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Stratégies thérapeutiques par conditionnement hypoxique : modalités pratiques et effets sur la santé cardio-respiratoire et métabolique

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THÈSE

Pour obtenir le grade de
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GRENOBLE ALPES**

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l'Autonomie**

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préparée au sein du **Laboratoire Hypoxie Physiopathologie (HP2)**,
INSERM U1042, Grenoble, France
dans l'**Ecole Doctorale Ingénierie pour la Santé, la Cognition et
l'Environnement**

Stratégies thérapeutiques par conditionnement hypoxique : modalités pratiques et effets sur la santé cardio- respiratoire et métabolique

Thèse soutenue publiquement le **29 Juin 2018**,
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RÉSUMÉ

L'hypoxie désigne une baisse de la biodisponibilité en oxygène au niveau tissulaire. La combinaison de l'hypoxie intermittente et de l'hypercapnie est identifiée dans le cadre de plusieurs maladies respiratoires comme un élément physiopathologique déterminant (BPCO, apnée du sommeil). Cependant, des travaux de recherche suggèrent qu'une exposition à l'hypoxie hypo- ou normocapnique à l'éveil peut améliorer la santé cardiovasculaire dans des modèles animaux et chez l'homme. La combinaison d'une exposition hypoxique et de l'entraînement à l'effort est utilisée depuis des années par les athlètes pour améliorer la performance sportive aérobie grâce entre autres à une amélioration de l'hématocrite et des effets sur la fonction cardiovasculaire et le muscle. Des études pilotes récentes y compris chez le malade chronique indiquent que l'exposition à l'hypoxie modérée au repos ou à l'effort chez le patient est faisable, bien tolérée, et susceptible d'induire des gains significatifs en termes de santé cardiovasculaire, de composition corporelle et de statut métabolique.

Au cours de ce travail de thèse, nous nous sommes intéressés aux effets cardiorespiratoires et tissulaires de l'exposition hypoxique normobarique chez le sujet sain et chez la personne en surpoids ou obèse présentant un risque ou des anomalies cardio-métaboliques. Nous avons étudié l'efficacité de 2 types de conditionnement au repos consistant en une hypoxie continue ou une hypoxie intermittente et un entraînement à l'effort hypoxique par comparaison à la situation normoxique. Nous avons tout d'abord étudié les effets d'une exposition hypoxique à court terme (3 séances) au repos chez 14 sujets sains. Nous avons ensuite étudié les effets cardiorespiratoires, tissulaires, vasculaires et métaboliques d'un programme de conditionnement hypoxique normobarique à moyen terme (8 semaines) au repos chez 35 patients en surpoids ou obèse. Nous avons de plus réalisé chez 24 sujets sains une étude préliminaire afin de vérifier la faisabilité et de caractériser les réponses cardiorespiratoires et l'oxygénation tissulaire au cours d'un exercice aigu à charge constante d'intensité modérée ou intermittent intense en hypoxie comparé à une condition placebo normoxique. La dernière étude de cette thèse a consisté à étudier les conséquences cardiorespiratoires, tissulaires, vasculaires et métaboliques d'un programme d'entraînement à l'effort (exercice continu d'intensité modérée) en hypoxie par rapport au même programme en normoxie chez 23 patients en surpoids ou obèses.

L'étude réalisée chez le sujet sain met en évidence l'intérêt à court terme (au bout de 3 séances) d'un conditionnement hypoxique intermittent au repos sur des variables associées aux risques cardiovasculaires (diminution de la pression artérielle systolique en normoxie et augmentation de la variabilité sinusale) et une modulation de l'hypoxie tissulaire. Nous avons montré également chez le sujet sain que l'hypoxie combiné à l'exercice aigu (à charge constante ou intermittent intense) provoque une diminution de l'oxygénation musculaire similaire mais une diminution de l'oxygénation du cortex préfrontal plus importante par comparaison à un effort normoxique à même intensité relative. Ensuite, chez le sujet en surpoids ou obèse, nous avons montré que le conditionnement hypoxique passif chronique induit une diminution de la pression artérielle diastolique de repos en normoxie, une augmentation de la réponse ventilatoire hypoxique et une diminution de la variabilité cardiaque (après conditionnement par hypoxie intermittente seulement) et que le conditionnement hypoxique actif (à l'effort) chronique améliore l'aptitude maximale aérobie par rapport à une situation placebo normoxique.

Les résultats obtenus au cours de cette thèse montrent la faisabilité de plusieurs conditionnements hypoxiques prometteurs au plan vasculaire y compris chez le sujet en surpoids ou obèse limité à l'exercice musculaire. Le conditionnement hypoxique actif montre également des bénéfices accrus sur l'aptitude aérobie. Ces protocoles de conditionnement doivent être affinés en vue d'optimiser leur efficacité en termes de perte de poids et d'amélioration du risque cardio-vasculaire et métabolique dans des populations présentant une obésité associée à une morbidité cardio-métabolique. Ils représentent également une piste thérapeutique innovante dans d'autres pathologies chroniques.

Mots-clés : Conditionnement hypoxique, hypoxie continue, hypoxie intermittente, repos, exercice, obésité, fonction cardiovasculaire, statut métabolique, oxygénation musculaire, oxygénation cérébrale

ABSTRACT

Hypoxia refers to a decrease in the oxygen bioavailability at the tissue level. The combination of intermittent hypoxia and hypercapnia is identified in several respiratory diseases as a critical pathophysiological element (COPD, sleep apnea). However, research suggests that exposure to hypo- or normocapnic hypoxia can improve cardiovascular health in animal models and in humans. The combination of hypoxic exposure and exercise training has been used over the years by athletes to improve aerobic exercise performance by improving hematocrit, the cardiovascular and muscle function. Recent pilot studies in patients with chronic diseases indicate that exposure to moderate hypoxia at rest or during exercise is feasible, well tolerated, and likely to induce significant gains in cardiovascular health, body composition and metabolic status.

In this thesis, we investigated the effects of normobaric hypoxic exposure on cardiorespiratory and tissue function in healthy subjects, overweight or obese subjects at risk or with cardio-metabolic abnormalities. We assessed the efficacy of 2 types of passive hypoxic conditioning consisting in sustained hypoxia or intermittent hypoxia and hypoxic exercise training in comparison with normoxic condition. First, we assessed the effects of short-term hypoxic exposure (3 sessions of intermittent or sustained hypoxia) at rest in 14 healthy subjects. Then, we evaluated the cardiovascular and metabolic effects of a 8-week normobaric hypoxic conditioning program at rest (intermittent or sustained hypoxia) in 35 overweight or obese patients, compared to placebo normoxic exposure. Next, we conducted a preliminary study in 24 healthy subjects to assess the acute responses to submaximal constant-load and high intensity interval cycling exercise performed in normoxia and in hypoxia. The last study in this thesis aimed to compare the effect of an 8-week exercise training program performed either in normoxia or hypoxia on maximal aerobic capacity in overweight or obese subjects.

In the healthy subject, we emphasized the rapid benefits of intermittent hypoxic conditioning on cardiovascular function (lower baseline systolic blood pressure and increased heart rate variability) and the modulation of tissue deoxygenation in response to hypoxia. We have also shown in healthy subjects that acute exercise (either constant-load or high intensity interval) combined with hypoxia causes a similar decrease in muscle oxygenation but a greater prefrontal cortex deoxygenation compared to normoxic condition. Then, in the overweight or obese subject, we have shown that chronic passive hypoxic conditioning induces a decrease in diastolic blood pressure at rest in normoxia, an increase in the hypoxic ventilatory response and a decrease in heart rate variability after intermittent hypoxic conditioning only. In addition, chronic active (exercise training) hypoxic conditioning improves the maximal aerobic capacity compared to placebo normoxic training.

Our results show the feasibility of several hypoxic conditioning strategies and their interesting effects on the vascular function in overweight/obese subjects presenting exercise limitations impeding exercise reconditioning. In addition, active hypoxic conditioning showed a greater effect on physical fitness than normoxic exercise training. These hypoxic conditioning strategies must be further optimized to improve their efficacy regarding weight loss and cardiometabolic morbidity in obese. They also represent promising therapeutic opportunities for other chronic diseases.

Keywords: Hypoxic conditioning, sustained hypoxia, intermittent hypoxia, rest, exercise, obesity, cardiovascular function, metabolic status, muscle oxygenation, brain oxygenation

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**“Quand on veut une chose, tout l'Univers conspire à nous
permettre de réaliser notre rêve” - *Paulo Coelho***

Tables des matières

INTRODUCTION GENERALE	16
PREMIERE PARTIE : REVUE DE LITTERATURE	20
1. Hypoxie.....	21
1.1. Définition.....	21
1.2. Conséquences physiologiques de l'hypoxie d'altitude	22
1.3. Principales adaptations à l'hypoxie d'altitude	23
1.3.1. Réponse ventilatoire.....	23
1.3.2. Réponse cardiovasculaire.....	24
1.3.3. Réponse hématologique	26
1.3.4. Réponses métaboliques et effet sur le poids.....	28
1.4. Conséquences physiopathologiques de l'hypoxie	28
1.5. Lien entre hypoxie et pathologie	29
2. Conditionnement hypoxique	33
2.1. Concept.....	33
2.2. Les mécanismes	36
2.3. Conditionnement hypoxique chez le sujet sain	39
2.3.1. Effets hématologiques.....	40
2.3.2. Effets ventilatoires	40
2.3.3. Effets sur le système cardiovasculaire	41
2.3.4. Effets sur le métabolisme et la prise alimentaire	42
2.4. Conditionnement hypoxique chez le patient	44
2.4.1. Maladies cardiovasculaires.....	44
2.4.2. Maladies respiratoires.....	46
2.4.3. Maladies métaboliques.....	46
3. Obésité	50
3.1. Définition.....	50
3.2. Epidémiologie	50
3.3. Causes de l'obésité	51
3.4. Conséquences physiopathologiques	52
3.5. Traitement de l'obésité.....	54
3.5.1. Effet de l'activité physique sur la santé métabolique	55
3.5.2. Effet de l'activité physique sur la santé cardiovasculaire	56
3.5.4. Recommandations.....	57
3.5.5. Hypoxie et obésité	58
DEUXIEME PARTIE : CONTRIBUTION PERSONNELLE.....	61
Objectifs scientifiques.....	62
1. Méthodologie générale	64
1.1. Exposition à l'hypoxie normobare.....	64
1.2. Mesure des variables cardiorespiratoires	65
1.2.1. Variabilité de la fréquence cardiaque (VFC).....	65
1.2.2. Mesure du tonus artériel périphérique par Pléthysmographie (PAT).....	66

1.2.3. Mesure de la rigidité artérielle	67
1.3. Mesure par NIRS	68
1.4. Mesures anthropométriques	70
1.4.1. Imagerie par Résonance Magnétique Nucléaire (IRM)	70
1.5. Epreuve d'effort maximale progressive	71
1.6. Epreuve sous maximale métabolique	73
1.7. Variables biologiques	74
1.8. Modalités d'exercice	75
2. Résultats	77
TROISIEME PARTIE: DISCUSSION GENERALE ET PRESPECTIVES	187
BIBLIOGRAPHIE	199
ANNEXE I	226

Publications

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Liste des figures

Figure 1. La réponse biphasique de la ventilation au cours de 15 minutes d'exposition à l'hypoxie chez l'adulte.

Figure 2. Moyenne \pm Ecart type en pourcentage des variations de débit cardiaque (Q), de fréquence cardiaque (HR), de volume systolique (SVI), de la pression partielle d'oxygène (PaO₂) et de l'apport en oxygène (TO₂) chez 24 sujets soumis à une courte durée d'hypoxie normobare

Figure 3. Fréquence cardiaque (HR), débit cardiaque (Q) et volume d'éjection systolique pendant les 8 premiers jours d'acclimatation à une altitude de 3800 m chez 8 sujets sains.

Figure 4. Valeurs individuelles et moyennes (trait épais) des concentrations d'hémoglobine ([Hb]) chez 8 sujets lors d'une exposition à 2340 m d'altitude durant 21 jours.

Figure 5. La taille de l'infarctus (I) exprimée en pourcentage de la masse ventriculaire (V) évaluée après une ischémie globale (30 min) -reperfusion (120 min) dans différents groupes de souris exposés 4h à la normoxie (S), à l'hypoxie intermittente (IH, FiO₂ = 5 ou 10 %), ou à l'hypoxie chronique (CH).

Figure 6. Modifications de la réponse ventilatoire à l'hypoxie (HVR) durant 12 jours d'exposition à l'hypoxie intermittente.

Figure 7. Variations des concentrations plasmatiques de triglycérides (TG), de cholestérol (CHOL), de HDL et de LDL après un entraînement en endurance réalisé en normoxie ou en hypoxie.

Figure 8. D'après l'enquête ObEpi-Roche 2012. Distribution de la population française en fonction de l'IMC en 2012.

Figure 9. Réalisation de la mesure du tonus artériel périphérique par pléthysmographie (PAT).

Figure 10. Valeurs normales de VOP en fonction de l'âge

Figure 11. Dispositif expérimental de la NIRS. Positionnement des optodes.

Figure 12. Dispositif expérimental de l'épreuve d'effort maximale.

Abréviations

AP	Activité physique
AVC	Accident vasculaire cérébral
BPCO	Broncho-pneumopathie chronique obstructive
CO₂	Dioxyde de carbone
ECG	Electrocardiogramme
EPO	Erythropoïétine
ERO	Espèces réactives de l'oxygène
EVA	Echelle Visuelle Analogique
FC	Fréquence cardiaque
FiO₂	Fraction inspirée d'oxygène
Hb	Hémoglobine
HbO₂	Oxyhémoglobine
HbTot	Hémoglobine totale
HC	Hypoxie continue
HF	Haute fréquence
HHB	Déoxyhémoglobine
HI	Hypoxie intermittente
HIF-1	Facteur inductible par l'hypoxie
HIIT	High intensity interval training. Exercice Intermittent Intense
HTA	Hypertension artérielle
HVR	Réponse ventilatoire à l'hypoxie
IMC	Indice de masse corporelle
IRM	Imagerie par Résonance Magnétique Nucléaire
LF	Basses fréquences
Lipoxmax	Point d'oxydation maximale lipidique
L-NA	<i>N</i> ^ω -nitro-L-arginine
MAM	Mal aigu des montagnes
MAPA	Monitoring Ambulatoire de la Pression Artérielle
NIRS	Spectroscopie Proche InfraRouge
NO	Monoxyde d'azote
NOS	Monoxyde d'azote synthase

O₂	Oxygène
OCHA	Œdème cérébral de haute altitude
OMS	Organisation mondiale de la santé
OPHA	Œdème pulmonaire de haute altitude
PaO₂	Pression partielle artérielle en oxygène
PAP	Pression artérielle pulmonaire
PAT	Tonus artériel périphérique
Pb	Pression barométrique
PCGL	Point de croisement glucido-lipidique
PetCO₂	Pression partielle de fin d'expiration en dioxyde de carbone
PetO₂	Pression partielle de fin d'expiration en oxygène
PHDs	Enzymes prolyl-hydroxylase
PMA	Puissance Maximale aérobie
PPC	Pression positive continue
QC	Débit cardiaque
SaO₂	Saturation artérielle en oxygène
SAOS	Syndrome d'apnées obstructives du sommeil
SV	Seuil ventilatoire
TSI	Index de Saturation Tissulaire
VE	Débit ventilatoire
VFC	Variabilité de la fréquence cardiaque
VLf	Très basses fréquences
VO_{2max}	Consommation maximale d'oxygène
VOP	Vitesse de l'onde de pouls

INTRODUCTION GENERALE

Introduction générale

L'exposition à l'hypoxie en milieu naturel (montagne : hypoxie hypobarique) ou artificielle (hypoxie normobare) déclenche des réponses physiologiques (respiratoires, cardio-circulatoires, hématologiques) adaptatives lorsqu'elle est modérée ou mal-adaptative (mal aigu des montagnes, voire œdème aigu du poumon de haute altitude) lorsqu'elle est sévère, brusque et/ou en cas de susceptibilité des sujets qui la subissent.

L'hypoxie sévère entraîne des effets délétères bien documentés dans le cadre pathologique et en particulier dans le syndrome d'apnées obstructives du sommeil (SAOS). En effet, une hypoxie sévère cyclique nocturne (de 30-90 s) constitue un mécanisme central à l'origine des effets délétères du SAOS. En particulier, les séquences de désaturation-ré-oxygénation entraînent un stress oxydatif et une inflammation systémique de bas grade ainsi qu'une hyper-activation sympathique, à l'origine de la morbidité cardio-métabolique associée au SAOS (hypertension, arythmie, accident vasculaire cérébral (AVC), coronaropathie, insulino-résistance/diabète de type 2...) [1].

Cependant de nombreux arguments suggèrent que l'exposition hypoxique entraîne des adaptations positives lorsque l'exposition est plus modérée (moins sévère, plus progressive). En effet, les sportifs l'utilisent depuis de nombreuses années en combinaison avec leur entraînement en vue d'améliorer la performance selon plusieurs modalités (ex : live high train low et vice versa) et des méthodes plus ou moins complexes [2] avec plus ou moins de succès [3].

D'autres études suggèrent également que l'exposition hypoxique est susceptible de protéger voire de traiter la pathologie. Par exemple, des données épidémiologiques suggèrent une moindre prévalence de l'obésité dans les populations vivant en altitude modérée [4-6]. Ainsi, la prévalence de l'obésité est abaissée de 24% environ chez les américains vivant au-delà de 3000 m par rapport à ceux vivant en dessous de 500 m [6]. D'autre part, il existe des données suggérant que l'hypoxie intermittente normalement considérée comme délétère dans le SAOS peut conférer une certaine protection vis-à-vis des événements ischémiques [7, 8] et notamment stimuler le développement de vaisseaux coronaires collatéraux [9]. Les effets potentiellement protecteurs de l'hypoxie intermittente ont également été mis en évidence par des études cliniques suggérant que les patients victimes d'un AVC avaient moins de séquelles quand l'AVC était précédé

d'accidents ischémiques transitoires (et donc d'un stress hypoxique relativement modéré) dans le même territoire vasculaire [10, 11].

Un certain nombre d'études animales ont décrit par le passé les effets protecteurs d'un préconditionnement hypoxique vis à vis de stimuli délétères tels que l'ischémie [12] (cf. annexe I). Ces effets protecteurs dépendent du modèle et de la sévérité de l'exposition. Cependant, ces études expérimentales de préconditionnement rapportent essentiellement des effets aigus (i.e. une seule séance d'exposition hypoxique) conférant une protection sur une période limitée. Malgré tous ces traitements ouvrent des perspectives sur la santé à plus long terme en cas d'exposition prolongée répétée, i.e. conditionnement hypoxique. Une recherche en physiopathologie humaine dans ce domaine pour traiter la morbidité cardio-métabolique notamment, est en plein essor depuis une dizaine d'années, même si peu d'études randomisées contrôlées avec des effectifs suffisants sont disponibles à l'heure actuelle. Les effets décrits sont souvent contrastés et obtenus avec une multitude de protocoles allant d'une exposition hypoxique continue, à une exposition intermittente, combinée ou non à l'exercice musculaire [12] (cf. tableau 1, annexe I). De plus la fréquence, la durée et la sévérité de l'exposition hypoxique sont extrêmement variables. Il semble important de rechercher les modalités d'exposition hypoxique optimales pour la santé parmi celles qui ont été proposées : exposition au repos continue, exposition au repos intermittente et exposition hypoxique combinée à l'exercice. Enfin, dans un cadre clinique il est important d'étudier l'effet de l'hypoxie sur des marqueurs représentatifs de la santé cardio-métabolique [12].

Cette thèse vise donc à étudier les effets de différents conditionnements hypoxiques pour améliorer la santé cardio-métabolique. Plusieurs expérimentations ont été menées afin d'examiner les effets de plusieurs stratégies de conditionnement hypoxique sur cette santé cardio-métabolique :

1. *réponses physiologiques à court terme à 2 stratégies de conditionnement hypoxique* (**publication 1**) – étude préliminaire physiologique chez le sujet sain permettant de vérifier l'impact à court terme (3 séances randomisées, plan d'étude croisé en simple aveugle) de 2 types de conditionnement i.e. hypoxie continue (**HC**) versus hypoxie intermittente (**HI**). Cette étude exploratoire chez le sujet sain avait pour but de caractériser les effets à court terme (3 séances) des adaptations cardio-

circulatoires (pression artérielle, variabilité sinusale), respiratoires et de l'oxygénation tissulaire (cerveau, muscle) de ces 2 modes de conditionnement hypoxique.

2. *Étude de recherche clinique HYPINT (modalités de repos) (publication 2)* – cette partie de l'étude de recherche clinique HYPINT vise à établir les modalités d'exposition chronique hypoxique optimales pour traiter la morbidité cardiovasculaire et métabolique associée à l'obésité. Dans ce but, nous avons étudié les effets à moyen terme (2 mois de supervision en milieu hospitalier, 24 séances) de 2 stratégies d'exposition hypoxique au repos : l'hypoxie continue (HC) versus intermittente (HI), comparé à une exposition « placebo » en normoxie (N). Nous avons fait l'hypothèse que ces 2 types de conditionnement hypoxique au repos entraîneraient des améliorations cardiovasculaire et métabolique de la personne en surpoids/obèse.
3. *Réponses physiologiques à la combinaison d'un exercice d'intensité modérée ou de haute intensité et d'une exposition hypoxique en aiguë (publication 3)* – étude préliminaire physiologique réalisée chez le sujet sain afin de vérifier la faisabilité et de caractériser la réponse cardio-respiratoire et l'oxygénation tissulaire au cours d'un exercice aigu à charge constante d'intensité modérée ou d'intervalle à haute intensité en hypoxie (EH). Ces réponses ont été comparées à celles induites par des exercices induisant une charge interne de travail similaire (fréquence cardiaque similaire) réalisés en normoxie (EN). Cette caractérisation a permis de fournir les éléments nécessaires au déroulement et à l'interprétation de l'étude 4.
4. *Étude de recherche clinique HYPINT (modalités d'exercice) (publication 4)* – cette partie de l'étude de recherche clinique vise à tester l'impact d'un programme d'entraînement à l'effort en hypoxie sur ergocycle (modalité d'exercice à charge constante d'intensité modérée similaire à l'étude 3) en vue d'améliorer la condition physique et de traiter la morbidité cardiovasculaire et métabolique associée à l'obésité (EH). Dans ce but, nous avons comparé chez le sujet en surpoids/obèse l'EH à un programme d'entraînement en normoxie (EN) imposant la même charge de travail interne (fréquence cardiaque similaire) que dans le programme EH.

PREMIERE PARTIE : REVUE DE LITTERATURE

1. Hypoxie

1.1. Définition

L'hypoxie désigne une baisse de la biodisponibilité en oxygène (O_2) d'un milieu par rapport à un autre où la disponibilité en oxygène est considérée comme normale [13]. Elle est produite par la diminution de la pression partielle inspirée en oxygène (PO_2), qui réduit la capacité de diffusion de l'oxygène dans le sang et dans les tissus. Cette PO_2 est égale à la fraction de l'oxygène dans l'air (FiO_2) multipliée par la pression barométrique (P_b): $PO_2 = FiO_2 \times P_b$. La FiO_2 en air ambiant équivaut à 20.93% [14].

Il existe deux types d'hypoxie:

- L'hypoxie hypobarique, est liée à la diminution de la P_b associée à l'altitude. En effet, cette pression égale à environ 760 mmHg au niveau de la mer, diminue d'une façon exponentielle pour atteindre la moitié de sa valeur à une altitude de 5500 m [14]. A noter que la FiO_2 reste stable quelle que soit l'altitude.

- L'hypoxie normobare, qui est créée artificiellement en diluant l'air ambiant par de l'azote, ce qui diminue la FiO_2 et donc la PO_2 inspirée.

Selon la durée de l'exposition, l'hypoxie peut être aiguë (quelques secondes voire minutes) ou chronique (plusieurs heures voire jours ou semaines)[13].

Bien que l'hypoxie chronique ait été considérée comme un facteur aggravant dans certaines maladies cardiovasculaire et respiratoire, plusieurs études ont montré des effets bénéfiques de l'exposition à l'hypoxie au niveau cardiorespiratoire dans les modèles animaux [12, 15, 16] et chez l'homme, particulièrement les hypertendus [17] et les sujets en surpoids [18].

Les études réalisées au cours de cette thèse mettent en œuvre une hypoxie intermittente (**HI**) / continue (**HC**), normobare, aiguë ou chronique.

1.2. Conséquences physiologiques de l'hypoxie d'altitude

L'exposition à l'environnement hypoxique déclenche des réponses physiologiques réflexes dont le but est de préserver l'apport d'oxygène aux tissus. La basse altitude (en dessous de 1000 m) n'entraîne aucune réponse physiologique significative ni au repos ni à l'exercice. Par contre, en haute montagne, entre 2000 m et 5500m, le manque de l'O₂ peut entraîner une hyperventilation et une tachycardie [14]. Ces effets sont très variables et dépendent de chaque individu. L'adaptation de l'organisme aux différentes contraintes hypoxiques se déroule selon plusieurs phases [14]:

Phase d'accommodation

Lors d'une exposition à l'altitude de quelques heures à deux-trois jours, on observe une phase d'accommodation. L'altitude provoque une hypoxémie (baisse de la pression partielle artérielle en oxygène dans le sang), qui induit des augmentations de fréquence (FC) et débit cardiaque (Qc) et de la ventilation. L'hyperventilation et la tachycardie sont des phénomènes immédiats en haute montagne (2000 à 5500m). Pendant les premières heures d'exposition (6-8 h), les symptômes de maladaptation, notamment le mal aigu des montagnes (MAM), ne sont pas encore apparus, on parle de « phase blanche ». En effet ce dernier n'apparaît qu'après 8 à 12 h d'exposition et est maximal au bout de 24-48 h.

Phase d'acclimatation

Si l'exposition se prolonge de plusieurs jours à plusieurs semaines, des phénomènes d'adaptation plus stables et plus « économiques » pour le métabolisme se mettent en place. On retrouve une stimulation de l'érythropoïèse avec une augmentation du nombre d'hématies permettant l'augmentation du transport de l'O₂ dans le sang. Parallèlement, l'hyperventilation augmente progressivement pour maintenir voire augmenter la pression partielle artérielle en O₂ (PaO₂), cette hyperventilation entraîne une alcalose liée à l'hypocapnie (alcalose respiratoire). Enfin, on peut constater une diminution de la distance de diffusion au niveau des tissus avec facilitation du passage de l'oxygène vers les tissus.

C'est dans les premiers jours d'exposition à une altitude donnée que peuvent se manifester des signes pathologiques plus graves, mais quand le processus arrive à un point d'équilibre on parle alors d'acclimatation.

Si le sujet reste trop longtemps à une altitude trop élevée, des signes d'intolérance et de dégradation (physiques et psychologiques) peuvent également apparaître.

1.3. Principales adaptations à l'hypoxie d'altitude

1.3.1. Réponse ventilatoire

L'acclimatation ventilatoire est un facteur important pour réduire la sévérité du stress hypoxique au niveau des tissus et participe au maintien relatif de la performance physique en altitude [19-21]. L'hyperventilation réflexe se manifeste immédiatement lors de l'ascension; le débit ventilatoire (VE) augmente, et donc la pression partielle artérielle en gaz carbonique diminue (hypocapnie). Cette réponse ventilatoire qui vise à maintenir un apport d'O₂ adéquat [22] s'explique par la stimulation des chémorécepteurs carotidiens qui provoque une hyperventilation réflexe et une stimulation cardiovasculaire d'origine sympathique.

Après quelques minutes d'hyperventilation et suite à une désensibilisation des chémorécepteurs à l'hypoxie [23], le VE diminue progressivement pour atteindre un nouveau plateau après 15 à 20 minutes d'exposition (**figure 1**) [24]. Ceci peut être considéré comme un premier signe d'acclimatation ventilatoire à l'hypoxie. Dans le cas d'une exposition prolongée durant plusieurs heures, parfois plusieurs semaines, le débit ventilatoire ré-augmente d'une façon lente et progressive en raison d'une hausse de la sensibilité des chémorécepteurs carotidiens à l'hypoxie [25].

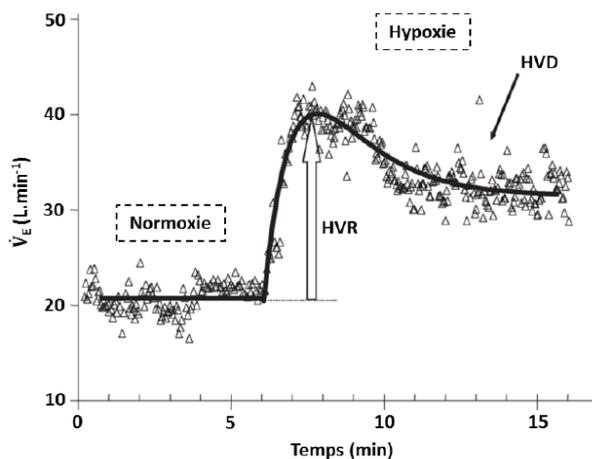


Figure 1. D’après Duffin et coll. [26]. La réponse biphasique de la ventilation au cours de 15 minutes d’exposition à l’hypoxie chez l’adulte représentée par le débit ventilatoire (\dot{V}_E , mesuré par la méthode cycle par cycle). L’intensité de la réponse ventilatoire à l’hypoxie (HVR) conditionne la diminution du \dot{V}_E (HVD).

Les facteurs génétiques peuvent influencer la réponse ventilatoire à l’hypoxie, expliquant les différences inter-individuelles au sein de la population [27].

1.3.2. Réponse cardiovasculaire

Après une exposition immédiate à l’hypoxie, hypobare ou normobare, le débit cardiaque de repos augmente. Chez 23 sujets sains respirant pendant 10 min une fraction d’oxygène inspiré $FiO_2 = 0,12$ (correspondant à 4000m d’altitude environ), la PaO_2 diminue de 40 ± 1 mmHg (**figure 2**), et le débit cardiaque augmente de 22%, essentiellement en raison d’une augmentation de fréquence cardiaque (+18%), alors que le volume d’éjection systolique est peu modifié. Cette augmentation de débit cardiaque compense la diminution du contenu artériel en oxygène afin que son apport aux tissus soit préservé. Cette observation suggère qu’en hypoxie, dans les conditions de repos, chez des sujets sains, l’apport d’oxygène aux tissus est assuré par une adaptation à court terme du débit cardiaque [28].

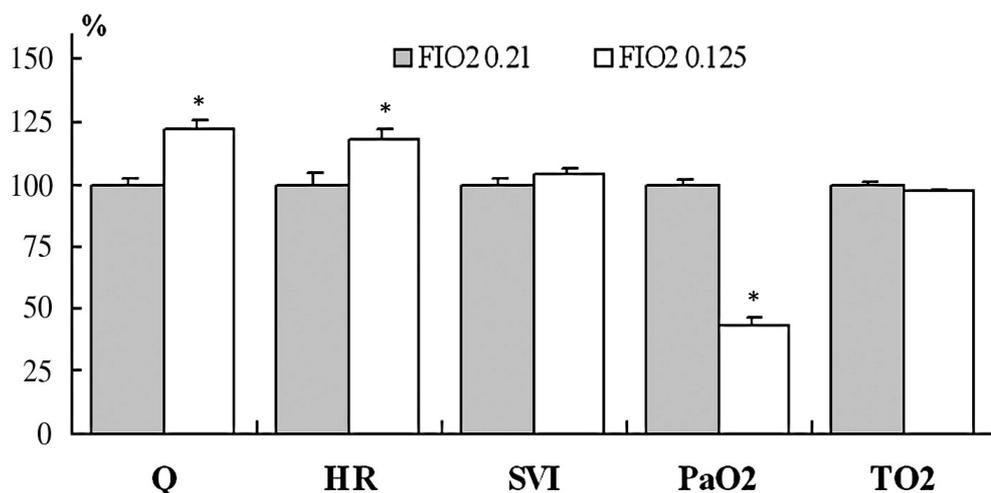


Figure 2. D'après Naeije [28]. Moyenne \pm Ecart type en pourcentage de variations de débit cardiaque (Q), de fréquence cardiaque (HR), de volume systolique (SVI), de la pression partielle d'oxygène (PaO₂) et de l'apport en oxygène (TO₂) chez 24 sujets soumis à une hypoxie normobare de courte durée.

Cependant, cette réponse cardiovasculaire à l'hypoxie est transitoire, car le débit cardiaque de repos retourne à son niveau de base voire légèrement en dessous en quelques jours [29, 30]. Ceci est rendu possible par une augmentation de l'extraction tissulaire en O₂. En revanche, la fréquence cardiaque continue d'augmenter et parfois le volume d'éjection systolique diminue en raison d'une diminution légère du volume sanguin. Puis ces 2 variables se stabilisent après 8 jours. Ceci est illustré sur la **figure 3** qui représente l'évolution du débit cardiaque, du volume d'éjection systolique et de la fréquence cardiaque chez 5 sujets pendant les 8 premiers jours d'exposition à une altitude de 3800 m [29]. Cette situation se stabilise dans le temps.

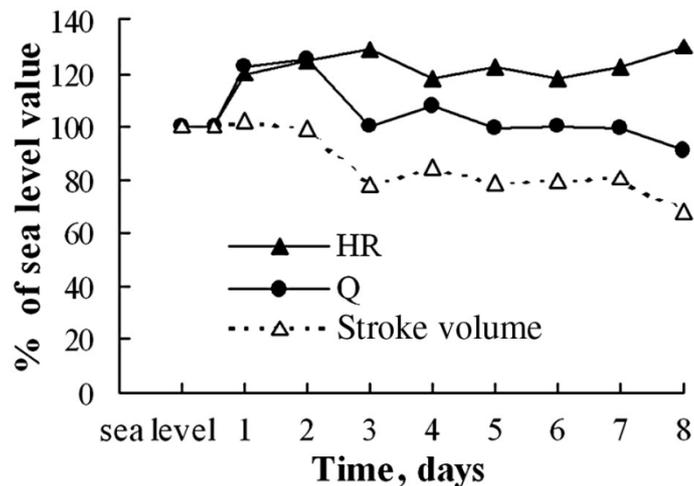


Figure 3. D'après Klausen [29]. Fréquence cardiaque (HR), débit cardiaque (Q) et volume d'éjection systolique pendant les 8 premiers jours d'acclimatation à une altitude de 3800 m. Q et HR augmentent dès le début de l'exposition. Après 8 jours, Q retourne à sa valeur de base pré-hypoxique, le volume d'éjection systolique a diminué et la fréquence cardiaque a augmenté.

Les résultats concernant l'évolution de la pression artérielle sont contrastés. Certaines études montrent une légère augmentation de pression artérielle dès le début du séjour alors que d'autres ne retrouvent pas de variation [31, 32].

Lors d'une exposition aiguë à l'hypoxie, on observe en revanche clairement une augmentation de la pression artérielle pulmonaire (PAP) en raison d'une vasoconstriction pulmonaire hypoxique qui peut provoquer une hypertension artérielle pulmonaire significative [28, 33].

1.3.3. Réponse hématologique

La synthèse d'érythropoïétine (EPO), est l'une des réponses naturelles qui se manifeste suite à une exposition à l'hypoxie. Cette réponse stimule l'érythropoïèse permettant l'augmentation du transport de l'O₂ dans le sang [14]. La sécrétion d'EPO dépend du niveau d'altitude, elle augmente de 20 à 30 % à 2000 m et de 300 % à 4500 m [34]. Les facteurs génétiques peuvent aussi influencer cette sécrétion, ce qui explique la variabilité interindividuelle [35]. L'altitude provoque immédiatement une diminution du volume plasmatique et une augmentation de la concentration en hémoglobine ([Hb]). Selon Schuler et coll. [36], la [Hb] augmente de 15% après 21 jours d'exposition à 2300 m d'altitude (**figure 4**). Cette augmentation est considérée comme un signe d'adaptation

à l'hypoxie chronique [37]. Plusieurs études montrent une élévation de 40% de la [Hb] après acclimatation en haute altitude (+ 5000 m) [38, 39].

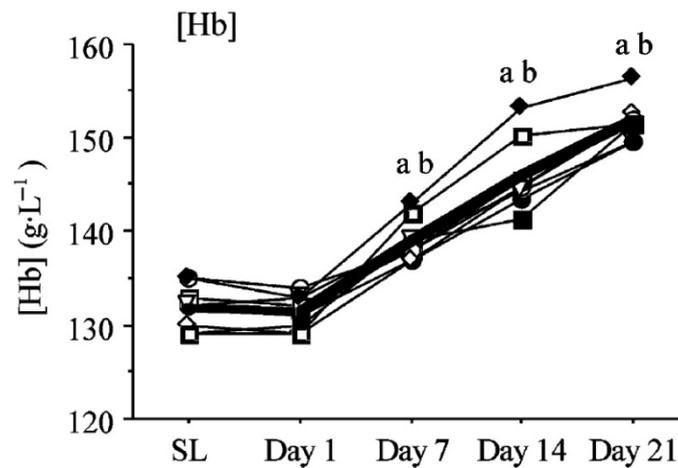


Figure 4. Valeurs individuelles et moyennes (trait épais) des concentrations d'hémoglobine ([Hb]) chez 8 sujets lors d'une exposition à 2340 m d'altitude durant 21 jours. Différence significative par rapport au niveau de la mer ($p < 0,05$) : **a** ; différence significative par rapport au jour précédent ($p < 0,05$) : **b**. La moyenne est représentée par la ligne épaisse [36].

Selon Sawka et coll. [40], la masse des globules rouges reste constante après un séjour de 3 semaines à 4000 m. De même, Gore et coll. [41] et Abellán et coll. [42] ne rapportent pas de variation dans la production de globules rouges après 4 semaines d'hypoxie intermittente (3 h/jour, 5 jours par semaine) entre 4000 – 5000 m. Par contre, après 6 mois d'exposition à une hypoxie intermittente (11 jours à 3550 m et 3 jours au niveau de la mer), la production de globules rouges augmente légèrement (11%) [43, 44]. De plus, cette production s'accroît de 40% après un séjour de 126 jours à 5500 m [45] ou 1 an à 4550 m [46]. On peut donc conclure que la production de globules rouges et l'augmentation de sa masse totale dépend du niveau d'altitude et du temps d'exposition.

1.3.4. Réponses métaboliques et effet sur le poids

L'hypoxie hypobarique continue peut induire une perte de poids [47]. Plusieurs études ont montré une diminution de 5% à 15% du poids corporel après une exposition en altitude [48, 49]. L'hypoxie normobare est susceptible aussi d'induire une perte de poids. Cependant, peu de données rapportent les effets de l'hypoxie normobare sur le poids et il existe peu de recherches comparant les effets de l'hypoxie normobare et hypobare [50].

Les causes de cette perte de poids sont multifactorielles. En haute altitude, l'appétit et l'apport énergétique sont souvent réduits. Plusieurs études rapportent une diminution de 30% à 50% de l'apport énergétique lors des séjours en altitude [49, 51]. Après acclimatation à 4300 m, l'appétit s'améliore généralement, mais la plupart des études suggèrent que l'appétit diminue aussi au-delà de 5000 m [52]. Les hormones de la satiété sont également influencées par l'hypoxie. La concentration plasmatique de leptine en particulier, une hormone anorexigène produite par le tissu adipeux augmente significativement après 17 h d'exposition nocturne à l'hypoxie normobare simulant 4100 m [52].

1.4. Conséquences physiopathologiques de l'hypoxie

Comme évoqué précédemment, le corps humain développe de nombreuses réponses physiologiques permettant de lutter contre le manque d'O₂ [53]. Lorsque ces réponses ne sont pas suffisantes, l'adaptation à l'hypoxie peut déclencher des pathologies liées à la haute altitude [54], telles que le mal aigu des montagnes (MAM), l'œdème pulmonaire de haute altitude (OPHA) et l'œdème cérébral de haute altitude (OCHA). D'après Richalet et Herry [14], l'altitude à partir de laquelle les effets pathologiques de l'altitude peuvent être déclenchés dans les conditions de repos est environ de 3000 m. Cependant, dès 2000 m des effets pathologiques peuvent être observés chez les sujets sensibles. Ils surviennent généralement après 6-8 heures d'hypoxie.

L'apparition d'un MAM est liée essentiellement à une diminution de la SaO₂ ainsi qu'à une sensibilité individuelle [32]. Le MAM est un syndrome qui regroupe un ensemble de symptômes non spécifiques qui peuvent apparaître au-delà de 2500 m. Il associe divers symptômes comme nausées, vomissements, céphalées, anorexie, asthénie,

vertiges, sensation d'ébriété, anorexie, insomnies, dyspnées, diminution de la diurèse. Une échelle et un score (le Lake Louise score en particulier) peuvent prédire l'apparition du MAM ou déterminer la gravité de celui-ci [55]. L'OCHA est la version exacerbée du MAM auquel se surajoute une hypertension intracrânienne conduisant à des troubles neuro-psychiques pouvant aller jusqu'au coma voire être létale. L'OPHA, associé entre autres à une hypertension artérielle pulmonaire, peut conduire à une insuffisance respiratoire aiguë grave pouvant également être fatale.

1.5. Lien entre hypoxie et pathologie

Plusieurs pathologies respiratoires obstructives peuvent être à l'origine d'une hypoxie chronique telle que le syndrome d'apnées obstructives du sommeil (SAOS) et la broncho-pneumopathie chronique obstructive (BPCO).

Le syndrome d'apnées obstructives du sommeil est une pathologie qui induit une succession de périodes d'hypoxie-ré-oxygénation brutales et donc une hypoxie intermittente (HI) qui affecte 15 à 24% des adultes [56], elle est caractérisée par une interruption (apnée) ou une diminution (hypopnée) du flux respiratoire répétés dans le temps au court du sommeil. Ce phénomène, lié à un collapsus pharyngé, entraîne une hypoxémie transitoire et une hypercapnie ainsi qu'une stimulation du système nerveux sympathique responsable de micro-éveils nocturnes et d'une altération de la structure du sommeil [57]. Cette dernière est responsable d'une somnolence diurne, des maux de tête et une diminution de la concentration [57]. L'hypoxie intermittente associée au SAOS lorsqu'elle est sévère peut induire une morbidité cardio-vasculaire métabolique voire cognitive. Les comorbidités associées au SAOS illustrent ainsi dans le contexte des pathologies respiratoires les effets potentiellement délétères de l'HI. Une quantité élevée d'espèces réactives de l'oxygène (ERO) sont produits suite à la variation de la pression partielle en oxygène pendant les apnées nocturnes [58-60] et induisent un stress oxydatif responsables des troubles cardio-vasculaires et métaboliques [61].

Plusieurs études ont été menées sur l'association entre le SAOS et le syndrome métabolique [62, 63]. Les sujets atteints de SAOS présentent, dans la majorité des cas, un surpoids ou une obésité, ce qui explique en partie les troubles du flux respiratoire [64]. Pour comprendre les mécanismes à l'origine de la corrélation entre l'HI chronique et les dysfonctions métaboliques, Drager et coll. [65] ont montré une augmentation de la synthèse lipidique hépatique chez les souris exposées de manière intermittente à

l'hypoxie sévère (FiO_2 minimale = 5%), pendant 12 heures (de 9h à 21h) avec une SpO_2 cible entre 70% et ~ 99%, ce qui est similaire au profil d'oxyhémoglobine en cas de SAOS sévère. Par ailleurs, l'HI diminue la sensibilité à l'insuline et perturbe donc l'équilibre glycémique [66-68].

Outre le syndrome métabolique, le SAOS est associé à des troubles cardiovasculaires, telles que l'augmentation de la pression artérielle, l'hypertrophie ventriculaire et un dysfonctionnement du ventricule gauche [69, 70]. Tous ces éléments augmentent le risque d'infarctus du myocarde, d'arythmie et d'hypertension (HTA) [71, 72].

En raison de l'importance du sommeil, les apnées du sommeil peuvent également conduire à une altération du système cognitivo-comportemental. En effet, cette pathologie a été reliée à une somnolence diurne excessive et à des troubles mentaux, de la personnalité et du comportement psycho-social [73, 74].

Nous pouvons donc considérer que l'hypoxie intermittente chronique telle qu'associée au syndrome d'apnées du sommeil entraîne un certain nombre de dysfonctions de l'organisme et que la sévérité de ces comorbidités est d'autant plus importante que la fréquence des cycles est élevée et que la SaO_2 est faible [75].

En outre, une hypoxémie peut survenir dans les stades avancés des maladies respiratoires chroniques telles que la broncho-pneumopathie chronique obstructive (BPCO). Cette dernière aggrave le tableau clinique et les symptômes dont les principaux sont la dyspnée, et la fatigue retentissant négativement sur la qualité de vie. Cette hypoxémie qui entraîne une insuffisance respiratoire, lorsque la PaO_2 devient inférieure à 55 mmHg entraîne notamment des complications cardiovasculaires qui peuvent être graves et conduire à une insuffisance cardiaque. En effet, contrairement à la circulation systémique dans laquelle l'hypoxie induit une vasodilatation artériolaire, l'hypoxie au niveau de la circulation pulmonaire entraîne une vasoconstriction artérielle [76]. Si elle persiste dans la durée, l'hypoxie entraîne une hypertension pulmonaire [77]. La vasoconstriction pulmonaire hypoxique est le résultat de différents mécanismes qui affectent les cellules musculaires lisses vasculaires [78, 79] : diminution de la libération de vasodilatateurs produits par l'endothélium [80] et sécrétion accrue de médiateurs vasoconstricteurs [81, 82]. Les principaux symptômes de l'hypertension pulmonaire sont la dyspnée, la fatigue, les douleurs thoraciques et la syncope dans sa forme la plus

sévère. La présence de symptômes est principalement liée à la dégradation progressive de la fonction ventriculaire droite à une insuffisance cardiaque résultante [83].

Par ailleurs, cette hypoxémie peut entraîner une dénutrition et une diminution de masse musculaire (diminution de la synthèse et activité protéolytique) avec un retentissement négatif sur la qualité de vie de ces patients [84]. Le stress oxydatif et l'inflammation qu'il génère (et qui entretient un stress oxydatif) joue un rôle important dans ces phénomènes délétères induits par l'hypoxie.

Synthèse

- ❖ L'hypoxie est une situation de stress cellulaire dans laquelle les tissus de l'organisme reçoivent une quantité insuffisante d'O₂.
- ❖ Il existe deux types d'hypoxie : hypobarique (liée à la diminution de la P_b associée à l'altitude) et normobare (créée artificiellement en réduisant la concentration en O₂).
- ❖ L'organisme s'adapte aux différentes contraintes hypoxiques selon deux phases successives : (i) l'accommodation pendant laquelle l'organisme déclenche immédiatement des mécanismes physiologiques pour contrebalancer la baisse de l'apport d'O₂, et (ii) la phase d'acclimatation pendant laquelle l'organisme développe des procédés plus économiques pour améliorer la capacité de transport de l'O₂.
- ❖ L'hypoxie déclenche des réponses physiologiques respiratoires, cardio-circulatoires et hématologiques.
- ❖ L'hypoxie peut également déclencher des réponses mal-adaptatives (mal aigu des montagnes, voire œdème pulmonaire de haute altitude et œdème cérébral de haute altitude) lorsqu'elle est sévère, brusque et/ou en cas de susceptibilité particulière des sujets qui la subissent.

2. Conditionnement hypoxique

2.1. Concept

Le principe du préconditionnement a été décrit pour la première fois à propos de l'ischémie myocardique [85] qui correspond en l'application d'une hypoxie sévère par réduction sévère du débit sanguin local. C'est une procédure par laquelle un stimulus délétère est appliqué à proximité mais en-deçà du seuil de dommages à l'organisme. En réponse à ce stimulus aigu, les tissus et les organes seront capables de répondre et mieux tolérer de futurs stimuli nocifs identiques ou similaires, empêchant ou réduisant les dommages qu'ils peuvent induire. Ce phénomène repose sur un principe de base : l'organisme développe des défenses complexes et actives pour lutter contre les conditions défavorables auxquelles il est exposé telles que la baisse d'oxygène [12].

Le préconditionnement provoque deux phases de protection : une précoce, 1 à 2 heures après le stimulus et l'autre retardée, environ 24 à 72 heures après le stimulus [86, 87]. Cette protection peut être induite par de nombreux stimuli, tels que l'ischémie, l'hypoxie, l'hypothermie. Les agents pharmacologiques peuvent également induire une réaction de préconditionnement et modifier les réponses de l'organisme à des conditions de stress ultérieures [88].

La stratégie de préconditionnement ischémique décrite par Murry et coll. [85] montre que les brefs épisodes d'ischémie (4 cycles de 5 min d'occlusion / reperfusion) avant une ischémie de 40 min rend la cellule plus résistante à l'ischémie prolongée et réduit la taille de l'infarctus. Récemment, des stratégies de conditionnement appliquées après une ischémie focale et une reperfusion (c'est-à-dire post-conditionnement) ont été proposées comme approche protectrice pour réduire la lésion et améliorer la récupération au lieu de pré-conditionner le cœur par de courtes séquences d'ischémie-reperfusion réalisées avant l'ischémie prolongée. Chez le chien par exemple, après une ischémie létale de l'artère interventriculaire antérieure, l'alternance de cycles ischémie/reperfusion entraîne une réduction de 44% de la taille de l'infarctus [89]. Le rétablissement peut non seulement être amélioré par une intervention dans les moments qui suivent une ischémie aiguë, mais aussi par des interventions appliquées au cours des jours suivant l'agression [12]. Chez le rat, après une lésion cérébrale d'origine ischémique, une exposition en hypoxie intermittente à raison de 4 h par jour pendant 7

jours à une $FiO_2 = 12\%$ augmente la neurogénèse et préserve l'apprentissage spatial et la mémoire [90].

L'hypoxie, étant le stimulus majeur du préconditionnement ischémique, a donc aussi été étudiée en tant que facteur potentiel susceptible d'induire un préconditionnement.

Il existe deux grands types d'exposition à l'hypoxie susceptibles d'induire un conditionnement. Certaines études ont utilisé l'hypoxie d'une façon intermittente pendant plusieurs séances, c'est-à-dire une variation de la FiO_2 consistant en une alternance de phases d'hypoxie et de normoxie [91] (3-7 min en hypoxie suivie de 3-5 min en normoxie répété 3-10 fois) [92-95]. En revanche, d'autres études ont utilisé l'hypoxie d'une façon continue pendant plusieurs séances, ce qui correspond à une diminution de la disponibilité en oxygène sur une certaine durée [96-98].

Plusieurs études présentent les effets de préconditionnement induits par l'hypoxie intermittente (HI) au niveau du cœur et du cerveau, notamment chez le rat soumis à différentes modalités d'exposition à l'hypoxie aiguë [15, 99, 100]. Par rapport à la normoxie, une hypoxie intermittente modérée à sévère ($FiO_2 = 10\%$) appliquée pendant 4 h améliore la tolérance myocardique à l'ischémie et réduit la taille de l'infarctus cardiaque induit 24 h après le conditionnement. Une hypoxie continue (HC) modérée à sévère appliquée pendant 4 h pourrait ne pas avoir d'effet protecteur équivalent à l'HI. De même, l'application d'une hypoxie intermittente très sévère ($FiO_2 = 5\%$) peut augmenter la taille de l'infarctus (**figure 5**). De ce fait, on constate que l'effet de l'hypoxie sur la taille de l'infarctus dépend du type (intermittente vs continue) et de la sévérité ($FiO_2 = 10$ vs 5%) de l'exposition hypoxique [15].

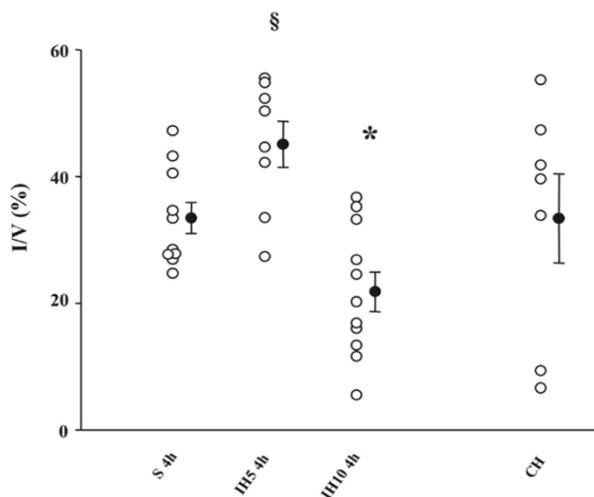


Figure 5. D'après Beguin et coll. [15]. La taille de l'infarctus (I) exprimée en pourcentage de la masse ventriculaire (V) évaluée après une ischémie globale (30 min) -reperfusion (120 min) dans différents groupes de souris exposés 4h à la normoxie (S), à l'hypoxie intermittente (IH, $FiO_2 = 5$ ou 10%), ou à l'hypoxie chronique (CH). * $P < 0.05$ vs les autres groups; § $P < 0.05$ vs S; 4 h, et IH10, 4 h.

En outre, les effets protecteurs du préconditionnement hypoxique ont également été décrits au niveau cérébral. La pré-exposition à l'hypoxie peut prolonger la survie après une anoxie en conservant le métabolisme cérébral [101], témoignant d'une faculté d'adaptation possible du cerveau à l'anoxie [102]. La plupart des expositions à l'hypoxie intermittente induisent une hypoxémie hypocapnique (à cause de l'hyperventilation), tandis que le syndrome d'apnées obstructives du sommeil induit une hypoxémie hypercapnique. Par conséquent, les différences entre les effets délétères du syndrome d'apnées obstructives du sommeil et les effets protecteurs du préconditionnement hypoxique pourraient tenir non seulement à la sévérité du stress induit par l'hypoxie intermittente, mais aussi à une différence relative au niveau de capnie.

En ce qui concerne le modèle d'hypoxie **intermittente** chronique **hypobarique**, les effets bénéfiques de l'adaptation à la haute altitude ont été caractérisés par une exposition prolongée à une altitude entre 5000-7000 m pendant plusieurs heures (4-8 h) suivie d'un retour à la normoxie. La répétition de ce stimulus a été réalisée sur plusieurs jours. Ainsi, chez des rats exposés à une haute altitude simulée (5000 m, 8 h/jour, 5 jours/semaine, 24-32 jours) la nécrose myocardique [103], l'arythmie ventriculaire [104-106] et l'apoptose [107] induites par une ischémie-reperfusion sont

diminuées. Globalement, l'exposition à ce modèle d'hypoxie améliore la tolérance à l'ischémie myocardique, et induit moins d'effets indésirables par rapport à l'hypoxie chronique continue [108, 109].

D'autre part, des études récentes ont étudié les effets protecteurs de l'exposition chronique répétée à l'hypoxie intermittente **normobare**. Chez la souris, Stowe et coll. [110] ont montré que le conditionnement hypoxique constitué par des cycles répétés d'hypoxie (2 ou 4 h, $FiO_2 = 8$ ou 11%) pendant 2 semaines protège contre l'accident vasculaire cérébral transitoire (AVC) pendant les 8 semaines suivantes, avec réduction de l'inflammation post-ischémique. Une série de stimuli de conditionnement hypoxique répétitifs (2 h, $FiO_2 = 8$ ou 11%) pendant 12 jours peut également induire une protection de la rétine qui peut perdurer jusqu'à 4 semaines [111].

L'exposition hypoxique peut donc être utilisée pour déclencher un phénomène de conditionnement lorsqu'elle est appliquée au repos, soit en aiguë (préconditionnement provoquant des effets disparaissant sous 72 h environ) soit de façon chronique (stimulus de conditionnement appliqué de façon répétée pendant plusieurs jours et induisant un état de protection capable de perdurer plusieurs semaines ensuite). L'exposition hypoxique peut également être combinée à l'exercice (entraînement physique en hypoxie). Sa combinaison à l'hyperoxie a également été proposée [112, 113] mais cet aspect n'a pas été étudié dans cette thèse.

2.2. Les mécanismes

La durée de la protection induite par le conditionnement hypoxique peut donc être prolongée en augmentant la durée du protocole de conditionnement [114]. Ceci peut être obtenu grâce à une exposition répétée à l'hypoxie qui déclenche des mécanismes de protection supplémentaires et perdurant dans le temps.

Quel que soit le type d'exposition, aigu ou répété, le conditionnement hypoxique s'accompagne de modifications dans l'expression des gènes. Le facteur inductible par l'hypoxie (HIF-1) est un activateur de transcription impliqué dans la régulation de l'expression de gènes activés dans des conditions d'hypoxie. Le facteur HIF-1 est une protéine constituée de deux sous-unités α et β . La sous-unité HIF-1 β est exprimée de

manière constante et indépendante de l'oxygène tandis que HIF-1 α est sensible à l'oxygène.

En normoxie, l'hydroxylation de la proline du HIF-1 α par les enzymes prolyl-hydroxylase (PHDs) entraîne l'ubiquitinylation et la dégradation du protéasome. En hypoxie, l'hydroxylation et la dégradation de HIF-1 α sont réduites à cause de la limitation du substrat (O₂), de sorte que HIF-1 α se déplace vers le noyau pour lier HIF-1 β et activer la transcription de divers gènes destinés à maintenir l'homéostasie cellulaire afin de lutter contre le manque de l'oxygène [115].

Le monoxyde d'azote (NO) en particulier est produit lors de l'oxydation par les NO synthases (NOS). Il joue un rôle essentiel dans le préconditionnement et la cytoprotection grâce à son effet vasodilatateur ainsi que sa capacité à moduler la fonction mitochondriale [116]. Le *N* ^{ω} -nitro-L-arginine (L-NA), un inhibiteur des NOS, fait disparaître l'effet protecteur du préconditionnement hypoxique sur la réduction de la taille de l'infarctus [117]. Ceci montre la relation entre la formation de NO et l'effet cardioprotecteur du préconditionnement hypoxique. Trois isoformes de la NOS ont été identifiées : la NOS neuronale, endothéliale et inducible. La NOS inducible est régulée par l'activation de HIF-1 suite à une exposition aiguë à l'hypoxie intermittente [100, 118]. Les médiateurs de l'inflammation peuvent induire une expression de la NOS inducible dans différentes cellules vasculaires notamment les cellules endothéliales, les cardiomyocytes, etc. De plus, GATA-4, un facteur de transcription impliqué dans la régulation de l'apoptose est connu pour activer l'expression génique des protéines anti-apoptotiques, bcl-2 et bcl-x (L) suite à une exposition à l'hypoxie intermittente aiguë chez les souris [119]. De même, d'autres médiateurs bien connus pour jouer un rôle dans le préconditionnement ischémique classique sont également déclenchés par une exposition aiguë à l'hypoxie intermittente. Beguin et coll. [15] ont mis en évidence le rôle des canaux potassiques (K_{ATP}) ATP dépendant et la protéine kinase C (PKC), la p38 mitogen-activated kinase (MAPK) et la kinase régulée par le signal extracellulaire (ERK1/2) [99] dans la cardio-protection induite par une exposition de 4 h à une hypoxie intermittente modérément sévère (FiO₂ = 10%).

Bien que les effets de HIF-1 s'estompent rapidement en phase de ré-oxygénation, nous avons démontré dans notre laboratoire que l'HI induit une activation marquée et soutenue de HIF-1 (ensemble de l'organisme) qui persiste même après l'exposition à l'HI. Ceci peut s'expliquer par le stress oxydatif produit par la répétition des séquences d'oxygénation-désoxygénation. En effet, les espèces réactives de l'oxygène (ERO) sont de puissants inducteurs de HIF-1 en augmentant l'expression de la protéine HIF-1 α et en inhibant sa dégradation dépendante de la prolyl-hydroxylase [115]. Une exposition répétée à l'HI pourrait donc entraîner l'activation de gènes cytoprotecteurs supplémentaires qui ne sont pas ou insuffisamment exprimés lors d'une hypoxie continue ou d'une exposition de courte durée à l'HI [12].

