM Statistics SPSS V.22 (IBM Corp, 2013, Chicago, Illinois, USA) and significance will be accepted at a two-sided α level of $p < 0.05$. After testing for normal distribution (Shapiro-Wilk test), data will be treated either by parametric or non-parametric analyses according to statistical assumptions. Means and SDs will be reported for descriptive statistics and used to summarise the data. In case of non-Gaussian distribution, median and IQR will be reported. For a GLM or a multilevel model, the data will be either log transformed or Box-Cox transformed, where appropriate.

Student's t-tests or Mann-Whitney U test (when assumptions of t-test are not met: normal distribution and homoscedasticity will be studied using the Fisher-Snedecor test) will be performed to compare adipose tissue (total, subcutaneous, visceral) variation reported to bone mass variation at the lumbar spine between groups at baseline. χ^2 tests or the Fisher's exact tests will be used for categorical variables. Group comparisons will be assessed using ANCOVA to compare quantitative parameters between obese and non-obese adolescents, as well as for gender comparisons after controlling for pubertal status. Moreover, Pearson's (or Spearman's when appropriate) correlation coefficients will be used and compared with the Fisher test (command `corcor` Stata) to measure the potential links between exercise-induced weight loss and changes in adipose tissue and bone mass. Longitudinal data will be treated using a mixed model analyses in order to treat fixed-effects group, time and group \times time interactions taking into account between and within participant variability. Moreover, the impact of covariates (ie, compliance, BMI, hormonal status) will be explored. A sensitivity analysis of missing data will be performed to ensure the relevance of the longitudinal data (missing at random or MCAR missing completely at random). In order to assess the problem caused by missing longitudinal data, estimation methods developed by Verbeke and colleagues will be proposed.

Ethical considerations and dissemination

The longitudinal study has been registered with a clinical trial number (NCT02626273). Ethics approval has been obtained from the Hospital Sud Est 1 committee (2015-33).

In accordance with ethical considerations, the principal investigator of each study is responsible for ensuring that the participants understand the potential risks and benefits of taking part in the study. Moreover, the principal investigator is responsible for obtaining written consent from the adolescents and their legal guardians/parents.

Link between studies

The ADIBOX protocol combines two studies: a cross-sectional study addressing gender and weight effects of the bone adipocyte cross-talk while the longitudinal study will focus on the effects of weight loss induced by physical activity and nutrition in obese adolescents. Since the actual literature remains unclear regarding the interactions between adipocyte and osteocyte, both studies appear necessary to advance the understanding of these interactions in obesity and how they change over time. As previously stated by our team¹¹ only limited and heterogeneous literature (ie, large heterogeneity: including gender, age, pubertal status, maturation stage) is available to date for explaining the inability to draw clear conclusions. Using the same

methodology, our studies will provide complementary approaches with transversal and longitudinal results.

Radiation

Both DXA and pQCT provide measures of body composition and bone properties by exposing participants to low-level radiation: 0.0056 mSv from DXA scans (whole body, lumbar and hip) and 0.0014 mSv from the pQCT scans (tibia and radius measures).³⁰ Over the duration of each study, the effective dose of 0.007 and 0.03 mSv will be administered, respectively, for both the cross-sectional study and the 10-month study.

Confidentiality

For both studies, data will be stored in the principal investigator's office in password-protected computer only accessible to members of our research team. Within the electronic database, participants' names will be replaced with numeric identity codes. Blood samples will also be labelled with the numeric identity codes and samples will be stored in our laboratories. Only aggregate results will be reported, ensuring participants' anonymity.

Dissemination

The results of both studies will be disseminated at several research conferences and published articles in peer-reviewed journals.

DISCUSSION

The ADIBOX protocol has been developed to advance the understanding of the bone adipocyte cross-talk in adolescents and to extend what is currently known about growth-related and body mass changes that may or may not influence obesity. The specific effects of weight loss on bone tissue are uncertain, particularly in young people. Growth responses cannot be overlooked. Targeting puberty stages should help to highlight growth-stimulated responses which are often masked by assessing young participants across puberty ranges. Adolescence is a very sensitive period, determined by positive and/or negative influences that may contribute or adversely alter adolescents' health. Despite the well-described regular exercise-induced benefits in terms of health improvement and maintenance, adolescence is characterised by a marked decline of physical activity level in men and women.

The ADIBOX protocol will attempt to clarify the impact of the bone adipocyte interaction in obese adolescents and should provide a better understanding about the bone strength indices and adiposity cross-talk in adolescent men and women. In addition, this will be the first protocol investigating the effects of physical activity-induced weight loss on bone, growth and adiposity markers. Findings from this protocol are expected to clarify the possible interactions between adiposity and bone in childhood obesity.

Current study status

The ADIBOX protocol began recruiting participants in September 2015. Data collection will be completed in January 2017. Regarding the longitudinal study, data collection started in September 2015 and will be running for 10 months.

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Contributors ADIBOX principal investigators DC, GN, DT and DG are responsible along with EC (PhD student) for identifying the research question, the design of the protocol. GN, DG and EC were responsible for obtaining ethics committee approval and recruiting participants for study I, while DC, DT, FD and EC were responsible for obtaining ethics committee approval and recruiting participants for study II. BP was responsible for all the statistical part of this protocol. All authors were responsible for the draft of this manuscript and have read and approved the final version.

Competing interests None declared.

Ethics approval Australian Catholic University Ethic Committee and French Hospital Sud Est 1 committee.

Provenance and peer review Not commissioned; externally peer reviewed.

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Appendix 10 - Article 3

Effects of physical activity interventions on bone health in obese children and adolescents: a systematic review and meta-analysis

7/7/2017

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Date: 28/06/2017
To: "Elodie CHAPLAIS" e.chaplais@live.fr
From: "Journal of Bone and Mineral Metabolism (JBMM)" jbmm@ac-square.co.jp
Subject: JBMM: Your manuscript entitled Effects of interventions with a physical activity component on bone health in obese children and adolescents: A systematic review and meta-analysis.

Ref.: Ms. No. JBMM-D-16-00362R2
Effects of interventions with a physical activity component on bone health in obese children and adolescents: A systematic review and meta-analysis.
Journal of Bone and Mineral Metabolism

Dear Miss CHAPLAIS,

It is a pleasure to inform you that your work has now been accepted for publication in Journal of Bone and Mineral Metabolism.

Thank you for submitting your work to this journal.

Yours sincerely,
Yoshiki Seino
Editor-in-Chief
Journal of Bone and Mineral Metabolism

Reviewer #2: I think that the manuscript entitled "Effects of physical activity interventions on bone health in obese children and adolescents: a systematic review and meta-analysis" is worth publication for Journal of Bone and Mineral Metabolism.

P.S.: Please cite this paper when you submit your next paper to JBMM or any other journal.

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Effects of interventions with a physical activity component on bone health in obese children and adolescents: A systematic review and meta-analysis.

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Abstract: Background. Given the rise in pediatric obesity, clarifications on the relationship between obesity and bone health and on the impact of structured intervention on this relationship are needed. This systematic review and meta-analysis investigated the effect of obesity on bone health and assessed the effect of structured intervention in children and adolescents with obesity. **Material and Methods.** Medline complete, OVID, CINAHL, EMBASE and PubMed databases were searched for studies on obesity and bone health variables up to September 2016, then an update occurred in March 2016. Search items included obesity, childhood, Dual Energy X Ray Absorptiometry and peripheral Quantitative Computed Tomography. **Results/Conclusion.** Twenty three studies (14 cross-sectional and 9 longitudinal) matched the inclusion criteria. Results from the meta-analysis (cross sectional studies) confirmed that children and adolescents with obesity have higher bone content and density than their normal weight peers. Results from longitudinal studies remain inconclusive as only 50% of the included studies reported a positive effect of a structured intervention program on bone health. As such, the meta-analysis reported that structured intervention did not influence bone markers despite having beneficial effects on general health in youth with obesity.

Keywords: pediatric obesity, bone mineral density, growth, structured intervention

Introduction

Globally, about 155 million school-aged children are overweight or obese; representing a 47% increase between 1980 and 2013 [42]. Public health strategies and multidisciplinary approaches are developed to prevent and reduce obesity by promoting increased physical activity, healthy eating habits and decreased sedentary behaviour. Despite national health policies to limit the risks associated with obesity, the success and sustainability of weight management interventions in children and adolescents can be questioned [38, 44]. Indeed, while the potential health benefits of lifestyle changes in young populations with obesity are well established (i.e. body composition and cardio-metabolic markers), the impact of a plethora of treatments remains modest and sustainability has not been extensively researched [40, 45]. Musculoskeletal health, the cornerstone of functional independence and metabolic health is rarely investigated in young populations with obesity [6].

Several different techniques exist to assess the material properties and geometric characteristics of bone. Dual energy X ray Absorptiometry (DXA) is the most common non-invasive technique for assessing pediatric bone strength. However, other methods, including peripheral quantitative computed tomography (pQCT) and quantitative ultrasonography (QUS) may provide important information on bone size, geometry and quality [55]. The main limitation associated with DXA stems from the two dimensional design and the inability of the machine to accurately determine and adjust results for bone depth [63]. Effectively, DXA is not capable of measuring volumetric bone mineral density (vBMD). The distinction between trabecular and cortical bone cannot be done without vBMD [43]. The p-QCT device provides a more detailed picture than DXA and permits investigations of bone structure [46].

Although at some stages of life obesity may be protective to the skeleton, adolescence seems to have deleterious effects [11]. Despite multiple investigations into the effect of fat mass on

bone health in lean and obese adolescents [13, 19, 21-24, 41], results remain inconclusive. While obese adolescents are likely to suffer from fracture [21], information related to bone health seems complicated to access as microstructure elements (vBMD) are not obtainable from DXA. Moreover, it has been highlighted that when assessed by DXA, bone mineral density during growth can be inaccurately estimated [29], [33]. Indeed, especially when comparing individuals with different body shapes and sizes it is recommended to normalized bone mineral density to the bone size. Estimates of vBMD can reduce the influence of growing bone [29].

In addition, adolescents who are overweight or obese are prone to fracture [21], and excessive adipose tissue may interfere with the pubertal spurt. The effect of fat and lean mass on bone accrual was investigated in 472 children and adolescents aged 9 to 18 years (with an obesity prevalence of 5.3%) [31]. Results showed that physical activity positively increased lean mass; the most important predictor of bone mineral density in both males and females. Lean mass rather than fat mass was identified as the most important contributor to bone parameters in a recent systematic review assessing a wide range of children and adolescents [54].

Rigorously designed and effective interventions are urgently needed for adolescents with obesity. Although some studies used physical activity and/or nutritional interventions in overweight and obese adolescents to demonstrate beneficial changes in body composition [4] or cardiovascular risks factors, the impact of lifestyle interventions on bone health remains unclear [6].

The aims of this review were (1) to determine the effect of obesity on bone health in children and adolescents and (2) to assess the effect of intervention that include physical activity on bone health children and adolescents with obesity.

Methods

Database searching

Studies were identified by searching electronic data bases and related article reference lists. To remain current, searches using “Medline complete”, “OVID”, “CINAHL”, “EMBASE” and “PubMed” were limited to publications from 2000 to present, without any language restrictions using the following terms to denote obesity (“obesity” OR “obes*” OR “obese” OR “pediatric obesity”), childhood (“adolescenc*” OR “teen*” OR “youth”), DXA (“DXA” OR “DEXA”; “dual energy X ray”), pQCT (“pQCT” OR “peripheral quantitative computed tomography”), in papers with the study design limitation of “randomized controlled trial”, “classical article”, “journal article”, and “observational study”. The search was conducted from March to September 2016, then an update occurred in March 2016.

Two reviewers (EC, DT) developed the review protocol, determined inclusion and exclusion criteria and assessed potential articles for inclusion into the review. Retrieved abstracts were independently assessed to be eligible for inclusion by these two reviewers. The two reviewers agreed on 95% of the selected article and reached consensus on all included studies through collective discussions with a third reviewers (GN).

Study eligibility

Inclusion criteria

The review sought to identify all studies providing information on the bone parameters of children and adolescents with obesity. The following inclusion criteria used were (i) studies targeting a young population with a mean age between 10 and 17 years for the cross sectional analysis while for the longitudinal analysis we expanded the mean age to 9.7 to 17 in order to widen our search; being inclusive lean and obese children and adolescents; (ii) non-smokers; (iii) not taking medications that affect bone metabolism; (iv) studies providing

information about bone health via DXA or pQCT. However, our meta-analysis is focusing on studies analysing bone health with DXA in order to compare studies results.

Exclusion criteria

Studies were excluded if they related to (i) overweight instead of obesity; (ii) obesity prevention; (iii) surgical intervention; (iv) pharmacology; (v) genetic rather than lifestyle-related obesity; (vi) supplementation (vii) obesity as a secondary outcome (e.g. diabetes studies); and or (viii) if the publication was a trial protocol only. Studies by authors who did not reply to emailed requests for more complete data were also excluded.

Data synthesis

Articles retrieved by the search strategy were first selected based on the title of the papers, a second reviewer independently assessed the studies' eligibility based on titles. Each author independently coded included and excluded papers using Covidence (www.covidence.org) software as "yes" or "no" or "maybe" for eligibility. Once this first round of selection was completed (based on title only), any disagreement was discussed and a common decision taken. The identical procedure was followed a second time based on the abstract of the previously included papers. Any disagreement regarding eligibility for inclusion was discussed until consensus emerged made among all reviewers. Full texts from the articles were imported from reference manager software (EndNote - Thompson Reuters, San Francisco, CA). Each author completed data extraction files for every paper included. Issues identified as confusing or contentious were discussed collectively during the data extraction process. The process for trial inclusion is shown in the PRISMA diagram (Figure 1).

Synthesis of the results

Tables were composed on a priori established data extraction strategy chosen by the authors. Table 1 lists the included cross-sectional studies as follows: article, age, body mass index (BMI), blood sample, densitometric measures, other measures, bone-fat interactions. Table 2 displays the included longitudinal studies with categories of data extraction as follows: article, age, BMI, duration of the study, blood sample, densitometric measures, other measures, physical activity intervention, nutritional intervention, psychologic intervention, and intervention results.

Quality of reporting

Quality of the reporting within studies (Table 3) was independently evaluated by two authors using the PEDro scale [10]. Risk of bias in reporting was assessed for: eligibility criteria, randomization, allocation, baseline measures, blinding, dropout and statistical analysis. Any discrepancies in bias coding were resolved with a third reviewer. Studies remained included despite the quality reporting scores from PEDro.

Meta-analysis procedure

Following data extraction of relevant common values, the bone health parameter data were entered into software designed specifically for meta-analyses (Comprehensive Meta-Analysis, version 2; Biostat, Englewood, NJ).

Cross-sectional analysis

Included data for the meta-analysis of the cross-sectional analysis were sample size, whole body BMD (g/cm^2) and BMC (g), lumbar spine BMD, and lumbar spine BMAD. Some studies included subgroup analyses for gender. Each subgroup was considered separately. The standardized mean differences (SMD) were calculated to determine Cohen's d for each

study and Hedge's g was used to account for potential bias in small sample sizes. When data could be pooled, these effect sizes (ES) and 95% confidence interval (95%CI) were calculated using random-effects models (DerSimonian and Laird approach), that account for true variation in effects between studies, as well as random error within single study. Statistical significance was set at $p < 0.05$. The effect sizes were interpreted according to Cohen (1992): <0.2 trivial, $0.2-0.3$ small, 0.5 moderate, and >0.8 large [7]. A negative effect size value indicated that obesity decreased bone markers, while a positive effect size indicated that obesity increased bone markers. Cochrane's Q and the I^2 index were used to calculate heterogeneity with 25%, 50% and 75%, respectively indicating low, moderate and high heterogeneity according to the I^2 analysis [27]. Also a Cochrane's Q value above the degrees of freedom (df) denoted significant heterogeneity [28].

Longitudinal analysis

Included data for the longitudinal analyses were: sample size, along with pre- and post-intervention values for whole body BMD, BMC, fat mass and fat free mass. Some studies included different subgroups (receiving different interventions as described in the results below), and each subgroup was considered separately. Paired standardized mean differences were estimated, as described previously using random-effects models which were preferred to fixed-effect models, as some experimental parameters such as the measurement of whole body parameters had wide variation [8].

Negative effects sizes indicated that decreased body composition markers (bone, muscle and fat tissue) occurred in association with the intervention while positive effect sizes indicated that an intervention was positively associated with selected bone parameters. Again, Cochrane's Q and the I^2 index were used to calculate heterogeneity. Sensitivity analyses were

performed to assess bias [14], which allows for the possibility of computing and accounting for potentially missing studies to create symmetry about the overall mean ES. Statistical significance was set at $p < 0.05$ in a Z-test analysis, used to examine if ES were significantly different from zero.

Moreover, meta-regression analyses were performed in order to assess the potential effects of intervention length, body mass index, fat mass and fat free mass variations on bone mineral density and bone mineral content. Results were expressed as regression coefficients and 95%CI.

Results

The search strategy initially yielded a total of 1258 references after removing the duplicates. Titles and abstracts of potentially relevant articles were screened and 1105 articles were excluded. Full text copies were obtained for 153 articles, of which 22 studies matched the inclusion criteria; 12 from cross-sectional designs and 10 from the longitudinal studies. Manual searching of reference lists from the obtained articles were scanned for additionally relevant articles. The main reasons for trial exclusion among the remaining trials were: (i) unrelated outcomes for the cross-sectional analysis (n=28) and for the longitudinal analysis (ii) no DXA whole body measure of BMD and/or BMC (n=29), (iii) hormonal markers only (n=1) and, (iv) non-selected study designs (n=1). As detailed in the methods section, an update was conducted in March 2016. Two additional publications were then added to the initial 12 selected cross-sectional studies (Fig. 1).

Cross sectional analysis

Table 1 details the results from cross-sectional studies related to bone health.

Population characteristics

All studies included both obese and a comparative group of normal weight children and adolescents. Three [17, 18, 25, 48] of the 14 studies differentiated between males and females, two recruited females [52] or males [48] exclusively. Mean age of participants was 11-12 years for seven studies [13, 17, 25, 26, 49-51], 13-15 years for three other studies [18, 37, 52], and older than 16 years in the remaining one [16]. One study described participants' mean age as being between 12 to 13 years old [12]. Another study did not provide the mean age of both groups (normal weight and obese) but all participants were aged between 12 to 17 years [48]. Finally, one study described their participants with obesity as being aged around 12 years while the age of their control participants 14 years [36].

Measures

Twelve studies assessed whole body composition via dual energy X-ray absorptiometry (DXA) [13, 16-18, 25, 26, 37, 48-52]. Lumbar spine [13, 16, 18, 26, 48, 50-52], hip [16, 52] – including hip geometry measures [51] and radius [13, 16, 51] were also measured in some studies. Only one study [50] included ultrasound at the calcaneus and another used magnetic resonance imaging (MRI) [52] to determine subcutaneous and visceral fat tissue. Two studies matched the inclusion criteria for the assessment of bone via pQCT [12, 36]. Sites of measures for the pQCT were performed for the distal tibia and radius [12, 36] and for the proximal tibia and radius [36].

Blood biomarker analyses included: glucose [37], leptin [12, 37, 51, 52], insulin [26, 37, 49], adiponectin [12, 49, 51, 52], estradiol, testosterone [12, 18], calcium, alkaline phosphatase,

inorganic phosphorus, parathormone, calcitonin [26], osteocalcin [12, 26, 49, 51], sclerostin [12], vitamin D [36, 37, 51], high sensitivity CRP [36], IGF1 [37], IL6 - sICAM 1,e-selectin [52]. Physical activity was assessed using questionnaires [16, 51] or ACTigraphs [36, 37], while nutrition estimates were derived from questionnaires [16, 36, 51].

Bone measures via DXA

Whole body analysis

Four of the studies did not report differences in bone mineral density between young people with and without obesity [16, 18, 48, 52]. Four others found that children and adolescents with obesity had higher values of BMD [37, 49, 51] including two studies [18, 37] showing a gender influence on BMD; with higher values in females than males with obesity. Only one study found lower whole body BMD values for obese adolescents [13]. Even if results were inconsistent in WB BMD findings [16, 37], all studies agreed on higher values for whole body BMC in adolescents with obesity [16, 17, 25, 37, 50]. Two studies assessed volumetric whole body mineral density and reported that adolescents with obesity had lower values while there was no significant difference reported in the WB BMD between obese and normal weight groups [16, 50].

Bone density was sometimes analyzed relative to pubertal stages and showed that males with obesity could be predicted to have either higher (Tanner stage 3-4) or lower (Tanner stage 5) whole body BMD; depending on maturation [18]. Alternatively, in females with obesity, BMD was likely to increase linearly with advancing puberty (Tanner Stages 3-4 and TS 5). Only four studies adjusted bone values to body weight in order to reduce the risk of variance due to body size and shapes [16, 17, 50, 51]. Each of these studies reported lower WB BMC and/or BMD values in adolescents with obesity than their normal weight peers.

Regional analysis

With the exception of three studies [13, 18, 48], all studies assessing lumbar spine demonstrated higher BMD or BMC values in the young people with obesity than their non-obese peers [18, 26, 50, 51]. Similar to whole body BMD, maturation stages influenced lumbar spine. Young males with obesity had higher lumbar spine BMD values at Tanner stage 3-4 than at Tanner stage 5 [18]. Results from bone volumetric comparisons showed three studies with higher LS BMAD in the obese groups than their normal weight peers [13, 18, 50]. Gender and Tanner stages seem to influence the volumetric bone mineral density at lumbar spine. Indeed, in adolescent males with obesity and their normal weight peers did not differ in LS BMAD. However, those results varied according to maturation stages. Specifically at Tanner stage 3-4 LS BMAD was higher in males with obesity than normal weight peers and at Tanner stage 4-5, the group with obesity was lower than the comparison group [18]. Overall result for females showed that the obese group had higher LS BMAD than the comparison group only for females at Tanner stage 3-4 of their pubertal development (Russell's study Tanner stage 4.3 ± 1.0). After this stage LS BMAD differences between obese and normal weight groups disappeared [18, 52].

The two studies assessing femoral neck and total hip showed higher BMD in adolescents with obesity than their normal weight peers [16, 51]. Additional investigations into structural geometry of cross-sections traversing the proximal femur occurred in only one study via hip structural analysis (HSA) [51]. Compared with their normal weight peers, adolescents with obesity showed higher values for the cross-sectional area (CSA) at the femoral neck without differences for other structural descriptors such as section modulus at the femoral neck and femoral shaft, and CSA at the femoral shaft [51]. When adjusted for body weight, all bone parameters were significantly lower in obese adolescents than their normal weight peers.

Influence of fat tissue and hormones on bone health

Explorations of the influence of obesity on bone parameters have included, the impact of fat localization in females with obesity on bone mass and showed that subcutaneous fat tissue was positively associated with bone mass while visceral fat tissue had a negative association with bone mass [52].

Only two included studies [51, 52] focused on the influence of hormones on bone variables. One of the two studies showed no influence of obesity on hormones linked to bone variables [51]. In contrast, the other one demonstrated a positive influence of leptin and a negative influence of adiponectin on bone mineral density [52].

Bone measures via pQCT

Regional analysis

Bone geometry and volumetric density assessed by pQCT revealed that at the tibial site similar results were observed between an obese and normal non-obese groups for trabecular and cortical volumetric density [12, 36]. Adolescents with obesity had a greater cortical section modulus, and a greater cortical periosteal circumference than their non-obese participants at the tibia [36]. Also, one study found lower trabecular thickness and cortical pore diameter in adolescents with obesity [12]. Similar results were found for trabecular and cortical volumetric density between groups at the radius [12, 36]. Cortical periosteal circumference (higher) at the radial site was higher among adolescents with obesity than their normal weight peers [36] and cortical porosity and cortical pore diameter were lower in obese than non-obese groups [12].

Influence of muscle and hormones on bone health

Studies using pQCT showed that high sensitive CRP or vitamin D were not associated with bone measures [36]. However, leptin was negatively correlated with trabecular thickness at the tibial and radial sites and with cortical porosity at the radial site [12]. Also, lean mass (assessed at the gastrocnemius) was associated with bone markers such as the tibial cortical section modulus [36].

Meta-analysis

Results from the meta-analysis for whole body BMC, whole body BMD, lumbar spine BMD and BMAD are reported in Table 4 with the following data extracted: analysis, number of groups, minimal and maximal value of the effect size, mean value of the effect size, 95% CI, significance and heterogeneity. Figure 2 shows the effects size plot for the influence of obesity on whole body BMD and lumbar spine BMAD in children and adolescents from the included studies.

Longitudinal analysis

Table 2 details the results from longitudinal studies related to an intervention based on physical activity on bone health.

Population characteristics

All studies included adolescents with obesity who were either male or female. Ages within studies ranged from 9.7 to 12 years [34, 39, 49], 13 to 14 years [56, 58] and 15 to 17 years [3-5, 16, 39].

Design of the studies

Five studies involved randomized clinical trials [4, 5, 49, 56, 58] and four were experimental interventions only [3, 15, 34, 39]. Three studies recruited a comparison group of normal weight young people [15, 49, 56] while two studies recruited a control group with obesity [49, 58]. Four studies [4, 5, 49, 58] used a randomized trial design comparing two groups randomly assigned to lifestyle interventions with a physical activity component or physical activity intervention. One study described an observational design however, their protocol was presented as a randomized clinical trial [56].

The duration of intervention ranged across 6 weeks [34], 8 weeks [39], 12 weeks [15], 26 weeks [49, 58] and 52 weeks [3-5, 56]. Interventions were a combination of supervised physical activity performed three times [3-5, 16, 34, 39, 58] or twice [49] a week and nutrition [3-5, 56], or supervised physical activity alone [15, 34, 39, 49, 58]. Overall, physical activity included 60 minutes of supervised aerobic [4], 30 minutes of aerobic plus 30 minutes of resistance training [3-5], 60 minutes of supervised resistance training [34], or martial arts (Kung Fu or Tai Chi) [58], 60 to 90 minutes of supervised aerobic training [15], or 90 minutes [49] or 120 minutes [56] of supervised aerobic training. Only one study did not set duration for the physical activity program [39]. Their training was composed of 3 sets of 8 exercises per session going from 8 to 10 rep with 90s rest, to 10 to 12 rep with 60 s rest and 5 to 8 rep with 3 min rest between series [39]. Nutritional components of intervention, varied: prescribing a hypo caloric diet in combination with a once weekly nutrition education session [5], restricting adolescents to 1200/1500Kcal per day [56], and generic descriptions of food restriction or a food restriction [4] plus weekly nutrition education sessions [3].

Additional measures

Body composition analysis involved DXA in all studies [3-5, 15, 34, 39, 49, 56, 58]. In addition to DXA, ultrasound was used to differentiate subcutaneous and visceral fat tissue

[3-5]. Blood analyses included insulin [5, 34, 49], adiponectin [3-5, 49], leptin [3-5, 34], ghrelin [3, 4], osteocalcin [49]. Nutrition was assessed using 3 or 5 days food records [3, 5, 39, 58]. Physical activity was typically evaluated using either questionnaires [39, 49, 58] or a sports activity record [58].

Whole body analysis

Five studies recorded higher BMC following the intervention [4, 5, 15, 34, 39, 56]. From these five studies, only three focused on weight loss [4, 5, 56]. The one study without a weight loss focus, showed no difference in whole body BMC after the intervention [39]. Weight loss was associated with decreased BMD in adolescents with obesity in only one study [3]. Three studies observed higher values of BMD after the lifestyle intervention [15, 49, 58]. However, one of these three studies lacked differences between the trained and untrained groups; thus an intervention effect was not supported [58].

Regional analysis

Higher BMC at the leg [15] and BMD at the lumbar spine [58] were reported following interventions but again, these results occurred without a group effect between trained and untrained participants.

Meta-analysis

Table 5 summarizes key data comparisons. Key data included the results from the meta-analysis for whole body BMC and BMD. Descriptions also included the type of analysis conducted, the number of groups, the effect size (minimal, maximal and mean value), 95% CI, significance and heterogeneity. Figure 3 shows the effects size plot for the structured lifestyle interventions on whole body BMD and percentage of FM. Table 6 reports the results

from the meta-regression on the influence of the length of the intervention on BMC and BMD, the influence of BMI variation on BMC, fat mass variation on BMC and BMD as well as fat free mass variation on BMC and BMD.

Discussion

The present review examined the available evidence reporting the impact of obesity on bone health in children and adolescents and assessed the impact of structured lifestyle interventions that included physical activity on bone health in children and adolescents with obesity. The meta-analysis quantified whether obesity influenced bone health in adolescents, and whether interventions that included physical activity could modify bone health indicators in this population. We also explored possible influences of the length of the interventions, the BMI, and specific body composition measures of fat mass and fat free mass impacting bone health.

First, the meta-analysis confirmed that children and adolescents with obesity had higher bone content and density than their normal weight peers. When systematically reviewed, 70% of the included studies found higher values in bone mineral content or density in children and adolescents with obesity than their non-obese peers. However, when adjusted for the confounder of body weight, bone mineral density values for the whole body were lower among young people with obesity than their non-obese peers. Five studies regionalizing scans (hip, lumbar), reported higher bone density at lumbar spine and/or hip in children and adolescents with obese than their normal weight peers. Also, BMAD at lumbar spine was higher in groups with obesity than normal weight groups.

Nowadays we can highlight a lack of literature regarding three-dimensional analysis. Indeed, only two pQCT studies included obese adolescents only (not obese plus overweight). In obesity results demonstrated no significant differences at the radial and tibial volumetric bone

mineral density between adolescents with obesity and normal weight controls. Yet, an impact on the important structural parameter of bone strength remained unclear. One study suggested greater estimates of bone strength in children and adolescents with obesity while the other one highlighted no adaptations in biomechanical properties of the radius or the tibia in obese children and adolescents. Moreover, both studies did not differentiate between males and females, while it is well known that peak high velocity and growth spurt timing differ between sexes [61], and that dimorphic differences in tibia and radius are readily observed in micro and macro-structure [61]. The absence of difference in vBMD and bone strength may be partially attributed to an advanced skeletal maturation and greater cortical structure at weight bearing sites. Adolescents with obesity usually demonstrate more advanced biological maturation for the same chronological age than their normal weight peers; underlying the complex and strong associations between maturation phases on bone accrual. Indeed, the effects of fat mass on peak bone mass and bone mass accrual are sex and maturation dependent [11, 53, 62]. Also this relationship appears more significant within specific growth phases [11]. A positive relationship between obesity and bone has been observed during pre-puberty while this effect tends to be somewhat attenuated or reversed during puberty and after puberty [11].

Despite the large heterogeneity of the included studies, our results are in line with a recent systematic review and meta-analysis [59] and others studies [2, 31] showing greater BMD in adolescents with obesity or overweight than their lean peers. The recent systematic review and meta-analysis included 27 studies (only one with a longitudinal design) ranging from 2 to 18 years. Also, others [54] using systematic review of the literature have shown positive effects of physical activity on lean mass; highlighting the greater influence of lean mass on bone parameters in a wide range of children and adolescents. Growth and obesity are postulated to be two factors that further complicate interpretation for BMD [20, 32];

distinguishing this article and as highlighted by the results of the current systematic review and meta-analysis. Indeed our systematic review and meta-analysis narrowed the targeted recruitment by focusing on obesity (BMI >95th percentile) and pubertal stage (albeit using a surrogate of selective mean age). Moreover this article aimed at better understanding the effects of lifestyle interventions with a physical activity component (including targeted weight loss strategies) on bone health.

Besides the complications associated with obesity, a wide range of other serious complications and side effects may appear in adolescents with obesity such as the metabolic syndrome (MetS). A potential link between bone health and metabolic syndrome can be speculated. In the last decade, the influence of MetS on bone health has been explored however, without conclusive results. Investigations into the impact of obesity on the metabolic and musculoskeletal health of adolescents are required. The existing literature highlights that children and adolescents with obesity had lower values of osteocalcin. It could be hypothesized that during adolescence, obesity could induce metabolic syndrome which in turn might be associated with bone fragility. Indeed, Lee et al (2007) demonstrated using an animal model that administration of osteocalcin corrected metabolic abnormalities [35].

In this review five of nine interventional studies reported a gain in bone density following their obesity management programs [4, 5, 15, 34, 56, 58]. However, one reported lower post-intervention bone strength [3], one found no differences before and after the intervention [39], and two studies found the observed gains in bone mass were not due to the intervention [49, 58]. Despite equivocal results on bone mass (with only 50% reporting positive effects), our meta-analysis revealed that structured lifestyle interventions that included physical activity did not influence BMC and BMD among adolescents with obesity. However,

heterogeneity among interventions was evident, with only half of the interventions involving only physical activity. The diversity of the proposed interventions may contribute to the difficulties to evaluate the exclusive effect of physical activity. Moreover, no information regarding the intensity of physical activities was provided, which might be of particular importance since light to moderate physical activity do not influence bone mineral parameters [30]. Nonetheless, these interventions reported beneficial effects on general health as an increase in fat free mass and reduced fat mass were consistently observed. The meta-regression results indicated that longer interventions were associated with positive BMC WB outcomes. While a greater loss of fat mass was associated with improved WB BMC and BMD outcomes, increased muscle mass was also associated with trends for increase in WB BMC and BMD. However, none of our results reached significance.

These inconclusive results may be explained by significant weight loss being observed in only 50% of the included studies. Specifically, the magnitude of weight loss might be suggested as key factors to induce bone changes in response to physical activity. In all of these studies [4, 5, 58] the interventions combined caloric restriction and physical activity (aerobic training or aerobic plus resistance training).

In a recent systematic review and meta-analysis, the effects of exercise training versus caloric restriction on body weight were compared [60]. Results showed that even in the absence of weight loss, exercise training was associated with decreased fat tissue; especially visceral adipose tissue [60]. These results highlight the fact that health benefits can occur independently of body weight changes and that lifestyle intervention that includes physical activity beyond the effects on body weight entails health benefits. Although, the central role of weight bearing physical activity to enhance bone parameters in children and adolescents is well known [57], little is known regarding bone specific responses between bone mass and structure and their contribution to bone strength. Mechanical stress on bone tissue from

excess weight as well as growth and development appear to stimulate similar gains to physical activity training. Indeed, in some studies comparing the impact of weight bearing exercise programs, no benefits from physical activity on bone have been shown [49, 58] . Heterogeneity within descriptions of physical activity training remains problematic.

As previously stated, little is known about the effectiveness of physical activity interventions alone on bone density in children and adolescents. The effectiveness of lifestyle interventions on specific criteria such as weight loss, comorbidities, health behavior, side effects and quality of life has been assessed in overweight children [47] and extensively reviewed in the Cochrane literature [44]. Reviews highlighted a lack of efficiency regarding lifestyle interventions [44, 47]. Indeed, most of the studies were interested in weight loss only, ignoring the impact of the interventions' duration on long term outcomes (sustainability of weight loss, comorbidities, etc.). Some of the included studies were short term interventions. It is well known that long term interventions (6-12 months) rather than short term interventions are more efficient to sustain weight loss [44, 47]. In addition, when it comes to bone health, short term interventional studies provide some bias as the bone remodeling cycle takes 4 to 6 months [53].

Limitations

There are several important limitations in the literature available for this review. The most critical limitation is the number of studies that have been specifically designed and adequately powered to explore structured interventions on bone health. Four studies out of nine coupled nutrition with physical activity in delivering weight management interventions as previously recommended in the Cochrane review [44]. Moreover, only four of them targeted their intervention on weight loss. In addition, even if DXA is the gold standard

method to evaluate bone health, limitations can apply due to an overestimation of bone mineral density. Normalization of BMD values is important especially when comparing growing individuals of different sizes and shapes [32, 33]. Another limitation is the inconsistent statistical treatment of body weight as a covariate to bone changes within studies that precluded a clearer understanding of the impact of obesity, structured intervention on bone parameters. Also, when assessing bone strength, whole body scans should be pooled with specific areas affected by loss of bone mass such as hip and lumbar spine as they provide important complementary information. Hip and lumbar spine have predominantly trabecular bone. Trabecular sites are shown to be more sensitive to bone turnover factors responses [1]. Gains of bone strength are most often associated with bone structure. Furthermore, most of the included studies did not followed the recommendations from the International Society of Clinical Densitometry [9].

