



Polymorphismes génétiques et perception gustative des lipides alimentaires chez les sujets obèses

Jiri Plesník

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MASARYK UNIVERSITY

FACULTY OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY



AND

UNIVERSITY OF BURGUNDY - FRANCHE-COMTÉ



FACULTE DES SCIENCES DE LA VIE

INSERM UMR 1231



Inserm

Genetic polymorphisms and gustatory perception of dietary lipids in the obese subjects

Ph.D. Disertation

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Genetic polymorphisms and gustatory perception of dietary lipids in the obese subjects

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Titre: Polymorphismes génétiques et perception gustative des lipides alimentaires chez les sujets obèses

Mots clés : CD36, goût gras, obésité, polymorphismes génétiques

Résumé : L'obésité est devenue l'un des problèmes majeurs de santé publique du notre siècle. Un certain nombre de facteurs génétiques et environnementaux contribuent au développement de cette pathologie. L'apport alimentaire quotidien est également l'un des facteurs cruciaux. Une consommation excessive de graisse alimentaire a été jugée essentielle dans le développement de l'obésité. Au cours des deux dernières décennies, plusieurs études ont montré que la détection de graisse orale joue un rôle important dans le développement de l'obésité. Pour prouver une relation entre l'obésité, la perception orale des graisses et les polymorphismes génétiques, nous avons recruté des enfants, des adolescents et des sujets adultes de trois populations différentes. À ce jour, le gène CD36 code le récepteur du goût gras le plus prometteur. De plus, nous avons étudié un lien possible entre le goût gras et le goût amer. Les résultats globaux montrent que les goûts amers et gras sont modifiés chez les personnes obèses. De plus, les polymorphismes CD36 ont une influence significative à la fois sur la perception orale et sur l'obésité. Nos résultats pourraient aider à mieux comprendre le lien entre l'obésité, le goût et les polymorphismes génétiques.

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Abstrakt

Obezita je ve 21. století jedním z nejdůležitějších problémů veřejného zdraví. Mnoho genetických a enviromentálních faktorů přispívá k rozvoji této choroby. Jedním z rozhodujících činitelů je také denní kalorický příjem. Bylo prokázáno, že nadměrný příjem tuků v potravě je kritickým faktorem pro rozvoj obezity. Několik studií v posledních letech ukázalo, že perorální vnímání tuků hraje důležitou roli v patogenezi obezity. Abychom prokázali spojitost mezi obezitou, perorálním vnímáním tuků a genovými polymorfizmy, provedli jsme nábor dětí, adolescentů a dospělých osob ze tří různých populací. V současné době se předpokládá, že gen pro CD36 kóduje nejdůležitější chuťový receptor tuků. Dále jsme studovali možný vztah mezi chutí tuků a hořkou chutí. Souhrnné výsledky ukazují, že je vnímání chuti tuků a hořké chuti pozměněno u obézních osob. Polymorfizmy v genu pro CD36 mají navíc významný vliv jak na perorální vnímání tuků, tak na obezitu. Dosažené výsledky mohou pomoci lépe pochopit vztah mezi obezitou, chutí a genetickými polymorfizmy.

Abstract

Obesity has become one of most important public health issue in this century. A number of genetic and environmental factors contribute to development of this pathology. Daily dietary intake is also one of the crucial factors. Excessive dietary fat intake has been shown to be critical in the development of obesity. In the last two decades, several studies have shown that oral fat sensing plays an important role in the development of obesity. To prove a relationship between obesity, oral fat sensing and genetic polymorphisms, we recruited children, adolescents and adult subjects from three different populations. To date, CD36 gene encodes the most promising fat taste receptor. Furthermore, we studied a possible relationship between fat taste and bitter taste. Overall results show that bitter and fat tastes are altered in obese individuals. Moreover, CD36 polymorphisms have a significant influence in both, oral sensing and obesity. Our results might help better understand a relationship between obesity, taste and genetic polymorphisms.

Table of contents

1. Literature Review	15
1.1 Obesity	15
1.1.1 Introduction	15
1.1.2 History	15
1.1.3 Socioeconomic Aspect	16
1.1.4 Epidemiology.....	16
1.1.4.1 Prevalence.....	16
1.1.4.2 Mortality	17
1.1.5 Obesity Description.....	18
1.1.5.1 BMI	18
1.1.5.2 BMI Z-score	19
1.1.5.3 Waist Circumference.....	20
1.1.5.4 Waist-to-Hip Ratio.....	20
1.1.5.5 Waist-to-Height Ratio	20
1.1.6 Risk Factors	21
1.1.6.1 Physical Activity.....	21
1.1.6.2 Eating Habits	21
1.1.6.3 Socioeconomic Factors.....	21
1.1.6.4 Genetic Factors	22
1.1.6.5 Gut Bacteria	22
1.2 Taste.....	22
1.2.1 Introduction	22
1.2.2 Taste Papillae	23
1.2.3 Taste Buds	23
1.2.4 Taste Cells.....	24
1.2.4.1 Type I Cells	24
1.2.4.2 Type II Cells	24
1.2.4.3 Type III Cells	24
1.2.4.4 Type IV Cells	24
1.2.5 Basic Taste Modalities.....	24
1.2.5.1 Salty.....	25
1.2.5.2 Sour	25