Il a également été montré que l'HI chronique favorise l'expression du gène de l'érythropoïétine (EPO) [120]. L'EPO est un puissant agent protecteur contre les lésions ischémiques. Sa liaison aux récepteurs de l'EPO active de nombreuses voies de signalisation protectrices, telles que la voie Janus kinase 2 (JAK2) / transducteur de signal et activateur de transcription 3 (STAT3), la voie phosphatidylinositol 3-kinase (PI3K) / protéine kinase B (Akt), et la voie MAPK, toutes connues pour conférer une cytoprotection, en particulier une cardioprotection [121], une néphroprotection [122] et une neuroprotection [123]. Une étude récente confirme le rôle central de la voie JAK2 / STAT3 dans un modèle d'HI chronique cardioprotecteur. L'activation de cette voie par une exposition de 4 semaines à l'HI chronique était directement liée à l'amélioration de la récupération fonctionnelle après reperfusion via le maintien de l'homéostasie intracellulaire du Ca²⁺ et de la fonction mitochondriale [124]. L'implication d'autres voies de signalisation liées à l'EPO, telles que la voie PI3K/Akt, a également été confirmée dans la limitation de la taille d'un infarctus suite à un conditionnement hypoxique intermittent chronique [125, 126].

Parmi les autres gènes protecteurs régulés par HIF-1 lors d'une exposition hypoxique à long terme on peut citer ceux codant pour des facteurs de croissance angiogéniques et des cytokines, tels que le facteur de croissance de l'endothélium vasculaire (VEGF), les angiopoïétines et les facteurs de croissance plaquettaires. Ainsi, les rats exposés à l'HI chronique présentent une augmentation marquée (1,5) de la capillarisation ventriculaire gauche, un flux coronarien pré-ischémique sur cœur isolés plus élevé et une meilleure récupération faisant suite à l'ischémie/reperfusion [127].

Par ailleurs, dans notre laboratoire, nous avons également observé une augmentation de la densité capillaire myocardique et de l'expression du VEGF chez des rats exposés à 35 jours de normobaric HI [128]. Cette augmentation de l'angiogenèse pourrait non seulement contribuer à la cytoprotection conférée par le conditionnement hypoxique répété, mais pourrait aussi expliquer pourquoi la protection dure plus longtemps que celle obtenue avec les protocoles de conditionnement hypoxique aigu [12].

Enfin, les protéines de stress thermique (HSP), en particulier HSP70, également régulée par HIF-1 relaient les effets cytoprotecteurs de l'HI chronique [129]. Les HSP70 agissent comme des chaperons moléculaires pour réparer ou éliminer les protéines dénaturées par des stress tels que ischémie / reperfusion, conduisant à la protection et / ou à la restauration de la fonction cellulaire [130]. Ainsi, il a été démontré que le préconditionnement hypoxique renforce la signalisation HSP70 dépendant de HIF-1 avec des effets bénéfiques sur l'apoptose rénale ischémique et l'autophagie [129] ainsi que sur l'apparition d'arythmies ventriculaires induites par ischémie/reperfusion [131].

Les ERO occupent jouent probablement un rôle important dans les mécanismes qui président aux effets protecteurs de l'hypoxie intermittente à condition que cette dernière soit modérée. En effet, chez le rongeur, dans un modèle d'HI sévère mimant l'apnée du sommeil, un excès de stress oxydatif a été impliqué dans l'aggravation de la taille d'un infarctus lors de l'ischémie / reperfusion (sur cœur isolé) par rapport à la normoxie. En effet, la sensibilité myocardique à l'ischémie est diminuée grâce après un traitement au Tempol, un anti-oxydant [132]. Par contre, une exposition à l'HI plus modérée entraîne une augmentation plus mesurée des ERO permettant une augmentation des défenses anti-oxydantes comme après un programme d'exercice chronique [133]. Ce dernier permet d'endiguer un éventuel excès d'ERO. Les séquences d'oxygénation/désoxygénation qui caractérisent une HI modérée peuvent renforcer les défenses anti-oxydantes [134, 135].

2.3. Conditionnement hypoxique chez le sujet sain

Depuis plus de 40 ans, l'entraînement en hypoxie est utilisé pour améliorer la performance aérobie chez les athlètes [2]. Vivre et/ou s'entraîner dans des conditions hypoxiques peut améliorer les performances physiques en favorisant des adaptations en particulier hématologiques et musculaires. Plusieurs études sur ces types

d'entraînement hypoxique ont rapporté des résultats prometteurs, notamment en termes de gain de performance [136], même si beaucoup de ces effets restent encore débattus. Le conditionnement hypoxique chez les sujets sains a été évalué comme une intervention potentiellement utile pour améliorer certaines fonctions physiologiques et les facteurs de risque cardiovasculaires aigu et chronique.

2.3.1. Effets hématologiques

D'un point de vue hématologique, l'exposition à l'hypoxie intermittente hypobarique (altitude simulée entre 4000 et 5500 m, 3-5 h/jour) durant neuf jours est suffisante pour induire une augmentation significative de l'hématocrite, du nombre de globules rouges, des réticulocytes et de la concentration en hémoglobine [96]. Chez l'athlète d'endurance on observe également une augmentation significative du nombre de réticulocytes (30%), de l'hémoglobine (4%) et de l'hématocrite (5%) après une exposition à l'hypoxie intermittente normobare pendant 18 jours (5 min en hypoxie à une $FiO_2 = 9-10\%$ suivies de 5 min en normoxie, 60 min par jour, deux fois par jour) [137]. De plus, même les niveaux d'hypoxie intermittente les plus faibles (2 heures par jour à une $FiO_2 = 13\%$ durant 12 jours) semblent suffisants pour améliorer l'érythropoïèse [138].

2.3.2. Effets ventilatoires

La réponse ventilatoire à l'hypoxie augmente suite à une exposition préalable à l'hypoxie au repos (**figure 6**) [97, 98, 138, 139] et à l'exercice chez des sportifs de haut niveau ainsi que chez des sujets peu entraînés [140, 141]. Cette augmentation peut être maintenue de 5 jours à deux mois en raison de la plasticité des chémorécepteurs. Par contre, l'exposition à une heure d'hypoxie par jour pendant 7 jours ($FiO_2 = 15.5\%$ correspond à une altitude d'environ 3000 m) ne semble pas affecter la chémosensibilité à l'hypoxie [139]. Par contre, Wille et coll. [142] montrent contrairement une élévation de la réponse ventilatoire lors d'un test de tolérance à l'hypoxie en suivant un protocole de durée identique mais pour une hypoxie plus sévère (7 x 1 h à 4500 m). D'autre part, ces auteurs observent une baisse du score de Lake Louise (questionnaire pour évaluer et diagnostiquer le mal aigu des montagnes), et une diminution des symptômes liés aux pathologies d'altitude. Ces résultats ont été confirmés suite à une exposition de quatre

heures à l'hypoxie (4300 m), cinq jours par semaine durant 3 semaines [143]. Ceci suggère l'importance du niveau de la dose d'hypoxie puisqu'une hypoxie modérée appliquée de façon intermittente ne semble pas avoir d'effet significatif sur la sensibilité des chémorécepteurs.

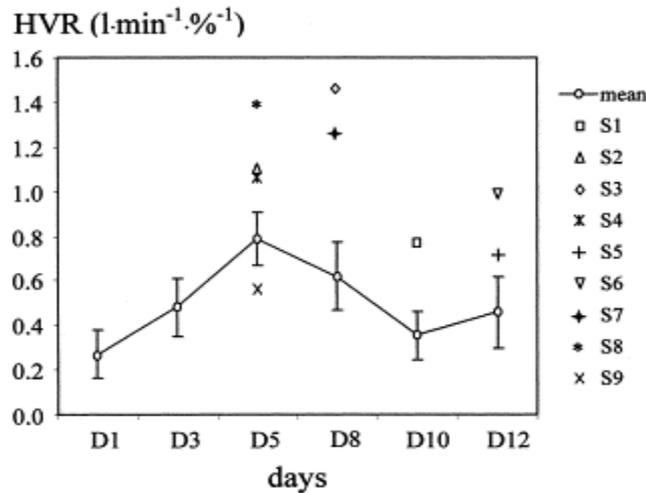


Figure 6. D'après Garcia et coll. [138]. Modifications de la réponse ventilatoire à l'hypoxie (HVR) durant 12 jours d'exposition à l'hypoxie intermittente (2 h par jour à $FiO_2 = 13\%$, équivalent à 3800 m d'altitude). Les symboles isolés indiquent la HVR maximale atteinte par chaque sujet durant le protocole. La valeur moyenne au 5^{ème} jour était significativement supérieure à celle du 1^{er} jour ($P < 0,05$).

2.3.3. Effets sur le système cardiovasculaire

Malgré les effets néfastes de l'hypoxie intermittente sévère chronique sur le système cardiovasculaire observés chez les patients qui souffrent de syndrome d'apnées obstructives du sommeil (SAOS) sévère, l'hypoxie intermittente modérée pourrait induire des adaptations cardiovasculaires positives. Quatorze sujets actifs et 21 sédentaires ont participé à l'étude de Shatilo et coll. [144]. Les sujets inclus dans cette étude étaient des hommes en bonne santé, âgés de 60 à 74 ans. Ils ont été exposés à une série de séances d'hypoxie normobare pendant 10 jours (cycles d'hypoxie $FiO_2 = 12\%$, 5 min, suivis par 5 min de normoxie, répétés quatre fois par jour). Chez les sujets sédentaires, les résultats montrent une diminution de la pression artérielle de $7,9 \pm 3,1$ mmHg et une augmentation de la capacité d'effort sous-maximale (+ 12,7%), soulignant les effets bénéfiques du conditionnement hypoxique au niveau cardiovasculaire chez les hommes sains âgés et sédentaires.

De plus la combinaison d'une exposition hypoxique et d'un entraînement en endurance (34 sujets physiquement actifs randomisés soit en normoxie soit en hypoxie: 20-30 min d'exercice x 3 séances/sem à 70-85% de la fréquence cardiaque maximale précédemment déterminée, pendant 4 semaines) en hypoxie (n = 18, FiO₂ ≈ 16%) montre une diminution de la pression artérielle systolique maximale d'exercice de 10 ± 9 mmHg et une augmentation de la consommation maximale d'oxygène (VO_{2max}) de 0,47 ± 0,77 L/min non observées en normoxie (n = 14, FiO₂ ≈ 20,9%) [145].

L'augmentation de la rigidité artérielle est associée à un risque cardiovasculaire important [146]. La rigidité artérielle chez l'adulte sain exposé à une seule session d'hypoxie (n = 12, SpO₂ ≈ 80% pendant 20 min) est diminuée par rapport à une situation normoxique [147]. Plusieurs chercheurs ont étudié les effets bénéfiques de la combinaison de l'exposition hypoxique et de l'entraînement physique sur la rigidité artérielle, surtout chez les femmes. Seize femmes ménopausées ont été randomisées en deux groupes (hypoxique hypobarique, simulant une altitude de 2000 m, 2 h/séance, x 4 jours par semaine pendant 8 semaines vs normoxie). Chaque groupe a réalisé des activités physiques en milieu aquatique sollicitant environ 50% de la consommation maximale d'oxygène, pendant 30 min. Seul l'entraînement en hypoxie a réduit de façon significative la rigidité artérielle chez les femmes ménopausées [148].

Finalement, ces résultats confirment les effets bénéfiques du conditionnement hypoxique sur la fonction cardiovasculaire chez le sujet adulte sain, quel que soient son âge et son sexe.

2.3.4. Effets sur le métabolisme et la prise alimentaire

Plusieurs études révèlent les effets bénéfiques de l'exposition à l'hypoxie intermittente modérée sur la glycémie, la cholestérolémie, l'activité enzymatique mitochondriale, la glycolyse et l'oxydation des acides gras [149-151]. Ainsi l'hypoxie semble jouer un rôle important sur la régulation de la glycémie et la sensibilité des cellules à l'insuline [152, 153]. Kelly et coll. [154] ont montré chez des sujets sains qu'une exposition continue à l'altitude simulée (4300 m) durant une semaine améliore la tolérance des cellules au glucose. Par ailleurs, il a été montré que l'exposition durant quelques jours voire quelques semaines à l'hypoxie hypobarique améliore le métabolisme basal dès les premiers jours d'ascension en montagne [155-157]. Cette

augmentation dépend de l'altitude à laquelle les sujets sont exposés : +6% à 3650 m, +10% et +27% à 3800 m et 4300 m, respectivement [155, 158, 159].

Il semblerait ainsi que l'hypoxie intermittente, soit en conditions de repos, soit combinée à l'entraînement à l'exercice, puisse améliorer l'état métabolique. Un entraînement en endurance en hypoxie (60 minutes de course sur tapis roulant à une intensité relative voisine du seuil lactique, trois fois par semaine pendant 4 semaines, $FiO_2 = 15\%$) entraîne chez le sujet normo-pondéré une réduction plus importante de la masse grasse, des concentrations plasmatiques de triglycérides et d'insuline à jeun qu'en normoxie (**Figure 7**) [160]. Ces effets bénéfiques ont été observés malgré une charge de travail absolue en hypoxie inférieure à celle de l'entraînement en normoxie.

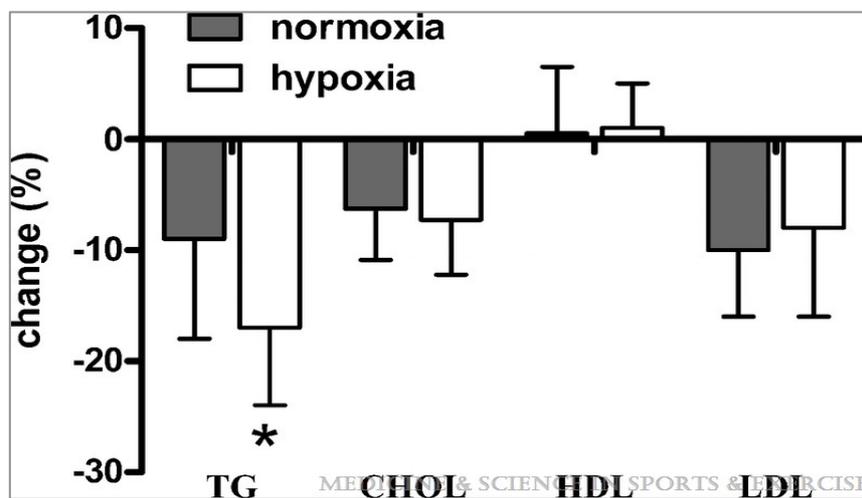


Figure 7. D'après Haufe et coll. [160]. Variations des concentrations plasmatiques de triglycérides (TG), de cholestérol (CHOL), de HDL et de LDL après un entraînement en endurance réalisé en normoxie ou en hypoxie. Les données représentent les moyennes \pm SEM. * $P < 0,05$.

L'exposition intermittente à l'hypoxie aiguë peut également modifier de façon significative la régulation de l'appétit. Bailey et coll. [161] ont rapporté chez 12 hommes en bonne santé, que 3 heures d'exposition hypoxique normobare incluant un exercice continu d'intensité modéré ou intermittent intense (pas de différence entre ces 2 modalités d'exercice) de 50 minutes, provoquait une suppression de l'appétit et une réduction des concentrations plasmatiques de ghréline acylée, une hormone orexigène.

Par contre, une heure par jour de conditionnement hypoxique modéré ($FiO_2 = 15\%$) ou sévère ($FiO_2 = 12\%$), 5 fois par semaine pendant 4 semaines ne modifie pas le poids de sujets sédentaires normo-pondérés ($20-23 \text{ kg/m}^2$) [162].

2.4. Conditionnement hypoxique chez le patient

Alors que certaines études réalisées chez le sujet sain montrent des effets bénéfiques de l'exposition à l'hypoxie intermittente, des études pilotes ont été menées dans diverses conditions pathologiques pour évaluer l'application clinique du conditionnement hypoxique.

2.4.1. Maladies cardiovasculaires

L'hypoxie intermittente a été proposée comme un moyen pour traiter *l'hypertension* chez l'homme. Après une exposition à l'hypoxie modérée de quelques séances ($FiO_2 = 10-14\%$) de 15 min à 4 h par séance, pendant 10 à 30 jours, Serebrovskaya et coll. [17] indiquent des améliorations notables de la pression artérielle systolique : $-10-30 \text{ mmHg}$. Il en est de même pour la pression artérielle diastolique qui diminue de $10-15 \text{ mmHg}$. Dans une autre étude plus récente, 37 sujets jeunes, de sexe masculin hypertendus (grade 1) vs 20 sujets contrôles sains, ont été exposés à 20 séances d'hypoxie intermittente (3 minutes à $FiO_2 = 10\%$ suivies de 3 min en air ambiant, le tout répété 4 à 10 fois). Une diminution significative et durable (3 mois chez 28 patients initialement hypertendus) de la pression artérielle a été observée suite à cette intervention. Les valeurs de pression artérielle post programme étaient normalisées puisque non différentes de celles d'un groupe contrôle normo-tendu [94]. Ainsi, le protocole de conditionnement hypoxique modéré était bien toléré par ces patients. Il pourrait représenter une stratégie thérapeutique intéressante, complémentaire aux traitements médicamenteux et prometteuse pour réduire la pression artérielle. Kong et coll. [163] ont proposé un entraînement en endurance-force combiné à un régime hypocalorique, pendant 4 semaines à 22 sujets jeunes en surpoids/obèses (17-25 ans, indice de masse corporelle $> 27,5 \text{ kg/m}^2$) randomisés soit en condition d'hypoxie normobare ($FiO_2 = 16,4 - 14,5\%$, trois séances de 2 h par semaine) soit en normoxie ($FiO_2 = 21\%$). Cette étude montre que l'exposition hypoxique améliore la tension artérielle systolique (-7.6%) et la pression artérielle moyenne (-7.1%).

Les stratégies de conditionnement hypoxique pourraient également améliorer la fonction cardiaque. Elles pourraient aussi prévenir les conséquences délétères précoces et retardées de l'**infarctus du myocarde**. Chez des souris, avant une ligature de l'artère coronaire gauche, 40 jours d'exposition à l'hypoxie intermittente hypobarique permettent de réduire la taille de l'infarctus du myocarde et de diminuer la mortalité [164]. La même intervention appliquée après la ligature peut réduire également le risque d'apparition d'une arythmie cardiaque [105]. Les protocoles d'hypoxie intermittente normobare induisent les mêmes effets car ils diminuent la taille de l'infarctus de 43% suite à une ischémie provoquée [165]. En revanche, chez l'homme, l'infarctus du myocarde n'est pas un événement prévisible, ce qui rend l'utilisation des stratégies similaires plus compliquée.

Zhuang et coll. [108] ont étudié l'effet bénéfique de l'hypoxie intermittente dans la **coronaropathie**. Un développement de vaisseaux coronaires collatéraux a été observé chez les patients atteints d'un syndrome d'apnées obstructives du sommeil sévère (indice d'apnées - hypopnées > 10 événements / h), ce qui suggère qu'une certaine dose d'hypoxie intermittente peut induire des adaptations de la circulation coronaire [8]. Dans une étude prospective contrôlée réalisée chez 16 hommes coronariens âgés de 50 à 70 ans, (8 avec antécédents d'infarctus du myocarde), les sujets ont été randomisés en double aveugle dans deux groupes [93]. Le premier groupe reçoit 15 séances d'hypoxie intermittente modérée pendant 3 semaines. Chaque séance comporte une variation de la FiO₂ permettant d'alterner des phases d'hypoxie et de normoxie : 3-5 min en hypoxie suivies de 3 min en normoxie, le tout répété 3 à 5 fois. L'autre groupe restait en normoxie (témoin, 3 semaines d'exposition). Seuls les patients ayant bénéficié du conditionnement hypoxique ont augmenté leur consommation maximale d'oxygène (+ 6.2%). Cette observation est remarquable tant chez les sujets avec ou sans antécédent d'infarctus du myocarde [93]. En outre, la fréquence cardiaque, la pression artérielle systolique, la lactatémie et la difficulté de l'effort perçue pendant un exercice sous-maximal (vélo à 1 W/kg) ont été diminuées chez les sujets exposés à l'hypoxie intermittente. Par conséquent, l'exposition à l'hypoxie intermittente pourrait améliorer les variables cardiovasculaires dans la coronaropathie.

Une amélioration de la fonction cardiaque a été observée après 4 semaines d'exposition à l'hypoxie intermittente chez les souris saines [166] et chez la souris

transgénique *insuffisante cardiaque* [167]. Par contre, chez les patients atteints d'insuffisance cardiaque, l'hypoxie intermittente n'a pas encore été testée cliniquement, mais des résultats encourageants concernant le conditionnement ischémique à distance (remote ischemic conditioning) sont actuellement disponibles [168].

Les résultats précliniques et cliniques actuels suggèrent que l'hypoxie intermittente peut être une option thérapeutique innovante et non pharmacologique dans les maladies cardiovasculaires. Des études cliniques supplémentaires, randomisées contrôlées, sont cependant nécessaires pour confirmer l'utilisation du conditionnement hypoxique en tant qu'outil thérapeutique [12].

2.4.2. Maladies respiratoires

Certaines données suggèrent que le conditionnement hypoxique peut également être envisagé chez les patients atteints de maladies respiratoires.

Selon Mateika et coll. [16] l'hypoxie intermittente peut être proposée comme traitement complémentaire pour le *Syndrome d'apnées obstructives du sommeil*, bien sûr en combinant l'exposition à l'hypoxie et les traitements classiques, notamment la ventilation nocturne en Pression Positive Continue (PPC). Des travaux de recherche supplémentaires sont nécessaires pour optimiser les protocoles d'exposition à l'hypoxie intermittente.

Burtscher et coll. [169] étudient l'intérêt de l'exposition à l'hypoxie intermittente modérée chez les malades souffrant de *bronchopneumopathie chronique obstructive (BPCO)* modérée. Les résultats montrent des effets bénéfiques tels que, l'augmentation de la masse totale d'hémoglobine, et les améliorations de la sensibilité du baroréflexe ainsi que la tolérance à l'effort. Par ailleurs, la réponse ventilatoire à l'hypercapnie est améliorée sans modification de la réponse ventilatoire à l'hypoxie [170].

2.4.3. Maladies métaboliques

L'hypoxie peut augmenter la dépense énergétique et est susceptible d'induire une perte de poids [18, 171]. Les sujets obèses soumis à une hypoxie hypobarique (1 semaine à 2650 m) présentent une perte de poids, une augmentation du métabolisme basal et une diminution de la prise alimentaire [172]. La combinaison de l'exposition

hypoxique et de l'activité physique pourrait potentialiser la perte de poids. Vingt sujets obèses (IMC = 33,1 kg/m²) ont participé à un programme d'entraînement durant 8 semaines (trois séances de 90 minutes par semaine) à une intensité proche de 60% de VO_{2max}. L'entraînement se déroulait en hypoxie normobare (FiO₂ = 15%) ou en normoxie (placebo, FiO₂ = 20,1%), sans intervention diététique. Les sujets du groupe hypoxique ont perdu significativement plus de poids que le groupe témoin (1,14 vs 0,03 kg) [173]. Une autre étude a été réalisée chez des sujets sédentaires, non diabétiques ou insulino-résistants, en surpoids ou obèses. Ils se sont entraînés sur tapis roulant, 60 min par jour, trois fois par semaine durant 4 semaines à environ 65% de leur consommation maximale d'oxygène. L'entraînement a été réalisé en normoxie (groupe normoxique, n = 21, FiO₂ = 21%) ou en hypoxie normobare (groupe hypoxique, n = 24, FiO₂ = 15%). Par rapport à la normoxie, l'intensité absolue d'exercice dans le groupe hypoxique était plus faible. Cependant, l'amélioration de la consommation maximale d'oxygène était similaire dans les deux groupes. La composition corporelle s'est améliorée davantage (c'est-à-dire une plus grande réduction de la masse grasse) dans le groupe hypoxique [174]. Récemment, Kong et coll. [163] ont proposé un entraînement en endurance-force combiné à un régime hypocalorique, pendant 4 semaines à 22 sujets jeunes obèses (17-25 ans, indice de masse corporelle > 27,5 kg/m²) randomisés soit à l'entraînement en hypoxie normobare (FiO₂ = 16,4-14,5%, trois séances de 2 h par semaine) soit à l'entraînement en normoxie (FiO₂ = 21%). La perte de poids a été significativement plus importante dans le groupe hypoxique. Pour cette population, dans leur revue de littérature, Urdampilleta et coll. [175] suggère un protocole de conditionnement hypoxique ciblant à la fois la perte de poids et l'amélioration de la l'aptitude aérobie. Ce protocole consisterait en une exposition à l'hypoxie intermittente trois fois par semaine, pendant 3-6 semaines (FiO₂ = 10-12%), avec entraînement physique. Par ailleurs, Hobbins et coll. [176] en conclusion de leur revue de littérature conseillent des intensités d'hypoxie, des durées d'exposition, des fréquences hebdomadaires, des durées de programme ainsi que des intensités d'exercice qu'il s'agissent d'un conditionnement passif ou actif (**Tableau 1**).

Variable	Type of exposure	
	Passive	Active
Level of hypoxia (FiO ₂ %)	10.0–12.0	13.0–14.0
Number of cycles	5–15	N/a
Intensity	N/a	55–65% VO _{2max} / 60–70% HR _{max}
Duration (hours)	1–1.5	1–1.5
Frequency	Daily	2–3 times per week
Periodisation (weeks)	2–4	4–6

FiO₂ = fraction of inspired oxygen; HR = heart rate; N/a = not applicable; VO_{2max} = maximal oxygen uptake.

Tableau 1. D'après Hobbins et coll. [176]. Résumé des protocoles de conditionnement hypoxique actif et passif pour l'amélioration de la santé cardio-métabolique et favoriser la perte de poids chez les sujets en surpoids ou obèses.

En se basant sur des données précliniques, le conditionnement hypoxique a été testé aussi chez les diabétiques de type 2. L'exposition hypoxique aiguë au repos et à l'exercice augmentent la sensibilité à l'insuline [177]. Cependant, cette sensibilité à l'insuline a été mesurée dans les 12 à 24 h suivant la dernière séance d'exposition hypoxique, suggérant un effet aigu de la dernière séance et non une amélioration liée à la chronicité du programme. Des études cliniques supplémentaires sont nécessaires afin de vérifier les effets bénéfiques du conditionnement hypoxique prolongé chez les patients diabétiques

Synthèse

- ❖ Le préconditionnement hypoxique permet à l'organisme de développer des défenses complexes et actives pour lutter contre la baisse d'oxygène.
- ❖ Il existe deux types d'exposition à l'hypoxie : intermittente (variation de la FiO_2 consistant en une alternance de phases d'hypoxie et de normoxie) et continue (diminution de la FiO_2 maintenue sur une certaine durée).
- ❖ Les différences entre les effets délétères du syndrome d'apnées obstructives du sommeil et les effets protecteurs du préconditionnement hypoxique pourraient tenir non seulement à la sévérité du stress induit par l'hypoxie intermittente, mais aussi aux différences de capnie.
- ❖ Les effets du préconditionnement hypoxique au niveau du cœur et du cerveau semblent dépendre du type : intermittente vs continue. Des études sont nécessaires pour déterminer la modalité la plus efficace.
- ❖ La persistance de la protection induite par le conditionnement hypoxique peut être prolongée en augmentant la durée du protocole de conditionnement.
- ❖ Le conditionnement hypoxique chez les sujets sains a été évalué comme une intervention potentiellement utile pour améliorer certaines fonctions physiologiques et les facteurs de risque cardiovasculaires aigu et chronique.
- ❖ Les résultats précliniques et cliniques actuelles dans diverses conditions pathologiques suggèrent que l'hypoxie intermittente peut être une option thérapeutique innovante et non pharmacologique dans plusieurs maladies cardiovasculaires et métaboliques.
- ❖ Des études cliniques supplémentaires sont cependant nécessaires pour confirmer l'utilisation du conditionnement hypoxique en tant qu'outil thérapeutique.

3. Obésité

3.1. Définition

Selon l'Organisation mondiale de la santé (OMS), le surpoids et l'obésité se définissent comme une « accumulation anormale et excessive de graisse corporelle qui peut nuire à la santé ». La sévérité de l'obésité est basée sur l'indice de masse corporelle (IMC) = poids/taille² exprimé en kg/m². On parle d'un surpoids si l'IMC se situe entre 25 et 29,9 kg/m² et d'une obésité si l'IMC est égal ou supérieur à 30 kg/m² (tableau 2) [178]. Cet indice a été critiqué car il ne permet pas de distinguer la masse musculaire de la masse grasse. Cependant, il peut être complété par la mesure du tour de taille [179], prédictif de la graisse viscérale, un facteur de morbidité cardio-métabolique important de l'obésité.

Classification	IMC (kg/m ²)
Maigreur	< 18.5
Normal	18.5 – 24.9
Surpoids	25 – 29.9
Obésité, classe I	30 – 34.9
Obésité, classe II	35 – 39.9
Obésité, classe III	> 40

Tableau 2. Classification du surpoids et de l'obésité chez l'adulte en fonction de l'indice de masse corporelle d'après l'OMS [178].

3.2. Epidémiologie

L'obésité est devenue un problème majeur de la santé publique. Selon l'OMS, le nombre de cas d'obésité dans le monde a triplé depuis 1975. En effet, en 2016, presque 2 milliards d'adultes étaient en surpoids parmi lesquels 650 millions avaient atteint le stade de l'obésité, soit 13% de la population adulte mondiale. Dans la plupart des régions du monde, les femmes sont plus sujettes à l'obésité que les hommes ; 11% des hommes et 15% des femmes étaient obèses en 2016. En 2012,

l'enquête ObEpi-Roche [180] montrait que 32,3% des Français adultes présentaient un surpoids et que 15% étaient obèses (**figure 8**).

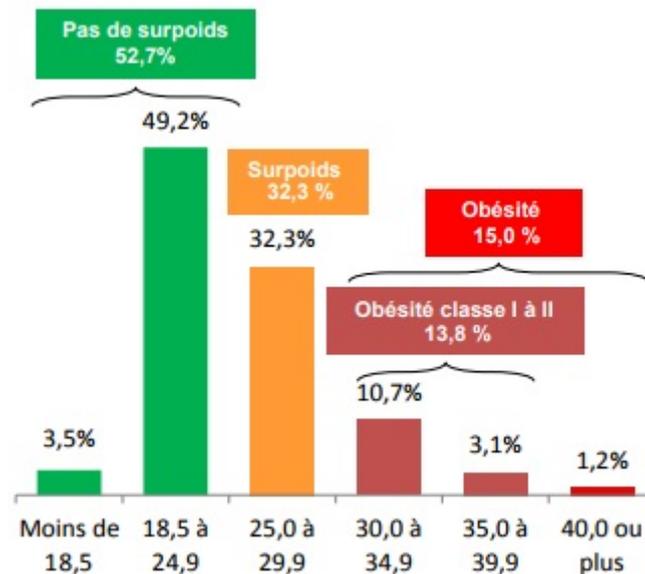


Figure 8. D'après l'enquête ObEpi-Roche [180]. Distribution de la population française en fonction de l'IMC en 2012.

Un nombre très élevé de décès annuels dans le monde est attribué au surpoids et à l'obésité. Le risque de mortalité augmente progressivement avec l'augmentation de l'IMC particulièrement lorsqu'il dépasse 30 kg.m⁻² [181-183]. La relation entre l'IMC et la mortalité est généralement une courbe en forme de U (la mortalité est plus faible autour de 23 kg.m⁻²) avec une mortalité accrue aux niveaux des extrêmes (valeurs très faibles et très élevées).

Plusieurs maladies associées au surpoids et à l'obésité sont responsables de cette mortalité : les maladies cardiovasculaires, le diabète, les maladies rénales et le cancer [184]. La perte de poids faisant par exemple suite à une chirurgie bariatrique réduit la mortalité liée aux maladies cardiaques, au diabète et au cancer [185, 186].

3.3. Causes de l'obésité

L'obésité est un phénomène complexe qui résulte d'un déséquilibre chronique de la balance énergétique [187], c.à.d. lorsque l'apport énergétique est supérieur à la dépense énergétique (trop grande sédentarité, niveau d'activité physique, insuffisant, régime alimentaire non équilibré).

Un certain nombre de facteurs contribuent de manière importante à cette prise de poids [187]:

- Facteurs environnementaux et comportementaux: la nourriture riche et abondante, la diminution des dépenses énergétiques en raison du style de vie moderne, les relations familiales, l'arrêt du tabac non accompagné de mesures adaptées...
- Facteurs endocrinologiques: dérèglements hormonaux et/ou glandulaires, notamment la grossesse et la ménopause,
- Facteurs biologiques et particulièrement la génétique : effectivement, si les deux parents sont obèses, l'enfant a un risque majoré de 80% de devenir obèse et si l'un des deux parents est obèse, il a un risque majoré de 40% [188]. La part des facteurs génétiques dans l'obésité est estimée à 25-40% voir 50 à 70% pour certains auteurs.
- Statut socio-économique et les facteurs psychologiques [189]

3.4. Conséquences physiopathologiques

Du point de vue cellulaire, le tissu adipeux assure le stockage et la libération d'énergie, l'isolation thermique et la protection des organes internes contre les traumatismes [190]. Les adipocytes sont les cellules de stockage des graisses qui stockent l'énergie sous forme de gouttelettes lipidiques riches en triglycérides. La régulation de l'absorption et du stockage de l'énergie est principalement maintenue par l'insuline. L'accumulation de graisse caractérisant l'obésité résulte d'un bilan positif de la balance énergétique (excès calorique). Les adipocytes subissent une hypertrophie et/ou une hyperplasie, ce qui entraîne une expansion du tissu adipeux pour permettre le stockage des calories excédentaires. Cette hypertrophie et son hypoxie [191] du tissu adipeux et son dépôt ectopique notamment autour des viscères sont à l'origine d'une inflammation bas grade et d'un stress oxydant qui portent une lourde responsabilité dans la morbidité cardio-métabolique associée à l'obésité.

L'inflammation bas grade liée à l'excès de graisse infiltrée par les macrophages en particulier dans le tissu adipeux viscéral (hypertrophie adipocytaire) mais aussi dans le muscle, se traduit par la sécrétion de cytokines pro-inflammatoires (telles que le TNF- α) qui contribuent à l'insulino-résistance musculaire [192, 193]. La présence de cette

infiltration macrophagique (et donc de la présence d'une architecture inflammatoire) du tissu adipeux conditionne l'insulino-résistance et par voie de conséquence une augmentation du risque cardio-vasculaire [194]. Un stress oxydant peut résulter de l'inflammation et l'entretenir. Il peut être amplifié par un dysfonctionnement mitochondrial et exerce des effets délétères directs sur la signalisation insulinique [193]. Il est en partie responsable de la dysfonction endothéliale prédictive du risque cardio-vasculaire [195].

Il existe une relation étroite entre l'insulino-résistance et la dysfonction endothéliale résultant d'une diminution de la production de NO (limitation de la vasodilatation) due à l'inflammation ou à l'excès d'acide gras libres circulants [196]. Les mécanismes de cette altération semblent liés à l'inactivation de la NO Synthase endothéliale (blocage de la phosphorylation de la sérine 1177) par l'inflammation ou l'excès d'acides gras libres circulants [197]. Cette altération de la cascade de signalisation de l'insuline au niveau vasculaire peut aussi tenir à une diminution de la biodisponibilité du NO par un excès d'espèces oxygénées radicalaires (NO « quenching ») [198]. L'insuline est d'autre part particulièrement impliquée dans l'activation de la microcirculation musculaire et notamment dans la stimulation du réseau capillaire musculaire nutritif [199], améliorant ainsi l'imprégnation et la distribution musculaires du glucose. Ainsi l'insulino-résistance peut altérer la fonction microcirculatoire qui peut à la fois aggraver et expliquer une partie de l'IR du sujet obèse ou diabétique de type 2 [200].

L'inflammation chronique et le stress oxydatif sont donc responsables de l'apparition des problèmes cardio-métaboliques associés à l'obésité [201], comme l'insulino-résistance, le diabète type 2, l'athérosclérose, l'insuffisance cardiaque etc... [202, 203]. La protéine C-réactive ultra-sensible est le biomarqueur clinique de l'inflammation le plus souvent mesuré car c'est un bon marqueur du risque cardio-vasculaire [204, 205].

Selon Farrell et coll. [206], le système cardio-circulatoire des sujets obèses est moins efficace que celui de sujets normo-pondérés. En effet, l'aptitude cardiorespiratoire à l'effort est inversement proportionnelle à l'IMC. D'autre part, il a été montré que le risque d'insuffisance cardiaque augmente avec la sévérité de l'obésité [207]. De plus, selon l'OMS [208], le diabète de type 2 a été défini comme une des principales complications de l'obésité. D'autres études [209-212] se sont intéressées aux

liens entre syndrome inflammatoire et IMC. Le syndrome inflammatoire provoque des lésions de l'endothélium vasculaire [213], tout comme la répétition de régimes alimentaires au cours du temps [214]. Ces altérations structurelles sont responsables d'une dysfonction vasculaire pouvant rendre compte de l'apparition d'un diabète de type 2 [215]. De nombreux chercheurs ont étudié la relation entre la dysfonction endothéliale et l'obésité [216-220]. La vasodilatation endothéliale est altérée dans l'obésité et particulièrement dans l'obésité viscérale [221], expliquant son lien avec le risque cardiovasculaires.

3.5. Traitement de l'obésité

La perte de poids devrait être recommandée en présence d'affections comorbides chez tous les patients obèses ou en surpoids. Les traitements doivent être adaptés à la gravité du surpoids, aux maladies chroniques associées et aux limitations fonctionnelles. Pour la plupart des patients qui ont besoin de perdre du poids pour des raisons médicales, l'objectif initial devrait être une perte de poids de 5 à 10% au cours des 6 premiers mois [222].

En dehors des traitements chirurgicaux, comme la chirurgie bariatrique, la lutte contre l'obésité repose sur l'association d'une normalisation des apports caloriques et d'une augmentation de la quantité d'activité physique, qui permet, d'une part, de limiter l'accumulation des graisses et, d'autre part, de maintenir la masse maigre [223]. Un programme bien adapté entraîne une perte de poids moyenne de 5 à 8% [224] et environ 60 à 65% des patients perdent 5% ou plus de leur poids initial [225]. Cette faible perte de poids peut suffire à entraîner des améliorations significatives des marqueurs de la santé métabolique, tout en diminuant les risques de comorbidité [226].

Bien que, contrairement aux idées reçues, l'activité physique régulière ne soit pas un facteur direct et important de perte poids important, elle constitue un adjuvant essentiel à la prise en charge de l'obésité [227]. D'autre part, en plus des bénéfices psychologiques sur l'humeur, le bien-être et l'estime de soi [228], l'activité physique améliore la santé cardio-métabolique du sujet obèse.

3.5.1. Effet de l'activité physique sur la santé métabolique

Shook et coll. [229] ont récemment examiné la relation entre l'apport énergétique, l'activité physique, l'appétit et la prise de poids pendant un an. Les personnes pratiquant une faible activité physique avaient un appétit supérieur comparativement à ceux qui avaient des niveaux élevés d'activité physique. De façon intéressante, les auteurs indiquent que le seuil d'équilibre énergétique entre les apports et la dépense caloriques correspond à 7116 pas par jour.

La masse musculaire joue un rôle important dans le métabolisme de repos, la locomotion et l'équilibre glycémique [230, 231]. Elle permet aussi de réaliser les mouvements et les activités de la vie quotidienne [232]. Il a été montré que l'activité physique permet de limiter la perte de masse musculaire entraînée par une restriction calorique. En effet, 12% seulement du poids perdu correspond à la perte de cette masse maigre lorsque qu'une activité est pratiquée contre 25% si ce n'est pas le cas [233].

Un des effets importants de l'exercice musculaire régulier est l'augmentation des possibilités d'oxydation lipidique [234], expliquant la diminution du risque de développer un diabète de type 2 [235] si on considère la théorie lipo-centrique du diabète 2 qui consiste en une accumulation ectopique de graisse autour des viscères, dans le foie et les muscles [236]. A noter que ces effets bénéfiques peuvent se produire indépendamment d'une perte de poids [237] même si l'insulino-sensibilité s'améliore plus lorsque l'exercice induit une diminution de masse grasse viscérale [238]. Ainsi, 2.5 h par semaine de marche rapide permet une réduction du risque de Diabète de type 2 de 30% [239].

Plusieurs pistes peuvent être évoquées pour expliquer les effets bénéfiques de l'exercice sur la sensibilité à l'insuline chez le sujet obèse: (i) en premier lieu, l'amélioration de la capacité de transport et d'oxydation des acides gras au niveau mitochondrial entraîne une diminution du contenu musculaire mais aussi du niveau de saturation des lipides intra-myocytaires [240] (ii) l'AP peut diminuer les niveaux de TNF-a produit par les monocytes [241] et d'autres cytokines pro-inflammatoires [242], pouvant constituer un des mécanismes explicatifs de l'effet protecteur de l'exercice vis-à-vis du diabète de type 2 et donc des maladies cardiovasculaires et [242, 243] (iii) l'AP peut diminuer le stress oxydatif, en excès dans l'obésité et le diabète de type II, contribuant également à l'insulino-résistance [244].

L'activité physique améliore également les variables métaboliques classiquement impliqués dans la santé cardio-vasculaire. Un programme de réentraînement, permet d'augmenter le niveau de HDL-cholestérol (+5%) [245, 246] et de diminuer le niveau de triglycérides. La diminution des triglycérides dépend de la concentration initiale : la diminution la plus importante a été observée chez les sujets inactifs présentant un taux élevé de triglycérides à l'état basal [245, 247]. En revanche, l'exercice a peu d'effets sur le niveau de LDL-cholestérol en l'absence de perte de poids [245, 248]. En effet, il existe une corrélation entre les variations de LDL-cholestérol et le poids [248] : pour chaque kilogramme de poids perdu, on observe une diminution de 0,8 mg.dL⁻¹ de LDL-cholestérol [249].

3.5.2. Effet de l'activité physique sur la santé cardiovasculaire

Comme évoqué précédemment, l'obésité joue un rôle important dans le développement des maladies cardiovasculaires [250], la pratique d'une activité physique régulière diminue la mortalité des sujets obèses, qui devient équivalente à celle des sujets normo-pondérés [251, 252]. En effet, le risque de mortalité lié aux capacités cardio-vasculaires des sujets obèses en bonne condition physique est même moindre que celui des sujets normo-pondérés sans pratique sportive [253, 254]. De plus, il a été montré que les sujets actifs diminuent le risque de développer une maladie cardio-vasculaire d'environ 30 à 35% [255]. Même chez les sujets très inactifs, une augmentation légère de l'activité physique réduit le risque d'événements coronariens et cardio-vasculaires. Ainsi, il a été montré que l'exercice réduit la tension artérielle chez les personnes hypertendues, normo-tendues [256] et obèses [257]. Chez la personne obèse cette amélioration de la pression artérielle peut résulter d'une diminution de l'insulino-résistance et de la cholestérolémie totale [258].

L'amélioration du profil inflammatoire constitue une des explications plausibles des effets protecteurs de l'AP régulière (modérée à intense) sur le risque cardio-vasculaire. Classiquement, un programme d'exercice modéré à intense peut entraîner une diminution de protéine C réactive, et de stress oxydatif [259, 260]. L'obésité, comme de nombreuses pathologies mettant en jeu une inflammation de bas grade et un excès de stress oxydatif s'accompagne d'une altération de la fonction endothéliale. Comme évoqué précédemment celle-ci est objectivée par une diminution de la réactivité

vasculaire liée à une diminution du taux de NO circulant en raison d'une réaction avec les radicaux. Cette réactivité vasculaire peut être améliorée par la perte de poids mais également par l'exercice [261].

En effet, à l'exercice aigu, l'augmentation du débit sanguin augmente les forces de frottement (« shear stress») sur l'endothélium vasculaire qui sécrète du NO. Ce dernier permet une vasodilatation locale et contribue ainsi à la redistribution du débit sanguin à l'exercice [262, 263]. La répétition de cet exercice (chronique) permet des modifications durables de la vasodilatation endothélium dépendante [264]. Cet effet peut apparaître dès 5 semaines d'entraînement en endurance modérée (60 % de VO_{2max} à raison de 5 séances de 30 min par semaine). Chez l'obèse, une modification du style de vie intégrant une restriction calorique et un programme d'exercice (3 séances de 30 minutes par semaine à 60-80 % de FC_{max}), améliore la fonction endothéliale d'environ 100 % [265].

L'exercice chronique peut corriger bon nombre des anomalies vasculaires par sa contribution à la diminution des concentrations d'acides gras libres circulants voire de $TNF-\alpha$ [266] susceptibles d'altérer la réactivité vasculaire et par une restauration de la production et NO [267] et/ou de sa biodisponibilité qui à terme peut entraîner des modifications structurales vasculaires. Le stimulus « exercice chronique » chez le sujet obèse a surtout été appliqué dans une perspective d'amaigrissement, ou dans le but d'améliorer les facteurs liés au risque cardiovasculaire (vasodilatation flux-médiée). Sur la base des études réalisées chez le rat Zucker (modèle de rat obèse insulino-résistant) [268, 269] et chez l'homme adulte [270, 271], il semble que l'exercice chronique augmente la densité capillaire dans l'obésité.

3.5.4. Recommandations

Selon l'association internationale pour l'étude de l'obésité, 30 minutes d'activité d'intensité modérée par jour sont efficaces et peuvent favoriser un maintien de la santé chez les sujets sains, obèses ou en surpoids [272]. En revanche, en vue de maintenir une perte de poids à long terme, ce volume d'activité est insuffisant. Ainsi il a été recommandé d'augmenter l'activité physique habituelle à 45-60 minutes d'activité d'intensité modérée ou de diminuer les apports énergétiques [272, 273]. De plus, Melzer et coll. [274] recommandent une pratique de 30 à 60 min d'effort quotidien avec une intensité comprise entre 3 et 6 MET (équivalents métaboliques) [274]. Par contre,

Donnelly et coll. [275] pensent que ce volume d'activité est toujours insuffisant pour favoriser la perte ou le maintien de poids et de bénéficier des effets favorables de l'activité physique sur les comorbidités associées. Ils recommandent de pratiquer 60 à 90 minutes par jour d'activité d'intensité modérée.

Il est intéressant de chercher des nouvelles modalités d'entraînement pour motiver la personne obèse. L'exercice prolongé d'intensité modérée à raison de 60 à 90 minutes suscite peu d'adhésion de la part de la population concernée (4 à 10%) [276]. Les raisons de ce manque d'adhésion sont en cours d'exploration (approche psychologique). Entre temps, des modalités plus ludiques et plus efficaces d'un point de vue physiopathologique ont été proposées notamment pour faire face au manque de temps invoqué par les personnes obèses pour expliquer leur manque d'engagement à l'activité physique. L'entraînement intermittent intense ou « High Intensity Interval Training » (**HIIT**) a été suggérée comme une stratégie prometteuse pour induire un certain nombre d'adaptations métaboliques habituelles (oxydation des graisses dans les muscles et amélioration de la tolérance au glucose) [277].

D'autres types d'interventions plus originales sont également à l'étude et notamment une exposition hypoxique passive ou active (combinaison entraînement physique en hypoxie). Cette modalité d'intervention (conditionnement hypoxique) constitue le cœur de cette thèse.

3.5.5. Hypoxie et obésité

La prévalence du surpoids ou de l'obésité dans la population vivant à haute altitude est plus faible [6]. L'hypoxie influence l'équilibre énergétique et elle s'accompagne souvent d'une perte de poids. Wu et coll. [278] ont constaté que les personnes obèses travaillant à une altitude supérieure à 4000 m pendant 3 à 5 mois perdent du poids. De plus, les sujets ayant un IMC plus élevé ont tendance à perdre plus de poids en altitude [279]. En effet, l'hypoxie affecte le système nerveux et les concentrations d'hormones tels que la leptine et modifie le métabolisme glucidique et le contrôle de l'appétit [18, 280]. Lors d'un séjour de 3 semaines à 1700 m d'altitude mettant en œuvre des activités physiques d'intensité modérée, Schobersberger et coll. [281] démontrent des effets favorables à court terme sur le système cardiovasculaire (pression artérielle) et sur plusieurs indicateurs métaboliques chez des sujets touchés par un syndrome métabolique. Des effets protecteurs de l'hypoxie / altitude ont

également été rapportés sur le développement du diabète [282], la maladie coronaire et l'accident vasculaire cérébral [283, 284], signifiant que l'hypoxie peut réduire l'incidence de ces maladies. Suite à un séjour d'une semaine à 2650 m, Lippl et coll. [172] rapportent une diminution du poids, de pression artérielle diastolique et une augmentation du métabolisme de base chez des sujets obèses. Les effets bénéfiques de l'altitude perdurent ensuite 4 semaines. Cependant, lorsque l'exposition à l'hypoxie était associée à une activité physique, Netzer et coll. [173] rapportent une perte de poids chez le sujet obèse plus importante qu'en normoxie. Par ailleurs, Haufe et coll. [160] ont étudié de manière approfondie de nombreux facteurs de risque cardiovasculaire en réponse à l'entraînement en hypoxie ($FiO_2 = 15\%$). Ils montrent des effets favorables sur la masse grasse, la concentration plasmatique de triglycérides, l'insulinémie, et la sensibilité à l'insuline, par rapport à un entraînement en normoxie ($FiO_2 = 21\%$). L'entraînement a consisté en 3 séances d'une heure/semaine pendant 4 semaines à la même intensité relative pour chaque condition (intensité d'exercice induisant une concentration de 3 mmol.L^{-1} de lactate).

L'entraînement en hypoxie semble donc induire des effets bénéfiques sur les facteurs de risque cardiovasculaires traditionnels, ce qui peut avoir des implications cliniques importantes [145, 285]. L'hypoxie pourrait en effet agir comme un outil thérapeutique potentiel pour lutter contre l'obésité.

Synthèse

- ❖ Le surpoids et l'obésité sont devenus un problème majeur de santé publique, ils sont associés à plusieurs maladies telles que les maladies cardiovasculaires, le diabète, les maladies rénales et le cancer...
- ❖ Les facteurs environnementaux et comportementaux, les facteurs endocrinologiques, les facteurs biologiques, le statut socio-économique et les facteurs psychologiques contribuent de manière importante à la prise de poids.
- ❖ L'accumulation de graisse provoque une hypertrophie du tissu adipeux qui est l'origine d'une inflammation bas grade et d'un stress oxydant qui portent une lourde responsabilité dans la morbidité cardio-métabolique associée à l'obésité.
- ❖ La perte de poids devrait être recommandée en présence d'affections comorbides chez tous les patients obèses ou en surpoids. L'exercice déclenche des adaptations favorables qui permettent de lutter contre le stress oxydatif.
- ❖ La prévalence du surpoids ou de l'obésité dans la population vivant à haute altitude est plus faible, l'hypoxie affecte le système nerveux et les hormones tels que la leptine et modifie le métabolisme glucidique et le contrôle de l'appétit.
- ❖ Il semblerait que l'hypoxie, soit en conditions de repos, soit combinée à l'entraînement, puisse améliorer l'état métabolique et les facteurs de risque cardiovasculaires. Elle pourrait en effet agir comme un outil thérapeutique potentiel pour lutter contre l'obésité.

DEUXIEME PARTIE : CONTRIBUTION PERSONNELLE

Objectifs scientifiques

La revue de la littérature présentée ci-dessus montre des effets potentiels bénéfiques de l'exposition à l'hypoxie non sévère sur la santé. Cependant, ces effets ont souvent été démontrés au cours d'études pilotes sur de petits effectifs, en particulier dans les populations atteintes de maladies cardio-métaboliques. Ces études cliniques, parfois critiquables dans leur conception, mettent en œuvre des modalités d'exposition hypoxique assez variées. Il semble donc important en vue d'une application clinique optimale d'étudier la faisabilité, et l'efficacité de plusieurs d'entre elles (exposition de repos, continue intermittente, entraînement à l'effort en hypoxie). Les indicateurs cliniques (ou le moment de leur mesure) utilisés dans les études antérieures ne sont pas toujours très solides. Ainsi, certaines études [286] semblent indiquer que les effets obtenus, notamment au plan métabolique, tiennent plus à un effet aigu de la dernière séance du programme qu'à son effet chronique. Au regard de la littérature, une exposition à l'hypoxie intermittente semble procurer notamment dans les populations atteintes de troubles cardio-métaboliques des effets bénéfiques similaires à ceux induits par l'activité physique régulière. Ceci est probablement en partie lié au déclenchement de réponses géniques permettant de moduler le stress oxydatif voire l'inflammation, bien documentés dans ces pathologies. Les effets potentiels de l'hypoxie sont tout à fait intéressants d'un point de vue clinique car ce traitement peut constituer une alternative intéressante à l'exercice musculaire, comme adjuvant à l'éducation alimentaire chez des patients chez lesquels le mouvement est limité voire contre-indiqué. Ce travail doctoral a pour objectif d'examiner les effets cardio-respiratoires et métaboliques à court (1 ou 3 séances) et moyen terme (2 mois) de différentes modalités d'exposition hypoxique normobare (repos continu, versus intermittent, entraînement à l'effort en conditions hypoxique versus normoxique). Après une présentation des matériels et méthodes, la présentation des résultats s'organisera autour de quatre études expérimentales dont les grandes lignes sont décrites ci-dessous.

Si le conditionnement hypoxique semble efficace sur des variables physiologiques ou physiopathologiques, il paraît important dans le but d'une utilisation clinique d'étudier la modalité la plus efficace, ainsi que sa tolérance, à court et moyen terme. Nous avons donc mené une première étude préliminaire physiologique, qui visait à

caractériser les effets cardio-respiratoires de 2 modalités d'exposition hypoxique normobare (hypoxie intermittente i.e. **HI** versus hypoxie continue i.e. **HC**) à court terme (3 séances).

Compte tenu des effets potentiels du conditionnement hypoxique sur la santé cardio-métabolique, nous avons testé dans le cadre d'une étude de recherche clinique (HYPINT) les effets chroniques (24 séances sur 8 semaines) de ces 2 stratégies de conditionnement (HI vs HC) dans une population de sujets en surpoids ou obèses. Nous avons fait l'hypothèse que l'HC et l'HI entraîneraient des améliorations significatives sur la santé cardiovasculaire, et métabolique de la personne en surpoids/obèse.

D'après la littérature, la combinaison d'une exposition hypoxique et de l'exercice musculaire peut potentialiser les effets sur des indicateurs de santé cardio-métabolique. Avant d'appliquer cette modalité de traitement chez le sujet obèse, nous avons réalisé une étude préliminaire en aiguë chez le sujet sain pour vérifier la bonne tolérance de ce conditionnement et étudier les réponses cardio-respiratoires ainsi qu'au niveau de l'oxygénation tissulaire à des exercices sur cycloergomètre à charge constante et intensité modérée ou sous forme d'intervalles à haute intensité réalisés en hypoxie ont été comparées à des exercices similaires réalisés en normoxie aussi sollicitant en termes de fréquence cardiaque.

Enfin, dans le cadre de l'étude clinique HYPINT, nous avons examiné l'impact d'un programme d'entraînement (24 séances en 8 semaines) à charge constante d'intensité modérée sur ergocycle sur la condition physique et la santé cardiovasculaire et métabolique dans une population de sujets en surpoids ou obèses. Nous avons fait l'hypothèse que l'entraînement hypoxique induirait une amélioration de la santé cardio-circulatoire plus importante que l'entraînement normoxique.

1. Méthodologie générale

1.1. Exposition à l'hypoxie normobare

L'hypoxie normobare a été créée artificiellement à l'aide d'un mélangeur de gaz AltiTrainer® (SMTEC S.A., Nyon, Suisse) qui permet de diluer la fraction d'oxygène inspiré ambiante en augmentant la fraction d'azote délivrée par une bouteille d'azote branchée au mélangeur de gaz. Cet appareil permet de simuler une altitude comprise entre 0 m et 6000 m, de façon continue ou intermittente, au repos ou à l'exercice. L'air inspiré par le patient via ce dispositif est conduit par un tuyau branché au masque naso-buccal (taille adaptée au visage du sujet, masque V2Mask® Hans Rudolph, USA) relié à une valve bidirectionnelle permettant d'inspirer l'air par un côté et de l'expirer par l'autre. La saturation pulsée en oxygène (SpO_2) cible (entre 70 et 80% dans ce travail de thèse) est mesurée à l'aide d'un oxymètre de pouls (fixé au doigt) et intégré à l'AltiTrainer®. Le système informatique de l'AltiTrainer® permet un enregistrement et un affichage en temps réel de la saturation pulsée en oxygène, de la FiO_2 , de la ventilation, du niveau d'altitude correspondant permettant à l'expérimentateur de faire des ajustements en temps réel. Dans le cadre des différents programmes de conditionnement hypoxique au repos utilisés dans ce travail de thèse, les sujets recevant l'hypoxie continue respirent un air appauvri en oxygène, équivalent à peu près à celui que l'on respire à 5000 m d'altitude ($FiO_2 = 0.10-0.11$ pour la plupart des sujets) pour atteindre une SpO_2 cible entre 70-80%, tandis que les sujets du groupe hypoxie intermittente subissent une alternance de phases d'hypoxie et de normoxie : 7 cycles de 5 min en hypoxie (avec une FiO_2 diminuée individuellement de façon à atteindre une SpO_2 cible de 70-80%) suivies de 3 min en normoxie ($FiO_2 = 0.21$). En fonction des études réalisées dans cette thèse, l'exposition hypoxique est réalisée soit en condition de repos, sujet confortablement installé sur une chaise longue, soit au cours de l'exercice sur un cycloergomètre (Corvial, Lode B.V., Groningen, Pays Bas).

1.2. Mesure des variables cardiorespiratoires

1.2.1. Variabilité de la fréquence cardiaque (VFC)

Il existe différentes méthodes pour analyser la VFC, en particulier les méthodes temporelles et les méthodes spectrales. La VFC est extraite d'une mesure de la fréquence cardiaque « battement à battement » et elle reflète l'état du système nerveux autonome. Elle est considérée comme un marqueur du contrôle sympathique et parasympathique [287]. L'augmentation de l'activité sympathique ou la diminution de l'activité parasympathique entraîne une accélération cardio-vasculaire. Par contre, une faible activité sympathique ou une forte activité parasympathique entraîne une cardio-décélération.

L'analyse temporelle permet d'étudier les indices quantitatifs parce qu'elle est capable de déterminer la FC à tout instant et d'isoler les intervalles RR. A partir des intervalles RR, un certain nombre de paramètres sont calculés:

- SDNN est l'écart type de l'intervalle RR sur toute la période d'enregistrement exprimé en ms. Il représente la variabilité globale,
- RMSSD représente la racine carrée des différences au carré des intervalles R-R successifs en ms. Il est influencé par la respiration et exprime la variabilité de haute fréquence d'origine parasympathique,
- pNN50 est le nombre d'intervalles RR successifs différents de plus de 50 ms exprimé en pourcentage du nombre total de cycles cardiaques analysés.