Moreover, the heterogeneous nature of population and method is a limiting factor to this review. Similarly, only 55% of the interventional studies had a control group, and the comparative group was not always obese. Finally, as we analyzed interventional studies, it would have been interesting to have information regarding participants' compliance to the lifestyle intervention, daily records of the duration and quality of compliance and any adverse events. It would also be ideal to be able to separate the impact of multidisciplinary interventions.

Conclusion

To conclude, our systematic review and meta-analysis confirmed that (1) children and adolescents with obesity had higher bone mineral content and density than their normal weight peers. Results from our meta-analysis revealed that (2) structured interventions did not influence BMC and BMD, but had positive effects on general health markers (fat free

mass and fat mass). Moreover, understanding the microstructural changes of bone during growth remains challenging and requires a (3) normalization of bone density values (i.e. whole body less head BMD, adjustment for cofounders affecting bone thickness and calculated parameters for bone mineral apparent density).

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Conflict of Interest:

None of the authors has any conflicts of interest to declare.

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Figures' legend:

Figure 1. Study inclusion flow diagram

Figure 2. Effect size forest plot for the effects of obesity on lumbar spine bone mineral apparent density (a) and whole body bone mineral density (b).

Figure 3. Effect size forest plot for the effects of structured physical activity intervention on bone mineral density (a) and percentage of fat mass (b)

Table 1

Table 1. Participants characteristics in cross sectional studies

Article	Age	BMI	Blood sample	Densitometric measures	Other measures	Bone – fat interactions (crude value)
<i>Dimitri et al. 2010</i>	Ob 11.7 ± 3 Cntrl 10 ± 3.2	Ob 3.3 (0.6) SDS Cntrl 0.2 (1.0) SDS		DXA (WB, LS, radius)		WB BMD, WB BMC, LS BMC, LS BMD, LS BMAD ↑ Regional BMC, BMD ↑
<i>El Hage et al. 2013</i>	Ob 17.1 ± 1.9 Cntrl 16.8 ± 2.1	Ob 33.8 ± 3.3 Cntrl 20.4 ± 2.1		DXA (WB, LS, radius, hip)	Calcium (questionnaire), physical activity (questionnaire)	WB BMC, TH BMD, FN BMD, LS BMD ↑ WB BMAD ↓ WB BMD ↔
<i>Ellis et al. 2003</i>	Ob ♀ 12.1 ± 3.4 Ob ♂ 11.5 ± 2.4 Cntrl ♀ 11.4 ± 3.3 Cntrl ♂ 11.2 ± 4.1			DXA (WB)		♀ WB BMC ↑ ♂ WB BMC ↔
<i>Fintini et al. 2011</i>	Ob ♂ 14.2 ± 1.6 Ob ♀ 14.2 ± 2.7 Cntrl ♂ 15.1 ± 2.4 Cntrl ♀ 14.6 ± 2.6	Ob ♂ 31.6 ± 4.3 Ob ♀ 31.9 ± 4.7 Cntrl ♂ 21 ± 3.1 Cntrl ♀ 21.3 ± 2.5	Estradiol, testosterone	DXA (WB, LS)		♂ LS BMD, LS BMAD, WB BMD ↔ ♂ TS 3 – 4: LS BMD, LS BMAD, WB BMD ↑ ♂ TS 5: LS BMD, LS BMAD, WB BMD ↓ ♀ LS BMD, LS BMAD, WB BMD ↑ ♀ TS 3 – 4: LS BMD, LS BMAD ↔ ♀ TS 3 – 4: WB BMD ↑ ♀ TS 5: LS BMD ↑ ♀ TS 5: WB BMD ↔
<i>Haroun et al. 2005</i>	Ob ♂ 11.9 ± 2 Ob ♀ 10.8 ± 2.2 Cntrl ♂ 11.1 ± 1.6 Cntrl ♀ 10.4 ± 2.1	Ob ♂ 26.3 ± 4.4 Ob ♀ 27.9 ± 6.0 Cntrl ♂ 17.1 ± 1.6 Cntrl ♀ 18.2 ± 2.5		DXA (WB)	Total body water (saliva), body volume (air-displacement plethysmography)	WB BMC ↑

<i>Hasanoglu et al. 2000</i>	Ob 10.2 ± 3.2 Cntrl 10.5 ± 2.8	Ob 27.0 ± 3.7 Cntrl 19.2 ± 3.1	Calcium, alkaline phosphatase, inorganic phosphorus, parathormone, calcitonin, osteocalcin, insulin	DXA (LS)		LS BMD ↑
<i>Maggio et al. 2014</i>	Ob 13.9 ± 1.2 Cntrl 13.2 ± 1.7	Ob 30.3 ± 5.4 Cntrl 18.9 ± 1.9	Vitamin D, leptin, IGF1, glucose, insulin	DXA (WB)	Physical activity (actigraph)	WB BMD ↑ WB BMC ↑ ♀ Ob WB BMD ↑
<i>Ripka et al. 2016</i>	Ob, cntrl 12 - 17 years	Ob >95 th percentile Cntrl <85 th percentile		DXA (WB, LS, legs, arms, pelvis)		WB BMD ↔ LS BMS ↔
<i>Rocheftort et al. 2011</i>	Ob 10.3 ± 4.8 Cntrl 10.6 ± 3.7	Ob 25.56 ± 2.91 Cntrl 16.48 ± 1.89	Insulin, total adiponectin, osteocalcin	DXA (WB)		WB BMD ↔
<i>Rocher et al. 2008</i>	Ob 10.72 ± 1.24 Cntrl 10.90 ± 1.14	Ob 28.02 ± 4.47 Cntrl 16.66 ± 1.79		DXA (WB, LS) QUS (n.d. calcaneus)		WB BMC, LS BMD, LS BMAD, LS BMC, BUA ↑ WB BMD, SOS ↔ WB BMAD ↓
<i>Rocher et al. 2013</i>	Ob 10.3 ± 1.4 Cntrl 10.4 ± 1.5	Ob 25.6 ± 4.3 Cntrl 16.5 ± 2.0	Leptin, adiponectin, osteocalcin, vitamin D,	DXA (WB, LS, forearm, hip/HSA)	Physical activity (questionnaire), nutrition (4-d food record)	WB BMD, LS BMD, TH BMD, FN BMD, FN CSA ↑ FN section modulus, FS section modulus, FS CSA ↔ Leptin ↑ Adiponectin ↔ Hormones ∅ bone variables
<i>Russell et al. 2010</i>	Ob♀ 14.0 ± 1.9 Cntrl♀ 15.9 ± 1.7	Ob 34.4 ± 7.1 Cntrl 21.7 ± 1.9	Leptin, IL-6, sICAM-1, adiponectin, e-selectin	DXA (WB, LS, hip) MRI (VAT, SAT) Bone age		(LS, hip, WB) BMD, LS BMAD ↔ SAT + BMD VAT – BMD Leptin ↑ ♀ leptin + BMD Adiponectin ↔ Adiponectin – BMD

Leonard et al. 2015	Ob 12.2 ± 1.2 Cntrl 14.5 ± 2.0	Ob 33.9 ± 4.9 Cntrl 19.7 ± 2.0	High sensitivity CRP, vitamin D	pQCT (z-score) tibia 3 - 38% radius 3 - 30%	Muscle strenght (biodex, handgrip), physical activity (actigraph), dietary and calcium intake (questionnaires)	<u>Tibia</u> Cort section modulus ↑ Periosteal circumference ↑ Endosteal circumference ↔ Trabecular vBMD ↔ Cort vBMD ↔ Hormones ∅ bone variables <u>Radius</u> Cort section modulus ↔ Periosteal circumference ↑ Endosteal circumference ↔ Trabecular vBMD ↔ Cort vBMD ↔ Hormones ∅ bone variables
Dimitri et al. 2015	Ob 12.6 ± 1.9 Cntrl 12.9 ± 2.0	Ob 3.14 (0.68) SDS Cntrl 0.08 (0.87) SDS	Leptin, adiponectin, testosterone, estrogen, osteocalcin, sclerostin	HR pQCT tibia distal radius distal		<u>Tibia</u> Trabecular vBMD ↔ Cort vBMD ↔ Trabecular thickness ↓ Cort porosity ↔ Cort pore diameter ↓ Leptin - trabecular thickness <u>Radius</u> Trabecular vBMD ↔ Cort vBMD ↔ Trabecular thickness ↔ Cort porosity ↓ Cort pore diameter ↓ Leptin - cort porosity Leptin - trabecular thickness

^o Data for ages are presented in mean ± standard deviation

♀ indicates female, ♂ indicates male, Ob obese, cntr control, DXA Dual energy X-ray Absorptiometry, pQCT peripheral Quantitative Computed Tomography, MRI Magnetic Resonance Imaging, QUS Quantitative UltraSound, WB whole body, LS lumbar spine, TH total hip, FN femoral neck, FS femoral shaft, CSA cross sectional area, BMD bone mineral density, BMC bone mineral content, BMAD bone mineral apparent density, SAT subcutaneous adipose tissue, VAT visceral adipose tissue, n.d. non dominant, Cort cortical, vBMD volumetric BMD, ↔ indicates no differences, ↑ indicates higher, ↓ indicates lower, + indicates positive, - indicates negative, ∅ not interact

Table 2

Table 2. Participants characteristics and study interventions

Article	Age	BMI	Duration (weeks)	Blood sample	Densitometric measures	Other measures	PA intervention	Nutr intervention	Psy intervention	Intervention results
<i>Campos et al. 2012</i>	Ob 17 ± 1.7	B 35.8 ± 5.3 E 32.2 ± 5.0	52	Insulin, leptin, adiponectin	DXA (WB) US (VAT, SAT)	Nutr : 3 days record Psy: BES questionnaire	3/week supervised AT (30min): treadmill RT (30min)	Hypo caloric diet Diet lesson 1/week	1/week	↑BMC, lean mass ↓BMI, FM
<i>Campos et al. 2013</i>	Ob 16 ± 1.5	B 37.2 ± 4.8 E 34 ± 4.2	52	Leptin, ghrelin, adiponectin	DXA (WB) US (VAT, SAT)	Nutr: 3 days record	3/week supervised AT (30min): treadmill RT (30min)	Food restriction Diet lesson 1/week		↓BMD, BMI, FM ↑Adiponectin B ∅ E ghrelin levels Ghrelin – BMD
<i>Campos et al. 2014</i>	Ob <u>AT</u> B 16.1 ± 1.2 Ob <u>AT</u> E 16.9 ± 1.8	B 35.8 ± 4.5 E 32.1 ± 4.9 B 37.6 ± 5.4 E 32.6 ± 4.5	52	Leptin, ghrelin, adiponectin	DXA (WB) US (VAT, SAT)		3/ week supervised AT(60min): treadmill AT (30min)+RT (30min) : training treadmill, cycle, strength	Food restriction Diet lesson 1/week	Body image, self esteem, family problems, eating disorders 1/week	↑ BMC ↓ BMI, FM AT + RT ↓ VAT/SAT ratio AT + RT ↑ lean mass B AT + RT ↓ ghrelin E AT + RT ↓ ghrelin
<i>El Hage et al. 2009</i>	Ob <u>AT</u> 15.8 ± 0.8 Cntrl 16.8 ± 0.2	B Ob 33.5 ± 0.8 B cntrl 20.9 ± 0.7	12		DXA (WB, pelvis, leg)		3/ week supervised AT (60 to 90min)			Ob ↑ BMC leg + whole body Cntrl + Ob ↑ BMD
<i>Lau et al. 2010</i>	Ob RT 12.4 ± 1.8	B 30.5 ± 4.9 E 30.4 ± 4.8	6	Leptin, insulin	DXA (WB)		3/ week supervised RT (60min) (10 exercises on machine 3 sets 5-8 rep 75-85% 1RM and 3 sets circuit training)			B ∅ E FM, FFM ↓ leptin/FM ↑ BMC

<i>McGuigan et al. 2009</i>	Ob RT 7-12 years	B 25.6 ± 3.1 E 25.9 ± 3.2	8		DXA (WB)	Nutr: 3 days record PA: 3 days record Strenght, power, muscular endurance	3/ week supervised (1/ 3 sets 8- 10 rep, 90s rests; 2/ high volume moderate intensity: 3 sets 10-12 rep, 60s rest; 3/ moderate-high intensity: 3 sets 3-5 rep 3mn rest)				B ∅ E BMC, FM
<i>Rochefort et al. 2011</i>	Ob 10.3 ± 0.9 Cntrl 10.6 ± 0.9	B 25.5 ± 2.9 B cntrl 16.5 ± 1.9	26	B insulin, total adiponectin, osteocalcin	DXA (WB)	PA: Self reported questionnaire	2/week supervised AT (90min): cycling, rowing, jumping, games, hip-hop				B Ob ↔ BMD 6mths trained Ob & untrained Ob ↔ BMD trained Ob vs cntrl ↑ osteocalcin (↑ uncarboxylated osteocalcin) trained Ob vs cntrl ↓ adiponectin
<i>Stettler et al. 2008</i>	Ob 14.5 ± 1.1 Cntrl 13.3 ± 2.7	B 36.8±3.7 E 21.1±5.4	52		DXA (WB, LS)		120min aerobic /week	1200 – 1500 kcal/day	Group sessions		↑BMC ↓BMI, FM
<i>Tsang et al. 2009</i>	Ob KF 13.4 ± 2.1 Ob cntrl 13.1 ± 1.6	B Ob KF 32.1 ± 6.7 B Ob TC 34 ± 7 E Ob KF 32.7 ± 7.8 E Ob TC 34.2 ± 7.2	26		DXA (WB, LS, FN)	Illnesses, injuries questionnaire, satisfaction questionnaire Nutr: 5 days record PA: 7 days record + PACE+ questionnaire	3/ week supervised KF (60min) TC (60min)				↑ WB BMD no group effect ↑ LS BMD no group effect

^o Data for ages are presented in mean ± standard deviation

♀ indicates female, ♂ indicates male, Ob obese, cntr control, B baseline, E end, AT aerobic training, RT resistance training, PA physical activity, Nutr nutrition, Ps psychology, DXA Dual energy X-ray Absorptiometry, US UltraSound, n.d. non dominant, KF Kung Fu, TC Tai Chi, ↔ indicates no differences, † indicates higher, ‡ indicates lower, + indicates positive, – indicates negative, ∅ not interact

Table 3a

Table 3a. Study design quality – cross sectional

<i>Study</i>	<i>Eligibility criteria</i>	<i>Randomisation</i>	<i>Concealed Allocation</i>	<i>Similar Baseline</i>	<i>Blinding of Subjects</i>	<i>Trainer Blinding</i>	<i>Invest Blinding</i>	<i><15% Dropout</i>	<i>Intention to Treat</i>	<i>Between Groups Stats</i>	<i>Means Std Dev</i>	<i>Total</i>
<i>Dimitri et al. 2010</i>	●									●	●	3
<i>El Hage et al. 2013</i>	●									●	●	3
<i>Ellis et al. 2003</i>										●	●	2
<i>Fintini et al. 2011</i>	●									●	●	3
<i>Haroun et al. 2000</i>										●	●	2
<i>Hasanoglu et al. 2000</i>										●	●	2
<i>Maggio et al. 2014</i>	●									●	●	3
<i>Ripka et al. 2016</i>	●									●	●	3
<i>Rochefort et al. 2011</i>	●	●								●	●	4
<i>Rocher et al. 2008</i>	●									●	●	3
<i>Rocher et al. 2013</i>	●									●	●	3
<i>Russell et al. 2010</i>										●	●	2
<i>Leonard et al. 2015</i>	●	●								●	●	4
<i>Dimitri et al. 2015</i>	●									●	●	3

Table 3b

Table 3b. Study design quality – longitudinal

<i>Study</i>	<i>Eligibility criteria</i>	<i>Randomisation</i>	<i>Concealed Allocation</i>	<i>Similar Baseline</i>	<i>Blinding of Subjects</i>	<i>Trainer Blinding</i>	<i>Invest Blinding</i>	<i><15% Dropout</i>	<i>Intention to Treat</i>	<i>Between Groups Stats</i>	<i>Means Std Dev</i>	<i>Total</i>
<i>Campos et al. 2012</i>	●	●*								●	●	4
<i>Campos et al. 2013</i>	●										●	2
<i>Campos et al. 2014</i>	●	●*		●						●	●	5
<i>El Hage et al. 2009</i>											●	1
<i>Lau et al. 2010</i>											●	1
<i>McGuigan et al. 2009</i>											●	1
<i>Rocheport et al. 2011</i>	●	●								●	●	4
<i>Stettler et al. 2008</i>	●	● [■]		●	● [■]	● [■]			● [■]	●	●	8
<i>Tsang et al. 2009</i>	●	●		●				●		●	●	6

* from <https://clinicaltrials.gov/ct2/show/NCT01358773>; [■] from Berkowitz Rl, Wadden TA, Tershakovec AM, Cronquist JL. Behavior therapy and sibutramine for the treatment of adolescent obesity: a randomized controlled trial. JAMA 2003;289:1805–1812.

Table 4

	n	Effect size			95% CI		p-value	Heterogeneity			
		Min	Max	Mean	Min	Max		I ²	Q	df	p-value
<i>Whole body BMC</i>	8	0.155	1.750	1.019	0.629	1.409	<0.001	81.75	38.36	7	<0.001
<i>Whole body BMD</i>	10	-0.131	1.154	0.568	0.273	0.863	<0.001	67.14	27.39	9	<0.001
<i>Lumbar spine BMD</i>	13	-0.169	1.121	0.529	0.260	0.798	<0.001	72.87	44.23	12	<0.001
<i>Lumbar spine BMAD</i>	5	0.000	1.100	0.653	0.292	1.013	<0.001	58.29	9.59	4	0.048

BMC bone mineral content, BMD bone mineral density, BMAD bone mineral apparent density

Table 5

	n	Effect size			95% CI		p-value	Heterogeneity			
		Min	Max	Mean	Min	Max		I ²	Q	df	p-value
<i>WB BMC</i>	8	-0.457	0.713	0.159	-0.039	0.356	0.116	6.28	7.47	7	0.38
<i>WB BMD</i>	9	-1.000	0.600	-0.031	-0.319	0.258	0.834	37.67	12.84	8	0.12
<i>FM_g</i>	9	-1.074	0.736	-0.369	-0.814	0.076	0.104	82.61	46.00	8	0.00
<i>FM_pct</i>	8	-1.198	-0.017	0.552	-0.820	-0.284	<0.001	37.90	11.27	7	0.13
<i>FFM_g</i>	8	0.014	0.477	0.236	0.033	0.438	0.023	0.00	2.40	7	0.93

BMC bone mineral content, BMD bone mineral density, FM fat mass, g grams, pct percentage, FFM fat free mass

Table 6

Table 6. Meta-regression results from longitudinal studies

	n	Coef.		95% CI		t	p-value	Heterogeneity	
		Mean	SD	Min	Max			I ²	Q
<i>Intervention's length on BMC</i>	8	0.001	0.005	-0.012	0.014	0.27	0.79	18.35	7.35
<i>Interv.'s length on BMD</i>	9	-0.015	0.018	-0.057	0.028	-0.83	0.44	0.00	6.76
<i>BMI on BMC</i>	4	-0.218	0.345	-1.704	1.267	-0.63	0.59	0.00	0.01
<i>FM on BMC</i>	7	-0.118	0.182	-0.585	0.349	-0.65	0.54	26.67	6.82
<i>FM on BMD</i>	6	-1.543	1.245	-4.999	1.914	-1.24	0.28	0.00	3.83
<i>FFM on BMC</i>	6	1.297	0.653	-0.515	3.109	1.99	0.12	0.00	3.25
<i>FFM on BMD</i>	6	1.430	0.932	-1.156	4.015	1.53	0.20	0.00	3.12

BMC bone mineral content, BMD bone mineral density, BMI body mass index, FM fat mass, FFM fat free mass

Figure 1

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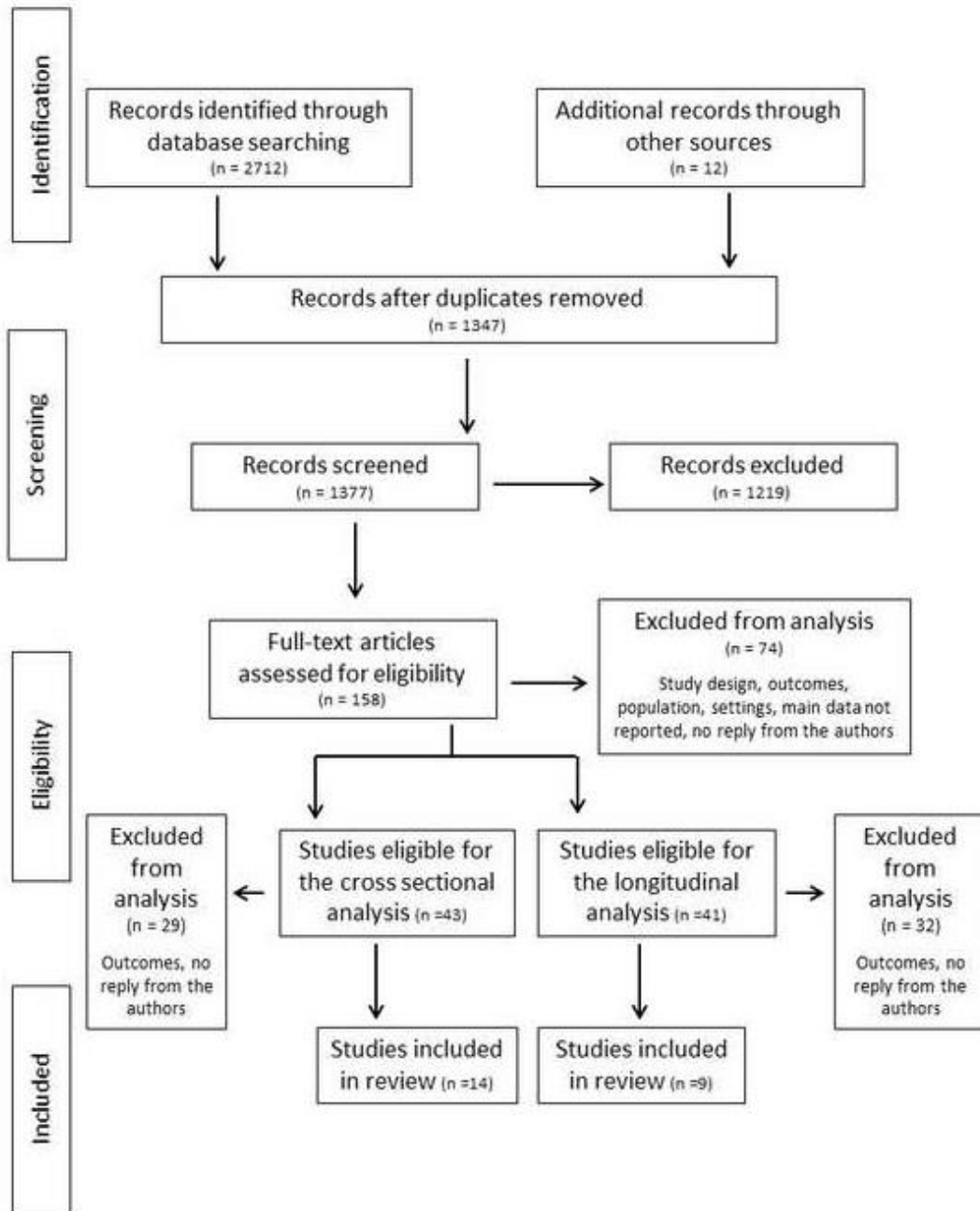
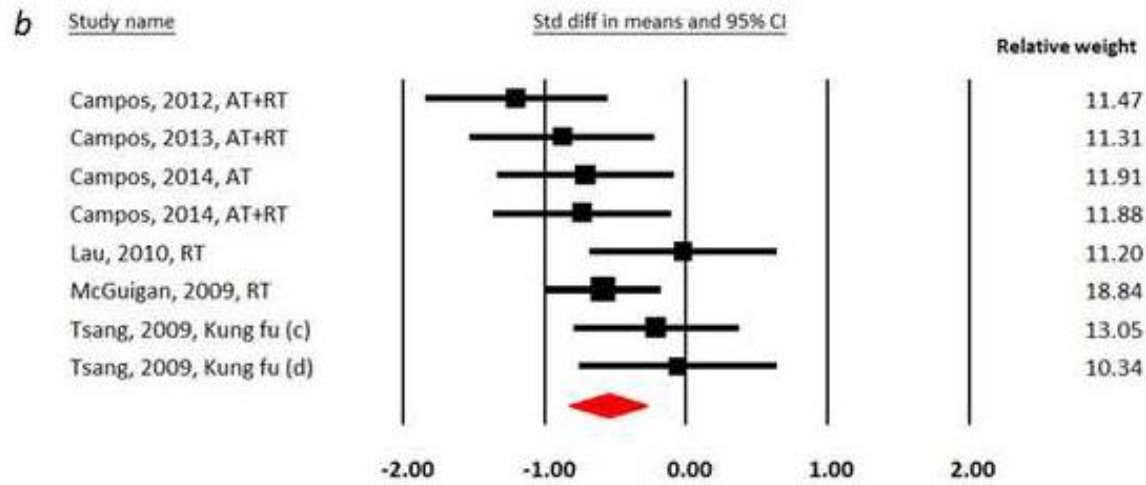
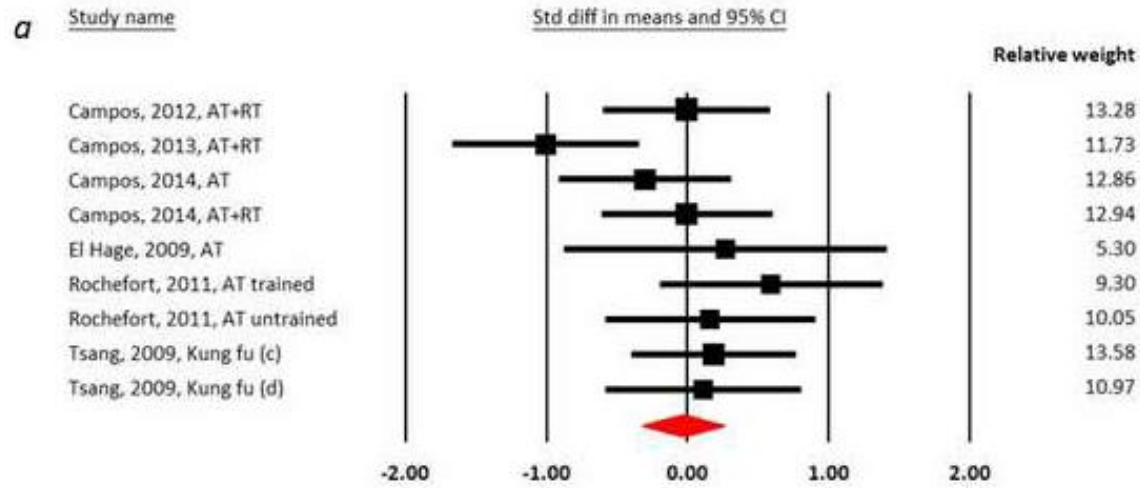


Figure 3

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An author or coauthor shall have:

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2. Written the report or reviewed successive versions and shared in their revisions;
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Journal of Bone and Mineral Metabolism

Manuscript's Title: "Effects of Physical Activity interventions on bone health in obese children and adolescents: a SR and RA"

Authors' Names: E. CHAPLAIN ; G. NAUGHTON ; D. GREENE ; F. DUTHEIL ; B. PEREIRA ; D. THIVEL ; D. COURTEIX .

I (We) hereby certify that the authors of the above manuscript have all:

1. conceived, planned, and performed the work leading to the report, or interpreted the evidence presented, or both;
2. written the report or reviewed successive versions and shared in their revisions; and
3. approved the final version.

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1. this work has neither been published nor is under consideration elsewhere; and
2. humane procedures have been utilized in the treatment of experimental animals.

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Chapman

Appendix 11 - Article 4

Bone responses to a multidisciplinary weight loss intervention in adolescents with obesity: The ADIBOX longitudinal study

Manuscript Details

Manuscript number NR_2017_404

Title "Bone responses to a multidisciplinary weight loss intervention in adolescents with obesity: The ADIBOX longitudinal study"

Article type Research Paper

Abstract

The aim of this intervention study was to investigate the impact of a structured weight loss intervention on bone health in adolescents with obesity. We hypothesized that weight loss induced by physical activity and nutrition would result in positive bone adaptations in adolescents with obesity. Thirty-one adolescents with obesity (OBESE, mean (SD) 13.61 (1.27) years, zBMI 2.26 (0.30)) following an 8-month weight loss program were compared with 23 matched controls (NW, 15.90 (0.43) years, zBMI -0.12 (0.48)). Body composition and bone densitometry (total body less head, lumbar spine, hip, neck and femoral neck using the Hip Structural Analysis program) was assessed by DXA. Plasma concentration of leptin, estradiol, CTx and P1NP were measured. Compared with the control group before intervention OBESE displayed lower bone density. By the end of the successful period of weight loss when adjusted for fat mass changes, OBESE displayed lower bone density at the whole body (mean (95%CI) 0.966 (0.927-1.005) $p < 0.001$), neck (0.933 (0.867-0.999) $p = 0.031$) and hip (1.003 (0.932-1.073) $p = 0.008$) compared with NW. Both the intertrochanteric and femoral shaft sites also showed lower cortical thickness (IT 0.449 (0.404-0.495) $p = 0.005$, FS 0.564 (0.502-0.606) $p = 0.011$) and density (IT 1.043 (0.953-1.134) $p = 0.006$, FS 1.523 (1.434-1.611) $p = 0.003$) in OBESE compared to NW. To conclude, from a clinical perspective, bulking ratio at the narrow neck suggests higher risks of fracture in OBESE. Although the induced weight loss favored positive adaptations in bone parameters in OBESE, geometric indices and bone strength at weight bearing sites remained compromised.

Keywords obesity, adolescents, weight reduction programs, bone mass, bone remodeling

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Suggested reviewers Lauren Burt, Rachel Duckham, Nicolas Bonnet, Eric LESPESSAILLES

Cover letter

June, 6th 2017

"Bone responses to a multidisciplinary weight loss intervention in adolescents with obesity: The ADIBOX longitudinal study"

Dear Editor in Chief Bruce A. Watkins,

We would like to thank you for allowing us to resubmit our work in your journal. We do hope that our article is now in line with the journal guidelines.

We are pleased to submit an original article entitled "**Bone responses to a multidisciplinary weight loss intervention in adolescents with obesity: The ADIBOX longitudinal study**" for publication in your journal. The overall purpose of this innovative work was to investigate the effects of body mass and body mass changes on the bone-adipocyte cross-talk in adolescents with obesity during a weight loss program induced by physical activity and nutrition.

We believe that our article will contribute to the existing literature. Currently, a major limitation of the literature is the heterogeneity in studies' design. The main strength of our article is to recruit maturation-matched participants. Also, this is the first time in longitudinal studies on weight loss and bone health that data collection were performed 3 times with a mid-intervention data collection point. In addition, we believe our article is innovative with the analysis of bone markers over the 8 months.

Given the increasing interest of obesity among pediatric populations, we hope this study's results will be of interest to your readership. We note strong Scopus citation records for recent publications in your journal on obesity related (O'Neil et al. 2011; Gonzalez et al. 2015) and bone related (Shen et al. 2012; Cao et al. 2015) issues.

The name/title, institution and email addresses of the 4 suggested experts are provided on the following page. Page 3 of this document provides all authors printed name, affiliation, email address and signature as requested.

We sincerely thank you for the attention that will be given to this article.

Kind regards,
Miss Elodie CHAPLAIS

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Highlights:

- Adolescents with obesity displayed lower baseline unadjusted and adjusted bone parameters compared with normal weight.
- Adolescents with obesity geometric indices of bone strength at baseline are altered when adjusted for cofounders.
- Adolescents with obesity geometric indices of bone strength at weight bearing site (narrow neck) remain unadapted to excess body weight with scores close to the fracture prediction threshold.
- Bone accretion in adolescents with obesity undergoing a weight loss program appear to follow an androgen growth through a stimulation of periosteal expansion and endocortical resorption.

1 Bone responses to a multidisciplinary weight loss intervention in adolescents with
2 obesity: The ADIBOX longitudinal study.

3

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26 **Abbreviations**

- 27 ACT; average cortical thickness,
28 aFM; android fat mass,
29 ANOVA; analysis of variance,
30 BMAD; bone mineral apparent density,
31 BMC; bone mineral content,
32 BMD; bone mineral density,
33 BMI; body mass index,
34 BW; body weight,
35 CSA; cross-sectional area,
36 CSMI; cross sectional moment of inertia,
37 CTX; C-terminal telopeptide of type 1 collagen,
38 DXA; dual energy x ray,
39 ED; endocortical diameter,
40 FM; fat mass,
41 FS; femoral shaft,
42 gFM; gynoid fat mass,
43 HSA; hip structure analysis,
44 IT; intertrochanteric,
45 LM; lean mass,

- 46 LS; lumbar spine,
- 47 NN; narrow neck,
- 48 NW; normal weight,
- 49 P1NP; N-terminal propeptide of type 1 procollagen,
- 50 SD; standard deviation,
- 51 TBLH; Total body less head
- 52 TS; Tanner stage,
- 53 UI; uncoupling index,
- 54 VFAT; visceral fat,
- 55 WIDTH; width,
- 56 WB; whole body,
- 57 Z; section modulus
- 58
- 59
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- 65

66 **Abstract**

67 The aim of this intervention study was to investigate the impact of a structured
68 weight loss intervention on bone health in adolescents with obesity. We
69 hypothesized that weight loss induced by physical activity and nutrition would result
70 in positive bone adaptations in adolescents with obesity. Thirty-one adolescents with
71 obesity (OBESE, mean (SD) 13.61 (1.27) years, zBMI 2.26 (0.30)) following an 8-
72 month weight loss program were compared with 23 matched controls (NW, 15.90
73 (0.43) years, zBMI -0.12 (0.48)). Body composition and bone densitometry (total
74 body less head, lumbar spine, hip, neck and femoral neck using the Hip Structural
75 Analysis program) was assessed by DXA. Plasma concentration of leptin, estradiol,
76 CTx and P1NP were measured. Compared with the control group before intervention
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81 intertrochanteric and femoral shaft sites also showed lower cortical thickness (IT
82 0.449 (0.404-0.495) $p = 0.005$, FS (0.564 (0.502-0.606) $p = 0.011$) and density (IT
83 1.043 (0.953-1.134) $p = 0.006$, FS (1.523 (1.434-1.611) $p = 0.003$) in OBESE
84 compared to NW. To conclude, from a clinical perspective, bulking ratio at the
85 narrow neck suggests higher risks of fracture in OBESE. Although the induced
86 weight loss favored positive adaptations in bone parameters in OBESE, geometric
87 indices and bone strength at weight bearing sites remained compromised.

88 **Keywords:** obesity, adolescents, weight reduction programs, bone mass, bone
89 remodeling

90 **1. Introduction**

91 Childhood obesity has dramatically increased over the past four decades [1]
92 resulting in a higher prevalence of health-related disorders [2]. Visceral adiposity
93 associated with obesity increases the risk of comorbidities such as type 2 diabetes,
94 hypertension and dyslipidaemia [3]. Despite the elaboration of national health
95 strategies to limit obesity and its associated complications, the success and
96 sustainability of weight management interventions among children and adolescents
97 remain questionable. Indeed, weight loss programs to improve obese youth's overall
98 health may inadvertently compromise the critical growth-related benefit of bone mass
99 [4].

100 The traditional understanding is that obesity can benefit bone density [5] [6] [7] [8] [9]
101 [10]. The increase of skeletal load and greater conversion of adrenal androgen to
102 estrogens in the presence of obesity seem to be the primary mechanisms behind
103 higher bone formation [11] [12]. However, recent studies have challenged the
104 concept of a protective effect of obesity on bone, indicating that fat accumulation
105 may be detrimental to bone quality during the growing years. A mismatch between
106 body weight and bone development during growth [13] as well as micro-architectural
107 bone deficits [14] increase the risk of fracture [13] and might possibly favour an early
108 onset of osteoporosis [14]. Recent investigations of the systemic relationships
109 between bone and fat challenge the passive role of adipocytes (storage cells) on
110 bone tissue [15]. Researchers have isolated the relative contribution of
111 subcutaneous versus visceral fat on bone mass [16] [17] [18]. Altered pro-
112 inflammatory cytokines may negatively affect bone metabolism by increasing bone
113 resorption [19] [20] [21] [22] leading to increased risk of fracture especially during the
114 peripubertal period [5].

115 A common treatment strategy for obesity during adolescence involves lifestyle
116 interventions aimed to reduce weight [23]. Yet, bone metabolism might be altered
117 because of weight loss strategies. Caloric restriction and decreased weight on the
118 skeleton might have a negative impact on bone health [4] [24] [25]. However, when
119 caloric restriction and physical activity occur concurrently, reduction of bone loss
120 may be exacerbated. In adults, studies have highlighted the necessity to add
121 exercise interventions to caloric restrictions when inducing weight loss to prevent the
122 loss of bone mass [26] [27]. Also, in adolescents with obesity, a study comparing the
123 effect of two types of exercise plus caloric restriction (normo-caloric diet) on
124 adipokines levels and bone density suggested an improvement in both when
125 physical activity combined aerobic and resistance training [28]. Results support the
126 mechanostat theory suggesting that bone fragility from a compromised relationship
127 between density, geometry and strength might theoretically be prevented if lean
128 mass is maintained during a successful weight loss program [29] [28]. The impact of
129 a multidisciplinary weight loss program combining nutrition and physical activity on
130 bone quality remains uncertain, especially in adolescents with obesity. To date, the
131 evaluation of bone parameters during weight loss program has been restricted to the
132 assessment of bone density [30] [28] [31] [32] [33]. Thus, we hypothesized that
133 weight loss induced by physical activity and nutrition would result in positive bone
134 adaptations in adolescents with obesity. The present study aimed to investigate (1)
135 the impact of a multidisciplinary weight loss program combining nutrition and
136 physical activity on the bone health of adolescents with obesity and (2) the effects of
137 body weight status and changes (obesity versus normal weight) on bone parameters
138 in adolescents.

139

140 **2. Methods and materials**

141 2.1- Participants

142 Thirty-one adolescents with obesity (OBESE, mean (SD) 13.61 (1.27) years
143 including 6 males) and 23 normal weight participants (NW, 15.90 (0.43) years -
144 female) were enrolled in the study (Figure 1). The inclusion criteria were as follows:
145 (1) aged between 12 to 16 years, (2) Tanner Stage \leq 4, (3) BMI < 95th percentile or
146 between the 5th and 85th percentiles for the normal weight control [34], (4) had
147 reached menarche at least one year prior to the study. The protocol was approved
148 by the Hospital Sud Est 1 Ethics committee (2015-33) and registered as a Clinical
149 Trial (NCT02626273).

150

151 2.2- Study design

152 After a medical inclusion by a Pediatrician to ensure the ability of the adolescents to
153 complete the whole study, participants from the obese intervention (OBESE) group
154 completed an 8-month multidisciplinary weight loss program. Assessments were
155 performed at the beginning of the intervention (T0), at 4 months (T1) and 8 months
156 (T2). In order to cover the whole period of the weight loss program and to minimize
157 the inconvenience of hospital-based testing for the control group, we only assessed
158 the normal weight control group (NW) twice: at T0 (baseline) and 8 months. The
159 protocol has been previously detailed [35].

160

161 2.3- The 8-month multidisciplinary weight loss program

162 Adolescents from the intervention group followed an 8-month weight loss program
163 combining dietary education and physical activity, at the Pediatric Obesity clinical
164 center (Tzanou, la Bourboule, France). Every week, adolescents completed two 60-

165 minute exercise sessions combining resistance and aerobic exercises as well as one
166 water-based 60-minute session and 1 session composed of collective and sport
167 games (i.e. ball and racquet games or recreational activities such as trekking or
168 snowshoeing). Regarding the nutritional intervention, the adolescents followed a
169 normo-caloric diet set to reach recommended levels of dietary intake based on
170 physical activity level, age and gender [36]. Adolescents also followed nutritional
171 education once a week.

172

173 2.4- Anthropometric measurements and maturation

174 Body mass was measured with participants wearing light clothing on a digital
175 electronic scale (0.1 kg precision) and stature on a stadiometer (0.1 cm precision).
176 BMI was converted into BMI z-score relative to age using the references
177 recommended by the Centers for Diseases Control and Prevention [37]. Body
178 composition of the whole body was measured using Dual X-ray Absorptiometry
179 (DXA, QDR-4500A, Hologic, Inc., Waltham, MA) for lean mass (LM, g) and fat mass
180 (FM, % and g). From the scan obtained the software characterized android fat mass
181 (aFM, %), gynoid fat mass (gFM, %) and estimated visceral fat (VFAT %, g and cm³)
182 [38]. Tanner stage (TS) were determined at baseline using the self-assessment
183 questionnaire developed by Morris and Udry [39]. Similarly, the age of menarche
184 (defined as the achievement of regular cycles) was self-reported for female.

185

186 2.5- Bone densitometry assessment

187 Bone mineral density (BMD, g/cm²), bone mineral content (BMC, g), and bone area
188 (cm²) were determined using the same DXA device. DXA measurements were: total
189 body less head (TBLH), lumbar spine (LS, L2-L4) and non-dominant hip (including

190 the femoral neck, trochanteric and intertrochanteric regions). The DXA reproducibility
191 assessment was based on two repeated measurements over 2 weeks in 3 subjects.
192 The in vivo coefficients of variability using this technique were 0.35 and 0.9% for
193 BMD, and 0.57 and 1.2% for BMC at the lumbar spine and total body, respectively.
194 Bone mineral apparent density (BMAD, $\text{g}\cdot\text{cm}^{-3}$) was calculated using the following
195 equation: $\text{WB BMAD} = \text{BMC}/(\text{WB bone area}^2/\text{body weight})$ and $\text{LS BMAD} = \text{BMC}$
196 $(\text{LS})/\text{spine bone area}$ [40].
197 Hip structure analysis (HSA) was performed at the narrow neck (NN), the femoral
198 shaft (FS) and the intertrochanteric region (IT). At each region we analyzed the
199 cross-sectional area (CSA, cm^2), the BMD ($\text{g}\cdot\text{cm}^{-2}$), the endocortical diameter (ED,
200 cm), the average cortical thickness (ACT, cm), the width (WIDTH, cm), the cross
201 sectional moment of inertia (CSMI, cm^4), the section modulus (Z, cm^3) and the
202 Bulking ratio (BR).

203

204 2.6- Blood analyses

205 Blood samples were collected by a qualified paediatric nurse after a fasted overnight.
206 The blood was centrifuged and aliquots were frozen for subsequent analyses. The
207 markers of bone formation (PINP) and resorption (CTx) (Cloud-Clone Corp, Houston,
208 US), leptin (BioVendor, Czech Republic) and estradiol (BioVendor, Czech Republic)
209 were assessed by a trained technician from the local University Hospital following
210 manufacturers' recommendations. Intra, inter-assay coefficient of variations and
211 sensitivity were respectively: P1NP <10%, <12%, <12.3 pg/ml; CTx <10%, <12%
212 <44.3pg/ml; leptin <8%, <7%, 0.2 ng/ml and estradiol <10%, <12%, 10pg/ml. In order
213 to assess the bone remodelling balance, we calculated the uncoupling index (UI) as
214 described by Eastell [41].

10

215

216 2.7- Statistical analysis

217 Sample size estimation centered on the variation index of body fat relative to the
218 variation of a marker of bone mass (bone mineral density) measured at the lumbar
219 spine. The index difference between groups was at 1.3 (standard deviations) based
220 on existing research [28]. To highlight statistically significant differences with a
221 statistical power of 90% and a type 1 error less than 5%, and anticipating a potential
222 20% drop-out among participants, a minimum of 25 volunteers per body composition
223 group will be invited to take part in the study.

224 Statistical analyses were performed using Stata software (version 13, StataCorp,
225 College Station, US). The tests were two-sided with a type I error set at $\alpha = 0.05$.

226 Data are displayed as the mean \pm standard deviation or median \pm interquartile.

227 Assumption of normality was assessed by Shapiro-Wilks test. A cross tabs chi-
228 square test was performed to test the homogeneity of the sample. At baseline,

229 comparisons between groups (OBESE group and normal weight groups) were
230 performed using ANOVA or Kruskal-Wallis (KW) test if assumptions of ANOVA were

231 not met ((i) normality and (ii) homoscedasticity using Bartlett test). The study of

232 relationships between body composition, bone, biologic parameters was explored

233 using correlation coefficient (Pearson or Spearman, according to data distribution).

234 Repeated correlated data (parameters measured longitudinally) were investigated

235 using mixed models to take into account between and within participant variability

236 (as random effect) while studying the impact of fixed effects: group, time point

237 evaluation and their interaction. In multivariate analysis, these regression models

238 were used to adjust for covariates (body weight or fat mass or lean mass)

239 determined according to univariate results and clinical relevance: delta weight, delta

240 whole body fat mass). The normality of residuals from these models was studied as
241 described above.

242 Using the control values as reference data, z-scores of formation and resorption
243 markers were calculated for each subject. Then the UI was calculated as the
244 average of the z-score for the bone formation marker minus the bone resorption
245 marker. A positive UI indicates that bone remodeling was unbalanced in favor of
246 formation. A negative UI indicates an imbalanced favoring resorption [42].

247

248 **3. Results**

249 **3.1- Baseline**

250 The study enrolled a total of 54 participants: 31 obese and 23 normal-weight
251 adolescents. As illustrated by the Figure 1, 47 (87%) of them completed the whole
252 study including three boys. For the longitudinal analysis, only adolescents who
253 completed the whole study were considered for statistical analysis. Baseline
254 descriptive characteristics (body composition) did not differ between the adolescents
255 who dropped out of the weight loss intervention and the rest of the intervention
256 sample. However, significant differences were observed in bone parameters. Indeed,
257 regarding bone density lower TBLH BMD ($p=0.005$), WB BMC ($p=0.006$), WB BMAD
258 ($p=0.002$), LS BMD ($p=0.020$), LS BMC ($p=0.010$), hip BMC ($p=0.046$), neck BMC
259 ($p=0.029$) were observed in OBESE who dropped out the study compared with the
260 one who completed it. Results in geometry parameters showed lower BMD
261 ($p=0.007$), cortical thickness ($p=0.029$) at the shaft. In addition, strength parameters
262 were lower for cross section area at IT ($p=0.043$) and FS, for cross sectional moment
263 of inertia ($p=0.018$) at the shaft and for section modulus at the narrow neck
264 ($p=0.020$), the intertrochanteric ($p=0.023$) and the femoral shaft ($p=0.008$) sites in the

265 adolescent with obesity who dropped out the study compared with the one who
266 completed it. The chi-square test showed no influence of male ($p=0.080$) even if the
267 estradiol level are lower: groups are homogenous.

268 The Table 1 presents the whole characteristics of the obese and lean sample. Body
269 Mass Index and body weight were both higher in OBESE ($p<0.001$ for all). Estradiol
270 levels did not differ between the two groups. Height and years since menarche of
271 girls was similar in both groups, despite the older chronological age of the NW
272 ($p<0.001$). At baseline, compared with NW, OBESE had higher lean mass, higher
273 whole body FM, android FM and gynoïd FM ($p<0.001$ for all). Measures of visceral
274 tissue (% , g and cm^3) were also higher in OBESE than NW ($p<0.001$ for all).

275 Bone measurements expressed as unadjusted and adjusted values (body weight or
276 fat mass or lean mass) are presented in Table 2.

277 Adolescents with obesity had lower total body less head bone density (TBLH BMD)
278 ($p<0.001$), lower hip BMD ($p=0.022$), lower bone mineral content at whole body
279 ($p=0.048$) spine ($p<0.001$) and hip ($p=0.008$) than the normal weight control. In
280 addition, OBESE had a lower bone mineral apparent density at the whole body
281 ($p<0.001$). Once differences in body weight (BW), or fat mass (FM), or lean mass
282 (LM) were accounted for, OBESE compared with NW, displayed lower quantitative
283 bone at all sites, except LS BMAD ($p=NS$) when adjusted to FM ($p<0.001$ when
284 adjusted for BW except LS BMAS $p=0.022$, $p<0.005$ when adjusted for FM except
285 WB BMAD and neck BMD ($p=0.020$) and $p<0.008$ when adjusted for LM).

286 OBESE had lower femoral shaft (FS) density ($p=0.008$), lower FS cortical thickness
287 ($p=0.009$) and higher FS endocortical diameter ($p=0.040$) and bulking ratio ($p=0.028$)
288 than NW. When adjusted for body weight, or fat mass, or lean mass, OBESE
289 displayed lower bone density at the intertrochanteric (IT) ($p<0.005$ adjusted BW,

290 p=0.012 adjusted LM and p=0.038 adjusted FM) and femoral shaft (p=0.001
291 adjusted BW or LM, p=0.022 adjusted FM), a tendency of lower density at NN was
292 observed after adjustment for BW (p=0.051). In addition, results showed lower width
293 at all sites (NN, IT FS) in Ob (p=0.001 adjusted BW, p<0.009 adjusted FM and
294 p<0.010 adjusted FM at NN and FS, p=0.002 at IT). At the narrow neck, lower
295 endocortical diameter after adjustment for BW (p=0.002) and FM (p=0.001) were
296 observed as well as a tendency after adjustment for LM (NN p=0.052). Similarly, at
297 IT a tendency of lower endocortical diameter was seen after adjustment for BW
298 (p=0.052). Finally, results showed lower cortical thickness at the intertrochanteric
299 and the femoral shaft in OBESE than NW after adjustment for body weight or lean
300 mass (p<0.008 adjusted BW and p<0.005 adjusted LM).

301 At baseline, OBESE had a higher raw bulking ratio at the shaft (p=0.028) than NW.
302 Otherwise, all data were similar between both groups. Once differences in body
303 weight, fat mass, or lean mass were accounted for, OBESE compared with NW,
304 displayed lower cross sectional area (all sites p<0.001 adjusted BW, p<0.009
305 adjusted FM, p<0.006 adjusted LM), cross sectional moment of inertia (all sites
306 p<0.001 adjusted BW, p<0.004 adjusted FM, p<0.004 adjusted LM) and section
307 modulus at all site (NN, IT, FS) (p<0.001 adjusted BW, adjusted FM: NN p=0.014, IT,
308 FS p<0.002, p<0.009 adjusted LM).

309

310 3.2- Weight loss intervention

311 Longitudinal analysis revealed that OBESE reduced their body weight, BMI, fat mass
312 (total (g, %), android, gynoid, visceral (g, %, cm³) during the 8-month weight loss
313 program (Figure 2) (p<0.007).

314 Bone mineral density increased within the first four months of the weight loss therapy
315 program (Δ mean variation (SD)) LS BMD Δ 2.66 (2.94) % $p < 0.001$) as well as after
316 8 months (WB BMD (Δ 3.22 (3.58) % $p < 0.001$, LS BMD (Δ 3.67 (4.04) % $p < 0.001$)).
317 Variations in BMD at TBLH and LS, at mid intervention, might be explained by body
318 composition changes (Δ BW or Δ FM) as data became non-significant when adjusted
319 to those variables.

320 At 4 months, reduced NN BMD (Δ -4.35 (6.19) % $p < 0.001$) and NN cortical thickness
321 (ACT) (Δ -7.19 (8.79) % $p < 0.001$) were observed. Also, the NN endocortical diameter
322 (ED) (Δ 2.85(0.26) % $p = 0.009$) and width (WIDTH (Δ 5.48(10.84) % $p = 0.016$)
323 increased. At the IT we observed reduced BMD (Δ -1.64 (3.24) % $p = 0.020$) and
324 increased ED (Δ 4.24 (5.24) % $p = 0.010$) and WIDTH (Δ 3.49 (4.40) % $p = 0.010$).

325 Also, the FS endocortical diameter (Δ 5.15 (8.09) % $p = 0.010$) and WIDTH increased
326 (Δ 3.14 (2.68) % $p < 0.001$). Similarly at the end of the 8 month program, OBESE
327 continued to show a reduction in NN BMD (Δ -4.74 (6.07) % $p < 0.001$) and an
328 increased in endocortical diameter (Δ 6.20 (6.77) % $p < 0.001$) and width (Δ 6.16
329 (7.69) % $p < 0.001$) compared with their baseline scores. Moreover, IT BMD reduced
330 (Δ -3.43 (4.62) % $p < 0.001$) while intertrochanteric ED (Δ 4.00 (4.31) % $p < 0.001$) and
331 width (Δ 3.06 (2.79) % $p < 0.001$) continued to increase. Finally, the FS cross
332 sectional area (CSA) (Δ 4.49 (5.21) % $p = 0.002$) increased (Figure 3). Variations of
333 narrow neck cortical thickness was at least partially explained by each of the body
334 composition changes and NN bone mineral density by changes in fat mass.

335 However, at the end of the weight loss program, variations of NN BMD, NN cortical
336 thickness, intertrochanteric BMD, IT endocortical diameter were explained by
337 changes in body weight or fat mass.

338 At 4 months, bone strength variables showed increases in FS bulking ratio (BR) (Δ
339 4.95 (9.28) % $p=0.020$), IT BR (Δ 3.66 (7.09) % $p=0.020$), NN section modulus (Z) (Δ
340 1.70 (0.39) $p=0.039$) and femoral shaft cross sectional moment of inertia (CSMI) (Δ
341 3.88 (1.27) $p=0.030$). At program completion, narrow neck BR (Δ 8.24 (2.00) %
342 $p=0.005$), intertrochanteric BR (Δ 9.25 (6.43) % $p<0.001$), femoral shaft cross-
343 sectional area (Δ 4.67 (5.29) % $p<0.001$) and FS cross sectional moment of inertia
344 (CSMI) (Δ 7.09 (15.99) % $p=0.030$) also increased (Figure 4). When adjusted for Δ
345 BW or Δ FM it appeared that Δ IT BR was associated with all body composition
346 changes at the mid-point of the program. At the end of the program, bone strength
347 parameters were explained by changes in body weight or fat mass.

348 Leptin, estradiol and P1NP concentrations remained similar throughout the weight
349 loss program. However, a significant reduction in marker CTx was observed at 4
350 months ($p=0.037$) before reaching its baseline secretion level ($p=0.013$) (Table 5).
351 At the end of the program, leptin was positively associated with bone density (LS)
352 ($p=0.020$) and cortical thickness (narrow neck, intertrochanteric, femoral shaft)
353 ($p=0.002$, $p=0.046$, $p=0.049$ respectively). In contrast, the bone resorption marker
354 displayed an inverse correlation with bone density (WB $p=0.002$; LS $p<0.001$; neck
355 $p<0.001$, hip $p=0.012$, NN $p<0.001$, IT $p=0.046$), cortical thickness (NN $p<0.001$, IT
356 $p=0.026$), cross sectional area (NN $p<0.001$, IT $p=0.013$) and section modulus (NN
357 $p=0.004$, IT $p=0.016$) and was positively correlated with bulking ratio (NN $p=0.016$)
358 (data and coefficients are detailed in Table 4 A.).

359 The uncoupling index and z- scores were calculated based on the OBESE baseline
360 data. The weight loss intervention program combining physical activity and nutrition
361 may profit to bone health with a metabolic balance favoring bone formation.
362 However, despite an acute formation phase observed in the UI at 4 months (0.98

363 (2.19)) before declining at 8 months (0.32 (1.80)) no significant differences regarding
364 the uncoupling index of bone remodeling activity were observed over the weight loss
365 program.

366 Correlational analysis at the completion of the weight loss therapy program showed
367 no influence of VFAT (% or g) on bone parameters. However, body weight (g), fat
368 mass (g) was moderately correlated with all bone parameters, except for NN BR
369 (BW $p=0.006$) (data and coefficients are detailed in Table 4 B.).

370

371 3.3- Time-related differences

372 Bone measurements expressed as unadjusted and adjusted values are presented
373 Table 6.

374 Differences in bone density crude values were observed for WB BMAD ($p<0.001$)
375 and hip BMD ($p=0.017$) between OBESE and NW. Once the longitudinal changes in
376 body weight (BW), or fat mass (FM) were accounted for, OBESE showed lower
377 density at TBLH ($p<0.008$ for all), neck ($p<0.001$ adjusted BW, $p=0.031$ adjusted
378 FM), hip ($p<0.008$ for all) and lower bone mineral apparent density ($p<0.008$ for all)
379 at WB than NW. Differences in the evolution of bone parameters (Δ LS BMAD
380 ($p=0.020$), Δ neck BMD ($p=0.030$), Δ hip BMD ($p<0.001$)) between both groups were
381 explained by variations in BW and Δ FM. Only Δ hip BMD remained significantly
382 different after adjustment Δ BW.

383 Compared with changes over time in NW, at the end of the weight loss program
384 OBESE showed greater values in narrow neck endocortical diameter (ED) ($p=0.005$),
385 NN width ($p=0.003$), intertrochanteric endocortical diameter ($p=0.005$), IT width
386 ($p=0.017$) and femoral shaft ED ($p=0.006$). In contrast, lower values were observed
387 in adolescents with obesity in IT BMD ($p=0.009$), IT cortical thickness (ACT)

388 (p=0.031), FS BMD (p=0.004) and FS ACT (p=0.001) (Figure 5). When data were
389 corrected for longitudinal changes in body weight (BW), or fat mass (FM) OBESE
390 had lower bone density and cortical thickness at IT (p<0.010 for all) and FS (p<0.010
391 for all) than NW. Lower cortical thickness was observed at NN (p=0.050) after
392 adjustment for body weight changes. In addition, higher endocortical diameter after
393 correction for BW changes (FS p=0.014) and FM changes (IT p<0.050, FS p=0.018)
394 were seen in OBESE compared with NW. Time-related changes in bone parameters
395 between OBESE and NW can be explained by variations in BW or Δ FM. Only the
396 Δ IT BMD maintained significance when adjusted for Δ BW.
397 Observed differences in raw strength indices were observed for NN bulking ratio
398 (BR) (p=0.008), IT BR (p=0.004) and FS BR (p=0.004). When adjusted for Δ BW,
399 cross-sectional area (IT and FS) was higher than NW (p<0.003 for all). Cross-
400 sectional area at IT (p=0.020) was also higher when corrected for Δ FM. Higher
401 bulking ratio at IT and FS for OBESE than NW were observed once the longitudinal
402 changes in BW or FM (p<0.004 for all) were adjusted. Time-related changes of bone
403 parameters between groups for Δ IT BR (p< 0.001), Δ FS BR (p= 0.022), were
404 explained by variations in BW or Δ FM.
405 At the end of the weight loss program, no difference was observed in estradiol level
406 between groups. Results from correlation coefficient analyses showed estradiol
407 negatively correlated with IT BMD (r= -0.228, p= 0.048).

408

409 **4. Discussion**

410 The aim of the study was first to assess the effects of a multidisciplinary weight loss
411 program combining nutrition and physical activity on bone parameters in adolescents
412 with obesity. A weight status comparison was also performed over the same

413 timeframe. This study showed site-specific bone changes (density, geometry and
414 strength) and positive adaptation in axial rather than appendicular sites in response
415 to the weight loss program. The major finding of the present study is that geometric
416 indices of bone strength at weight bearing site (NN) remain unadapted to excess
417 body weight despite positive adaptation of bone parameters induced by the weight
418 loss intervention in adolescents with obesity. Moreover, our results highlight that
419 bone accretion in adolescents with obesity follow an androgen growth through a
420 stimulation of periosteal expansion and endocortical resorption.

421

422 The current literature shows inconsistency regarding bone mineral density in
423 adolescents with obesity [43] [44] [13] [45] [46] [45] [44] [18] [46] [10]. In accordance
424 with some studies [43] [44] [13] [45] [46], we observed at baseline lower unadjusted
425 data for bone mineral density measures involving WB (less head) BMD, WB BMAD,
426 LS BMAD and no differences at LS BMD, neck BMD, hip BMD in OBESE compared
427 with NW. When adjusted to the variation of body weight (kg), or fat mass (g), OBESE
428 had lower values than NW for bone mineral density measures of the whole body,
429 lumbar spine and hip. Although the influence of lean mass (g) on bone is well
430 demonstrated [47], lean mass did not change through the weight loss program
431 justifying why we only adjusted to lean mass at baseline. During the weight loss
432 program, trabecular and cortical bone appeared to adapt to body weight changes.
433 Contrary to the negative associations between weight loss and BMD [48], OBESE
434 increased their BMD (WB and LS). However, when compared with changes in NW,
435 OBESE had similar unadjusted bone density values at WB, LS and neck but lower
436 values at the hip. With adjustments for body weight changes, BMD became lower in
437 OBESE than NW at all sites except the LS. When adjusted for fat mass variations,

438 similar LS BMD was found in both groups but adolescents with obesity showed lower
439 BMD at WB, neck and hip than their NW peers. Despite the well-known effects of
440 high body weight-stimulating mechanical loading [3], OBESE seemed to have
441 insufficient WB and site-specific bone density to cope with excess of body weight.

442 The discordances (BMD) observed in the literature might be related to in-between
443 study inconsistencies in pubertal [14] [5] and maturation status [49], genders [4] or
444 degree of obesity [50] [51] [16] [52]. In the present study, adolescents shared a
445 similar maturation status despite a younger age in the OBESE group. Indeed, both
446 groups had similar estradiol levels, age at menarche and all participants from this
447 study were at least at Tanner Stage 4. In addition, our study only included
448 adolescents with a BMI percentile above 95th (BMI percentile mean and SD of the
449 adolescents in the weight-loss program group was 98.25 (1.89)). Moreover, the
450 OBESE adolescents enrolled in the present work showed an early onset history of a
451 BMI percentile ranking above 97th by the age of 3.76 (1.54) years. The incidence of
452 obesity in the early childhood years of ages 2 to 7 are critical times for developing
453 bone parameter weaknesses [14]. We might postulate that the mineralization delay
454 potentially occurring in prepubertal years due to the early onset of obesity was too
455 substantial to be corrected during pubertal years.

456

457 Even if the fat-bone mechanisms remain to be elucidated, fat mass has an important
458 role during bone growth depending of the degree of obesity [50] [51] [16] [52].
459 Growing bone are fashioned into a structure adapted to support the skeleton. During
460 adolescence, changes in modular volume depends on endocortical resorption
461 enlarging the marrow cavity stimulated by bone growth and endocortical apposition
462 of bone mineral often in response to physical activity [53]. Moreover, most of the

463 increases of cortical thickness are attributed to gains in bone width [54]. Impairment
464 of periosteal apposition might result in smaller bones and increased fracture risks
465 due to bending loads [54]. In our study, we observed that OBESE have lower
466 quantitative bone parameters. Reduced load appeared to cause higher fragility at the
467 narrow neck than the shaft. This could be explained by modifications in compressive
468 and tensile stresses attributed to weight loss. Results from investigations with
469 peripheral Quantitative Computed Tomography (pQCT) [51] [55] support our finding
470 that skeletal adaptations may be compromised during growth independent of the
471 weight bearing effects of excess weight relative to body mass and levels of fat mass.
472 In addition, when focusing on the shaft the present study highlighted a significant
473 increase in cortical area with a similar magnitude in both group. However, only the
474 normal weight group increased cortical thickness and bone density. It is of interest as
475 the normal bone growth during late puberty is stimulated by periosteal apposition
476 and medullary contraction (endocortical apposition) increasing cortical thickness [56]
477 [57] [58]. In the adolescents with obesity from our study we observed that reduced
478 weight leads to periosteal expansion and endocortical resorption. Interestingly,
479 during pubertal growth, estrogen is known to inhibit periosteal expansion but
480 stimulate endocortical acquisition [57]. OBESE and normal weight adolescents from
481 this study have similar estradiol level. Because of this observation (androgen growth)
482 we question if the presence of males (three boys) influenced geometric
483 development. We performed complementary analysis and withdrew the males from
484 the data set. As shown in figure 5, similar results were observed between groups.
485 However, we expected that the decline observed in cortical thickness would be
486 minimized. Surprisingly, the decline observed worsened. We speculate that the 8-

487 month weight loss intervention produced a similar pattern in pubertal growth in boys
488 with a stimulation of periosteal expansion and endocortical resorption.
489

490 HSA analysis reflects bone's ability to withstand forces generated during walking or
491 during a fall. The impact of weight loss on local modification at specific bone sites is
492 of interest. Section modulus (Z) represents an endpoint in mechanical homeostasis
493 in long bone, able to change itself in order to adapt bone geometry, depending on
494 the demand of environmental load [59]. Conflicting findings about bone strength
495 have been reported in adolescents with obesity. Cross-sectional pQCT studies
496 reported higher bone strength in adolescents with obesity, which was mainly
497 explained by higher maturational levels [60] [61], while HSA studies reported similar
498 [62] or lower [44] [63] bone strength at the hip. In our study, baseline data showed
499 that OBESE had lower resistance to bending, torsional and axial stresses at the
500 narrow neck, the intertrochanteric site and femoral shaft after adjustment to body
501 weight or fat mass. The 8-month WL program worsen adolescents with obesity BR at
502 narrow neck, with scores close to the fracture prediction threshold. In addition, higher
503 BR values at IT and FS were observed after adjusting for Δ body weight or Δ fat
504 mass. With impaired values, OBESE are predisposed to a high risk of hip fractures in
505 long term. Our results suggest that the hip structure of the adolescents with obesity
506 might be insufficient to compensate for their excess of body weight. The evolution of
507 HSA parameters during those 8 months of successful weight loss seemed to be
508 induced by variations in body weight and fat mass. Indeed, observations from NW
509 suggest that unchanged loading (normal weight group) produced significant
510 increases in shaft bending strength, while a significantly reduced weight (OBESE)
511 favors the variation of bone strength parameters. Changes in hip loading are

512 associated with mechanical alteration of section modulus and are likely to weaken
513 femoral neck (cortical and trabecular bone) than shaft (cortical bone) [59].

514

515 Correlation analyses confirmed the observed associations between body
516 composition parameters and bone mass parameters [64] [65] [66]. In the current
517 study, lean mass and bone parameters (density, geometry and strength) correlated
518 strongly, while body weight and fat mass were associated with geometry and
519 strength parameters. In a pQCT study [55] assessing the relationship between WB
520 fat mass and bone geometry on pre-pubertal girls it was highlighted that girls with
521 higher WB FM have diminished volumetric bone mineral density, geometry and bone
522 strength at femur and tibia. In addition, others [16] [18] demonstrated a link between
523 fat mass location and bone mineral density suggesting that visceral fat or visceral
524 fat/subcutaneous fat ratio were negative predictors of BMD in OBESE post pubertal
525 adolescents. Whole body fat mass is inversely correlated to lean mass making it
526 difficult to determine whether bone parameters are influenced by either the fat mass
527 or the lean mass percentages [67]. However, during the weight loss program,
528 OBESE did not significantly change their lean mass and significantly decrease their
529 fat mass suggesting that the observed effect after adjustment for weight change
530 might be linked to the improvement of the fat mass/lean mass ratio. The absence of
531 variation of lean mass during the weight loss program was unexpected. Indeed, most
532 of the studies report an increase in lean mass during WL programs [28] [31] [33].
533 Although this study did not aim at identifying causes of lean mass loss, a possible
534 explanation might be that the physical activity program was more focused on
535 physical practice than on specific osteogenic modalities.

536

537 Finally, bone growth observed by DXA has been observed by calculation of bone
538 marker z-scores as well as by the uncoupling index. Indeed, the uncoupling index,
539 calculated based on OBESE baseline values, showed an acute formation phase at 4
540 months, then, at 8-month a reduced formation occurred. However, without reaching
541 significance. These acute adaptations might be linked with the weight lost by the
542 adolescents with obesity. Indeed, the first 4 months of the WL program correspond
543 to the greatest weight lost.

544

545 We acknowledge several limitations in this study. First, although the adolescents
546 took part in a structured physical activity intervention, it has not been possible to
547 individually track the volume and intensity of the intervention. This could have been
548 of importance to explore a potential individual dose-response effect. Second, the
549 physical activity program did not include enough osteogenic activities. Third, it would
550 have be interesting to have an obese control group. Although these techniques have
551 already shown their good reliability [68], the use of 2D technologies to assess bone
552 strength and bone trabecular microarchitecture may also comprise a limitation.
553 Fourth, we were not able to control for menstrual cycle phases regarding bone
554 markers. Finally, we did not measure the SHBG hormone (Sex hormone-binding
555 globulin), which could provide information regarding the androgen bone growth.

556

557 **Conclusion**

558 Despite positive adaptation of bone parameters induced by the 8-month
559 multidisciplinary weight loss program, fracture risks remained higher in the
560 adolescents with obesity especially at the narrow neck. Moreover, bone accretion in
561 adolescents with obesity undergoing a weight loss program appear to follow an

24

562 androgen growth through a stimulation of periosteal expansion and endocortical
563 resorption.