L'analyse temporelle est complétée par l'analyse fréquentielle ou spectrale qui est plus qualitative et estime la modulation sympathique. Cette méthode transforme le signal des intervalles RR en signal fréquentiel à l'aide d'une transformée de Fourier rapide. Trois bandes de fréquences peuvent être distingué [288]:

- Les hautes fréquences (HF : 0.015 Hz à 0.40 Hz) : reflète essentiellement l'activité parasympathique,
- Les basses fréquences (LF : 0.04 Hz à 0.15 Hz) : reflètent l'activité sympathique et parasympathique,
- Les très basses fréquences (VLF : 0.00 à 0.04 Hz) : sont probablement associées à la thermorégulation, à la vasomotricité et à la dépense énergétique.

A partir de ces bandes, on peut calculer le ratio LF/HF pour évaluer la balance sympatho-vagale. On trouve une prédominance parasympathique si ce ratio est inférieur à 1 et une prédominance sympathique si cette valeur dépasse 1.

Au cours de cette thèse, nous avons utilisé l'Holter (AFT 1000, Holter supplies, Paris, France) qui enregistre l'électrocardiogramme (ECG) sur 2 voies et 3 électrodes placés sur la cage thoracique. La mesure est effectuée au repos, en position allongée, en particulier tout au long de la première et la dernière séance de conditionnement hypoxique. L'interprétation de l'ECG et les intervalles RR a été réalisée à l'aide du logiciel Kubios HRV analysis [289, 290].

1.2.2. Mesure du tonus artériel périphérique par Pléthysmographie (PAT)

La mesure du tonus artériel périphérique est réalisée à l'aide d'un endo-PAT 2000 (Itamar Medical Ltd) [291]. C'est une méthode non invasive qui mesure la fonction endothéliale [292]. Deux sondes de pléthysmographie sont placées sur les index des deux mains (**figure 9**), elles permettent de mesurer les variations pulsatiles du volume artériel. La sonde est composée d'une membrane en latex placée dans un doigtier. La membrane est gonflée par un micro-compresseur intégré à l'appareil et maintenu à une pression proche de la pression artérielle diastolique. Les sondes sont ainsi parfaitement adaptées au volume du doigt et permettent de mesurer les variations de l'amplitude de l'onde de pouls, qui sont un reflet des variations de volume sanguin capillaire. L'examen dure 15 minutes. Il est réalisé au repos, le sujet est en position allongée. Il s'agit d'étudier la réponse vasculaire à une ischémie de 5 min de l'avant-bras gauche. Celle-ci est obtenue par l'occlusion complète de l'artère brachiale à l'aide d'un brassard (**figure 9**) gonflé au moins 60 mmHg au-dessus de la pression artérielle systolique de repos. L'amplitude de l'onde de pouls est enregistrée, en continu des deux côtés pendant 15 min : 5 min avant occlusion, 5 min pendant l'occlusion et 5 min après l'occlusion. La réactivité vasculaire est calculée automatiquement par un logiciel intégré. Il s'agit du rapport entre la moyenne de l'amplitude de l'onde de pouls calculée entre la 90^{ème} et la 120^{ème} seconde après le relâchement de l'occlusion et la moyenne de l'amplitude de pouls des 210 secondes qui précèdent l'occlusion. Afin d'éliminer un effet vasomoteur

neurovégétatif systémique confondant, cet index est normalisé par rapport au côté controlatéral. Une valeur inférieure à 1.67 indique une dysfonction endothéliale [293].



Figure 9. Réalisation de la mesure du tonus artériel périphérique par pléthysmographie (PAT).

La mesure de réactivité vasculaire NO-dépendante par EndoPAT est reproductible et indépendante de l'examineur. Tierney et coll. [294] ont montré une différence moyenne des indices Endo-PAT de 0,12 (IC à 95%, -0,09-0,33, $P = 0,24$, coefficient de corrélation intraclass, 0,78) et une variation intra-sujet de 0,16.

1.2.3. Mesure de la rigidité artérielle

La vitesse de l'onde de pouls (VOP) permet de mesurer la rigidité artérielle. Il a été démontré que la VOP carotido-fémorale est un bon reflet de la rigidité aortique. Elle représente donc un indicateur important du risque cardiovasculaire [295, 296]. La VOP est mesurée de façon non invasive à l'aide de deux capteurs placés simultanément sur la peau en regard des artères carotide primitive et fémorale commune droite. La mesure est réalisée au repos en position allongée, depuis au moins 15 minutes. Chaque capteur enregistre l'onde de pression dans l'artère sous-jacente. L'intervalle de temps séparant le début de l'onde carotidienne de celui de l'onde fémorale correspondante (temps de transit carotido-fémoral) est mesuré de façon automatique (Complior®, Colson, Garges-les-Gonnesse, France). La VOP est définie par la distance séparant les deux capteurs, mesurée par un mètre ruban, rapportée au temps de transit carotido-fémoral moyenné sur 10 battements. La société Européenne de cardiologie classe les valeurs normales de VOP en fonction de l'âge. Chez les sujets moins 40 ans la VOP se situe autour de 6.1 m/s (5.3-7.1) tandis que chez les sujets de plus de 70 ans elle est à environ à 10.6 m/s (8,0-14.6) (**figure 10**) [297].

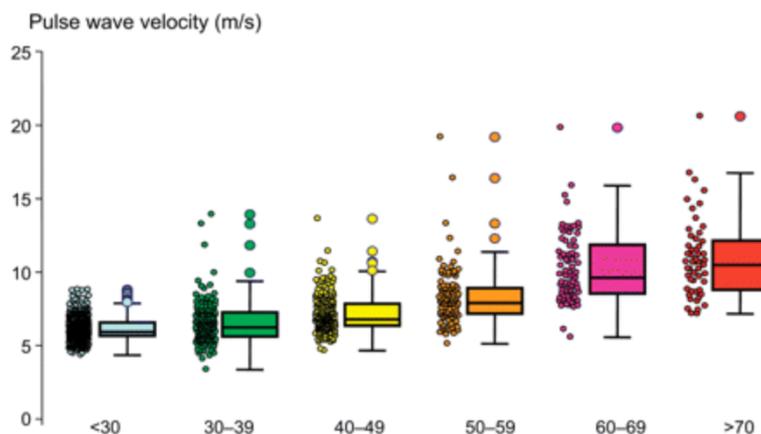


Figure 10. Valeurs normales de VOP en fonction de l'âge [297].

La VOP mesurée sur Complior (Colson, Garges-les-Gonesse, France) est un indicateur valide et reproductible de la rigidité artérielle [298]. Le coefficient de reproductibilité inter-observateur est de 0,947 pour la méthode manuelle et de 0,890 pour les coefficients de répétabilité automatique. Les coefficients de reproductibilité intra-observateur sont respectivement de 0,938 et de 0,935 [298].

1.3. Mesure par NIRS

La spectroscopie proche infrarouge (NIRS) mesure les variations d'oxygénation tissulaire et de l'hémodynamique. Cette technique optique d'oxymétrie non invasive repose sur l'utilisation d'une lumière infrarouge (650-950 nm). Au cours de cette thèse, un système commercial de NIRS (Oxymon Mark III, Artinis Medical Systems, Pays-Bas) a permis d'étudier les variations en micromoles de la concentration en hémoglobine oxygénée (oxyhémoglobine [HbO₂]), en hémoglobine réduite (déoxyhémoglobine ([HHb])), et en hémoglobine totale ([HbTot], qui correspond à la somme de [HbO₂] et [HHb]). L'estimation par le système NIRS Oxymon de la saturation en oxygène TSI (Tissue Saturation Index) a été mesurée également. Cette saturation est généralement comprise entre 60 et 80%, et calculée selon l'équation ci-après :

$$TSI [\%] = \frac{c_{O_2Hb}}{c_{tHb}} \times 100\% = \frac{c_{O_2Hb}}{c_{O_2Hb} + c_{HHb}} \times 100\%$$

Le principe physique de ce dispositif repose sur l'absorption des photons infrarouges qui traversent la peau en comparant l'intensité de la lumière avant et après le passage. Des optodes émettrices envoient une lumière infrarouge qui traverse la peau, cette lumière est détectée par les optodes réceptrices NIRS. Les photons du proche infrarouge sont capables de traverser les tissus (y compris l'os) sur plusieurs centimètres. Cette lumière infrarouge est cependant sensible à l'hémoglobine, la myoglobine et le cytochrome oxydase [299]. Chez l'homme, les variations des spectres d'absorption en infrarouge représentent l'état d'oxygénation de l'hémoglobine [300].

L'analyse de l'absorption repose sur le principe de la loi de Beer-Lambert, à savoir que l'absorption de la lumière est proportionnelle à 3 éléments : la concentration du chromatophore étudié, la distance parcourue par le faisceau lumineux et le coefficient d'extinction du chromatophore [301]. La différence entre l'intensité émise et l'intensité transmise à une certaine longueur d'onde permet d'étudier la concentration des différents chromatophores d'un tissu. De façon logique, plus l'intensité de la lumière reçue par le détecteur est faible, plus la concentration du chromatophore concerné est élevée.

Au cours de cette thèse, la mesure de la NIRS a été réalisée durant la première et la dernière séance d'hypoxie au repos (**publication 1 et 2**), nous avons mesuré l'oxygénation cérébrale au niveau du cortex préfrontal gauche et l'oxygénation musculaire au niveau du biceps brachial (**figure 11**). D'autre part, au cours de l'étude 3, nous avons réalisé la même mesure au niveau du cortex préfrontal gauche et au niveau musculaire, sur le tiers inférieur du vaste externe du quadriceps (**figure 11**). Le support de la sonde est fixé sur la peau grâce à du ruban double-face. Il est maintenu au niveau du crâne par un bandeau et recouvert au niveau musculaire par une bande pour protéger les optodes de la lumière ambiante.



Figure 11. Dispositif expérimental de la NIRS. Positionnement des optodes.

Le logiciel Oxysoft a été utilisé pour acquérir les signaux NIRS. La fréquence d'échantillonnage au cours des différentes expérimentations était de 10 Hz. [HbO₂], [HHb] et [HbTot] ont été moyennées sur les 30 dernières secondes de chaque période de 5 min durant **HC**, et à la fin de chaque cycle (5 min **H**, 3 min **N**) durant **HI**. [HbO₂], [HHb] et [HbTot] ont ensuite été calculées comme la différence entre les valeurs moyennées obtenues dans la période d'intérêt et la période de repos initiale de la session. Les valeurs de [HbO₂], [HHb] et [HbTot] représentent donc des valeurs relatives, c'est-à-dire des modifications de concentration par rapport à un état initial.

1.4. Mesures anthropométriques

- Poids : les patients sont pesés en sous-vêtements, à $\pm 0,1$ kg.
- Taille : ils sont mesurés pieds nus, à $\pm 0,1$ cm à l'aide d'une toise.
- Tours de taille et de hanche : sont mesurés avec un mètre-ruban, les patients étant debout, en position anatomique. Mesuré sur un plan horizontal, le tour de taille est situé à mi-distance entre l'épine iliaque antéro-supérieure et le bord inférieur de la dernière côte. Le tour de hanche est situé au milieu de l'épine iliaque.

1.4.1. Imagerie par Résonance Magnétique Nucléaire (IRM)

L'IRM est une des méthodes de référence pour apprécier la composition corporelle incluant la masse maigre, la masse grasse et sa répartition régionale et notamment la masse grasse viscérale, un déterminant important du risque cardiovasculaire.

Lors des mesures, le patient est couché sur le ventre, les bras en extension et les mesures de coupes de scanner sont effectuées des mains aux pieds. Afin de standardiser les profils des patients, ces derniers étant de taille et de compositions corporelles différentes, l'échantillonnage a été divisé en trois sections corporelles. La première section correspond à une zone allant des talons jusqu'à la tête du fémur, la seconde zone part de la tête du fémur jusqu'au tronc et enfin, la dernière et troisième zone débute à la tête de l'humérus et se termine aux poignets en passant par la tête et les extrémités supérieures. Les images obtenues sont traitées par un programme spécifique (Matlab®, Mathworks, Inc.). Après élimination du bruit, la distinction entre les tissus maigres et gras est réalisée par le niveau de luminosité (le niveau de luminosité le plus fort correspond aux tissus adipeux). Nous avons cherché la masse grasse et la masse totale en pourcentage et en kg chez le sujet en surpoids et obèse (**publication 4**). La grasse sous cutanée ainsi que celle présente dans la moelle osseuse sont incluses dans la quantification du tissu adipeux. Le volume des tissus gras et maigre est calculé en multipliant le nombre de pixels par la dimension de la surface et l'épaisseur de la coupe analysée.

1.5. Epreuve d'effort maximale progressive

Cette épreuve qui a été réalisée sur cycloergomètre (Corvial, Lode B.V., Groningen, Pays Bas) jusqu'au maximum tolérable, permet de mesurer la consommation maximale d'oxygène (VO_{2max}), la fréquence cardiaque maximale, la puissance maximale nécessaire pour programmer le protocole de l'épreuve maximale métabolique, les seuils ventilatoires et les échanges gazeux.

Ce test a été réalisé en normoxie (à l'altitude de Grenoble) avec mesure des échanges gazeux (MetaMax 3B, Cortex Biophysik GmbH, Leipzig, Allemagne), surveillance continue de l'ECG 12 pistes (Électrocardiographe 12 canaux Custo cardio 110 BT, Custo med GmbH, Ottobrunn, Allemagne) (**figure 12**). Toutes les deux minutes, la tension artérielle à l'effort, la SaO_2 , la dyspnée et la fatigue musculaire (échelle visuelle analogique ou EVA : cotation à 0 : pas de dyspnée / pas de fatigue, cotation à 10 : dyspnée maximale / fatigue maximale). Avant de débiter le test, nous avons bien défini aux sujets la dyspnée comme une sensation désagréable pour respirer et la fatigue musculaire comme une sensation perçue au niveau des membres inférieurs. A la fin du

test et après 2 minutes de récupération, un micro-prélèvement de sang a été réalisé afin de mesurer la concentration d'acide lactique (Lactate Plus®, Nova Biomedical Corporation, Innovation House, Watham, MA, USA).

L'épreuve consiste en un protocole d'intensité croissante. Le test débute par 2 min de repos, suivies de 2 min d'échauffement à 50 W pour les hommes et 30 watt pour les femmes, puis la puissance est augmentée de 20 W chez les hommes et de 15 W chez les femmes toutes les 2 minutes jusqu'à épuisement. La vitesse de pédalage est maintenue entre 70 et 80 rpm tout au long du test.

Les critères de maximalité de cette épreuve sont:

- Fréquence cardiaque voisine de la fréquence cardiaque maximale théorique ($220 - \text{âge}$)
- Quotient respiratoire égal ou supérieur à 1.1
- Lactatémie égale ou supérieure à 8 mmol.L^{-1}

Les deux seuils ventilatoires SV1 et SV2 sont déterminés visuellement. Selon les critères de Wasserman et coll. [302], pour déterminer le SV1 « seuil d'adaptation ventilatoire », on identifie une augmentation brusque et non linéaire de la ventilation (VE) et de l'équivalent respiratoire en oxygène (VE/VO_2) tandis que l'équivalent respiratoire en dioxyde de carbone (VE/VCO_2) et la pression partielle de fin d'expiration en CO_2 ($PetCO_2$) restent stables. La pression partielle d'oxygène de fin d'expiration ($PETO_2$) augmente aussi. Ensuite, on observe une deuxième augmentation de la VE accompagnée d'une élévation de VE/VO_2 et une diminution importante de $PetCO_2$ ce qui correspond à SV2 « seuil d'inadaptation ventilatoire ».



Figure 12. Dispositif expérimental de l'épreuve d'effort maximale.

1.6. Epreuve sous maximale métabolique

Ce test est réalisé le matin à jeun pour la détermination du comportement métabolique à l'effort. Cette épreuve commence par 8 min de repos, ensuite 3 min d'échauffement à 20% suivi des paliers de 6 minutes sur cycloergomètre (Corvial, Lode B.V., Groningen, Pays Bas) à 30%, 40%, 50% et 60% de la puissance maximale aérobie (PMA) déterminée lors de l'épreuve d'effort maximale décrite ci-dessus. Les échanges gazeux sont mesurés en continu tout au long de l'épreuve (MetaMax 3B, Cortex Biophysik GmbH, Leipzig, Allemagne). La fréquence cardiaque est enregistrée à l'aide d'une ceinture Polar H7 (Capteur de fréquence cardiaque Polar H7, Polar Electro Oy, Kempele, Finland).

La calorimétrie indirecte (mesure des échanges gazeux) permet de déterminer le profil métabolique des patients à l'effort. Les données qui représentent la consommation d'oxygène et le débit de CO₂ rejeté sont moyennées sur la dernière minute d'échauffement et la dernière minute de chaque palier. Suite à ce test le point de croisement glucido-lipidique, le point d'oxydation maximal des lipides (Lipoxmax) ainsi que le taux d'oxydation maximal lipidique (en mg.min⁻¹) sont déterminés. Ces variables sont calculées grâce à un programme (gracieusement prêté par le Pr brun) conçu dans le

logiciel Excel 2003 (Microsoft office, France) par l'équipe du laboratoire CERAMM (Pr J. Mercier ; Pr J.-F. Brun, Montpellier, France). Le calcul de l'oxydation des glucides et des lipides est réalisé à partir de la méthode du QR non protidique qui néglige la participation des protéines à la production énergétique [303]:

- Oxydation glucidique ($\text{mg}\cdot\text{min}^{-1}$) = $4.210 \times \text{VCO}_2 - 2.962 \times \text{VO}_2$
- Oxydation lipidique ($\text{mg}\cdot\text{min}^{-1}$) = $-1.701 \times \text{VCO}_2 + 1.695 \times \text{VO}_2$

Les débits d'oxydation sont convertis en Kcal à partir de l'équivalent calorique des glucides ($4 \text{ kcal}\cdot\text{g}^{-1}$) et des lipides ($9 \text{ kcal}\cdot\text{g}^{-1}$). Ceci permet de déterminer la contribution énergétique des glucides et des lipides (en %) à la dépense énergétique totale. Conformément au concept défini par Brooks et Mercier [304], le point de croisement (PCGL) est défini comme la puissance à partir de laquelle l'énergie provenant des glucides devient prédominante par rapport à celle des lipides. Plus précisément, lorsque l'énergie provient des glucides pour 70% et 30% des lipides. Le point de croisement survient pour un QR voisin de 0,91 [305]. Ce point a été déterminé par l'intrapolation de la relation linéaire établie sur 4 points entre le pourcentage de glucides participant à la dépense énergétique totale et la puissance mécanique de l'exercice (dont l'évolution est linéaire). Ceci permet d'amortir les variations de cette relation liées aux points qui s'écarteraient de la linéarité. Le point d'oxydation lipidique maximal (Lipoxmax) correspond à l'intensité d'exercice pour laquelle l'oxydation lipidique est maximale (taux d'oxydation lipidique maximal). À partir de cette intensité, l'oxydation lipidique diminue alors que celles des glucides devient prédominante. Ce Lipoxmax est calculé à partir de l'équation : oxydation lipidique = $1.6946 \text{ VO}_2 - 1.7012 \text{ VCO}_2$ qui peut être simplifié en : oxydation lipidique = $1.7 (1-\text{QR}) \text{ VO}_2$. Cette équation permet de déterminer le taux d'oxydation lipidique maximal qui est le maximum de cette fonction mathématique. Il correspond donc au point auquel la dérivée de cette fonction s'annule [305].

1.7. Variables biologiques

Un échantillon sanguin veineux a été prélevé afin de doser les éléments suivants : insulinémie et glycémie (calcul de HOMA2-IR : valeur normale <1), profil lipidique, protéine-C réactive ultra-sensible.

La glycémie, la cholestérolémie, la triglycéridémie ont été déterminées par

spectrophotométrie par réflectance colorimétrique. L'insulinémie a été mesurée déterminée par radio-immunologie IRMA (Bio Rad). L'insulino-résistance a été calculée grâce aux concentrations plasmatiques de glucose et d'insuline à jeun à l'aide du "homeostasis model assessment of insulinresistance", version 2 (HOMA2-IR) de 1998 (logiciel disponible à <http://www.dtu.ox.ac.uk/>) [306]. La protéine C réactive ultrasensible (un marqueur de l'inflammation bas grade) est mesurée par néphlométrie. Les normes biologiques sont présentées dans le tableau 3 ci-dessous.

Cholestérol Total (g·L⁻¹)	1.6 à 2.4
Triglycérides (g·L⁻¹)	0.35 à 1.25
HDL (g·L⁻¹)	0.35 à 0.75
LDL (g·L⁻¹)	1.2 à 1.7
Total chol/HDL	< 4.5
CRP-us (mg.L⁻¹)	< 5.0
Glycémie (mmol.L⁻¹)	4.2 à 6.0
Insuline (μUI. mL⁻¹)	2.0 à 15.0

Tableau 3. Normes biologiques [307]

1.8. Modalités d'exercice

Toutes les séances d'exercice ont été réalisées sur un cycloergomètre ; nous avons utilisé un ergocycle Kettler E3 (Ense-parsit, Germany) dans l'étude 3 et un Corvial Lode B.V. (Groningen, Pays Bas) dans l'étude 4. L'intensité de l'exercice a été réglée manuellement sur la base des recommandations de « American College of Sports Medicine » [308] c'est-à-dire entre 50 et 70% de VO_{2pic} ou entre 60 et 80% de la FCmax. Nous avons choisi une charge interne de 75% de la FCmax (très légèrement supérieur à celle recommandée par Hobbin et coll. [176]. Ainsi pour une charge interne similaire, la puissance développée en normoxie était supérieure à celle réalisée en hypoxie. Lors de l'exercice, la saturation artérielle en oxygène et la FC ont été mesurées en continu pendant toutes les séances d'entraînement. La perception de l'effort a été évaluée toutes les 5 min.

Etude 3 :

Lors de l'exercice continu, le sujet pédale à charge constante à une puissance correspondant à 75% ($\pm 2\%$) de la FC maximale pendant 30 min (la puissance est ajustée si besoin toutes les 5 min pour respecter la cible de FC). Les séances d'exercice intermittent intense débutent par 5 min d'échauffement à 40% (en normoxie) ou 30% (en hypoxie) de la puissance maximale normoxique, suivies d'une alternance de 15 sessions d'1 minute à PMA (normoxie) ou 85% de PMA (hypoxie) [309] entrecoupées d'1 min de récupération passive . Pour ce qui concerne la correspondance entre l'intensité d'exercice en normoxie et en hypoxie (FC cible), nous avons également réalisé une étude préliminaire sur le personnel du laboratoire. En hypoxie la SpO₂ cible de 75% ($\pm 2\%$), était assurée par un mélangeur de gaz (Altitrainer, Suisse) comme précédemment décrit Par contre, les sujets exposés à la normoxie, respirait l'air ambiant délivré également par Altitrainer afin de préserver l'aveugle.

Etude 4

Le sujet est au repos sur ergocycle pendant 5 min. Lors de l'entraînement à charge constante modérée en normoxie, le sujet pédale à une puissance sollicitant 75% ($\pm 2\%$) de la FC maximale mesurée pendant l'épreuve d'effort maximale progressive durant 45 min (la puissance est ajustée si besoin toutes les 5 min pour maintenir la cible de FC). Lors de l'entraînement à charge constante modérée en hypoxie, de la même façon le sujet pédale pendant 45 min à charge constante, la puissance est ajustée toutes les 5 min pour être à 75% de la FC maximale. Pendant ce test, la SpO₂ cible est maintenue à 80%, grâce au mélangeur de gaz (Altitrainer, Suisse).

2. Résultats

-Publication n °1-

**Physiological responses to two hypoxic conditioning strategies
in healthy subjects**

Hypoxic conditioning strategies

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Résumé

Contexte: L'exposition à l'hypoxie peut être utilisée comme un outil thérapeutique en induisant des adaptations cardiovasculaires, neuromusculaires et métaboliques. Différentes stratégies de conditionnement hypoxique ont été proposées : une exposition hypoxique continue (HC) ou intermittente (HI). L'objectif de cette étude préliminaire était de caractériser de façon précise les réponses physiologiques (cardio-vasculaire, respiratoire, oxygénation tissulaire) à ces 2 types de conditionnement passif (en conditions de repos).

Méthodes: Quatorze sujets sains actifs (7 femmes, âge 25 ± 8 ans, IMC $21,5 \pm 2,5$ kg·m⁻²) ont bénéficié de 2 types de conditionnement hypoxique (3 séances sur 48 h) dans une étude croisée randomisée : HC ou HI, séparés par 2 semaines de « washout ». Les séances HC consistaient à respirer un mélange gazeux avec une FiO₂ ajustée en continu pour atteindre une SpO₂ cible entre 70 et 80% pendant 1 h. Les séances HI consistaient en des périodes de 5 minutes à une FiO₂ réduite (cible de SpO₂ = 70-80%), suivies de périodes de 3 minutes en normoxie, répétée sept fois. Pendant la première (S1) et la troisième (S3) séance de chaque intervention, les variables cardiorespiratoires et l'oxygénation musculaire et cérébrale (par spectroscopie proche infrarouge) ont été évaluées en continu.

Résultats: La ventilation augmente significativement durant les séances d'HI seulement ($+2 \pm 2$ L·min⁻¹), tandis que la fréquence cardiaque augmente au cours de HC ($+11 \pm 4$ bpm) et d'HI ($+13 \pm 5$ bpm). La tension artérielle a augmenté durant toutes les séances d'hypoxie. La tension artérielle systolique de base est plus basse à S3 qu'à S1 uniquement après HI (-8 ± 11 mmHg). L'oxygénation musculaire a diminué de manière significative au cours de S3 seulement dans les deux types d'exposition (S3: HC $-6 \pm 5\%$, HI $-3 \pm 4\%$). L'oxygénation préfrontale diminue au cours de S1 et S3 de façon plus marquée en HC qu'en HI ($-13 \pm 3\%$ contre $-6 \pm 6\%$). Les indices de variabilité de la fréquence cardiaque indiquent une augmentation significative de l'activité sympathique au cours des séances de HC vs HI (valeurs inférieures de SDNN, PNN50 et RMSSD en HC). De S1 à S3, une diminution de la variabilité de la fréquence cardiaque a été observée en HC (SDNN, PNN50 et RMSSD) tandis que la variabilité de la fréquence cardiaque a augmenté en HI (augmentation de SDNN et RMSSD).

Conclusions: Ces résultats montrent une influence à court terme (en 3 séances) des 2 conditionnements hypoxiques sur la variabilité de la fréquence cardiaque, la pression artérielle, et l'oxygénation tissulaire. La variabilité de la fréquence cardiaque pourrait fournir des informations utiles sur les adaptations précoces induites par une telle intervention.

Mots-clés: conditionnement hypoxique, hypoxie continue, hypoxie intermittente, pression artérielle, variabilité de la fréquence cardiaque, oxygénation tissulaire



Physiological Responses to Two Hypoxic Conditioning Strategies in Healthy Subjects

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Objective: Hypoxic exposure can be used as a therapeutic tool by inducing various cardiovascular, neuromuscular, and metabolic adaptations. Hypoxic conditioning strategies have been evaluated in patients with chronic diseases using either sustained (SH) or intermittent (IH) hypoxic sessions. Whether hypoxic conditioning via SH or IH may induce different physiological responses remains to be elucidated.

Methods: Fourteen healthy active subjects (7 females, age 25 ± 8 years, body mass index $21.5 \pm 2.5 \text{ kg}\cdot\text{m}^{-2}$) performed two interventions in a single blind, randomized cross-over design, starting with either 3 x SH (48 h apart), or 3 x IH (48 h apart), separated by a 2 week washout period. SH sessions consisted of breathing a gas mixture with reduced inspiratory oxygen fraction (FI_{O_2}), continuously adjusted to reach arterial oxygen saturations (SpO_2) of 70–80% for 1 h. IH sessions consisted of 5 min with reduced FI_{O_2} ($\text{SpO}_2 = 70\text{--}80\%$), followed by 3-min normoxia, repeated seven times. During the first (S1) and third (S3) sessions of each hypoxic intervention, cardiorespiratory parameters, and muscle and pre-frontal cortex oxygenation (near infrared spectroscopy) were assessed continuously.

Results : Minute ventilation increased significantly during IH sessions ($+2 \pm 2 \text{ L}\cdot\text{min}^{-1}$) while heart rate increased during both SH ($+11 \pm 4 \text{ bpm}$) and IH ($+13 \pm 5 \text{ bpm}$) sessions. Arterial blood pressure increased during all hypoxic sessions, although baseline normoxic systolic blood pressure was reduced from S1 to S3 in IH only ($-8 \pm 11 \text{ mmHg}$). Muscle oxygenation decreased significantly during S3 but not S1, for both hypoxic interventions (S3: SH $-6 \pm 5\%$, IH $-3 \pm 4\%$); pre-frontal oxygenation decreased in S1 and S3, and to a greater extent in SH vs. IH ($-13 \pm 3\%$ vs. $-6 \pm 6\%$). Heart rate variability indices indicated a significantly larger increase in sympathetic activity in SH vs. IH (lower SDNN, PNN50, and RMSSD values in SH). From S1 to S3, further reduction in heart rate variability was observed in SH (SDNN, PNN50, and RMSSD reduction) while heart rate variability increased in IH (SDNN and RMSSD increase).

Conclusions: These results showed significant differences in heart rate variability, blood pressure, and tissue oxygenation changes during short-term SH vs. IH conditioning interventions. Heart rate variability may provide useful information about the early adaptations induced by such intervention.

Keywords: hypoxic conditioning, sustained hypoxia, intermittent hypoxia, blood pressure, heart rate variability, tissue oxygenation

INTRODUCTION

Although chronic hypoxia is known to be an aggravating factor in several cardiovascular and respiratory diseases, hypoxic exposure may also be of significant benefits to one's cardiorespiratory status as demonstrated in animal models (e.g., Béguin et al., 2005) and as reviewed recently by our group (Verges et al., 2015) and others (Almendros et al., 2014; Navarrete-Opazo and Mitchell, 2014; Mateika et al., 2015). Hypoxic exposure as a potential therapeutic aid is a promising rehabilitation method for many clinical populations, including hypertensive (Serebrovskaya et al., 2008) and overweight (Kayser and Verges, 2013) patients. Unlike in chronic respiratory failure where hypoxia is most often associated with hypoventilation and hypercapnia, the combination of hypoxia, hyperventilation (and slight resultant hypocapnia) can be used to exploit certain physiological benefits of hypoxic exposure e.g., decreased blood pressure observed in animal models and hypertensive patients after repeated hypoxic exposures (Serebrovskaya et al., 2008). Indeed, exposure to either normobaric or hypobaric hypoxia has historically been implemented to improve athletic performance through various physiological mechanisms, including increased hemoglobin mass and enhanced muscle capillarization and metabolic capacities (reviewed in Millet et al., 2010). From this perspective, and using methods previously employed in sport medicine, repeated hypoxic exposures, i.e., hypoxic conditioning, may be seen as a new preventive, and therapeutic strategy, especially for deconditioned individuals.

Unfortunately, current research in the field of hypoxic conditioning does not provide the optimal hypoxic exposure required to obtain protective or therapeutic effects. It is now recognized that the consequences of hypoxic exposure depend on a dose–response relationship ranging from normoxia to deleterious severe hypoxia [intermittent inspiratory oxygen fraction (FiO_2) < 0.08–0.10 and arterial oxygen saturation (SpO_2) < 70%, with short cycles < 2 min], with intermediate doses (moderate hypoxia, FiO_2 > 0.10 or SpO_2 \geq 70–80%) and patterns (intermittent hypoxia with > 5-min cycles or sustained hypoxia) of hypoxia that can have positive cardiovascular and metabolic effects (Navarrete-Opazo and Mitchell, 2014; Verges et al., 2015). Previous studies on hypoxic conditioning in healthy subjects and patients used two main types of hypoxic exposure, with repetitive sessions of either intermittent [IH, e.g., 3–7 min hypoxia and 3–5 min normoxia, repeated 3–10 times (Bernardi et al., 2001a; Burtcher et al., 2004; Lyamina et al., 2011; Zhang et al., 2014)] or sustained [SH, e.g., 1 h under sustained hypoxia (Rodríguez et al., 1999; Katayama et al., 2001, 2009; Lusina et al., 2006)] hypoxia. Whether these two types of hypoxic

conditioning may induce different adaptations needs to be elucidated in order to establish optimal hypoxic conditioning strategies.

The possible deleterious side-effects of hypoxia [cardiovascular and metabolic dysfunctions as observed in sleep apnoea syndrome for instance: increased blood pressure, impaired glucose, and lipid metabolism (Lévy et al., 2011)] have to be considered when applying hypoxic conditioning strategy, especially to higher-risk, clinical populations. The hypoxic dose and pattern used for hypoxic conditioning should probably be adapted depending on patient characteristics and individual responses. The determination of the optimal hypoxic dose should occur during the beginning of the hypoxic conditioning program (i.e., over the first sessions) in order to avoid either insufficient (and therefore no treatment effect) or too severe (and its potential deleterious side-effects) hypoxic stimuli. Monitoring the efficiency and safety of hypoxic conditioning strategies requires to identify physiological biomarkers reflecting the early responses (during the first session and over the following sessions) to hypoxic conditioning.

Acute hypoxia induces within the first minutes and hours of exposure typical cardiorespiratory and autonomic nervous system responses including hyperventilation, tachycardia, and increased sympathetic activity. These mechanisms have been especially studied in the context of acute and chronic altitude exposure, i.e., prolonged sustained hypoxia (several hours and days staying at altitude, Mazzeo, 2008). While some of these mechanisms may be helpful in monitoring the effect of hypoxic conditioning strategies, few data are available in the literature regarding the main physiological responses during and after repetitive SH or IH conditioning sessions. Changes in blood pressure and autonomic nervous system are potential relevant markers of the physiological changes induced by repetitive hypoxic exposure. Severe intermittent hypoxia as encountered in sleep apnoea alters baroreflex activity and increases blood pressure (Lévy et al., 2011) while hypoxic conditioning strategy may improve baroreflex sensitivity (Haider et al., 2009) and reduce blood pressure (Serebrovskaya et al., 2008). Changes in heart rate variability (a useful marker of the autonomic nervous system activity, Malik, 1996) induced during and after hypoxic conditioning sessions remain to be elucidated. For example, it is known that after 12 h of sustained hypoxia (4000 m), a pronounced (dampened) effect on heart rate variability is evident, even 1 h after returning to normoxic ambient air (Guger et al., 2008), while after a brief hypoxic exposure of 15 min (FiO_2 = 0.11), the hypoxia-induced reduction in heart rate variability disappears almost immediately (Roche et al., 2002). Hence, in order to provide potential physiological biomarkers for the

implementation of hypoxic conditioning strategies, the effects of repetitive SH or IH exposure over several days need to be clarified especially on important cardiovascular mechanisms such as the autonomic nervous system.

Another issue to consider when applying hypoxic conditioning strategies is the determination of tissue-specific responses to hypoxic exposure which are generally characterized based on the magnitude of arterial blood deoxygenation. Important differences in oxygenation levels between tissues have been shown when FiO_2 is reduced both in animal models (Almendros et al., 2011; Reinke et al., 2011) and in healthy subjects (Rupp et al., 2011, 2016). Rupp et al. (2016) demonstrated that healthy subjects exposed to intermittent hypoxemia for 45 min showed significant cyclical prefrontal cortex deoxygenation while the biceps brachialis muscle remained normoxic. Hence, changes in tissue (especially muscle and brain) oxygenation as measured non-invasively by near-infrared spectroscopy (NIRS) need to be characterized in order to better determine the organ-specific hypoxic stress applied during hypoxic conditioning sessions.

Therefore, the purpose of this study was to assess the acute and short-term cardiorespiratory and tissue oxygenation effects of two hypoxic conditioning strategies (i.e., sustained vs. intermittent hypoxic exposures as previously used in the literature) in order to determine the most efficient strategy and to identify physiological biomarkers able to provide early assessment of each hypoxic conditioning program, with emphasis on distinguishing protective vs. deleterious responses. We hypothesized that IH conditioning would induce larger cardiorespiratory (i.e., improved blood pressure and heart rate variability) benefits due to repetitive deoxygenation-reoxygenation cycles compared to SH conditioning.

MATERIALS AND METHODS

Subjects

Fourteen (7 females) active, healthy volunteers (age: 25 ± 8 years, body mass: 72 ± 11 kg, height: 179 ± 7 cm, body mass index 21.5 ± 2.5 kg·m⁻²) were included in the present study after routine medical assessment consisting of a short clinical examination, a 12-lead electrocardiogram and a respiratory function test. All subjects were non-smokers and had no history of cardiorespiratory or neuromuscular disease. They lived at sea level (Grenoble, 212 m) and were not exposed to altitude >1000 m over the past 3 months. Subjects were engaged 1–3 times a week in low- to moderate-intensity endurance activities such as walking, jogging, or cycling. They were instructed to keep their diet, hydration status, and physical activity routine identical throughout the study. Subjects refrained from physical exercise the days prior to the tests, abstained from drinking caffeinated beverages on test days, and had their last meal at least 2 h prior to the tests. The study was approved by the local ethics committee (CPP Sud-Est V) and performed according to the Declaration of Helsinki. Subjects were fully informed of the procedure and risks involved and gave their written consent prior to all assessments.

Experimental Design

In this prospective, randomized, cross-over, controlled, single-blind study performed in Grenoble (altitude: 210 m), all participants performed first a full familiarization trial in normoxia including sitting and breathing quietly through a facemask, with continuous electrocardiogram and blood pressure measurements. Then, they were randomized (using block randomization) into either the sustained hypoxia (SH) or intermittent hypoxia (IH) condition. After performing the first hypoxic condition (three ~1-h sessions separated by 48 h), all subjects completed the second hypoxic condition (three ~1-h sessions separated by 48 h) after a minimum washout period of 15 days. Cardiorespiratory and NIRS signals were continuously recorded on session 1 (S1), and session 3 (S3) of each hypoxic condition (described below). On session 2, only SpO_2 and FiO_2 were recorded. This study focused on physiological responses over three sessions of each hypoxic conditioning strategy since this represents a reasonable time frame (1 week) to assess the early effects of this intervention (Rodway et al., 2007) and to adapt, if required, the hypoxic dose.

In all testing sessions subjects seated in a semi-supine position, in a quiet environment, at rest but without sleeping. They were blinded for the gas mixture composition they were inhaling. Subjects were told that this study aimed to compare the effect of two different types of hypoxic exposure, without further details. The hypoxic stimulus was obtained by having subjects breathe via a facemask a nitrogen-enriched gas mixture provided by a gas-mixing device (Altitrainer[®], SMTEC S.A., Nyon Switzerland). FiO_2 was individually adjusted to reach the targeted SpO_2 of 70–80% in both hypoxic conditioning interventions (see **Figure 1**).

Sustained Hypoxia (SH) Sessions

SH sessions started with an initial phase of 5 min breathing normoxic, ambient air for baseline data collection. Then, the hypoxic phase began with a progressive decrease of FiO_2 from 0.21 to 0.10 over 10 min. Afterwards, FiO_2 was automatically adjusted to reach 70–80% SpO_2 . This target SpO_2 level was sustained until 55 min of hypoxic exposure. There was a final normoxic recovery phase where participants breathed ambient air for an additional 10 min, for a total testing duration of 70 min.

Intermittent Hypoxia (IH) Sessions

IH sessions consisted of a 5 min baseline breathing normoxic, ambient air, followed by seven cycles of 5-min hypoxia (breathing a gas mixture with reduced FiO_2 to reach the target SpO_2 of 70–80%) and 3-min normoxic breathing. The initial 5-min hypoxia was performed with a FiO_2 of 0.10 for all subjects, and FiO_2 was individually adjusted for the subsequent 5-min hypoxic phases in order to reach the target SpO_2 . After the seven hypoxia–normoxia cycles, 7 min of normoxic recovery were performed for a total session duration of 68 min.

Cardiorespiratory Measurements

Ventilation and gas exchanges were monitored continuously breath-by-breath using the MetaMax 3B (Cortex Biophysik GmbH, Leipzig, Germany). Gas analyzers and volume transducers were calibrated prior to each test with a 3-L syringe

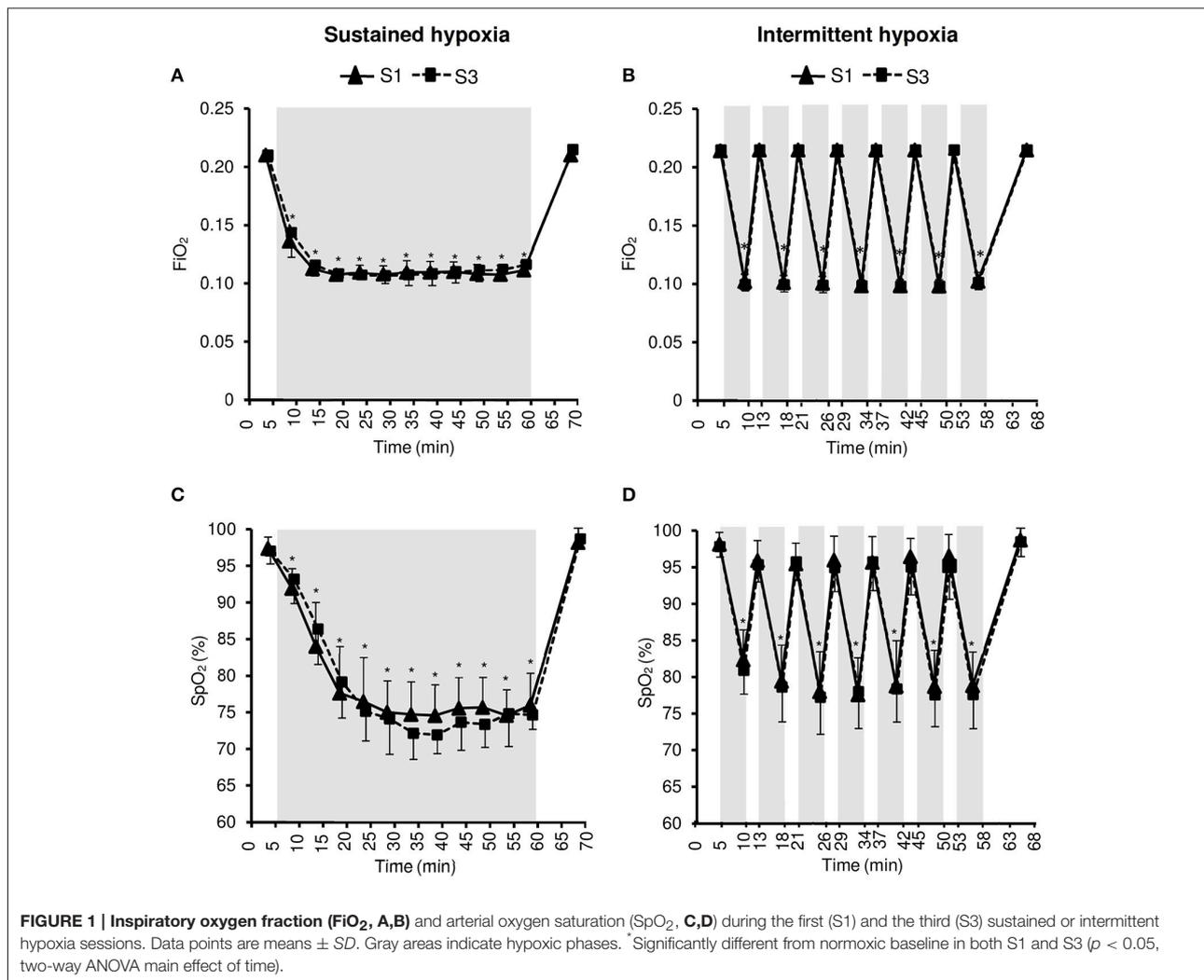


FIGURE 1 | Inspiratory oxygen fraction (F_{iO_2} , A,B) and arterial oxygen saturation (SpO_2 , C,D) during the first (S1) and the third (S3) sustained or intermittent hypoxia sessions. Data points are means \pm SD. Gray areas indicate hypoxic phases. *Significantly different from normoxic baseline in both S1 and S3 ($p < 0.05$, two-way ANOVA main effect of time).

and references gases, respectively, according to manufacturer's instructions. Blood pressure was measured on the dominant arm twice at each time point with a digital pressure monitor system (A&D Medical, Kitamoto-Shi, Japan). ECG was continuously recorded using a 2-lead ECG at a sampling rate of 1000 Hz (Holter AFT 1000, Holter supplies, Paris, France).

NIRS Measurements

Oxy[HbO₂]-, deoxy[HHb]-, and total[HbTot]-hemoglobin concentration changes and tissue oxygenation index (TSI) were estimated continuously throughout testing sessions over multiple sites using a two-wavelength (780 and 850 nm) multichannel, continuous wave NIRS system (OxyMon MkIII, Artinis Medical Systems, Netherlands). Muscle hemodynamics were assessed at the middle of right *biceps brachialis* using a 4-cm interoptode distance. Left pre-frontal cortex hemodynamics were assessed between Fp1 and F3 locations according to the international 10–20 EEG system with 3.5-cm inter-optode distance. The probe holders were secured to the skin with double-sided

tape and maintained with Velcro bands. Data were recorded continuously at 10 Hz and filtered with a 2-s width moving Gaussian smoothing algorithm before analysis.

Data Analysis

ECG data was transferred to the QuickReader software (Holter supplies) and R–R intervals were analyzed by means of the Kubios HRV Analysis Software 2.2 (Tarvainen et al., 2002, 2014). Heart rate variability parameters were recorded and calculated in accordance with the task force of the European society of cardiology and the North American society of pacing and electrophysiology (Malik, 1996). Data were visually inspected to remove artifacts and heart rate variability parameters were calculated over 5-min periods. The following parameters were used to analyze the heart rate variability within the time domain: standard deviation of R–R interval (SDNN), percentage of successive R–R that varied by 50 ms or more (PNN50) and the root-mean-square difference of successive normal R–R intervals (RMSSD). In the frequency domain, the following parameters

were calculated using a Fast Fourier Transform: low frequency (LF; 0.04–0.15 Hz), high frequency (HF; 0.15–0.40 Hz), and LF/HF ratio.

During SH sessions, minute ventilation (V_E), gas exchanges, heart rate, SpO_2 , and NIRS parameters were averaged over the last 30 s of the baseline normoxic phase, of each 5-min periods in hypoxia and of the normoxic recovery phase. During IH sessions, the same parameters were averaged over the last 30 s of the baseline normoxic phase, of each 5-min hypoxic, and 3-min normoxic periods, and of the normoxic recovery phase. Arterial blood pressure was calculated as the average of two measurements at each of these time points. Heart rate variability parameters were calculated over the 5-min baseline normoxic phase, every 5 min during the hypoxic phase of SH sessions and during every 5-min hypoxic phases of IH sessions, and over the last 5 min of the normoxic recovery phase.

Statistical Analysis

All statistical procedures were completed on Statistica version 10 (Statsoft, Tulsa, OK). Normality of distribution and homogeneity of variances of the main variables were confirmed using a Shapiro-Wilk normality test and the Levene's test, respectively. For each hypoxic condition (SH and IH), in order to assess changes over time within the sessions and changes from S1 to S3, a two-way ANOVA (time \times session) with repeated measures was performed for each dependent variable (see statistical results provided in figures). In order to assess differences between hypoxic conditions (SH vs. IH), a three-way ANOVA (time \times session \times condition) with repeated measures was performed for each dependent variable, using four time points only [baseline normoxia, min 25 (mid-session) and 60 (end-session) in SH and min 26 (mid-session) and 58 (end-session) in IH, and normoxic recovery). These four time points were selected in order to compare SH and IH under similar hypoxemic conditions, i.e., at mid and end hypoxic phase, when SpO_2 remained at the same 70–80% target value for at least 5 min in both SH and IH (see statistical results provided in tables). *Post-hoc* Tukey's tests were applied to determine a difference between two mean values if the ANOVA revealed a significant main effect or interaction effect. For all statistical analyses, a two-tailed alpha level of 0.05 was used as the cut-off for significance. All data are presented as mean values \pm SD within text and tables.

RESULTS

Subjects reported no significant discomfort and no adverse effect was observed during all SH and IH sessions.

Inspiratory Oxygen Fraction and Arterial Oxygenation

Changes in FiO_2 and SpO_2 during the hypoxic conditioning sessions are presented in **Figure 1** and **Table 1**. FiO_2 was significantly reduced from baseline normoxic values throughout SH sessions (two-way ANOVA main effect of time $F = 807.5$, $p < 0.001$) and during the hypoxic phases of IH sessions (two-way ANOVA main effect of time $F = 4762.0$, $p < 0.001$). FiO_2 reduction magnitude was similar between S1 and S3 in both

conditions (SH two-way ANOVA, main effect of session $F = 1.4$, $p = 0.262$; IH two-way ANOVA main effect of session $F = 1.0$, $p = 0.340$) and was significantly larger at mid- and end-session in IH vs. SH (three-way ANOVA condition \times time interaction $F = 30.2$, $p < 0.001$; **Table 1**). SpO_2 was reduced from baseline normoxic values throughout SH sessions (two-way ANOVA main effect of time $F = 171.5$, $p < 0.001$) and during the hypoxic phases of IH sessions (two-way ANOVA main effect of time $F = 213.7$, $p < 0.001$). SpO_2 reduction (SH 77.5 ± 1.7 and IH $78.2 \pm 3.2\%$, on average during the hypoxic phases) was similar between S1 and S3 in both conditions (SH two-way ANOVA main effect of session $F = 0.5$, $p = 0.482$; IH two-way ANOVA main effect of session $F = 1.7$, $p = 0.211$) and was significantly larger at the end of the session in SH vs. IH (three-way ANOVA condition \times time interaction $F = 3.1$, $p = 0.040$; **Table 1**).

Cardiorespiratory Responses

Changes in V_E and $PetCO_2$ during the hypoxic conditioning sessions are presented in **Figure 2** and **Table 1**. A significant increase in V_E from baseline normoxic values was observed during the hypoxic phases of IH sessions both in S1 and S3 (two-way ANOVA main effect of time $F = 6.1$, $p < 0.001$) but not during SH sessions (two-way ANOVA main effect of time $F = 1.6$, $p = 0.108$). $PetCO_2$ decreased significantly from baseline normoxic values during SH sessions both in S1 and S3 (two-way ANOVA main effect of time $F = 9.0$, $p < 0.001$) and during the hypoxic phases of IH sessions both in S1 and S3 (two-way ANOVA main effect of time $F = 10.2$, $p < 0.001$). V_E and $PetCO_2$ changes did not differ between SH and IH at mid- and end-session (V_E three-way ANOVA condition \times time interaction $F = 0.6$, $p = 0.617$; $PetCO_2$ three-way ANOVA condition \times time interaction $F = 0.6$, $p = 0.612$; **Table 1**).

Changes in heart rate and arterial blood pressure during the hypoxic conditioning sessions are presented in **Figure 3** and **Table 1**. A significant increase in heart rate from baseline normoxic values was observed throughout SH sessions (two-way ANOVA main effect of time $F = 30.0$, $p < 0.001$) and during the hypoxic phases of IH sessions (two-way ANOVA main effect of time $F = 57.5$, $p < 0.001$) both in S1 and S3. Heart rate increase was similar in SH and IH (three-way ANOVA main condition effect $F = 1.4$, $p = 0.251$; **Table 1**). Systolic blood pressure (SBP) increased significantly from baseline normoxic values during SH both in S1 and S3 (two-way ANOVA main effect of time $F = 21.0$, $p < 0.001$) while it increased during IH in S3 only (two-way ANOVA session \times time interaction $F = 2.1$, $p = 0.013$). Baseline normoxic SBP was significantly reduced at S3 compared to S1 in IH ($p = 0.009$). Diastolic blood pressure (DBP) increased significantly from baseline normoxic values during SH and IH both in S1 and S3 (SH two-way ANOVA main effect of time $F = 5.5$, $p < 0.001$; IH two-way ANOVA main effect of time $F = 6.5$, $p < 0.001$). Changes in SBP and DBP did not differ between SH and IH conditions (**Table 1**).

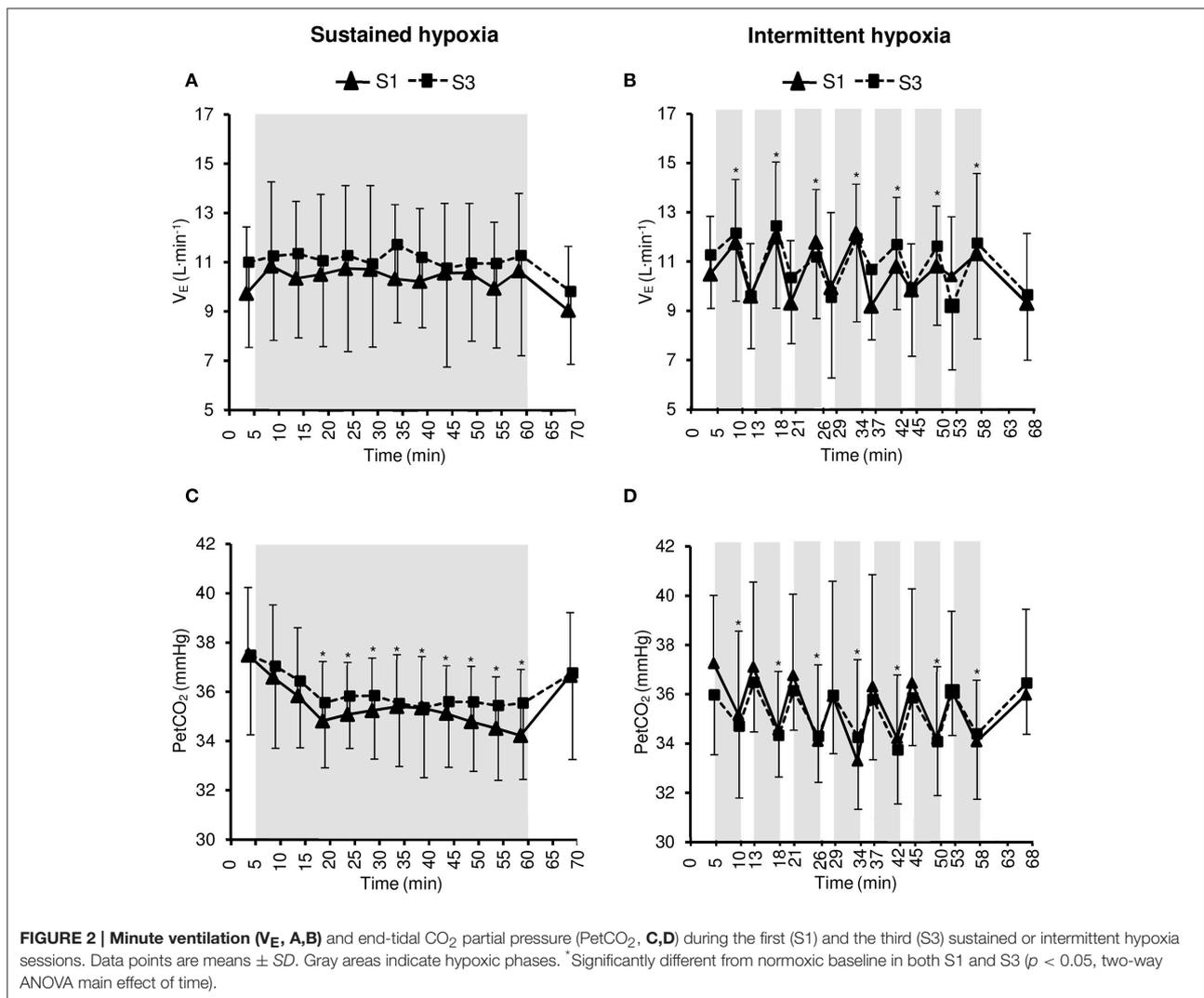
Heart Rate Variability

Changes in heart rate variability parameters during the hypoxic conditioning sessions are presented in **Figures 4, 5** and **Table 2**. SDNN decreased significantly in SH during S3 (two-way ANOVA

TABLE 1 | Inspiratory oxygen fraction, arterial oxygen saturation, ventilation and cardiovascular responses during the first and the third sustained or intermittent hypoxia sessions.