564

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569

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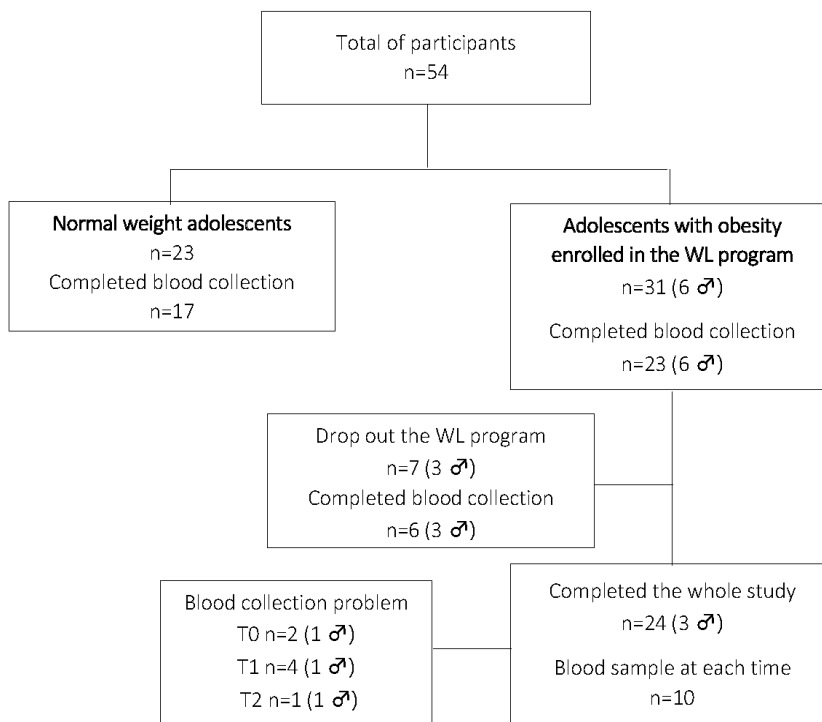
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Figure 1. Flow char of participants

WL weight loss



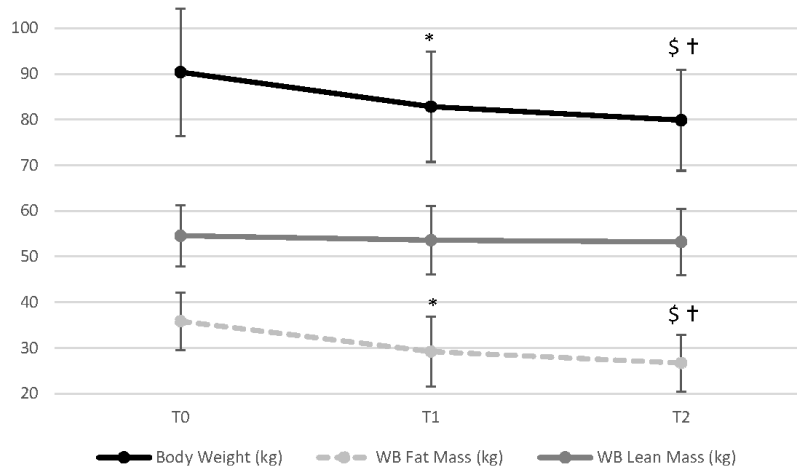


Figure 2. Body composition changes of the OBESE interventional group during the weight loss program

* Significant difference between T0 and T1; \$ significant differences between T1 and T2; † significant differences between T0 and T2

WB whole body

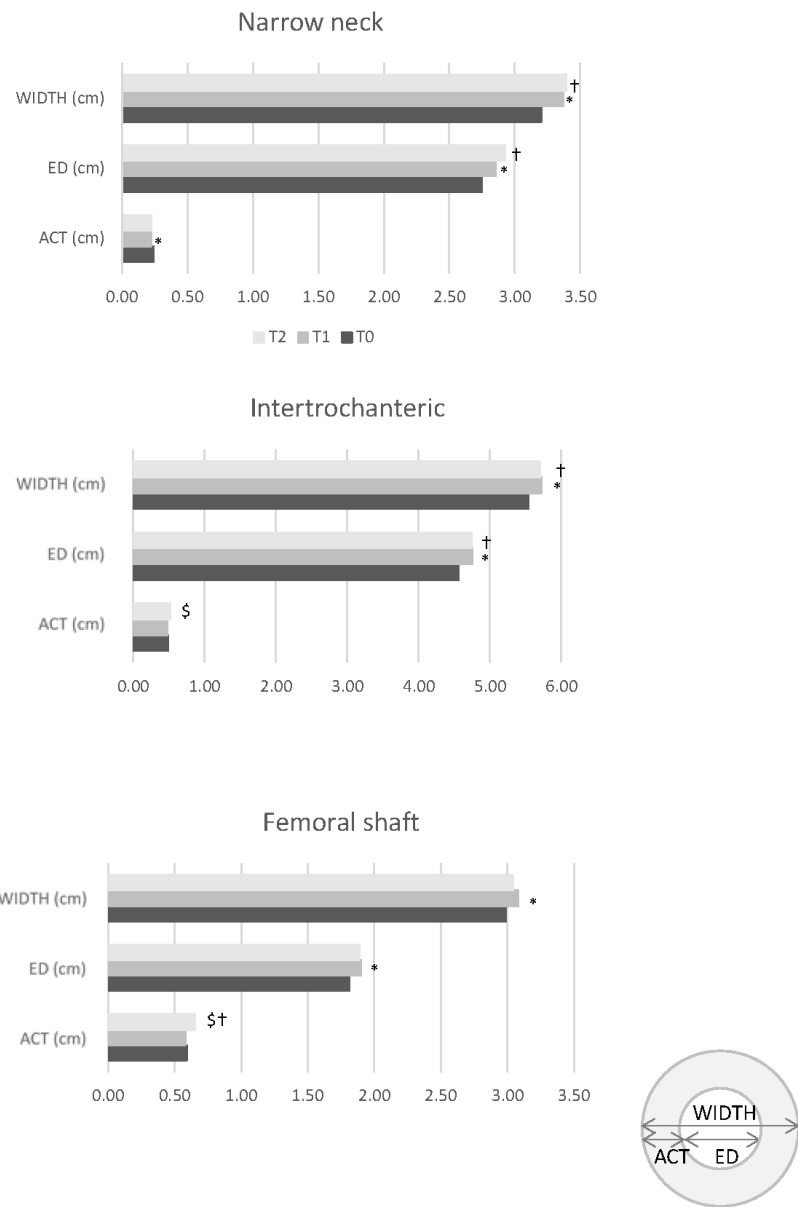


Figure 3 - Bone geometric evolution at NN, IT and FS of the OBESE interventional group during the weight loss program

* Significant difference between T0 and T1; \$ significant differences between T1 and T2; † significant differences between T0 and T2

ACT average cortical thickness, ED endocortical diameter, WIDTH width

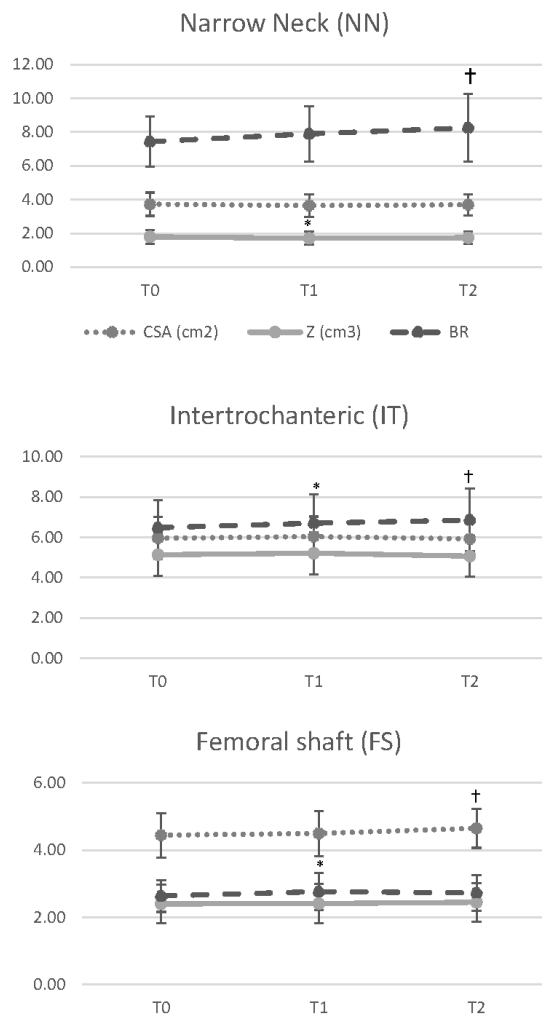


Figure 4. Bone strength evolution at NN, IT and FS of the OBESE interventional group during the weight loss program

* Significant difference between T0 and T1; † significant differences between T1 and T2; ‡ significant differences between T0 and T2

CSA cross sectional area, Z section modulus, BR bulkiness ratio

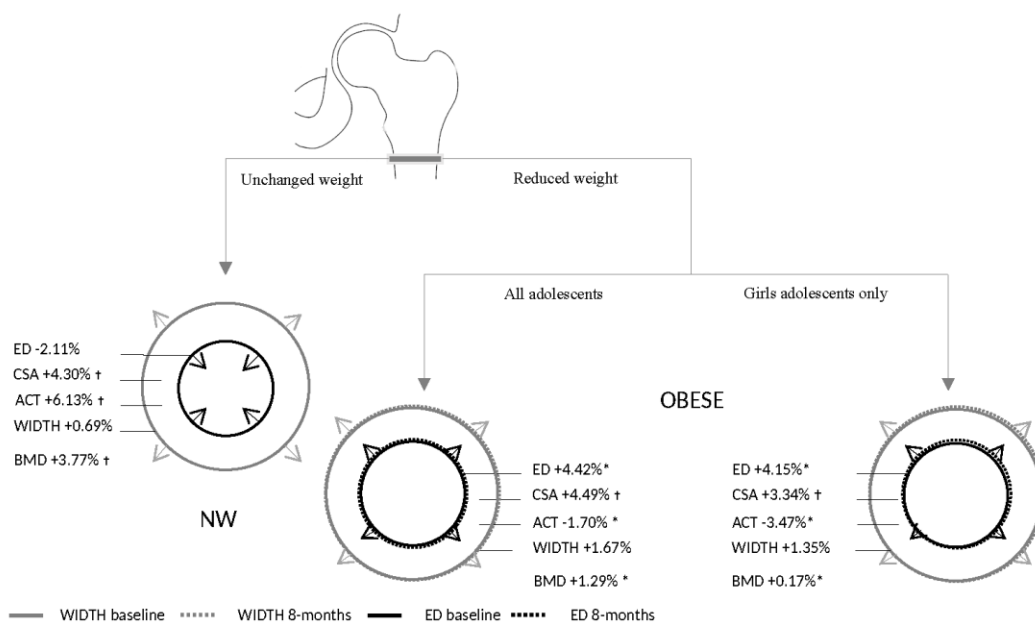


Figure 5 - Schematic representation of unadjusted geometric changes at the femoral shaft at 8-months. Over the 8 months, CSA significantly increased with a similar magnitude in both group. ACT and BMD significantly increased only for the NW group.

† $p < 0.05$ between baseline and 8 months; * $p < 0.05$ at 8-months between OBESSE and NW when adjusted to fat mass

NW normal weight control group, OBESSE obese group, ED endocortical diameter, CSA cross-sectional area, ACT average cortical thickness, WIDTH subperiosteal width, BMD bone mineral density

	OBESE (n= 31)		NW (n= 23)	
	Mean	SD	Mean	SD
Age (years)	13.61	1.27 *	15.90	0.434 *
Menarche age (years)	12.50	0.76	13.21	1.31
BMI	32.30	4.15 *	20.48	1.32 *
zBMI	2.26	0.30 *	-0.12	0.48 *
Height (cm)	161.38	8.62	164.48	5.48
Body weight (Kg)	86.32	15.21 *	55.91	5.90 *
WB Lean Mass (g)	51995.47	8380.66 *	42194.69	4200.64 *
WB FM (%)	39.49	3.82 *	20.33	3.82 *
WB FM (g)	34330.76	7942.64 *	11436.73	2807.79 *
Android (%)	42.31	4.56 *	18.53	4.80 *
Gynoid (%)	41.17	3.78 *	26.19	4.15 *
V FAT (%)	43.33	4.22 *	19.37	5.06 *
V FAT (g)	315.71	97.67 *	128.25	54.35 *
V FAT (cm ³)	341.31	105.59 *	138.64	58.76 *
Estradiol pg.ml ⁻¹	123.90	170.71	75.96	84.81

Table 1. Descriptive statistics of adolescents with obesity and their normal weight peers at baseline

* p<0.05 in comparison between OBESSE and NW

OBESSE obese intervention group, NW normal weight control group, SD standard deviation, BMI body mass index, WB LM whole body lean mass, WB FM whole body fat mass, V FAT visceral fat

A	WB (TBLH BMD)				LS				Hip				Neck			
	OBESSE n=31		NW n=23		OBESSE n=31		NW n=23		OBESSE n=31		NW n=23		OBESSE n=31		NW n=23	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
BMD (g/cm2)	0.941	0.088	1.054	0.071	0.964	0.150	1.030	0.107	1.021	0.137	1.102	0.100	0.953	0.139	1.005	0.102
BMC (g)	2082.98	379.55	2275.94	285.84	49.62	11.69	63.55	10.79	33.42	5.83	38.00	5.41	4.71	0.85	5.05	0.60
BMAD (g/cm3)	0.09	0.01	0.1	0.01	0.97	0.15	1.03	0.11								

	NN				IT				FS			
	OBESSE n=31		NW n=23		OBESSE n=31		NW n=23		OBESSE n=31		NW n=23	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
BMD (g/cm2)	1.179	0.187	1.183	0.137	1.104	0.177	1.178	0.133	1.515	0.171	1.651	0.152
ED (cm)	2.75	0.22	2.75	0.3	4.50	0.39	4.46	0.4	1.81	0.30	1.65	0.23
ACT (cm)	0.23	0.05	0.23	0.03	0.48	0.09	0.52	0.06	0.58	0.08	0.65	0.09
WIDTH (cm)	3.20	0.25	3.21	0.27	5.45	0.39	5.51	0.38	2.99	0.25	2.91	0.2
CSA (cm2)	3.60	0.68	3.6	0.42	5.75	1.06	6.17	0.69	4.27	0.67	4.58	0.56
CSMI (cm4)	2.90	0.84	2.81	0.72	14.94	4.04	15.16	3.12	3.49	1.12	3.51	0.80
Z (cm3)	1.69	0.42	1.67	0.31	4.93	1.07	4.96	0.76	2.27	0.56	2.31	0.38
BR	7.70	1.56	7.41	1.4	6.48	1.22	5.87	0.81	2.70	0.51	2.36	0.39

B	WB (TBLH BMD)		LS		Hip		Neck	
	OBESSE n=31		OBESSE n=31		OBESSE n=31		OBESSE n=31	
	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI
BMD (g/cm2)	0.891	(0.858 - 0.925)	0.890	(0.834 - 0.946)	0.966	(0.914 - 1.018)	0.908	(0.855 - 0.960)
BMC (g)	1847.56	(1727.28 - 1967.83)	44.73	(40.04 - 49.41)	30.57	(28.33 - 32.82)	4.32	(4.03 - 4.61)
BMAD (g/cm3)	0.09	(0.08 - 0.09)	0.93	(0.86 - 0.99)				

	NN		IT		FS	
	OBESSE n=31		OBESSE n=31		OBESSE n=31	
	mean	95% CI	mean	95% CI	mean	95% CI
BMD (g/cm2)	1.130	(1.056 - 1.204)	1.066	(0.995 - 1.137)	1.467	(1.392 - 1.541)
ED (cm)	2.59	(2.48 - 2.70)	4.32	(4.14 - 4.51)	1.65	(1.52 - 1.78)
ACT (cm)	0.22	(0.21 - 0.24)	0.46	(0.42 - 0.49)	0.57	(0.52 - 0.61)
WIDTH (cm)	3.02	(2.92 - 3.12)	5.24	(5.08 - 5.40)	2.77	(2.67 - 2.86)

CSA (cm2)	3.27	(3.06 - 3.50)	5.17	(4.684 - 5.51)	3.87	(3.62 - 4.12)
CSMI (cm4)	2.34	(2.07 - 2.61)	12.50	(11.17 - 13.82)	2.78	(2.41 - 3.15)
Z (cm3)	1.47	(1.32 - 1.61)	4.19	(3.84 - 4.52)	1.91	(1.73 - 2.10)
BR	7.49	(6.77 - 8.20)	6.32	(5.78 - 6.85)	2.60	(2.38 - 2.81)

C	WB (THLH BMD)		LS		Hip		Neck	
	OBESSE n=31		OBESSE n=31		OBESSE n=31		OBESSE n=31	
	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI
BMD (g/cm2)	0.901	(0.857 - 0.945)	0.894	(0.823 - 0.964)	0.974	(0.909 - 1.039)	0.914	(0.849 - 0.980)
BMC (g)	1863.54	(1695.02 - 2032.05)	45.59	(39.63 - 51.53)	31.25	(28.30 - 34.21)	4.37	(3.99 - 4.75)
BMAD (g/cm3)	0.09	(0.09 - 0.10)	0.95	(0.87 - 1.02)				

	NN		IT		FS	
	OBESSE n=31		OBESSE n=31		OBESSE n=31	
	mean	95% CI	mean	95% CI	mean	95% CI
BMD (g/cm2)	1.144	(1.053 - 1.235)	1.070	(0.984 - 1.156)	1.485	(1.395 - 1.576)
ED (cm)	2.55	(2.42 - 2.67)	4.32	(4.10 - 4.60)	1.63	(1.48 - 1.78)
ACT (cm)	0.23	(0.20 - 0.25)	0.47	(0.42 - 0.51)	0.58	(0.53 - 0.62)
WIDTH (cm)	2.97	(2.85 - 3.09)	5.24	(5.04 - 5.44)	2.76	(2.64 - 2.88)
CSA (cm2)	3.28	(2.99 - 3.57)	5.21	(4.75 - 5.66)	3.91	(3.59 - 4.24)
CSMI (cm4)	2.29	(1.93 - 2.66)	12.62	(10.82 - 14.42)	2.80	(2.30 - 3.30)
Z (cm3)	1.48	(1.29 - 1.66)	4.21	(3.73 - 4.69)	1.93	(1.68 - 2.18)
BR	7.16	(6.34 - 7.98)	6.28	(5.65 - 6.91)	2.54	(2.28 - 2.80)

D	WB (TBLH BMD)		LS		Hip		Neck	
	OBESSE		OBESSE		OBESSE		OBESSE	
	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI
BMD (g/cm2)	0.910	(0.8862 - 0.934)	0.922	(0.877 - 0.967)	0.991	(0.950 - 1.032)	0.934	(0.893 - 0.976)
BMC (g)	1958.88	(1872.87 - 2044.89)	47.20	(43.54 - 50.86)	31.90	(30.25 - 33.56)	4.50	(4.28 - 4.73)
BMAD (g/cm3)	0.09	(0.08 - 0.09)	0.95	(0.89 - 0.99)				

	NN		IT		FS	
	OBESSE		OBESSE		OBESSE	
	mean	95% CI	mean	95% CI	mean	95% CI

BMD (g/cm ²)	1.159	(1.099 - 1.218)	1.096	(1.038 - 1.154)	1.490	(1.430 - 1.549)
ED (cm)	2.67	(2.57 - 2.76)	4.40	(4.25 - 4.55)	1.71	(1.60 - 1.82)
ACT (cm)	0.23	(0.21 - 0.24)	0.47	(0.44 - 0.50)	0.57	(0.53 - 0.60)
WIDTH (cm)	3.11	(3.02 - 3.20)	5.33	(5.20 - 5.45)	2.84	(2.77 - 2.92)
CSA (cm ²)	3.44	(3.27 - 3.61)	5.45	(5.21 - 5.70)	4.05	(3.86 - 4.23)
CSMI (cm ⁴)	2.61	(2.40 - 2.83)	13.63	(12.66 - 14.59)	3.09	(2.82 - 3.37)
Z (cm ³)	1.57	(1.46 - 1.68)	4.51	(4.27 - 4.75)	2.07	(1.94 - 2.20)
BR	7.61	(7.02 - 8.20)	6.30	(5.85 - 6.75)	2.64	(2.45 - 2.82)

Table 2. Bone variables at baseline for adolescents with obesity and their normal weight peers. **2.A.** Unadjusted mean. **2.B.** Body weight adjusted. **2.C.** Fat mass adjusted. **2.D.** Lean mass adjusted.

* p<0.05 OBESE significantly different than NW.

OBESE Obese intervention group, NW normal weight control group, SD standard deviation, WB whole body, TBLH total body less head, LM lean mass, FM fat mass, BMD bone mineral density, BMC bone mineral content, BMAD bone mineral apparent density, NN narrow neck, IT intertrochanteric, FS femoral shaft, ED endocortical diameter, ACT average cortical thickness, WIDTH width, CSA cross sectional area, CSMI cross sectional moment of inertia, Z section modulus, BR bulking ratio

	OBESE		NW	
	Median	IQR	Median	IQR
Leptin ng.ml ⁻¹ T0	29.83	19.63		
Leptin ng.ml ⁻¹ T2	25.14	18.04		
Estradiol pg.ml ⁻¹ T0	56.67	97.49	49.00	46.00
Estradiol pg.ml ⁻¹ T2	80.08	72.47	42.00	30.00

Table 3. Biochemical characteristics of adolescents with obesity and their normal weight peers at baseline (T0) and 8 months (T2)

* p<0.05 in comparison between OBESE and NW

OBESE obese intervention group, NW normal weight control group, IQR interquartile

A

	BMD								CTx	
	TBLH		LS		neck		hip		r	p
	r	p	r	p	r	p	r	p		
Leptin			0.397	0.02					-0.419	0.02
CTx	-0.552	0.002	-0.625	<0.001	-0.565	<0.001	-0.462	0.012		

	BMD				ACT				CSA				Z		BR					
	NN		IT		NN		IT		FS		NN		IT		NN					
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p				
Leptin					0.502	0.002	0.345	0.046	0.339	0.049										
CTx	-0.614	<0.001	-0.374	0.046	-0.579	<0.001	-0.414	0.026			0.594	<0.001	-0.457	0.013	-0.517	0.004	-0.443	0.016	0.443	0.016

B

	WB (TBLH BMD)				LS				Hip				Neck							
	BW		LM (g)		BW		LM (g)		BW		FM (g)		LM (g)		BW		FM (g)		LM (g)	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
BMD (g/cm ²)	0.511	<0.001	0.735	<0.001	0.455	0.001	0.533	<0.001	0.568	<0.001	0.334	0.025	0.695	<0.001	0.562	<0.001	0.330	0.027	0.687	<0.001
BMAD (g/cm ³)					0.305	0.035	0.450	<0.001												

	NN				IT				FS									
	BW		FM (g)		LM (g)		BW		FM (g)		LM (g)		BW		FM (g)		LM (g)	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
BMD (g/cm ²)	0.553	<0.001	0.349	0.017	0.648	<0.001	0.505	<0.001			0.625	<0.001	0.562	<0.001	0.319	0.033	0.705	<0.001
ACT (cm)	0.341	0.021			0.324	0.028											0.316	0.033
WIDTH (cm)					0.349	0.017	0.342	0.020			0.498	<0.001	0.389	0.008			0.503	<0.001
CSA (cm ²)	0.635	<0.001	0.401	0.006	0.743	<0.001	0.587	<0.001	0.314	0.034	0.754	<0.001	0.623	<0.001	0.347	0.019	0.791	<0.001
Z (cm ³)	0.678	<0.001	0.462	<0.001	0.753	<0.001	0.606	<0.001	0.325	0.027	0.778	<0.001	0.585	<0.001	0.355	0.015	0.702	<0.001
BR	-0.398	0.006			-0.480	<0.001												

Table 4. Correlation analysis. **4.A.** Between hormones and bone parameters. **4.B.** Between body composition parameters and bone parameters.
TBLH total body less head, BW body weight, FM fat mass, LM lean mass, BMD bone mineral density, BMAD bone mineral apparent density, WB whole body, LS lumbar spine, CTx collagen type 1 cross-linked C-telopeptide, NN narrow neck, IT intertrochanteric, FS femoral shaft, CSA cross sectional area, ACT average cortical thickness, WIDTH width, Z section modulus, BR bulking ratio.

	OBESE					
	T0		T1		T2	
	Median	IQR	Median	IQR	Median	IQR
Leptin ng.ml ⁻¹	29.83	19.63	19.01	29.85	25.14	18.04
Estradiol pg.ml ⁻¹	56.67	97.49	80.77	104.59	80.08	72.47
P1NP ng.ml ⁻¹	41.30	4.09	38.55	9.46	41.22	7.74
CTx ng.ml ⁻¹	4.42	1.50	3.47	0.94 *	4.34	1.31 †

Table 5. Biochemical characteristics of the OBESE during the weight loss program

* Significant difference between T0 and T1; † significant differences between T1 and T2; ‡ significant differences between T0 and T2

OBESE obese intervention group, IQR interquartile range, P1NP Procollagen type 1 N-terminal propeptide, CTx collagen type 1 cross-linked C-telopeptide

A	WB (TBLH BMD)				LS				Hip				Neck			
	OBESE		NW		OBESE		NW		OBESE		NW		OBESE		NW	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
BMD (g/cm ²)	0.988	0.078	1.092	0.065	1.037	0.128	1.048	0.099	1.039	0.140	1.130	0.101	0.969	0.132	1.021	0.093
BMC (g)	2305.14	362.92	2363.88	276.79	58.56	10.43	64.95	10.72	35.10	5.88	38.10	5.21	4.92	0.79	5.09	0.49
BMAD (g/cm ³)	0.093	0.005	0.102	0.005	1.037	0.128	1.037	0.086								

B	WB (TBLH BMD)				LS				Hip				Neck	
	OBESE		NW		OBESE		NW		OBESE		NW		OBESE	
	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI
BMD (g/cm ²)	0.966	(0.926 - 1.006)	0.969	(0.917 - 1.021)	0.972	(0.910 - 1.035)	0.910	(0.850 - 0.969)						
BMC (g)	2024.60	(1915.29 - 2133.90)	51.38	(46.87 - 55.88)	31.43	(28.73 - 34.13)	4.47	(4.15 - 4.78)						
BMAD (g/cm ³)	0.094	(0.091 - 0.097)	0.975	(0.924 - 1.025)										

C	WB (TBLH BMD)				LS				Hip				Neck	
	OBESE		NW		OBESE		NW		OBESE		NW		OBESE	
	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI
BMD (g/cm ²)	0.966	(0.926 - 1.006)	1.019	(0.955 - 1.083)	1.003	(0.932 - 1.073)	0.933	(0.867 - 0.999)						
BMC (g)	2240.34	(2061.47 - 2419.20)	57.90	(51.98 - 63.82)	33.08	(29.89 - 36.27)	4.82	(4.42 - 5.21)						
BMAD (g/cm ³)	0.092	(0.089 - 0.095)	1.023	(0.961 - 1.084)										

D	WB (TBLH BMD)				LS				Hip				Neck	
	OBESE		NW		OBESE		NW		OBESE		NW		OBESE	
	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI	mean	95% CI
BMD (g/cm ²)	1.128	(1.036 - 1.220)	1.043	(0.953 - 1.134)	1.523	(1.434 - 1.611)								
ED (cm)	2.84	(2.68 - 3.00)	4.76	(4.51 - 5.01)	1.94	(1.75 - 2.12)								
ACT (cm)	0.22	(0.20 - 0.23)	0.44	(0.40 - 0.49)	0.56	(0.50 - 0.62)								
WIDTH (cm)	3.31	(3.17 - 3.46)	5.66	(5.44 - 5.88)	3.07	(2.88 - 3.25)								
CSA (cm ²)	3.52	(3.23 - 3.81)	5.61	(5.13 - 6.08)	4.49	(4.16 - 4.81)								
CSMI (cm ⁴)	2.83	(2.44 - 3.23)	15.19	(13.18 - 17.20)	3.85	(3.22 - 4.46)								
Z (cm ³)	1.63	(1.44 - 1.81)	4.80	(4.29 - 5.31)	2.37	(2.07 - 2.66)								
BR	8.10	(7.16 - 9.03)	7.34	(6.63 - 8.04)	2.86	(2.58 - 3.15)								

Table 6. Bone variables of adolescents with obesity and their normal weight peers at 8 months. **6.A.** Unadjusted mean. **6.B.** Body weight changes adjusted. **6.C.** Fat mass changes adjusted.

* p<0.05 OBESE significantly different than NW.

OBESE Obese intervention group, NW normal weight control group, SD standard deviation, WB whole body, TBLH total body less head, LM lean mass, FM fat mass, BMD bone mineral density, BMC bone mineral content, BMAD bone mineral apparent density, NN narrow neck, IT intertrochanteric, FS femoral shaft, ED endocortical diameter, WIDTH width, CSA cross sectional area, CSMI cross sectional moment of inertia, ACT average cortical thickness, Z section modulus, BR bulking ratio

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Appendix 12 - Baseline data of the Ob intervention group

T	Gpe	ID num	SEX	Age	BMI	HEIG HT	DXA WB mass	E2	Leptin	WB (%) FM	WB FM (g)	WB FFM (g)	WB Lean mass (g)	a FM (%)	g FM (%)	VFAT (%)	VFAT (g)	VFAT (cm3)
1	Ob	1	2	15.93	36.11	166	97.74	47.1	34.34	42.68	41714.35	56022.97	53778.45	44.66	43.14	44.99	362.24	391.61
1	Ob	2	2	12.05	33.79	149	51.70	25.0	5.849	36.94	19098.35	32597.04	31332.84	39.02	40.09	38.06	228.97	247.53
1	Ob	3	2	15.12	35.42	165	92.71			35.84	33224.90	59481.54	56708.20	39.65	38.53	40.30	243.60	263.36
1	Ob	4	2	13.99	30.33	173	110.07			44.05	48491.05	61581.81	59347.25	50.49	43.78	51.15	471.72	509.97
1	Ob	5	2	13.13	22.97	167	85.28	668.2	9.884	31.93	27231.96	58047.24	55704.45	29.52	35.70	32.67	138.58	149.82
1	Ob	6	1	15.61	48.18	163	129.45			46.03	59585.00	69868.53	67829.31	50.03	42.45	49.81	290.66	314.23
1	Ob	7	2	13.70	34.14	172	101.39			39.76	40319.24	61075.67	58707.43	40.02	42.23	41.52	274.63	296.90
1	Ob	8	2	15.10	38.62	168	109.25	62.9	37.2	43.60	47634.41	61616.76	59140.96	46.27	47.31	46.79	230.08	248.74
1	Ob	9	2	13.99	31.98	164	85.43	71.9	49.38	45.49	38861.03	46569.66	44489.65	47.64	47.42	49.87	215.12	232.56
1	Ob	10	1	14.67	38.86	158	100.23	37.9	22.45	42.18	42273.69	57953.71	56047.85	46.97	40.42	47.14	289.73	313.22
1	Ob	11	2	13.67	33.27	157	84.64	50.4	22.84	38.55	32623.03	52013.02	49797.03	45.95	42.54	45.89	291.21	314.82
1	Ob	12	2	13.16	22.09	154	58.76			33.15	19480.15	39283.08	37618.22	35.51	37.66	35.92	167.84	181.45
1	Ob	13	1	12.97	27.99	168	80.25	15.4	10.19	32.87	26377.02	53874.95	51884.61	37.71	36.02	38.51	364.67	394.24
1	Ob	14	2	15.46	35.29	170	104.14			41.38	43095.28	61045.04	58429.22	45.03	43.75	44.34	379.17	409.91
1	Ob	15	2	12.18	33.15	162	89.73	36.3	37.72	39.34	35299.27	54429.42	52389.11	42.40	41.16	43.90	298.02	322.19
1	Ob	16	2	13.72	31.62	163	75.49			34.31	25903.70	49587.89	47533.57	36.93	36.69	40.12	143.55	155.19
1	Ob	17	1	13.38	33.06	165	92.24			39.41	36346.16	55889.10	54108.51	41.64	40.29	42.51	363.50	392.97
1	Ob	18	2	12.16	33.05	141	67.04			43.29	29022.25	38020.86	36373.24	39.79	46.29	41.44	292.20	315.89
1	Ob	19	2	13.30	25.99	159	67.81		33.44	34.54	23424.66	44384.58	42624.34	37.52	36.65	40.24	250.74	271.07
1	Ob	20	2	13.48	33.96	175	106.82	146.8	43.88	41.60	44437.16	62384.78	59652.56	45.54	43.15	46.85	301.25	325.67
1	Ob	21	2	15.55	30.11	169	85.97	118.3	23.61	41.17	35396.86	50570.60	48268.43	44.28	45.18	42.18	302.30	326.81
1	Ob	22	1	13.93	32.03	160	85.31	55.2	18.87	36.94	31511.26	53793.89	51687.62	40.01	37.14	40.40	617.10	667.13
1	Ob	23	2	11.74	28.32	153	68.26			36.40	24844.53	43418.41	41867.44	39.60	40.08	40.38	241.40	260.97
1	Ob	24	2	15.03	31.60	164	97.77	123.9	35.06	36.01	35212.01	62560.69	60145.92	39.54	35.65	40.07	325.26	351.64
1	Ob	25	2	14.06	37.20	168	105.53	36.8	25.34	39.14	41306.63	64222.60	61746.91	44.90	38.59	45.44	371.30	401.41
1	Ob	26	1	12.34	35.88	161	94.26			47.24	44528.96	49726.84	47728.62	49.65	48.79	50.19	357.11	386.07
1	Ob	27	2	13.90	29.90	167	84.07			39.54	33236.55	50831.63	48315.01	44.09	37.93	44.75	393.61	425.52
1	Ob	28	1	11.19	26.90	143	57.48			39.05	22446.77	35033.96	33789.68	41.05	38.94	42.73	427.92	462.61
1	Ob	29	2	15.51	39.88	146	86.51	181.7	43.78	44.70	38668.36	47845.52	46135.37	49.31	45.92	50.28	310.07	335.21
1	Ob	30	2	12.88	34.05	158	84.85			41.64	35329.09	49522.86	47805.72	43.92	45.99	44.22	359.45	388.60
1	Ob	31	2	11.04	32.44	150	74.69			41.11	30709.82	43983.95	42217.13	45.43	39.15	46.19	286.28	309.49

WB_B MAD	TBLH_ BMD	WB BMC (g)	WB BMD (g/cm ²)	L1-L4 BMC	L1-L4 BMD	LS BMA D	LS TBS	NECK _BMC	NECK _BM D	HTOT_ BMC	HTO T_B MD	AXIS_LE NGTH	NN_ BMD	NN_ CSA	NN_C SMI	NN_WI DTH	NN_ ED	NN_ ACT	NN_ PCD	NN_C MP	NN_Z
0.096	0.989	2244.52	1.137	51.27	1.00	1.00	1.34	5.54	1.11	39.44	1.21	95.68	1.38	4.15	3.38	3.15	2.60	0.28	1.55	0.49	2.10
0.097	0.774	1264.20	0.908	24.80	0.60	0.60	1.10	3.06	0.70	20.91	0.75	97.45	0.81	2.18	1.27	2.88	2.56	0.16	1.46	0.50	0.81
0.102	1.140	2773.33	1.308	59.47	1.19	1.19	1.33	5.25	1.33	38.22	1.36	89.66	1.87	4.70	2.67	2.64	1.84	0.40	1.33	0.50	1.99
0.089	0.945	2234.56	1.071	56.09	1.01	1.01	1.34	5.03	0.92	36.06	1.12	100.68	1.09	3.46	3.25	3.32	2.89	0.21	1.61	0.49	1.90
0.092	0.990	2342.79	1.136	61.02	0.99	0.99	1.40	4.64	0.89	33.67	0.93	106.04	1.10	3.49	3.20	3.33	2.91	0.21	1.64	0.49	1.89
	0.911	2039.22	1.008	45.67	0.85		1.21	4.67	1.02	35.99	1.02	97.32	1.21	3.90	3.15	3.69	3.18	0.25	2.09	0.53	1.32
0.091	0.940	2368.24	1.122	59.17	1.02	0.85	1.39	4.85	0.97	34.90	0.98	103.79	1.23	3.66	2.87	3.13	2.65	0.24	1.51	0.48	1.77
0.094	1.047	2475.80	1.178	63.28	1.12	1.02	1.37	5.55	1.10	34.53	1.16	87.22	1.34	4.81	5.40	3.78	3.26	0.26	1.95	0.51	2.71
0.090	0.917	2080.01	1.069	49.95	1.01	1.12	1.46	4.93	0.92	28.97	0.97	100.21	1.14	3.39	2.71	3.13	2.68	0.22	1.51	0.48	1.68
0.084	0.914	1905.86	1.004	43.44	0.86	1.01	1.19	3.87	0.86	34.82	1.03	93.90	0.98	3.09	2.38	3.33	2.95	0.19	1.71	0.51	1.33
0.096	1.032	2215.99	1.165	57.34	1.21	0.86	1.48	5.87	1.10	32.66	1.09	96.71	1.37	4.04	3.19	3.10	2.55	0.27	1.47	0.47	1.96
0.089	0.884	1664.86	0.983	43.92	0.89	1.21	1.28	3.96	0.86	29.89	0.99	96.64	1.08	2.90	1.71	2.81	2.39	0.21	1.28	0.46	1.11
0.087	0.926	1990.34	1.013	42.60	0.81	0.89	1.33	4.40	0.91	37.12	1.03	113.79	1.11	3.36	2.79	3.16	2.73	0.22	1.56	0.49	1.74
0.094	1.044	2615.81	1.202	65.57	1.10	0.81	1.43	5.83	1.13	42.93	1.08	116.71	1.42	4.47	3.80	3.31	2.75	0.28	1.60	0.48	2.22
0.097	0.958	2040.31	1.107	52.71	1.05	1.10	1.30	4.77	0.97	34.74	1.07	96.60	1.17	3.45	2.28	3.13	2.67	0.23	1.63	0.51	1.41
0.096	0.923	2054.32	1.098	50.49	0.92	1.05	1.24	4.26	0.86	31.20	0.94	99.52	1.09	3.17	2.41	3.05	2.62	0.21	1.47	0.48	1.53
0.082	0.817	1780.59	0.941	36.93	0.75	0.92	1.07	4.62	0.86	29.70	0.87	105.95	1.05	3.54	3.55	3.54	3.14	0.20	1.66	0.47	1.88
		1647.62	0.937	48.03	0.97			4.56	0.95	31.84	1.00	126.40	1.24	3.60	2.37	3.06	2.57	0.25	1.49	0.49	1.51
0.088	0.854	1760.24	0.984	57.45	0.96	0.97	1.24	4.56	0.85	32.54	0.93	105.89	1.08	3.23	2.33	3.14	2.71	0.21	1.48	0.47	1.41
0.094	1.065	2732.22	1.210	65.66	1.08	0.96	1.34	6.24	1.09	43.67	1.16	101.33	1.39	4.43	3.98	3.35	2.80	0.28	1.58	0.47	2.24
0.084	0.917	2302.17	1.071	63.30	1.04	1.08	1.35	4.32	0.93	30.11	0.85	110.78	1.19	3.50	2.85	3.10	2.64	0.23	1.43	0.46	1.71
0.090	0.966	2106.27	1.090	32.40	0.74	1.04	1.15	5.24	0.96	35.62	1.00	100.68	1.17	3.90	3.70	3.49	3.04	0.23	1.64	0.47	1.99
		1550.97	0.940	40.61	0.87			4.10	0.84	26.62	0.89	102.37	1.02	2.98	2.32	3.07	2.67	0.20	1.50	0.49	1.47
0.100	1.072	2414.78	1.215	53.92	1.12	0.87	1.24	6.26	1.21	45.32	1.30	105.28	1.56	4.74	3.75	3.19	2.55	0.32	1.60	0.50	2.32
0.093	1.012	2475.68	1.172	62.16	1.07	1.12	1.26	5.43	1.07	40.40	1.09	101.55	1.36	4.22	3.34	3.27	2.73	0.27	1.66	0.51	2.01
0.086	0.905	1998.21	1.035	41.38	0.93	1.07	1.11	3.22	0.87	24.87	0.92	98.43	1.00	3.05	2.23	3.25	2.86	0.19	1.70	0.52	1.26
		2516.62	1.182	69.11	1.13			5.25	1.18	42.43	1.24	104.37	1.52	4.14	2.58	2.85	2.23	0.31	1.37	0.48	1.74
0.088	0.758	1244.27	0.877	25.83	0.61	1.13	1.14	3.38	0.76	25.31	0.84	92.62	0.96	2.47	1.35	2.71	2.33	0.19	1.30	0.48	0.96
0.087	0.884	1710.15	1.010	40.61	0.96	0.61	1.35	3.51	0.81	24.91	0.90	89.00	1.11	3.34	2.76	3.17	2.74	0.22	1.48	0.47	1.64
0.101	0.930	1717.14	1.046	35.95	0.79	0.96	1.10	4.65	0.95	28.69	0.97	91.30	1.04	3.37	2.60	3.47	3.06	0.20	1.86	0.53	1.38
0.083	0.853	1766.82	0.990	39.41	0.84	0.79	1.10	4.36	0.89	29.68	0.93	103.75	1.09	3.18	2.43	3.06	2.63	0.21	1.48	0.48	1.54

NN_B R	IT_BM D	IT_CS A	IT_CS MI	IT_WI DTH	IT_ED IT_ED	IT_AC T	IT_P CD	IT_C MP	IT_Z	IT_BR	FS_B MD	FS_CS A	FS_CS MI	FS_W IDTH	FS_E D	FS_A CT	FS_P CD	FS_C MP	FS_SE CT_M OD	FS_BR
5.84	1.40	7.00	16.44	5.25	4.07	0.59	2.30	0.44	5.58	4.99	1.73	4.90	3.79	2.98	1.62	0.68	1.46	0.49	2.51	2.23
10.84	0.76	3.50	6.36	4.81	4.16	0.33	2.11	0.44	2.36	8.22	1.25	3.03	1.80	2.55	1.63	0.46	1.21	0.47	1.34	2.91
3.36	1.58	7.68	16.69	5.10	3.74	0.68	2.22	0.44	5.80	4.25	1.81	5.00	3.65	2.91	1.45	0.73	1.43	0.49	2.47	2.02
8.02	1.31	6.68	16.66	5.35	4.23	0.56	2.29	0.43	5.45	5.44	1.68	4.74	4.01	2.96	1.66	0.65	1.41	0.48	2.58	2.38
7.95	0.99	5.54	17.51	5.88	5.09	0.40	2.68	0.46	5.48	8.08	1.50	4.77	5.03	3.35	2.26	0.54	1.60	0.48	2.88	3.22
11.29	1.20	6.40	16.85	5.62	4.61	0.50	2.55	0.45	5.49	6.09	1.35	4.01	3.58	3.11	2.13	0.49	1.46	0.47	2.18	3.38
6.73	0.99	5.46	14.99	5.77	4.89	0.44	2.51	0.44	4.60	7.42	1.66	4.73	4.04	3.00	1.72	0.64	1.47	0.49	2.65	2.39
7.83	1.36	7.72	22.70	5.95	4.62	0.67	2.64	0.44	6.85	4.97	1.60	4.41	3.34	2.90	1.67	0.61	1.41	0.49	2.25	2.42
7.23	1.12	5.73	14.52	5.37	4.45	0.46	2.24	0.42	4.64	6.85	1.47	4.26	3.52	3.05	1.97	0.54	1.52	0.50	2.29	2.85
10.04	1.11	5.50	12.72	5.21	4.21	0.50	2.57	0.49	4.82	5.29	1.64	4.24	2.78	2.72	1.41	0.65	1.35	0.50	2.03	2.09
5.98	1.20	6.69	19.26	5.83	4.73	0.55	2.62	0.45	6.00	5.87	1.54	4.42	3.67	3.01	1.85	0.58	1.44	0.48	2.34	2.70
7.20	1.04	4.91	9.77	4.97	4.02	0.48	2.29	0.46	3.64	5.63	1.61	3.88	2.22	2.54	1.22	0.66	1.24	0.49	1.71	1.97
7.37	1.27	6.21	13.25	5.15	4.04	0.55	2.31	0.45	4.67	5.11	1.42	3.77	2.63	2.80	1.74	0.53	1.32	0.47	1.78	2.78
6.08	1.09	6.06	17.41	5.85	4.97	0.44	2.59	0.44	5.35	7.41	1.80	5.69	5.86	3.32	1.95	0.69	1.65	0.50	3.50	2.44
7.65	1.21	6.39	16.06	5.55	4.36	0.59	2.62	0.47	5.48	4.94	1.53	3.91	2.42	2.69	1.50	0.59	1.34	0.50	1.79	2.27
7.38	0.99	5.21	14.84	5.51	4.66	0.43	2.48	0.45	4.89	7.11	1.50	4.21	3.67	2.95	1.82	0.56	1.45	0.49	2.44	2.67
9.30	0.97	5.36	15.80	5.79	4.93	0.43	2.59	0.45	4.94	7.43	1.25	3.57	3.15	2.99	2.10	0.45	1.40	0.47	1.97	3.58
6.40	1.34	4.18	3.88	3.27	2.27	0.50	1.53	0.47	2.23	3.49	1.35	2.82	1.42	2.20	1.12	0.54	1.02	0.46	1.20	2.18
7.85	1.02	5.05	12.06	5.18	4.22	0.48	2.35	0.45	4.25	5.88	1.55	3.80	2.36	2.57	1.33	0.62	1.29	0.50	1.83	2.07
6.43	1.18	6.88	21.95	6.11	5.15	0.48	2.76	0.45	6.56	7.01	1.74	5.78	6.59	3.48	2.18	0.65	1.74	0.50	3.77	2.69
7.18	0.88	4.96	15.36	5.89	5.20	0.35	2.43	0.41	4.43	10.01	1.20	3.88	4.13	3.39	2.56	0.42	1.64	0.48	2.36	4.21
8.13	1.12	6.25	17.83	5.83	4.86	0.48	2.60	0.45	5.52	6.69	1.46	4.36	3.96	3.14	2.07	0.53	1.50	0.48	2.42	3.07
7.94	1.03	5.15	12.81	5.27	4.34	0.47	2.40	0.46	4.47	6.16	1.27	3.28	2.30	2.71	1.78	0.47	1.30	0.48	1.63	3.03
5.13	1.47	7.75	20.11	5.55	4.27	0.64	2.54	0.46	6.69	4.71	1.85	5.40	4.48	3.06	1.57	0.74	1.54	0.50	2.91	2.07
6.20	1.17	6.65	20.23	5.97	4.97	0.50	2.76	0.46	6.31	6.43	1.62	4.99	5.03	3.24	2.04	0.60	1.62	0.50	3.08	2.71
9.74	1.02	4.95	11.32	5.07	4.26	0.41	2.37	0.47	4.18	6.64	1.31	3.66	2.75	2.94	2.00	0.47	1.40	0.48	1.79	3.27
4.77	1.39	6.72	13.92	5.07	3.81	0.63	2.31	0.46	5.04	4.38	1.85	4.79	3.05	2.72	1.13	0.79	1.35	0.50	2.23	1.72
7.50	0.90	3.96	6.86	4.62	3.86	0.38	2.19	0.47	2.83	6.41	1.39	3.36	1.97	2.54	1.47	0.53	1.21	0.48	1.48	2.49
7.85	0.91	4.42	10.31	5.13	4.35	0.39	2.37	0.46	3.73	7.06	1.43	3.72	2.44	2.73	1.64	0.54	1.36	0.50	1.78	2.54
10.31	1.00	4.96	11.07	5.20	4.36	0.42	2.33	0.45	3.87	6.88	1.47	4.03	2.85	2.88	1.78	0.55	1.38	0.48	1.90	2.72
7.40	1.11	5.46	13.52	5.17	4.27	0.45	2.38	0.46	4.85	6.18	1.30	3.66	2.82	2.96	2.02	0.47	1.46	0.49	1.89	3.20

Appendix 13 - Baseline and 8 months data of the NW group

Gpe	SEX	menar che	E2_1_pg/ ml	E2_2_pg/ ml	zBMI_1	DXAWBm asskg_1	DXAWB masskg_ 3	WBFMg_1	WBFMg_3	WBLearnMas sg_1	WBLearnMas sg_3	VFATg_1	VFATg_3	TBLH BMCg_1	TBLH BMCg_3
3	2		19	472	-0.52	50.268	51.6045	7929.056	8089.782	42338.608	43514.767	83.240	74.357	1561.11	1695.59
3	2		49	78	0.14	54.279	55.1669	12677.042	11534.449	41601.723	43632.403	134.714	93.640	1750.78	1779.86
3	2	11	82	34	-1.02	49.032	53.0965	7407.116	8794.207	41624.846	44302.306	96.378	108.181	1812.88	1997.50
3	2	12	124	19	0.18	57.750	60.313	11835.045	12454.229	45915.098	47858.820	149.292	158.505	1810.34	1958.03
3	2	13	46	33	0.4	59.365	61.92	15468.265	15080.042	43896.592	46839.970	147.598	103.199	1821.10	1874.70
3	2		52	109	-0.65	52.371	53.9131	9327.050	9610.705	43043.497	44302.418	87.569	64.852	1728.14	1871.05
3	2	13	39	46	0.72	65.876	68.7113	16150.771	15068.299	49725.199	53643.041	269.950	122.303	2095.42	2145.50
3	2		25	20	0.1	54.985	58.045	13545.771	13840.368	41439.260	44204.641	223.583	165.737	1772.64	1878.96
3	2		28	129	0.31	55.037	55.7058	12293.356	10594.856	42743.467	45110.901	75.215	54.203	1784.67	1879.53
3	2		39	63	-0.36	56.748	59.3885	12708.068	12582.880	44039.500	46805.649	175.761	124.203	1779.18	1866.79
3	2	12	69	347	0.19	52.287	54.3794	8348.965	9120.342	43938.449	45259.025	82.151	138.063	1517.10	1612.27
3	2	13	70	60	0.31	61.751	63.736	14378.899	13756.473	47371.866	49979.565	198.763	161.682	1852.68	1920.40
3	2	14	342	59	-1.03	40.551	43.278	8992.562	9146.968	31558.868	34131.060	145.454	140.555	1078.29	1269.50
3	2	13	24	30	-0.02	59.616	61.8906	14741.234	14176.486	44874.753	47714.162	123.283	143.475	1940.24	1985.10
3	2		326	8	0.25	61.048	61.5054	16217.045	15771.795	44831.265	45733.635	142.987	85.598	1738.77	1867.80
3	2		77	42	-0.84	53.097	56.3783	8066.900	8934.172	45030.134	47444.156	156.585	96.608	1853.30	1988.11
3	2	16	64	45	0	60.377	63.4701	10374.909	11245.256	50002.520	52224.817	77.932	131.109	2123.99	2294.76
3	2	14	33	37	-0.38	58.196	58.3971	8630.256	7775.097	49565.579	50622.017	101.335	61.346	2184.69	2323.42
3	2	14	42	278	0.34	66.572	71.1658	12985.024	14065.354	53586.572	57100.425	79.509	76.774	2073.76	2241.50
3	2	15	55	34	-0.44	52.503	54.4503	8297.830	9085.789	44205.644	45364.553	43.701	67.662	1570.04	1700.85
3	2	13	38	312	-0.01	52.211	58.7577	11016.724	13283.246	41194.267	45474.503	81.291	99.894	1563.98	1706.52
3	2		69	50	-0.52	50.526	55.6629	9823.411	11171.038	40703.076	44491.876	108.194	85.132	1414.38	1669.65
3	2	12	35	45	0.14	61.423	66.0843	11829.497	11721.386	49593.723	54362.928	165.186	141.364	1836.28	1982.25

TBLH BMD_1	TBLH BMD_3	WBBMCg _1	WBBMCg_ 3	WBBMA D_1	WBBMA D_3	WBBMD_ 1	WBBMD_ 3	LS BMAD_ 1	LS BMAD_ 3	L1L4BMC	L1L4BM C_3	L1L4B MD	L1L4BM D_3	NECK_ BMC	NECK_ BMC_ 3	NECK_B MD	NECK_B MD_3
1.034	1.076	2083.486	2142.404	0.105	0.106	1.170	1.193	0.978	0.999	57.658	61.293	0.978	0.999	4.175	4.437	1.012	0.989
1.053	1.072	2318.619	2279.998	0.099	0.101	1.203	1.205	0.950	0.986	54.050	57.571	0.950	0.986	4.918	5.101	0.980	1.026
1.100	1.144	2369.450	2561.892	0.109	0.110	1.252	1.302	1.102	1.108	70.031	71.549	1.102	1.108	5.804	5.449	1.222	1.248
1.065	1.104	2266.052	2416.828	0.098	0.099	1.167	1.208	1.026	1.033	62.261	63.057	1.026	1.033	4.926	5.332	1.105	1.157
1.023	1.051	2287.278	2300.479	0.092	0.095	1.125	1.148	1.101	1.110	70.055	71.513	1.101	1.110	4.923	4.965	0.913	0.919
1.058	1.113	2271.802	2355.907	0.105	0.108	1.201	1.237	1.047	1.036	58.405	58.922	1.047	1.036	4.879	4.893	1.006	1.022
1.129	1.123	2588.701	2630.682	0.098	0.096	1.231	1.232	1.195	1.025	83.251	81.238	1.195	1.182	5.596	5.280	0.983	0.994
1.044	1.068	2225.914	2298.693	0.097	0.098	1.151	1.169	1.071	1.073	68.455	69.551	1.071	1.073	5.273	5.323	0.989	0.976
1.150	1.149	2337.243	2350.759	0.104	0.107	1.238	1.261	1.038	1.057	58.380	59.376	1.038	1.057	4.525	5.026	1.202	1.143
0.985	1.034	2230.328	2299.402	0.090	0.095	1.091	1.137	1.009	0.942	65.594	67.207	1.009	1.050	5.337	4.851	0.928	0.960
0.973	1.016	2080.235	2113.651	0.098	0.101	1.142	1.166	1.007	1.191	63.083	65.140	1.007	1.038	4.334	4.737	0.920	0.993
1.058	1.085	2283.188	2285.718	0.094	0.098	1.134	1.157	1.033	1.032	60.365	60.789	1.033	1.032	5.588	5.553	0.975	0.996
0.879	0.947	1469.030	1634.692	0.107	0.104	1.018	1.062	0.736	0.811	35.499	37.580	0.736	0.811	3.842	4.019	0.858	0.898
1.066	1.079	2568.019	2568.717	0.100	0.101	1.231	1.242	1.013	1.036	60.269	61.237	1.013	1.036	5.200	5.222	0.974	1.006
1.049	1.062	2218.892	2339.879	0.102	0.100	1.168	1.185	0.969	0.997	58.378	57.265	0.969	0.970	4.945	4.842	0.924	0.957
1.110	1.138	2354.962	2471.487	0.107	0.106	1.230	1.250	1.117	1.143	74.088	76.414	1.117	1.143	5.526	5.633	1.073	1.106
1.181	1.240	2690.360	2826.559	0.112	0.112	1.321	1.358	1.118	1.139	71.536	72.932	1.118	1.139	5.621	5.891	1.071	1.078
1.145	1.217	2838.816	2974.044	0.103	0.108	1.306	1.368	1.290	1.147	89.887	92.574	1.290	1.320	6.359	6.336	1.211	1.190
1.082	1.142	2569.558	2685.923	0.094	0.098	1.175	1.226	1.008	1.021	66.223	69.724	1.008	1.021	5.354	5.198	0.937	0.941
1.004	1.039	2110.034	2194.799	0.101	0.099	1.146	1.152	0.946	0.956	56.046	57.055	0.946	0.956	4.922	4.837	0.945	0.944
1.034	1.072	2048.805	2151.574	0.106	0.105	1.156	1.183	0.963	0.934	60.595	56.408	0.963	0.934	5.166	4.968	1.009	1.002
0.922	1.017	1824.239	2075.436	0.094	0.097	1.028	1.107	0.895	0.970	53.215	57.921	0.895	0.970	4.009	4.449	0.861	0.904
1.097	1.129	2311.568	2409.754	0.106	0.105	1.198	1.222	1.087	1.119	64.232	67.724	1.087	1.119	4.951	4.880	1.025	1.054

HTOT_B MC	HTOT_B MC_3	HTOT_B MD	HTOT_B MD_3	AXIS_LEN GTH	AXIS_LE NGTH_3	shaft neck angle	shaft neck angle_3	NN_BM D	NN_BM D_3	NN_CS A	NN_CSA _3	NN_CSM I	NN_CS MI_3	NN_ED	NN_ED_ 3	NN_WID TH.1	NN_WI DTH.3
34.728	34.855	1.162	1.182	103.050	106.701	135.741	131.186	1.181	1.218	3.038	3.186	1.638	2.004	2.232	2.262	2.702	2.748
39.579	41.578	1.081	1.107	110.287	110.287	141.618	139.478	1.199	1.238	3.680	3.729	2.793	2.901	2.751	2.674	3.221	3.162
42.899	38.258	1.283	1.319	102.802	101.897	132.030	127.062	1.449	1.549	4.007	3.991	2.500	2.319	2.319	2.066	2.905	2.705
36.296	39.184	1.181	1.244	105.887	104.078	126.726	122.840	1.179	1.373	3.709	3.884	2.635	2.661	2.904	2.422	3.367	2.972
38.026	36.041	1.016	1.028	111.042	113.619	136.022	135.000	1.060	1.142	3.488	3.507	3.320	2.946	3.047	2.779	3.457	3.225
32.637	35.722	1.145	1.181	104.540	102.141	127.117	125.395	1.266	1.236	3.290	3.295	1.962	2.179	2.221	2.307	2.729	2.800
41.727	40.719	1.070	1.102	111.518	111.703	136.543	135.340	1.127	1.143	3.776	3.878	3.736	3.962	3.082	3.119	3.519	3.563
39.227	39.474	1.187	1.199	102.731	102.525	130.020	130.259	1.149	1.158	3.668	3.687	3.177	3.221	2.909	2.890	3.357	3.342
37.686	37.574	1.258	1.261	97.448	93.865	131.186	134.604	1.457	1.272	3.829	3.809	2.107	2.265	2.166	2.705	2.760	3.211
36.074	37.475	0.970	1.034	113.979	114.537	130.236	131.082	1.044	1.143	3.513	3.398	3.406	2.616	3.131	2.675	3.534	3.123
29.409	31.687	0.970	1.004	100.855	102.257	133.091	136.364	1.111	1.181	3.172	3.426	2.210	2.318	2.562	2.580	2.997	3.045
37.554	31.967	1.110	1.090	108.970	107.453	133.813	124.262	1.121	1.120	3.811	3.673	3.774	3.387	3.136	3.007	3.570	3.442
27.198	27.617	0.912	0.938	100.836	96.887	134.061	132.532	0.934	1.068	2.644	2.850	1.582	1.713	2.662	2.383	3.025	2.803
42.734	41.789	1.073	1.101	109.358	103.867	137.083	135.625	1.243	1.203	3.847	3.812	3.287	3.177	2.760	2.858	3.249	3.329
31.504	30.877	0.992	1.000	109.548	106.517	137.726	129.053	1.082	1.109	3.246	3.279	2.536	2.448	2.729	2.671	3.150	3.104
40.928	42.694	1.137	1.179	108.045	107.306	132.274	132.357	1.317	1.306	4.125	4.095	3.344	3.250	2.768	2.777	3.289	3.292
44.765	46.449	1.263	1.296	115.205	114.400	137.440	132.510	1.256	1.347	4.085	4.090	3.525	3.243	2.923	2.652	3.415	3.187
51.059	50.192	1.184	1.209	119.299	117.879	136.219	135.000	1.422	1.414	4.506	4.406	3.545	3.596	2.763	2.709	3.328	3.272
43.919	43.012	1.106	1.127	114.101	116.707	130.642	126.670	1.061	1.066	3.700	3.663	4.013	3.806	3.254	3.196	3.663	3.608
38.225	39.037	1.071	1.095	105.284	108.653	134.812	137.695	1.115	1.118	3.477	3.502	2.718	2.781	2.841	2.853	3.275	3.288
37.896	37.516	1.029	1.051	105.155	106.404	132.542	129.879	1.217	1.203	3.733	3.665	2.752	2.745	2.742	2.725	3.220	3.198
31.668	32.329	0.990	1.046	100.836	101.635	136.169	136.975	0.993	1.073	2.938	3.164	1.859	2.097	2.723	2.679	3.108	3.098
38.355	40.331	1.148	1.205	107.370	107.306	123.690	125.557	1.222	1.255	3.527	3.576	2.410	2.412	2.546	2.493	3.030	2.991

NN_A CT.1	NN_ACT. 3	NN_PCD .1	NN_PC D.3	NN_CM P.1	NN_CMP .3	NN_SECT_ MOD	NN_SEC T_MOD 3	NN_BR	NN_BR_3	IT_BMD	IT_BMD _3	IT_CSA	IT_CSA _3	IT_CS MI	IT_CSMI_ 3	IT_ED	IT_ED_ 3	IT_WID TH.1
0.235	0.243	1.315	1.340	0.487	0.487	1.181	1.421	5.896	5.797	1.218	1.268	5.685	5.646	10.500	9.736	3.708	3.434	4.898
0.235	0.244	1.493	1.496	0.463	0.473	1.616	1.741	7.345	6.828	1.174	1.192	5.795	5.877	12.158	12.604	4.234	4.148	5.184
0.293	0.319	1.486	1.415	0.512	0.523	1.682	1.639	5.072	4.428	1.388	1.519	7.399	7.320	18.382	16.356	4.293	3.744	5.595
0.232	0.275	1.834	1.470	0.536	0.495	1.464	1.771	8.610	5.463	1.369	1.457	6.747	7.210	16.087	17.431	4.068	3.977	5.176
0.205	0.223	1.620	1.546	0.469	0.479	1.807	1.754	8.977	7.531	1.043	1.071	5.599	5.628	14.685	15.043	4.735	4.631	5.634
0.254	0.247	1.318	1.360	0.483	0.486	1.391	1.512	5.557	5.841	1.229	1.253	5.711	5.769	11.260	11.318	3.936	3.871	4.881
0.219	0.222	1.628	1.653	0.463	0.464	1.976	2.074	8.651	8.613	1.080	1.139	6.467	6.633	19.700	20.020	5.288	5.098	6.285
0.224	0.226	1.708	1.712	0.509	0.512	1.858	1.880	7.651	7.574	1.242	1.226	6.458	6.516	16.156	16.634	4.411	4.519	5.463
0.297	0.253	1.341	1.762	0.486	0.541	1.485	1.302	4.779	7.594	1.391	1.380	7.305	7.146	17.555	16.097	4.203	4.160	5.511
0.201	0.224	1.722	1.563	0.487	0.500	1.877	1.668	9.003	7.005	1.031	1.133	5.926	5.875	18.194	15.269	5.101	4.489	6.035
0.218	0.233	1.495	1.527	0.499	0.502	1.471	1.511	6.893	6.590	0.976	1.030	5.473	5.867	15.309	16.304	4.898	4.904	5.886
0.217	0.218	1.702	1.670	0.477	0.485	2.017	1.908	8.610	8.147	1.141	1.192	5.974	6.092	15.113	15.050	4.599	4.541	5.497
0.182	0.210	1.590	1.371	0.517	0.489	0.994	1.197	9.667	6.819	0.944	0.976	4.983	4.815	11.647	10.273	4.612	4.264	5.544
0.245	0.235	1.550	1.626	0.477	0.488	1.935	1.866	6.947	7.236	1.165	1.174	6.101	6.383	15.201	16.402	4.407	4.640	5.499
0.211	0.217	1.550	1.548	0.492	0.499	1.579	1.555	7.608	7.256	1.104	1.083	5.536	5.499	11.959	12.567	4.301	4.437	5.266
0.260	0.258	1.636	1.673	0.497	0.508	2.024	1.943	6.354	6.490	1.271	1.292	6.580	6.797	15.528	17.052	4.361	4.383	5.435
0.246	0.268	1.631	1.544	0.478	0.485	1.975	1.973	7.248	6.142	1.383	1.455	7.217	7.298	16.360	15.633	4.243	3.955	5.478
0.283	0.281	1.616	1.637	0.486	0.500	2.071	2.180	6.058	5.861	1.228	1.251	6.916	7.079	19.111	19.711	4.776	4.797	5.916
0.204	0.206	1.740	1.734	0.475	0.481	2.086	2.030	9.413	9.114	1.141	1.191	6.791	7.090	22.251	23.662	5.220	5.228	6.251
0.217	0.218	1.577	1.584	0.482	0.482	1.601	1.632	7.819	7.826	1.151	1.194	6.033	6.133	14.346	13.629	4.440	4.326	5.502
0.239	0.236	1.530	1.538	0.475	0.481	1.628	1.653	7.069	7.028	1.069	1.094	5.640	5.807	13.231	13.948	4.573	4.545	5.541
0.192	0.209	1.428	1.457	0.459	0.470	1.106	1.279	8.730	7.846	1.039	1.085	5.070	5.316	10.340	11.129	4.148	4.153	5.122
0.242	0.249	1.447	1.441	0.478	0.482	1.522	1.556	6.552	6.224	1.312	1.393	6.411	6.717	13.601	13.768	4.082	3.968	5.129

IT_WI DTH.3	IT_AC T.1	IT_ACT. 3	IT_PC D.1	IT_PCD .3	IT_CMP .1	IT_CM P.3	IT_SEC T_MO D	IT_SECT _MOD_ 3	IT_BR IT_BR	IT_BR _3	FS_BM D	FS_BM D_3	FS_CSA	FS_CS A_3	FS_CSM I	FS_CS MI_3	FS_ED	FS_ED_ 3	FS_WID TH.1
4.677	0.595	0.621	2.210	2.137	0.451	0.457	3.903	3.833	4.519	4.090	1.792	1.919	4.137	4.269	2.165	2.212			2.423
5.176	0.475	0.514	2.313	2.332	0.446	0.451	4.236	4.432	6.043	5.531	1.626	1.759	4.633	4.736	3.683	3.555	1.747	1.399	2.993
5.059	0.651	0.658	2.532	2.302	0.452	0.455	5.996	5.934	4.712	4.202	1.823	1.908	4.977	5.182	3.247	3.716	1.370	1.240	2.866
5.196	0.554	0.610	2.324	2.344	0.449	0.451	5.642	6.111	5.153	4.682	1.502	1.669	4.348	4.744	3.475	3.712	1.925	1.694	3.041
5.516	0.449	0.442	2.411	2.443	0.428	0.443	4.554	4.895	7.174	6.949	1.501	1.554	4.311	4.561	3.711	4.092	1.899	1.921	3.016
4.833	0.472	0.481	2.164	2.135	0.443	0.442	4.145	4.195	5.755	5.615	1.681	1.705	4.774	4.818	3.884	3.901	1.678	1.636	2.982
6.112	0.499	0.507	2.737	2.668	0.436	0.437	5.552	5.813	7.115	6.789	1.914	1.847	5.721	5.587	4.849	4.978	1.601	1.725	3.139
5.579	0.526	0.530	2.493	2.504	0.456	0.449	5.443	5.409	5.646	5.800	1.808	1.844	5.211	5.313	3.967	4.093	1.590	1.545	3.027
5.435	0.654	0.638	2.571	2.487	0.466	0.457	5.965	5.453	4.504	4.631	1.739	1.805	4.676	4.863	3.194	3.373	1.421	1.346	2.824
5.444	0.467	0.478	2.705	2.379	0.448	0.437	5.461	4.981	7.134	6.419	1.456	1.591	4.248	4.550	3.892	4.008	1.995	1.796	3.065
5.979	0.494	0.538	2.726	2.807	0.463	0.469	4.845	5.140	6.402	5.899	1.544	1.618	3.964	4.115	2.564	2.670	1.491	1.375	2.696
5.366	0.449	0.412	2.514	2.364	0.457	0.441	5.067	5.015	6.645	7.288	1.632	1.602	5.019	5.441	4.691	6.135	2.008	2.407	3.229
5.181	0.466	0.459	2.568	2.340	0.463	0.452	3.909	3.613	6.390	6.193	1.440	1.461	3.568	3.568	2.099	2.068	1.492	1.427	2.602
5.710	0.546	0.535	2.498	2.613	0.454	0.458	5.065	5.295	5.498	5.788	1.853	1.771	4.878	4.946	3.395	3.849	1.194	1.516	2.764
5.330	0.482	0.447	2.425	2.413	0.461	0.453	4.209	4.307	5.892	6.530	1.347	1.358	3.613	3.873	2.964	3.514	1.824	2.008	2.816
5.525	0.537	0.571	2.481	2.522	0.456	0.456	5.257	5.678	5.504	5.257	1.602	1.708	4.623	4.810	3.850	3.862	1.816	1.618	3.031
5.266	0.618	0.656	2.493	2.416	0.455	0.459	5.481	5.486	4.832	4.347	1.819	1.952	5.083	5.267	3.807	3.743	1.462	1.148	2.934
5.940	0.570	0.571	2.607	2.604	0.441	0.438	5.775	5.908	5.804	5.840	1.820	1.834	5.184	5.335	4.161	4.375	1.531	1.593	2.991
6.254	0.516	0.513	2.709	2.664	0.433	0.426	6.282	6.591	6.871	7.003	1.736	1.729	5.287	5.527	5.017	5.804	1.871	2.056	3.199
5.395	0.531	0.534	2.388	2.370	0.434	0.439	4.602	4.504	5.863	5.663	1.629	1.707	4.346	4.615	2.981	3.231	1.520	1.477	2.801
5.573	0.484	0.514	2.439	2.455	0.440	0.441	4.265	4.472	6.408	6.067	1.608	1.699	4.357	4.487	3.033	2.958	1.595	1.406	2.845
5.146	0.487	0.497	2.286	2.243	0.446	0.436	3.647	3.832	5.825	5.852	1.561	1.629	3.809	4.097	2.221	2.540	1.311	1.325	2.563
5.063	0.524	0.547	2.314	2.254	0.451	0.445	4.826	4.898	5.376	5.132	1.546	1.690	4.472	4.932	3.976	4.365	1.879	1.765	3.038

FS_WIDT	FS_ACT	FS_ACT.	FS_PC	FS_PCD	FS_C	FS_CM	FS_SECT	FS_SEC	FS_BR	FS_BR_3
H.3	.1	3	D.1	.3	MP.1	P.3	_MOD	T_MOD 3		
2.336	0.823	1.072	1.200	1.193	0.495	0.511	1.769	1.854	1.487	1.115
2.828	0.623	0.714	1.411	1.326	0.471	0.469	2.328	2.367	2.544	2.107
2.852	0.748	0.806	1.381	1.353	0.482	0.474	2.186	2.478	1.986	1.860
2.986	0.558	0.646	1.515	1.536	0.498	0.514	2.276	2.417	2.739	2.380
3.082	0.558	0.580	1.411	1.439	0.468	0.467	2.311	2.490	2.877	2.832
2.968	0.652	0.666	1.440	1.437	0.483	0.484	2.517	2.548	2.366	2.298
3.177	0.769	0.726	1.527	1.533	0.486	0.483	3.008	3.029	2.099	2.266
3.025	0.719	0.740	1.468	1.492	0.485	0.493	2.545	2.670	2.169	2.072
2.829	0.702	0.741	1.429	1.422	0.506	0.503	2.236	2.372	2.037	1.918
3.003	0.535	0.604	1.470	1.446	0.480	0.481	2.441	2.573	2.983	2.581
2.671	0.603	0.648	1.329	1.286	0.493	0.481	1.876	1.927	2.270	2.141
3.567	0.610	0.580	1.516	1.621	0.470	0.454	2.738	3.151	2.808	3.357
2.566	0.555	0.569	1.242	1.236	0.477	0.482	1.543	1.555	2.453	2.339
2.933	0.785	0.708	1.389	1.452	0.503	0.495	2.440	2.599	1.773	2.094
2.994	0.496	0.493	1.361	1.416	0.483	0.473	2.037	2.226	2.935	3.201
2.957	0.607	0.669	1.465	1.455	0.483	0.492	2.458	2.571	2.580	2.245
2.833	0.736	0.843	1.409	1.370	0.480	0.483	2.496	2.557	2.072	1.738
3.055	0.730	0.731	1.452	1.487	0.486	0.487	2.703	2.791	2.109	2.146
3.357	0.664	0.650	1.519	1.561	0.475	0.465	2.985	3.232	2.532	2.763
2.839	0.640	0.681	1.376	1.389	0.491	0.489	2.091	2.228	2.226	2.130
2.774	0.625	0.684	1.392	1.354	0.489	0.488	2.087	2.083	2.326	2.078
2.641	0.626	0.658	1.230	1.266	0.480	0.480	1.665	1.848	2.130	2.090
3.066	0.579	0.650	1.433	1.474	0.472	0.481	2.477	2.740	2.772	2.450

Appendix 14 - Baseline, 4 months and 8 months data of the adolescents that completed the whole WL intervention