	Sustained hypoxia						Intermittent hypoxia						3-way ANOVA				
	Baseline normoxia		Middle hypoxia		End hypoxia		Recovery normoxia		Middle hypoxia		End hypoxia		Recovery normoxia		Condition main effect	Condition x time interaction	Condition x session interaction
	Session	Baseline normoxia	Middle hypoxia	End hypoxia	Recovery normoxia	Middle hypoxia	End hypoxia	Recovery normoxia	Baseline normoxia	Middle hypoxia	End hypoxia	Recovery normoxia					
FIO ₂	S1	0.21 (0.00)	0.11 (0.01)	0.11 (0.01)	0.21 (0.00)	0.10 (0.01)	0.10 (0.01)	0.21 (0.00)	0.10 (0.01)	0.10 (0.01)	0.10 (0.01)	0.21 (0.00)	0.21 (0.00)	<i>p</i> = 0.002	<i>p</i> < 0.001	<i>p</i> = 0.114	
	S3	0.21 (0.00)	0.11 (0.00)	0.12 (0.01)	0.21 (0.00)	0.10 (0.01)	0.10 (0.01)	0.21 (0.00)	0.10 (0.01)	0.10 (0.01)	0.10 (0.01)	0.21 (0.00)	0.21 (0.00)				
SpO ₂ (%)	S1	97.4 (1.6)	76.5 (6.0)	75.9 (4.4)	98.1 (2.5)	77.9 (5.5)	78.8 (4.6)	98.7 (1.6)	77.9 (5.5)	78.8 (4.6)	78.8 (4.6)	98.7 (1.6)	98.4 (2.0)	<i>p</i> = 0.008	<i>p</i> = 0.040	<i>p</i> = 0.939	
	S3	97.0 (1.8)	75.1 (4.0)	74.6 (1.9)	98.7 (1.3)	77.2 (5.0)	77.6 (4.6)	98.4 (2.0)	77.2 (5.0)	77.6 (4.6)	77.6 (4.6)	98.4 (2.0)	98.4 (2.0)				
PetCO ₂ (mmHg)	S1	37.5 (2.7)	35.0 (2.1)	34.2 (2.7)	36.7 (2.5)	34.0 (3.1)	34.0 (2.5)	35.9 (3.4)	34.0 (3.1)	34.0 (2.5)	34.0 (2.5)	35.9 (3.4)	36.4 (2.0)	<i>p</i> = 0.131	<i>p</i> = 0.612	<i>p</i> = 0.581	
	S3	37.5 (2.2)	35.8 (2.1)	35.5 (3.0)	36.8 (3.5)	34.2 (1.9)	34.4 (2.7)	36.4 (2.0)	34.2 (1.9)	34.4 (2.7)	34.4 (2.7)	36.4 (2.0)	36.4 (2.0)				
V _E (L·min ⁻¹)	S1	9.7 (2.7)	10.7 (3.4)	10.7 (3.1)	9.0 (2.6)	11.8 (2.1)	11.3 (3.2)	9.3 (2.8)	11.8 (2.1)	11.3 (3.2)	11.3 (3.2)	9.3 (2.8)	9.7 (2.6)	<i>p</i> = 0.428	<i>p</i> = 0.617	<i>p</i> = 0.451	
	S3	11.0 (3.6)	11.2 (3.9)	11.2 (4.0)	9.8 (2.9)	11.1 (2.5)	11.7 (3.9)	9.7 (2.6)	11.1 (2.5)	11.7 (3.9)	11.7 (3.9)	9.7 (2.6)	9.7 (2.6)				
HR (bpm)	S1	65 (15)	73 (15)	74 (12)	61 (12)	71 (11)	73 (10)	60 (10)	71 (11)	73 (10)	73 (10)	60 (10)	59 (10)	<i>p</i> = 0.251	<i>p</i> = 0.564	<i>p</i> = 0.834	
	S3	64 (8)	76 (11)	74 (10)	60 (10)	74 (10)	73 (8)	59 (10)	74 (10)	73 (8)	73 (8)	60 (10)	59 (10)				
SBP (mmHg)	S1	115 (14)	119 (15)	120 (16)	125 (18)	115 (14)	118 (13)	116 (11)	115 (14)	118 (13)	118 (13)	116 (11)	116 (14)	<i>p</i> = 0.195	<i>p</i> = 0.899	<i>p</i> = 0.633	
	S3	110 (13)	119 (18)	119 (18)	120 (16)	114 (16)	114 (13)	116 (14)	120 (16)	114 (16)	114 (13)	116 (14)	116 (14)				
DBP (mmHg)	S1	67 (11)	68 (9)	72 (7)	75 (8)	64 (12)	68 (10)	68 (9)	63 (12)	64 (12)	68 (10)	68 (9)	70 (9)	<i>p</i> = 0.295	<i>p</i> = 0.779	<i>p</i> = 0.143	
	S3	62 (12)	65 (16)	69 (10)	70 (10)	65 (10)	69 (9)	70 (9)	70 (10)	65 (10)	69 (9)	68 (9)	70 (9)				

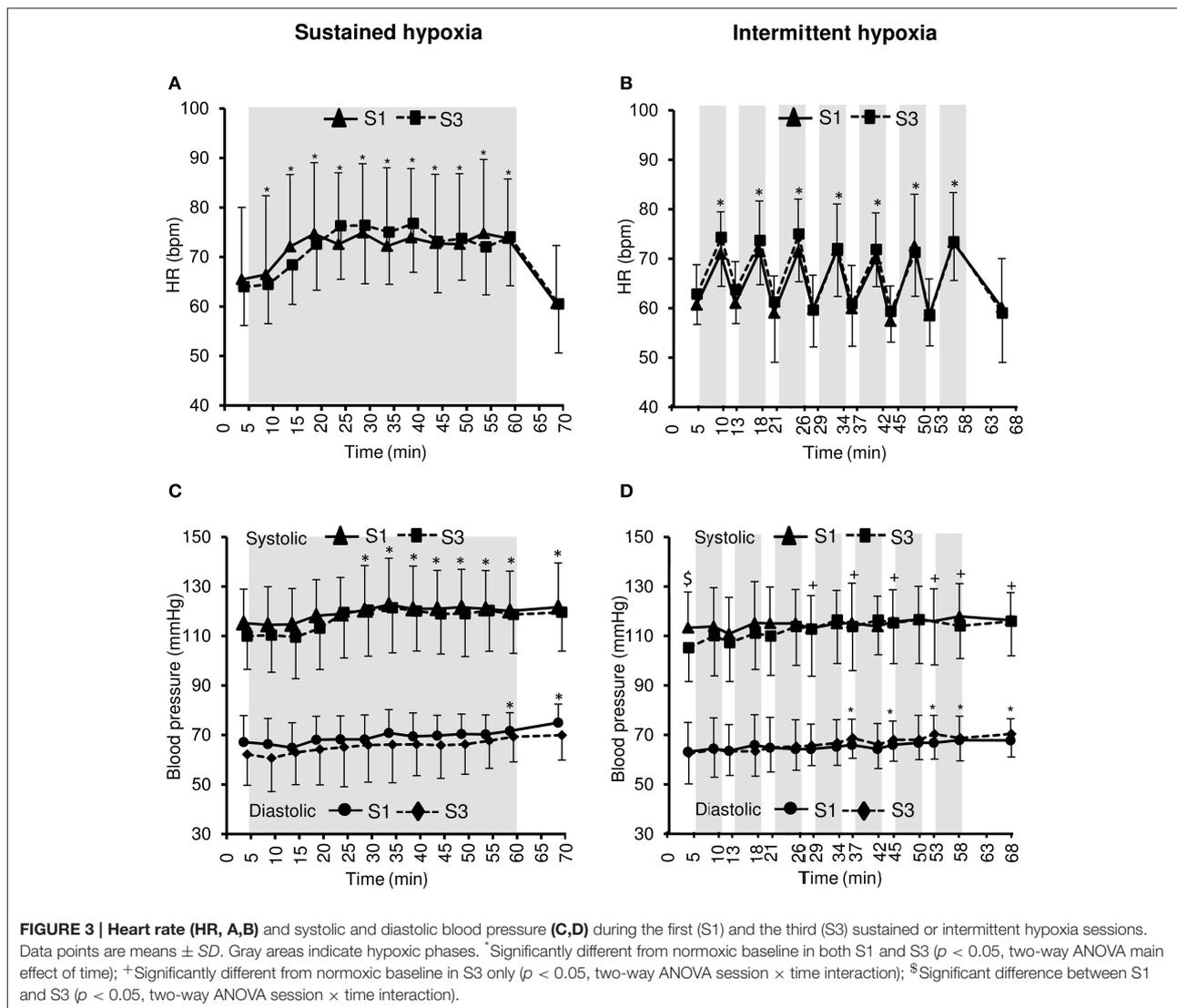
Values are provided for the normoxic phases before and after hypoxic exposure and in the middle and the end of the hypoxic exposure. Data are means (SD); FIO₂, inspiratory oxygen fraction; SpO₂, arterial oxygen saturation; PetCO₂, end tidal carbon dioxide partial pressure; V_E, minute ventilation; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; S1, first session; S3, third session.



condition \times time interaction $F = 2.9, p = 0.001$) and increased significantly in IH during both S1 and S3 (two-way ANOVA main effect of time $F = 3.2, p = 0.003$). SDNN in IH was significantly larger during S3 compared to S1 (two-way ANOVA main effect of session $F = 4.9, p = 0.045$). SDNN was significantly lower in SH compared to IH (three-way ANOVA main effect of condition $F = 23.4, p < 0.001$; three-way ANOVA condition \times time interaction $F = 5.8, p = 0.002$; **Table 2**). PNN50 decreased during both S1 and S3 in SH (two-way ANOVA main effect of time $F = 10.1, p < 0.001$) and IH (two-way ANOVA main effect of time $F = 5.9, p < 0.001$) sessions. PNN50 in SH decreased to a greater extent during S3 compared to S1 (two-way ANOVA condition \times time interaction $F = 5.8, p = 0.002$). PNN50 was significantly lower in SH compared to IH (three-way ANOVA main effect of condition $F = 8.7, p = 0.011$; **Table 2**). RMSSD decreased during S3 in SH (two-way ANOVA session \times time interaction $F = 3.3, p < 0.001$) and during both S1 and S3 in IH (two-way ANOVA main effect

of time $F = 4.6, p < 0.001$) sessions. RMSSD in SH decreased to a greater extent during S3 compared to S1 (two-way ANOVA session \times time interaction $F = 3.3, p < 0.001$) while in IH it was larger during S3 compared to S1 (two-way ANOVA main effect of session $F = 4.2, p = 0.046$). RMSSD was significantly lower in SH compared to IH (three-way ANOVA main effect of condition $F = 8.9, p = 0.010$; **Table 2**).

LF did not change during both S1 and S3 in SH (two-way ANOVA main effect of time $F = 1.8, p = 0.054$) and IH (two-way ANOVA main effect of time $F = 1.4, p = 0.207$). HF decreased significantly during both S1 and S3 in SH (two-way ANOVA main effect of time $F = 8.4, p < 0.001$) and IH (two-way ANOVA main effect of time $F = 6.6, p < 0.001$) sessions. LF/HF increased significantly during both S1 and S3 in SH (two-way ANOVA main effect of time $F = 4.6, p < 0.001$) but not in IH (two-way ANOVA main effect of time $F = 0.5, p = 0.868$). LF/HF did not differ significantly between SH and IH (three-way ANOVA



main effect of condition $F = 0.2, p = 0.658$; three-way ANOVA condition \times time interaction $F = 1.5, p = 0.231$; **Table 2**).

Tissue Oxygenation

Changes in muscle NIRS parameters during the hypoxic conditioning sessions are presented in **Figures 6, 7** and **Table 3**. Muscle TSI decreased significantly in S3 but not in S1 during both SH (two-way ANOVA session \times time interaction $F = 3.1, p = 0.001$) and IH (two-way ANOVA session \times time interaction $F = 2.3, p = 0.006$) sessions. Muscle TSI reduction was similar between SH and IH conditions (three-way ANOVA condition \times time interaction $F = 2.2, p = 0.109$; **Table 3**). Muscle HHb (SH two-way ANOVA session \times time interaction $F = 3.1, p = 0.001$; IH two-way ANOVA session \times time interaction $F = 2.3, p = 0.007$) but not HbO₂ (SH two-way ANOVA session \times time interaction $F = 1.7, p = 0.077$; IH two-way ANOVA session \times

time interaction $F = 1.1, p = 0.395$) nor HbTot (SH two-way ANOVA session \times time interaction $F = 1.8, p = 0.052$; IH two-way ANOVA session \times time interaction $F = 1.2, p = 0.311$) showed larger changes in S3 compared to S1 in both SH and IH conditions. HbO₂ reduction (three-way ANOVA condition \times time interaction $F = 3.1, p = 0.037$) and HHb increase (three-way ANOVA condition \times time interaction $F = 3.8, p = 0.017$) were larger during SH compared to IH conditions (**Table 3**).

Changes in pre-frontal cortex NIRS parameters during the hypoxic conditioning sessions are presented in **Figures 6, 8** and **Table 3**. Pre-frontal cortex TSI decreased significantly during both S1 and S3 in SH (two-way ANOVA main effect of time $F = 75.4, p < 0.001$) and IH (two-way ANOVA main effect of time $F = 9.8, p < 0.001$) sessions. Pre-frontal cortex HbO₂ significantly decreased (SH two-way ANOVA main effect of time $F = 36.7, p < 0.001$; IH two-way ANOVA main effect of time

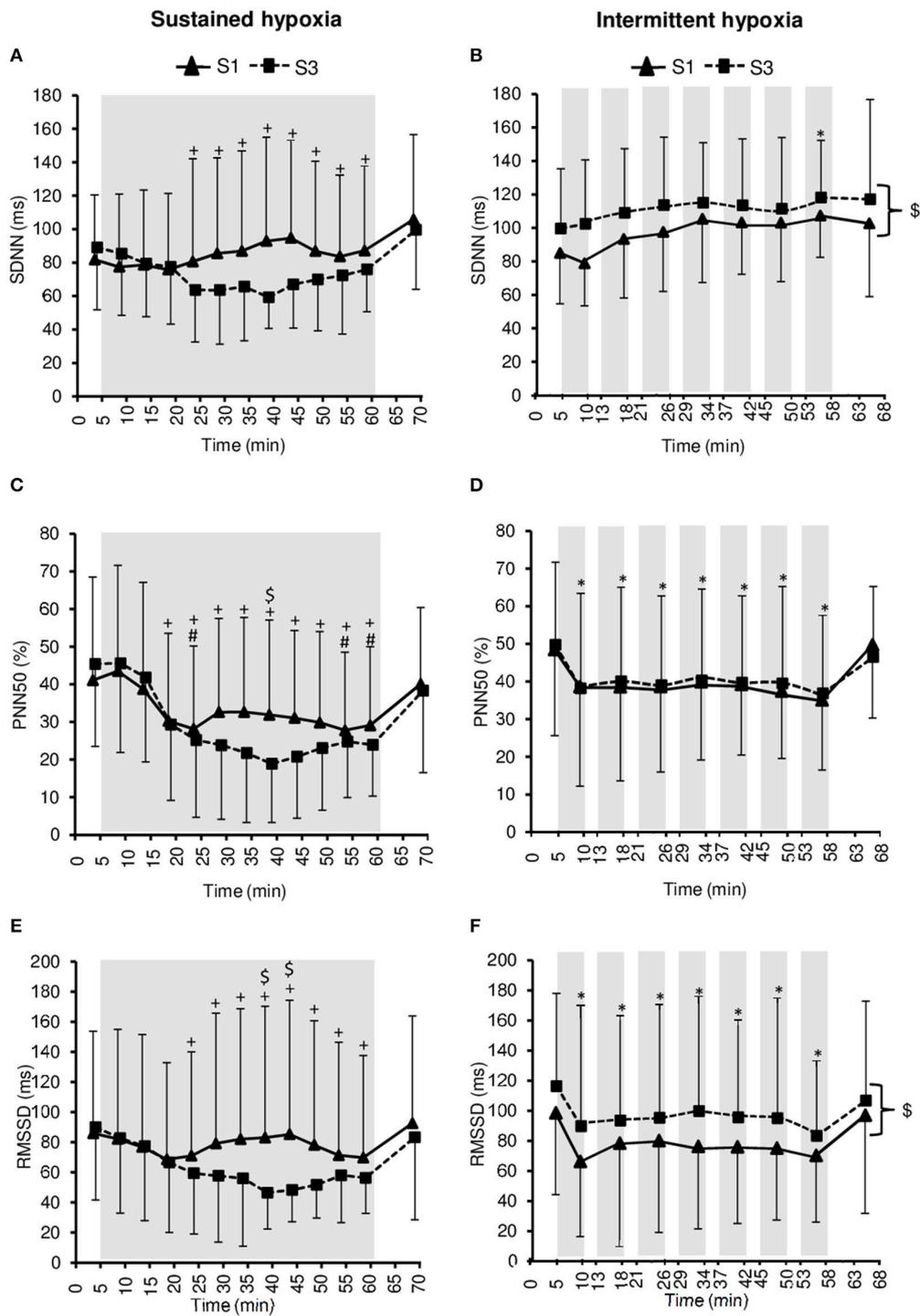


FIGURE 4 | Heart rate variability indices within the time domain [standard deviation of R-R interval (SDNN), (A,B); percentage of successive R-R that varied by 50 ms or more (PNN50), (C,D); root-mean-square difference of successive normal R-R intervals (RMSSD), (E,F)] during the first (S1) and the third (S3) sustained or intermittent hypoxia sessions. Data points are means \pm SD. Gray areas indicate hypoxic phases. *Significantly different from normoxic baseline in both S1 and S3 ($p < 0.05$, two-way ANOVA main effect of time); +Significantly different from normoxic baseline in S3 only ($p < 0.05$, two-way ANOVA session \times time interaction); #Significantly different from normoxic baseline in S1 only ($p < 0.05$, two-way ANOVA session \times time interaction); \$Significant difference between S1 and S3 ($p < 0.05$, two-way ANOVA main effect of session or session \times time interaction).

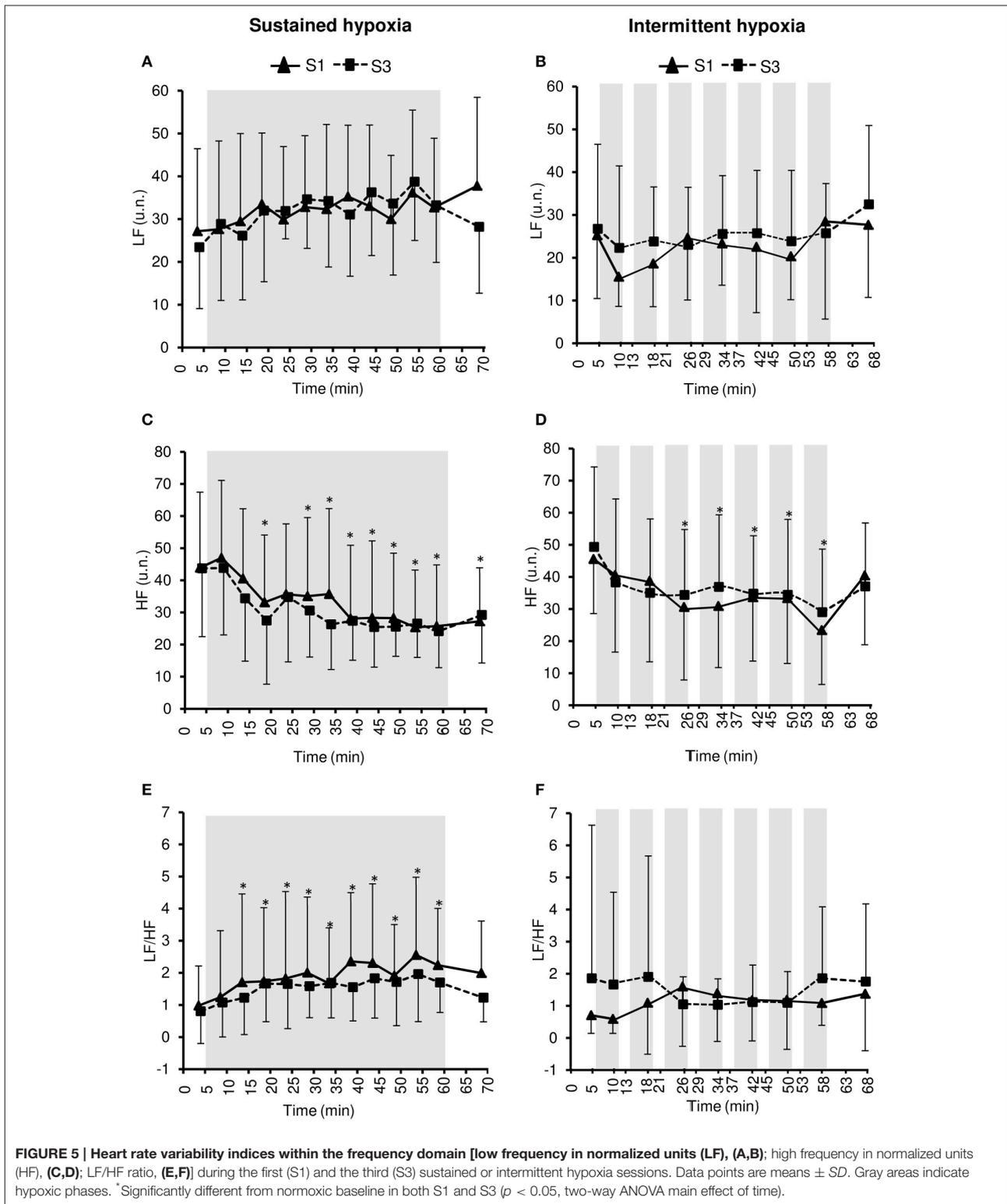
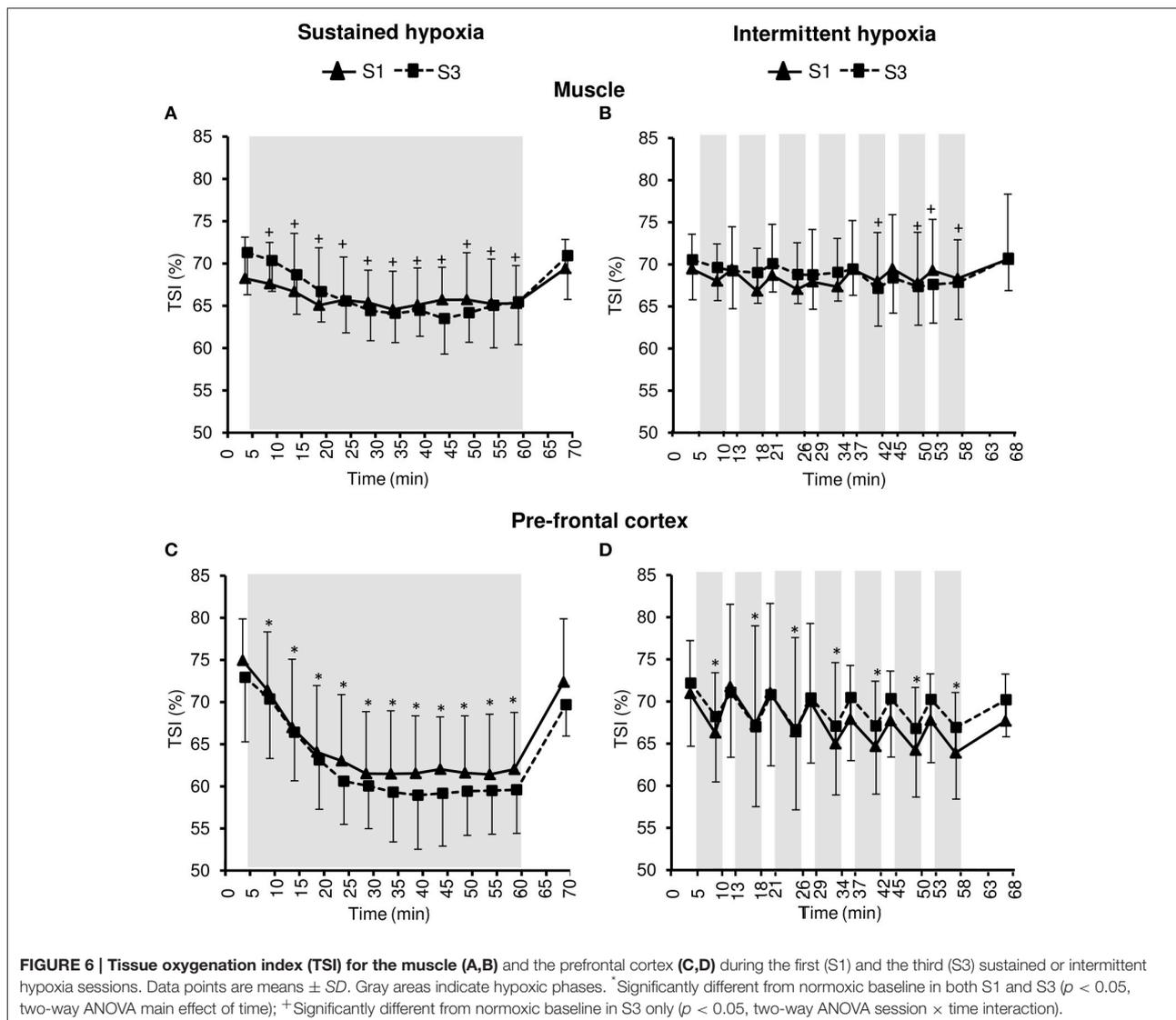


TABLE 2 | Heart rate variability indices during the first and the third sustained or intermittent hypoxia sessions.

	Session	Sustained hypoxia						Intermittent hypoxia						ANOVA				
		Baseline normoxia		Middle hypoxia		End hypoxia		Recovery normoxia		Middle hypoxia		End hypoxia		Recovery normoxia		Condition main effect	Condition x time interaction	Condition x session interaction
		normoxia	hypoxia	normoxia	hypoxia	normoxia	hypoxia	normoxia	hypoxia	normoxia	hypoxia	normoxia	hypoxia	normoxia	hypoxia			
SDNN (ms)	S1	81.7 (38.6)	80.5 (61.4)	87.7 (50.6)	105.7 (50.8)	84.9 (30.3)	97.5 (35.5)	107.2 (24.7)	102.6 (43.7)	$p < 0.001$	$p = 0.002$	$p = 0.100$						
	S3	89.0 (37.1)	63.5 (31.0)	75.8 (25.2)	99.5 (35.5)	99.6 (35.7)	113.5 (40.6)	118.1 (34.1)	117.0 (59.6)									
PNN50 (%)	S1	41.0 (27.4)	28.1 (22.0)	29.0 (20.9)	40.0 (20.3)	48.4 (22.7)	38.6 (22.6)	35.0 (18.5)	49.7 (19.4)	$p = 0.011$	$p = 0.362$	$p = 0.783$						
	S3	45.0 (21.9)	25.2 (20.5)	23.9 (13.5)	38.3 (21.8)	49.7 (21.9)	39.0 (23.6)	36.8 (20.7)	46.4 (18.7)									
RMSSD (ms)	S1	85.8 (67.7)	70.9 (69.0)	69.6 (67.9)	92.5 (71.3)	98.5 (54.2)	80.2 (60.9)	70.4 (44.4)	97.03 (61.5)	$p = 0.010$	$p = 0.638$	$p = 0.156$						
	S3	90.0 (48.3)	59.3 (40.2)	56.2 (23.5)	83.3 (54.8)	116.3 (61.5)	95.3 (75.2)	83.3 (49.7)	106.7 (66.1)									
LF (u.n.)	S1	27.1 (19.2)	29.8 (17.0)	32.6 (16.1)	37.7 (20.6)	25.2 (14.7)	24.6 (14.5)	28.4 (22.8)	27.6 (16.9)	$p = 0.167$	$p = 0.115$	$p = 0.478$						
	S3	23.4 (14.3)	31.8 (6.4)	33.2 (13.3)	28.1 (15.4)	26.7 (19.7)	22.9 (13.4)	25.7 (11.6)	32.4 (18.4)									
HF (u.n.)	S1	43.9 (23.5)	35.6 (21.9)	25.7 (19.0)	27.2 (16.6)	45.5 (16.9)	30.61 (22.7)	23.4 (16.9)	40.4 (21.5)	$p = 0.180$	$p = 0.123$	$p = 0.407$						
	S3	43.7 (21.2)	34.7 (20.1)	24.1 (11.3)	29.1 (14.9)	49.3 (24.9)	34.3 (20.4)	29.0 (19.6)	37.0 (19.7)									
LF/HF	S1	0.97 (1.23)	1.82 (2.70)	2.23 (1.77)	1.99 (1.62)	0.70 (0.56)	1.59 (1.86)	1.08 (0.69)	1.37 (1.77)	$p = 0.658$	$p = 0.231$	$p = 0.239$						
	S3	0.80 (0.77)	1.65 (1.38)	1.69 (0.92)	1.22 (0.74)	1.85 (4.77)	1.04 (0.86)	1.85 (2.23)	1.75 (2.42)									

Values are provided for the normoxic phases before and after hypoxic exposure and in the middle and the end of the hypoxic exposure. Data are means (SD); SDNN, standard deviation of R-R interval; PNN50, percentage of successive R-R that varied by 50 ms or more; RMSSD, root-mean-square difference of successive normal R-R intervals; LF, low frequency in normalized units; HF, high frequency in normalized units; S1, first session; S3, third session.



$F = 12.7$, $p < 0.001$) whereas HHb significantly increased (SH two-way ANOVA main effect of time $F = 98.7$, $p < 0.001$; IH two-way ANOVA main effect of time $F = 16.4$, $p < 0.001$) during both S1 and S3 in SH and IH. Pre-frontal cortex HbTot did not change over time both in SH (two-way ANOVA main effect of time $F = 0.9$, $p = 0.519$) and IH (two-way ANOVA main effect of time $F = 1.7$, $p = 0.056$). Pre-frontal cortex TSI (three-way ANOVA condition \times time interaction $F = 15.4$, $p < 0.001$) and HbO₂ (three-way ANOVA condition \times time interaction $F = 10.2$, $p < 0.001$) reduction as well as HHb increase (three-way ANOVA condition \times time interaction $F = 13.2$, $p < 0.001$) were larger during SH compared to IH conditions (Table 3).

Changes in pre-frontal cortex TSI, HbO₂, and HHb during SH and IH conditions were larger than changes at the muscle level (all $p < 0.05$).

DISCUSSION

The present study aimed to assess the acute and short-term (3 sessions) cardiorespiratory and tissue oxygenation responses to two hypoxic conditioning strategies, i.e., SH and IH, in order to determine their respective effects and potential physiological biomarkers able to detect early adaptations. During SH and IH sessions (acute effects), hyperventilation, tachycardia, increased blood pressure, and reduced heart rate variability were observed. SH sessions were associated with larger reduction in heart rate variability and greater muscle and prefrontal cortex deoxygenation compared to IH sessions. From S1 to S3 (short-term adaptations), IH reduced normoxic SBP and increased heart rate variability, SH sessions induced further reduction in heart rate variability, while both SH and IH sessions induced larger muscle deoxygenation. Hence, these results emphasize

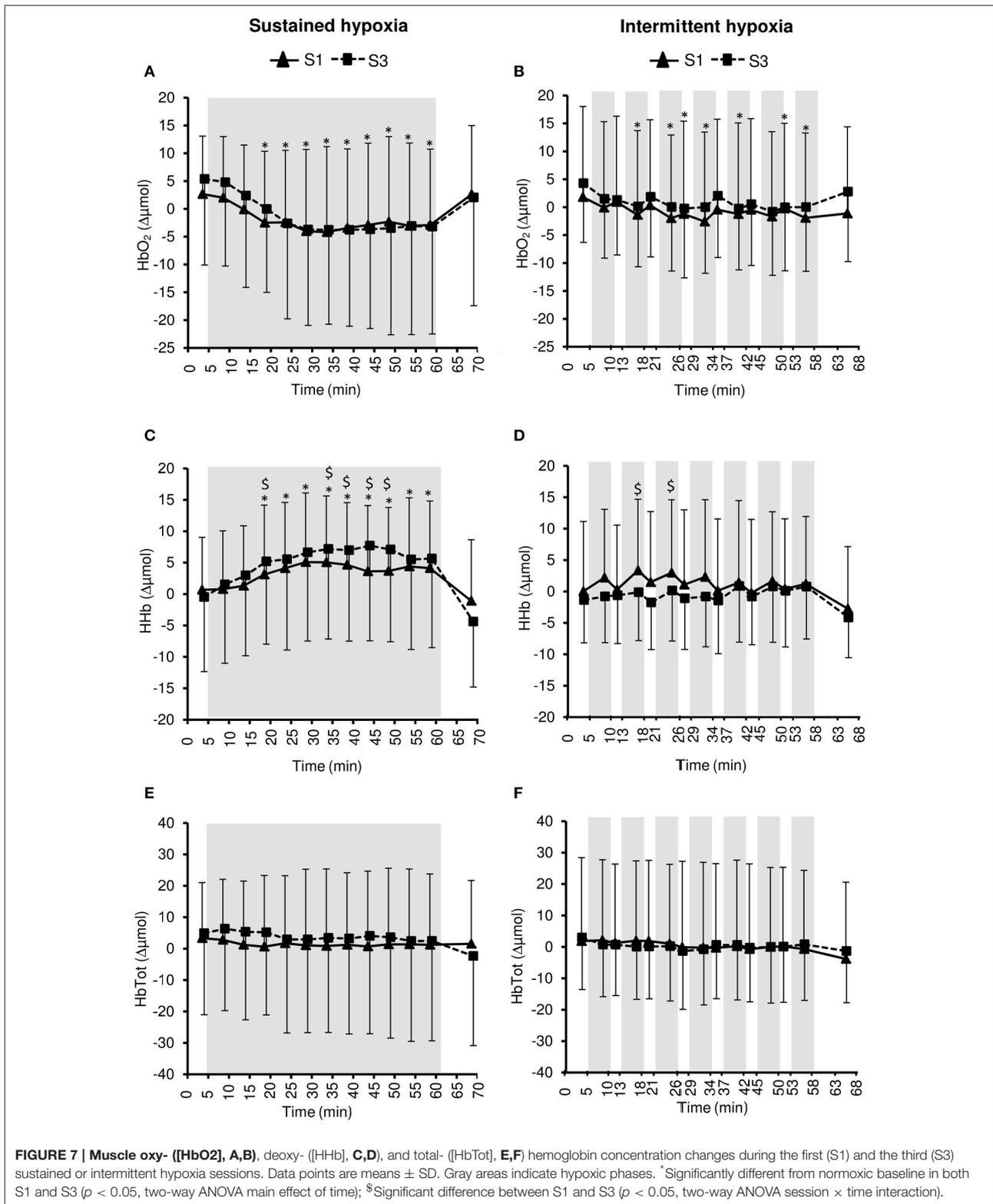
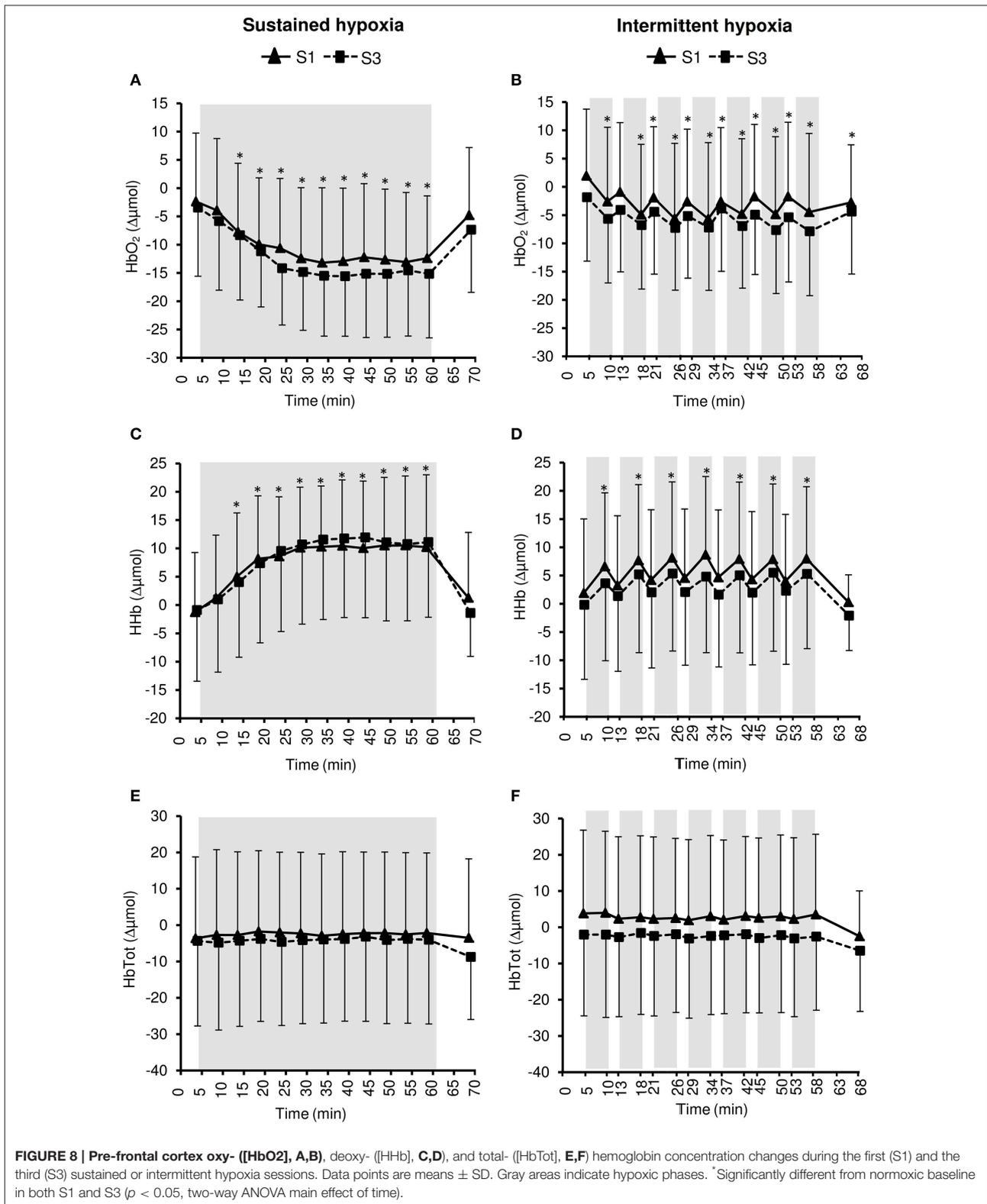


TABLE 3 | Muscle and pre-frontal cortex oxygenation during the first and the third sustained or intermittent hypoxia sessions.

	Session	Sustained hypoxia						Intermittent hypoxia						ANOVA			
		Baseline normoxia			End hypoxia			Recovery normoxia			End hypoxia			Condition x time interaction			Condition x session interaction
		Baseline normoxia	Middle hypoxia	End hypoxia	Recovery normoxia	End hypoxia	Recovery normoxia	Baseline normoxia	Middle hypoxia	End hypoxia	Recovery normoxia	End hypoxia	Recovery normoxia	Condition main effect	Condition x time interaction		
T _{Sm} (%)	S1 S3	68.2 (4.8) 71.2 (4.9)	65.6 (5.0) 65.5 (3.7)	65.2 (4.4) 65.4 (5.0)	69.4 (3.4) 70.9 (5.1)	67.0 (5.5) 68.7 (3.4)	68.3 (4.5) 67.8 (4.3)	70.6 (7.7) 70.7 (3.8)						<i>p</i> = 0.130	<i>p</i> = 0.109	<i>p</i> = 0.582	
HbO _{2m} (Δμmol)	S1 S3	2.6 (10.4) 5.3 (15.4)	-2.4 (12.9) -2.6 (17.1)	-2.9 (13.6) -3.1 (19.3)	2.6 (12.3) 2.0 (19.4)	1.8 (16.2) 0.0 (11.4)	-1.9 (15.1) 0.0 (11.5)	-1.1 (15.5) 2.7 (12.5)						<i>p</i> = 0.280	<i>p</i> = 0.037	<i>p</i> = 0.195	
HHbm (Δμmol)	S1 S3	0.6 (8.3) -0.4 (11.8)	4.1 (10.4) 5.5 (14.4)	4.1 (10.6) 5.6 (14.1)	-1.0 (9.7) -4.3 (10.4)	0.0 (11.1) -1.3 (6.8)	1.1 (10.7) 0.7 (8.3)	-2.7 (9.8) -4.1 (6.4)						<i>p</i> = 0.160	<i>p</i> = 0.017	<i>p</i> = 0.247	
HbTotm (Δμmol)	S1 S3	3.3 (17.6) 4.9 (25.9)	1.6 (21.5) 2.9 (29.7)	1.2 (22.5) 2.4 (31.8)	1.5 (20.1) -2.3 (28.4)	1.8 (26.5) 2.9 (16.4)	-0.7 (25.0) 0.8 (17.8)	-3.8 (24.4) -1.3 (16.4)						<i>p</i> = 0.967	<i>p</i> = 0.995	<i>p</i> = 0.741	
T _{Sc} (%)	S1 S3	74.9 (4.9) 72.9 (7.6)	63.9 (7.8) 60.6 (5.1)	62.0 (6.7) 59.5 (5.1)	72.3 (7.5) 69.6 (3.7)	70.9 (6.2) 72.1 (7.4)	63.8 (7.1) 66.9 (8.4)	67.6 (5.5) 70.2 (4.3)						<i>p</i> = 0.213	<i>p</i> < 0.001	<i>p</i> = 0.128	
HbO _{2c} (Δμmol)	S1 S3	-2.3 (12.0) -3.4 (12.1)	-10.6 (12.3) -14.1 (10.0)	-12.3 (11.0) -15.1 (11.3)	-4.7 (11.9) -7.3 (11.0)	1.95 (11.7) -1.8 (11.3)	-4.4 (13.9) -7.8 (11.3)	-2.7 (10.1) -4.3 (11.0)						<i>p</i> = 0.023	<i>p</i> < 0.001	<i>p</i> = 0.249	
HHbc (Δμmol)	S1 S3	-1.2 (10.5) -0.9 (12.5)	8.5 (10.5) 9.5 (14.2)	10.2 (12.7) 11.1 (13.2)	1.2 (11.5) -1.4 (7.6)	1.8 (13.1) -0.1 (13.1)	7.9 (12.7) 5.3 (13.2)	0.2 (4.8) -2.1 (6.1)						<i>p</i> = 0.001	<i>p</i> < 0.001	<i>p</i> = 0.111	
HbTotc (Δμmol)	S1 S3	-3.6 (22.4) -4.3 (23.3)	-2.0 (22.1) -4.6 (22.9)	-2.1 (22.0) -4.0 (23.1)	-3.5 (21) -8.7 (17.1)	3.8 (22.9) -2.02 (22.4)	3.5 (22.1) -2.5 (20.3)	-2.4 (12.5) -6.4 (16.7)						<i>p</i> = 0.200	<i>p</i> = 0.580	<i>p</i> = 0.485	

Values are provided for the normoxic phases before and after hypoxic exposure and in the middle and the end of the hypoxic exposure. Data are means (SD); T_{Si}, tissue saturation index; HbO₂, oxyhemoglobin concentration; HHb, deoxy/hemoglobin concentration; HbTot, total hemoglobin concentration; -m, muscle; -c, prefrontal cortex; S1, first session; S3, third session.



differences between SH and IH conditioning strategies regarding cardiovascular and tissue oxygenation responses and suggest some early adaptations after three hypoxic conditioning sessions only.

Acute Cardiorespiratory Responses

Acute hypoxic exposure is known to induce an increase in ventilation within the first minutes of exposure due to stimulation of peripheral chemoreceptors *Mazzeo (2008)*. In the present study, a significant increase in V_E was observed during the 5-min hypoxic phases of IH sessions but not during SH sessions (**Figure 1**). One potential reason for this difference between SH and IH is the progressive reduction in FiO_2 over the first 10 min of hypoxic exposure during SH sessions while during IH subjects were switched instantaneously from normoxia to a gas mixture with a FiO_2 of 0.10, possibly inducing greater chemoreceptor stimulation. Significant reductions in $PetCO_2$ during SH and IH sessions indicate however that subjects hyperventilated during both types of hypoxic exposure (**Figure 2**). The hypoxic hyperventilatory response was nevertheless relatively modest since V_E increased by <2 L·min⁻¹ and $PetCO_2$ decreased by <3 mmHg on average whatever the hypoxic condition, which is consistent with previous observations during SH and IH conditioning sessions [~ 50 -min IH with 5–6 min $FiO_2 = 0.10$ –4 min $FiO_2 = 0.21$ (Zhang et al., 2014); 1-h SH or IH at $SpO_2 = 80$ –90% (Rodway et al., 2007)]. This modest increase in ventilation probably contributed to the absence of significant discomfort reported by all subjects during both SH and IH sessions.

Acute hypoxic exposure is also known to induce tachycardia as observed during both SH and IH sessions (**Figure 3**), due to a combination of factors including aortic bodies stimulation and modulation of the autonomous nervous system *Mazzeo (2008)*. Changes in arterial blood pressure during acute hypoxic exposure result from the opposite effect of hypoxia-induced peripheral vasodilation (Crawford et al., 2006) and sympathetic activation (Halliwill et al., 2003). Previous studies reported inconsistent changes in arterial blood pressure during acute SH or IH exposure with no change [~ 50 -min IH with 5–6 min $FiO_2 = 0.10$ –4 min $FiO_2 = 0.21$ (Zhang et al., 2014)] or slight increase [1-h SH at $SpO_2 = 80\%$ (Lusina et al., 2006), 1-h SH or IH at $SpO_2 = 80$ –90% (Rodway et al., 2007), 1-h IH with 5–7 min normocapnic rebreathing–5–7 min $FiO_2 = 0.21$ (Bernardi et al., 2001b)]. In the present study both SH and IH induced a significant increase in blood pressure (**Figure 3**) which may be of larger magnitude compared to some previous studies due to the relatively low target SpO_2 (70–80%).

Heart rate variability is generally increased during acute hypoxic exposure which is thought to reflect sympathetic activation and parasympathetic withdrawal (Roche et al., 2002). In the present study, heart rate variability decreased during SH and IH sessions (from baseline normoxic exposure) suggesting a shift in the sympathovagal balance due to sympathetic activation as suggested by the increased LF/HF ratio in SH (**Figure 5**) and vagal withdrawal as suggested by reduced PNN50 and RMSSD in both SH and IH (**Figure 4**; Malik, 1996). Because changes in ventilation were modest in the present study

and more pronounced in the hypoxic condition associated with smaller changes in heart rate variability (i.e., IH), it is likely that changes in heart rate variability reflected alterations in the sympathovagal balance rather than respiratory sinus node modulation (Hirsch and Bishop, 1981). Interestingly, the reduction in heart rate variability was larger during SH compared to IH as shown by lower SDNN, PNN50, and RMSSD values during SH compared to IH sessions (**Table 2**). A greater effect of SH might be explained by the larger dose of hypoxia characterizing SH sessions compared to IH sessions due to the sustained hypoxic pattern of exposure (without reoxygenation phases as in IH) and the slightly lower SpO_2 (2–3% lower at mid- and end-session) in SH compared to IH sessions (**Table 1**). The importance of the total hypoxic dose has also been suggested by Tamisier et al. (2005) who observed a larger increase in muscle sympathetic nerve activity after 2 h of SH ($SpO_2 \sim 85\%$) vs. 2 h of IH (~ 2 -min cycles with minimum $SpO_2 \sim 85\%$). The more severe reduction of heart rate variability during SH compared to IH observed in the present (**Table 2**) and previous studies is however in contrast to previous observations suggesting greater cardiorespiratory alterations due to intermittent vs. sustained hypoxia in rats (8-h/day IH with 15 s $FiO_2 = 0.05$ –5 min $FiO_2 = 0.21$; 4-h/day SH at ~ 6500 m), possibly due to oxidative stress associated with repetitive deoxygenation–reoxygenation cycles (Peng and Prabhakar, 2004). In the later study, the cycle duration and hypoxia severity were nevertheless shorter and more severe, respectively, (and therefore potentially more harmful) which may explain these contrasting results when comparing SH and IH exposures.

Under similar hypoxemic levels (i.e., similar target SpO_2 in SH and IH), the present study demonstrates that (i) SH induces larger reductions in muscle (larger HbO_2 and HHb but not TSI changes) and even more pre-frontal cortex (larger HbO_2 , HHb, and TSI changes) oxygenation compared to IH sessions (**Table 3**) and (ii) larger reductions in pre-frontal cortex than muscle oxygenation are induced during both SH and IH sessions (**Figure 6**). Of note, these changes in tissue oxygenation occurred together with unchanged $HbTot$ both at the pre-frontal cortex and muscle levels (**Figures 7, 8**), suggesting no significant change in local blood flow. Although the slightly greater reduction in SpO_2 during SH compared to IH ($\sim 2\%$ difference, **Table 1**) may have contributed to the greater tissue deoxygenation during SH sessions, it is unlikely to explain the large difference in tissue oxygenation between both hypoxic conditions (**Table 3**). Since we have previously shown that both muscle and cerebral deoxygenations display slower kinetics than arterial deoxygenation when FiO_2 is reduced (Rupp et al., 2013), it can be suggested that 5-min hypoxic phases during IH sessions did not allow maximal muscle and pre-frontal cortex deoxygenation compared to prolonged and sustained arterial oxygenation reduction characterizing SH sessions (where maximal deoxygenation required up to 30 min of hypoxic exposure, **Figure 6**). Whether the larger cerebral deoxygenation induced by SH compared to IH sessions contributed to some differences in cardiorespiratory responses observed between the two types of hypoxic exposure in the present study (e.g., the

greater reduction in heart rate variability in SH compared to IH) remains to be elucidated.

The larger reduction in pre-frontal cortex oxygenation compared to muscle oxygenation during both SH and IH sessions (**Figure 6**) is in accordance with the greater cerebral oxygenation sensitivity to reduction in arterial oxygenation previously reported by our group under both sustained [$\text{FiO}_2 = 0.12$ (Rupp et al., 2013)] and intermittent [100 s $\text{FiO}_2 = 0.12$ –20 s $\text{FiO}_2 = 1.0$ (Rupp et al., 2016)] hypoxia, possibly due to greater basal metabolic activity and the inability to recruit additional capillaries at the cerebral compared to the muscle level. In order to induce significant deoxygenation and therefore potential adaptations within the muscle, coupling hypoxic exposure and physical exercise may be an attractive hypoxic conditioning strategy to consider (Millet et al., 2016).

Short-Term Cardiorespiratory Adaptations

While chronic hypoxic exposure such as at high altitude is known to increase the hypoxic ventilatory response [e.g., 5 days at 4350 m (Rupp et al., 2014)], repeated bouts of intermittent hypoxia have also been shown to increase hypoxic chemosensitivity [7 days, 1–3-h/day SH with $\text{FiO}_2 = 0.12$ (Katayama et al., 2009); 10 days, 1-h/day SH at $\text{SpO}_2 = 80\%$ (Lusina et al., 2006); 14 days, 1-h/day IH with 5–7 min normocapnic rebreathing—5–7 min $\text{FiO}_2 = 0.21$ (Bernardi et al., 2001b); 10 days, 1-h/day IH with 5 min $\text{FiO}_2 = 0.12$ –5 min $\text{FiO}_2 = 0.21$ (Foster et al., 2005)]. In the present study, three sessions of hypoxic conditioning did not change the ventilatory response during SH or IH (**Figure 2**) while previous studies reported increased hypoxic ventilatory responses after at least seven hypoxic sessions (Bernardi et al., 2001b; Foster et al., 2005; Lusina et al., 2006; Katayama et al., 2009). Changes in hypoxic ventilatory response may require a larger amount of hypoxic conditioning sessions than performed in the present study. Hypoxic heart rate response has been reported to be reduced [~50-min IH with 5–6 min $\text{FiO}_2 = 0.10$ –4 min $\text{FiO}_2 = 0.21$, (Zhang et al., 2014)] or unchanged [7 days, 1-h/day SH at 4500 m (Katayama et al., 2001); 10 days, 1-h/day IH with 5 min $\text{FiO}_2 = 0.12$ –5 min $\text{FiO}_2 = 0.21$ (Foster et al., 2005)] by repeated exposure to intermittent hypoxia. In the present study, the SH- or IH-induced increases in HR were similar at S1 and S3 (**Figure 3**), indicating no change in heart rate hypoxic response after three hypoxic conditioning sessions.

While SH induced similar increase in blood pressure during S1 and S3, a significant increase in SBP was observed in IH during S3 only, due to a reduction in baseline normoxic value compared to S1 (**Figure 3**). This ~8 mmHg SBP reduction is of significance in healthy young subjects and in terms of cardiovascular prevention, and may represent a true effect of IH conditioning on vascular tone. Shatilo et al. (2008) reported similar reduction in blood pressure in healthy older individuals (61 ± 2 years) following 10 days of IH conditioning (40 min/day IH with 5-min $\text{FiO}_2 = 0.12$ –5-min $\text{FiO}_2 = 0.21$). Several studies also suggested that in patients with hypertension, hypoxic conditioning programs can significantly reduce blood pressure [see for a review (Serebrovskaya et al., 2008)]. Lyamina et al. (2011) for instance reported in patients with stage 1 hypertension

a significant decrease in blood pressure (>20 and >15 mmHg systolic and DBP reduction, respectively) after 20 days of IH conditioning (30–60 min/day IH with 3 min $\text{FiO}_2 = 0.10$ –3 min $\text{FiO}_2 = 0.21$). Hence, the present study suggests that even in healthy young subjects with normal blood pressure and no cardiovascular risk factor, early improvements in blood pressure can occur after only three IH conditioning sessions. Improvements in blood pressure after hypoxic conditioning may be due to changes in the autonomic function (with augmented parasympathetic tone), increased endothelial NO production and enhanced vasoactive factors such as vascular endothelial growth factor (Serebrovskaya et al., 2008). The results of the present study suggesting that IH conditioning may be more efficient in reducing blood pressure compared to SH conditioning are in accordance with the study by Rodway et al. (2007) showing some specific effects of IH (3 days, 1-h IH with 10 min $\text{SpO}_2 = 80$ –90%—10 min $\text{FiO}_2 = 0.21$) compared to SH (3 days, 1-h SH with $\text{SpO}_2 = 80$ –90%) in young healthy subjects, i.e., an increase in nitric oxide synthases expression in circulating lymphocytes negatively correlated with blood pressure after three IH conditioning sessions only.

Interestingly, from S1 to S3, opposite changes in heart rate variability were observed in SH compared to IH conditions (**Table 2**). Greater reduction in overall heart rate variability (as suggested by lower SDNN) and larger vagal withdrawal (as suggested by lower PNN50 and RMSSD) were observed in SH after three sessions. Conversely, IH was associated with larger overall heart rate variability (as suggested by larger SDNN) and greater parasympathetic activity (as suggested by larger RMSSD) at S3 compared to S1. Previous studies have reported that similar IH protocols than the present study but over longer durations also increased heart rate variability [14 days, ~50-min IH with 5–6 min $\text{FiO}_2 = 0.10$ –4 min $\text{FiO}_2 = 0.21$ (Zhang et al., 2014); 16 days, 1-h/day IH with 5 min $\text{FiO}_2 = 0.16$ –0.10–5 min $\text{FiO}_2 = 0.21$ (Lizamore et al., 2016)], while Lusina et al. (2006) reported that 10 daily 1-h SH exposures with $\text{SpO}_2 = 80\%$ in healthy young subjects increased muscle sympathetic nerve activity both during and after hypoxic exposure. These previous results together with the present data suggest important differences regarding changes in heart rate variability induced by repetitive SH vs. IH exposures. Improved heart rate variability and possibly increased parasympathetic activity following three sessions of IH in the present study (**Figure 4**) may underlie the reduction in normoxic SBP observed at S3 in this condition. Although the significance of these early and opposite changes in heart rate variability indices observed in SH and IH needs to be further investigated, the present data suggest that measurements of heart rate variability may provide useful physiological biomarkers to monitor the effects of hypoxic conditioning strategies.

From S1 to S3, and according to the study design, similar reductions in arterial oxygenation were induced in both SH and IH conditions (**Figure 1**). The similar reductions in pre-frontal cortex oxygenation from S1 to S3 (**Figure 8**) suggest that, as opposed to the effect of several days of sustained hypoxia [5 days at 4350 m (Rupp et al., 2014)] or intermittent hypoxic exposure [10 days, 1-h/day IH with 5 min $\text{FiO}_2 = 0.12$ –5 min $\text{FiO}_2 = 0.21$ (Foster et al., 2005)], no early changes in hypoxic cerebrovascular

responses were induced. In contrast to the pre-frontal cortex, muscle deoxygenation was larger in S3 compared to S1 both in SH and IH (Figure 7). The reduction in muscle TSI, an index of tissue oxygenation, reached significance at S3 only both during SH and IH (Figure 6). These results suggest that while during the first session of hypoxic conditioning the muscle is minimally exposed to hypoxia despite substantial reduction in arterial oxygenation level, the repetition of hypoxic sessions accentuates muscle deoxygenation for identical reduction in SpO₂. This may suggest that the potential effects of hypoxic conditioning on the muscle (if any) may require several sessions to be induced. The reasons for this reduction in muscle oxygenation triggered after several hypoxic conditioning sessions remain to be determined but larger muscle sympathetic nerve activity as observed by Lusina et al. (2006) after 10 daily 1-h SH exposures with SpO₂ = 80% may contribute to reduced muscle perfusion and oxygenation, at least in SH condition.

Limitations

Although the present study provides several important insights into the acute and short-term responses to SH and IH conditioning strategies, several other potential adaptations remain to be evaluated such as hematological and metabolic changes or exercise cardiorespiratory responses. The effects of SH and IH conditioning protocols also need to be compared over more prolonged duration (e.g., several weeks) as usually performed to induce health benefits. The present study focused however on acute differences and early adaptations to hypoxic conditioning strategies in order to provide potential physiological biomarkers able to monitor the benefits and potential deleterious consequences of these hypoxic conditioning interventions. Females were included in the present study without taking account of potential effects of menstrual cycles on hypoxic responses. Although recent results indicate that the menstrual cycle may not affect hypoxic ventilatory responses for instance (Macnutt et al., 2012), future studies on hypoxic conditioning should clarify the effect of menstrual cycle. Finally, the present data applies to healthy young subjects and further studies are needed to evaluate the acute and short-term responses to similar hypoxic conditioning strategies in older individuals and in patients at risk of or with cardiovascular or cerebrovascular abnormalities.

Practical Considerations

The present study aimed to provide practical information useful to design hypoxic conditioning interventions for healthy subjects. These results also give a rationale to further develop and investigate hypoxic conditioning interventions for patients, especially regarding the type of hypoxic exposure (SH vs. IH) and the potential physiological biomarkers that could be used during the first sessions in order to detect early adaptations. During SH sessions, larger homeostasis disruption seemed to occur compared to IH sessions, at least when considering the larger reduction in heart rate variability and greater tissue deoxygenation in SH compared to IH sessions (Tables 2, 3). Although this larger physiological stress in SH

was well tolerated in the present population, it seemed to be associated with less favorable adaptations over three sessions compared to IH, at least regarding the improved heart rate variability and normoxic SBP observed from S1 to S3 in IH only. The lack of muscle deoxygenation during S1 and the relatively modest muscle deoxygenation level (compared to the pre-frontal cortex especially) at S3 suggest that both SH and IH at rest may not provide sufficient muscle homeostasis disruption in order to induce significant muscular adaptations (e.g., metabolic). Combining hypoxic exposure and muscular exercise (i.e., hypoxic exercise training) might be an attractive strategy especially to induce muscle adaptations. At last, the present study suggests that monitoring heart rate variability during and after hypoxic conditioning sessions may provide a useful physiological biomarker able to detect early adaptations and to indicate whether the hypoxic dose is adequate for a given individual. These practical considerations should help to implement hypoxic conditioning strategies to more fragile populations such as older individuals or patients with chronic diseases for instance.

CONCLUSIONS

The present results showed significant differences in heart rate variability and tissue (especially cerebral) oxygenation changes during acute SH vs. IH exposures in healthy young subjects. In addition, after three hypoxic conditioning sessions, IH only induced significant reduction in normoxic SBP while SH and IH induced opposite effects (i.e., a reduction and an increase, respectively) on heart rate variability. Hence this study indicates that the pattern of hypoxic exposure (i.e., sustained vs. intermittent) during hypoxic conditioning intervention should be carefully selected and suggests that heart rate variability may provide useful information about the early adaptations induced by such intervention.

AUTHOR CONTRIBUTIONS

SC, PF, SD, and SV contributed to the conception and design of the work; SC, AB, SM, SB, PF, SD, and SV contributed to data acquisition, analysis, and interpretation; SC, AB, SM, SB, PF, SD, and SV drafted the work and revised it critically; SC, AB, SM, SB, PF, SD, and SV approved the final version to be published; SC, AB, SM, SB, PF, SD, and SV agree to be accountable for all aspects of the work and ensure that questions related to the accuracy and integrity of all parts of the work are appropriately investigated and resolved.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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-Publication n °2-

**Cardiovascular and metabolic responses to passive hypoxic conditioning
in overweight and obese individuals**

Hypoxic conditioning and obesity

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Résumé

Alors que l'hypoxie intermittente sévère a des conséquences cardiovasculaires et métaboliques délétères (e.g. syndrome d'apnées obstructives du sommeil), une exposition modérée à l'hypoxie intermittente pourrait induire des effets positifs chez les personnes obèses. La présente étude visait à évaluer l'effet de 2 programmes de conditionnement hypoxique sur la santé cardiovasculaire et métabolique de sujets en surpoids ou obèses.

Dans cette étude randomisée contrôlée en simple aveugle, 35 sujets ($54 \pm 9,3$ ans, indice de masse corporelle $31,7 \pm 3,5$ $\text{kg}\cdot\text{m}^{-2}$) ont été randomisés en 3 groupes (3 séances d'une heure par semaine durant 8 semaines): hypoxie continue (HC, saturation artérielle en oxygène cible, $\text{SpO}_2 = 70\text{-}80\%$), hypoxie intermittente (HI, 5 min à SpO_2 cible = $70\text{-}80\%$ - 3 min de normoxie) et normoxie (N, groupe placebo). La ventilation, la fréquence cardiaque, la pression artérielle et l'oxygénation tissulaire (musculaire et cérébrale par spectroscopie proche infrarouge) ont été mesurées lors de la première et de la dernière séance de conditionnement hypoxique. La fonction vasculaire (rigidité vasculaire par la mesure de la vitesse de l'onde de pouls et réactivité vasculaire par la mesure du tonus artériel périphérique par pléthysmographie), la glycémie et l'insulinémie (et l'insulino-résistance: HOMA2-IR) et le profil lipidique ont été évalués avant et après les interventions.