Gpe	SEX	Age	menar che	E2_1_pg /ml	E2_2_pg/ ml	E2_3_pg/ ml	Leptin_ 1	Leptin_ 2	Leptin_ 3	BMI_1	BMI_2	BMI_3	DXAWB masskg_ 1	DXAWB masskg_ 2	DXAWB masskg_ 3	WBFM% _1	WBFM% _2	WBFM% _3
1	2	15.93	14	47.10	52.76	34.26	34.34	27.85	23.65	36.11	30.48	29.00	97.74	86.73	82.56	42.68	36.95	35.26
1	2	12.05	12	25.02	49.38	91.36	5.85	2.75	3.46	33.79	31.60	18.54	51.70	45.85	43.94	36.94	29.88	25.04
1	2	15.12	13							35.42	30.69	31.22	92.71	89.45	87.11	35.84	31.09	31.22
1	2	13.99	12							30.33	28.69	28.41	110.07	96.95	89.82	44.05	39.80	37.66
1	2	13.13	12	668.19	338.63	433.88	9.88	15.89	22.03	22.97	28.70	29.76	85.28	83.15	86.32	31.93	31.48	33.34
1	2	13.81	14	41.45		34.49	44.69	24.01	47.75	31.20	29.04	29.06	89.98	84.03	84.95	40.33	38.37	37.56
1	2	13.70	12							34.14	30.76	29.75	101.39	93.81	89.91	39.76	37.06	34.56
1	2	15.10	12	62.94	46.09	68.81	37.20	46.46	43.15	38.62	35.14	32.56	109.25	101.19	95.86	43.60	39.76	39.38
1	2	13.99	11	71.85	94.24	114.05	49.38	46.46	49.68	31.98	29.19	28.46	85.43	81.23	78.95	45.49	42.05	40.84
1	1	14.67		37.92	72.47	47.35	22.45	11.85	9.08	38.86	33.56	30.49	100.23	90.73	83.73	42.18	34.58	28.54
1	2	13.67	12	50.41	193.30	46.59	22.84	13.62	14.47	33.27	28.04	26.11	84.64	73.00	67.37	38.55	32.96	27.80
1	1	12.97		15.39		52.76	10.19		7.54	27.99	25.26	23.12	80.25	75.45	72.26	32.87	26.40	21.07
1	2	15.46	13							35.29	32.18	29.41	104.14	95.57	88.24	41.38	38.10	35.72
1	2	12.18	12	36.30	67.32	60.39	37.72	22.14	28.41	33.15	30.75	28.98	89.73	81.84	79.87	39.34	34.24	33.91
1	2	13.72	12		43.38			27.88		31.62	26.35	26.40	75.49	72.94	73.57	34.31	31.06	32.32
1	2	13.30	12			71.85	33.44		46.77	25.99	26.11	26.56	67.81	67.86	69.62	34.54	35.51	35.05
1	2	13.48	13	146.77		64.38	43.88		45.38	33.96	31.02	29.71	106.82	98.43	94.48	41.60	35.54	35.82
1	2	15.55	12	118.27	139.77	100.55	23.61	13.01	28.16	30.11	27.31	27.31	85.97	79.25	80.28	41.17	36.85	35.44
1	1	13.93	13	55.16		51.19	18.87		9.85	32.03	28.76	27.68	85.31	75.63	75.91	36.94	29.54	25.09
1	2	15.03		123.93	79.45	97.92	35.06	31.22	22.41	31.60	31.60	30.49	97.77	87.86	85.46	36.01	32.84	30.03
1	2	14.06	13	36.76	45.10	44.12	25.34	10.58	12.77	37.20	29.41	26.99	105.53	88.59	80.61	39.14	32.59	30.29
1	2	15.51	13	181.74	122.61	131.66	43.78	40.86	26.64	39.88	31.58	29.63	86.51	75.22	70.12	44.70	41.19	38.56
1	2	12.88								34.05	31.24	28.73	84.85	79.68	75.98	41.64	37.90	35.99

WBFMg_1	WBFMg_2	WBFMg_3	WBLearnMas sg_1	WBLearnMa ssg_2	WBLearnMas sg_3	aFM_1	aFM_2	aFM_3	gFM_1	gFM_2	gFM_3	VFAT%_ 1	VFAT%_ 2	VFAT%_ 3	VFATg_1
41714.35	32044.40	29113.08	56022.97	54685.72	53445.27	44.66	37.94	37.51	43.14	36.08	35.21	44.99	38.44	37.89	362.24
19098.35	13697.56	11003.56	32597.04	32150.25	32933.67	39.02	28.58	23.29	40.09	32.96	28.70	38.06	30.33	22.85	228.97
33224.90	27810.37	27192.45	59481.54	61642.43	59912.61	39.65	35.63	36.97	38.53	33.05	34.05	40.30	35.91	39.88	243.60
48491.05	38588.51	33821.58	61581.81	58365.48	55993.67	50.49	45.40	42.49	43.78	38.93	36.96	51.15	46.77	43.65	471.72
27231.96	26172.51	28780.47	58047.24	56974.49	57536.23	29.52	32.33	36.21	35.70	35.37	38.67	32.67	34.42	37.20	138.58
36287.32	32243.03	31902.37	53694.36	51788.91	53044.16	40.12	39.86	38.45	41.74	40.97	39.49	43.18	41.75	39.84	375.68
40319.24	34765.41	31069.38	61075.67	59042.76	58836.25	40.02	39.52	36.70	42.23	39.99	38.96	41.52	40.89	40.05	274.63
47634.41	40235.03	37752.97	61616.76	60950.67	58108.56	46.27	41.95	39.68	47.31	42.30	41.62	46.79	40.52	37.78	230.08
38861.03	34155.11	32248.62	46569.66	47074.17	46705.53	47.64	43.73	42.90	47.42	43.37	43.03	49.87	41.69	45.07	215.12
42273.69	31376.09	23895.76	57953.71	59350.08	59832.38	46.97	38.76	30.42	40.42	32.97	27.55	47.14	39.79	32.90	289.73
32623.03	24058.41	18730.55	52013.02	48945.27	48641.04	45.95	38.12	31.25	42.54	36.42	33.43	45.89	37.99	31.59	291.21
26377.02	19920.57	15227.52	53874.95	55524.56	57027.86	37.71	28.30	21.54	36.02	28.57	23.05	38.51	26.70	22.18	364.67
43095.28	36413.00	31515.57	61045.04	59159.52	56720.85	45.03	38.80	35.59	43.75	38.22	37.40	44.34	42.10	37.30	379.17
35299.27	28023.94	27085.52	54429.42	53812.87	52779.66	42.40	35.11	34.44	41.16	37.60	36.52	43.90	37.27	36.39	298.02
25903.70	22657.94	23773.64	49587.89	50285.26	49792.10	36.93	37.01	37.05	36.69	35.47	35.03	40.12	38.71	38.58	143.55
23424.66	24097.83	24401.77	44384.58	43764.18	45215.60	37.52	40.84	39.91	36.65	35.43	35.26	40.24	42.18	41.71	250.74
44437.16	34979.16	33843.26	62384.78	63446.31	60638.18	45.54	38.30	35.25	43.15	39.65	39.02	46.85	40.05	35.97	301.25
35396.86	29201.49	28450.56	50570.60	50051.44	51829.87	44.28	37.83	39.52	45.18	41.49	40.80	42.18	38.20	40.54	302.30
31511.26	22340.55	19046.89	53793.89	53288.60	56861.13	40.01	33.97	29.15	37.14	30.85	27.28	40.40	33.09	29.96	617.10
35212.01	28857.83	25668.03	62560.69	59006.65	59794.32	39.54	35.36	31.81	35.65	32.00	32.48	40.07	35.88	34.59	325.26
41306.63	28875.37	24419.46	64222.60	59715.07	56194.68	44.90	35.41	34.25	38.59	32.85	31.57	45.44	34.99	37.21	371.30
38668.36	30983.49	27036.13	47845.52	44233.93	43085.28	49.31	44.52	42.81	45.92	42.85	40.77	50.28	46.57	43.30	310.07
35329.09	30196.84	27348.82	49522.86	49481.68	48631.67	43.92	40.23	34.64	45.99	42.27	38.91	44.22	41.62	37.72	359.45

VFATg_2	VFATg_3	VFATcm3 _1	VFATcm3 _2	VFATcm3 _3	SOS_1	SOS_2	SOS_3	BUA_1	BUA_2	BUA_3	TBLH BMCg_1	TBLH BMCg_2	TBLH BMCg_3	TBLH BMD_1	TBLH BMD_2
309.95	259.49	391.61	335.08	280.53	1698.00	1685.00	1708.00	99.00	115.00	105.00	1731.27	1798.61	1832.56	0.99	1.01
230.91	137.06	247.53	249.63	148.17	1540.00	1538.00	1551.00	85.00	84.00	84.00	929.49	963.97	967.81	0.77	0.78
245.78	347.31	263.36	265.71	375.47	1712.00	1684.00		142.00	135.00		2146.21	2164.45	2212.17	1.14	1.13
409.30	327.06	509.97	442.49	353.58	1550.00	1548.00		103.00	106.00		1751.32	1783.17	1833.33	0.94	0.94
177.77	143.09	149.82	192.18	154.69	1579.00	1557.00	1580.00	110.00	107.00	116.00	1793.89	1828.18	1847.00	0.99	0.99
179.05	207.81	406.14	193.57	224.66	1557.00	1545.00	1520.00	102.00	105.00	93.00	1611.92	1611.93	1721.45	0.91	0.92
160.99	163.63	296.90	174.04	176.90	1531.00	1562.00		117.00	127.00		1747.45	1780.62	1858.04	0.94	0.94
210.09	190.45	248.74	227.12	205.89	1648.00	1615.00	1664.00	129.00	120.00	139.00	1956.19	2065.68	2089.79	1.05	1.03
161.41	205.34	232.56	174.50	221.99	1541.00	1551.00	1567.00	113.00	118.00	106.00	1592.80	1606.34	1630.07	0.92	0.92
295.66	243.92	313.22	319.63	263.70	1588.00	1603.00	1642.00	113.00	108.00	114.00	1520.89	1575.27	1695.91	0.91	0.94
171.99	65.72	314.82	185.93	71.04	1625.00	1627.00	1659.00	112.00	132.00	109.00	1719.44	1740.62	1781.89	1.03	1.05
203.24	153.00	394.24	219.72	165.41	1549.00	1566.00	1580.00	107.00	114.00	120.00	1590.94	1658.27	1844.09	0.93	0.94
294.79	163.06	409.91	318.69	176.29	1591.00	1601.00		128.00	134.00		2021.61	2052.17	2130.70	1.04	1.04
233.92	213.11	322.19	252.88	230.39	1585.00	1621.00	1630.00	140.00	137.00	146.00	1545.32	1625.16	1823.05	0.96	1.00
208.49	196.94	155.19	225.39	212.91		1588.00			129.00		1510.80	1510.71	1598.12	0.92	0.92
215.62	188.64	271.07	233.10	203.93	1549.00	1507.00	1524.00	104.00	98.00	107.00	1325.81	1389.61	1471.19	0.85	0.85
232.97	167.05	325.67	251.86	180.60	1606.00	1632.00	1656.00	136.00	129.00	123.00	2116.68	2088.32	2263.48	1.06	1.07
259.16	243.39	326.81	280.18	263.13	1563.00	1558.00	1608.00	126.00	116.00	141.00	1699.55	1718.09	1782.14	0.92	0.89
358.69	243.53	667.13	387.77	263.28	1566.00	1566.00	1608.00	128.00	118.00	123.00	1615.54	1508.47	1795.13	0.97	0.94
168.62	170.98	351.64	182.29	184.84	1698.00	1688.00	1716.00	126.00	124.00	130.00	1889.51	1950.25	2018.27	1.07	1.09
213.84	172.32	401.41	231.18	186.30	1556.00	1553.00	1575.00	114.00	114.00	122.00	1908.66	1944.93	1987.71	1.01	1.02
215.21	179.80	335.21	232.66	194.37	1564.00	1566.00	1596.00	102.00	114.00	112.00	1319.06	1320.28	1360.60	0.88	0.87
204.86	148.86	388.60	221.48	160.93	1593.00	1605.00		111.00	126.00		1311.59	1384.19	1597.69	0.93	0.90

TBLH BMD_3	WBBMCg_ 1	WBBMCg_ 2	WBBMCg_ 3	WBBM AD_1	WBBM AD_2	WBBM AD_3	WBBM D_1	WBBM D_2	WBBM D_3	LS BMAD_ 1	LS BMAD_ _2	LS BMAD_ _3	LS TBS_1	LS TBS_2	LS TBS_3	L1L4BM C	L1L4BM C	L1L4B MC_3
1.03	2244.52	2340.28	2379.24	0.10	0.10	0.10	1.14	1.17	1.20	1.00	0.84	1.01	1.34	1.38	1.41	51.27	53.40	53.38
0.80	1264.20	1322.89	1363.57	0.10	0.09	0.10	0.91	0.91	0.96	0.60	1.01	0.64	1.10	1.02	1.00	24.80	23.51	26.31
1.15	2773.33	2856.54	2902.31	0.10	0.10	0.10	1.31	1.29	1.33	1.19	0.63	1.21	1.33	1.30	1.37	59.47	59.20	58.86
0.94	2234.56	2290.28	2343.57	0.09	0.09	0.09	1.07	1.08	1.08	1.01	1.21	1.02	1.34	1.39	1.40	56.09	59.81	60.57
0.99	2342.79	2423.19	2399.54	0.09	0.09	0.09	1.14	1.15	1.14	0.99	1.04	1.00	1.40	1.39	1.43	61.02	63.69	63.43
0.95	2087.60	2118.17	2213.99	0.09	0.09	0.09	1.04	1.07	1.09	1.01	1.15	1.05	1.17	1.23	1.25	57.21	59.99	62.52
0.97	2368.24	2399.63	2492.50	0.09	0.09	0.09	1.12	1.12	1.15	0.85	1.05	1.03	1.39	1.41	1.36	59.17	61.77	62.86
1.03	2475.80	2626.26	2678.84	0.09	0.09	0.09	1.18	1.17	1.18	1.02	1.03	1.17	1.37	1.38	1.38	63.28	67.54	66.31
0.92	2080.01	2109.88	2158.96	0.09	0.09	0.09	1.07	1.07	1.09	1.12	1.14	1.03	1.46	1.50	1.53	49.95	52.09	53.04
1.01	1905.86	1973.31	2116.46	0.08	0.09	0.09	1.00	1.03	1.11	1.01	1.00	0.99	1.19	1.32	1.30	43.44	51.90	57.46
1.05	2215.99	2235.98	2279.52	0.10	0.10	0.10	1.16	1.18	1.19	0.86	0.95	1.23	1.48	1.49	1.54	57.34	59.10	60.59
1.04	1990.34	2074.76	2268.85	0.09	0.09	0.10	1.01	1.03	1.12	0.89	0.91	0.86	1.33	1.29	1.33	42.60	47.66	50.41
1.06	2615.81	2641.09	2775.12	0.09	0.09	0.09	1.20	1.21	1.24	0.81	0.82	1.11	1.43	1.44	1.45	65.57	68.42	70.72
1.03	2040.31	2158.23	2387.50	0.10	0.10	0.10	1.11	1.16	1.20	1.10	1.11	1.20	1.30	1.39	1.36	52.71	60.36	65.70
0.95	2054.32	2063.31	2170.40	0.10	0.10	0.10	1.10	1.10	1.13	1.05	1.12	0.95	1.24	1.21	1.25	50.49	53.93	55.33
0.90	1760.24	1832.90	1939.65	0.09	0.09	0.09	0.98	0.99	1.05	0.97	1.02	1.02	1.24	1.33	1.34	57.45	60.77	63.94
1.08	2732.22	2729.63	2930.46	0.09	0.10	0.09	1.21	1.23	1.24	0.96	0.74	1.10	1.34	1.41	1.42	65.66	69.90	70.36
0.94	2302.17	2310.74	2361.69	0.08	0.08	0.09	1.07	1.06	1.10	1.08	1.11	1.05	1.35	1.33	1.35	63.30	66.55	64.20
1.04	2106.27	2023.65	2310.40	0.09	0.09	0.10	1.09	1.08	1.16	1.04	1.05	1.18	1.15	1.14	1.22	32.40	42.92	75.92
1.10	2414.78	2648.87	2580.51	0.10	0.09	0.10	1.21	1.21	1.25	0.87	0.90	1.11	1.24	1.30	1.31	53.92	52.48	57.61
1.03	2475.68	2540.41	2624.67	0.09	0.09	0.09	1.17	1.19	1.21	1.12	1.02	1.08	1.26	1.31	1.36	62.16	65.06	64.96
0.90	1710.15	1716.95	1744.57	0.09	0.09	0.09	1.01	1.01	1.03	0.61	0.97	0.97	1.35	1.29	1.38	40.61	42.83	42.21
0.92	1717.14	1818.69	2025.42	0.10	0.09	0.08	1.05	1.03	1.03	0.96	1.00	0.87	1.10	1.22	1.20	35.95	42.60	45.65

L1L4B MD	L1L4BM D	L1L4BM D_3	NECK_B MC	NECK_B MC	NECK_B MC_3	NECK_ BMD	NECK_ BMD_2	NECK_ BMD_3	HTOT_B MC	HTOT_B MC_2	HTOT_B MC_3	HTOT_ BMD	HTOT_ MD_2	HTOT_ BMD_3	AXIS_LEN GTH	AXIS_LEN GTH_2	AXIS_LEN GTH_3	shaft neck angle
1.00	1.01	1.01	5.54	5.24	5.55	1.11	1.06	1.07	39.44	38.57	39.23	1.21	1.19	1.22	95.68	95.97	96.71	122.91
0.60	0.63	0.64	3.06	2.98	2.92	0.70	0.67	0.68	20.91	22.11	20.73	0.75	0.76	0.73	97.45	98.30	97.01	138.31
1.19	1.21	1.21	5.25	6.31	5.80	1.33	1.28	1.26	38.22	37.14	37.71	1.36	1.35	1.33	89.66	96.13	94.10	120.64
1.01	1.04	1.02	5.03	4.61	4.77	0.92	0.97	1.00	36.06	37.44	38.90	1.12	1.09	1.10	100.68	104.85	104.13	126.76
0.99	1.00	1.00	4.64	4.69	4.86	0.89	0.90	0.87	33.67	30.20	32.05	0.93	0.90	0.92	106.04	106.04	109.36	130.73
1.01	1.05	1.05	4.26	5.22	4.86	0.84	0.92	0.86	32.41	33.34	32.03	0.92	0.95	0.95	113.62	107.23	107.26	138.58
1.02	1.03	1.03	4.85	5.04	5.00	0.97	0.95	0.96	34.90	34.12	35.44	0.98	0.98	1.00	103.79	97.79	106.04	132.78
1.12	1.14	1.17	5.55	4.32	4.26	1.10	1.05	1.01	34.53	36.42	36.77	1.16	1.16	1.16	87.22	100.37	99.98	111.80
1.01	1.00	1.03	4.93	4.85	4.56	0.92	0.93	0.90	28.97	29.91	27.89	0.97	0.98	0.96	100.21	101.63	100.95	128.19
0.86	0.95	0.99	3.87	3.64	4.20	0.86	0.84	0.91	34.82	35.04	35.61	1.03	1.05	1.06	93.90	100.21	101.03	133.64
1.21	1.20	1.23	5.87	5.15	5.48	1.10	1.05	1.05	32.66	35.63	34.16	1.09	1.11	1.10	96.71	93.90	98.31	120.19
0.81	0.82	0.86	4.40	4.67	4.62	0.91	0.93	0.97	37.12	38.32	44.15	1.03	1.05	1.07	113.79	115.09	117.24	135.03
1.10	1.11	1.11	5.83	5.69	6.14	1.13	1.08	1.13	42.93	37.52	40.79	1.08	1.05	1.05	116.71	106.74	116.62	139.81
1.05	1.12	1.20	4.77	4.57	4.96	0.97	0.94	0.99	34.74	33.18	34.71	1.07	1.06	1.09	96.60	100.21	98.79	137.85
0.92	0.95	0.95	4.26	4.33	4.63	0.86	0.88	0.88	31.20	32.08	31.51	0.94	0.93	0.95	99.52	99.52	99.35	132.18
0.96	0.98	1.02	4.56	4.43	4.58	0.85	0.88	0.89	32.54	33.46	34.14	0.93	0.94	0.97	105.89	105.84	102.26	139.61
1.08	1.11	1.10	6.24	6.26	6.27	1.09	1.13	1.13	43.67	42.84	42.42	1.16	1.16	1.15	101.33	99.93	108.68	129.20
1.04	1.05	1.05	4.32	4.36	4.76	0.93	0.89	0.86	30.11	30.28	29.06	0.85	0.85	0.83	110.78	110.78	110.10	137.60
0.74	0.82	1.18	5.24	5.02	4.76	0.96	0.94	0.94	35.62	38.13	35.37	1.00	0.99	0.98	100.68	106.14	104.08	128.33
1.12	1.11	1.11	6.26	5.93	5.94	1.21	1.15	1.12	45.32	41.85	42.40	1.30	1.27	1.28	105.28	102.45	99.12	139.98
1.07	1.06	1.08	5.43	5.59	5.78	1.07	1.01	1.08	40.40	39.96	41.73	1.09	1.07	1.07	101.55	104.42	106.04	137.15
0.96	1.00	0.97	3.51	3.29	3.74	0.81	0.77	0.77	24.91	26.68	25.47	0.90	0.91	0.90	89.00	89.95	97.32	131.82
0.79	0.87	0.87	4.65	4.58		0.95	0.92		28.69	31.86		0.97	1.00		91.30	100.21		129.21

shaft neck angle_2	shaft neck angle_3	NN_B MD_1	NN_B MD_2	NN_B MD_3	NN_CS A_1	NN_C SA_2	NN_CS A_3	NN_C SMI_1	NN_C SMI_2	NN_C SMI_3	NN_E D_1	NN_E D_2	NN_E D_3	NN WID TH_1	NN WIDT H_2	NN WIDT H_3	NN ACT_ 1	NN ACT_ 2	NN ACT_ 3	NN_S ECT_ MOD_1	NN_S ECT_ MOD_2	NN_SE CT_M OD_3
129.30	131.47	1.38	1.27	1.30	4.15	3.91	4.08	3.38	3.01	3.39	2.60	2.73	2.79	3.15	3.23	3.30	0.28	0.25	0.24	2.10	1.82	2.01
138.97	137.75	0.81	0.77	0.77	2.18	2.08	2.06	1.27	1.31	1.26	2.56	2.54	2.51	2.88	2.84	2.81	0.16	0.15	0.24	0.81	0.85	0.85
133.61	128.92	1.69	1.76	1.60	5.16	5.19	4.64	3.46	3.44	2.99	2.53	2.38	2.41	2.64	3.92	3.52	0.40	0.27	0.29	2.03	2.15	1.87
133.32	130.41	1.09	1.13	1.14	3.46	3.40	3.54	3.25	2.50	2.90	2.89	2.71	2.81	3.32	3.16	3.25	0.21	0.22	0.23	1.90	1.58	1.77
132.56	135.76	1.10	1.08	1.06	3.49	3.47	3.48	3.20	3.03	3.17	2.91	2.96	3.03	3.33	3.38	3.44	0.21	0.21	0.20	1.89	1.77	1.83
135.36	138.58	1.05	1.16	1.05	3.03	3.69	3.03	2.12	3.21	2.12	2.61	2.87	2.61	3.02	3.33	3.02	0.21	0.23	0.25	1.33	1.76	1.33
132.67	134.55	1.23	1.13	1.14	3.66	3.61	3.76	2.87	3.13	3.42	2.65	2.92	3.04	3.13	3.36	3.48	0.24	0.22	0.22	1.77	1.78	1.81
133.15	131.20	1.34	1.21	1.20	4.81	4.81	4.51	5.40	6.05	4.91	3.26	3.70	3.49	3.78	4.17	3.96	0.26	0.23	0.22	2.71	2.75	2.30
126.61	126.52	1.14	1.13	1.07	3.39	3.45	3.43	2.71	2.72	2.66	2.68	2.77	2.98	3.13	3.21	3.39	0.22	0.22	0.30	1.68	1.64	1.50
136.76	135.49	0.98	0.95	0.95	3.09	2.99	3.48	2.38	2.24	3.19	2.95	2.94	3.50	3.33	3.93	3.87	0.19	0.15	0.20	1.33	1.32	1.49
130.45	120.19	1.37	1.25	1.37	4.04	3.66	4.04	3.19	2.59	3.19	2.55	2.57	2.55	3.10	3.06	3.10	0.27	0.25	0.22	1.96	1.64	1.96
132.58	131.19	1.11	1.07	1.14	3.36	3.22	3.46	2.79	2.43	2.97	2.73	2.75	2.76	3.16	3.17	3.20	0.22	0.21	0.22	1.74	1.52	1.84
135.44	140.15	1.42	1.32	1.30	4.47	4.29	4.50	3.80	3.67	4.65	2.75	2.88	3.12	3.31	3.41	3.63	0.28	0.26	0.18	2.22	2.11	2.42
136.75	136.75	1.17	1.17	1.18	3.45	3.37	3.56	2.28	2.43	2.56	2.67	2.58	2.72	3.13	3.04	3.18	0.23	0.23	0.24	1.41	1.55	1.55
135.29	135.59	1.09	1.06	1.09	3.17	3.20	3.24	2.41	2.40	2.37	2.62	2.77	2.69	3.05	3.18	3.11	0.21	0.21	0.21	1.53	1.44	1.46
141.30	140.39	1.08	1.05	1.07	3.23	3.27	3.35	2.33	2.42	2.47	2.71	2.86	2.87	3.14	3.27	3.28	0.21	0.20	0.25	1.41	1.40	1.42
131.91	139.39	1.39	1.34	1.37	4.43	4.34	4.55	3.98	3.69	4.17	2.80	2.87	2.96	3.35	3.40	3.50	0.28	0.26	0.28	2.24	2.08	2.29
135.00	133.36	1.19	1.09	1.07	3.50	3.46	3.32	2.85	3.01	2.76	2.64	2.91	2.86	3.10	3.34	3.27	0.23	0.21	0.20	1.71	1.68	1.59
131.15	131.15	1.17	1.12	1.12	3.90	3.74	3.74	3.70	3.18	3.18	3.04	3.08	3.08	3.49	3.52	3.52	0.23	0.22	0.22	1.99	1.74	1.74
135.71	130.41	1.56	1.40	1.38	4.74	4.43	4.35	3.75	3.55	3.44	2.55	2.76	2.77	3.19	3.32	3.32	0.32	0.28	0.24	2.32	2.12	2.06
136.70	134.41	1.36	1.26	1.35	4.22	4.04	4.40	3.34	3.20	3.66	2.73	2.86	2.90	3.27	3.36	3.43	0.27	0.25	0.19	2.01	1.89	2.10
135.94	140.40	1.11	0.86	0.84	3.34	2.88	2.82	2.76	2.27	2.36	2.74	3.21	3.26	3.17	3.54	3.58	0.22	0.17	0.24	1.64	1.19	1.19
134.82		1.04	1.04	0.97	3.37	3.26	3.58	2.60	2.38	3.40	3.06	2.93	3.52	3.47	3.33	3.89	0.20	0.20	0.26	1.38	1.35	1.56

NN_B R_1	NN_B R_2	NN_BR _3	IT_BM D_1	IT_BM D_2	IT_BM D_3	IT_CS A_1	IT_CSA _2	IT_CS A_3	IT_CS MI_1	IT_CS MI_2	IT_CS MI_3	IT_ED _1	IT_ED _2	IT_E D_3	IT WIDT H_1	IT WIDT H_2	IT WIDTH _3	IT ACT_1	IT ACT_2	IT ACT_3	IT_SEC T_MO D_1	IT_SE CT_M OD_2	IT_SE CT_M OD_3
5.84	6.61	6.58	1.40	1.34	1.29	7.00	7.29	6.88	16.44	18.85	16.56	4.07	4.53	4.47	5.25	5.69	5.60	0.59	0.58	0.59	5.58	5.78	5.17
10.84	10.39	10.02	0.76	0.78	0.76	3.50	3.72	3.69	6.36	7.36	7.47	4.16	4.33	4.46	4.81	5.01	5.11	0.33	0.34	0.48	2.36	2.66	2.63
5.05	4.46	5.12	1.45	1.42	1.42	7.13	7.01	7.11	14.96	14.42	15.05	3.97	3.99	4.04	5.10	5.27	5.24	0.68	0.63	0.65	5.15	4.96	5.01
8.02	7.16	7.35	1.31	1.17	1.22	6.68	6.28	6.33	16.66	16.00	15.27	4.23	4.63	4.42	5.35	5.62	5.45	0.56	0.50	0.55	5.45	5.00	5.00
7.95	8.19	8.45	0.99	0.98	0.94	5.54	5.53	5.40	17.51	17.26	17.15	5.09	5.21	5.30	5.88	5.93	6.02	0.40	0.36	0.45	5.48	5.24	5.21
7.73	8.03	7.73	0.94	0.98	0.94	5.24	5.26	5.24	17.07	15.89	17.07	5.01	4.83	5.01	5.82	5.63	5.82	0.41	0.40	0.47	5.43	5.14	5.43
6.73	8.01	8.55	0.99	0.99	0.98	5.46	5.86	5.50	14.99	17.55	15.64	4.89	5.28	5.01	5.77	6.20	5.87	0.44	0.46	0.50	4.60	5.17	4.67
7.83	9.46	9.30	1.36	1.31	1.24	7.72	7.12	6.67	22.70	18.69	17.07	4.62	4.48	4.58	5.95	5.68	5.66	0.67	0.60	0.53	6.85	5.96	5.51
7.23	7.53	9.06	1.12	1.13	1.01	5.73	5.70	5.28	14.52	14.14	13.18	4.45	4.44	4.72	5.37	5.31	5.51	0.46	0.44	0.65	4.64	4.62	4.16
10.04	9.60	12.03	1.11	1.09	1.06	5.50	5.57	5.41	12.72	13.07	12.99	4.21	4.14	4.50	5.21	5.40	5.38	0.50	0.49	0.47	4.82	4.67	4.52
5.98	6.35	5.98	1.20	1.20	1.20	6.69	6.50	6.69	19.26	17.26	19.26	4.73	4.59	4.73	5.83	5.68	5.83	0.55	0.55	0.49	6.00	5.53	6.00
7.37	7.69	7.27	1.27	1.23	1.37	6.21	6.71	7.28	13.25	15.99	19.26	4.04	4.58	4.33	5.15	5.70	5.56	0.55	0.56	0.45	4.67	4.97	6.22
6.08	6.67	7.55	1.09	1.12	1.05	6.06	6.75	5.98	17.41	21.09	17.36	4.97	5.42	5.18	5.85	6.34	5.99	0.44	0.46	0.47	5.35	6.15	5.07
7.65	6.84	7.17	1.21	1.17	1.17	6.39	6.23	6.29	16.06	16.10	16.21	4.36	4.49	4.54	5.55	5.60	5.64	0.59	0.56	0.55	5.48	5.38	5.36
7.38	8.13	7.58	0.99	0.99	0.94	5.21	5.56	5.15	14.84	16.65	14.84	4.66	5.04	4.95	5.51	5.91	5.76	0.43	0.43	0.48	4.89	5.24	4.60
7.85	8.49	8.40	1.02	1.01	1.01	5.05	5.51	5.17	12.06	15.23	12.58	4.22	4.76	4.43	5.18	5.74	5.35	0.48	0.49	0.62	4.25	5.00	4.25
6.43	6.71	6.78	1.18	1.22	1.19	6.88	7.08	7.09	21.95	22.33	23.39	5.15	5.12	5.31	6.11	6.08	6.28	0.48	0.48	0.57	6.56	6.63	6.82
7.18	8.45	8.41	0.88	0.84	0.84	4.96	4.91	5.00	15.36	15.84	16.01	5.20	5.45	5.57	5.89	6.12	6.25	0.35	0.34	0.52	4.43	4.43	4.41
8.13	8.46	8.46	1.12	1.11	1.11	6.25	6.39	6.39	17.83	19.27	19.27	4.86	5.03	5.03	5.83	6.03	6.03	0.48	0.50	0.53	5.52	5.77	5.77
5.13	6.00	6.13	1.47	1.36	1.34	7.75	7.43	7.31	20.11	20.40	19.61	4.27	4.57	4.60	5.55	5.74	5.74	0.64	0.58	0.48	6.69	6.62	6.37
6.20	6.81	6.60	1.17	1.15	1.07	6.65	7.14	6.57	20.23	25.66	23.15	4.97	5.40	5.45	5.97	6.49	6.44	0.50	0.54	0.49	6.31	7.56	6.68
7.85	12.28	13.09	0.91	0.89	0.89	4.42	4.35	4.43	10.31	9.96	10.03	4.35	4.41	3.89	5.13	5.15	5.23	0.39	0.37	0.52	3.73	3.61	3.57
10.31	9.10	12.02	1.00	0.98	1.05	4.96	4.91	5.31	11.07	10.46	11.97	4.36	4.44	4.48	5.20	5.24	5.32	0.42	0.40	0.57	3.87	3.76	4.09

IT_BR _1	IT_BR 2	IT_BR _3	FS_B MD_1	FS_B MD_2	FS_BM D_3	FS_CS A_1	FS_CSA _2	FS_CSA _3	FS_CS MI_1	FS_CS MI_2	FS_CS MI_3	FS_E D_1	FS_ED _2	FS_ED _3	FS WIDT H_1	FS WIDT H_2	FS WIDT H_3	FS ACT_1	FS ACT_2	FS ACT_3	FS_S ECT_1 MOD	FS_S ECT_2 MOD	FS_SE CT_M OD_3
4.99	5.65	5.64	1.73	1.67	1.67	4.90	4.98	4.78	3.79	4.16	3.65	1.62	1.86	1.72	2.98	3.13	3.01	0.68	0.64	0.82	2.51	2.61	2.35
8.22	8.15	8.75	1.25	1.26	1.22	3.03	3.13	3.15	1.80	1.94	2.08	1.63	1.69	1.81	2.55	2.62	2.70	0.46	0.46	0.62	1.34	1.41	1.45
4.80	4.81	5.01	1.75	1.78	1.79	4.94	4.96	5.10	3.52	3.48	3.74	1.59	1.50	1.55	2.91	2.97	2.99	0.73	0.70	0.75	2.34	2.33	2.44
5.44	6.42	5.91	1.68	1.62	1.70	4.74	4.76	4.87	4.01	4.13	4.14	1.66	1.85	1.70	2.96	3.08	3.01	0.65	0.62	0.56	2.58	2.59	2.63
8.08	9.17	9.05	1.50	1.39	1.46	4.77	4.82	4.91	5.03	5.81	5.23	2.26	2.68	2.48	3.35	3.65	3.53	0.54	0.48	0.56	2.88	2.89	2.76
7.77	7.68	7.77	1.44	1.48	1.44	4.24	4.41	4.24	3.71	4.10	3.71	2.05	2.05	2.05	3.10	3.13	3.10	0.53	0.54	0.65	2.37	2.53	2.37
7.42	7.38	7.82	1.66	1.60	1.64	4.73	4.67	4.81	4.04	3.97	4.07	1.72	1.85	1.84	3.00	3.06	3.09	0.64	0.61	0.77	2.65	2.52	2.58
4.97	5.20	5.73	1.60	1.58	1.66	4.41	4.43	4.81	3.34	3.35	3.97	1.67	1.74	1.76	2.90	2.94	3.04	0.61	0.60	0.72	2.25	2.19	2.55
6.85	7.00	8.08	1.47	1.42	1.40	4.26	4.27	4.30	3.52	3.70	3.86	1.97	2.14	2.22	3.05	3.16	3.23	0.54	0.51	0.70	2.29	2.31	2.32
5.29	4.51	6.49	1.64	1.22	1.62	4.24	3.29	4.58	2.78	2.14	3.52	1.41	1.30	1.72	2.72	2.89	2.97	0.65	0.63	0.53	2.03	1.51	2.31
5.87	5.72	5.87	1.54	1.57	1.54	4.42	4.38	4.42	3.67	3.30	3.67	1.85	1.74	1.85	3.01	2.94	3.01	0.58	0.60	0.60	2.34	2.21	2.34
5.11	5.74	5.03	1.42	1.45	1.58	3.77	4.07	4.47	2.63	3.10	3.60	1.74	1.88	1.75	2.80	2.95	2.96	0.53	0.54	0.61	1.78	1.98	2.28
7.41	7.47	8.50	1.80	1.63	1.68	5.69	5.47	5.66	5.86	6.43	6.64	1.95	2.34	2.31	3.32	3.53	3.54	0.69	0.59	0.56	3.50	3.56	3.72
4.94	5.37	5.52	1.53	1.58	1.60	3.91	4.16	4.30	2.42	2.77	2.84	1.50	1.54	1.60	2.69	2.77	2.83	0.59	0.61	0.79	1.79	1.97	1.99
7.11	7.33	8.04	1.50	1.50	1.50	4.21	4.41	4.38	3.67	4.09	4.06	1.82	1.97	1.97	2.95	3.08	3.08	0.56	0.56	0.50	2.44	2.57	2.57
5.88	6.22	6.47	1.55	1.51	1.58	3.80	3.93	4.14	2.36	2.66	2.81	1.33	1.59	1.51	2.57	2.74	2.75	0.62	0.58	0.74	1.83	1.93	2.03
7.01	7.03	7.07	1.74	1.72	1.60	5.78	5.85	5.40	6.59	7.06	6.11	2.18	2.31	2.36	3.48	3.58	3.53	0.65	0.63	0.73	3.77	3.90	3.43
10.01	10.63	10.70	1.20	1.20	1.23	3.88	3.92	4.05	4.13	4.15	4.41	2.56	2.61	2.62	3.39	3.44	3.47	0.42	0.41	0.66	2.36	2.33	2.45
6.69	6.68	6.68	1.46	1.53	1.53	4.36	4.52	4.52	3.96	3.67	3.67	2.07	1.98	1.98	3.14	3.11	3.11	0.53	0.57	0.64	2.42	2.29	2.29
4.71	5.27	5.37	1.85	1.87	1.92	5.40	5.62	5.76	4.48	4.87	4.91	1.57	1.67	1.62	3.06	3.15	3.16	0.74	0.74	0.63	2.91	3.03	3.08
6.43	6.26	7.02	1.62	1.69	1.72	4.99	5.16	5.27	5.03	4.75	5.04	2.04	1.91	1.90	3.24	3.20	3.21	0.60	0.64	0.63	3.08	2.96	3.12
7.06	7.39	4.20	1.43	1.46		3.72	3.90		2.44	2.54	1.35	1.64	1.70	1.12	2.73	2.80	1.69	0.54	0.55	0.58	1.78	1.80	0.95
6.88	6.98	6.95	1.47	1.52	1.51	4.03	4.29	4.39	2.85	3.16	3.61	1.78	1.83	1.93	2.88	2.97	3.05	0.55	0.57	0.64	1.90	2.04	2.24

FS_B	FS_BR	FS_B
R_1	_2	R_3
2.23	2.51	2.42
2.91	2.97	3.24
2.18	2.09	2.15
2.38	2.60	2.40
3.22	4.15	3.65
2.97	2.99	2.97
2.39	2.60	2.53
2.42	2.53	2.44
2.85	3.13	3.31
2.09	2.03	2.45
2.70	2.50	2.70
2.78	2.92	2.61
2.44	3.04	2.90
2.27	2.28	2.31
2.67	2.86	2.86
2.07	2.39	2.24
2.69	2.87	3.12
4.21	4.33	4.25
3.07	2.83	2.83
2.07	2.16	2.08
2.71	2.50	2.46
2.54	2.56	1.96
2.72	2.73	2.87

Appendix 15 - Baseline and 4 months data of the adolescents with obesity in the control group

WBBMAD_2	WBBMD	WBBMD_2	LS BMAD	LS BMAD_2	LS TBS	LS TBS_2	L1L4BMC	L1L4BMC_2	L1L4BMD	L1L4BMD_2	NECK_BMC
0.098	1.151	1.206	0.785	0.810	1.500	1.490	68.546	67.583	1.105	1.074	4.776
0.110	1.106	1.156	1.047	1.061	1.489	1.497	58.998	60.742	1.047	1.061	5.216
0.092	0.987	1.036	0.776	0.794	1.256	1.154	34.942	34.921	0.776	0.794	4.856
0.085	0.991	1.024	0.762	0.750	1.134	1.107	38.628	36.073	0.762	0.750	4.672
0.078	1.054	1.053	0.853	0.880	1.057	1.064	50.578	53.465	0.853	0.880	5.122
0.089	1.068	1.094	1.066	1.096	1.255	1.193	60.108	60.106	1.066	1.096	4.570
0.094	1.131	1.150	1.082	1.148	1.369	1.356	59.147	59.355	1.082	1.148	4.755
0.099	1.185	1.224	1.101	1.128	1.365	1.343	64.117	67.085	1.101	1.128	6.531
0.092	1.098	1.130	1.007	0.991	1.476	1.384	58.888	58.070	1.007	0.991	4.908
0.087	0.945	0.970	0.810	0.864	1.181	1.231	37.355	40.431	0.810	0.864	3.832
0.087	1.011	1.023	0.748	0.817	1.025	1.051	32.258	41.204	0.748	0.817	4.421

NECK_BMC_2	NECK_BMD	NECK_BMD_2	HTOT_BMC	HTOT_BMC_2	HTOT_BMD	HTOT_BMD_2	AXIS_LENGTH_2	AXIS_LENGTH_ shaft neck angle	shaft neck angle_2	
4.626	1.037	1.000	32.990	32.288	1.083	1.047	104.468	104.468	136.698	137.257
4.641	0.960	1.006	31.328	28.983	0.997	0.953	106.037	102.450	131.055	128.006
5.044	0.975	0.983	29.880	30.530	0.976	0.969	100.762	103.050	130.662	135.047
4.719	0.968	0.931	35.645	34.904	0.962	0.939	110.206	116.459	140.278	143.746
5.617	0.888	0.881	39.383	41.790	1.000	1.040	117.879	117.196	135.000	133.452
4.366	0.951	0.961	28.077	30.762	0.970	0.996	100.836	103.875	137.726	146.657
5.080	1.037	1.064	33.938	34.336	1.042	1.041	105.777	107.306	128.089	137.672
5.752	1.260	1.112	36.850	37.369	1.387	1.181	100.372	101.251	131.668	137.840
5.069	0.957	0.998	33.851	34.924	1.052	1.083	109.535	108.788	140.184	127.610
4.142	0.849	0.880	26.381	28.001	0.939	0.947	90.671	94.337	133.690	136.841
4.685	0.881	0.877	32.091	33.021	1.008	1.005	106.814	104.213	130.601	136.985

NN_BMD	NN_BMD_2	NN_CSA	NN_CSA_2	NN_CSMI	NN_CSMI_2	NN_ED	NN_ED_2	NN_SECT_MO		NN_BR	NN_BR_2
								D	D_2		
1.323	1.195	3.641	3.448	2.350	2.335	2.360	2.559	1.612	1.493	5.504	6.644
1.196	1.038	3.497	3.519	2.563	2.538	2.599	3.239	1.603	1.239	6.784	11.520
1.217	1.242	3.543	3.653	2.494	2.577	2.577	2.599	1.550	1.578	6.699	6.656
1.215	1.122	3.610	3.443	2.652	2.505	2.642	2.785	1.622	1.495	6.830	7.648
1.061	1.079	3.697	3.842	3.633	4.308	3.253	3.328	1.936	2.232	9.198	9.349
1.163	1.139	3.231	3.253	2.053	1.975	2.458	2.552	1.375	1.273	6.512	6.944
1.304	1.337	3.613	3.621	2.477	2.381	2.389	2.309	1.687	1.661	5.640	5.350
1.533	1.408	5.620	4.037	3.620	3.010	3.247	2.445	1.410	1.985	5.440	5.363
1.274	1.158	3.776	3.483	3.016	2.718	2.615	2.705	1.893	1.658	6.353	7.235
1.046	1.099	2.794	3.037	1.737	1.969	2.394	2.470	1.194	1.319	7.090	6.916
1.071	1.006	3.275	3.238	2.721	2.788	2.795	2.995	1.581	1.544	8.262	9.316

IT_BMD	IT_BMD_2	IT_CSA	IT_CSA_2	IT_CSMI	IT_CSMI_2	IT_ED	IT_ED_2	IT_SECT_MOD		IT_BR	IT_BR_2	FS_BMD
								IT_SECT_MOD_2				
1.122	1.052	5.831	5.449	14.571	12.730	4.505	4.569	4.743	4.133	6.446	7.073	1.555
1.115	0.997	5.636	5.136	13.481	12.485	4.303	4.660	4.670	4.182	5.734	7.985	1.467
1.143	1.097	5.767	6.007	14.319	17.047	4.321	4.775	4.785	5.285	6.114	6.638	1.397
1.055	0.961	5.531	5.269	13.205	13.302	4.619	4.955	4.240	4.073	7.044	8.163	1.330
1.056	1.130	6.274	6.857	21.496	25.198	5.357	5.465	6.376	7.119	7.683	7.793	1.522
1.017	1.028	5.208	5.449	12.392	12.967	4.503	4.726	4.203	4.202	6.743	7.377	1.439
1.102	1.069	5.969	5.845	17.012	17.424	4.723	4.816	5.599	5.639	6.297	6.692	1.483
1.140	1.275	6.766	6.672	17.955	15.807	5.104	4.371	5.923	5.163	5.365	5.436	1.708
1.231	1.172	6.408	5.963	15.467	14.072	4.372	4.353	5.016	4.866	5.639	5.847	1.608
1.014	0.997	4.899	4.990	10.599	12.209	4.178	4.326	3.971	4.456	5.955	5.900	1.524
1.109	1.108	5.867	6.213	14.447	16.840	4.565	4.913	4.794	5.124	6.086	6.733	1.548

FS_BMD_2	FS_CSA	FS_CSA_2	FS_CSMI	FS_CSMI_2	FS_ED	FS_ED_2	FS_SECT_MO D	FS_SECT_MO D_2	FS_BR	FS_BR_2
1.633	4.423	4.713	3.971	4.125	1.814	1.785	2.485	2.600	2.723	2.546
1.364	3.853	4.271	2.773	4.039	1.643	2.317	1.926	2.443	2.583	3.404
1.414	3.847	4.055	2.892	3.450	1.861	1.977	1.854	2.150	3.025	3.102
1.351	3.838	4.056	3.375	3.764	2.073	2.186	2.145	2.308	3.288	3.373
1.567	4.964	5.408	5.517	6.512	2.326	2.498	3.136	3.394	3.201	3.411
1.391	3.961	4.155	3.104	3.551	1.818	2.132	2.025	2.121	2.858	3.333
1.531	4.255	4.473	3.545	3.925	1.911	1.926	2.271	2.433	2.836	2.829
1.674	4.779	4.689	3.862	3.747	1.596	1.638	2.539	2.475	2.267	2.325
1.540	4.444	4.341	3.594	3.567	1.661	1.799	2.329	2.324	2.488	2.643
1.511	3.877	3.780	2.557	2.389	1.483	1.443	1.872	1.796	2.299	2.251
1.488	4.270	4.377	3.211	3.554	1.720	1.992	2.040	2.165	2.673	2.994

Appendix 16 - Excel file calculation of baseline bone turnover normalised to NW baseline median

Based on Bieglmayer et al. 2009

										equation (1)							
										MoM Formation et Resorption							
										MoM = marker/median(marker)							
n=	5		n=	22		n=	11										
TO	P1NP	CTX		P1NP	CTX		P1NP	CTX	MoM_F	Mom_R		MoM_F					
NW cntrl	BASO	168	10695	TN 8mths	ABAU	36.60	5365.25	Ob cntrl	ALAL	38.51	4173.02	NW cntrl	1.400	1.518	TN 8mths	0.886	
Clairfontaine	BUAN	91	5254	WL	BEHE	41.18	6763.58		ALAU	40.84	5424.62	Clairfontaine	0.758	0.746	WL	0.997	
	CHSO	184	8602		BOAU	40.01	5191.54		BAHA	41.77	5607.21		1.533	1.221		0.969	
	DESI	135	7177		DAAL	41.43	3002.58		BELO	37.68	6303.24		1.125	1.019		1.003	
	DJME	113	8060		DEMA	39.34	4823.51		CHBE	42.94	7057.89		0.942	1.144		0.952	
	GAKE	111	5828		FLCH	37.43	4784.48		COCO	37.68	5927.20		0.925	0.827		0.906	
	HUYS	120	10152		GUMA	41.85	3999.94		COMA	45.21	4401.53		1.000	1.441		1.013	
	JAIN	123	6626		MASA	44.54	3158.94		FLSO	42.27	3134.90		1.025	0.941		1.078	
	KACH	193	11005		PEMA	44.54	3916.49		GRCA	37.43	4378.04		1.608	1.562		1.078	
	MAMA	93	5626		SCAM	42.44	4063.86		MEEM	44.96	7938.47		0.775	0.799		1.027	
	MEDY	26	6502						POEN	46.48	6356.57		0.217	0.923		0.000	
	MESA	75	5247										0.625	0.745		0.000	
	MEEL	80	7045										0.667	1.000			
	PRKE	203	8060										1.692	1.144			
	POCH	103	6285										0.858	0.892			
	RULE	155	6673										1.292	0.947			
	THCI	171	7479										1.425	1.062			
	median:	120	7045		median:	41.30	4424.17		median:	41.77	5607.21		mean	1.051	1.130	mean	0.826
	mean	126.117647	7430.35294		mean	40.94	4507.02		mean	41.43	5518.43						
	SD	47.76	1801.82		sd	2.66	1123.35		sd	3.29	1405.99						
					zscore TN	-1.78	-1.62		zscore Ob	-1.77	-1.06						
	UI	=			UI TN	-0.16			UI Ob	-0.71							
	mean 4 subject	122.75	8629.5			40.86	3842.26			40.81	6098.24						
	SD 4 subject	35.98	2090.37			4.00	1321.27			2.26	743.38						
					zscore TN 4suj	-2.28	-2.29		zscore Ob 4suj	-2.28	-1.21						
					avec moyenn	-1.79	-1.99			-1.78634828	-0.7393152						
					UI TN 4	0.01			UI Ob 4	-1.07							
					avec moyenn	0.21				-1.04703307							
	1 subject	135	7177		1 subject	37.43	4784.48		1 subject	37.68	5927.20						
	zscore 1 NW	0.19	-0.14		zscore 1 Ob	-1.86	-1.47		zscore 1 Ob c1	-1.85	-0.83						
	UI 1 NW	0.33			UI 1 Ob	-0.39			UI 1 Ob cntrl	-1.02							

Balance form: bone turnover
Balance = Mo Turnover = $\frac{racine(MoM)^2}{4}$

Mom_R	MoM_F	Mom_R	MoM_F_norr	Mom_R_normalized to NW	MoM_F_norma	Mom_R_normalized to NW	MoM_F_norr	Mom_R_normalized to NW	Balance	Turnover rate		
1.213 Ob cntrl	0.922	0.744 NW	1.400	1.518 TN	0.305	0.762 Ob cntrl	0.321	0.592	NW cntrl	0.922	2.065 TN 8mths	
1.529	0.978	0.967	0.758	0.746 WL	0.343	0.960	0.340	0.770	Clairfontaine	1.017	1.064 WL	
1.173	1.000	1.000	1.533	1.221	0.333	0.737	0.348	0.796		1.256	1.960	
0.679	0.902	1.124	1.125	1.019	0.345	0.426	0.314	0.895		1.104	1.518	
1.090	1.028	1.259	0.942	1.144	0.328	0.685	0.358	1.002		0.823	1.482	
1.081	0.902	1.057	0.925	0.827	0.312	0.679	0.314	0.841		1.118	1.241	
0.904	1.083	0.785	1.000	1.441	0.349	0.568	0.377	0.625		0.694	1.754	
0.714	1.012	0.559	1.025	0.941	0.371	0.448	0.352	0.445		1.090	1.391	
0.885	0.896	0.781	1.608	1.562	0.371	0.556	0.312	0.621		1.030	2.242	
0.919	1.077	1.416	0.775	0.799	0.354	0.577	0.375	1.127		0.970	1.113	
0.000	1.113	1.134	0.217	0.923			0.387	0.902		0.235	0.948	
0.000			0.625	0.745						0.839	0.972	
			0.667	1.000						0.667	1.202	
			1.692	1.144						1.479	2.042	
			0.858	0.892						0.962	1.238	
			1.292	0.947						1.364	1.602	
			1.425	1.062						1.342	1.777	
0.849	mean	0.992	0.984		0.341	0.640	0.345	0.783	mean	0.995	1.506	mean

equation (4)

Cartesian coordinates

$$x = \log(\text{balance}) - \log(\text{rate}) - \text{mean}[\log(\text{rate})] / \#$$

	Log_balance	Log_rate		Log_balance	Log_rate	normalized to NV	Log_balance	normalized to NW	mediang_rate	Log_x	Log_y		Log_x	Log_y		Log_x
Ob cntrl	0.093	0.074	TN 8mths	-0.397	-0.086	Ob cntrl	-0.266	-0.172					-0.135	0.022	Ob cntrl	0.077
	0.005	0.138	WL	-0.447	0.008		-0.355	-0.075		NW cntrl	0.007	0.027	WL	0.107		-0.185
	0.000	0.151		-0.344	-0.092		-0.359	-0.061		Clairfontaine	0.099	0.292		0.028		-0.082
	-0.096	0.159		-0.091	-0.261		-0.455	-0.023			0.043	0.181		-0.071		-0.112
	-0.088	0.211		-0.320	-0.120		-0.447	0.027			-0.085	0.171		0.006		-0.058
	-0.069	0.143		-0.338	-0.126		-0.428	-0.047			0.049	0.094		-0.005		-0.076
	0.140	0.126		-0.212	-0.176		-0.220	-0.137			-0.159	0.244		-0.021		0.050
	0.258	0.063		-0.082	-0.235		-0.102	-0.246			0.037	0.143		0.242		0.180
	0.060	0.075		-0.175	-0.175		-0.299	-0.158			0.013	0.351		0.044		0.087
	-0.119	0.250		-0.213	-0.170		-0.478	0.075			-0.013	0.046		-0.135		0.049
	-0.008	0.201					-0.367	-0.008			-0.629	-0.023		-0.024		
											-0.076	-0.012				
											-0.176	0.080				
											0.170	0.310				
											-0.017	0.093				
											0.135	0.205				
											0.128	0.250				

mean 0.016 0.145 -0.262 -0.143 -0.343 -0.075 mean -0.028 0.153 mean 0.000 0.000 mean 0.000

Log_y
 -0.071
 -0.006
 0.006
 0.014
 0.066
 -0.002
 -0.018
 -0.002
 -0.070
 0.105
 0.056

0.000

Appendix 17 - Excel file calculation of the 4 months bone turnover normalised to NW baseline median

Based on Bieglmayer et al. 2009

equation (1)
MoM Formation et Resorption
MoM = marker/median(marker)

n= 5			n= 22			n= 11			equation (1)		
T1	P1NP	CTX	TN 8mths	P1NP	CTX	Ob cntrl	P1NP	CTX	MoM_F	Mom_R	TN 8mths
NW cntrl	BASO	163	10230	ABAU	35.77	3482.65	ALAL	43.95	5894.29	1.199	1.512
Clairfontaine	BUAN	101	4983	BEHE	37.93	5560.92	ALAU	41.68	5439.57	0.743	0.736
	CHSO	169	8602	BOAU	36.52	1306.49	BAHA	39.42	6303.24	1.243	1.271
	DESI	176	7502	DAAL	38.76	3402.66	BELO	38.51	6744.45	1.294	1.109
	DJME	142	5750	DEMA	39.34	3564.81	CHBE	39.93	7961.44	1.044	0.850
	GAKE	94	5456	FLCH	38.34	3473.66	COCO	36.93	7472.94	0.691	0.806
	HUYS	108	8292	GUMA	45.38	2118.24	COMA	31.82	5454.57	0.794	1.226
	JAIN	114	6502	MASA	48.52	4010.51	FLSO	34.78	4983.29	0.838	0.961
	KACH	233	12245	PEMA	36.76	3111.07	RCA	44.54	4694.80	1.713	1.810
	MAMA	92	3030	SCAM	51.25	3735.91	MEEM	39.68	8007.59	0.676	0.448
	MEDY	136	6479				POEN	39.68	7515.93	1.000	0.958
	MESA	96	4681						0.706	0.692	
	MEEL	94	7518						0.691	1.111	
	PRKE	193	7370						1.419	1.089	
	POCH	97	4906						0.713	0.725	
	RULE	137	7246						1.007	1.071	
	THCI	148	6766						1.088	1.000	
	median:	136	6766	median:	38.55	3478.16	median:	39.68	6303.24	mean	0.992
	mean	134.88	2353	mean	40.86	3376.69	mean	39.17	6406.56	1.022	mean
	SD	41.32	2192.12	sd	5.48	1120.43	sd	3.71	1205.54		
	zscore NW	0.18	-0.29	zscore TN	-1.79	-2.25	zscore Ob	-1.82	-0.57		
	UI NW	0.47		UI TN	0.46		UI Ob	-1.25			
	mean 4 subj	119.75	8135.5		41.02	3631.94		39.89	6612.17		
	sd 4 subj	30.026	1577.028		6.667	330.283		1.33	1050.15		
	zscore 4 subj	-0.08	-0.24		-2.27	-2.39		-2.30	-0.97		
	UI 4 subj	0.15			-1.78	-2.11		-1.34			
					0.12						
					0.33						
	1 subject	176	7502	1 subject	38.34	45.38	1 subject	36.93	7472.94		
	zscore 1 NW	1.04	0.04	zscore 1 Ob	-1.84	-4.10	zscore 1 Ob cr	-1.87	0.02		
	UI 1 NW	1.00		UI 1Ob	2.26		UI 1 ob cntrl	-1.89			

Balance form: bone turnover
 Balance = Mo Turnover = ra

MoM_F	Mom_R		MoM_F	Mom_R	MoM_F_norm	Mom_R_normalized to NW	MoM_F_norm	Mom_R_normalized to NW	MoM_F_norm	Mom_R_normalized to NW	MoM_F_norm	Mom_R_normalized to NW	Balance	Turnover rate		
0.928	1.001	Ob cntrl	1.108	0.935	NW cntrl	1.358	1.452	TN 8mths	0.298	0.494	Ob cntrl	0.366	0.837	NW cntrl	0.793	1.929
0.984	1.599		1.051	0.863	Clairfontaine	0.842	0.707	WL	0.316	0.789		0.347	0.772	Clairfontaine	1.008	1.046
0.947	0.376		0.994	1.000		1.408	1.221		0.304	0.185		0.329	0.895		0.977	1.778
1.005	0.978		0.971	1.070		1.467	1.065		0.323	0.483		0.321	0.957		1.167	1.704
1.021	1.025		1.006	1.263		1.183	0.816		0.328	0.506		0.333	1.130		1.229	1.346
0.995	0.999		0.931	1.186		0.783	0.774		0.320	0.493		0.308	1.061		0.857	1.062
1.177	0.609		0.802	0.865		0.900	1.177		0.378	0.301		0.265	0.774		0.648	1.460
1.259	1.153		0.877	0.791		0.950	0.923		0.404	0.569		0.290	0.707		0.872	1.275
0.954	0.894		1.123	0.745		1.942	1.738		0.306	0.442		0.371	0.666		0.947	2.492
1.329	1.074		1.000	1.270		0.767	0.430		0.427	0.530		0.331	1.137		1.511	0.811
			1.000	1.192		1.133	0.920					0.331	1.067		1.044	1.385
						0.800	0.664								1.020	0.988
						0.783	1.067								0.622	1.309
						1.608	1.046								1.303	1.789
						0.808	0.696								0.984	1.017
						1.142	1.029								0.941	1.470
						1.233	0.960								1.088	1.478
1.060	0.971	mean	0.987	1.016										mean	1.001	1.432

equation (2) equation (3)
Log formation

r
cine(MoM_p² + MoM_r²)

a	Balance	Turnover rate	Balance	Turnover rate	Balance	Turnover rate	Balance	Turnover rate	Balance	Turnover rate	Balance	Turnover rate	Balance	Turnover rate	Log_balance	
TN 8mths	0.927	1.365	Ob cntrl	1.185	1.450	NW cntrl	0.935	1.988	TN 8mths	0.603	0.577	Ob cntrl	0.438	0.913	NW cntrl	-0.101
WL	0.615	1.877		1.217	1.360	Clairfontaine	1.190	1.099	WL	0.400	0.850		0.450	0.847	Clairfontaine	0.004
	2.522	1.019		0.994	1.410		1.153	1.864		1.641	0.356		0.367	0.953		-0.010
	1.028	1.403		0.907	1.445		1.377	1.812		0.669	0.581		0.335	1.010		0.067
	0.996	1.446		0.797	1.615		1.450	1.438		0.648	0.603		0.294	1.178		0.089
	0.996	1.409		0.785	1.507		1.011	1.102		0.648	0.588		0.290	1.104		-0.067
	1.933	1.325		0.927	1.180		0.765	1.482		1.258	0.483		0.342	0.818		-0.188
	1.092	1.707		1.109	1.180		1.029	1.324		0.710	0.698		0.410	0.764		-0.059
	1.066	1.307		1.507	1.347		1.117	2.606		0.694	0.537		0.557	0.763		-0.024
	1.238	1.709		0.787	1.617		1.783	0.879		0.805	0.681		0.291	1.184		0.179
				0.839	1.556		1.232	1.460					0.310	1.117		0.019
							1.204	1.040								0.009
							0.734	1.324								-0.206
							1.537	1.919								0.115
							1.161	1.067								-0.007
							1.110	1.537								-0.027
							1.284	1.563								0.037
mean	1.241	1.457	mean	1.005	1.424										mean	-0.010

equation (4)

Cartesian coordinates

$$x = \log(\text{balanc}_y - \text{mean}[\log(\text{rate})]) / \# \#$$

Log_x	Log_y		Log_x	Log_y		Log_x	Log_y
-0.091	0.146	TN 8mths	-0.094	-0.023	Ob cntrl	0.081	0.010
0.014	-0.120	WL	-0.272	0.116		0.093	-0.018
0.000	0.111		0.341	-0.150		0.005	-0.002
0.077	0.092		-0.049	-0.011		-0.035	0.008
0.099	-0.010		-0.063	0.003		-0.091	0.057
-0.057	-0.113		-0.063	-0.009		-0.098	0.027
-0.178	0.025		0.225	-0.035		-0.026	-0.079
-0.049	-0.034		-0.023	0.075		0.052	-0.079
-0.014	0.257		-0.033	-0.041		0.185	-0.022
0.189	-0.230		0.032	0.075		-0.097	0.057
0.029	0.002					-0.069	0.041
0.019	-0.144						
-0.196	-0.022						
0.125	0.113						
0.003	-0.132						
-0.017	0.028						
0.047	0.030						
0.000	0.000	mean	0.000	0.000	mean	0.000	0.000

Appendix 18 - Excel file calculation of the 8 months bone turnover normalised to NW baseline median

Based on Bieglmayer et al. 2009

n=

equation (1)

MoM Formation et Resorption

MoM = $\text{marker}_i / \text{median}(\text{marker})$

		P1NP	CTX		MoM_F	Mom_R		MoM_F_norn
TN 8mths	ABAU	36.35	4240.12	TN 8mths	0.882	0.976	TN 8mths	0.303
WL	BEHE	35.77	5910.72	WL	0.868	1.360	WL	0.298
	BOAU	49.03	5394.84		1.189	1.242		0.409
	DAAL	41.51	3957.96		1.007	0.911		0.346
	DEMA	39.76	3555.58		0.965	0.818		0.331
	FLCH	37.68	3835.02		0.914	0.883		0.314
	GUMA	40.93	4150.92		0.993	0.955		0.341
	MASA	43.78	4448.92		1.062	1.024		0.365
	PEMA	43.19	4902.66		1.048	1.128		0.360
	SCAM	50.99	5191.54		1.237	1.195		0.425
	median:	41.22	4344.52	mean	1.016	1.049		
	mean	41.90	4558.83					
	sd	5.06	761.33					
	zscore TN	-1.76	-1.59					
	UI TN	-0.17						
	1 subject	37.68	3835.02					
	zscore 1 Ob	-1.85	-2.00					
	UI 1Ob	0.14						

equation (2)

Balance form: bone turnover
 $\text{Balance} = \text{Mo} - \text{Turnover} = \text{racine}(\text{MoM}^2 + \text{MoM}^2)$

Mom_R_normalized to NW median		Balance	Turnover rate		Balance	Turnover rate_normalized to NW median	
0.602	TN 8mths	0.904	1.315	TN 8mths	0.503	0.674	TN 8mths
0.839	WL	0.638	1.614	WL	0.355	0.890	WL
0.766		0.958	1.720		0.534	0.868	
0.562		1.105	1.358		0.616	0.660	
0.505		1.179	1.265		0.656	0.604	
0.544		1.035	1.271		0.577	0.628	
0.589		1.039	1.378		0.579	0.681	
0.632		1.037	1.475		0.578	0.729	
0.696		0.929	1.540		0.517	0.783	
0.737		1.035	1.720		0.577	0.851	
	mean	0.986	1.466				mean

equation (3)
Log formation et resorption

equation (4)
Cartesian coordinates
 $x = \log(\text{balance}) - \text{mean}[\log(\text{rate})]$
 $y = \log(\text{rate}) - \text{mean}[\log(\text{rate})]$

Log_balance	Log_rate		Log_balance_	Log_rate_normalized to NW median		Log_x	Log_y
-0.044	0.119	TN 8mths	-0.298	-0.171	TN 8mths	-0.033	-0.044
-0.195	0.208	WL	-0.449	-0.050	WL	-0.184	0.045
-0.019	0.235		-0.273	-0.062		-0.007	0.072
0.044	0.133		-0.211	-0.181		0.055	-0.030
0.071	0.102		-0.183	-0.219		0.083	-0.061
0.015	0.104		-0.239	-0.202		0.026	-0.059
0.017	0.139		-0.237	-0.167		0.028	-0.024
0.016	0.169		-0.238	-0.137		0.027	0.006
-0.032	0.188		-0.286	-0.106		-0.021	0.024
0.015	0.236		-0.239	-0.070		0.026	0.072
-0.011	0.163		-0.265	-0.137	mean	0.000	0.000

Appendix 19 - Excel file calculation of baseline Ob bone turnover normalised to Ob baseline median

Based on Bieglmayer et al. 2009							equation (1) MoM Formation et Resorption MoM = marker/median(marker)	Balance form: bone turnover Balance = Mo Turnover = ra			
n=	10										
	P1NP	CTX	zf	zr	ui		MoM_F	Mom_R	Balance		
TN 8mths	ABAU	36.60	5365.25	-1.63	0.76	-2.39	TN 8mths	0.886	1.213	TN 8mths	0.731
WL	BEHE	41.18	6763.58	0.09	2.01	-1.92	WL	0.997	1.529	WL	0.652
	BOAU	40.01	5191.54	-0.35	0.61	-0.96		0.969	1.173		0.825
	DAAL	41.43	3002.58	0.19	-1.34	1.53		1.003	0.679		1.478
	DEMA	39.34	4823.51	-0.60	0.28	-0.88		0.952	1.090		0.874
	FLCH	37.43	4784.48	-1.32	0.25	-1.56		0.906	1.081		0.838
	GUMA	41.85	3999.94	0.34	-0.45	0.79		1.013	0.904		1.121
	MASA	44.54	3158.94	1.35	-1.20	2.55		1.078	0.714		1.510
	PEMA	44.54	3916.49	1.35	-0.53	1.88		1.078	0.885		1.218
	SCAM	42.44	4063.86	0.56	-0.39	0.96		1.027	0.919		1.118
	median:	41.30	4424.17				mean	0.991	1.019	mean	1.037
	mean	40.94	4507.02								
	sd	2.66	1123.35								
	zscore TN	0.00	0.00	0.00	0.00	0.00					
	UI TN	0.00									
	1 subject	37.43	4784.48								
	zscore 1 Ob	-1.32	0.25								
	UI 1 Ob	-1.56									

r cine(MoM σ^2 + MoM κ^2)	equation (2)	equation (3)	equation (4)			
		Log formation et resorption	Cartesian coordinates $x = \log(\text{balance}) - \text{mean}[\log(\text{rate})]$			
Turnover rate		Log_balance	Log_rate	Log_x	Log_y	
1.502	TN 8mths	-0.136	0.177	TN 8mths	-0.135	0.022
1.825	WL	-0.186	0.261	WL	-0.185	0.107
1.522		-0.083	0.182		-0.082	0.028
1.211		0.170	0.083		0.170	-0.071
1.448		-0.059	0.161		-0.058	0.006
1.411		-0.077	0.149		-0.076	-0.005
1.358		0.049	0.133		0.050	-0.021
1.293		0.179	0.112		0.180	-0.043
1.395		0.086	0.145		0.087	-0.010
1.378		0.049	0.139		0.049	-0.015
1.434	mean	-0.001	0.154	mean	0.000	0.000

Appendix 20 - Excel file calculation of 4 months Ob bone turnover normalised to Ob baseline median

Based on Bieglmayer et al. 2009

equation (1)

MoM Formation et Resorption

MoM = marker_i/median(marker)

n=		10					equation (1)				
		P1NP	CTX	zf	zr	ui	MoM_F	Mom_R	MoM_F_norn		
TN 8mths	ABAU	35.77	3482.65	-1.94	-0.91	-1.03	TN 8mths	0.928	1.001	TN 8mths	0.866
WL	BEHE	37.93	5560.92	-1.13	0.94	-2.07	WL	0.984	1.599	WL	0.918
	BOAU	36.52	1306.49	-1.66	-2.85	1.19		0.947	0.376		0.884
	DAAL	38.76	3402.66	-0.82	-0.98	0.17		1.005	0.978		0.938
	DEMA	39.34	3564.81	-0.60	-0.84	0.24		1.021	1.025		0.952
	FLCH	38.34	3473.66	-0.97	-0.92	-0.05		0.995	0.999		0.928
	GUMA	45.38	2118.24	1.67	-2.13	3.80		1.177	0.609		1.099
	MASA	48.52	4010.51	2.85	-0.44	3.29		1.259	1.153		1.175
	PEMA	36.76	3111.07	-1.57	-1.24	-0.32		0.954	0.894		0.890
	SCAM	51.25	3735.91	3.87	-0.69	4.56		1.329	1.074		1.241
	median:	38.55	3478.16				mean	1.060	0.971		0.989
	mean	40.86	3376.69								
	sd	5.48	1120.43								
	zscore Ob	-0.03	-1.01	-0.03	-1.01	0.98					
	UI Ob	0.98									
	1 subject	38.34	3473.66								
	zscore 1 Ob	-0.97	-0.92								
	UI 1 Ob	-0.05									