HC et HI augmentent la réponse ventilatoire hypoxique ($+ 0,5 \pm 3,6$ et $+ 1,1 \pm 2,7$ $\text{l}\cdot\text{min}^{-1}$, respectivement, $p < 0,05$) et réduisent la tension artérielle diastolique mesurée en normoxie (-12 ± 15 et -13 ± 10 mmHg, respectivement, $p < 0,05$). HI seulement réduit la variabilité de la fréquence cardiaque (par exemple RMSSD $-21 \pm 35\%$, $p < 0,05$). HC et HI n'ont modifié ni l'indice de masse corporelle, ni la fonction vasculaire, ni l'équilibre glycémique, ni le profil lipidique.

Cette étude indique qu'un conditionnement hypoxique passif induit certaines adaptations cardiovasculaires et respiratoires positives chez les personnes obèses qui pourraient être amplifiées par la combinaison d'une exposition hypoxique avec des mesures diététiques et une activité physique.

Mots-clés: obésité, hypoxie, conditionnement, fonction cardiovasculaire, état métabolique

ABSTRACT

While severe intermittent hypoxia is well known to induce deleterious cardiovascular and metabolic consequences in sleep apnea syndrome, moderate intermittent hypoxia exposure may induce positive effects in obese individuals. The present study aimed to evaluate the effect of two hypoxic conditioning programs on cardiovascular and metabolic health status of overweight or obese individuals.

In this randomized single-blind controlled study, 35 subjects (age 54 ± 9.3 yr, body mass index 31.7 ± 3.5 $\text{kg}\cdot\text{m}^{-2}$) were randomized into three 8-week interventions (three 1-hour sessions per week): sustained hypoxia (SH, target arterial oxygen saturation, $\text{SpO}_2=70\text{-}80\%$), intermittent hypoxia (IH, 5 min target $\text{SpO}_2=70\text{-}80\%$ - 3 min normoxia) and normoxia (N, sham condition). Ventilation, heart rate, blood pressure and tissue oxygenation (near-infrared spectroscopy) measurements were performed before and during the first and last hypoxic conditioning sessions. Vascular function (pulse-wave velocity and reactive hyperemia), blood glucose and insulin (and insulin resistance: HOMA2-IR) and lipid profile were evaluated before and after the interventions.

Both SH and IH increased the hypoxic ventilatory response ($+0.5\pm 3.6$ and $+1.1\pm 2.7$ $\text{l}\cdot\text{min}^{-1}$, respectively; $p<0.05$) and reduced normoxic diastolic blood pressure (-12 ± 15 and -13 ± 10 mmHg, respectively; $p<0.05$). IH only reduced heart rate variability (e.g. RMSSD in normoxia $-21\pm 35\%$; $p<0.05$). Both SH and IH induced no significant change in body mass index, vascular function, blood glucose and insulin and lipid profile.

This study indicates that passive hypoxic conditioning induces some positive cardiovascular and respiratory adaptations in obese individuals suggesting that future strategies for obesity management may combine hypoxic exposure with dietary restriction and physical activity.

Keywords: Obesity, hypoxia, conditioning, cardiovascular function, metabolic status

Introduction

Obesity is characterized by an excess of body fat stores and is considered as the global epidemic of the 21st century. Obesity is defined based on a body mass index (BMI) $>30 \text{ kg}\cdot\text{m}^{-2}$, while individuals with a BMI between 25 and $30 \text{ kg}\cdot\text{m}^{-2}$ are considered overweight. In 2016, more than 1.9 billion adults were overweight and 650 million of them were obese (56). Obesity is a major health burden because of associated comorbidities such as increased risk of insulin resistance, diabetes, hypertension, cardiovascular diseases, cancers and possibly higher mortality rate (12, 14). Various interventions can be considered to manage obesity and its comorbidities, including diet manipulation, caloric restriction, increased physical activity and in the most severe patients, bariatric surgery (1). Current strategies often failed however to induce clinically significant and sustained weight loss (16) and innovative strategies are warranted for both weight loss and improvements in cardiovascular and metabolic health status of overweight and obese individuals.

Hypoxia is defined as reduced oxygen availability at the lung, blood or tissue levels. While it is considered as a key pathophysiological mechanism in several diseases such as respiratory diseases, several arguments also suggest that hypoxia can induce positive adaptations of the human body able to prevent or even contribute to the treatment of several pathologies (53). Intermittent hypoxia is well known as a major mechanism responsible for adverse systemic consequences of sleep apnea syndrome leading to the production of reactive oxygen species, systemic inflammation and cardiovascular and metabolic morbidities (25). While intermittent hypoxia in sleep apnea refers to repetitive (severe) désaturation-reoxygenation within 30-90 s cycles, intermittent (moderate) hypoxia consisting of hypoxic exposure for several minutes or hours while living the rest of the day in normoxia has been shown in animal and humans studies to induce positive cardiovascular and metabolic responses (35). Hence, hypoxic conditioning refers to systemic (whole body) or local (organ) exposure to hypoxia in order to induce protective or recovery processes. In addition to the cardio- (4, 36) and cerebral- (19, 47) protection of hypoxic conditioning, animal studies also demonstrated that intermittent hypoxic exposure may have positive metabolic consequences (9, 39, 57). Qin et al. (39) showed in mice submitted to a fatty-diet that intermittent hypoxia exposure (daily $4 \times 30 \text{ min}$ in hypobaric hypoxia equivalent to 3000 m, 40 days) reduced body weight and blood glucose and increased insulin and leptin levels compared to normoxic

exposure. These and other results led to consider hypoxia as a two-sided sword for obesity and its comorbidities, potentially playing a role both in the pathogenesis (e.g. inflammation, sleep apnea) and therapy of the disease (22).

Although the potential of hypoxic conditioning as a new therapeutic tool in overweight and obese individuals has been underlined in several recent reviews (17, 22, 51), randomized control studies evaluating the effect of passive hypoxic conditioning in this context are still missing. Two uncontrolled pilot studies exposed overweight and obese individuals (6 patients in each study) to passive normobaric sustained (55) or intermittent (10) hypoxic sessions (target mean arterial oxygen saturation, $SpO_2 = 80\%$; 7-10 sessions). Hypoxic exposure appeared to increase basal energy expenditure and induced a shift in substrate utilization towards lipid sources (55) and to reduce low- to high-density lipoprotein ratio and systolic blood pressure (10). In healthy lean subjects, we have recently shown that three 1-hour intermittent (5 min hypoxia – 3 min normoxia) or sustained hypoxic ($SpO_2 = 70-80\%$) sessions over one week induced significant reduction in baseline systolic pressure and decreased (sustained hypoxia) or increased (intermittent hypoxia) heart rate variability (7). These results suggest that repetitive hypoxic exposure might be an efficient intervention to improve metabolic and cardiovascular status in overweight or obese individuals. Comparing hypoxic conditioning programs with different patterns of hypoxic exposure (i.e. intermittent *versus* sustained) to placebo normoxic exposure remains however to be performed to confirm the potential therapeutic interest of such intervention for obesity management.

Hence, the present study aimed to evaluate the metabolic and cardiovascular consequences of two (intermittent and sustained) hypoxic conditioning programs over 8 weeks in overweight and obese individuals. We hypothesized that both intermittent and sustained hypoxic conditioning would improve cardiovascular and metabolic health status compared to placebo normoxic exposure. Because our previous results in healthy subjects indicated changes in tissue (muscle and prefrontal cortex) deoxygenation responses after only 3 sessions of hypoxic conditioning (7), we also assessed muscle and prefrontal cortex oxygenation during hypoxic conditioning sessions.

Methods

Subjects

Thirty five (11 females), overweight or obese, sedentary subjects (age: 54 ± 9.3 yr, body mass index: 31.7 ± 3.5 $\text{kg} \cdot \text{m}^{-2}$) were included in the present study after routine medical assessment consisting of a short clinical examination, a 12-lead electrocardiogram (ECG) and a respiratory function test. The main inclusion criteria were as follows: age between 18 and 65 yrs, $\text{BMI} > 27$ $\text{kg} \cdot \text{m}^{-2}$, no type I or II diabetes, no heart diseases, no hypertension and no respiratory disease (except treated sleep apnea). All subjects lived at sea level (< 300 m) and were not exposed to altitude > 1000 m over the past three months. After inclusion, they were randomized into three groups: sustained hypoxia (SH), intermittent hypoxia (IH) and normoxia (sham condition, N). The study was approved by the local ethics committee (CPP Sud-Est V) and performed according to the Declaration of Helsinki. Subjects were fully informed of the procedure and risks involved and gave their written consent prior to all assessments. Subjects were asked not to make any significant lifestyle changes throughout the study (e.g. initiating physical activity or dietary restriction programs).

Experimental design

In this prospective, randomized, controlled, single-blind study performed in Grenoble (altitude: 210 m), all participants performed first a full familiarization trial in normoxia including sitting and breathing quietly through a facemask, with continuous ECG and blood pressure measurements. Then, according to the randomization, subjects performed twenty-four 1-hour sustained/intermittent hypoxic conditioning or normoxic (sham) sessions over 8 weeks. Subjects in the SH group inhaled a gas mixture with reduced inspiratory oxygen fraction (FiO_2) to induce a sustained target SpO_2 between 70-80% for 1 hour. Subjects in the IH group were submitted during each session to 7 cycles of 5 min of hypoxia (reduced FiO_2 to reach a target $\text{SpO}_2 = 70\text{-}80\%$) followed by 3 min of normoxia ($\text{FiO}_2 = 0.21$). Subjects in the N group breathed normoxic air ($\text{FiO}_2 = 0.21$) throughout each session. Subjects were blinded for the gas mixture composition they were inhaling. Cardiorespiratory and tissue oxygenation responses (see below) were continuously recorded during session 1 (S1) and session 24 (S24). During all other sessions, FiO_2 and SpO_2 only were recorded.

In all sessions, subjects seated comfortably in a semi-supine position, in a quiet environment. The hypoxic stimulus was obtained by having subjects breathe via a facemask a nitrogen-enriched gas mixture provided by a gas-mixing device (Altitrainer®, SMTEC S.A.,

Nyon, Switzerland). FiO_2 was individually adjusted to reach the targeted SpO_2 of 70-80% in both hypoxic conditioning interventions.

Sustained hypoxia (SH) sessions. SH sessions started with an initial phase of 5 min (10 min for S1 and S24) breathing normoxic, ambient air for baseline data collection. Then, the hypoxic phase began with a progressive decrease of FiO_2 from 0.21 to 0.10 over 10 min. FiO_2 was then automatically adjusted to reach 70-80% SpO_2 . This targeted SpO_2 level was sustained until 55 min of hypoxic exposure. There was a final normoxic recovery phase where participants breathed ambient air for an additional 5 min (10 min for S1 and S24).

Intermittent hypoxia (IH) sessions. IH sessions consisted of a 5 min (10 min for S1 and S24) baseline breathing normoxic, ambient air, followed by 7 cycles of 5-min hypoxia (breathing a gas mixture with reduced FiO_2 to reach the target SpO_2 of 70-80%) and 3-min normoxic breathing. The initial 5-min hypoxia was performed with a FiO_2 of 0.10 for all subjects, and FiO_2 was individually adjusted for the subsequent 5-min hypoxic phases in order to reach the target of SpO_2 . After the seven hypoxia-normoxia cycles, 7 min of normoxic recovery were performed.

Normoxia (N) sessions. During N sessions, subjects breathed continuously normoxic ambient air.

Cardiorespiratory measurements (S1 and S24)

Ventilation and end-tidal CO_2 partial pressure were monitored continuously breath-by-breath using a metabolic cart (MetaMax 3B, Cortex Biophysik GmbH, Leipzig, Germany). Gas analyzers and volume transducers were calibrated prior to each test with a 3-L syringe and reference gases, respectively, according to manufacturer's instructions. Blood pressure was measured twice on the dominant arm after 5 and 10 min of baseline normoxia with a digital pressure monitor system (A&D Medical, Kitamoto-Shi, Japan). Arterial blood pressure was measured continuously beat-by-beat using finger pulse photoplethysmography (Finometer, Finapres Medical Systems BV; Arnhem, The Netherlands). The digital pressure monitor system was used to calibrate the Finometer during baseline normoxic conditions. ECG was continuously recorded using a 2-lead ECG at a sampling rate of 1000 Hz (Holter AFT 1000, Holter supplies, Paris, France). FiO_2 and SpO_2 were recorded continuously during all session by using the Altitrainer® device (SMTEC S.A.).

Near-infrared spectroscopy (NIRS) measurements (S1 and S24)

Oxy[HbO₂]-, deoxy[HHb]- and total[HbTot]-hemoglobin concentration changes and tissue oxygenation index (TSI) were estimated continuously throughout testing sessions over two main sites using a two-wavelength (780 and 850 nm) multichannel, continuous wave NIRS system (Oxymon MkIII, Artinis Medical Systems, the Netherlands). Muscle hemodynamic was assessed at the middle of right *biceps brachialis* using a 4-cm interoptode distance. Left pre-frontal cortex hemodynamic was assessed between Fp1 and F3 locations according to the international 10–20 EEG system with 3.5-cm inter-optode distance. The probe holders were secured to the skin with double-sided tape and maintained with Velcro bands. Data were recorded continuously at 10 Hz and filtered with a 2-s width moving Gaussian smoothing algorithm before analysis. NIRS data are relative values expressed as changes from the start of each session.

Vascular function and biological analysis

Arterial stiffness was assessed by carotid-femoral pulse-wave velocity as measured with a Complior device (Artech Medical; Pantin, France) (3). Endothelial function was assessed by reactive hyperemia using finger plethysmography with an Endo-PAT device (Itamar Medical Ltd) (11). After an overnight fast, blood samples were drawn for plasma analysis of glucose, insulin, triglycerides, total and high-density lipoprotein cholesterol (HDL-C), high-sensitive C-reactive protein (hsCRP). The low-density lipoprotein cholesterol (LDL-C) was calculated according to the Friedewald's formula. The insulin resistance was calculated according to the homeostasis model assessment of insulin resistance (HOMA2-IR; (24)), based on fasting blood glucose and insulin concentrations (<http://www.dtu.ox.ac.uk/>).

Data analysis

ECG data was transferred to the QuickReader software (Holter supplies) and R-R intervals were analyzed by means of the Kubios HRV Analysis Software 2.2 (49, 50). Heart rate variability parameters were recorded and calculated in accordance with the task force of the European society of cardiology and the North American society of pacing and electrophysiology (2). Data were visually inspected to remove artifacts and heart rate variability parameters were calculated over 5-min periods. The following parameters were used to analyze the heart rate variability within the time domain: standard deviation of R-R interval (SDNN), percentage of successive R-R that varied by 50 ms or more (PNN50) and the root-mean-square difference of successive normal R-R intervals (RMSSD). In the

frequency domain, the following parameters were calculated using a Fast Fourier Transform: low frequency (LF; 0.04–0.15 Hz), high frequency (HF; 0.15–0.40 Hz) and LF/HF ratio.

During SH and N sessions, minute ventilation (V_E), gas exchange heart rate, arterial blood pressure, SpO₂ and NIRS parameters were averaged over the last 30 s of the 5th and 10th min of baseline normoxic phase, of each 5-min periods in hypoxia and of the 5th and 10th min of the normoxic recovery phase. During IH sessions, the same parameters were averaged over the last 30 s of the 5th and 10th min of the baseline normoxic phase, of each 5-min hypoxic and 3-min normoxic periods and after the 5th and 10th min of the normoxic recovery phase. Heart rate variability parameters were calculated over 5-min periods during the baseline normoxic phase, every 5 min during the hypoxic phase of SH sessions and during every 5-min hypoxic phases of IH sessions, and during the normoxic recovery phase.

Statistical Analysis

All statistical procedures were completed on Statistica version 10 (Statsoft, Tulsa, OK). Normality of distribution and homogeneity of variances of the main variables were confirmed using a Shapiro-Wilk normality test and the Levene's test, respectively. For each hypoxic condition (SH, IH and N), in order to assess changes overtime within the sessions and changes from S1 to S24, a two-way ANOVA (time \times session) with repeated measures was performed for each dependent variable. Post-hoc Tukey's tests were applied to determine a difference between two mean values if the ANOVA revealed a significant main effect or interaction effect. For all statistical analyses, a two-tailed alpha level of 0.05 was used as the cut-off for significance. All data are presented as mean values \pm SD within text and tables.

Results

Patients randomized in the SH, IN and N groups did not differ between the intervention regarding age (SH 53 ± 11 yrs, IH 55 ± 7 yrs, N 52 ± 11 yrs) and anthropometric characteristics (Table 1, all $p > 0.05$). Patient attendance to the hypoxic conditioning/normoxic sessions was excellent in each group (SH 23 ± 1 sessions, IH 22 ± 1 sessions, N 21 ± 2 sessions). No adverse effect of hypoxic exposure was observed throughout the protocol.

Cardiorespiratory measurements during S1 and S24

Figure 1 shows the FiO_2 and SpO_2 values recorded during S1 and S24 of each group. According to the protocol design, mean SpO_2 was $76 \pm 5\%$ during the hypoxic phase of SH sessions, $76 \pm 5\%$ during the hypoxic phases of the IH sessions and $96 \pm 2\%$ during the N sessions. These SpO_2 values were obtained by reducing FiO_2 on average to 0.11 ± 0.01 during the SH sessions and 0.10 ± 0.01 during the IH sessions. FiO_2 was 0.21 throughout the N sessions. FiO_2 and SpO_2 did not differ between S1 and S24 in each group (all $p > 0.05$).

V_E throughout S1 and S24 in each group is provided in Figure 2 for each group. V_E was significantly higher in S24 compared to S1 in the SH group (ANOVA session main effect, $F(1, 11) = 9.4$, $p = 0.011$) as well as in the IH group (ANOVA interaction effect, $F(16, 176) = 1.8$, $p = 0.029$), while no significant change was observed in the N group (ANOVA interaction effect, $F(14, 112) = 1.1$, $p = 0.399$). Mean end-tidal CO_2 partial pressure did not differ between S1 and S24 in the hypoxic phase of SH (33 ± 3 versus 32 ± 2 mmHg; ANOVA interaction effect, $F(14, 154) = 0.7$, $p = 0.731$), in the hypoxic phases of IH (33 ± 3 versus 32 ± 3 mmHg; ANOVA interaction effect, $F(16, 176) = 1.2$, $p = 0.248$) and during N sessions (37 ± 2 versus 36 ± 2 mmHg; ANOVA interaction effect, $F(14, 112) = 0.8$, $p = 0.694$).

Heart rate and blood pressures throughout S1 and S24 are provided in Figure 2 for each group. Heart rate did not differ between S1 and S24 in all groups (SH, ANOVA interaction effect, $F(14, 154) = 0.8$, $p = 0.598$; IH, ANOVA interaction effect, $F(16, 176) = 0.9$, $p = 0.200$; N, ANOVA interaction effect, $F(14, 112) = 1.3$, $p = 0.202$). Systolic blood pressure did not differ between S1 and S24 in all groups (SH, ANOVA interaction effect, $F(14, 154) = 1.1$, $p = 0.367$; IH, ANOVA interaction effect, $F(16, 176) = 0.8$, $p = 0.705$; N, ANOVA interaction effect, $F(14, 112) = 0.8$, $p = 0.434$). Diastolic blood pressure was significantly reduced in S24 compared to S1 during the initial normoxic phase as well as during the beginning of the hypoxic phase in the SH group (ANOVA interaction effect, $F(14, 154) = 1.9$, $p = 0.030$) and the IH group (ANOVA interaction effect, $F(16, 176) = 2.8$, $p < 0.001$), while no significant change was observed in the N group (ANOVA interaction effect, $F(14, 112) = 1.7$, $p = 0.071$).

Heart rate variability parameters throughout S1 and S24 are provided in Figure 3 for each group. Heart rate variability did not differ between S1 and S24 in the SH and N groups. In the IH group, SDNN (ANOVA interaction effect, $F(10, 100) = 2.8, p = 0.004$; ANOVA main session effect, $F(1, 10) = 28.7, p < 0.001$), PNN50 (ANOVA interaction effect, $F(10, 100) = 4.0, p < 0.001$; ANOVA main session effect, $F(1, 10) = 13.0, p = 0.005$) and RMSSD (ANOVA interaction effect, $F(10, 100) = 3.6, p < 0.001$; ANOVA main session effect, $F(1, 10) = 24.3, p < 0.001$) were lower and LF/HF (ANOVA interaction effect, $F(10, 100) = 4.1, p < 0.001$; ANOVA main session effect, $F(1, 10) = 12.5, p = 0.005$) was higher in S24 compared to S1.

NIRS measurements during S1 and S24

Muscle NIRS parameters throughout S1 and S24 are provided in Figure 4 for each group. In the SH group, $[HbO_2]$ and $[HbTot]$ decreased less in S24 compared to S1 (ANOVA interaction effect, $F(14, 154) = 1.9, p = 0.026$ and $F(14, 154) = 3.5, p < 0.001$, respectively) while $[HHb]$ (ANOVA interaction effect, $F(14, 154) = 1.4, p = 0.165$) and TSI (ANOVA interaction effect, $F(14, 154) = 1.6, p = 0.099$) did not differ significantly between sessions. In the IH group, $[HbO_2]$ decreased less in S24 compared to S1 (ANOVA interaction effect, $F(16, 176) = 3.4, p < 0.001$) while $[HHb]$ (ANOVA interaction effect, $F(16, 176) = 0.4, p = 0.976$), $[HbTot]$ (ANOVA interaction effect, $F(16, 176) = 1.4, p = 0.138$) and TSI (ANOVA interaction effect, $F(16, 176) = 0.8, p = 0.653$) did not differ significantly between sessions. In the N group, no significance difference in NIRS parameters was observed between S1 and S24 (all $p > 0.05$).

Pre-frontal cortex NIRS parameters throughout S1 and S24 are provided in Figure 5 for each group. In the SH group, TSI was significantly lower in S24 compared to S1 (ANOVA interaction effect, $F(14, 154) = 2.0, p = 0.022$) while $[HbO_2]$ (ANOVA interaction effect, $F(14, 154) = 0.8, p = 0.714$), $[HHb]$ (ANOVA interaction effect, $F(14, 154) = 1.6, p = 0.099$) and $[HbTot]$ (ANOVA interaction effect, $F(14, 154) = 1.0, p = 0.484$) did not differ significantly between sessions. In the IH group, $[HbTot]$ was significantly higher in S24 compared to S1 (ANOVA interaction effect, $F(16, 176) = 2.2, p = 0.006$) while $[HbO_2]$ (ANOVA interaction effect, $F(16, 176) = 1.5, p = 0.111$), $[HHb]$ (ANOVA interaction effect, $F(16, 176) = 1.6, p = 0.085$) and TSI (ANOVA interaction effect, $F(16, 176) = 1.0, p = 0.437$)

did not differ significantly between sessions. In the N group, no significance difference in NIRS parameters was observed between S1 and S24 (all $p > 0.05$).

Anthropometric characteristics, vascular function and biological analysis

Anthropometric characteristics, vascular function and biological analysis performed in each group pre- and post-intervention are provided in Table 1. No difference was observed from pre- to post-intervention for any parameter in each group (all $p > 0.05$).

Discussion

This is the first randomized controlled study to evaluate the effect of two passive hypoxic conditioning programs on cardiovascular and metabolic health status of overweight and obese individuals. Both sustained and intermittent hypoxic conditioning induced a significant increase in hypoxic ventilatory response and a reduction in diastolic blood pressure. Intermittent hypoxic conditioning only induced a significant reduction in heart rate variability. Muscle oxygenation tended to be improved during hypoxic exposure after both SH and IH programs while prefrontal cortex oxygenation worsened and prefrontal cortex blood volume increased during hypoxic exposure following the SH and IH programs, respectively. Both hypoxic conditioning programs induced no change in anthropometric values, vascular function and blood glucose and lipid profiles.

Altitude and normobaric hypoxic exposure is increasingly used in athletic populations in an attempt to improve competitive performances (34). In addition to athletes and coaches, the potential of hypoxic exposure to induce positive physiological adaptations has become over the past years a subject of interest for clinical scientists (27, 53). Obesity and its associated comorbidities have been underlined as a clinical condition where hypoxic conditioning could potentially improve several aspects of the disease (17, 22, 51). Passive hypoxic conditioning may be an attractive treatment strategy especially in patients with impaired exercise capacity and musculoskeletal limitations (e.g. ankle and knee disorders) as frequently encountered in overweight and obese individuals (46). In the present study we evaluated the effect of two passive hypoxic conditioning programs (sustained and intermittent

hypoxic sessions potentially inducing distinct responses, (35)) on respiratory, cardiovascular, tissue oxygenation and metabolic parameters in order to provide an overview of the potential clinical interest of this treatment strategy in overweight and obese individuals. Because for a given reduction in inspiratory oxygen pressure there is a large inter-individual variability in SpO₂ reduction and therefore potentially in hypoxic conditioning responses (17), the same target SpO₂ was used during hypoxic conditioning sessions for all subjects by individually adjusting FiO₂ throughout all sessions (figure 1).

Cardiorespiratory responses to hypoxic conditioning

In humans and mammals, the carotid body senses the arterial levels of oxygen, carbon dioxide, pH and initiates the systemic ventilatory and cardiovascular adjustments to hypoxic exposure. It is well known that chronic exposure to sustained hypoxia (for hours and days, e.g. at high altitude (41)) augments the ventilatory response to acute hypoxia in humans, a phenomenon known as ventilatory acclimatization. In healthy subjects, repeated bouts over several days of intermittent hypoxia have also been shown to increase hypoxic chemosensitivity (5, 13, 20, 29, 54) despite the total dose of hypoxic exposure being a lot smaller compared to chronic sustained hypoxia exposure. In the present study only 3 times 1 hour of sustained or intermittent hypoxic exposure per week for 8 weeks induced a slight but significant increase in hypoxic ventilatory response (as shown by higher V_E during identical reduction in SpO₂ in S24 compared to S1, figures 1 and 2). V_E in the sham N group did not change between S1 and S24 indicating that the increased ventilatory response observed in SH and IH groups represent a true physiological effect. Obese patients can develop some degree of hypoventilation (37, 52) and it remains to evaluate whether an increase in hypoxic ventilatory response as observed in the present study might be of clinical significance to improve ventilation and blood gases either during the day or at night in these patients (33).

Hypoxic heart rate response has been reported to be reduced [~50-min IH with 5-6 min FiO₂ = 0.10 - 4 min FiO₂ = 0.21, (58)] or unchanged [7 days, 1-h/day SH at 4500 m (21); 10 days, 1-h/day IH with 5 min FiO₂ = 0.12 – 5 min FiO₂ = 0.21 (13)] following repeated exposure to intermittent hypoxia in healthy subjects. Pilot studies in overweight and obese individuals reported no change in heart rate following 7-10 sessions of SH or IH (10, 55). In the present study, 24 sessions of both SH and IH did not change heart rate during hypoxic exposure, suggesting that hypoxic conditioning programs are less likely to induce changes in heart rate hypoxic responses than hypoxic ventilatory responses. IH has however been shown to reduce heart rate and to improve baroreflex sensitivity in patients with chronic obstructive

pulmonary diseases showing impaired baroreflex sensitivity at baseline (15). Hence, in obese patients with more severe cardiovascular function impairments than in the present study, hypoxic conditioning might lead to significant improvement in cardiovascular function.

Hypoxic conditioning has been shown to reduce systolic and diastolic blood pressure in healthy subjects (7, 28), to reduce systolic blood pressure in healthy older individuals (45) and in subjects with chronic obstructive pulmonary diseases symptoms (6). In overweight or obese subjects, Workman et al. (55) reported no change in blood pressure after 7 sessions of IH while Costalat et al. (10) reported a reduction in systolic blood pressure after 10 IH sessions. In the present study, both the SH and the IH programs led to significant reductions in normoxic diastolic blood pressure (-12 ± 15 and -13 ± 10 mmHg after SH and IH, respectively; figure 2) of similar amplitude to previous observations reported by Serebrovskaya et al. (44) in their review of the Russian literature on hypoxic conditioning. Conversely, the sham N intervention did not lead to any reduction in blood pressure, confirming that hypoxic conditioning (rather than any other placebo effect) was responsible for the reduction in diastolic blood pressure. The hypotensive effect of the hypoxic conditioning programs in the present study is unlikely the consequence of an improved autonomic nervous system since heart rate variability parameters indicate no change (following SH) in sympathetic/parasympathetic balance or even (following IH) an increase in sympathetic activity and a reduction in parasympathetic activity (see below, figure 3). The reduction in diastolic blood pressure could be due to mechanisms at the endothelium level (44). Lyamina et al. (30) showed that a 20-day IH program in patients with stage 1 arterial hypertension significantly reduced blood pressure together with an increased in urine nitrate and nitrite concentrations. Hence, an increase in NO bioavailability may be responsible for the improvement in blood pressure observed after hypoxic conditioning strategies (44). In the present study however, parameters known to reflect vascular health status (pulse wave velocity and reactive hyperemia) did not change after both SH and IH programs. Reactive hyperemia assessed by peripheral arterial tonometry is known to reflect the endothelial function (23). Hence, the present results cannot confirm that hypoxic conditioning reduces blood pressure by improving endothelial function. Additional measurement of NO bioavailability and other vasoactive molecules (e.g. VEGF, (44)) would be required to identify the mechanisms responsible for blood pressure reduction following hypoxic conditioning programs in obese patients. Although hcCRP did not change after hypoxic conditioning in the present study, future studies should also investigate the effect of hypoxic

conditioning on low grade inflammation and oxidative stress as potential mechanisms responsible for improved cardiovascular health status.

Several previous studies in healthy subjects have reported that IH programs increased heart rate variability (28, 58) while others rather suggested increased sympathetic nervous activity (29, 48). One of the reasons for these discrepant results might be the severity of hypoxia and the pattern of exposure (SH *versus* IH, duration of the IH cycles) as reviewed by Mateika et al. (33). In a previous study where we assessed the effect of two short (3 sessions) hypoxic conditioning programs (SH and IH) in healthy subjects, SH and IH induced opposite effects on heart rate variability, 3 sessions of SH inducing a reduction of heart rate variability while IH increased it (8). In the present study with 24 sessions of similar SH or IH exposure, IH only influenced heart rate variability mostly during hypoxic exposure (figure 3), with an increased sympathetic nervous activity (as suggested by the increased LF/HF ratio) and some vagal withdrawal (as suggested by reduced PNN50 and RMSSD). These data suggest that the pattern of exposure to hypoxia during IH sessions (seven 5 min hypoxia – 3 min normoxia cycles) may induce specific sympathetic nervous system alterations at least in obese subjects. These changes in sympathovagal balance were however not associated with deleterious cardiovascular consequences (i.e. increased heart rate, blood pressure, arterial stiffness, endothelial function).

Tissue oxygenation responses to hypoxic conditioning

Although the physiological consequence of hypoxic exposure is usually characterized by the reduction in SpO₂, a given level of hypoxemia does not result in similar levels of hypoxia for all tissues, which is an important factor to consider when aiming to induce hypoxic conditioning mechanisms within target organs. We have previously shown for instance in healthy individuals exposed to acute intermittent hypoxia (one 45-min session) that the prefrontal cortex and the muscle (*biceps brachialis*) exhibit very different pattern of deoxygenation (43). In the present study, NIRS parameters recorded at the muscle level indicated that for both SH and IH, muscle oxygen delivery was less impaired (as suggested by higher [HbO₂] values) in response to a similar SpO₂ reduction at S24 compared to S1 (figure 4). This effect was associated with a larger local blood volume ([HbTot]) in SH only. This suggests that hypoxic conditioning strategies might induce significant improvement in muscle perfusion (e.g. increased blood flow, increased capillarization) able to improve local oxygen delivery at rest. Whether this effect might have some functional consequences during exercise for instance remains to be determined.

As previously reported (7, 42, 43), hypoxic exposure led to greater prefrontal cortex than muscle deoxygenation (figures 4 and 5). Moreover, after 24 sessions of SH, prefrontal cortex oxygenation (TSI) decreased to a greater extent in response to hypoxia, suggesting a larger cerebral hypoxic stress. In contrast, after 24 sessions of IH, [HbTot] was enhanced during hypoxic exposure suggesting larger local cerebral blood volume and improved cerebrovascular reactivity to hypoxia. These changes in prefrontal cortex NIRS signals occurred despite similar end-tidal CO₂ partial pressure in S1 and S24. Future studies should focus on the cerebrovascular consequences of hypoxic conditioning strategies since they may represent a powerful tool to improve brain perfusion and oxygenation in older individuals or in pathologies associated with impaired cerebral blood flow such as Alzheimer disease for instance (18, 32).

Metabolic responses to hypoxic conditioning

While Qin et al. (39) showed that IH exposure (daily 4 × 30 min in hypobaric hypoxia equivalent to 3000 m, 40 days) reduced body weight in mice submitted to a fatty-diet, other studies using IH protocols with more severe hypoxia and shorter hypoxic-normoxic cycles in obese animals did not lead to significant weight loss (e.g. (38, 40)). In six overweight and obese individuals, Workman et al. (55) reported that 7 SH sessions (3 hours, SpO₂ = 80%) increased basal energy expenditure. Such an increase in energy expenditure over a prolonged duration might promote weight loss. The present study tested for the first time the effects of prolonged (8 weeks) passive hypoxic conditioning strategies on body weight in obese individuals and showed that neither SH nor IH program induced a significant reduction in body mass index (Table 1). Similarly, waist and hip circumferences did not change significantly after hypoxic conditioning. Although hypoxic exposure might influence the energy balance by increasing energy expenditure and reducing energy intake (possibly due to endocrine mechanisms such as increased leptin levels) (22, 26), the absence of specific dietary intervention in the present study probably prevents the induction of any significant weight loss. Future studies should evaluate the effect of combining hypoxic conditioning to dietary or physical activity interventions in order to reduce body weight in obese individuals.

Animals studies suggest that hypoxic exposure might improve glucose and lipid profiles (see (17) for a review) and pilot data in overweight and obese individuals suggested that hypoxic conditioning might improve blood lipid profile with a reduction in LDL and LDL/HDL ratio (10). In diabetic patients, a single session of passive exposure was also shown to increase insulin sensitivity (31). In the present study however, both 8 weeks of SH and IH

induced no significant change in blood glucose and lipid profiles (Table 1). This lack of effect may be due to the normal baseline metabolic values exhibited by our population and future studies should focus on the effect of hypoxic conditioning interventions in patients with abnormal glucose and lipid profiles.

Limitations

Although the present study investigated several important responses to hypoxic conditioning in obese individuals, further studies are required to assess additional mechanisms such as baroreflex sensitivity, cerebrovascular reactivity (using transcranial Doppler for instance), NO metabolism and oxidative stress. Whether hypoxic conditioning leads to significant functional improvement in terms of exercise capacity, sleep quality, energy intake, appetite, and quality of life also remains to be addressed. Overweight or obese individuals without major comorbidities were included, therefore reducing the ability of hypoxic conditioning to improve their cardiovascular and metabolic health status.

In conclusion, this study showed that passive hypoxic conditioning using either SH or IH in overweight or obese individuals significantly increased hypoxic ventilatory response, decreased diastolic blood pressure while IH only reduced heart rate variability. These effects were not associated with improvement in anthropometric characteristics, vascular function or blood glucose and lipid profiles. SH and IH also induced significant changes in muscle and prefrontal oxygenation responses to hypoxia. Hence, passive hypoxic conditioning strategies alone induced some positive cardiovascular and respiratory adaptations in obese individuals. Future strategies for obesity management may combine hypoxic exposure with dietary restriction and physical activity interventions in order to induce synergetic effects on body composition and cardiovascular health status.

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Table 1. Anthropometric, vascular and biological characteristics in each group pre- and post-interventions.

	Sustained hypoxia		Intermittent hypoxia		Normoxia	
	Pre	Post	Pre	Post	Pre	Post
BMI (kg·m ⁻²)	31.0 ± 2.9	30.8 ± 2.6	32.5 ± 3.1	32.4 ± 3.3	32.5 ± 3.1	32.4 ± 3.2
Waist circumference (cm)	103.0 ± 9.7	102.5 ± 9.7	108.3 ± 10.2	107.2 ± 9.6	111.0 ± 6.2	109.5 ± 7.6
Hip circumference (cm)	104.3 ± 7.9	103.4 ± 8.2	106.6 ± 10.0	106.2 ± 10.7	109.1 ± 8.0	108.6 ± 8.5
PWV (m·s ⁻¹)	8.18 ± 1.35	8.07 ± 1.21	8.21 ± 1.62	7.88 ± 1.76	9.53 ± 2.20	8.66 ± 1.85
Reactive hyperemia index	2.05 ± 0.39	1.79 ± 0.25	2.21 ± 0.62	2.36 ± 0.43	2.17 ± 0.62	1.85 ± 0.47
Cholesterol (g·l ⁻¹)	2.22 ± 0.44	2.14 ± 0.46	2.10 ± 0.35	2.02 ± 0.31	2.06 ± 0.45	2.32 ± 1.01
Triglyceride (g·l ⁻¹)	1.69 ± 0.99	1.62 ± 1.30	1.15 ± 0.48	1.04 ± 0.41	1.81 ± 1.06	2.00 ± 1.33
HDL (g·l ⁻¹)	0.48 ± 0.10	0.49 ± 0.10	0.54 ± 0.11	0.53 ± 0.12	0.43 ± 0.13	0.49 ± 0.24
LDL (g·l ⁻¹)	1.40 ± 0.35	1.32 ± 0.39	1.33 ± 0.33	1.27 ± 0.27	1.21 ± 0.34	1.50 ± 0.72
Glycemia (mmol·l ⁻¹)	5.67 ± 0.84	5.70 ± 0.78	5.74 ± 0.69	5.93 ± 1.02	6.75 ± 2.21	6.39 ± 1.67
Insulin (μUI·ml ⁻¹)	9.08 ± 4.52	8.83 ± 5.08	10.77 ± 10.06	10.35 ± 7.44	16.73 ± 11.40	10.65 ± 5.59
HOMA2-IR	1.21 ± 0.62	1.18 ± 0.70	1.34 ± 1.05	1.41 ± 1.00	1.91 ± 0.85	1.43 ± 0.72
hsCRP (mg·l ⁻¹)	8.24 ± 5.06	8.69 ± 5.23	9.97 ± 10.29	9.65 ± 7.55	16.73 ± 11.40	10.59 ± 5.58

Data are mean ± SD. BMI, body mass index; PWV, pulse-wave velocity; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; HOMA2-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitive C-reactive protein.

Figure legends

Figure 1. Inspiratory oxygen fraction (FiO_2) and arterial oxygen saturation (SpO_2) during the first (S1) and the last (S24) sessions of the sustained hypoxia, intermittent hypoxia and normoxic (placebo) conditioning programs. * significantly different from normoxic baseline (5 min).

Figure 2. Minute ventilation (V_E), heart rate (HR) and blood pressure during the first (S1) and the last (S24) sessions of the sustained hypoxia, intermittent hypoxia and normoxic (placebo) conditioning programs. * significantly different from normoxic baseline (5 min); + significantly different between S1 and S24.

Figure 3. Heart rate variability indices (SDNN, standard deviation of R–R interval; PNN50, percentage of successive R–R that varied by >50 ms; RMSSD, root-mean-square difference of successive normal R–R intervals; LF/HF, ratio of low-to-high frequency) during the first (S1) and the last (S24) sessions of the sustained hypoxia, intermittent hypoxia and normoxic (placebo) conditioning programs. * significantly different from normoxic baseline (5 min); + significantly different between S1 and S24.

Figure 4. Muscle NIRS parameters (TSI, tissue saturation index; $[HbO_2]$, oxyhemoglobin concentration; $[HHb]$, deoxyhemoglobin concentration; $[HbTot]$, total hemoglobin concentration) during the first (S1) and the last (S24) sessions of the sustained hypoxia, intermittent hypoxia and normoxic (placebo) conditioning programs. * significantly different from normoxic baseline (5 min); + significantly different between S1 and S24.

Figure 5. Prefrontal cortex NIRS parameters (TSI, tissue saturation index; $[HbO_2]$, oxyhemoglobin concentration; $[HHb]$, deoxyhemoglobin concentration; $[HbTot]$, total hemoglobin concentration) during the first (S1) and the last (S24) sessions of the sustained hypoxia, intermittent hypoxia and normoxic (placebo) conditioning programs. * significantly different from normoxic baseline (5 min); + significantly different between S1 and S24.

Figure 1.

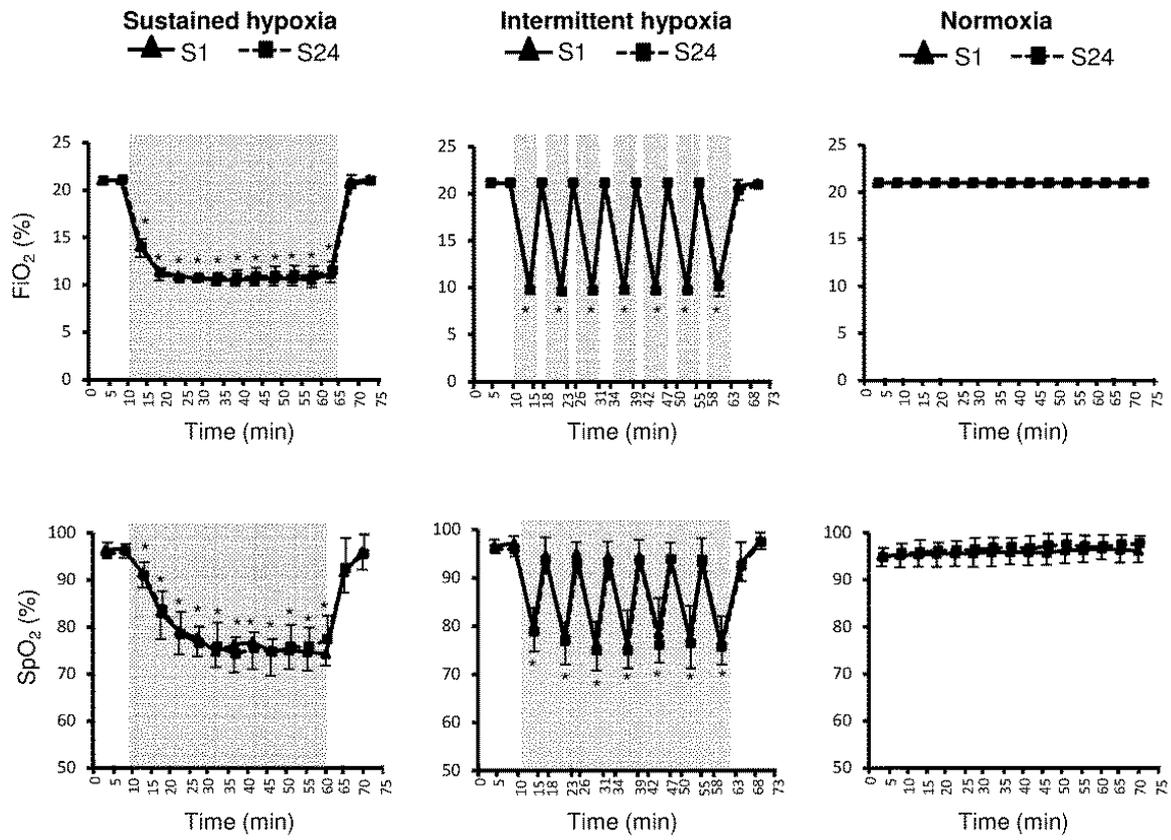


Figure 2.

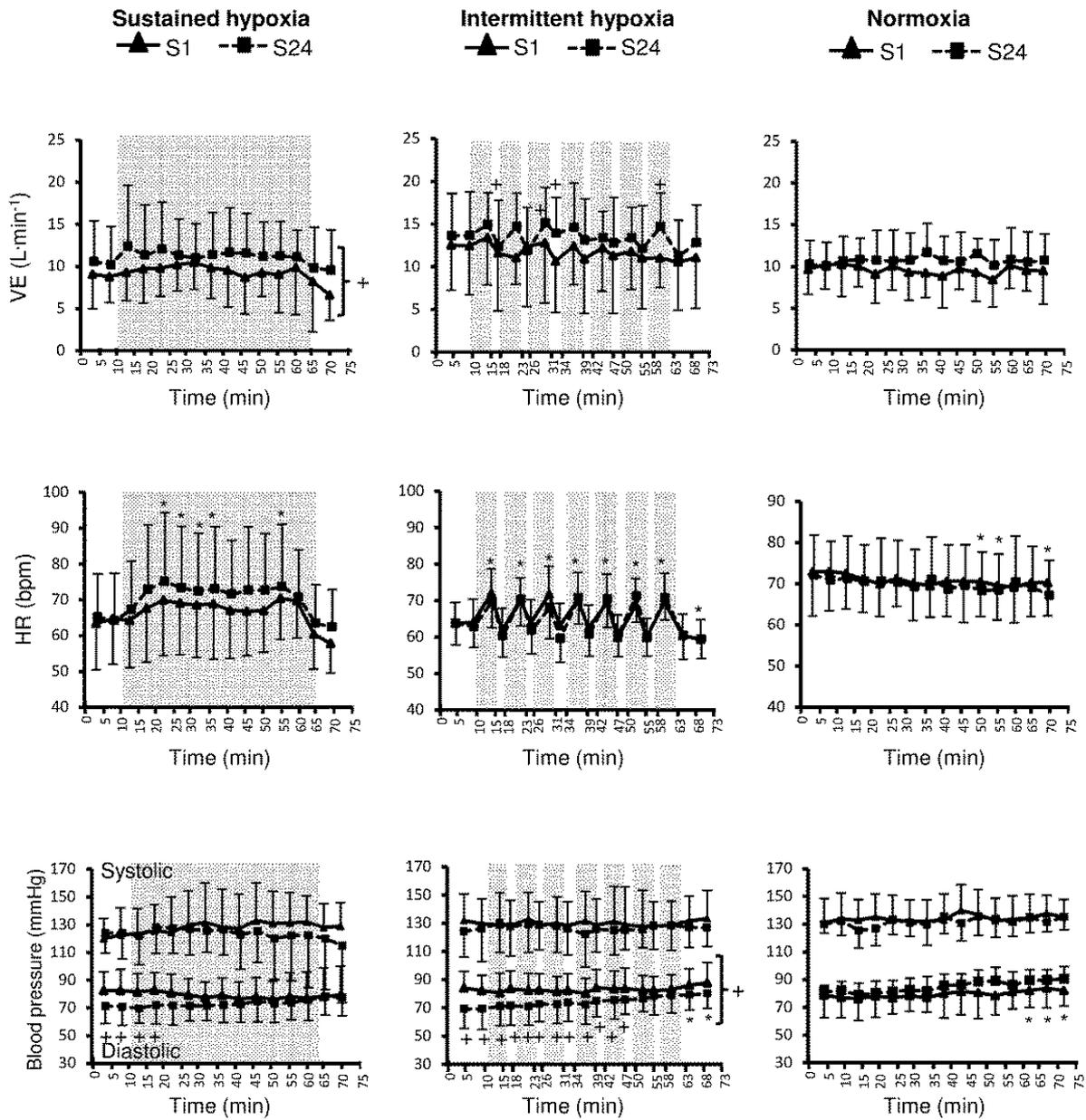


Figure 3.

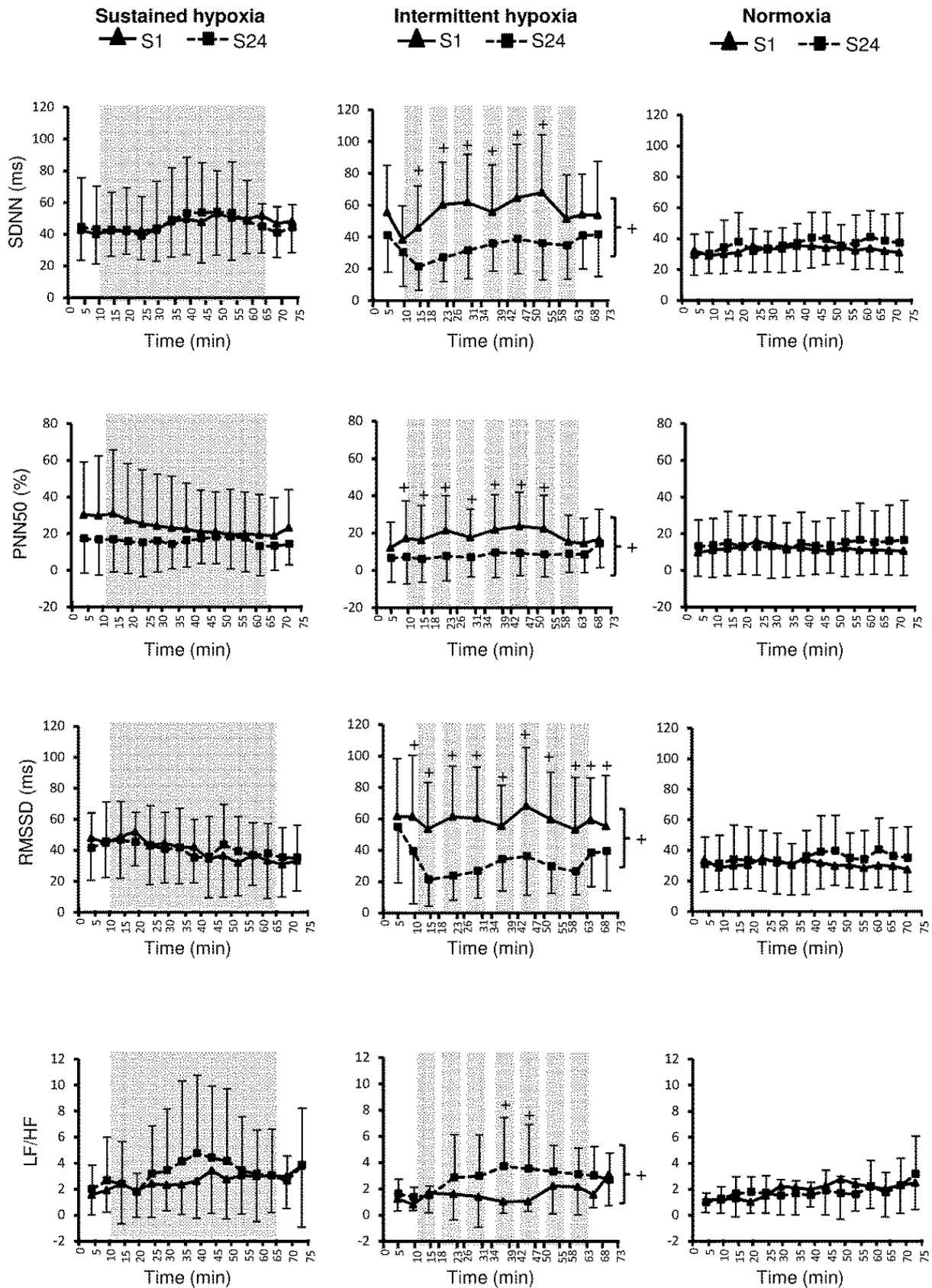


Figure 4.

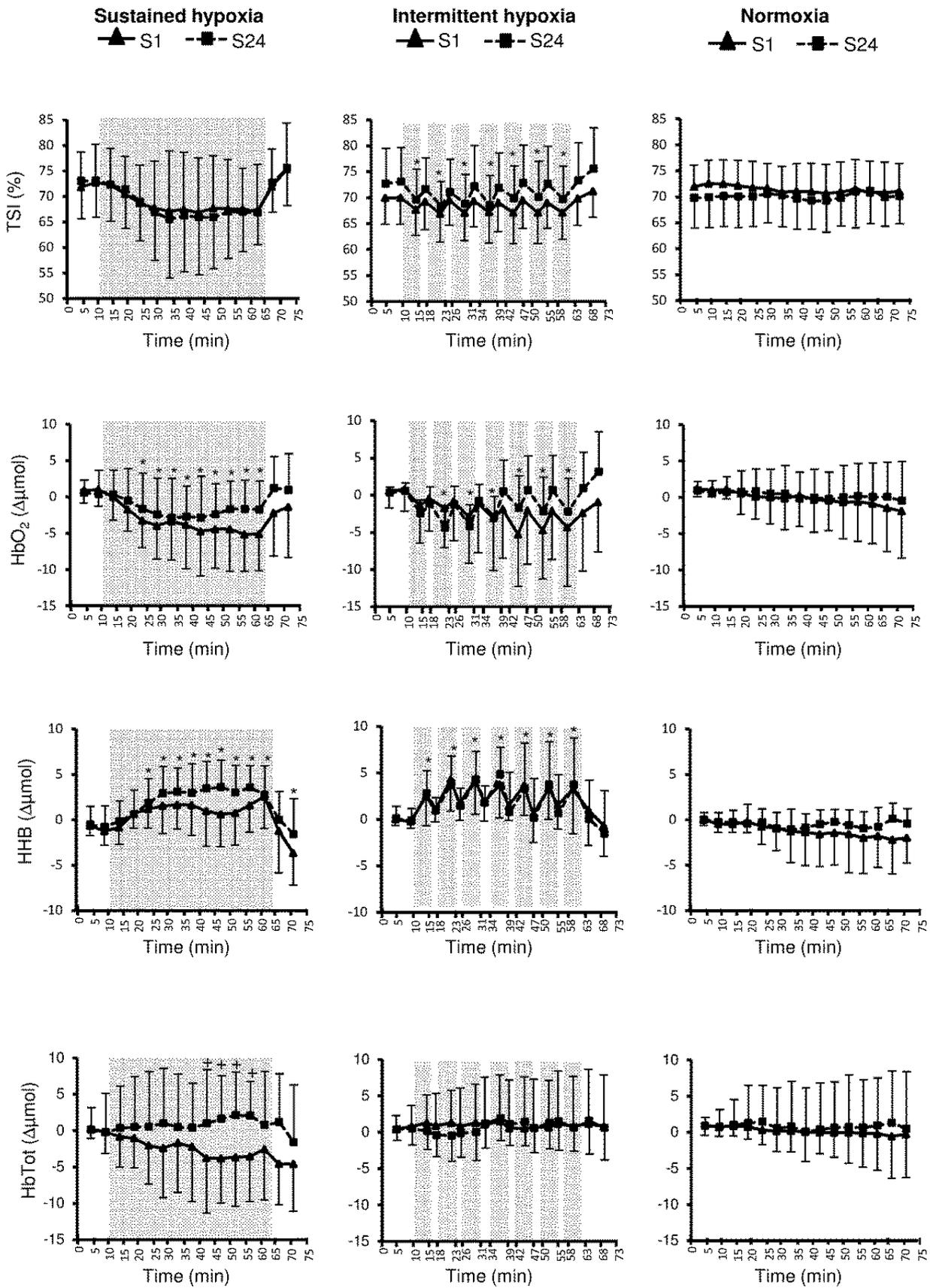
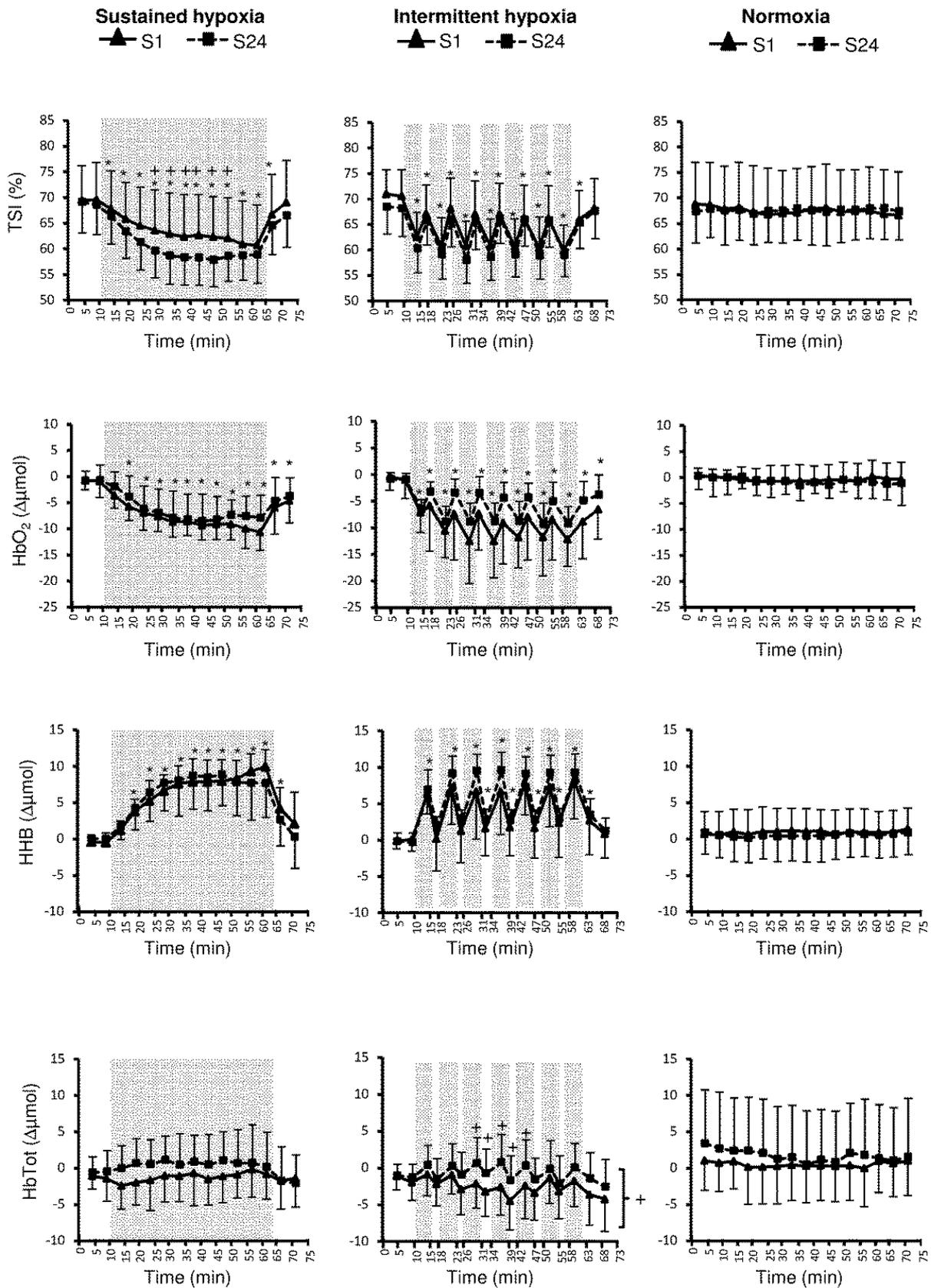


Figure 5.



-Publication n °3-

**Physiological responses to hypoxic constant-load and high-intensity interval
exercise training session in healthy subjects**

Hypoxic exercise responses

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Résumé

L'objectif de cette étude, réalisée chez le sujet sain, était d'évaluer les réponses cardiorespiratoires, tissulaires et cérébrales au cours d'un exercice sous-maximal à charge constante (CL) et d'un exercice intermittent intense (HII) aigus réalisés en normoxie et en hypoxie à une intensité relative similaire, reproduisant des séances types d'entraînement en endurance cardio-respiratoire pratiquées dans des populations sédentaires et cliniques. Les sujets ont effectué deux exercices CL (30 min, 75% de la fréquence cardiaque maximale, n = 12) et deux exercices HII (quinze fois 1 min d'exercice intense - 1 min récupération passive, n = 12) en normoxie et en hypoxie (saturation artérielle en oxygène $76 \pm 1\%$ pendant l'exercice CL et $77 \pm 5\%$ durant l'exercice HII). Les réponses cardiorespiratoires, les variables de spectroscopie proche infrarouge ainsi que la difficulté de l'effort perçue ont été évaluées au cours de chaque exercice. La fréquence cardiaque ne diffère pas entre les exercices normoxiques et hypoxiques tandis que la ventilation est plus élevée en hypoxie à l'exercice HII seulement ($+ 13 \pm 29\%$, $p < 0,05$). L'indice de saturation tissulaire au niveau du quadriceps ne diffère pas significativement entre la normoxie et l'hypoxie (CL $60 \pm 8\%$ versus $59 \pm 5\%$, HII $59 \pm 10\%$ versus $56 \pm 9\%$, $p > 0,05$) tandis que la désoxygénation du cortex préfrontal est significativement plus importante en hypoxie durant les deux types d'exercices (CL $66 \pm 4\%$ versus $56 \pm 6\%$; HII $58 \pm 5\%$ versus $55 \pm 5\%$; $p < 0,05$). Le niveau de la difficulté d'effort perçue ne diffère pas entre les exercices normoxiques et hypoxiques CL ($2,4 \pm 1,7$ versus $2,9 \pm 1,8$) et HII ($6,9 \pm 1,4$ versus $7,5 \pm 0,8$). Cette étude indique qu'à une intensité relativement identique, l'hypoxie n'amplifie pas la désoxygénation musculaire pendant les exercices CL et HII par rapport à la normoxie. Ainsi, l'absence d'augmentation du stress hypoxique musculaire lors d'un exercice hypoxique pourrait limiter les améliorations physiologiques musculaires attendues après un programme d'entraînement en hypoxie.

Mots-clés: Exercice à charge constante; exercice intermittent intense; hypoxie; oxygénation musculaire; oxygénation cérébrale

ABSTRACT

The purpose of this study was to assess the acute cardiorespiratory and muscle and cerebral tissue oxygenation responses to submaximal constant-load (CL) and high-intensity interval (HII) cycling exercise performed in normoxia and in hypoxia at similar relative intensity, reproducing whole-body endurance exercise training sessions as performed in sedentary and clinical populations. Healthy subjects performed two CL (30 min, 75% of maximal heart rate, n=12) and two HII (fifteen times 1-min high-intensity exercise – 1-min passive recovery, n=12) cycling exercises in normoxia and in hypoxia (arterial oxygen saturation $76\pm 1\%$ during CL exercise and $77\pm 5\%$ during HII exercise). Cardiorespiratory and near-infrared spectroscopy parameters as well as the rate of perceived exertion were recorded during all exercises. Heart rate did not differ between normoxic and hypoxic exercises while minute ventilation was higher in hypoxia during HII exercise only ($+13\pm 29\%$, $p<0.05$). Quadriceps tissue saturation index did not differ significantly between normoxia and hypoxia (CL $60\pm 8\%$ versus $59\pm 5\%$; HII $59\pm 10\%$ versus $56\pm 9\%$; $p>0.05$) while prefrontal cortex deoxygenation was significantly larger in hypoxia during both CL ($66\pm 4\%$ versus $56\pm 6\%$) and HII ($58\pm 5\%$ versus $55\pm 5\%$; $p<0.05$) exercises. The rate of perceived exertion did not differ between normoxic and hypoxic CL (2.4 ± 1.7 versus 2.9 ± 1.8) and HII (6.9 ± 1.4 versus 7.5 ± 0.8) exercises. This study indicates that, at identical relative intensity, hypoxia does not accentuate muscle deoxygenation during both CL and HII exercises compared to normoxia. Hence, hypoxic exercise training may not provide greater improvements compared to normoxic training due to larger muscle hypoxic stress.

Keywords: Constant load exercise; high-intensity exercise; hypoxia, muscle oxygenation; cerebral oxygenation

INTRODUCTION

Current physiological models suggest that muscle responses to acute and chronic endurance exercise are due to homeostatic perturbations provoked by exercise which are integrated into gene expression and protein synthesis (1). In this context, reduced oxygen tension within the exercising muscle (2) is recognised as an important stimulus related to exercise response triggering the hypoxia-inducible factor 1 alpha (HIF-1 α) pathway (3, 4). This pathway lead to the expression of many genes involved in a wide range of adaptations associated with exercise training such as enhanced tissue perfusion linked to angiogenesis (5), improved mitochondrial efficiency and control of mitochondrial respiration (6, 7) and enhanced hydrogen ion buffering capacity (8).

Because of the important role of hypoxia regarding the muscle responses to exercise, it has been proposed to amplify the exercise-induced reduction in muscle oxygen tension by reducing the inspiratory oxygen pressure, i.e. by performing exercise in hypobaric (e.g. altitude) or normobaric (reduced inspiratory oxygen fraction, FiO₂) hypoxic conditions (9-11). The main rationale for using hypoxic conditions during exercise sessions is to increase the homeostatic stress on skeletal muscle tissue (9, 10). Hence, these kind of hypoxic training protocols are expected to induce improvements beyond those achieved under normoxic conditions by increasing the cellular disturbance and thus inducing larger muscle adaptive responses. While the effect of hypoxic training on maximal exercise performance in healthy sedentary subjects and in athletes is somehow controversial (9, 10), performing exercise training under hypoxic conditions has been recently proposed as a new strategy to improve health status in older individuals with various comorbidities (12) as well as in several pathologies including obesity (13-15) and type II diabetes (16).

Because of the well-known reduction in maximal aerobic capacity in hypoxic condition (17), hypoxic exercise training sessions have to be performed at a lower absolute workload than normoxic exercise training sessions (9, 18). This is a potential limitation of hypoxic exercise training since lower absolute workload means lower muscle homeostatic stress (e.g. smaller reduction in muscle oxygen tension) and therefore potentially reduced exercise training muscle responses. Hence, regarding muscle tissue hypoxic stress, it is unknown whether the lower absolute workload will be compensated by the reduced muscle oxygen delivery during hypoxic exercise and finally results in greater muscle tissue hypoxia as expected.

Near infrared spectroscopy (NIRS) is a useful tool in exercise physiology providing non-invasive and continuous evaluation of tissue oxygenation both at rest and during exercise (19). Previous studies by using NIRS compared muscle oxygenation during exercise in normoxia and in hypoxia. When compared at identical absolute power output, muscle oxygenation during exercise was reported to be reduced in hypoxia compared to normoxia in some (20-23) but not all (24-26) studies. These contrasting results may be explained by the NIRS parameters being considered (changes in oxy- and deoxy-haemoglobin concentrations or changes in tissue oxygenation index) and by the type of exercise (e.g. constant-load exercise *versus* intermittent exercise). Whether muscle oxygenation during exercise at identical relative intensity (i.e. as performed in hypoxic exercise training programs) is reduced in hypoxia compared to normoxia remains to be elucidated. Moreover, in order to implement hypoxic exercise training regimen in older individuals or patients, it remains to clarify whether hypoxic exercise training at the same relative intensity leads to similar ventilatory responses and similar rate of perceived exertion. This is an important issue since ventilatory stress and sensations associated with physical effort represent critical factors for tolerance and adherence to hypoxic exercise training, especially in patients (18).

Therefore, the purpose of this study was to assess the acute responses to submaximal constant-load and high-intensity interval cycling exercise performed in normoxia and in hypoxia at similar relative intensity (i.e. at similar heart rate), simulating whole-body endurance exercise training sessions as performed in sedentary and clinical populations. We hypothesized that despite lower absolute workload, hypoxic constant-load and high-intensity interval cycling exercise will induce lower muscle tissue oxygenation (assessed by NIRS) compared to normoxic exercise. We also hypothesized that hypoxic and normoxic constant-load and high-intensity interval cycling exercise at similar relative intensity will be associated with similar ventilatory response and rate of perceived exertion.

SUBJECTS AND METHODS

Subjects

Twenty-one (12 females) active, healthy volunteers (age: 29 ± 8 yr, body mass: 64 ± 10 kg, height: 171 ± 7 cm) were included in the present study after routine medical visit consisting of a short clinical examination, 12-lead ECG and respiratory function test. Twelve

subjects (6 females) took part in part 1 of the study (constant-load exercise, CL) and 12 (6 females) subjects took part in part 2 of the study (high-intensity interval exercise, HII), 3 subjects participated in both parts of the study. Part 1 and part 2 of the study were conducted more than 6 months apart. All subjects were non-smokers and had no history of cardiorespiratory or neuromuscular disease. Subjects refrained from physical exercise the days prior to the tests, abstained from drinking caffeinated beverages on test days, and had their last meal at least 2 h prior to the tests. The study was approved by the local ethics committee (CPP Sud-Est V) and performed according to the Declaration of Helsinki. Subjects were fully informed of the procedure and risks involved and gave their written consent prior to all assessments.

Experimental design

Subjects first performed a maximal incremental cycling test in normoxic condition in order to determine maximal power output and maximal heart rate. Each part of the study followed a prospective, randomized, cross-over, controlled, single-blind design and consisted in two experimental sessions. In each part of the study, participants performed the hypoxic and the normoxic exercise sessions in a randomized order (using block randomization), with a minimum period of 48 h between both sessions. Cardiorespiratory and NIRS measures were continuously recorded during each exercise session (described below). The study has been performed in Grenoble, France, at an altitude of 210 m.

Subjects were blinded for the composition of the gas mixture they were inhaling during all experimental sessions. The hypoxic stimulus was obtained by having subjects breathe a nitrogen-enriched gas mixture provided by a gas-mixing device (Altitrainer®, SMTEC S.A., Nyon Switzerland). During hypoxic CL exercise, FiO_2 was individually adjusted to reach the targeted arterial oxygen saturation (SpO_2) of 75% ($\pm 2\%$). Standardizing the SpO_2 between subjects allows minimizing the heterogeneous reduction in SpO_2 obtained for a given reduction in inspiratory oxygen pressure. However, because during HII exercise the rapid and cyclical changes in power output do not allow to target a given SpO_2 , a FiO_2 of 0.135 was used based on preliminary experiments showing that HII exercise in this condition led to a SpO_2 close to 75%. FiO_2 was 0.21 in the normoxic condition.

Subjects were installed on an electrically-braked cycle ergometer (Kettler E3, Enseparsit, Germany). All experimental sessions started with an initial resting phase of 5 min while breathing ambient air for baseline data collection. Then, the subjects breathed the gas mixture of the experimental condition (normoxic or hypoxic) for 5 min at rest before starting

the exercise. The CL exercise sessions lasted 30 min and the workload was continuously adjusted to obtain a heart rate of 75% (\pm 2%) of maximal heart rate, i.e. an intensity corresponding to standard recommendations for exercise training (27). The HII exercise sessions started with 5 min of warm-up at 40% (normoxic condition) or 30% (hypoxic condition) of maximal normoxic power output and then fifteen 1-min bouts of high-intensity exercise were performed with 1 min of passive recovery in between. In normoxia, HII bouts were performed at maximal normoxic power output. In hypoxia, HII bouts were performed at 85% of maximal normoxic power output since maximal power output with an FiO_2 of 0.135 has been previously shown to be reduced by about 15% (17).

Cardiorespiratory and rate of perceived exertion measurements

Ventilation was monitored continuously breath-by-breath by using a pneumotachograph connected to the gas mixing device (Altitrainer®, SMTEC S.A.). SpO_2 and heart rate were continuously recorded using a portable pulse oximetry (Nonin® 3150 WristOx2, Minnesota, USA) and a heart rate monitor (Polar Electro Oy, Kempele, Finland), respectively. The rate of perceived exertion (RPE) was assessed with a standard 100-mm visual analog scale every 5 min during CL exercise and at the end of warm-up and of each 1-min exercise bout during HII exercise.

NIRS measurements

Oxy[HbO₂]-, deoxy[HHb]- and total[HbTot]-hemoglobin concentration changes and tissue oxygenation index (TSI) were estimated throughout testing sessions over multiple sites using a two-wavelength (780 and 850 nm) multichannel, continuous wave NIRS system (OxyMon MkIII, Artinis Medical Systems, the Netherlands). Muscle hemodynamic was assessed on the lower third of the vastus lateralis muscle of the left quadriceps using a 4-cm interoptode distance. The position of the probe was marked on the skin to use identical site of recording on the next session. Left pre-frontal cortex hemodynamic was assessed between Fp1 and F3 locations according to the international 10–20 EEG system with 3.5-cm interoptode distance. The probe holders were secured to the skin with double-sided tape and maintained with Velcro bands. Data were recorded continuously at 10 Hz and filtered with a 2-s width moving Gaussian smoothing algorithm before analysis. In addition to muscle NIRS signals, prefrontal cortex NIRS signals were also recorded as the cerebral hypoxic stress during hypoxic exercise session is an important parameter to consider in the context of hypoxic training for healthy subjects and patients (12, 13, 16).

Statistical analysis

Minute ventilation, heart rate, SpO₂ and NIRS parameters were averaged over the last 30 s of the 5-min resting period while breathing the gas mixture, of each 5-min periods during CL exercise and of the 1st, 3rd, 6th, 9th, 12th and 15th 1-min exercise bout and 1-min passive rest during HII exercise. All statistical procedures were completed on Statistica version 10 (Statsoft, Tulsa, OK). Normality of distribution and homogeneity of variances of the main variables were confirmed using a Shapiro-Wilk normality test and the Levene's test, respectively. A two-way ANOVA (time × condition) with repeated measures was performed for each dependent variable. Post-hoc Tukey's tests were applied to determine a difference between two mean values if the ANOVA revealed a significant main effect or interaction effect. For all statistical analyses, a two-tailed alpha level of 0.05 was used as the cut-off for significance. All data are presented as mean values ± SD.

RESULTS

No adverse effect was observed during both normoxic and hypoxic conditions of either CL or HII sessions. Individual questioning of the subjects after each session on whether they thought to have carried out exercise in normoxia or hypoxia revealed that in only 60% of the exercise sessions the subjects indicated the right condition.

Inspiratory oxygen fraction, arterial oxygenation and exercise power output

FiO₂ and SpO₂ during CL and HII exercise in normoxia and hypoxia are provided in figure 1. Mean SpO₂ was 76 ± 1% during CL exercise and 77 ± 5% during HII exercise bouts. Mean FiO₂ was 0.13 ± 0.02 during CL exercise and 0.135 during HII according to protocol design. Mean power output during CL exercise was 125 ± 61 W in normoxia and 99 ± 49 W in hypoxia (p < 0.001), i.e. 21 ± 11% lower in hypoxia. Mean power output during HII exercise was 261 ± 72 W in normoxia and 222 ± 61 W in hypoxia (p < 0.001), i.e. 15 ± 1% lower in hypoxia.

Cardiorespiratory responses and RPE

Minute ventilation and heart rate during CL and HII exercise in normoxia and hypoxia are provided in figure 2. Minute ventilation and heart rate did not differ significantly between CL normoxic and hypoxic exercise ($p > 0.05$). Minute ventilation was significantly higher throughout the HII hypoxic exercise session compared to the normoxic exercise session (ANOVA main condition effect, $p < 0.05$), while heart rate was higher during the 1-min passive recovery periods only of the HII hypoxic exercise session compared to the normoxic exercise session ($p < 0.05$).

RPE during CL and HII exercise in normoxia and hypoxia is provided in figure 3. RPE did not differ between normoxic and hypoxic conditions both during CL and HII exercise sessions ($p > 0.05$).

Tissue oxygenation

Muscle NIRS parameters during CL and HII exercise in normoxia and hypoxia are provided in figures 4 and 5. Muscle TSI did not differ significantly between normoxia and hypoxia both during CL and HII exercise sessions ($p > 0.05$). Muscle $[\text{HbO}_2]$ and $[\text{HHb}]$ were significantly lower and higher, respectively, in hypoxia compared to normoxia both during CL and HII exercise (all $p < 0.05$). Muscle $[\text{HbTot}]$ did not differ significantly between normoxia and hypoxia both during CL and HII exercise sessions ($p > 0.05$).

Prefrontal cortex NIRS parameters during CL and HII exercise in normoxia and hypoxia are provided in figures 6 and 7. Prefrontal cortex TSI was significantly lower in hypoxia compared to normoxia throughout CL exercise and at the beginning of HII exercise sessions ($p < 0.05$). Prefrontal cortex $[\text{HbO}_2]$ and $[\text{HHb}]$ were significantly lower and higher, respectively, in hypoxia compared to normoxia both during CL and HII exercise (all $p < 0.05$). Prefrontal cortex $[\text{HbTot}]$ was lower in hypoxia compared to normoxia both during CL and HII exercise sessions (ANOVA condition \times time interaction $p < 0.05$).

DISCUSSION

The present study aimed to assess the acute cardiorespiratory and tissue oxygenation responses to two types exercise training sessions (CL and HII) performed in hypoxia

compared to similar sessions performed at the same relative intensity (i.e. similar heart rate) in normoxia. The results indicate that muscle tissue oxygenation as assessed by the TSI NIRS parameter did not differ between normoxia and hypoxia during both CL and HII exercise. These similar muscle TSI values were associated with reduced $[HbO_2]$ and larger $[HHb]$ increases together with similar $[HbTot]$ in hypoxia compared to normoxia during both CL and HII exercises. In contrast to muscle TSI, prefrontal cortex TSI was significantly reduced in hypoxia compared to normoxia during both CL and HII exercise. In addition, minute ventilation was significantly higher in hypoxia compared to normoxia during HII exercise only, while RPE did not differ between hypoxia and normoxia both during CL and HII exercises.

Exercise power output and hypoxia severity

In order to compare normoxic and hypoxic exercise sessions, we aimed to define exercise intensities in normoxia and hypoxia for both types of exercise (constant-load and high-intensity intermittent) that i) allow a physiologically relevant comparison of both conditions (normoxia-hypoxia) and ii) correspond to intensities feasible in clinical practice for training purpose. The main objective of the present study was to compare muscle oxygenation during cycling exercise performed in normoxia and hypoxia. Comparing normoxic and hypoxic whole body exercise at the same absolute intensity leads to compare exercise responses at different relative intensities due to the reduction in maximal power output under hypoxic condition. This situation has the inconvenient to compare conditions with different cardiorespiratory constraints (higher ventilation and cardiac output in hypoxia) and rate of perceived exertion (greater rate of perceived exertion in hypoxia) (28). The practical consequence of this higher relative intensity in hypoxia when planning to perform hypoxic exercise training program is also that exercise is more difficult to tolerate and therefore cannot be performed for the same duration and induces more discomfort and potentially less adherence for patients. Therefore, we compared constant-load and high-intensity interval exercise at the same target heart rate in normoxic and hypoxic conditions. During constant-load exercise, this could be performed by individually adjusting the power output during hypoxic exercise sessions in order to obtain the same target heart rate than in normoxia, i.e. 75% of maximal heart rate corresponding to standard recommendations for exercise training (27). During high-intensity exercise, the short duration of the 1-min high-intensity exercise bout did not allow adjusting the power output during the session, and therefore based on the

expected reduction in maximal power output with the level of hypoxia used in the present study and based on preliminary experimentations, we reduced the normoxic maximal power output by 15% during hypoxic HII session. Based on these adjustments of power output in hypoxia, we were able to compare both CL and HII exercise at identical heart rate in normoxia and in hypoxia (Figure 2). Interestingly, the only difference in heart rate observed between normoxia and hypoxia was observed during passive recovery phases of HII exercise, with significantly higher heart rate values in hypoxia compared to normoxia. This suggests a lower rate of recovery in hypoxia as suggested by others having investigated the effect of hypoxia on repeated sprint exercises (29).

Another important factor to consider when comparing normoxic and hypoxic exercise is the severity of the hypoxic stress (28). In order to minimize the well-known inter-subjects variability in arterial oxygenation in response to a given reduction in inspiratory oxygen pressure, the FiO_2 during hypoxic CL exercise was individually and continuously adjusted to induce a target SpO_2 of 75% ($\pm 2\%$) (figure 1). During HII exercise, because of rapid cyclical (2 min cycles) changes from high-intensity exercise to passive resting phases inducing large metabolic variations, it was not possible to continuously adapt FiO_2 in order to maintain a given target SpO_2 level. Therefore based on preliminary experiments, we defined a FiO_2 (0.135) that induced on average a SpO_2 close to 75%. This FiO_2 (and the corresponding inspiratory oxygen partial pressure close to 100 mmHg) is very similar to previous studies having investigated intermittent exercise in hypoxia (26, 30-33). Also, despite there are no international recommendations on hypoxic exercise training, it has been proposed in obese individuals for instance to train in hypoxia at FiO_2/SpO_2 levels closed to those used in the present studies (18).

Tissue oxygenation during hypoxic exercise

Because muscle hypoxia is a critical factor triggering adaptations to physical exercise (3, 4) and since hypoxic exercise is thought to accentuate muscle hypoxia and potentially subsequent adaptations to training (9, 10), this study aimed to evaluate changes in muscle oxygenation status non-invasively and continuously during whole body exercise sessions similar to those used for hypoxic exercise training. Providing the detailed knowledge regarding this kind of exercise and training is critical since an increasing number of publications propose hypoxic conditioning strategies as a new therapeutic opportunities in various diseases (e.g. (18, 34-37)). NIRS is unique tool to provide non-invasive and

continuous evaluation of muscle oxygenation during exercise (19). NIRS parameters reflect the dynamic balance between O₂ demand and supply in the tissue microcirculation. [HbO₂] and [HbTot] are mostly sensitive to blood flow and O₂ delivery whereas [HHb] is closely associated with changes in venous O₂ content and therefore tissue oxygen extraction (38, 39). The TSI is calculated by the NIRS system based on [HbO₂], [HHb] and [HbTot] and provides an index of tissue oxygenation.

During both CL and HII exercises in hypoxia, the reduced muscle [HbO₂] with similar [HbTot] mainly reflect the reduction in oxygen delivery due to reduced arterial blood oxygenation, while the unchanged [HbTot] may suggest that local blood flow was similar between normoxia and hypoxia (40). The larger muscle [HHb] during CL exercise in hypoxia compared to normoxia suggests a greater muscle oxygen extraction. Hence, the reduced oxygen delivery during hypoxic exercise appears to be compensated by a greater extraction, leading to similar TSI values during both CL and HII exercises in normoxia and hypoxia. If TSI is considered as the most meaningful parameter regarding muscle oxygen tension within the muscle, the present results suggest that both CL and HII exercises in hypoxia performed at the same relative intensity (i.e. heart rate) lead to similar reduction in muscle oxygenation than normoxic exercise condition. This similar muscle oxygenation status may be due to an opposite effect of reduced muscle oxygen delivery (due to the reduced FiO₂) and reduced muscle oxygen consumption (due to reduced power output) in hypoxia compared to normoxia. Hence, these results suggest that hypoxic exercise training cannot rely on greater muscle hypoxic stress in order to induce larger muscle adaptations as compared to normoxic exercise training, at least in healthy subjects when using the same relative intensity (characterized by similar heart rate) and a target SpO₂ level closed to 75%.

Cerebral hypoxia during hypoxic exposure can lead to important functional impairments, both cognitive and physical (41). Because the brain is known to be specifically sensitive to hypoxia during exercise (42), we assessed in addition to muscle oxygenation, prefrontal cortex oxygenation continuously during exercise. Hypoxia during exercise is known for instance to exacerbate the central component of neuromuscular fatigue, both during CL (20) and HII (30) exercises. We have also shown previously that prefrontal cortex deoxygenation is significantly larger than muscle deoxygenation during exposure to reduced FiO₂ in healthy subjects at rest (43, 44). In the present study, reduced [HbO₂] and increased [HHb] during hypoxic exercise were observed during both CL and HII exercise at the prefrontal cortex level, similar to observations within the muscle. In contrast to the muscle however, prefrontal cortex [HbTot] was significantly reduced during hypoxic CL and HII

exercise compared to normoxia, suggesting that prefrontal cortex blood volume and potentially blood flow may be reduced during hypoxic exercise. Hyperventilation and subsequent hypocapnia (since minute ventilation was similar or higher during hypoxic exercise despite reduced power output and therefore lower CO₂ production) may have been responsible for cerebral vasoconstriction. End-tidal CO₂ partial pressure were measured during HII exercise only and indicated average values 6 ± 4 mmHg lower during hypoxic compared to normoxic exercise ($p < 0.05$; results not shown). Hence, the reduced prefrontal cortex oxygen delivery due to both a reduction in arterial oxygenation and cerebral blood flow led to lower prefrontal cortex TSI during both hypoxic CL and HII exercise compared to normoxia, indicating greater cerebral hypoxic stress. Whether such a cerebral hypoxic stress should be considered as potentially deleterious (e.g. a >13% reduction in prefrontal cortex oxygenation during neurosurgical intervention has been considered as possibly harmful (45)) or as a stress able to induce positive cerebral adaptations (e.g. at the cerebrovascular and neuronal levels (46)) has to be considered in future studies.

Cardiorespiratory responses and sensations during hypoxic exercise

According to the protocol design and as discussed previously, heart rate did not differ significantly during exercise in normoxia and hypoxia (figure 2). Minute ventilation was similar during normoxic and hypoxic CL exercise while it was significantly higher during hypoxic compared to normoxic HII exercise (figure 2). At least two potential reasons can be suggested to explain this difference between CL and HII exercise regarding the effect of hypoxic exposure on minute ventilation. Firstly, the power output in hypoxia was reduced by 15% compared to normoxia during HII exercise, while it was reduced by 21% during hypoxic CL exercise. This higher power output relative to normoxia may have been responsible for the larger minute ventilation only during HII exercise in hypoxia compared to normoxia. Secondly, HII exercise was performed by definition above the respiratory compensation point where hypoxia chemosensitivity and acidosis may be important factors influencing ventilation (47). Hence the higher intensity during HII compared to CL exercise may accentuate the effect of hypoxia on minute ventilation.

Interestingly, despite the significantly higher minute ventilation during hypoxic compared to normoxic HII exercise, RPE did not differ significantly between hypoxic and normoxic conditions for both CL and HII exercises. This result indicates that by reducing the power output and by targeting the hypoxic levels as performed in the present study, the

subjects reported similar sense of effort during hypoxic and normoxic exercise, which is of interest regarding tolerance and adherence to exercise training programs that may implement hypoxic conditions for both healthy subjects and patients. The fact that only 60% of the subjects were able to identify whether they carried out exercise in normoxia or hypoxia further emphasizes that sensations during exercise at identical relative intensity in normoxia and hypoxia were very similar. These results regarding respiratory responses and sensations during hypoxic versus normoxic exercise remain however to be confirmed in different populations than the present one, e.g. older individuals and patients with cardiorespiratory or metabolic diseases.

Study limitations

An important limitation to acknowledge relates to the use of NIRS parameters to investigate tissue oxygenation. NIRS signals are known to be influenced by blood flow in superficial layers (48). By standardizing the placement of the probes as well as room air temperature in normoxic and hypoxic sessions, we believe that superficial skin layers influenced to a similar extent normoxic and hypoxic NIRS recordings and therefore that differences in tissue oxygenation and hemodynamic between conditions were due to the reduced FiO_2 *per se*. NIRS parameters assessed however oxygenation at the microcapillary blood level and one cannot directly infer the intra-muscular (e.g. mitochondrial) oxygen tension which is the most relevant regarding gene expression and protein synthesis in response to muscle exercise. More invasive evaluations or methods not easily compatible with whole body exercise (e.g. nuclear resonance magnetic spectroscopy (2)) would be required to approach the intra-muscular oxygen tension during hypoxic whole-body exercise in human.

While the present study focused on acute responses to hypoxic exercise in order to provide important knowledge to consider hypoxic exercise training in healthy subjects and in patients, the results do not provide insight into the adaptations to chronic hypoxic exercise. In particular, whether after repetitive hypoxic exercise sessions muscle oxygenation would remain similar to the first session of hypoxic exercise and to normoxic exercise condition as in the present study remains to be evaluated. We have shown for instance that after 3 sessions of passive hypoxic exposure, the muscle deoxygenation in response to reduced SpO_2 was larger compared to the first hypoxic session (49).

Conclusions

These results indicate that while muscle oxygen delivery is reduced ([HbO₂]) during hypoxic *versus* normoxic exercise, oxygen extraction ([HHb]) is simultaneously enhanced and blood volume ([HbTot]) is unchanged, leading to similar muscle TSI in normoxia and hypoxia during both CL and HII exercises. This suggests that under the condition of the present study (identical relative intensity during normoxic and hypoxic exercise and SpO₂ closed to 75% during hypoxic exercise), muscle oxygenation tension may not be reduced to a greater extent during hypoxic compared to normoxic exercise. Therefore, larger improvement in muscle function cannot be expected following hypoxic exercise training because of greater muscle oxygen stress. This does not prevent however hypoxic training to provide additional benefits compared to normoxic training because of alternative mechanisms. As opposed to muscle oxygenation, prefrontal cortex oxygenation appears to be significantly reduced during hypoxic compared to normoxic exercise. The effect of repetitive cerebral dextoxygenation during hypoxic exercise training remains to be elucidated. In addition, the results indicate that the rate of perceived exertion was similar during normoxic and hypoxic CL and HII exercises (at the same heart rate), which may contribute to the feasibility and adherence to hypoxic exercise training in various populations.

Conflict of interest: All authors declare to have no conflict of interest.

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FIGURE LEGENDS

Figure 1. Inspiratory oxygen fraction (FiO_2 , panels A and C) and arterial oxygen saturation (SpO_2 , panels B and D) during constant-load and high-intensity intermittent cycling exercise sessions performed in normoxia and in hypoxia. * significantly different from resting baseline in normoxia and hypoxia; # significantly different from resting baseline in hypoxia only; \$ significantly different between normoxia and hypoxia.

Figure 2. Minute ventilation (V_E , panels A and C) and heart rate (HR, panels B and D) during constant-load and high-intensity intermittent cycling exercise sessions performed in normoxia and in hypoxia. * significantly different from resting baseline in normoxia and hypoxia; # significantly different from resting baseline in hypoxia only; \$ significantly different between normoxia and hypoxia.

Figure 3. Rate of perceived exertion (RPE) during constant-load (panel A) and high-intensity intermittent (panel B) cycling exercise sessions performed in normoxia and in hypoxia. * significantly different from resting baseline in normoxia and hypoxia; # significantly different from resting baseline in hypoxia only; \$ significantly different between normoxia and hypoxia.

Figure 4. Muscle NIRS parameters during constant-load cycling exercise sessions performed in normoxia and in hypoxia. Panel A, tissue saturation index (TSI); panel B, oxyhemoglobin concentration ($[\text{HbO}_2]$); panel C, deoxyhemoglobin concentration ($[\text{HHb}]$); panel D, total hemoglobin concentration ($[\text{HbTot}]$). * significantly different from resting baseline in normoxia and hypoxia; # significantly different from resting baseline in hypoxia only; \$ significantly different between normoxia and hypoxia.

Figure 5. Muscle NIRS parameters during high-intensity interval cycling exercise sessions performed in normoxia and in hypoxia. Panel A, tissue saturation index (TSI); panel B, oxyhemoglobin concentration ($[\text{HbO}_2]$); panel C, deoxyhemoglobin concentration ($[\text{HHb}]$); panel D, total hemoglobin concentration ($[\text{HbTot}]$). * significantly different from resting baseline in normoxia and hypoxia; # significantly different from resting baseline in hypoxia only; \$ significantly different between normoxia and hypoxia.

Figure 6. Pre-frontal cortex NIRS parameters during constant-load cycling exercise sessions performed in normoxia and in hypoxia. Panel A, tissue saturation index (TSI); panel B, oxyhemoglobin concentration ($[\text{HbO}_2]$); panel C, deoxyhemoglobin concentration ($[\text{HHb}]$); panel D, total hemoglobin concentration ($[\text{HbTot}]$). * significantly different from resting baseline in normoxia and hypoxia; # significantly different from resting baseline in hypoxia only; § significantly different between normoxia and hypoxia.

Figure 7. Pre-frontal cortex NIRS parameters during high-intensity interval cycling exercise sessions performed in normoxia and in hypoxia. Panel A, tissue saturation index (TSI); panel B, oxyhemoglobin concentration ($[\text{HbO}_2]$); panel C, deoxyhemoglobin concentration ($[\text{HHb}]$); panel D, total hemoglobin concentration ($[\text{HbTot}]$). * significantly different from resting baseline in normoxia and hypoxia; # significantly different from resting baseline in hypoxia only; § significantly different between normoxia and hypoxia.

Figure 1.

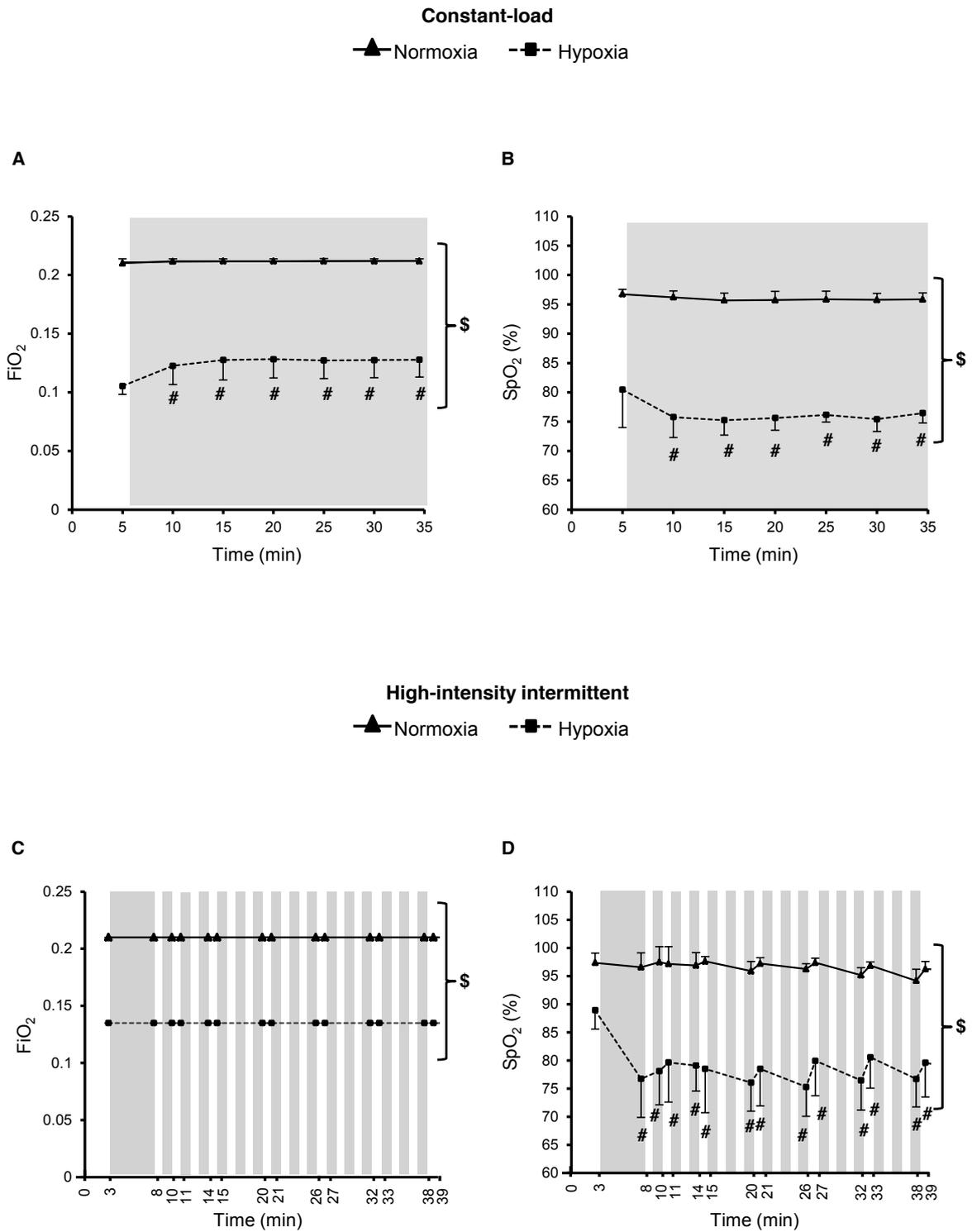


Figure 2.

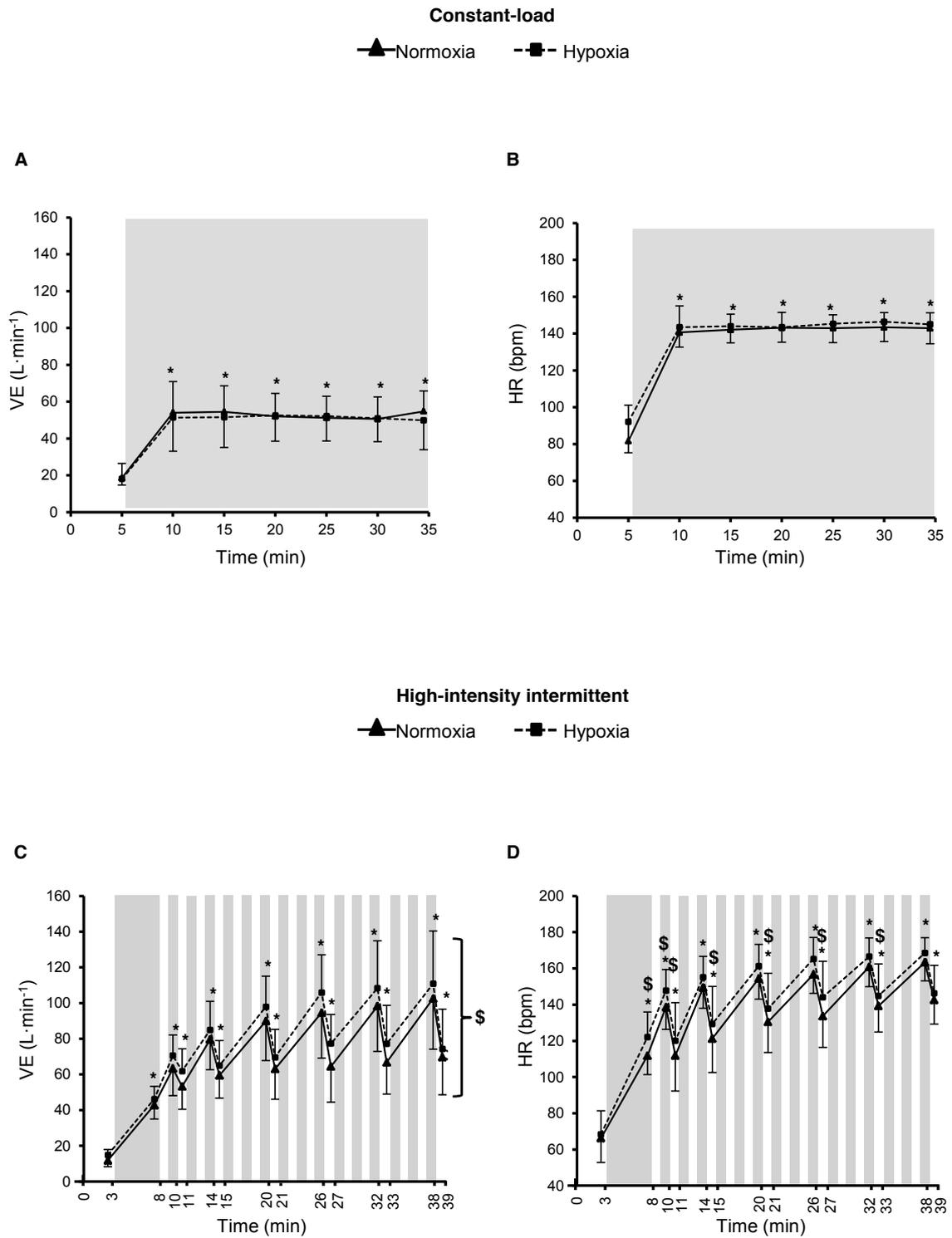


Figure 3.

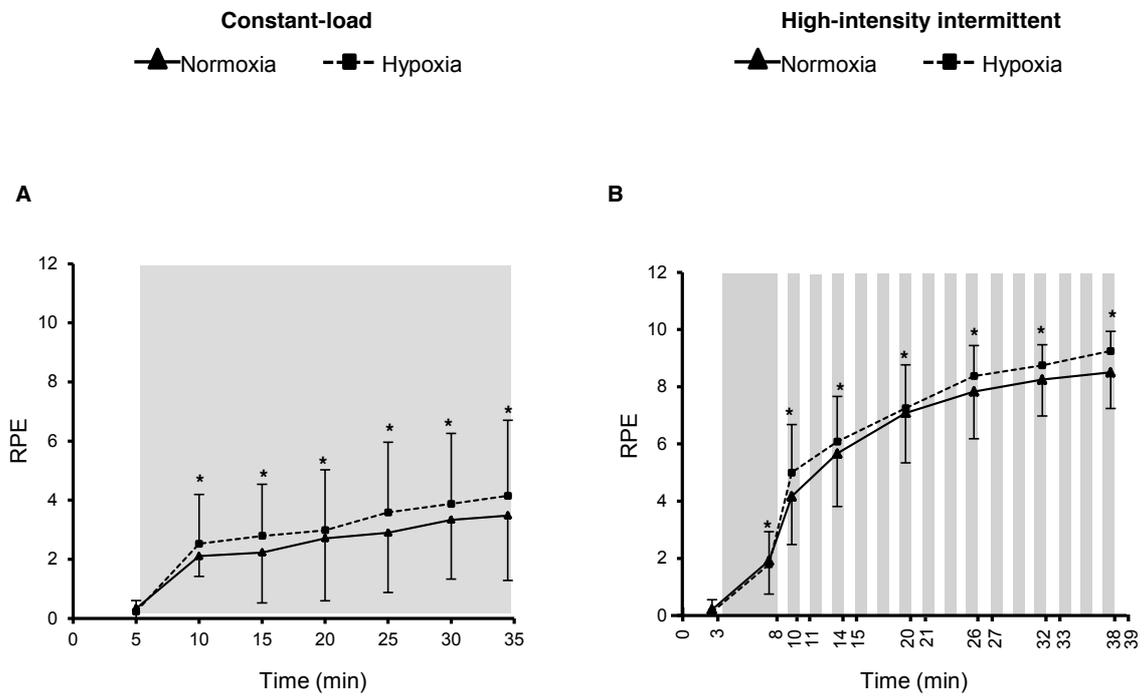


Figure 4.

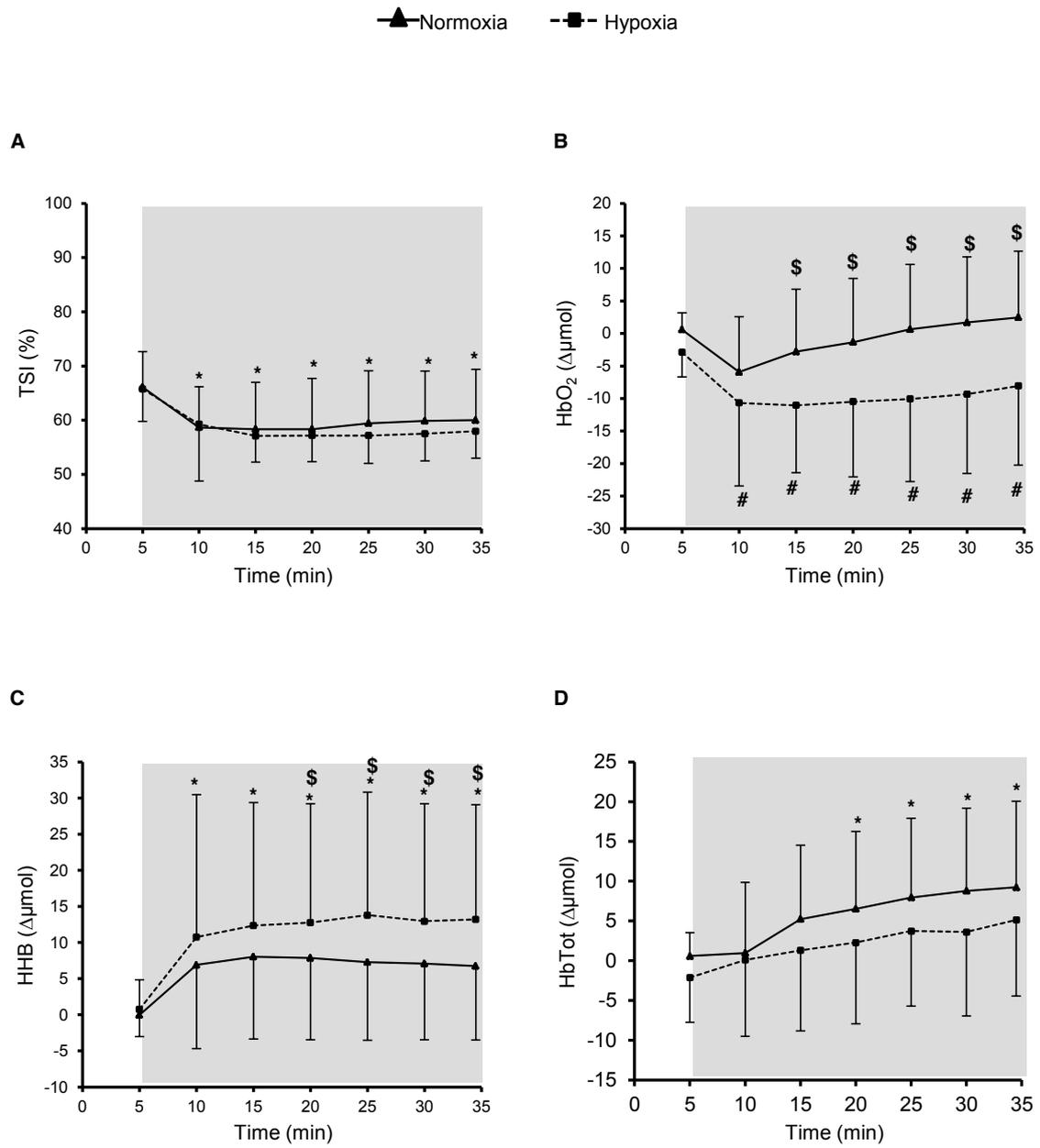


Figure 5.

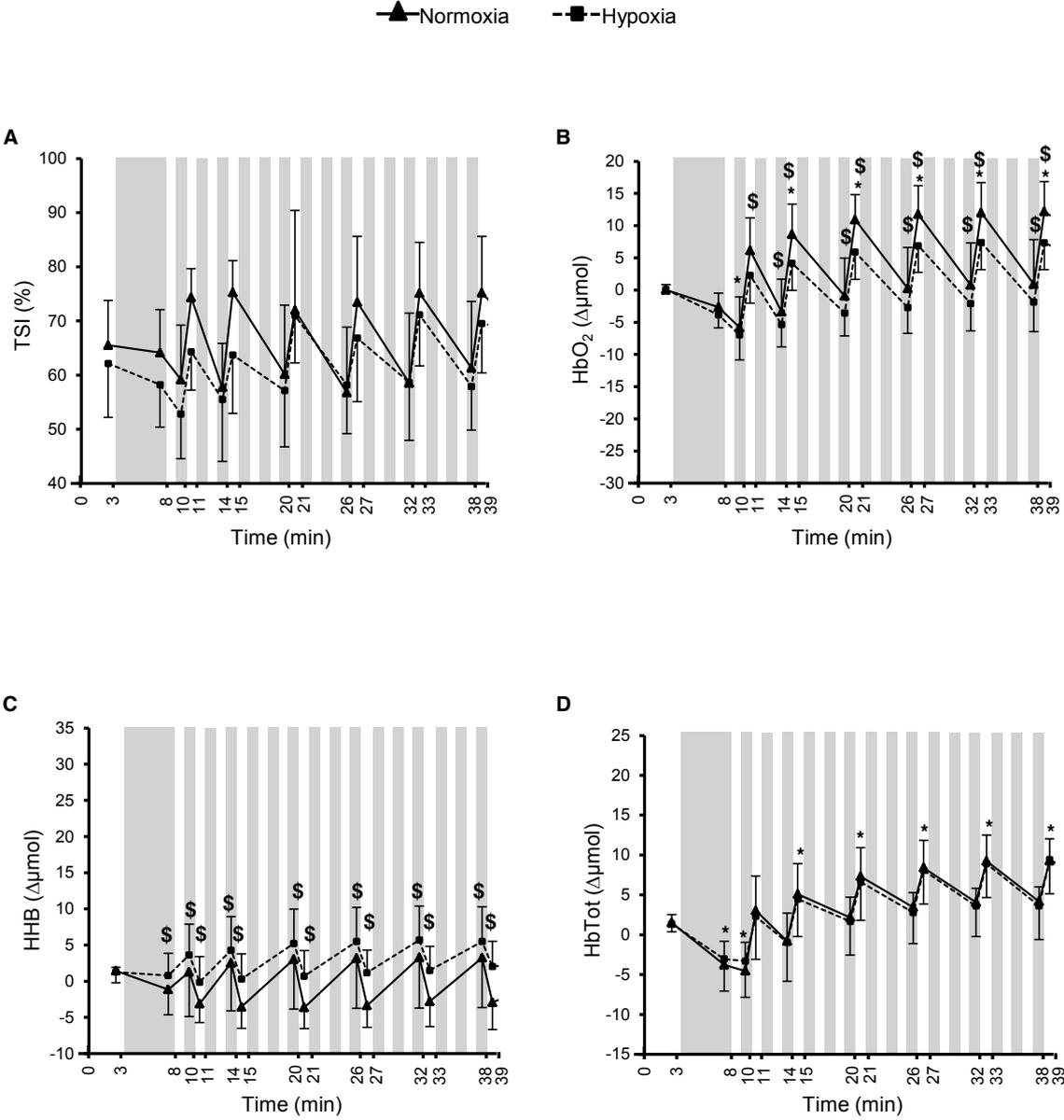


Figure 6.

▲ Normoxia ■ Hypoxia

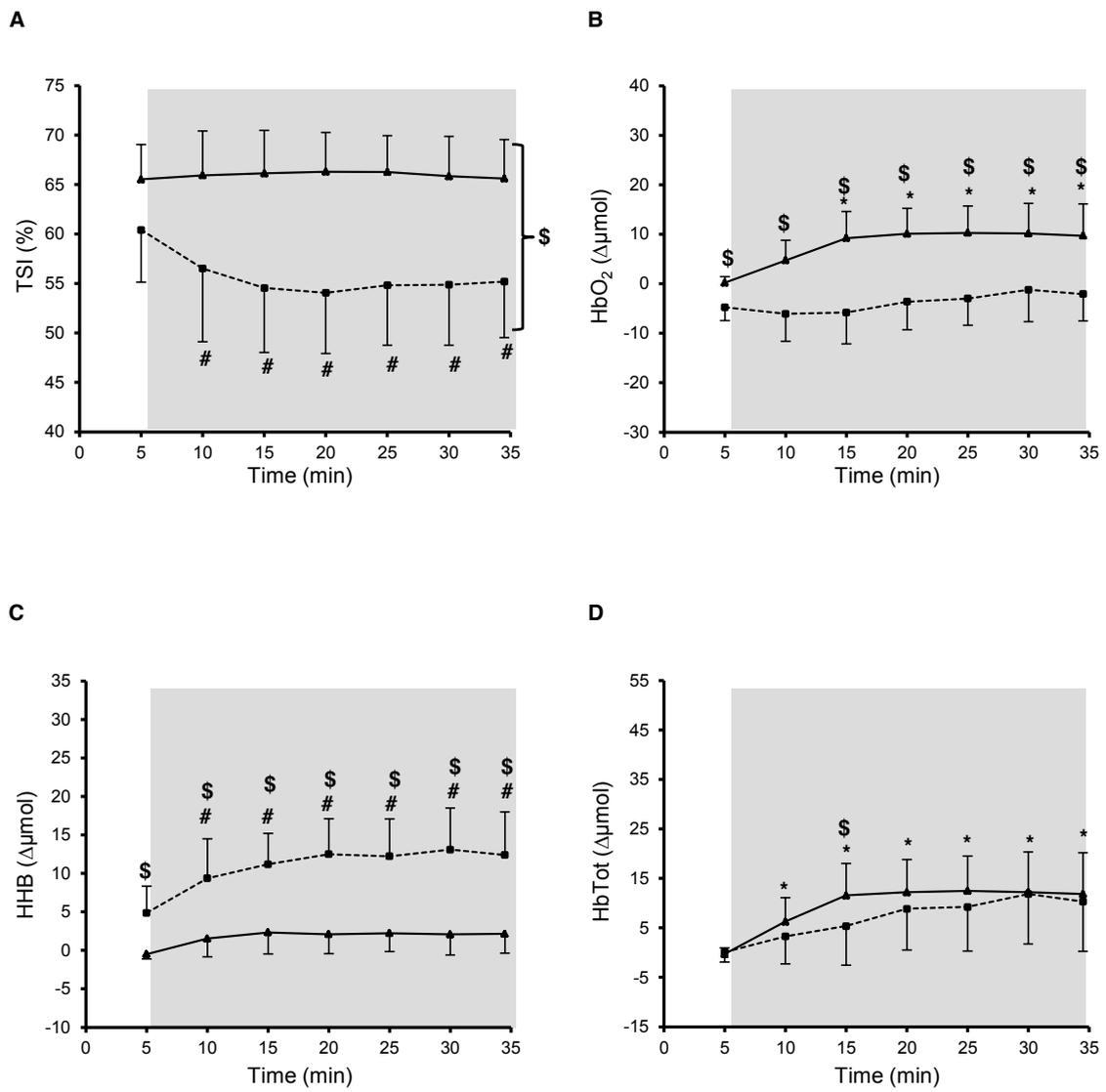
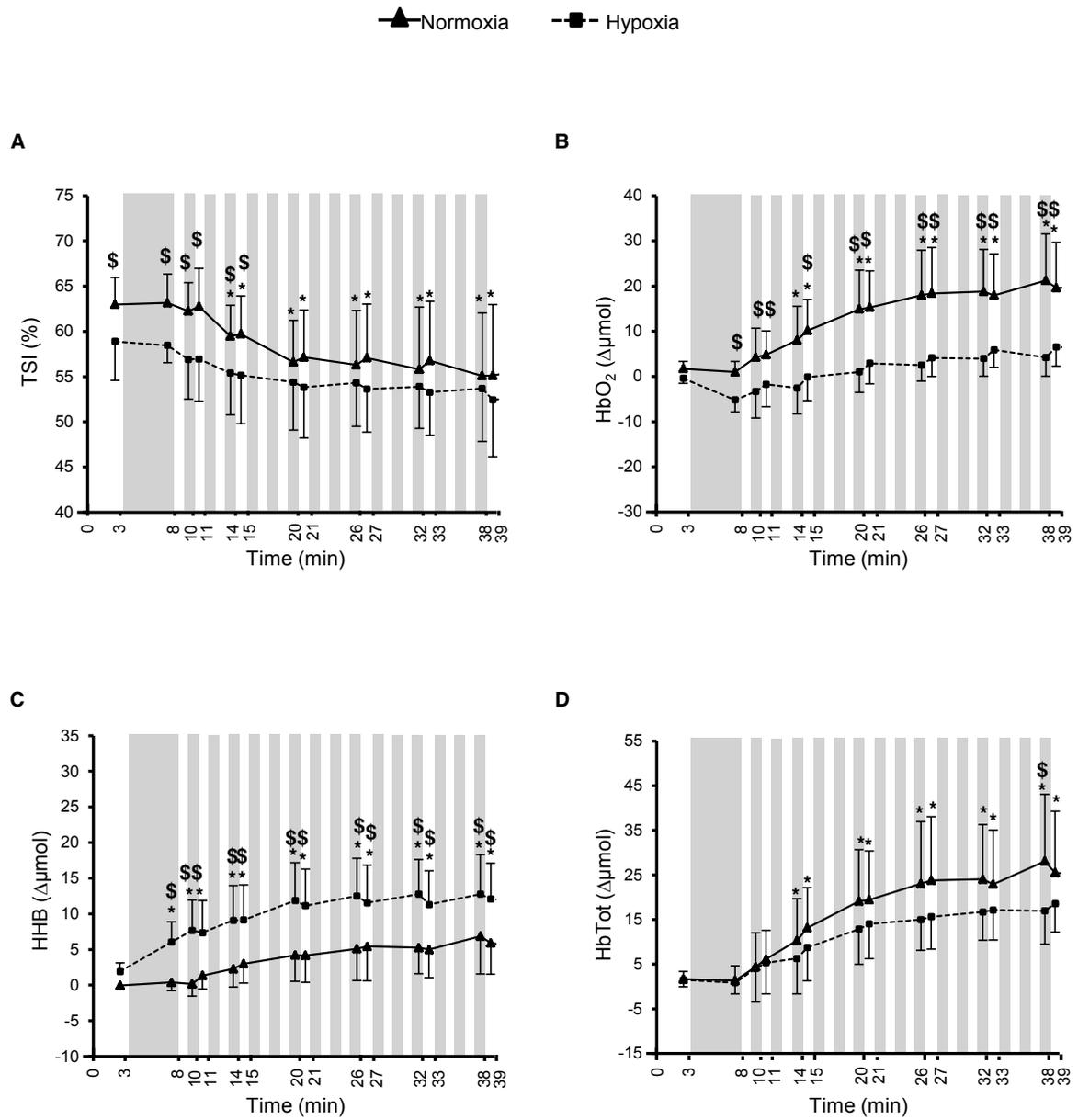


Figure 7.



-Publication n °4-

**Hypoxic exercise training to improve fitness status
in overweight and obese individuals**

Hypoxic training and obesity

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Résumé

La combinaison d'un entraînement physique et d'une exposition hypoxique a été récemment proposée comme une nouvelle stratégie thérapeutique pour améliorer l'état de santé des personnes obèses. Cependant, les avantages sur la composition corporelle et le statut cardiométabolique de la combinaison d'un entraînement en hypoxie (HET) par rapport à un entraînement normoxique (NET) sont à clarifier. Nous avons émis l'hypothèse que HET induirait une amélioration de la performance aérobie maximale supérieure à NET chez le sujet en surpoids/obèse. Vingt-trois sujets (54 ± 11 ans, IMC $31,5 \pm 2,8$ kg·m⁻²) ont été randomisés dans un programme HET ou NET de 8 semaines (3 séances / semaine, à charge constante équivalente à 75% de la fréquence cardiaque maximale, saturation artérielle en oxygène cible pour HET 80%). Les évaluations suivantes ont été réalisées avant et après entraînement : épreuve d'effort maximale progressive, épreuve sous-maximale métabolique, rigidité artérielle par la mesure de la vitesse de l'onde de pouls, réactivité vasculaire par mesure du tonus artériel périphérique par pléthysmographie, glycémie, insulinémie et profil lipidique, détermination de la composition corporelle par imagerie par résonance magnétique nucléaire. La consommation maximale d'oxygène et la puissance maximale ont augmenté significativement dans le groupe HET seulement (pic de consommation d'oxygène HET $+ 10 \pm 11\%$ versus NET $+ 1 \pm 10\%$ et puissance maximale HET $+ 11 \pm 7\%$ versus NET $+ 3 \pm 10\%$, $p < 0.05$). Les réponses sous-maximales à l'effort se sont améliorées de la même manière dans les deux groupes HET et NET. À l'exception de la pression artérielle diastolique qui a diminué de façon significative après HET et NET, aucune modification de la fonction vasculaire, de l'état métabolique et de la composition corporelle n'a été observée après entraînement. La combinaison de l'entraînement physique et de l'exposition hypoxique peut apporter des avantages supplémentaires sur l'état de santé de la personne en surpoids/obèse par rapport à un entraînement réalisé en normoxie classique.