Balance form: bone turnover
Balance = Mo Turnover = $\frac{MoM_{\sigma}^2}{MoM_{\pi}^2}$

equation (2) equation (3)
 Log formation et resorption

Mom_R_normalized to Ob median T0		Balance	Turnover rate		Balance	Turnover rate_normalized to Ob median	Log_balance	Log_rate
0.787	TN 8mths	0.927	1.365 TN 8mths		1.100	1.170	-0.033	0.135 TN 8mths
1.257	WL	0.615	1.877 WL		0.731	1.557	-0.211	0.274 WL
0.295		2.522	1.019		2.994	0.932	0.402	0.008
0.769		1.028	1.403		1.220	1.213	0.012	0.147
0.806		0.996	1.446		1.182	1.248	-0.002	0.160
0.785		0.996	1.409		1.182	1.216	-0.002	0.149
0.479		1.933	1.325		2.295	1.199	0.286	0.122
0.907		1.092	1.707		1.296	1.484	0.038	0.232
0.703		1.066	1.307		1.266	1.134	0.028	0.116
0.844		1.238	1.709		1.469	1.501	0.093	0.233
0.763	mean	1.241	1.457				mean 0.061	0.158

equation (4)

Cartesian coordinates

$$x = \log(\text{balance}) - \text{mean}[\log(\text{rate})]$$

Log_balance	Log_rate	normalized to Ob median T0	Log_x	Log_y
0.041	0.068	TN 8mths	-0.094	-0.023
-0.136	0.192	WL	-0.272	0.116
0.476	-0.031		0.341	-0.150
0.086	0.084		-0.049	-0.011
0.073	0.096		-0.063	0.003
0.073	0.085		-0.063	-0.009
0.361	0.079		0.225	-0.035
0.113	0.171		-0.023	0.075
0.102	0.055		-0.033	-0.041
0.167	0.176		0.032	0.075
0.136	0.098	mean	0.000	0.000

Appendix 21 - Excel file calculation of 8 months Ob bone turnover normalised to Ob baseline median

Based on Bieglmayer et al. 2009

n=

equation (1)
 MoM Formation et Resorption
MoM = marker_z/median(marker)

		P1NP	CTX	zf	zr	ui		MoM_F	Mom_R
TN 8mths	ABAU	36.35	4240.12	-1.72	-0.24	-1.48	TN 8mths	0.882	0.976
WL	BEHE	35.77	5910.72	-1.94	1.25	-3.19	WL	0.868	1.360
	BOAU	49.03	5394.84	3.04	0.79	2.25		1.189	1.242
	DAAL	41.51	3957.96	0.22	-0.49	0.71		1.007	0.911
	DEMA	39.76	3555.58	-0.44	-0.85	0.41		0.965	0.818
	FLCH	37.68	3835.02	-1.22	-0.60	-0.62		0.914	0.883
	GUMA	40.93	4150.92	0.00	-0.32	0.31		0.993	0.955
	MASA	43.78	4448.92	1.07	-0.05	1.12		1.062	1.024
	PEMA	43.19	4902.66	0.85	0.35	0.50		1.048	1.128
	SCAM	50.99	5191.54	3.78	0.61	3.17		1.237	1.195
	median:	41.22	4344.52				mean	1.016	1.049
	mean	41.90	4558.83						
	sd	5.06	761.33						
	zscore Ob	0.36	0.05	0.36	0.05	0.32			
	UI Ob	0.32							

Balance form: bone turnover

$$\text{Balance} = \text{Mo Turnover} = \text{racine}(\text{MoM}_F^2 + \text{MoM}_R^2)$$

	MoM_F_norn	Mom_R_normalized to Ob median	Balance	Turnover rate		Balance	
TN 8mths	0.880	0.958	TN 8mths	0.904	1.315	TN 8mths	0.918
WL	0.866	1.336	WL	0.638	1.614	WL	0.648
	1.187	1.219		0.958	1.720		0.973
	1.005	0.895		1.105	1.358		1.123
	0.963	0.804		1.179	1.265		1.198
	0.912	0.867		1.035	1.271		1.052
	0.991	0.938		1.039	1.378		1.056
	1.060	1.006		1.037	1.475		1.054
	1.046	1.108		0.929	1.540		0.944
	1.235	1.173		1.035	1.720		1.052
			mean	0.986	1.466		

equation (2) equation (3)
 Log formation et resorption

Cartesian coord
 x = log(balanc

Turnover rate_normalized to Ob median	Log_balance	Log_rate	Log_balance_	Log_rate_normalized to Ob median			
1.301	TN 8mths	-0.044	0.119	TN 8mths	-0.037	0.114	TN 8mths
1.592	WL	-0.195	0.208	WL	-0.188	0.202	WL
1.702		-0.019	0.235		-0.012	0.231	
1.346		0.044	0.133		0.051	0.129	
1.254		0.071	0.102		0.078	0.098	
1.258		0.015	0.104		0.022	0.100	
1.365		0.017	0.139		0.024	0.135	
1.461		0.016	0.169		0.023	0.165	
1.524		-0.032	0.188		-0.025	0.183	
1.703		0.015	0.236		0.022	0.231	
	mean	-0.011	0.163		-0.004	0.159	mean

equation (4)

ordinates

$$y = \log(\text{rate}) - \text{mean}[\log(\text{rate})]$$

Log_x	Log_y
-0.033	-0.044
-0.184	0.045
-0.007	0.072
0.055	-0.030
0.083	-0.061
0.026	-0.059
0.028	-0.024
0.027	0.006
-0.021	0.024
0.026	0.072
0.000	0.000

Appendix 22 - Excel file calculation of baseline Ob control bone turnover normalised to Ob control baseline median

Based on Bieglmayer et al. 2009

equation (1)

MoM Formation et Resorption

MoM = markerz/median(marker)

n=	11	P1NP	CTX	zf	zr	ui	MoM_F	Mom_R
Ob cntrl	ALAL	38.51	4173.02	-0.89	-0.96	0.07	0.922	0.744
	ALAU	40.84	5424.62	-0.18	-0.07	-0.11	0.978	0.967
	BAHA	41.77	5607.21	0.10	0.06	0.04	1.000	1.000
	BELO	37.68	6303.24	-1.14	0.56	-1.70	0.902	1.124
	CHBE	42.94	7057.89	0.46	1.09	-0.64	1.028	1.259
	COCO	37.68	5927.20	-1.14	0.29	-1.43	0.902	1.057
	COMA	45.21	4401.53	1.15	-0.79	1.94	1.083	0.785
	FLSO	42.27	3134.90	0.25	-1.70	1.95	1.012	0.559
	GRCA	37.43	4378.04	-1.22	-0.81	-0.40	0.896	0.781
	MEEM	44.96	7938.47	1.07	1.72	-0.65	1.077	1.416
	POEN	46.48	6356.57	1.53	0.60	0.94	1.113	1.134
	median:	41.77	5607.21				mean	0.992
	mean	41.43	5518.43					0.984
	sd	3.29	1405.99					
	zscore Obcntr	0.000	0.000	0.000	0.000	0.000		
	UI TN	0.000						
	1 subject	37.68	5927.20					
	zscore 1 Ob	-1.14	0.29					
	UI 1 Ob	-1.43						

Balance form: bone turnover

$$\text{Balance} = \text{Mo Turnover} = \text{racine}(\text{MoM}^2 + \text{MoM}^2)$$

equation (2) equation (3)

Log formation et resorption

	Balance	Turnover rate		Log_balance	Log_rate
	1.239	1.185		0.093	0.074
	1.011	1.376		0.005	0.138
	1.000	1.414		0.000	0.151
	0.803	1.441		-0.096	0.159
	0.817	1.625		-0.088	0.211
	0.853	1.390		-0.069	0.143
	1.379	1.337		0.140	0.126
	1.810	1.156		0.258	0.063
	1.148	1.189		0.060	0.075
	0.760	1.779		-0.119	0.250
	0.982	1.589		-0.008	0.201
mean	1.073	1.407	mean	0.016	0.145

equation (4)

Cartesian coordinates

$$x = \log(\text{balance}) - \text{mean}[\log(\text{rate})]$$

Log_x	Log_y
0.077	-0.071
-0.011	-0.006
-0.016	0.006
-0.112	0.014
-0.104	0.066
-0.085	-0.002
0.124	-0.018
0.242	-0.082
0.044	-0.070
-0.135	0.105
-0.024	0.056

mean **0.000** **0.000**

Appendix 23 - Excel file calculation of 4months Ob control bone turnover normalised to Ob control baseline median

n=	11								
		P1NP	CTX	zf	zr	ui		MoM_F	Mom_R
Ob control	ALAL	43.950	5894.288	0.76	0.27	0.50		1.108	0.935
	ALAU	41.682	5439.572	0.08	-0.06	0.13		1.051	0.863
	BAHA	39.425	6303.235	-0.61	0.56	-1.17		0.994	1.000
	BELO	38.509	6744.451	-0.89	0.87	-1.76		0.971	1.070
	CHBE	39.925	7961.439	-0.46	1.74	-2.20		1.006	1.263
	COCO	36.931	7472.943	-1.37	1.39	-2.76		0.931	1.186
	COMA	31.821	5454.575	-2.92	-0.05	-2.87		0.802	0.865
	FLSO	34.781	4983.292	-2.02	-0.38	-1.64		0.877	0.791
	RCA	44.539	4694.797	0.94	-0.59	1.53		1.123	0.745
	MEEM	39.675	8007.594	-0.53	1.77	-2.30		1.000	1.270
	POEN	39.675	7515.934	-0.53	1.42	-1.95		1.000	1.192
	median:	39.68	6303.24						
							mean	0.987	1.016
	mean	39.17	6406.56						
	sd	3.71	1205.54						
	zscore Ob	26.59	0.63	-0.69	0.63	-1.32			
	UI Ob	25.96							
	1 subject	36.93	7472.94						
	zscore 1 Ob	-1.37	1.39						
	UI 1 Ob	-2.76							

MoM_F_norn	Mom_R_normalized to Obcntrl median T(Balance	Turnover rate	Balance	Turnover rate_normalized 1	
1.052	1.051		1.185	1.450	1.001	1.487
0.998	0.970		1.217	1.360	1.029	1.392
0.944	1.124		0.994	1.410	0.840	1.468
0.922	1.203		0.907	1.445	0.767	1.516
0.956	1.420		0.797	1.615	0.673	1.712
0.884	1.333		0.785	1.507	0.663	1.599
0.762	0.973		0.927	1.180	0.783	1.236
0.833	0.889		1.109	1.180	0.937	1.218
1.066	0.837		1.507	1.347	1.274	1.356
0.950	1.428		0.787	1.617	0.665	1.715
0.950	1.340		0.839	1.556	0.709	1.643
0.938	1.143	mean	1.005	1.424		

to Obcntrl me	Log_balance	Log_rate	Log_balance_	Log_rate_normalized to	Obcntrl median *	Log_x	Log_y
	0.074	0.161	0.000	0.172		0.074	0.161
	0.085	0.133	0.012	0.144		0.085	0.133
	-0.003	0.149	-0.076	0.167		-0.003	0.149
	-0.042	0.160	-0.115	0.181		-0.042	0.160
	-0.099	0.208	-0.172	0.233		-0.099	0.208
	-0.105	0.178	-0.178	0.204		-0.105	0.178
	-0.033	0.072	-0.106	0.092		-0.033	0.072
	0.045	0.072	-0.028	0.086		0.045	0.072
	0.178	0.129	0.105	0.132		0.178	0.129
	-0.104	0.209	-0.177	0.234		-0.104	0.209
	-0.076	0.192	-0.150	0.216			
mean	-0.007	0.151	-0.080	0.169	mean	0.000	0.147

Appendix 24 - Excel file calculation of baseline Ob bone turnover normalised to Ob control baseline median

Based on Bieglmayer et al. 2009							equation (1) MoM Formation et Resorption MoM = markerz/median(marker)		
n=	10								
	P1NP	CTX	zf	zr	ui		MoM_F	Mom_R	
TN 8mths	ABAU	36.60	5365.25	-1.47	-0.11	-1.36	TN 8mths	0.876	0.957
WL	BEHE	41.18	6763.58	-0.08	0.89	-0.96	WL	0.986	1.206
	BOAU	40.01	5191.54	-0.43	-0.23	-0.20		0.958	0.926
	DAAL	41.43	3002.58	0.00	-1.79	1.79		0.992	0.535
	DEMA	39.34	4823.51	-0.64	-0.49	-0.14		0.942	0.860
	FLCH	37.43	4784.48	-1.22	-0.52	-0.69		0.896	0.853
	GUMA	41.85	3999.94	0.13	-1.08	1.21		1.002	0.713
	MASA	44.54	3158.94	0.94	-1.68	2.62		1.066	0.563
	PEMA	44.54	3916.49	0.94	-1.14	2.08		1.066	0.698
	SCAM	42.44	4063.86	0.30	-1.03	1.34		1.016	0.725
	median:	41.30	4424.17				mean	0.980	0.804
	mean	40.94	4507.02						
	sd	2.66	1123.35						
	zscore TN	-0.15	-0.72	-0.15	-0.72	0.57			
	UI TN	0.57							
	1 subject	37.43	4784.48						
	zscore 1 Ob	-1.22	-0.52						
	UI 1 Ob	-0.69							

Balance form: bone turnover
Balance = Mo Turnover = racine(MoM² + MoM²)

equation (2) equation (3)
 Log formation et resorption

equation (4)
 Cartesian coord
x = log(balanc

	Balance	Turnover rate
TN 8mths	0.916	1.297
WL	0.817	1.558
	1.035	1.332
	1.852	1.127
	1.095	1.276
	1.050	1.237
	1.405	1.230
	1.893	1.206
	1.527	1.275
	1.402	1.248
mean	1.299	1.279

	Log_balance	Log_rate
TN 8mths	-0.038	0.113
WL	-0.088	0.193
	0.015	0.125
	0.268	0.052
	0.039	0.106
	0.021	0.093
	0.148	0.090
	0.277	0.081
	0.184	0.105
	0.147	0.096
mean	0.097	0.105

	Log_x
TN 8mths	-0.135
WL	-0.185
	-0.082
	0.170
	-0.058
	-0.076
	0.050
	0.180
	0.087
	0.049
mean	0.000

ordinates
 $y = \log(\text{rate}) - \text{mean}[\log(\text{rate})]$

- Log_y**
- 0.008
 - 0.087
 - 0.019
 - 0.053
 - 0.000
 - 0.013
 - 0.015
 - 0.024
 - 0.000
 - 0.009

0.000

Appendix 25 - Excel file calculation of 4 months Ob bone turnover normalised to Ob control baseline median

Based on Bieglmayer et al. 2009		equation (1) MoM Formation et Resorption MoM = marker;/median(marker)							
n=	10	P1NP	CTX	zf	zr	ui			MoM_F_norn
TN 8mths	ABAU	35.77	3482.65	-1.72	-1.45	-0.27	TN 8mths		0.856
WL	BEHE	37.93	5560.92	-1.06	0.03	-1.10	WL	WL	0.908
	BOAU	36.52	1306.49	-1.49	-3.00	1.50			0.874
	DAAL	38.76	3402.66	-0.81	-1.50	0.69			0.928
	DEMA	39.34	3564.81	-0.64	-1.39	0.75			0.942
	FLCH	38.34	3473.66	-0.94	-1.45	0.52			0.918
	GUMA	45.38	2118.24	1.20	-2.42	3.62			1.087
	MASA	48.52	4010.51	2.15	-1.07	3.22			1.162
	PEMA	36.76	3111.07	-1.42	-1.71	0.29			0.880
	SCAM	51.25	3735.91	2.98	-1.27	4.25			1.227
	median:	38.55	3478.16						
							mean		0.978
	mean	40.86	3376.69						
	sd	5.48	1120.43						
	zscore Ob	-0.18	-1.52	-0.18	-1.52	1.35			
	UI Ob	1.35							
	1 subject	38.34	3473.66						

Balance form: bone turnover

$$\text{Balance} = \text{Mo} \sqrt{\text{Turnover}^2 + \text{MoM}^2}$$

equation (2) equation (3)

Log formation et resorption Cartesian coord

$$x = \log(\text{balance})$$

Mom_R_normalized to Ob median T0			Balance	Turnover rate_normalized to Ob median	Log_balance_	Log_rate_normalized to Ob	
0.621	TN 8mths	TN 8mths	1.379	1.058	TN 8mths	0.140	0.024
0.992	WL	WL	0.916	1.345	WL	-0.038	0.129
0.233			3.752	0.905		0.574	-0.043
0.607			1.529	1.109		0.184	0.045
0.636			1.482	1.136		0.171	0.056
0.619			1.482	1.108		0.171	0.044
0.378			2.876	1.150		0.459	0.061
0.715			1.624	1.364		0.211	0.135
0.555			1.586	1.040		0.200	0.017
0.666			1.842	1.396		0.265	0.145
0.602		mean				0.234	0.061

Appendix 26 - Excel file calculation of 8 months Ob bone turnover normalised to Ob control baseline median

Based on Bieglmayer et al. 2009

n=

		P1NP	CTX	zf	zr	ui		MoM_F_norn	Mom_R_norn
TN 8mths	ABAU	36.35	4240.12	-1.72	-0.24	-1.48	TN 8mths	0.870	0.756
WL	BEHE	35.77	5910.72	-1.94	1.25	-3.19	WL	0.856	1.336
	BOAU	49.03	5394.84	3.04	0.79	2.25		1.174	1.219
	DAAL	41.51	3957.96	0.22	-0.49	0.71		0.994	0.895
	DEMA	39.76	3555.58	-0.44	-0.85	0.41		0.952	0.804
	FLCH	37.68	3835.02	-1.22	-0.60	-0.62		0.902	0.867
	GUMA	40.93	4150.92	0.00	-0.32	0.31		0.980	0.938
	MASA	43.78	4448.92	1.07	-0.05	1.12		1.048	1.006
	PEMA	43.19	4902.66	0.85	0.35	0.50		1.034	1.108
	SCAM	50.99	5191.54	3.78	0.61	3.17		1.221	1.173
	median:	41.22	4344.52						
	mean	41.90	4558.83				mean		
	sd	5.06	761.33						
	zscore Ob	0.14	-0.68	0.36	0.05	0.32			
	UI Ob	0.82							

normalized to Ob median	Balance	Turnover rate_normalized to Ob median	Log_balance_	Log_rate_normalized to Ob median
TN 8mths	1.151	1.153	TN 8mths	0.061
WL	0.641	1.587	WL	-0.193
	0.963	1.693		-0.016
	1.111	1.337		0.046
	1.185	1.246		0.074
	1.041	1.251		0.017
	1.044	1.357		0.019
	1.042	1.453		0.018
	0.933	1.516		-0.030
	1.040	1.693		0.017
				0.001
				0.151

Appendix 27 - Bone variables of the 24 adolescents with obesity at 4 months adjusted to (A) BW changes and (B) fat mass changes

<u>A</u>	WB (TBLH BMD)		Lumbar Spine		Hip		Neck	
	Ob		Ob		Ob		Ob	
	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>
BMD (g/cm ²)	0.964	0.927 - 1.001	1.011	0.952 - 1.070	1.035	0.975 - 1.095	0.962	0.904 - 1.020
BMC (g)	1698.66	1572.35 - 1824.97	55.89	51.17 - 60.61	34.61	32.48 - 36.73	4.81	4.43 - 5.18
BMAD (g/cm ³)	0.092	0.090 - 0.094	0.992	0.931 - 1.053				

	Narrow Neck		Intertrochanteric		Femoral Shaft	
	Ob		Ob		Ob	
	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>
BMD (g/cm ²)	1.043	0.954 - 1.133	1.107	1.033 - 1.182	1.532	1.458 - 1.606
ED (cm)	3.00	2.86 - 3.13	4.74	4.56 - 4.93	1.90	1.75 - 2.04
ACT (cm)	0.22	0.20 - 0.23	0.48	0.44 - 0.51	0.58	0.55 - 0.61
WIDTH (cm)	3.21	3.03 - 3.40	5.72	5.55 - 5.89	3.08	2.96 - 3.20
CSA (cm ²)	3.64	3.34 - 3.93	6.03	5.59 - 6.47	4.49	4.19 - 4.79
CSMI (cm ⁴)	2.95	2.56 - 3.34	16.49	14.73 - 18.26	3.88	3.32 - 4.45
Z (cm ³)	1.70	1.52 - 1.87	5.21	4.75 - 5.67	2.41	2.15 - 2.67
BR	7.88	7.16 - 8.61	6.69	6.07 - 7.32	2.76	2.52 - 3.00

<u>B</u>	WB (TBLH BMD)		Lumbar Spine		Hip		Neck	
	Ob		Ob		Ob		Ob	
	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>
BMD (g/cm ²)	0.964	0.927 - 1.001	1.011	0.953 - 1.069	1.035	0.976 - 1.094	0.962	0.904 - 1.020
BMC (g)	1698.66	1572.39 - 1824.93	55.89	51.39 - 60.38	34.61	32.51 - 36.71	4.81	4.44 - 5.18
BMAD (g/cm ³)	0.092	0.09 - 0.09	0.992	0.93 - 1.05				

	Narrow Neck		Intertrochanteric		Femoral Shaft	
	Ob		Ob		Ob	
	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>
BMD (g/cm ²)	1.158	1.070 - 1.245	1.107	1.034 - 1.181	1.532	1.458 - 1.606
ED (cm)	2.85	2.73 - 2.96	4.74	4.56 - 4.93	1.90	1.75 - 2.05
ACT (cm)	0.22	0.20 - 0.23	0.48	0.44 - 0.51	0.58	0.55 - 0.61
WIDTH (cm)	3.36	3.23 - 3.50	5.72	5.55 - 5.89	3.08	2.96 - 3.19
CSA (cm ²)	3.64	3.34 - 3.93	6.03	5.60 - 6.46	4.49	4.19 - 4.79
CSMI (cm ⁴)	2.95	2.56 - 3.33	16.49	14.71 - 18.28	3.88	3.32 - 4.44
Z (cm ³)	1.70	1.52 - 1.87	5.21	4.74 - 5.67	2.41	2.15 - 2.67
BR	7.88	7.16 - 8.61	6.69	6.08 - 7.30	2.76	2.52 - 3.00

Ob obese intervention group, NW normal weight control group, SD standard deviation, WB whole body, TBLH total body less head, LM lean mass, FM fat mass, BMD bone mineral density, BMC bone mineral content, BMAD bone mineral apparent density, NN narrow neck, IT intertrochanteric, FS femoral shaft, ED endocortical diameter, WIDTH width, CSA cross sectional area, CSMI cross sectional moment of inertia, ACT average cortical thickness, Z section modulus, BR bulking ratio

Appendix 28 - Bone variables of the 24 adolescents with obesity at 8 months adjusted to (A) BW changes and (B) fat mass changes

<u>A</u>	WB (TBLH BMD)		Lumbar Spine		Hip		Neck	
	Ob		Ob		Ob		Ob	
	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>
BMD (g/cm ²)	0.993	0.959 - 1.027	1.038	0.980 - 1.096	1.040	0.979 - 1.100	0.962	0.905 - 1.019
BMC (g)	1788.79	1663.45 - 1914.13	58.79	54.16 - 63.43	35.10	32.53 - 37.66	4.92	4.56 - 5.29
BMAD (g/cm ³)	0.094	0.092 - 0.096	1.038	0.980 - 1.096				

	Narrow Neck		Intertrochanteric		Femoral Shaft	
	Ob		Ob		Ob	
	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>
BMD (g/cm ²)	1.153	1.070 - 1.236	1.091	1.071 - 1.166	1.572	1.505 - 1.640
ED (cm)	2.92	2.79 - 3.05	4.74	4.55 - 4.92	1.88	1.74 - 2.02
ACT (cm)	0.23	0.21 - 0.24	0.52	0.49 - 0.55	0.65	0.61 - 0.69
WIDTH (cm)	3.39	3.27 - 3.51	5.69	5.54 - 5.85	3.04	2.88 - 3.20
CSA (cm ²)	3.69	3.42 - 3.96	5.92	5.50 - 6.34	4.65	4.40 - 4.90
CSMI (cm ⁴)	3.09	2.76 - 3.42	16.10	14.43 - 17.77	3.94	3.43 - 4.45
Z (cm ³)	1.73	1.57 - 1.89	5.06	4.62 - 5.51	2.44	2.19 - 2.70
BR	8.24	7.36 - 9.12	6.85	6.22 - 7.48	2.72	2.51 - 2.94

<u>B</u>	WB (TBLH BMD)		Lumbar Spine		Hip		Neck	
	Ob		Ob		Ob		Ob	
	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>
BMD (g/cm ²)	0.993	0.959 - 1.028	1.038	0.981 - 1.095	1.040	0.976 - 1.104	0.969	0.909 - 1.029
BMC (g)	1788.79	1663.57 - 1914.01	58.79	54.28 - 63.31	35.10	32.45 - 37.75	4.92	4.56 - 5.28
BMAD (g/cm ³)	0.094	0.092 - 0.096	1.038	0.981 - 1.095				

	Narrow Neck		Intertrochanteric		Femoral Shaft	
	Ob		Ob		Ob	
	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>	<i>mean</i>	<i>95% CI</i>
BMD (g/cm ²)	1.153	1.069 - 1.237	1.091	1.015 - 1.167	1.572	1.499 - 1.646
ED (cm)	2.92	2.78 - 3.05	4.73	4.55 - 4.92	1.88	1.74 - 2.03
ACT (cm)	0.23	0.22 - 0.24	0.52	0.49 - 0.55	0.65	0.61 - 0.68
WIDTH (cm)	3.39	3.27 - 3.51	5.69	5.54 - 5.85	3.04	2.88 - 3.20
CSA (cm ²)	3.69	3.41 - 3.97	5.92	5.49 - 6.34	4.65	4.38 - 4.91
CSMI (cm ⁴)	3.09	2.74 - 3.45	16.10	14.43 - 17.77	3.94	3.43 - 4.44
Z (cm ³)	1.73	1.56 - 1.90	5.06	4.62 - 5.51	2.44	2.19 - 2.70
BR	8.24	7.36 - 9.12	6.85	6.20 - 7.50	2.72	2.49 - 2.95

Ob obese intervention group, NW normal weight control group, SD standard deviation, WB whole body, TBLH total body less head, LM lean mass, FM fat mass, BMD bone mineral density, BMC bone mineral content, BMAD bone mineral apparent density, NN narrow neck, IT intertrochanteric, FS femoral shaft, ED endocortical diameter, WIDTH width, CSA cross sectional area, CSMI cross sectional moment of inertia, ACT average cortical thickness, Z section modulus, BR bulking ratio

Appendix 29 - Baseline bone variables of the adolescents with obesity in the control group compared with the Ob intervention group

	WB (TBLH BMD)				Lumbar Spine				Hip				Neck			
	Ob		Ob control		Ob		Ob control		Ob		Ob control		Ob		Ob control	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
BMD (g/cm ²)	0.952	0.082	0.939	0.056	1.004	0.160	0.941	0.150	1.004	0.145	1.038	0.123	0.961	0.137	0.978	0.110
BMC (g)	1619.56	303.74	1662.27	223.40	52.64	12.19	51.23	13.05	32.03	6.04	37.31	16.81	4.76	0.91	4.88	0.66
BMAD (g/cm ³)	0.092	0.004	0.088	0.004	0.950	0.198	0.91	0.15								

	Narrow Neck				Intertrochanteric				Femoral Shaft			
	Ob		Ob control		Ob		Ob control		Ob		Ob control	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
BMD (g/cm ²)	1.196	0.17	1.217	0.14	1.101	0.21	1.100	0.06	1.486	0.16	1.507	0.10
ED (cm)	2.73	0.21	2.66	0.32	4.59	0.39	4.60	0.35	1.87	0.33	1.81	0.24
ACT (cm)	0.23	0.03	0.24	0.03	0.48	0.11	0.48	0.04	0.55	0.07	0.57	0.05
WIDTH (cm)	3.20	0.23	3.15	0.33	5.56	0.40	5.56	0.37	2.98	0.28	2.95	0.19
CSA (cm ²)	3.65	0.70	3.66	0.71	5.86	1.28	5.83	0.53	4.22	0.60	4.23	0.40
CSMI (cm ⁴)	3.04	1.04	2.67	0.58	15.87	4.75	14.99	2.96	3.51	1.07	4.23	0.40
Z (cm ³)	1.79	0.49	1.59	0.21	5.08	1.32	4.94	0.75	2.26	0.52	2.24	0.38
BR	7.45	1.43	6.85	1.07	6.74	1.66	6.28	0.67	2.80	0.58	2.75	0.34

* p<0.05 Ob significantly different than Ob control.

Ob obese intervention group, NW normal weight control group, SD standard deviation, WB whole body, TBLH total body less head, LM lean mass, FM fat mass, BMD bone mineral density, BMC bone mineral content, BMAD bone mineral apparent density, NN narrow neck, IT intertrochanteric, FS femoral shaft, ED endocortical diameter, WIDTH width, CSA cross sectional area, CSMI cross sectional moment of inertia, ACT average cortical thickness, Z section modulus, BR bulking ratio

Appendix 30 - Proposed future research question

The proposed research question could be addressed in future research.

- i. What is the ratio between the effects of physical activity/nutrition during the WL intervention on bone health?

The assessment of the relative contribution of physical activity versus nutrition on bone health among adolescents enrolled in a weight loss intervention is of interest. Indeed, in the current thesis it was not possible to individually track the volume and intensity of the exercise component. Moreover, nutrition intakes would need to be evaluated at the beginning of the WL intervention along with changes during the course of the residential program to better explain potential bone and appetite markers changes.

- ii. Does High Intensity Interval Training (HIIT) combining resistance and aerobic exercise plus nutrition induce WL have better protective effects on bone health among adolescents with obesity than traditional weight loss program?

Continuous moderate physical activity is the traditional recommendation for weight loss exercise intervention (Graf 2011). Yet, it is unclear which type of exercise can provide the greatest health benefits, including bone parameters during a weight loss intervention. Indeed, systematically reviewed, the results of HIIT on fat loss have been shown to be similar to moderate intensity (Keating et al. 2017) (Wewege et al. 2017). However, others using HIIT have demonstrated better efficiency in visceral fat loss (Dutheil et al. 2013), positive effects on cardiovascular fitness and weight status (Jung et al. 2014) (Little et al. 2014), cardiometabolic markers (Blucher et al. 2017), anorexigenic effects on energy intake (Thivel et al. 2012) (Thivel

et al. 2012), improvement of glycaemic control and pancreatic β cell function (Madsen et al. 2015) and inhibition of sclerostin levels (Macias et al. 2012). Although, not in populations with obesity, high-intensity training has been associated with an effective slowdown of bone loss (de Jong et al. 2004). Also, in older overweight adults with type 2 diabetes, it has been found that including high intensity progressive resistance training in a weight loss intervention optimised the effects on body composition without having a negative effect on bone health (Daly et al. 2005). Based on those observations it would be of interest to observe the effects of HIIT on bone health.

iii. Can the results of weight loss interventions (physical activity and nutrition) differentiate between adolescent males and females with obesity?

Observation in young mice study showed sexual dimorphism relating to bone responses to exercise training (Koenen et al. 2017). Indeed, after 8 weeks of high intensity interval training using a treadmill, male mice increased cortical bone but lost trabecular bone while no effects on bone were seen in female mice. Indeed, trabecular bone and cortical bone of female mice did not change in response to HIIT. Moreover, in human studies gender differences were observed in hormone circulating levels such as leptin and osteocalcin (Abseyi et al. 2012) (Do Prado et al. 2009) (Garanty-Bogacka et al. 2013), which have direct or indirect effects on bone health. Also, there is some evidence to support higher BMD values in female than male children with obesity (Nagasaki et al. 2004).

- ii.* Do adolescents with metabolic syndrome respond similarly to a weight loss program combining physical activity and nutrition in peers who demonstrate a “healthy” obesity?

A potential link between bone health and metabolic syndrome (MetS) can be speculated. In the last decade, the influence of MetS on bone health has been explored. However, results remain inconclusive. The existing literature highlights that children and adolescents with obesity have lower values of osteocalcin than their normal weight peers. It could be hypothesised that during adolescence, obesity inducing metabolic syndrome might be associated with bone fragility. Indeed, even if their results were inconclusive about a potential link between osteocalcin and MetS, some evidence using animal models have shown that the administration of osteocalcin can correct metabolic abnormalities (Lee et al. 2007).

Appendice 31- Ethics approval by the ACU Human Research Ethics

Ethic application

Principal Investigator: Prof Geraldine Naughton, A/Prof David Greene
Student Researcher: Elodie Chaplais (HDR student)
Ethics Register Number: 2014 320N
Project Title: Adiposity and Bone Metabolism: A Profile of Connections
Risk Level: Low Risk
Date Approved: 11/05/2015
Ethics Clearance End Date: 31/12/2016

This email is to advise that your application has been reviewed by the Australian Catholic University's Human Research Ethics Committee and confirmed as meeting the requirements of the National Statement on Ethical Conduct in Human Research.

The data collection of your project has received ethical clearance but the decision and authority to commence may be dependent on factors beyond the remit of the ethics review process and approval is subject to ratification at the next available Committee meeting. The Chief Investigator is responsible for ensuring that outstanding permission letters are obtained, interview/survey questions, if relevant, and a copy forwarded to ACU HREC before any data collection can occur. Failure to provide outstanding documents to the ACU HREC before data collection commences is in breach of the National Statement on Ethical Conduct in Human Research and the Australian Code for the Responsible Conduct of Research. Further, this approval is only valid as long as approved procedures are followed.

Clinical Trials: You are required to register it in a publicly accessible trials registry prior to enrolment of the first participant (e.g. Australian New Zealand Clinical Trials Registry <http://www.anzctr.org.au/>) as a condition of ethics approval.

If you require a formal approval certificate, please respond via reply email and one will be issued.

Researchers who fail to submit a progress report may have their ethical clearance revoked and/or the ethical clearances of other projects suspended. When your project has been completed please complete and submit a progress/final report form and advise us by email at your earliest convenience. The information researchers provide on the security of records, compliance with approval consent procedures and documentation and responses to special conditions is reported to the NHMRC on an annual basis. In accordance with NHMRC the ACU HREC may undertake annual audits of any projects considered to be of more than low risk.

It is the Principal Investigators / Supervisors responsibility to ensure that:

1. All serious and unexpected adverse events should be reported to the HREC with 72 hours.
2. Any changes to the protocol must be reviewed by the HREC by submitting a Modification/Change to Protocol Form prior to the research commencing or continuing. <http://research.acu.edu.au/researcher-support/integrity-and-ethics/>
3. Progress reports are to be submitted on an annual basis. <http://research.acu.edu.au/researcher-support/integrity-and-ethics/>
4. All research participants are to be provided with a Participant Information Letter and consent form, unless otherwise agreed by the Committee.
5. Protocols can be extended for a maximum of five (5) years after which a new application must be submitted. (The five year limit on renewal of approvals allows the Committee to fully re-review

research in an environment where legislation, guidelines and requirements are continually changing, for example, new child protection and privacy laws).

Researchers must immediately report to HREC any matter that might affect the ethical acceptability of the protocol eg: changes to protocols or unforeseen circumstances or adverse effects on participants.

Please do not hesitate to contact the office if you have any queries.

2014 320N Modification

Ethics Register Number: 2014 320N

Project Title: Adiposity and Bone Metabolism: A Profile of Connections End Date: 31/12/2016

Thank you for submitting the request to modify form for the above project.

The Chair of the Human Research Ethics Committee has approved the following modification(s):

1. Inclusion of 2 recruitment sites - NSW Catholic Independent schools.

Recruitment is subject to Principal permission. If schools are part of the Catholic Education Office system then CEO approval would be required.

2014 320N Modification

Ethics Register Number: 2014 320N

Project Title: Adiposity and Bone Metabolism: A Profile of Connections End Date: 31/12/2016

Thank you for submitting the request to modify form for the above project.

The Chair of the Human Research Ethics Committee has approved the following modification(s):

Recruitment extended to ACU staff - Strathfield campus - email to be sent to ACU staff inviting participation. A copy of the email to be provided for our files.