Mots-clés: Obésité, hypoxie, entraînement, performance, fonction cardiorespiratoire

ABSTRACT

Combining exercise training to hypoxic exposure has been recently proposed as a new therapeutic strategy to improve health status in obese individuals. Whether hypoxic exercise training (HET) provides greater benefits regarding body composition and cardiometabolic status than normoxic exercise training (NET) remains however unclear. We hypothesized that HET would induce greater increase in maximal aerobic performance than NET in overweight and obese individuals. Twenty-three subjects (age 54 ± 11 yr, BMI 31.5 ± 2.8 kg·m⁻²) were randomized into an 8-week HET or NET program (3 sessions/week, constant-load cycling at 75% of maximal heart rate, target arterial oxygen saturation for HET 80%). Before and after the training programs, the following evaluations were performed: incremental maximal and submaximal cycling tests, measurements of pulse-wave velocity, reactive hyperemia, blood glucose, insulin and lipid profile, determination of body composition by magnetic resonance imaging. Peak oxygen consumption and maximal power output increased significantly after HET only (peak oxygen consumption HET $+ 10 \pm 11\%$ *versus* NET $+ 1 \pm 10\%$ and maximal power output HET $+ 11 \pm 7\%$ *versus* NET $+ 3 \pm 10\%$, $p < 0.05$). Submaximal exercise responses improved similarly after HET and NET. Except diastolic blood pressure which decreased significantly after both HET and NET, no change in vascular function, metabolic status and body composition was observed after training. Combining exercise training and hypoxic exposure may provide some additional benefits to standard NET for obese individual health status.

Keywords: Obesity, hypoxia, training, exercise performance, cardiorespiratory function

Introduction

Obesity is characterized by an excess of body fat stores and is considered as the global epidemic of the 21st century. Obesity is defined based on a body mass index (BMI) $>30 \text{ kg}\cdot\text{m}^{-2}$, while individuals with a BMI between 25 and $30 \text{ kg}\cdot\text{m}^{-2}$ are considered overweight. In 2016, more than 1.9 billion adults were overweight and 650 million of them were obese (40). Obesity is a major health burden because of associated comorbidities such as increased risk of insulin resistance, diabetes, hypertension, cardiovascular diseases, cancers and possibly higher mortality rate (9, 12).

Obesity is typically the consequence of a chronically positive energy balance, i.e. a larger amount of calorie intake compared to the amount expended. In order to enhance the later, increasing spontaneous physical activity and getting involved in exercise training programs represent important interventions for the management of obesity (6). Current strategies often failed however to induce clinically significant and sustained weight loss (13) and innovative strategies are warranted for both weight loss and improvements in cardiovascular and metabolic health status of overweight and obese individuals.

Hypoxia is defined as reduced oxygen availability at the lung, blood or tissue levels. While it is considered as a key pathophysiological mechanism in several diseases such as respiratory diseases, several arguments also suggest that hypoxia can induce positive adaptations of the human body able to prevent or even contribute to the treatment of several pathologies (37). In order to accentuate the effect of exercise training, it has been proposed originally in the sports medicine community to combine exercise training and hypoxic exposure as a useful strategy to enhance exercise performance in athletes (26). While the effect of hypoxic training on maximal exercise performance in healthy sedentary subjects and in athletes remains somehow controversial (23, 26), performing exercise training under hypoxic conditions has been recently proposed as a new strategy to improve health status in older individuals with various comorbidities (30) as well as in several pathologies including obesity (10, 11, 20, 28, 39) and type II diabetes (34). These studies typically consist in 2-3 exercise training sessions per week for 4-8 weeks, performed with a reduced inspiratory oxygen fraction (FiO_2) of 0.14-0.16. In overweight or obese populations, body weight and body composition have been shown to be improved to a greater extent after hypoxic versus normoxic exercise training in several (10, 20, 28, 39) but not all (11) studies. Three studies only assessed in obese individuals maximal exercise performance changes after hypoxic

exercise training and did not show greater improvement in maximal aerobic capacity compared to normoxic exercise training (10, 11, 39). Hypoxic exercise training may also improve blood pressure (11, 20) while the effect on metabolic status is unclear (28). In their recent review on hypoxic conditioning in obesity, Hobbins et al. (16) emphasized that these contrasting and somehow unclear results may arise from methodological issues such as i) the use of fixed FiO_2 for all subjects which induces heterogeneous levels of arterial oxygen desaturation depending on subject individual hypoxic responses, and ii) an insufficient amount of training (training duration ≤ 4 weeks (20, 39) or < 3 training sessions per week (11)). Hence, although the potential of hypoxic conditioning as a new therapeutic tool in overweight and obese individuals has been underlined in several recent reviews (16, 17, 36), further randomized control studies are required to clarify the potential interest of hypoxic exercise training in obese individuals.

Reduced maximal exercise capacity has been associated with an increased risk of cardiovascular disease and all-cause mortality in healthy men and women, in patients with cardiovascular disease and comorbid conditions such as obesity, hypertension and lipid abnormalities (4, 18, 19, 27). It has been recently emphasized that each 1-MET (i.e. a multiple of the resting metabolic rate, approximating $3.5 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) increase in maximal exercise capacity is associated with considerable (10-25%) improvement in survival (31). Hence, the present study aimed at comparing the effect of an 8-week exercise training program performed either in normoxia or hypoxia on maximal aerobic capacity in overweight or obese individuals. We hypothesized that hypoxic exercise training would induce a greater increase in maximal aerobic performance together with a significant improvement in vascular and metabolic function compared to normoxic exercise training.

Methods

Subjects

23 (4 females), overweight or obese, sedentary subjects (age 54 ± 11 yr, BMI $31.5 \pm 2.8 \text{ kg}\cdot\text{m}^{-2}$) were included in the present study after routine medical assessment consisting of a clinical examination, a 12-lead electrocardiogram (ECG) and a respiratory function test. The main inclusion criteria were as follows: age between 18 and 65 yrs, BMI $> 27 \text{ kg}\cdot\text{m}^{-2}$, no type I or II diabetes, no heart diseases and no respiratory disease (except treated sleep apnea). All subjects lived at sea level (< 300 m) and were not exposed to altitude > 1000 m over the past

three months. The study was approved by the local ethics committee (CPP Sud-Est V) and performed according to the Declaration of Helsinki. Subjects were fully informed of the procedure and risks involved and gave their written consent prior to all assessments.

Experimental design

In this prospective, randomized, controlled, single-blind study performed in Grenoble (altitude: 210 m), all subjects were randomized into two groups: submaximal constant-load exercise training performed in normobaric hypoxia (hypoxic exercise training, HET; n = 12) and submaximal constant-load exercise training performed in normoxia (normoxic exercise training, NET; n = 11). They performed three 45-min sessions of exercise training per week for 8 weeks. Before and after the 8-week training period, all subjects performed a series of cardiorespiratory and metabolic tests (see details below).

Exercise training sessions

During all training sessions, subjects were installed on an electrically-braked cycle ergometer (Corival, Lode B.V., Groningen, Netherlands) with heart rate and arterial oxygen saturation (SpO_2) continuously recorded (Nonin® 3150 WristOx2, Minnesota, USA). During cycling, workload was continuously adjusted to obtain a heart rate of 75% (\pm 2%) of individual maximal heart rate, i.e. an intensity corresponding to standard recommendations for exercise training (1). In the HET group, the hypoxic stimulus was obtained by having subjects breathe via a facemask a nitrogen-enriched gas mixture provided by a gas-mixing device (Altitrainer®, SMTEC S.A., Nyon, Switzerland). FiO_2 was individually and continuously adjusted to reach a target SpO_2 of 80%. In the NET group, subjects inhaled normoxic ambient air ($FiO_2 = 0.21$).

Maximal exercise test

Subjects performed a maximal progressive exercise test to exhaustion on an electrically-braked cycle ergometer (Corival, Lode B.V.). The test started with 2 min of rest, followed by 2-min warm-up at 50 W for male and 30 W for female, and then power output increased by 20 W for male and 15 W for female every 2 min until exhaustion. The pedaling frequency has to be between 70 and 80 rpm. Heart rate (12-channel ECG, Custo cardio 110 BT, Custo med GmbH, Ottobrunn, Allemagne) and gas exchange (MetaMax 3B, Cortex Biophysik GmbH, Leipzig, Germany) were monitored continuously. Peak oxygen consumption was defined as the highest oxygen intake that was sustained for at least 30 s

during the cycling exercise. Blood pressure, SpO₂, leg fatigue and dyspnea (assessed with a standard 100-mm visual analog scale) were recorded every 2 minutes until the end of exercise. Capillary blood sample was taken after 2 min of recovery from the fingertip to determine blood lactate concentration (Lactate Plus®, Nova Biomedical Corporation, Waltham, MA, USA). The ventilatory thresholds (VT1 and VT2) were determined according to the criteria proposed by Wasserman et al. (38).

Submaximal exercise test

Subjects completed a submaximal exercise test on an electrically-braked cycle ergometer (Corival, Lode B.V.) in the morning after an overnight fast, in order to determine specific metabolic parameters during exercise. This test followed the procedures of Brun et al. (5) and consisted in a 3-min warm-up at 20% followed by four 6-min steady-state workloads at 30, 40, 50 and 60% of the individual maximal aerobic power, previously determined during the maximal exercise test. Gas exchange (MetaMax 3B, Cortex Biophysik GmbH) was monitored continuously throughout the test. The crossover point of substrate utilization, the maximal lipid oxidation rate and the maximal fat oxidation rate were assessed using indirect calorimetry as previously described (5).

Vascular function and biological analysis

Blood pressure was measured on the dominant arm after 5 min of rest with a digital pressure monitor system (A&D Medical, Kitamoto-Shi, Japan). Arterial stiffness was assessed by carotid-femoral pulse-wave velocity as measured with a Complior device (Artech Medical; Pantin, France) (2). Endothelial function was assessed by reactive hyperemia using finger plethysmography with an Endo-PAT device (Itamar Medical Ltd) (8). After an overnight fast, blood samples were drawn for plasma analysis of glucose, insulin, triglycerides, total and high-density lipoprotein cholesterol (HDL-C), high-sensitive C-reactive protein (hsCRP). The low-density lipoprotein cholesterol (LDL-C) was calculated according to the Friedewald's formula. The insulin resistance was calculated according to the homeostasis model assessment of insulin resistance (HOMA2-IR; (21)), based on fasting blood glucose and insulin concentrations (<http://www.dtu.ox.ac.uk/>).

Body composition

Standard measures of height, weight, waist circumference and hip circumference were performed. Whole body magnetic resonance imagery (MRI) allowing for total fat and lean mass determinations were obtained on a General Electric Signa Advantage 1.5-tesla scanner (General Electric Medical Systems, Milwaukee, WI, USA) using a validated protocol (33) previously used in our laboratory (25). A standardized protocol was used for the differentiation of abdominal fat within trunk fat mass. Two sets of 10 images extended from L4–L5 to the diaphragm domes and the other set extended from the diaphragm domes to C6–C7. The images were analyzed manually with a homemade software (Matlab R2006, The Math Works, Inc., Natick, MA, USA) allowing adipose tissue identification (25).

Statistical Analysis

All statistical procedures were completed on Statistica version 10 (Statsoft, Tulsa, OK). Normality of distribution and homogeneity of variances of the main variables were confirmed using a Shapiro-Wilk normality test and the Levene's test, respectively. A two-way ANOVA (group \times time) with repeated measures was performed for each dependent variable. Post-hoc Tukey's tests were applied to determine a difference between two mean values if the ANOVA revealed a significant main effect or interaction effect. For all statistical analyses, a two-tailed alpha level of 0.05 was used as the cut-off for significance. All data are presented as mean values \pm SD within text and tables.

Results

The HET and NET groups did not differ at baseline regarding age and anthropometric characteristics (Table 1, all $p > 0.05$). Patient attendance to the training programs was excellent in each group (HET 22 ± 1 sessions, NET 23 ± 1 sessions). No adverse effect of hypoxic exposure was observed throughout the protocol.

Anthropometric data

Body mass index, waist and hip circumferences and body composition (Table 1) did not change significantly after both HET and NET.

Maximal exercise responses

Peak oxygen consumption and maximal power output before and after training in the HET and NET groups are provided in figure 1. Peak oxygen consumption (ANOVA interaction effect $F = 5.1$, $p = 0.036$) and maximal power output (ANOVA interaction effect $F = 16.0$, $p = 0.001$) increased significantly after training in the HET group only (peak oxygen consumption HET + $10 \pm 11\%$ versus NET + $1 \pm 10\%$ and maximal power output HET + $11 \pm 7\%$ versus NET + $3 \pm 10\%$).

Maximal cardiorespiratory and metabolic responses during exercise are provided before and after training in the HET and NET groups in table 2. Maximal minute ventilation increased significantly after training in the HET group only (ANOVA interaction effect $F = 4.9$, $p = 0.037$) while maximal heart rate decreased significantly after training both in the HET and NET groups (ANOVA main effect of time $F = 4.7$, $p = 0.041$). A significant ANOVA group \times time interaction ($F = 8.5$, $p = 0.009$) was observed for maximal blood lactate concentration but the post-hoc comparisons revealed no significant difference. The respiratory exchange ratio, SpO₂, blood pressure, leg fatigue and dyspnea at maximal exercise did not change significantly after both HET and NET.

Submaximal exercise responses

Power outputs at SV1 and SV2 during the maximal exercise test before and after training in the HET and NET groups are provided in figure 2. Power output at SV2 but not at SV1 significantly increased after training in both HET and NET groups, with no significant difference between groups. Cardiorespiratory responses at SV1 and SV2 during the maximal exercise test are provided in table 3. Heart rate and respiratory exchange ratio at SV1 and SV2 and systolic and diastolic blood pressure and dyspnea (and leg fatigue tendentially, ANOVA main effect of time $F = 3.5$, $p = 0.077$) at SV1 decreased after training in both groups, with no significant difference between groups. No difference in oxygen consumption and minute ventilation was observed at SV1 and SV2.

The power outputs corresponding to the crossover point of substrate utilization and the maximal lipid oxidation rate during the submaximal exercise test before and after training in the HET and NET groups are provided in figure 3. Power outputs at the crossover point and the maximal lipid oxidation rate increased significantly in both groups, with no difference between groups. No change in maximal fat oxidation rate was observed (HET before training 248 ± 123 versus after training 290 ± 120 mg·min⁻¹; NET before training 221 ± 69 versus after training 220 ± 80 mg·min⁻¹; all $p > 0.05$).

Vascular function and biological analysis

Evaluations of vascular function, blood glucose, insulin and lipid status before and after training in the HET and NET groups are provided in table 1. Resting systolic blood pressure did not change significantly after training in both groups, while a significant reduction in diastolic blood pressure was observed after training in both groups (ANOVA main effect of time $F = 4.5$, $p = 0.045$). The pulse wave velocity and the reactive hyperemia index did not change significantly after training in both HET and NET groups. Similarly, no significant change in glucose, insulin, HOMA2-IR, triglycerides, total cholesterol, HDL-C, LDL-C and hsCRP was observed in both groups. No difference between groups was observed for all vascular function and biological parameters.

Discussion

This study aimed at comparing in overweight or obese individuals the effect on maximal aerobic exercise capacity of an 8-week constant-load cycling exercise training program performed either in normoxia or in hypoxia at a target SpO₂ of 80%. The results indicate that peak oxygen consumption and maximal power output increased significantly after the exercise training program performed in hypoxia only. Submaximal exercise responses improved significantly and to a similar extent after both normoxic and exercise training programs. Except diastolic blood pressure which decreased significantly after both normoxic and hypoxic training programs, no change in vascular function and metabolic status was observed after training.

Effect of hypoxic exercise training on exercise responses

Because of the strong negative relationship observed in several cardiometabolic diseases between maximal exercise aerobic capacity and mortality (18, 19, 27, 32), increasing maximal exercise performance by using exercise training programs has become a critical tool for prevention and therapeutic strategies against various chronic diseases (29). In obese individuals, increasing physical activity levels and performing exercise training should be useful for both reducing body weight and for improving cardiovascular and metabolic health status (6). The optimal exercise training strategies regarding both the health benefits and the adherence of the patients (and therefore long-term benefits) remain however a matter of

debate and research in the field of obesity (13). Based on hypoxic training strategies for athletes (26) and results in animal models (37), several authors evaluated over the past 10 years the effects in obese patients of exercise training performed in normobaric hypoxia compared to normoxia (10, 11, 20, 28, 39). Only three of them evaluated the effect of HET *versus* NET on maximal exercise performance and all of them reported no additional benefits of hypoxia on this parameter (10, 11, 39). This is in contrast to the present study demonstrating a significant increase in peak oxygen consumption and maximal power output after HET compared to NET (figure 1). The characteristics of the hypoxic training programs implemented in the different studies may explain these contrasting results. Gatterer et al. (10) and Gonzalez-Muniesa et al. (11) trained their patients only twice a week while Wiesner et al. (39) trained their patients three times a week but at 65% of maximal heart rate only. Because of the well-known effect of the amount and intensity of exercise on training-induced exercise capacity improvements, the training load in previous studies might have been insufficient to demonstrate an additional benefit of HET on maximal exercise performance. Training at a too low relative intensity (i.e. % of maximal heart rate) might be an issue especially during HET because power output has to be reduced compared to NET in order to elicit similar heart rate during both types of training. As a consequence, the absolute power output during training in patients with reduced maximal normoxic power output will be low and may therefore be insufficient (especially as the muscle level) to induce significant adaptations. In the present study, subjects trained 3 times per week at 75% of their individual maximal heart rate in both HET and NET groups which may have induced larger training load and stimulus compared to previous studies (10, 11, 39). Despite this relative intensity higher than previous studies (but in accordance with standard recommendations for constant load exercise training (1)), 9 out of 12 subjects in the HET group trained at ≤ 70 W at the beginning of the program, which may represent a relatively low muscle stimulus. Therefore, instead of constant-load hypoxic exercise training, one may consider interval-training protocol as a solution to increase absolute workload during hypoxic exercise training in future studies (7). Conversely, in obese patients with frequent joint pain, reducing the absolute work load during hypoxic training compared to normoxic training while inducing similar or even greater health benefits is of significant interest to reduce pain and improve adherence to exercise training intervention as previously emphasized (39).

Another difference between the present study and previous works having reported no change in maximal exercise performance after hypoxic exercise training in obese individuals is the control and the severity of hypoxemia during hypoxic exercise training. While in

previous studies (10, 11, 39) a fixed FiO_2 of 0.14-0.16 was used for hypoxic exercise training, inducing SpO_2 values around 85-90% with significant interindividual heterogeneity, in the present study a target SpO_2 level of 80% was used which corresponds to values recommended recently for hypoxic conditioning in obese patients (16). Using a target SpO_2 of 80% by individually and continuously adjusting FiO_2 during hypoxic exercise induces a more severe and homogeneous hypoxic stimulus which may have been more prone to induce larger maximal aerobic capacity improvements than normoxic training. This emphasizes the potential interest of adding a hypoxic stimulus to exercise training in order to improve fitness level in obese individuals but also the importance to calibrate precisely the severity of hypoxia and the intensity of exercise.

As opposed to maximal exercise performances, similar changes during submaximal intensities were observed after HET and NET. The increase in power output at SV1 and SV2 (figure 2) as well as the changes in cardiorespiratory responses observed at SV1 and SV2 (table 3) illustrate the positive effects of both HET and NET on submaximal exercise cardiorespiratory responses and therefore the efficiency of both training programs to improve important markers of fitness status. These improvements were however of similar amplitude in the HET and NET groups, suggesting no additional benefit of adding hypoxia to exercise training on these parameters.

The increase in maximal aerobic performance observe in the HET group only was associated with a significant increase in maximal minute ventilation (table 2). Although a causal relationship cannot be ascertain, it may be suggested that exercise training in hypoxia by inducing greater ventilatory response may specifically improve the ability of the respiratory system to reach high levels of minute ventilation. Obese individuals are indeed well-known to present ventilatory constraints during exercise which can contribute to exercise tolerance limitation (3, 35). Hence, allowing overweight and obese individuals to reach higher minute ventilation during maximal exercise by performing HET may contribute to their greater improvement in maximal aerobic performance after training compared to NET. The mechanisms responsible for the larger increase in maximal aerobic performance after HET remain however to be clarified in future studies.

Effect of hypoxic exercise training on vascular function and metabolic status

Intermittent hypoxia is well known as a major mechanism responsible for adverse systemic consequences of sleep apnea syndrome leading to the production of reactive oxygen species, systemic inflammation, cardiovascular and metabolic morbidities (22). However,

hypoxic conditioning based on exposure to less severe hypoxia and longer hypoxia-reoxygenation cycles than sleep apnea has been described as an intervention able to improve the vascular function and the metabolic status (37). Lyamina et al. (24) showed for instance that 20 days of passive intermittent hypoxia exposure in patients with stage 1 arterial hypertension significantly reduced blood pressure together with an increased in urine nitrate and nitrite concentrations. Haufe et al. (15) also reported in sedentary healthy subjects that compared to NET, HET induced greater improvements in fasting insulin, HOMA index and triglycerides. Hence, because of the cardiovascular and metabolic dysfunctions frequently associated with overweight and obesity, HET may be of interest to improve vascular and metabolic function in these patients. In the present study, resting diastolic blood pressure decreased after both HET and NET while no other significant change in vascular function or blood metabolic parameters was observed after training. The power outputs corresponding to the crossover point of substrate utilization and the maximal lipid oxidation rate during the submaximal exercise test increased significantly however after both HET and NET. These results indicate that HET and NET induced some improvements in blood pressure and in metabolism with a greater ability to use lipids as energy substrate during exercise which illustrates the positive effect of both training programs. Changes in vascular and metabolic functions did not differ however between the HET and the NET groups. Some previous results in obese individuals suggested that HET may improve blood pressure to a greater extent than NET (11, 20) while others did not observed such an effect of hypoxia (10). Kong et al. (20) also reported no significant effect of HET on pulse-wave velocity in obese subjects. Regarding blood metabolic parameters, Netzer et al. (28) only evaluated the effect of HET *versus* NET in obese individuals and reported non-statistically significant greater reduction of cholesterol, triglycerides and LDL after HET compared to NET. The subjects included in the present study had normal vascular and metabolic status according to the evaluations performed before training (table 1). Therefore, it might be that the overweight and obese subjects included would have shown less benefit compared to obese patients with vascular and metabolic dysfunctions. Hence, future studies should define the patient phenotypes who would benefit the most from hypoxic training strategies. The effect of HET on mechanisms previously shown to underline the effect of hypoxic conditioning strategies and playing an important role in obesity also need to be evaluated, e.g. oxidative stress, NO metabolism, inflammation, appetite regulatory mechanisms (17, 31).

Effect of hypoxic exercise training on body composition

Because of the potential effect of hypoxic exposure on energy balance (see for a review (17)), hypoxic training has been thought to potentially promote weight reduction in obese individuals. Qin et al. (31) showed in mice submitted to a fatty-diet that intermittent hypoxia exposure (daily 4×30 min in hypobaric hypoxia equivalent to 3000 m, 40 days) reduced body weight and increased leptin levels compared to normoxic exposure. Most (10, 20, 28, 39) but not all (11) previous studies having investigated the effect of HET in obese individuals reported greater improvement in body weight and composition than NET. In the present study both HET and NET induced no significant change in BMI. By using magnetic resonance imaging, we could also show that body composition remain stable throughout the study. The lack of effect of both training programs on body weight and composition in the present study may be due to the absence of diet control and further studies should combine hypoxic training and diet intervention (14).

In conclusion, the present study indicates that in overweight and obese individuals, constant-load exercise training performed in hypobaric hypoxia with a target SpO₂ of 80% leads to greater improvements in exercise responses than normoxic constant-load exercise training performed at the same relative intensity (i.e. identical % of maximal heart rate). This greater improvement in maximal aerobic capacity following HET compared to NET was not associated with larger improvement in vascular and metabolic functions and in body composition. These results suggest that combining hypoxic exposure and exercise training may be of interest for the management of obesity, although the optimal modalities and the underlying mechanisms remain to be clarified in future studies.

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Table 1. Anthropometric, vascular and biological characteristics in each group before and after training.

	HET		NET	
	Before	After	Before	After
Age	52.2 ± 12.1	-	55.5 ± 10.7	-
BMI (kg·m ⁻²)	31.2 ± 2.4	31.6 ± 2.6	31.7 ± 3.2	31.5 ± 3.2
Waist circumference (cm)	109.8 ± 6.1	109.5 ± 6.2	108.6 ± 8.0	108.0 ± 8.7
Hip circumference (cm)	106.8 ± 6.9	106.1 ± 8.0	105.5 ± 8.2	104.9 ± 8.2
Total lean mass (kg)	61.8 ± 7.4	62.9 ± 7.7	57.4 ± 9.7	57.7 ± 10.1
Total lean mass (% weight)	65.2 ± 7.7	65.4 ± 6.5	64.4 ± 9.3	64.2 ± 9.4
Total fat mass (kg)	33 ± 8.3	33.3 ± 7.2	32.1 ± 10.0	32.4 ± 10.1
Total fat mass (% weight)	34.8 ± 7.7	34.6 ± 6.5	35.6 ± 9.3	35.7 ± 9.4
SBP (mmHg)	131 ± 11	128 ± 13	120 ± 15	121 ± 10
DBP (mmHg)	84 ± 9	80 ± 8*	75 ± 10	73 ± 7*
PWV (m·s ⁻¹)	8.0 ± 0.9	7.5 ± 1.9	7.8 ± 1.3	7.2 ± 0.9
Reactive hyperemia index	2.0 ± 0.3	2.5 ± 2.2	2.1 ± 0.4	2.1 ± 0.5
Cholesterol (g·l ⁻¹)	2.1 ± 0.3	2.1 ± 0.3	1.9 ± 0.4	1.9 ± 0.4
Triglyceride (g·l ⁻¹)	1.3 ± 0.3	1.3 ± 0.3	1.5 ± 0.3	1.5 ± 1.0
HDL (g·l ⁻¹)	0.53 ± 0.19	0.46 ± 0.08	0.50 ± 0.13	0.53 ± 0.17
LDL (g·l ⁻¹)	1.42 ± 0.30	1.42 ± 0.30	1.15 ± 0.44	1.15 ± 0.38
Glycemia (mmol·l ⁻¹)	5.8 ± 0.8	5.8 ± 0.7	5.8 ± 1.0	5.8 ± 0.8
Insulin (μUI·ml ⁻¹)	9.7 ± 4.2	9.3 ± 4.1	11.7 ± 4.5	11.3 ± 6.9
HOMA2-IR	1.30 ± 0.57	1.27 ± 0.49	1.55 ± 0.59	1.50 ± 0.92
hsCRP (mg·l ⁻¹)	2.5 ± 1.9	1.9 ± 1.6	6.0 ± 8.8	3.3 ± 5.1

Data are mean ± SD. HET, hypoxic exercise training group; NET, normoxic exercise training group; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PWV, pulse-wave velocity; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; HOMA2-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitive C-reactive protein. * significantly different from before (p < 0.05).

Table 2. Cardiorespiratory and metabolic responses at maximal exercise during the incremental cycling test in each group before and after training.

	HET		NET	
	Before	After	Before	After
RER	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.0	1.1 ± 0.0
V _E (L·min ⁻¹)	99.5 ± 16.2	110.5 ± 17.3*	86.1 ± 12.5	84.3 ± 15.8
Heart rate (bpm)	156 ± 25	155 ± 22*	157 ± 17	148 ± 22*
[La] (mmol·L ⁻¹)	8.4 ± 2.3	9.2 ± 2.6	8.2 ± 1.8	7.2 ± 1.6
SpO ₂ (%)	95 ± 2	94 ± 2	96 ± 2	96 ± 2
SBP (mmHg)	208 ± 31	211 ± 21	196 ± 28	198 ± 27
DBP (mmHg)	90 ± 14	88 ± 15	82 ± 8	81 ± 11
Leg fatigue	7.7 ± 2	8.0 ± 2.0	7.8 ± 1.6	8.4 ± 1.0
Dyspnea	8.2 ± 1.6	8.3 ± 1.8	8.0 ± 1.7	8.4 ± 1.2

Data are mean ± SD. HET, hypoxic exercise training group; NET, normoxic exercise training group; RER, respiratory exchange ratio; V_E, minute ventilation; [La], blood lactate concentration; SpO₂, arterial oxygen saturation; SBP, systolic blood pressure; DBP, diastolic blood pressure. * significantly different from before (p < 0.05)

Table 3. Cardiorespiratory and metabolic responses at the ventilatory thresholds during the incremental cycling test in each group before and after training.

	HET		NET	
	Before	After	Before	After
SV1				
VO ₂ (L·min ⁻¹)	1.8 ± 0.4	1.9 ± 0.3	1.5 ± 0.2	1.4 ± 0.2
RER	1.00 ± 0.06	0.95 ± 0.09*	0.98 ± 0.06	0.95 ± 0.04*
V _E (L·min ⁻¹)	51.9 ± 11.3	58.0 ± 13.4	44.8 ± 8.6	46.2 ± 9.4
Heart rate (bpm)	125 ± 23	118 ± 19*	124 ± 18	113 ± 14*
SpO ₂ (%)	96 ± 1	96 ± 1	96 ± 1	96 ± 2
SBP (mmHg)	183 ± 28	174 ± 24*	185 ± 29	178 ± 26*
DBP (mmHg)	89 ± 12	83 ± 13*	88 ± 13	81 ± 8*
Leg fatigue	4.9 ± 2.2	3.8 ± 2	4.3 ± 2.2	3.8 ± 1.8
Dyspnea	4.2 ± 1.9	3.5 ± 1.5*	4.0 ± 1.7	2.9 ± 1.7*
SV2				
VO ₂ (L·min ⁻¹)	2.2 ± 0.5	2.3 ± 0.3	1.8 ± 0.3	1.8 ± 0.3
RER	1.08 ± 0.09	1.00 ± 0.09*	1.04 ± 0.05	1.02 ± 0.04*
V _E (L·min ⁻¹)	72.1 ± 12.3	74.3 ± 12.5	59.9 ± 10.6	58.7 ± 10.9
Heart rate (bpm)	141 ± 25	134 ± 21*	139 ± 15	127 ± 17*
SpO ₂ (%)	96 ± 2	95 ± 2	96 ± 2	96 ± 2
SBP (mmHg)	192 ± 28	185 ± 28	198 ± 28	198 ± 25
DBP (mmHg)	90 ± 13	88 ± 15	89 ± 11	86 ± 11
Leg fatigue	6.5 ± 2.3	5.9 ± 2.3	6.2 ± 2.0	5.6 ± 2.3
Dyspnea	6.3 ± 2	5.9 ± 2	5.6 ± 2.4	4.8 ± 2.2

Data are mean ± SD. HET, hypoxic exercise training group; NET, normoxic exercise training group; SV1, first ventilatory threshold; SV2, ventilatory thresholds; VO₂, oxygen consumption; RER, respiratory exchange ratio; V_E, minute ventilation; [La], blood lactate concentration; SpO₂, arterial oxygen saturation; SBP, systolic blood pressure; DBP, diastolic blood pressure. * significantly different from before (p < 0.05).

FIGURE LEGENDS

Figure 1. Peak oxygen consumption (VO_2 peak, panel A) and maximal power output (panel B) during the incremental cycling test in the hypoxic (HET) and normoxic (NET) exercise training groups before and after training. * significantly different from before ($p < 0.05$).

Figure 2. Power outputs at the first (SV1, panel A) and second (SV2, panel B) ventilatory thresholds during the incremental cycling test in the hypoxic (HET) and normoxic (NET) exercise training groups before and after training. * significantly different from before ($p < 0.05$).

Figure 3. Power outputs corresponding to the crossover point of substrate utilization (panel A) and the maximal lipid oxidation rate (panel B) during the submaximal exercise test in the hypoxic (HET) and normoxic (NET) exercise training groups before and after training. * significantly different from before ($p < 0.05$).

Figure 1.

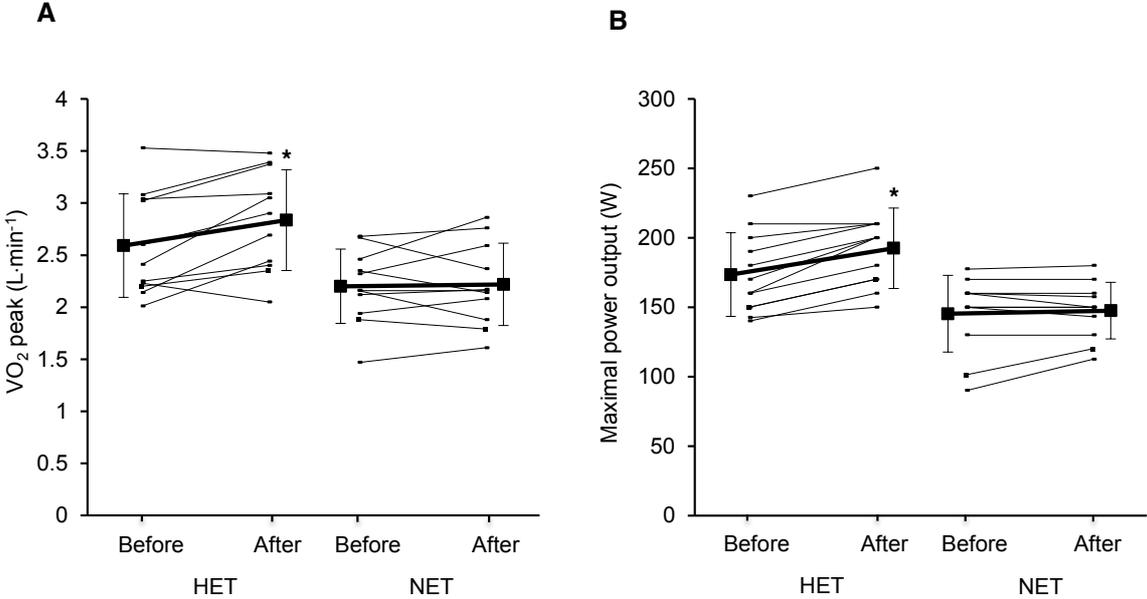


Figure 2.

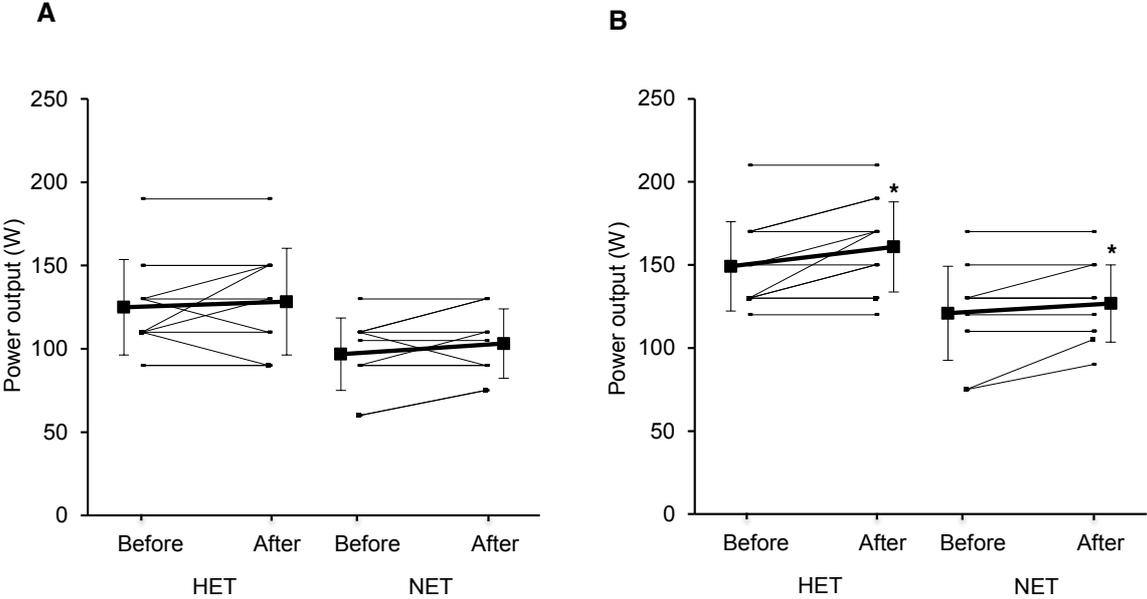
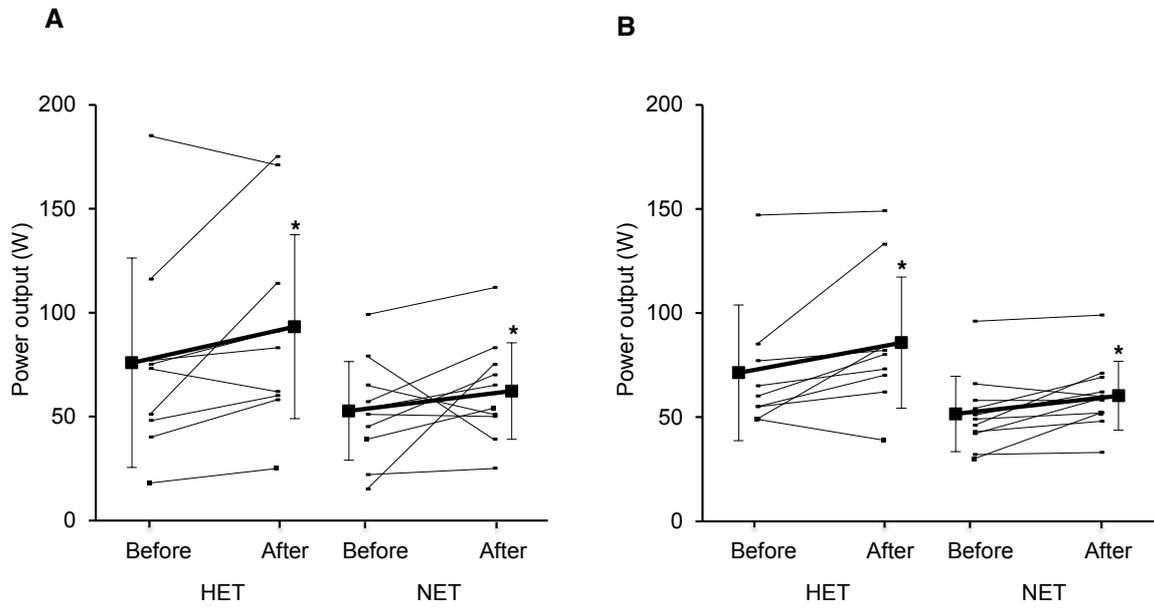


Figure 3.



**TROISIEME PARTIE: DISCUSSION GENERALE ET
PRESPECTIVES**

Les résultats principaux de cette thèse sont rappelés ci-dessous.

Etude 1. Nous avons étudié au cours d'une exposition hypoxique de repos, chez le sujet sain, l'effet de 3 séances (réponse à court terme au conditionnement hypoxique) sur la réponse cardiovasculaire et l'oxygénation musculaire et cérébrale (cortex pré-frontal gauche). Au cours de la troisième séance d'hypoxie intermittente (conditionnement passif), la pression artérielle systolique de repos (état basal normoxique qui précède l'exposition hypoxique) diminue. La variabilité sinusale au cours de l'exposition hypoxique intermittente est supérieure à celle déterminée lors de l'exposition hypoxique continue. Nous avons également observé une augmentation de la variabilité de la fréquence cardiaque lors de la 3^{ème} séance de conditionnement hypoxique intermittente par rapport à la première. En revanche, 3 séances d'hypoxie continue diminuent cette variabilité de la fréquence cardiaque. Ces deux types d'hypoxie provoquent une désoxygénation musculaire plus importante au cours de la séance 3. Par ailleurs, la diminution de l'oxygénation musculaire induite par l'hypoxie est accentuée lors de la 3^{ème} séance d'exposition hypoxique. Ces résultats préliminaires soulignent l'intérêt d'un conditionnement hypoxique intermittent sur des variables associées au risque cardiovasculaires avant et pendant une exposition hypoxique. On peut également souligner la rapidité des effets et l'aptitude de ce conditionnement à moduler l'hypoxie tissulaire.

Etude 2. Un conditionnement hypoxique passif chronique (intermittent ou continu), administré dans une population de sujets en surpoids ou obèses induit une diminution de la pression artérielle diastolique de repos en normoxie. Lors de la 24^{ème} séance d'exposition hypoxique, la désoxygénation cérébrale pré-frontale en réponse à l'hypoxie est accentuée par rapport à la première séance. On observe également une tendance à une moindre réduction de l'oxygénation musculaire au cours de cette 24^{ème} séance (vs la 1^{ère}). En revanche, le conditionnement hypoxique intermittent passif provoque une diminution de la variabilité de la fréquence cardiaque, résultats semblant opposé à ce qui a été observé dans l'étude 1 chez le sujet sain. Malgré des bénéfices intéressants du conditionnement hypoxique passif sur la pression artérielle chez la personne obèse, un certain nombre de questions sont à clarifier quant aux mécanismes impliqués.

Etude 3. Chez le sujet sain, un exercice aigu (continu (CL) ou intermittent intense (HII)) combiné à l'hypoxie provoque une diminution de l'apport et une augmentation de l'extraction en oxygène musculaire ([HbO₂] diminue et [HHb] augmente) sans modifier le niveau d'oxygénation musculaire (TSI) ni le volume sanguin local ([HbTot]) par comparaison à la situation normoxique réalisé à la même charge de travail interne (même fréquence cardiaque). De plus, l'oxygénation du cortex préfrontal est significativement diminuée en hypoxie par rapport à la normoxie pendant les exercices CL et HII. En outre, la ventilation était plus élevée durant l'exercice intermittent intense combiné à l'hypoxie par rapport à la normoxie. Au cours des périodes de récupération passive de l'HII, fréquence cardiaque était plus élevée en hypoxie qu'en normoxie. La

difficulté perçue durant les exercices ne différait en revanche pas entre l'hypoxie et la normoxie pendant les exercices CL et HII.

Etude 4. Un conditionnement hypoxique actif chronique (entraînement sur ergocycle à une intensité modérée) de 24 séances administré dans une population obèse montre une augmentation de l'aptitude maximale aérobie (puissance maximale aérobie, VO_{2pic}) plus importante qu'un entraînement à l'effort normoxique à même intensité relative. Cependant, malgré une amélioration de l'adaptation métabolique à l'exercice musculaire et une diminution de la pression artérielle diastolique de repos qui surviennent dans le groupe hypoxique pour une puissance mécanique d'entraînement inférieure à celle réalisée dans le groupe normoxique, le statut métabolique (glucose, insuline et statut lipidique sanguins) et vasculaire n'est pas plus amélioré dans le groupe hypoxique. Ces résultats suggèrent que les protocoles de conditionnement hypoxique combiné à l'exercice peuvent amener certains bénéfices supplémentaires aux prises en charge standard en normoxie.

Quelles modalités de conditionnement hypoxique ?

Conditionnement passif (repos) ou actif (exercice chronique)

Une exposition chronique à l'hypoxie normobarique ou hypobare a été mise en œuvre pour améliorer la performance des athlètes via des adaptations physiologiques, incluant des augmentations de masse d'hémoglobine et de capillarisation et des capacités métaboliques [2]. L'exposition chronique à l'hypoxie modérée peut induire également des réponses cardiovasculaires et métaboliques positives chez l'animal et chez l'homme [12, 15, 16, 135, 310].

Ce conditionnement hypoxique chronique qu'il soit passif ou actif pourrait constituer un nouvel outil thérapeutique face au surpoids et à l'obésité [18, 175, 176]. Par exemple, il pourrait induire une augmentation du métabolisme de base, et de l'oxydation des lipides [171] et diminuer le rapport LDL/HDL ainsi que la pression artérielle [311]. Mais, ce type de traitement n'a pas ou peu fait l'objet d'études randomisées contrôlées. Dans un premier temps, au cours de cette thèse, il nous a paru important de mieux caractériser au plan physiologique les effets des différentes stratégies de conditionnement hypoxique (passif ou actif, continu ou intermittent). Ceci a permis d'une part de tester leur innocuité en vue d'une application clinique mais aussi d'anticiper les stratégies les plus efficaces de ce type de conditionnement. Ainsi, toutes les procédures de conditionnement hypoxiques ont été testées au cours d'études réalisées chez le sujet sain. A noter que l'étude simultanée de plusieurs stratégies de conditionnement hypoxique (hypoxie continue versus hypoxie intermittente versus normoxie) constitue une des originalités de cette thèse par rapport à la littérature existante.

Nous avons d'abord étudié les conséquences cardiorespiratoires et tissulaires d'un conditionnement hypoxique de courte durée au repos. Cette étude nous a permis de montrer en moins de 3 jours des adaptations à l'exposition hypoxique aiguë bénéfiques sur la variabilité de la fréquence cardiaque, la pression artérielle, et l'oxygénation tissulaire. Ces adaptations prometteuses ont été testées en clinique dans une population de sujets obèses (protocole HYPINT, étude 2), au cours de programmes d'exposition hypoxique chronique passive (24 séances ; 3 séances d'environ une heure/semaine pendant 8 semaines à une SpO₂ moyenne d'environ 76 +/- 5 %) chez le sujet en surpoids ou obèse.

Compte tenu des effets limités notamment sur le poids d'une exposition chronique passive dans une population de sujets obèses observés dans cette étude randomisée contrôlée, il semble important d'envisager d'autres stratégies complémentaires au seul conditionnement hypoxique [176] parmi lesquelles une prise en charge diététique et l'exercice chronique. L'intérêt de la combinaison d'un conditionnement hypoxique à un exercice musculaire réside en particulier dans la recherche d'une amplification de l'hypoxie musculaire induite par l'exercice, et donc dans la recherche d'un stimulus des réponses adaptatives à l'exercice plus important.

Ainsi, nous avons testé dans l'étude 3, l'effet aigu d'un exercice (continu vs intermittent intense) combiné à l'hypoxie sur la réponse cardio-respiratoire et l'oxygénation cérébrale et musculaire chez le sujet sain, comparé à une séance d'exercice en normoxie. La finalité de cette étude préliminaire étant de préparer l'administration d'un programme d'exercice chronique en hypoxie dans une population de sujets obèses à risque cardio-métabolique. Nous avons notamment cherché à vérifier la tolérance et la faisabilité de cette stratégie de conditionnement et à caractériser précisément les réponses cardio-ventilatoires et d'oxygénation musculaire associées. Compte tenu de la diminution de la puissance maximale aérobie dans les conditions hypoxiques, l'exercice en hypoxie doit être réalisé à des intensités absolues inférieures à celles réalisées en normoxie de façon à obtenir une intensité relative équivalente (% de la fréquence cardiaque). Se pose alors la question de l'intensité du stress hypoxique musculaire, et donc de son efficacité potentielle. La réduction de l'apport musculaire en oxygène peut-elle compenser la diminution de l'intensité d'exercice et donc de l'utilisation d'oxygène par le muscle? La réduction de la fraction inspirée d'oxygène induit-elle un stress hypoxique musculaire supérieur à celui de l'exercice en normoxie? Cette étude montre qu'à une intensité d'exercice relative identique, quelle que soit la nature de l'exercice (charge constante ou intermittent intense), l'hypoxie n'accentue pas l'oxygénation musculaire par rapport à la normoxie, tout au moins dans le cadre de son évaluation par technique NIRS. Par conséquent, il peut sembler difficile d'attendre une amélioration plus importante de la fonction musculaire causée par un plus grand stress hypoxique (l'hypoxie étant reconnue comme l'élément central des adaptations musculaires). Ceci nécessite cependant une confirmation chez le malade, notamment insulino-résistant ou diabétique qui présente souvent des altérations de la circulation locale [199, 200].

Nous avons ensuite étudié les adaptations à un programme d'exercice continu de 8 semaines réalisé en normoxie ou en hypoxie chez le sujet en surpoids et obèse. Cette étude montre une amélioration de la consommation maximale d'oxygène et de la puissance maximale après le programme d'entraînement physique en hypoxie seulement malgré une puissance absolue à l'effort moins importante en hypoxie. Même si les mécanismes de cette amélioration nécessitent confirmation et clarification, ce résultat important semble confirmer l'intérêt du conditionnement hypoxique lorsqu'il est combiné à l'exercice musculaire. Ce type d'entraînement est particulièrement intéressant à proposer aux patients atteints de troubles ostéo-articulaires puisqu'il permet de diminuer la puissance absolue de l'exercice chronique. D'autres modalités d'exercice plus ludiques et reconnues comme plus efficaces à court terme [132, 277, 312] pourraient être associées à l'hypoxie. Nous avons testé les réponses en aigu à l'exercice intermittent combiné à l'hypoxie dans le cadre de l'étude 3 réalisée chez le sujet sain. Nous n'avons pas obtenu de différence en terme d'oxygénation musculaire par rapport à la normoxie et à l'exercice continu mais surtout la difficulté perçue par les sujets sur ce type d'exercice montre qu'il est bien toléré. Ceci reste à être confirmé chez le sujet obèse. De façon intéressante la fréquence cardiaque plus élevée au cours des phases de récupération suggère que le stimulus physique reste plus important que lors

d'un exercice continu avec une récupération légèrement plus difficile. Egalement, la sollicitation ventilatoire (et potentiellement les progrès induits au niveau du système respiratoire : fonction des muscles respiratoires par exemple) était plus importante lors de l'exercice aigu intermittent intense en hypoxie par rapport à la normoxie. Ces différences entre exercice aigu intermittent intense réalisé en hypoxie versus normoxie pourraient constituer un levier d'amélioration supplémentaire chez des patients réalisant un programme d'entraînement à l'exercice intermittent intense en hypoxie versus normoxie. Des études à venir au sein du laboratoire doivent tester cette hypothèse.

En conclusion, le conditionnement hypoxique passif chez le patient en surpoids ou obèse semble exercer certains effets potentiellement importants sur le système cardio-vasculaire (pression artérielle diastolique) alors que certaines absences d'effet significatif (par exemple sur le plan métabolique) contrastent avec ceux rapportés dans la littérature [176]. Nous avons pourtant largement respecté les suggestions de Hobbins et coll. [176] en terme de durée de prise en charge (8 semaines vs de 2 à 4 semaines conseillé), en terme de durée d'exposition (1 h pour 1-1.5 h), en terme de stress hypoxique (Fraction inspirée d'oxygène = 10-12%). La seule différence notable réside dans la fréquence d'exposition hypoxique. En effet, les sujets ont réalisé 3 séances dans notre étude alors que Hobbins et coll. [176] conseillent une séance quotidienne. Ce point mérite donc une clarification dans de futures études.

D'autre part, des études supplémentaires sont nécessaires notamment avec des populations présentant une morbidité cardio-métabolique supérieure à celle incluse dans cette thèse. Enfin, la persistance sur le long terme des effets sur la pression artérielle diastolique mérite d'être étudiée. Ainsi, des évaluations récurrentes à distance du conditionnement hypoxique (ex. chaque semaine) pourraient être proposées.

Les résultats concernant le conditionnement hypoxique actif sont plus probants notamment pour ce qui concerne la condition physique et la réponse ventilatoire. Ceci suggère un intérêt de cette stratégie pour améliorer la condition physique (bon prédicteur de la mortalité cardiovasculaire) de sujets limités au plan ostéo-articulaire puisque cette approche permet de diminuer la puissance absolue de l'exercice. A noter tout de même que la charge interne que nous avons utilisée (75% de la fréquence cardiaque maximale) est supérieure à celle conseillée par Hobbins et coll. [176] (60-70%). D'autres modalités d'exercice combiné à l'hypoxie sont à étudier et notamment l'exercice intermittent intense. Cette combinaison est un bras d'étude prévu et à venir dans le cadre de l'étude de recherche clinique HYPINT.

Quelle dose d'hypoxie pour obtenir des effets protecteurs et thérapeutiques optimaux ?

Les effets du conditionnement hypoxique dépendent de la dose administrée. Une dose sévère peut induire des effets délétères et pathologiques (voir SAOS) alors qu'une hypoxie modérée semble plus bénéfique. L'hypoxie est considérée sévère et délétère

notamment si elle est appliquée en cycles courts (< 2min) à $FiO_2 < 8-10\%$ et $SpO_2 < 70\%$, et modérée si la $FiO_2 > 10\%$ et la $SpO_2 \geq 70-80\%$ [135].

Hamlin et coll. [313] ont montré que pour une exposition au même niveau d'hypoxie ($FiO_2 = 10\%$), il existe une plus grande variabilité interindividuelle de la désaturation artérielle par rapport à une situation où une valeur de SpO_2 est ciblée (75%). Nous avons donc fixé une SpO_2 cible entre 70-80% pour les groupes repos (publication 1 et 2), 75% pour les sujets sains qui ont été exposés à une séance de conditionnement hypoxique actif (publication 3) et de 80% pour les sujets obèses recevant également un conditionnement hypoxique actif (publication 4). Hobbins et coll. [176] ont proposé de fixer une cible de SpO_2 pour minimiser la variabilité interindividuelle de la réponse à la baisse de FiO_2 . Le niveau de la SpO_2 que nous avons utilisé (entre 70% et 80%) a été bien toléré par tous nos sujets qu'ils soient sains ou en surpoids/obèses soit au repos soit à l'exercice.

A priori, l'intensité du stimulus que nous avons utilisé est suffisante. Cependant, le sujet obèse est parfois considéré comme «résistant à l'hypoxie» et présente une désaturation retardée / minimale lorsqu'il est exposé à une $FiO_2 \leq 12\%$ [314]. Ceci pourrait peut-être participer à l'absence d'effets marqués du conditionnement hypoxique passif chez les sujets obèses inclus dans l'étude 2 de cette thèse. Ainsi, cette absence d'effets notamment sur le poids et la masse grasse pourrait tenir à une dose d'hypoxie insuffisante. La revue de littérature de Hobbins et coll. [176] publiée trois ans après le démarrage de notre étude conseille plutôt une application quotidienne du conditionnement hypoxique alors que nous avons proposé 3 séances/sem lors de la conception de cette étude. A noter qu'une exposition quotidienne rend une telle prise en charge particulièrement lourde. Ainsi, même si notre programme est assez proche sur les caractéristiques conseillées par Hobbins et coll. [176], hormis une moindre fréquence d'exposition dans notre protocole, la dose totale d'hypoxie passive administrée pourrait être insuffisante pour induire des effets marqués sur la composition corporelle en particulier. Il se pourrait également que la durée de notre programme supérieure à celle recommandée par Hobbins et coll. [176] ait entraîné un effet plafond qui pourrait grever la possibilité d'observer des variations en début de protocole telles qu'observées chez l'animal dans notre laboratoire pour des hypoxies sévères [68]. Cette possibilité pourrait avoir été vérifiée par une mesure du poids à chaque séance ou chaque semaine par exemple.

En revanche dans le conditionnement actif chez le sujet obèse pour lequel nous avons ciblé une saturation de 80 %, nous avons obtenu des effets relativement importants et probants sur l'aptitude maximale aérobie suggérant que la dose est adaptée, comme conseillé par Hobbins et coll. [176]. Ceci suggère également que dans les conditions d'élévation du métabolisme (i.e telle que dans une situation d'exercice), la « résistance » à l'hypoxie du sujet obèse pourrait moins impacter l'efficacité de l'intervention par conditionnement hypoxique.

Il paraît utile de tester une cible d'hypoxie légèrement inférieure au cours de l'exercice, par exemple 75%, puisque les sujets la supportent bien au repos. Cela implique qu'il faudra abaisser encore la puissance de l'exercice par rapport à la condition normoxique. Il reste donc à conduire des études spécifiques quant aux doses d'hypoxie (saturation artérielle cible de 70, 75, 80%) pour optimiser le conditionnement hypoxique et potentiellement individualiser les doses en fonction des réponses individuelles.

Hypoxie continue ou hypoxie intermittente ?

Les modèles d'hypoxie intermittente (cycles > 5 min) ou d'hypoxie continue (1h) peuvent avoir des effets cardiovasculaires et métaboliques positifs [12, 135]. Des études antérieures sur le conditionnement hypoxique chez les sujets sains et les patients ont utilisé deux types principaux d'exposition hypoxique : soit des séances répétitives d'hypoxie intermittente, par exemple 3-7 min d'hypoxie et 3-5 min de normoxie, répétées 3-10 fois [92-95], soit continue (1 h) [96-98]. Deux études pilotes non randomisées non contrôlées de faible effectif ont exposé des sujets en surpoids et obèses (6 patients dans chaque étude) à des séances hypoxiques passives continues normobariques [171] ou intermittentes ($SpO_2 = 80\%$, 7-10 séances) [311]. L'exposition hypoxique semble entraîner des effets bénéfiques sur le plan métabolique (dépense énergétique basale et plus grande oxydation lipidique) [171] et vasculaire (pression artérielle systolique [311]). Un des aspects originaux de cette thèse, est l'utilisation de 2 types de conditionnement hypoxique en parallèle: intermittent (7 cycles de 5 min en hypoxie suivies de 3 min en normoxie) et continu (durant une heure). Chez le sujet sain, 3 séances d'hypoxie intermittente (conditionnement passif) induit des bénéfices au niveau vasculaire (une diminution de la pression artérielle de repos, et une augmentation de variabilité sinusale) supérieure à celle mesurée lors de l'exposition hypoxique continue. Cependant, chez la personne en surpoids/obèse, ces deux types d'exposition entraînent une diminution de la pression artérielle diastolique similaire. Au niveau de l'oxygénation tissulaire, l'hypoxie continue entraîne une diminution de l'oxygénation cérébrale plus marquée que l'hypoxie intermittente. Ces résultats préliminaires à court terme chez le sujet sain soulignent l'intérêt d'un conditionnement hypoxique intermittent sur des variables associées au risque cardiovasculaires avant et pendant une exposition hypoxique. On peut également souligner la rapidité des effets et l'aptitude de ce conditionnement à moduler l'oxygénation tissulaire en réponse à l'exposition hypoxique. Lors de l'exercice chronique, nous n'avons pas testé l'exposition hypoxique intermittente, combinaison qui n'a jamais été rapportée dans la littérature. Cependant, le caractère intermittent hypoxique pourrait être obtenu lors d'un exercice intermittent comme indiqué dans la figure 3 de l'article 3, ce qui pourrait être un élément important pour d'éventuelles réponses spécifique à l'entraînement à l'exercice intermittent intense en hypoxie (études à venir).

Le mode d'exposition hypoxique (intermittent ou continu) utilisé au cours du conditionnement hypoxique pourrait être sélectionné selon de type de patient et selon le

paramètre cible (pression artérielle, variabilité de la fréquence cardiaque, muscle, cerveau...). Par ailleurs il pourrait être envisagé de combiner le conditionnement hypoxique passif avec la réalisation d'un exercice en normoxie juste avant ou juste après l'exposition hypoxique de façon à induire d'autres interactions entre exposition hypoxique et réalisation d'un effort.

Application thérapeutique du conditionnement hypoxique : pour quel(s) sujets ?

Alors que certaines études réalisées chez le sujet sain montrent des effets bénéfiques du conditionnement hypoxique, des études pilotes ont été menées dans diverses conditions pathologiques pour évaluer l'application clinique du conditionnement hypoxique. Au cours de cette thèse nous avons d'abord testé le protocole de conditionnement hypoxique chez le sujet sain, afin d'établir la faisabilité et la tolérance de l'hypoxie avant de l'appliquer au sujet en surpoids ou obèse. En effet, les traitements doivent être adaptés à la gravité du surpoids/obésité à sa morbidité associée et aux limitations fonctionnelles. Il était donc nécessaire de bien caractériser les effets des traitements proposés. Nous avons montré que 24 séances de conditionnement hypoxique combiné à l'exercice continu entraînent une amélioration de la condition physique chez l'obèse (augmentation de VO_{2max} et puissance maximale aérobie), de plus une diminution de la pression artérielle diastolique a été observée après entraînement à l'effort (combiné à l'hypoxie et pas). Par contre, nous n'avons pas observé de modifications notables au plan cardio-métabolique (poids, composition corporelle, rigidité artérielle, réactivité vasculaire) qu'il s'agisse d'un conditionnement hypoxique passif (repos) ou actif (exercice). Cette absence d'effet s'oppose à la tendance décrite dans les revues de Vergès et coll. [12] et de Hobbins coll. [176] qui semblent montrer des effets chez des sujets hypertendus stade I, des sujets obèses et des diabétiques de type 2, même si les effectifs des études évoquées étaient souvent faibles. Dans nos études 2 et 4, malgré le surpoids et l'obésité, peu de sujets présentaient une morbidité cardiovasculaire telles qu'une hypertension ou une insulino-résistance (les sujets des différents groupes hypoxique présentaient en moyenne des HOMA2-IR compris entre 1,18 et 1,55). Ceci pourrait expliquer les effets parfois modestes ou non significatifs du conditionnement hypoxique dans cette population spécifique. Compte tenu des résultats positifs des différents conditionnements hypoxiques que nous avons appliqués, notre traitement semble particulièrement adapté aux sujets hypertendus.

Sur la base des données actuelles, il nous semble important de vérifier les effets de nos différents programmes de conditionnement chez des sujets présentant une plus grande morbidité mais nous risquons de nous heurter aux traitements médicamenteux de ces patients qu'il est difficile d'interrompre pour des aspects éthiques. Nous avons en projet d'étudier les effets d'un conditionnement hypoxique dans l'insuffisance cardiaque [315]. En effet, chez l'animal, une activation de la voie JAK/STAT agissant via le récepteur au $TNF\alpha_2$, dont le ligand ($TNF\alpha$) peut être activé par l'HI pourrait jouer un rôle protecteur sur le myocarde [167]. Il se pourrait également que l'HI induise une plus grande capillarisation myocardique [167].

Quels organes sont les plus sensibles au conditionnement hypoxique ?

Les résultats des conditionnements hypoxiques passifs réalisés chez l'obèse semblent indiquer un effet essentiellement vasculaire puisque seule la pression artérielle diastolique est impactée. Cette diminution de pression artérielle (4 à 12 mm Hg) n'est pas négligeable en terme d'impact épidémiologique puisque une diminution 5 mmHg permet de sauver 27600 vies aux USA [316]. Les mécanismes de cette diminution de pression artérielle ne sont pas encore élucidés. Compte tenu de la diminution de la variabilité sinusale observée après conditionnement par hypoxie intermittente seulement chez les sujets obèses (publication 2), il se pourrait que l'hypoxie agisse par d'autres mécanismes que ceux du système nerveux autonome via par exemple la production ou la potentialisation des effets de substances vaso-actives telles que le NO ou sa moindre inhibition par le stress oxydatif [18] mais aussi le VEGF [144]. Ainsi, une évaluation des biomarqueurs du NO (nitrites/nitrites), du stress oxydatif ou d'un VEGF dans la suite de nos travaux permettra de clarifier les mécanismes responsables de l'amélioration de pression artérielle. Ces mesures sont à venir dans le cadre des études présentées ici.

Par ailleurs, au niveau de l'oxygénation cérébrale, nous avons montré que l'exposition chronique à l'hypoxie continue provoque un stress hypoxique pour le cortex préfrontal plus important. En revanche, l'exposition chronique à l'hypoxie intermittente semble entraîner une augmentation du volume sanguin cérébral local et une meilleure réactivité cérébrovasculaire à l'hypoxie (étude 2). Il paraît essentiel de se concentrer à l'avenir sur les conséquences cérébrovasculaires des stratégies de conditionnement hypoxique, car elles peuvent constituer un outil puissant pour améliorer la perfusion cérébrale et l'oxygénation chez les personnes âgées ou dans des pathologies associées à un trouble cérébral comme la maladie d'Alzheimer [317, 318].

Ces résultats ouvrent des pistes de recherche dans le domaine du conditionnement hypoxique afin d'identifier et cibler les organes susceptibles de bénéficier le plus d'un conditionnement hypoxique. Le système cardio-vasculaire et cérébro-vasculaire pourraient être des cibles privilégiées et le conditionnement hypoxique pourrait ainsi devenir une option thérapeutique innovante et prometteuse.

Modalités pratique de mise en œuvre du conditionnement hypoxique

Un conditionnement hypoxique en routine clinique est tout à fait faisable mais il ne peut pas être réalisé à grande échelle si on prend le modèle appliqué au cours de cette thèse qui a nécessité plus de 1000 conditionnements hypoxiques. En effet, nous n'avons utilisé qu'un dispositif Altitrainer ce qui n'a pas permis de traiter plus d'un sujet par séance. D'autre part, le coût de ce conditionnement n'est pas négligeable car il nécessite le changement régulier (tous les 10 jours pour le conditionnement hypoxique actif, tous les 21 jours pour le conditionnement passif pour environ 6 sujets conditionnés par semaine en moyenne) de bouteilles d'azote.

Il est possible de regrouper les sujets dans des chambres / tentes hypoxiques qui permettent de simuler l'altitude mais ceci pose le problème de l'individualisation de l'hypoxie puisque pour un niveau pour une exposition à un même niveau d'hypoxie, il existe une plus grande variabilité interindividuelle sur la désaturation [313]. Ainsi, pour une utilisation à grande échelle de ce conditionnement, il sera nécessaire d'adapter les dispositifs commerciaux aux contraintes cliniques.

Compte tenu de la lourdeur et du coût de ce conditionnement, il semble important d'identifier rapidement les patients répondeurs ou non à un conditionnement hypoxique. Ceci pourrait permettre d'ajuster rapidement la dose efficace pour certains sujets, notamment dans le cadre d'une recherche clinique. En effet, un ajustement permanent du « design clinic » tel que proposé dans les « Adaptive Clinical Trial » permettrait un gain de temps et la réduction des coûts en évitant d'engager des patients dans des traitements longs et inefficaces. L'atteinte de ces objectifs nécessiterait en particulier l'identification de biomarqueurs physiologiques permettant d'identifier après quelques séances les bons et mauvais répondeurs et l'adaptation de la dose d'hypoxie au patient. Nous pensons évidemment à la pression artérielle mais aussi à la variabilité sinusale qui semblent assez sensibles au conditionnement hypoxique. Une automesure automatique de la pression artérielle le matin au lever pourrait constituer un indicateur intéressant de même qu'une mesure en début et fin de chaque séance.

La variabilité sinusale pourrait également constituer un moyen simple de mesurer la réponse au conditionnement hypoxique mais il reste à démontrer que l'amélioration de cette variabilité observée chez le sujet sain notamment au cours d'une exposition hypoxique intermittente aiguë constitue bien le reflet d'une amélioration cardiovasculaire des patients au plan clinique.

Conclusion générale

Les travaux présentés dans cette thèse mettent en évidence les effets de différents conditionnements hypoxiques pour améliorer la santé cardio-métabolique.

Les résultats obtenus suggèrent que le conditionnement hypoxique passif peut induire des adaptations vasculaires (tension artérielle diastolique) et respiratoires positives chez les personnes obèses. Un conditionnement hypoxique actif peut être une option intéressante pour la prise en charge de l'obésité, en induisant une amélioration de l'aptitude maximale aérobie surtout pour les sujets présentant une limitation à l'effort ne leur permettant pas de se ré-entraîner à des intensités d'exercice élevées. Le type (passif ou actif), la sévérité ainsi que la durée d'exposition (aiguë ou chronique) du conditionnement hypoxique modifient très probablement les adaptations cardio-métaboliques induites. Ces protocoles de conditionnement doivent donc être affinés en vue d'optimiser leur efficacité en termes de perte de poids et d'amélioration du risque

cardio-vasculaire dans des populations présentant une obésité associée à une morbidité cardio-métabolique.

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ANNEXE I

Hypoxic conditioning as a new therapeutic modality

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Preconditioning refers to a procedure by which a single noxious stimulus below the threshold of damage is applied to the tissue in order to increase resistance to the same or even different noxious stimuli given above the threshold of damage. Hypoxic preconditioning relies on complex and active defenses that organisms have developed to counter the adverse consequences of oxygen deprivation. The protection it confers against ischemic attack for instance as well as the underlying biological mechanisms have been extensively investigated in animal models. Based on these data, hypoxic conditioning (consisting in recurrent exposure to hypoxia) has been suggested a potential non-pharmacological therapeutic intervention to enhance some physiological functions in individuals in whom acute or chronic pathological events are anticipated or existing. In addition to healthy subjects, some benefits have been reported in patients with cardiovascular and pulmonary diseases as well as in overweight and obese individuals. Hypoxic conditioning consisting in sessions of intermittent exposure to moderate hypoxia repeated over several weeks may induce hematological, vascular, metabolic, and neurological effects. This review addresses the existing evidence regarding the use of hypoxic conditioning as a potential therapeutic modality, and emphasizes on many remaining issues to clarify and future researches to be performed in the field.

Keywords: intermittent hypoxia, conditioning, therapeutics, dose, humans, murines

Introduction

Intermittent hypoxia (IH) is well known in clinical physiopathology as a central characteristic of obstructive sleep apnea syndrome (OSAS). Chronic cyclical (30–90 s cycles) severe hypoxia is recognized as a major mechanism underlying the adverse systemic consequences of OSAS. Desaturation–reoxygenation sequences lead to oxidative stress and the production of reactive oxygen species (ROS) (1). Increased ROS levels lead to increased expression of adhesion molecules (2), activation of leukocytes (3), and production of systemic inflammation (4). Oxidative stress, systemic inflammation, and sympathetic activation underlie marked cardiovascular and metabolic morbidities in OSAS. There are now convincing data regarding the association between hypertension, arrhythmias, stroke, coronary heart disease, increased cardiovascular mortality, metabolic dysregulation, and OSAS (5). Nocturnal exposure to IH in healthy subjects has been shown to increase daytime arterial blood pressure and sympathetic activity (6, 7). OSAS should therefore be considered as a systemic disease with IH leading to deleterious consequences throughout the organism.

Several arguments suggest, however, that hypoxic exposure may also lead to some positive adaptations of the human body, able to protect him from several pathological conditions. Athletes have been using hypoxic training as a training strategy for decades, with increasingly complex methods

and technology aimed at improving sport performances (8). Interestingly, some epidemiologic data suggest that living at moderate altitude may be associated with lower prevalence of obesity (9–11). Voss et al. (11), for instance, found in a USA-wide representative sample of >400,000 subjects that after controlling for urbanization, temperature category, and behavioral and demographic factors, male and female Americans living <500 m above sea level had 5.1 [95% confidence interval (CI): 2.7–9.5] and 3.9 (95% CI: 1.6–9.3) times the odds of obesity, respectively, compared to their counterparts living >3000 m. Although IH is essentially considered as a deleterious factor in OSAS, some results suggest that moderate OSAS may be associated with some protection against ischemic-reperfusion events (12, 13). IH may in this case enhance the number and function of endothelial progenitor cells, promoting angiogenesis and coronary collateral vessels for instance (14). The potential protective effect of IH exposure also arose from clinical studies, suggesting that patients who had strokes preceded by transient ischemic attacks (implying some hypoxic stress) in the same vascular territory often had less severe deficits at the onset of their stroke and more favorable outcomes (15, 16).

A number of animal studies have described over the past decades the effect of ischemic and hypoxic preconditioning, demonstrating that some pattern and severity of hypoxic exposure can provide protective effects against various deleterious stimuli. While these experimental studies focusing on preconditioning mostly looked at the effect of acute hypoxic conditioning (i.e., a single session of hypoxic exposure) to induce protection over a limited period of time, they opened perspectives regarding the induction of a more prolonged state of protection based on recurrent hypoxic exposure, i.e., hypoxic conditioning (or intermittent hypoxic training). After summarizing the principles of hypoxic (pre)-conditioning, this review will present the available evidence regarding the use of hypoxic conditioning for several pathological conditions as well as highlight the remaining questions and perspectives for future research.

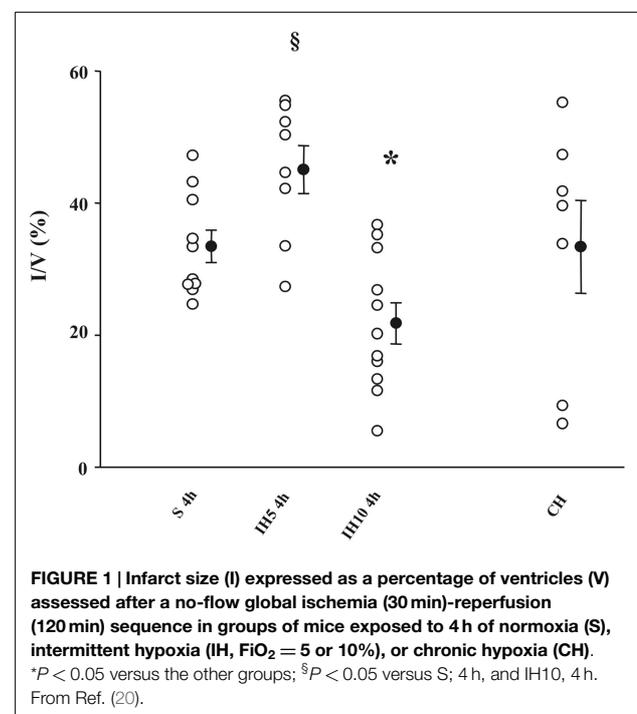
Principle of Hypoxic (Pre)-Conditioning

Preconditioning is a procedure by which a potentially deleterious stimulus is applied near to but below the threshold of damage to the organism. Shortly after this preconditioning procedure or after some time delay, tissues and organs can develop resistance or tolerance to the same or similar noxious stimuli, therefore preventing or reducing the damage it may induce. The preconditioning phenomenon relies on the basic principle that organisms have developed complex and active defenses to counter adverse conditions such as starvation and oxygen deprivation. Many stimuli, such as ischemia, hypoxia, hypothermia, and pharmacological agents can induce a preconditioning response and modify the responses of the organism to subsequent stress conditions. To identify endogenous mechanisms of protection and repair, and to potentially use these mechanisms therapeutically, preconditioning strategies have been tested over the past decades.

In 1986, Murry et al. (17) first described the principle of ischemic preconditioning. Four cycles of 5-min ischemia followed by short episodes of reperfusion in dogs resulted in a remarkable reduction of infarct size following a subsequently induced

myocardial ischemia-reperfusion (I/R) injury. A few years later, Shizukuda et al. (18) were able to demonstrate similar protective effects of hypoxia pre-treatment and ischemic preconditioning and introduced the term hypoxic preconditioning. Both the perfusion of dog hearts with severely hypoxic blood and a short pre-insult period of ischemia of 5 min followed by 10 min of reperfusion subsequently resulted in a comparable reduction of the size of an infarct induced by 60 min of coronary artery occlusion (18).

Most of the studies demonstrating IH-induced preconditioning effects in the heart and brain are derived from animal models mimicking OSAS. In a series of studies from our laboratory (19–21), rats were subjected to various paradigms (i.e., pattern and severity of exposure) of acute hypoxia. Compared to normoxia, intermittent moderately severe hypoxia (inspiratory oxygen fraction, $FiO_2 = 10\%$) applied for 4 h improved the tolerance of the myocardium to ischemia and induced delayed preconditioning by reducing infarct size in isolated rat hearts. Conversely, constant moderately severe hypoxia for 4 h had no protective effect while applying a more severe IH ($FiO_2 = 5\%$) enhanced infarct size, clearly demonstrating that the effect of IH on infarct size was dependent on the pattern (intermittent versus continuous) and severity (10 versus 5% FiO_2) of hypoxic exposure (20) (Figure 1). In addition to myocardial protection, similar protective effects of hypoxic preconditioning were also described in the brain, with the discoveries that pre-exposure to hypoxia can prolong anoxic survival by preserving brain metabolism (22), that brain can adapt to anoxia by hypoxic pre-exposure (23), and finally with the description of ischemic preconditioning against ischemic neuronal damage (24). It should be emphasized that most IH exposure used to trigger hypoxic preconditioning induces hypocapnic hypoxemia (because of the hypoxia-induced hyperventilation), while OSAS



induces hypercapnic hypoxemia. Hence, the differences between the deleterious consequences of OSAS described earlier and the protective effects of hypoxic preconditioning might result not only from the severity of the IH stress but also from the differences in CO₂ levels between both conditions.

Most of the studies mentioned above describe a preconditioning phenomenon consisting in acute ischemic or hypoxic exposure (i.e., a single exposure to hypoxia), leading to protection against subsequent ischemic insult for some hours or days only. However, several experimental studies suggest that conditioning strategies using repetitive exposure (several hypoxic exposures over days or weeks) to appropriate doses of hypoxia may induce a prolonged and sustained state of protection.

Hence, as early as 1960, the reported lower incidence of myocardial infarction in people living at high altitude (25) led to animal models describing protective effects of repetitive exposure to IH on the myocardium, more than 10 years (26) before the breakthrough study of Murry et al. on acute ischemic preconditioning (17). The chronic intermittent hypobaric hypoxia model was first designed to reproduce the beneficial effects of adaptation to high altitude. It was characterized by daily exposure to a sustained (4–8 h) period of hypobaric (5000–7000 m) hypoxia followed by a return to normoxic conditions. Intermittence was provided by repetition of this stimulus over several days. Thus, rats exposed to simulated high altitude (5000 m, 8 h/day, 5 days/week, 24–32 days) have reduced I/R-induced myocardial necrosis (27), ventricular arrhythmia (28–30), and apoptosis (31). Overall, chronic intermittent hypobaric hypoxia exposure has consistently been shown to improve cardiac ischemic tolerance, with less adverse effects than chronic continuous hypoxia (32, 33). Most importantly, the myocardial infarct size-limiting effect of chronic intermittent hypobaric hypoxia appears to have a much longer time-span than that obtained with acute preconditioning protocols (34).

Recent studies have been investigating the protective effects of chronic repeated exposure to normobaric hypoxia. Hence, Stowe et al. (35) showed in mice that hypoxic conditioning by repeated hypoxia sequences (2 or 4 h, FiO₂ = 8 or 11%) for 2 weeks protects against transient focal stroke for the following 8 weeks, together with reduced post-ischemic inflammation. A series of repetitive hypoxic conditioning stimuli (2 h, FiO₂ = 8 or 11%) for 12 days can induce neuroprotection in the retina that lasts up to 4 weeks (36). Repetitive conditioning stimuli may therefore be an attractive option to provide prolonged protection.

Other results also suggest that intermittent hypoxic exposure may improve regeneration following an organ insult. Hence, conditioning strategies applied after focal ischemia and reperfusion (i.e., post-conditioning) have been proposed as a protective approach to lessen injury and improve recovery. In dogs, following lethal ischemia of the left anterior descending artery, it has been shown that three cycles of 30 s reperfusion and 30 s occlusion at the start of reperfusion reduced myocardial infarct size by 44% (37). Recovery may not only be enhanced by intervention in the minutes following acute ischemia but also by interventions applied over the days following the insult. For instance, in rats after ischemic brain injury IH exposure for 7 days with moderately reduced inspiratory oxygen fraction (FiO₂ = 12%, 4 h per day)

has been shown to enhance neurogenesis and to preserve spatial learning and memory (38). Hence, while acute preconditioning to protect against a subsequent ischemic insult occurring during the following hours has been originally described and most deeply investigated, alternative conditioning strategies include repetitive conditioning stimuli in order to provide prolonged state of protection or regeneration. Such strategies may not only apply to protection or recovery associated with ischemic insults but also to other cardiovascular, metabolic, and neurological disorders (see below). Hypoxic exposure can be used to trigger conditioning phenomenon when applied at rest but also when combined with exercise (exercise training in hypoxia, see below) or with hyperoxic exposure [intermittent hypoxic–hyperoxic training, e.g., Ref. (39, 40)].

Potential Mechanisms Triggered by Hypoxic Conditioning

As mentioned above, the time-course of protection afforded by hypoxic conditioning can be extended by increasing the duration of the conditioning protocol (34). This could be due to the recruitment of additional protective mechanisms in response to repetitive exposure. In this section, we review the various mechanisms involved in the beneficial effects of both acute and long-term IH exposure in order to propose a potential explanation for this phenomenon.

Whether acute or repeated, hypoxic conditioning appears to be accompanied by substantial changes in gene expression. A crucial mediator of this genomic response is the hypoxia-inducible factor 1 (HIF-1) transcription factor, a key regulator of cellular oxygen homeostasis. HIF-1 is a complex protein composed of two subunits, the cytosolic HIF-1 α (O₂-sensitive) subunit and the nuclear HIF-1 β subunit, which dimerize in the nucleus to form the functional HIF-1 transcription factor and activate gene transcription. In well-oxygenated cells, proline hydroxylation of the HIF-1 α subunit by prolyl-hydroxylase enzymes (PHDs) leads to ubiquitination and proteasomal degradation. In sustained hypoxic conditions, HIF-1 α hydroxylation and subsequent degradation are reduced because of substrate (O₂) limitation, so that HIF-1 α translocates to the nucleus to bind HIF-1 β and activate the transcription of various genes geared to maintain cellular homeostasis under reduced oxygen availability. Upon return to normoxic conditions, cytosolic levels of HIF-1 α rapidly decline and the effects of HIF-1 decrease (41).

The major role of HIF-1 and its target genes in ischemic/hypoxic conditioning is evidenced by the complete loss of ischemia-induced cardioprotection in mice with partial HIF-1 deficiency (42). Similarly, we have shown that prevention of HIF-1 activation abolished the delayed cardio-protection provided by acute IH in the rat (21), and a recent study reported that HIF-1 inhibition prevented the acute IH-induced neuroprotection (43).

Nitric oxide (NO) is known to play a critical role in preconditioning and cytoprotection through its vasodilating effect as well as for its ability to modulate mitochondrial function (44). Others and we have shown that the inducible nitric oxide synthase (iNOS) gene, a HIF-1 target gene, is up-regulated along with HIF-1 activation in response to acute exposure to IH (21, 45) and

that NO is involved in its delayed protective effects (20, 21, 46). In addition to HIF-1, GATA-4, a transcription factor involved in the regulation of apoptosis, has been shown to activate the gene expression of the anti-apoptotic proteins, bcl-2 and bcl-x(L), following acute IH exposure in mice (47). Finally, well-known mediators of classic ischemic preconditioning are also triggered by acute IH exposure. Hence, we have evidenced the role of ATP-dependent potassium (K_{ATP}) channels (20) and of stress-activated kinases such as protein kinase C (PKC), p38 mitogen-activated protein kinase (MAPK), and extracellular signal-regulated kinase (ERK1/2) (19) in the cardio-protection induced by a 4-h exposure to moderately severe ($FiO_2 = 10\%$) IH.

Although the effects of HIF-1 rapidly decline upon re-oxygenation following sustained hypoxia, we have found that IH induces a strong and sustained whole-body activation of HIF-1 that persists after IH exposure. This can be explained by the oxidative stress produced by the repetition of oxygenation-desaturation sequences. Indeed, ROS are potent inducers of HIF-1 by increasing the expression of the HIF-1 α protein and by inhibiting its PHD-dependent degradation (41). Repeated IH exposure could thus result in activation of additional cytoprotective genes that are not or insufficiently expressed upon sustained hypoxia or short-term IH exposure.

Consequently, in addition to involving the iNOS gene (48) and the K_{ATP} channels (49), chronic IH has also been shown to promote the expression of the erythropoietin (EPO) gene (50). EPO is well recognized as a potent protective agent against ischemic injury since its binding to EPO receptors activates numerous protective signaling pathways, such as Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) pathway, the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway, and the MAPK pathway, all known to confer cytoprotection, in particular cardioprotection (51), nephroprotection (52), and neuroprotection (53). A recent study confirms the pivotal role of the JAK2/STAT3 pathway in a cardioprotective chronic IH model. Activation of the pathway by a 4-week exposure to chronic IH was directly linked to improvement of functional recovery upon reperfusion via maintenance of intracellular Ca^{2+} homeostasis and of mitochondrial function (54). The involvement of other EPO-related signaling pathways, such as the PI3K/Akt pathway, has also been confirmed in the infarct size-sparing effects of chronic IH exposure (55, 56). Interestingly, we observed that (PI3K)/Akt pathway was not involved in the cardioprotection afforded by acute IH exposure (19). This is in agreement with the notion that long-term exposure can trigger additional protective mechanisms, particularly through EPO production. In accordance, EPO has been shown to play a pivotal role in the cerebral infarct size reduction provided by chronic IH post-conditioning in mice (57).

Among the other tissue protective genes upregulated by HIF-1 upon long-term hypoxic exposure are genes encoding angiogenic growth factors and cytokines, such as vascular endothelial growth factor (VEGF), angiopoietins, and platelet-derived growth factors (41). In agreement, rats exposed to chronic IH display a 1.5-fold increase in left ventricular capillary, a higher pre-ischemic coronary flow of isolated hearts and an improved recovery after I/R (58). We also found an increased myocardial capillary density and VEGF expression in rats exposed to 35 days of normobaric

IH (59). This increase in angiogenesis might not only contribute to the cytoprotection conferred by repeated hypoxic conditioning but could also explain why the protection lasts longer than that obtained with acute conditioning protocols.

Finally, other important mediators of the cytoprotective effects of chronic IH are heat stress proteins (HSP), in particular the HSP70 family, also regulated by HIF-1 (60). HSP70 are thought to act as molecular chaperones to repair or remove proteins denatured by stresses such as I/R, leading to protection and/or restoration of cell function (61). Thus, hypoxic preconditioning has been shown to reinforce HIF-1-dependent HSP70 signaling with beneficial effects on ischemic renal apoptosis and autophagy (60) as well as occurrence of I/R-induced ventricular arrhythmias (62).

Hypoxic Conditioning in Healthy Subjects

Intermittent hypoxia training is recognized by the sports medicine community as a potentially useful strategy to enhance exercise performance in athletes (8). Living or training under hypoxic conditions may improve exercise performance probably by promoting hematological and muscle adaptations and without eliciting the detrimental effects of chronic hypoxic exposure. Several studies focusing on these types of hypoxic training programs reported promising results especially in terms of performance gain, but some debates remain regarding the usefulness of IH training in athletes (63). Beyond the enhancement of physical performances (8, 64), and on the basis of some established protective preclinical effects of IH, hypoxic conditioning in healthy subjects has been evaluated as a potential useful intervention to improve some physiological functions and risk factors for acute and chronic diseases.

Hematology

As demonstrated following environmental exposure to sustained hypobaric hypoxia (e.g., altitude) (65), moderate IH exposure can also trigger hematological changes. Nine days of hypobaric IH exposure (simulated altitude ranging from 4000 to 5500 m, 3–5 h/day) induced a significant increase in hematocrit, red blood cell count, reticulocytes, and hemoglobin concentration (66). Hellemans (67) showed that normobaric IH sessions (5 min $FiO_2 = 9\text{--}10\%$ alternating with 5 min of normoxia, 60 min per day, twice a day, for 18 days) in 10 elite endurance athletes triggered significant increases in reticulocyte count (30%), hemoglobin (4%), and hematocrit (5%). Hence, while continuous and prolonged exposure to hypoxia triggers significant and sustained hematological effects, IH protocols with substantially lower total hypoxic doses seem to be sufficient to enhance erythropoiesis (68).

Ventilation

Beyond the well-known increase in hypoxic ventilatory response associated with prolonged hypoxic exposure, IH may elicit specific forms of respiratory plasticity. Briefly, respiratory plasticity is defined as a persistent change in the neural pathways and synapses (morphology and/or function) of the nervous system involved in generating respiratory activity, in response to prior experience

(69). In the context of IH, respiratory plasticity is referred to long-term facilitation (LTF) (70). Ten brief bouts (3 min) of isocapnic hypoxia ($\text{FiO}_2 = 8\%$), interspersed with 5 min of normoxia applied during non-rapid eye movement sleep in healthy subjects (71) and snoring individuals (72) reduced upper airway resistance, suggesting LTF of upper airway dilators and more particularly hypoglossal muscle LTF (73). Hence, based on these results suggesting that IH exposure may trigger respiratory plasticity in physiological conditions, translating the use of hypoxic conditioning to pathological conditions seems to be relevant and will be discussed later in this review.

Cardiovascular System

In contrast to the harmful effects of chronic IH upon the cardiovascular system observed in severe OSAS, moderate IH may induce some positive cardiovascular adaptations. Shatilo et al. (74) submitted two groups of healthy 60- to 74-year-old men (14 physically active, 21 sedentary) to normobaric IH (cycles of 5 min hypoxia, $\text{FiO}_2 = 12\%$, followed by 5 min normoxia, repeated four times a day during 10 days). In sedentary subjects only, IH induced a decrease in blood pressure of 7.9 ± 3.1 mmHg and an increase in submaximal exercise capacity (workload at anaerobic threshold + 12.7%), highlighting the positive cardiovascular effects of hypoxic conditioning in healthy older inactive men. Supporting these results, and by combining hypoxic exposure and exercise, Bailey et al. (75) exposed, in a randomized controlled and double blind manner, 34 physically active subjects to either a normoxic ($n = 14$, $\text{FiO}_2 \approx 20.9\%$) or a hypoxic ($n = 18$, $\text{FiO}_2 \approx 16\%$) training (cycling three times per week for 20–30 min at 70–85% of maximum heart rate previously determined either in normoxia or hypoxia, during 4 weeks). Hypoxic exercise training only significantly decreased maximal exercise systolic blood pressure by 10 ± 9 mmHg and increased maximal oxygen uptake by 0.47 ± 0.77 L/min.

Increased arterial stiffness is known to be associated with an increase in the risk of occurrence of cardiovascular events (76). The beneficial aspects of combining hypoxic exposure and exercise training upon arterial stiffness have been studied in women. Sixteen postmenopausal women were randomly allocated either to a hypoxic exercise group ($n = 8$, hypobaric hypoxic chamber, 2000 m, 2 h exposure per session, 4 days per week, during 8 weeks) or a normoxic exercise group ($n = 8$, 2 h exposure per session, 4 days per week, during 8 weeks). Exercise (aquatic exercises) was performed in both groups under normoxic or hypoxic conditions at an intensity of $\approx 50\%$ peak oxygen uptake for 30 min. Mild hypoxic exposure combined with exercise training significantly reduced arterial stiffness in postmenopausal women, while exercise training performed at the same relative intensity under normoxic conditions did not induce any change (77). These results corroborate the work of Vedam et al. (78) showing an NO-mediated reduction in arterial stiffness among healthy adults men exposed to a single hypoxic session ($n = 12$, arterial oxyhemoglobin saturation $\approx 80\%$ during 20 min) compared to subjects evaluated under room-air conditions.

Taken together, these results confirm the potential positive effects of hypoxic conditioning on the cardiovascular function in healthy subjects, including older individuals and females.

Metabolic Status

Preclinical data indicate a potential beneficial impact of moderate IH exposure on blood glucose and cholesterol levels, mitochondrial enzyme activity, glycolysis, and fatty-acid oxidation (79–81). In healthy humans also, some data suggest that IH either at rest or combined with exercise training can improve metabolic status. In a single blind, randomized controlled trial, Haufe et al. (82) have shown in 20 healthy lean subjects that performing endurance training in hypoxia (60 min of treadmill running, three times a week during 4 weeks, $\text{FiO}_2 = 15\%$) compared to normoxia ($\text{FiO}_2 = 21\%$) at the same relative intensity elicit greater reduction in body fat mass as well as in circulating triglyceride and fasting insulin levels. Interestingly, these effects were observed despite of a smaller absolute workload during hypoxic exercise training.

Intermittent hypoxia exposure can also significantly modify appetite regulation. Bailey et al. (83) reported that a 3 h exposure to normobaric hypoxia while performing a 50 min exercise in 12 healthy males caused a suppression in appetite and a reduction in plasma levels of acylated ghrelin concentrations, the hunger-stimulating hormone.

Hypoxic Conditioning for Patients

Based on preclinical data as well as on studies in healthy subjects showing potential positive effects of IH exposure, pilot investigations have been conducted in various pathological conditions to assess the potential clinical application of hypoxic conditioning (Table 1).

Cardiovascular Diseases

Hypertension and Vascular Function

Murine models have demonstrated the antihypertensive effect of hypoxic conditioning as observed in spontaneously hypertensive rats. This effect seems to be mediated, at least in part, by NO metabolism, which may underlie hypoxia-induced prevention of endothelial dysfunction, a well-known major risk for hypertension (100–102). IH has been proposed as a mean to treat hypertension in humans. Serebrovskaya et al. (102) highlight in their review encouraging results reported in the Russian literature with noteworthy improvements in blood pressure (e.g., a decrease of 10–30 mmHg in systolic and 10–15 mmHg in diastolic blood pressure) following exposure to only a few episodes of moderate hypoxia ($\text{FiO}_2 = 10\text{--}14\%$) for a short duration (15 min to 4 h per session) for 10–30 days. In a more recent study, Lyamina et al. (85) exposed 37 young non-overweight men with stage 1 hypertension to 20 sessions of 4 to 10 cycles of 3-min hypoxia ($\text{FiO}_2 = 10\%$) and 3-min room air breathing. A significant decrease in blood pressure was observed following the intervention with values in patients not different anymore from the normotensive control group. Moreover, the reduction in blood pressure persisted for up to 3 months in 28 initially hypertensive patients. Thus, considering the good tolerance profile and apparently harmless aspect of moderate IH conditioning protocols, it could be considered as an interesting and promising adjunct therapeutic strategy to reduce systemic blood pressure.

TABLE 1 | Characteristics of hypoxic conditioning interventions in patients.

Author	Subjects	Conditioning stimulus			
		Duration	Hypoxic intervention	Control intervention	Exercise/rest
CARDIOVASCULAR DISEASES					
Burtscher et al. (84)	16 elderly men (50–70 years old, eight with prior myocardial infarction, and eight without)	3 weeks, 5 sessions a week (15 sessions)	Three to five times 3–5 min hypoxia (FiO ₂ = 10–14%) – 3 min normoxia	Normoxia (3 weeks of exposure, five sessions a week)	Rest
Lyamina et al. (85)	37 young non-overweight men with stage 1 hypertension (32 years old)	20 consecutive days, one session a day	Four to 10 times 3 min hypoxia (FiO ₂ = 10%) – 3 min normoxia	20 normotensive participants (35 years old), no exposure	Rest
NEUROLOGICAL DISEASES					
Trumbower et al. (86)	13 subjects with incomplete SCI, ASIA Score C or D	One single session	15 times 60 or 90 s hypoxia (FiO ₂ = 9%) – 60 s normoxia	Normoxia	Rest
Schega et al. (87)	34 healthy subjects (60–70 years), cognitively preserved	6 weeks, 3 sessions a week (18 sessions)	1 h/session, 10 min hypoxia (FiO ₂ adjusted to reach a SpO ₂ = 90% the first 2 weeks, 85% the third week and 80% the last three weeks) – 5 min normoxia	Normoxia	30 min strength-endurance training after each normoxia/hypoxia session
Hayes et al. (88)	19 subjects with incomplete chronic SCI, ASIA Score C or D	5 consecutive days, one session a day	15 times 90 s hypoxia (FiO ₂ = 9%) – 60 s normoxia	Normoxia	Conditioning at rest or combined with a 30-min over-ground walking training performed 1 h later
Tester et al. (89)	Eight individuals with cervical or thoracic incomplete SCI, ASIA Score ranging from A to D	10 consecutive days, one session a day	Eight times 2 min hypoxia (FiO ₂ = 8%) – 2 min normoxia, controlled end-tidal CO ₂ level (2 mmHg above resting values)	Normoxia in a subset of <i>n</i> = 4 subjects 1 day before and 10 days after conditioning	Rest
VENTILATION/RESPIRATORY DISEASES					
Aboubakr et al. (90)	11 severe OSAS patients	One night session during non-rapid-eye movement sleep before and after 4 weeks of CPAP treatment	10 times 3 min hypoxia (FiO ₂ = 8%) – 5 min normoxia, isocapnia	One-night normoxia, subset of <i>n</i> = 7	Rest
Rowley et al. (91)	13 OSAS patients	One night session during non-rapid-eye movement sleep	10 times 3 min hypoxia (FiO ₂ = 8%) – 5 min normoxia, isocapnia	One-night normoxia, subset of <i>n</i> = 8	Rest
Burtscher et al. (92)	18 eucapnic normoxic mild COPD patients	3 weeks, 5 sessions a week (15 sessions)	3 to 5 times 3–5 min hypoxia (FiO ₂ = 12–15%) – 3 min normoxia	Normoxia	Rest
Haider et al. (93)	18 eucapnic normoxic mild COPD patients	3 weeks, 5 sessions a week (15 sessions)	Three to five times 3–5 min hypoxia (FiO ₂ = 12–15%) – 3 min normoxia	Normoxia Age-matched healthy controls, <i>n</i> = 14	Rest
METABOLIC DISEASES					
Netzer et al. (94)	20 obese subjects	8 weeks, 3 sessions a week (24 sessions)	90 min hypoxia (FiO ₂ = 15%)	Normoxia, sham-exposure	Stepper, treadmill and bicycle ergometer-training combined with conditioning, without any dietary intervention
Wiesner et al. (95)	45 sedentary overweight or obese subjects, non-diabetic or insulin-resistant	4 weeks, 3 sessions a week (12 sessions)	60 min hypoxia (FiO ₂ = 15%)	Normoxia	Treadmill-training combined with conditioning
Mackenzie et al. (96)	Eight type 2 diabetic patients	One single session	60 min hypoxia (FiO ₂ = 15%)	Normoxia	Four conditions for all patients: Rest: hypoxia and normoxia; Cycling: hypoxia and normoxia

(Continued)

TABLE 1 | Continued

Author	Subjects	Conditioning stimulus			
		Duration	Hypoxic intervention	Control intervention	Exercise/rest
Mackenzie et al. (97)	Eight type 2 diabetic patients	One single 60, 40 and 20-min session, combined with exercise	60, 40 and 20 min hypoxia (FiO ₂ = 15%)	No control group	60, 40, and 20 min cycling
Workman and Basset et al. (98)	15 sedentary overweight males	One session. A subset of <i>n</i> = 6 underwent conditioning on 6 consecutive days	3 h hypoxia, targeted SpO ₂ ≈ 80%	3 h normoxia	Rest
Kong et al. (99)	18 young obese subjects	4 weeks, 3 sessions a week, cumulative exposure of 6 h/week	2 h hypoxia (FiO ₂ = 14.5–16.4%)	2 h normoxia	Endurance and strength training combined with a low-calorie intake diet

ASIA, American Spinal Injury Association; COPD, chronic obstructive pulmonary disease; FiO₂, fraction of inspired oxygen; h, hour(s); min, minute(s); Normoxia (FiO₂ = 21%); OSAS, obstructive sleep apnea syndrome; s, second(s); SCI, spinal cord injury; SpO₂, peripheral capillary oxygen saturation.

Acute Myocardial Infarction

In addition to its potential effect on the vascular function and blood pressure, hypoxic conditioning strategies may be useful to improve heart function and prevent the early and delayed deleterious consequences of myocardial infarction. Forty days of hypobaric IH exposure before ligation of the left coronary artery in mice reduced myocardial infarct size and mortality rates (26). The same intervention applied after ligation was also shown to reduce the risk of arrhythmia occurrence (29). Normobaric IH protocols induce the same effects, with reduced cardiac arrhythmias during ischemia and decreased infarct size by 43% following ischemia-reperfusion injury (103). However, in humans, since myocardial infarction is not a predictable event, similar hypoxic preconditioning strategies using IH are actually difficult to implement.

Besides hypoxic conditioning, ischemic conditioning, consisting in brief ischemic episodes, even remote (lower or upper limb intermittent ischemia), can enhance myocardial tolerance to subsequent ischemic or ischemia-reperfusion insults. Based on pre-clinical remote or perioperative ischemic conditioning studies (17, 104), clinical studies have been conducted to evaluate the effect of remote ischemic conditioning (RIC) in patients. In a randomized controlled study, 333 consecutive adult patients with a suspected first acute myocardial infarction were assigned to receive a primary percutaneous coronary intervention with (interventional group, unilateral intermittent upper-arm ischemia, four cycles of 5 min inflation up to 200 mmHg and 5 min deflation of a standard upper-arm pressure cuff) or without (control group) prior RIC during transport to hospital. In addition to the favorable safety profile of RIC, a significant increase in myocardial salvage, an established prognostic factor of mortality (105), has been shown when RIC was applied (106). More recently, and using the same study design and RIC paradigm in patients presenting with a ST-segment elevation myocardial infarction, the group treated with RIC prior to primary percutaneous coronary intervention showed reduced myocardial infarct size, increased myocardial salvage, and reduced myocardial edema (107).

Chronic Coronary Artery Disease

The beneficial role of IH in chronic coronary artery disease has also been considered (32). An augmented coronary collateral

vessel development has been reported in OSAS patients (apnea-hypopnea index >10 events/h) (13) suggesting that some degree of IH may induce positive myocardial adaptations. In a prospective controlled study in 16 elderly men (50–70 years old, eight with prior myocardial infarction and eight without), subjects were assigned randomly and in a double-blind fashion to moderate IH [3 weeks, 15 passive exposure sessions, three to five episodes (3–5 min) of hypoxia (FiO₂ = 10–14%) per session with 3-min normoxic interval] or normoxia (control group, 3 weeks of exposure). An increased peak-oxygen consumption following hypoxic conditioning was shown both in subjects with and without prior myocardial infarction compared to normoxic exposure (84). Furthermore, heart rate, systolic blood pressure, blood lactate concentration, and the rating of perceived exertion during submaximal exercise (cycling at 1 W/kg) were diminished in subjects exposed to IH. Hence, IH exposure may be considered in chronic coronary artery disease as a procedure to improve cardiovascular parameters.

Heart Failure

Preclinical studies in healthy lean mice (108) and in a mice model of heart failure (109) showed an improved cardiac function following 4 weeks of exposure to IH. In patients with heart failure, a syndrome resulting from several cardiac injuries, IH has not been clinically tested yet, but encouraging results regarding RIC are actually available. Kono et al. (110) reported increased coronary flow reserve both in subjects with heart failure (*n* = 10) and in healthy subjects (*n* = 10) following 1 week of upper-limb RIC (4 cycles of 5 min inflation and 5 min deflation of a blood pressure cuff).

While current preclinical and clinical results suggest that IH may be an innovative and non-pharmacological therapeutic option in cardiovascular diseases, additional clinical studies are required in patients with various cardiac conditions to confirm the potential of hypoxic conditioning as a therapeutic tool.

Neurological Diseases

Stroke

The protective effects of hypoxic conditioning are not restricted to the heart and may also apply to the brain. Remarkable clinical

and preclinical data support the ability of hypoxic/ischemic conditioning to enhance brain tolerance to ischemia. In humans, retrospective studies (15, 16) or prospective studies (111) and clinical evidence (112, 113) have reported lower severity and better functional outcome following stroke in patients who have previously experienced spontaneous transient ischemic attacks. Despite some discrepancies (114–116), these findings suggest an endogenous ischemic neuroprotective preconditioning triggered by transient ischemic attacks. As for myocardial infarction, the unpredictable nature of stroke actually restrains the clinical applicability of hypoxic/ischemic preconditioning. Post-conditioning might be a useful alternative paradigm. It refers to the application of the conditioning stimulus after the occurrence of the harmful event in order to treat or prevent its consequences. In rodents, 7 days after transient mild cerebral artery occlusion, exposure to moderate IH for 7 days induced hippocampal neurogenesis, synaptogenesis, increased brain-derived neurotrophic factor expression, and significantly improved functional outcomes regarding spatial learning and memory (117). RIC following acute ischemic stroke has also been studied in preclinical (118) and clinical (119) conditions alone or as an adjunct therapy to conventional fibrinolysis with encouraging results in both cases. Since hypoxic/ischemic conditioning seems to be harmless and relatively easy to implement at bedside, it may represent a promising adjunct therapy in stroke patients.

Spinal Cord Injury

Trumbower et al. (86) exposed, in a randomized controlled single blind crossover trial, 13 incomplete spinal cord injury (SCI) subjects to 15 repeated bouts of IH (60 or 90 s, $FiO_2 = 9\%$), separated by 60 s of normoxic exposure ($FiO_2 = 21\%$). Hypoxic sessions were compared to sessions in which subjects received sham exposure (i.e., room air). Changes in maximum isometric ankle plantar flexor torque generation were significantly increased by $82 \pm 33\%$ immediately after IH exposure and were maintained above baseline for more than 90 min. Increased ankle plantar flexor electromyogram activity was correlated with increased torque ($r^2 = 0.5$, $P < 0.001$). There was no change from baseline following sham experiments. Hayes et al. (88), in a randomized, double blind, placebo-controlled, crossover study, exposed 19 participants with chronic incomplete SCI to 15 repetitions of 90 s hypoxic exposure ($FiO_2 = 9\%$) interspersed by 60 s normoxia or to normoxic exposure ($FiO_2 = 21\%$, placebo) on five consecutive days, alone or combined with 30 min of over-ground walking 1 h later. In this study, IH improved both walking speed and endurance, and the impact of hypoxia exposure was enhanced when combined with walking (88). Beyond motor function enhancement, IH could also promote recovery of respiratory motor function: a recent case study has outlined an improvement in airflow in response to resistive loads applied in a 55-year-old woman with chronic SCI following acute exposure to IH (120). Furthermore, in a controlled trial, eight individuals with either cervical ($n = 6$) or thoracic ($n = 2$) incomplete SCI were exposed to IH [eight 2 min hypoxic bouts ($FiO_2 = 8\%$) interspersed with a 2 min recovery period (room air, $FiO_2 = 21\%$)] for 10 consecutive days, with controlled end-tidal CO_2 level (2 mmHg above resting values) (89). A subset of four participants received

additional sham exposure (room air). After each single IH session, an increase in minute ventilation 30 min after IH exposure was observed, whereas no change was observed following sham exposure to room air. These results occurred within the first 2 days of IH exposure, and persisted throughout the 10 days of exposure to IH. The magnitude of ventilatory LTF remained enhanced 10 days after the intervention in some ($n = 2$) but not all participants.

The effect of hypoxic conditioning in the field of cognitive performances has also been explored in healthy older individuals. In a recent randomized controlled trial, 34 retired healthy subjects (60–70 years), not physically active and cognitively preserved ($MMSE > 27/30$), were assigned either to a normoxic group (control group, $n = 17$, targeted $SpO_2 = 94\text{--}98\%$) or to a hypoxic group ($n = 17$, alternating 10 min hypoxia and 5 min of normoxia, FiO_2 in hypoxia was adjusted to reach a $SpO_2 = 90\%$ the first 2 weeks, 85% the third week, and 80% in the last 3 weeks during hypoxia). Both groups were subjected to 1-h sessions (normoxia/hypoxia), three times a week for 6 weeks. Thirty minutes strength-endurance training followed each normoxic/hypoxic session. Combining IH exposure and exercise training enhanced cognitive performance and quality of life to a greater extent than exercise training alone (87).

On the basis of these clinical findings and according for instance to promising results in animal models of Alzheimer's disease submitted to IH (121), further research is needed to evaluate the effect of IH exposure in neurological conditions and the potential of hypoxic conditioning as a preventive or therapeutic tool for these diseases. As physical activity, by triggering beneficial neurovascular adaptations (122), hypoxic conditioning may be a promising therapeutic strategy to prevent or slow down brain aging.

Respiratory Diseases

Although exposing patients with respiratory illnesses to IH may seem provocative, some data suggest that hypoxic conditioning may also be considered for these patients.

Obstructive Sleep Apnea Syndrome

Considering OSAS patients, a decrease in upper airway resistance following moderate IH protocol [11 subjects with severe OSAS, exposed during non-rapid eye movement to 10 cycles of 3-min hypoxia ($FiO_2 = 8\%$) followed by 5 min of room air] has been shown, indicating a LTF of upper airway dilators (90). The same IH paradigm does not alter upper airway critical closing pressure within the same population (91). This may support the use of IH as an adjunct therapy in OSAS, in combination with other conventional treatments (e.g., continuous positive airway pressure), as suggested by Mateika et al. in their recent review (123). Additional work is, however, required to optimize IH protocols before translating it to therapeutic interventions.

Chronic Obstructive Pulmonary Disease

The effects of repetitive bouts of mild acute IH [15 sessions over 3 weeks, three to five hypoxic bouts ($FiO_2 = 12\text{--}15\%$), each lasting 3–5 min, separated by 3 min normoxic intervals], were evaluated in 18 eucapnic normoxic mild chronic obstructive pulmonary disease (COPD) patients according to a randomized controlled

and double blind study design. Increased total hemoglobin mass, exercise time to anaerobic threshold, and total exercise time were demonstrated (92) as well as improved baroreflex sensitivity up to normal levels and enhanced hypercapnic ventilatory response without changes in hypoxic ventilatory response (93).

Metabolic Diseases

The use of IH to induce weight loss has been suggested since hypoxia can increase energy expenditure (98, 124). Obese subjects submitted to hypobaric hypoxia (1 week at 2650 m) showed weight loss, increased basal metabolic rate, and decreased food intake (125). Combining hypoxic exposure with physical activity has been reported to potentiate weight loss. Netzer et al. (94) randomly exposed 20 obese individuals (mean body mass index = 33.1 kg/m²) to an 8-week training program (three 90 min sessions per week) at 60% of their individual maximal oxygen uptake either in normobaric hypoxia (FiO₂ = 15%) or in normoxia (sham, FiO₂ = 20.1%), without any dietary intervention. Subjects in the hypoxic group lost significantly more weight (1.14 versus 0.03 Kg) than the sham group. Wiesner et al. (95) submitted sedentary, non-diabetic or insulin-resistant, overweight or obese subjects to a treadmill-training, 60 min a day, thrice weekly over a 4-week period at a heart rate corresponding to 65% of their maximum oxygen uptake under normoxia (normoxic group, *n* = 21, FiO₂ = 21%) or normobaric hypoxia (hypoxic group, *n* = 24, FiO₂ = 15%). Both groups showed similar improvement in maximal oxygen uptake, despite a smaller training workload in the hypoxic group. Body composition improved more (i.e., greater reduction in body-fat mass) in the hypoxic group. More recently, Kong et al. (99) randomly assigned 22 young obese subjects (17–25 years, body mass index >27.5 kg/m²) to either a normobaric hypoxic (FiO₂ = 16.4–14.5%, three 2 h sessions per week, cumulative exposure of 6 h/week) or a normoxic (FiO₂ = 21%) training for 4 weeks. Combined with a low-calorie intake diet and a strength-endurance training program, both groups exhibited weight loss, but weight loss was significantly greater in the hypoxic group. In addition, hypoxic exposure improved systolic blood pressure and mean blood pressure. Taken together, these arguments are supportive of IH as an adjunct therapy to enhance weight loss in obese patients. In a recent review, Urdampilleta et al. (126) proposed an IH conditioning protocol targeting both weight loss and aerobic capacity improvement. This protocol would consist in IH exposure three times per week, during 3–6 weeks, with a target FiO₂ of 10–12%, with or without physical training (20–30 min strength-resistance exercises and 30 min high-intensity aerobic exercise).

Based on preclinical data (127), hypoxic conditioning has been tested in type 2 diabetes. Eight type 2 diabetic patients completed four 60-min sessions (interspersed by 7–14 days) of normoxic rest, normoxic exercise, hypoxic rest, and hypoxic exercise (FiO₂ = 14.6 ± 0.4%) (41). Exercise intensity was set at 90% of lactate threshold. Acute hypoxic exposure both at rest and during exercise increased insulin sensitivity (96). In another study from the same group, eight type 2 diabetic males first performed 60 min of hypoxic exercise (FiO₂ = 14.7 ± 0.2%, exercise intensity at 90% of lactate threshold = 49 ± 4 Watts) and then 40- and 20-min exercise sessions at a higher intensity (at 70 ± 9 and 140 ± 12

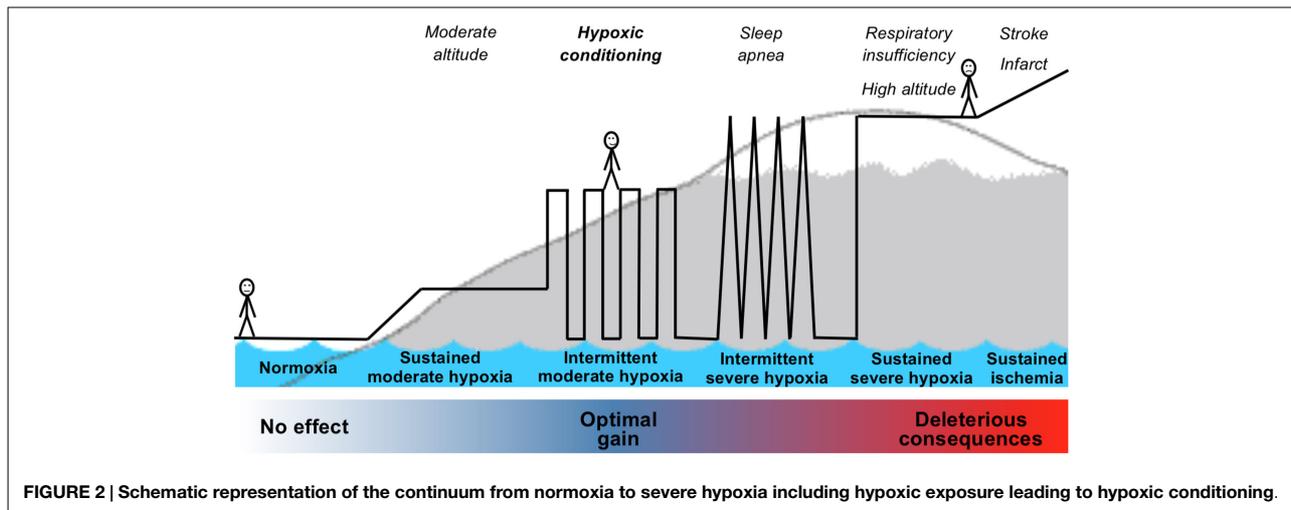
Watts, respectively) to provide similar total workload. This protocol induced an acute- (24 h) and moderate-term (48 h) improvement in insulin sensitivity following the 40- and 60-min hypoxic exercise sessions. This emphasizes the role of exercise duration or intensity (rather than the total workload completed) on glucose control in type 2 diabetics (97). Although encouraging, the beneficial effects of hypoxic conditioning for diabetic patients have nonetheless to be further evaluated by additional clinical studies.

Remaining Questions and Perspectives for Future Researches

Although the bench-based foundation for ischemic and hypoxic conditioning is strong, the translation of conditioning strategies into effective, validated therapies and clinical practice is so far mostly missing. The above results reported in patients, despite frequent important weakness in study design, suggest several promising applications for hypoxic conditioning as a non-pharmacological intervention for prevention or recovery in many pathological conditions. To successfully address the challenge of translating the strong experimental and preclinical data regarding conditioning strategies into potential clinical applications, several important issues have to be considered. Many questions remain regarding the optimal conditioning stimulus, the most favorable clinical setting to apply the conditioning phenomenon, and whether a conditioning response can always be induced in humans who, in contrast to laboratory animals, are elderly and have multiple comorbidities. Since hypoxic conditioning is part of a continuum from normoxia to deleterious severe hypoxia (Figure 2), suitable biomarkers to identify in humans the occurrence of hypoxic conditioning are needed.

Various conditioning stimuli such as exposure to ischemia, hypoxia, metabolic inhibition, or inflammation below the threshold of damage have been tested mostly in animal models and in some cases in humans. Several conditioning strategies involved drug administration and were potentially associated with important limitations in terms of clinical application due to tolerance issues, potential adverse effects, and clinical approval. Conversely, non-pharmacological ischemic or hypoxic conditioning strategies provide reasonable safety/toxicity profiles and appear to be well tolerated in patients. Among the non-pharmacological conditioning strategies, RIC (e.g., limb ischemia to induce organ protection) has been mostly tested in recent large clinical studies. Although hypoxic conditioning and RIC may induce some common mechanisms, these two attractive and relatively easy to implement conditioning strategies have to be compared in order to determine which one should be the most efficient and practical for the clinic.

Although preclinical studies demonstrated the striking ability of preconditioning strategies to protect against ischemic injuries, in most clinical settings demonstration of the therapeutic potential of preconditioning against major insults may not be readily achievable. Stroke as well as cardiac arrest is mostly of unpredictable nature, which makes clinical validation of anticipatory treatment with preconditioning strategy difficult. In addition, most preconditioning models induce short-term protection (i.e., <72 h), while more prolonged state of protection or regeneration are needed for many clinical settings. Hence, clinical validation



and application of new conditioning strategies inducing sustained state of protection and regeneration such as prolonged hypoxic conditioning may be most appropriate (i) to improve risk factors for cardiovascular, metabolic, and neurological diseases in populations not yet presenting acute insults or advanced degenerative diseases (e.g., older individuals), (ii) as an intervention aiming to antagonize and even reverse degenerative processes associated with chronic disorders (e.g., neurological disorders), and (iii) as a regenerative treatment following acute ischemic insult (e.g., following infarct).

The dose response of a conditioning stimuli is thought to range from no response at low intensities to a protected state at higher intensities while a further increase in stimulus intensity would cause tissue damage. The therapeutic range of conditioning may be therefore relatively narrow. Although grouped within the term hypoxic conditioning, the interventions used in the human studies described above vary greatly in terms of hypoxic exposure duration, frequency, and severity (Table 1). Since all stimuli are defined by their frequency, magnitude, and duration, optimal titration of the repetitive stimulus is critical in order to provide proofs of concept and practical guidelines for hypoxic conditioning.

Responses to conditioning stimuli might be specific to sex, dependent on age, and affected by medical comorbidities. Large inter-individual differences exist in the response to identical hypoxic stimulus due to variations in oxygen regulated gene expression. This was shown for VEGF and other downstream genes of HIF-1, suggesting that the source of this variation resides within the HIF system itself (128). There are also epigenetic

differences between individuals as well as environmental and life style related variables that may affect the hypoxic conditioning mechanisms. Titration to define the optimal individual dose of hypoxia for subsequent hypoxic conditioning program may be needed to obtain the largest benefits without adverse effects.

Appropriate titration of the conditioning stimulus will depend on reliable and validated biomarkers that remain to be determined. It is therefore critical to identify suitable biomarkers and most probably arrays of biomarkers to detect whether a conditioning response is actually occurring or conversely whether some deleterious consequences of IH are induced as well as to distinguish responders and non-responders to a given conditioning strategy. Further animal studies are needed to determine such biomarkers specific to hypoxic conditioning, which should subsequently be validated in humans.

There is an increasing number of commercially available devices permitting hypoxic exposure. These devices use technologies to reduce inhaled oxygen concentrations from the normal 21% to below 10%. Exposure can be done by hypoxic breathing through a mask or inside a confined space like tents or rooms while resting, sleeping, or exercising. These products are however mostly designed for athletes and healthy individuals. The use of hypoxic conditioning as a therapeutic strategy in patients would require the use of specific technologies permitting controlled, safe, and inexpensive IH exposure. Although the technologies mostly exist, their integration within validated and certified medical devices represents an important challenge for a large-scale use of hypoxic conditioning as a preventive and treatment strategy in various clinical settings.

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