1.2.5.3 Sweet.....	25
1.2.5.4 Umami.....	27
1.2.5.5 Bitter	27
1.2.5.6 Extraoral Taste Receptors	27
1.2.6 Taste Transduction.....	28
1.3 Fat taste.....	29
1.3.1 Introduction	29
1.3.2 Dietary Lipids.....	29
1.3.3 Lipids Chemoreception in Taste Buds Cells.....	29
1.3.3.1 DRK Channels	30
1.3.3.2 GPCRs	30
1.4 CD36	31
1.4.1 CD36 Ligands.....	31
1.4.2 Function	32
1.4.4 Structure	32
1.4.4.1 Post-Translational Modifications	32
1.4.5 CD36 as Taste Receptor	33
1.4.6 CD36 and Obesity.....	34
1.4.6.7 CD36 Genetic Variations	34
2 Aim of the Dissertation	35
3 Material and methods.....	36
3.1 Participants	36
3.1.1. Algerian Population.....	36
3.1.2 Tunisian Population.....	36
3.1.3 Czech Population.....	37
3.2 Anthropometric Parameter.....	37
3.3 Determination of Blood Parameters.....	38
3.3.1 Algerian Adolescents.....	38
3.3.2 Tunisian Population.....	38
3.4 Taste Sensitivity Analysis.....	39
3.4.1 Fat Taste	39
3.4.2 Bitter Taste	40
3.5 Genetic Analysis	40
3.5.1 Polymorphisms Genotyping	40
3.5.2 CD36 Sequencing	42
3.6 Questionnaires	43

3.6.1 Algerian Children.....	43
3.6.2 Czech Adults	43
3.7 Statistical Analysis	44
4. Results	45
4.1 Characteristics of the Participants	45
4.1.1 Algerian Children.....	45
4.1.2 Algerian Adolescents.....	45
4.1.3 Tunisian Cohort Study	46
4.1.4 Tunisian Case-Control Study	47
4.1.5 Czech Cohort Study	48
4.2 Taste Sensitivity.....	49
4.2.1 Fat Taste and Obesity.....	49
4.2.2 PROP.....	53
4.2.3 Cholesterol and Triglyceride Levels after Tasting Session	54
4.3 Genetic Analysis	55
4.3.1 CD36 Polymorphisms	55
4.3.1.1 Rs1761667 and Obesity.....	56
4.3.1.2 Rs1761667 and Fatty Acid Detection Threshold	57
4.3.1.4 Rs1527483 and Corpulence	58
4.3.2 CD36 and Fatty Acid Non-Tasters	60
4.3.3 T2R28 SNPs.....	60
4.4 Nutrition Questionnaires	61
5 Discussion.....	63
6 Conclusion.....	70
7 Bibliography	71
8 Appendices.....	86

List of Abbreviations

AceK	Acesulfame K
AcLDL	Acetylated LDL
AFC	Alternative-forced choice
ALA	Alpha-linolenic acid
ALAT	Alanine aminotransferase
ANOVA	Analysis of variance
ApoA1	Apolipoprotein A1
ApoB	Apolipoprotein B
ASAT	Aspartate transaminase
ATP	Adenosine 5'-triphosphate
BF%	Percentage of body fat
BMI	Body mass index
CD36	Cluster of differentiation 36
CNS	Central nervous system
CRD	Cysteine-rich domain
CRP	C-reactive protein
DAG	Diacylglycerol
DHA	Docosahexaenoic acid
DIO	Diet-induced obese
DRK	Delayed rectifying K ⁺
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ENaC	Epithelial sodium channel
EPA	Eicosapentaenoic acid
FAT	Fatty acid translocase
FCI	Food craving inventory
FFAR1	Free fatty acid receptor
FFAR4	Free fatty acid receptor 4
gDNA	Genomic DNA
GPCR	G protein-coupled receptor
HbA1C	Glycosylated hemoglobin
HCN	Hyperpolarization-activated cyclic nucleotide-gated channels
HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol
HOMA	Homeostasis model assessment
IL-6	Interleukin 6
IP ₃	Inositol trisphosphate
kDa	Kilodalton
LA	Linoleic acid
LCFA	Long-chain fatty acids
LD	Linkage disequilibrium
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol

MAP	Mitogen-activated protein
MCFA	Medium-chain fatty acids
NAFLD	Nonalcoholic fatty liver disease
OA	Oleic acid
OxLDL	Oxidized low density lipoprotein
PCR	Polymerase chain reaction
PIP ₂	Phosphatidylinositol 4,5-bisphosphate
PKD1L3	Polycystic kidney disease 1-like 3
PKD2L1	Polycystic kidney disease 2-like 1
PLCβ2	Phospholipase Cβ2
PROP	6-n-propylthiouracil
PTC	Phenylthiocarbamide
PUFA	Polyunsaturated fatty acids
RFLP	Restriction fragment-length polymorphism
SAP	Shrimp alkaline phosphatase
SD	Standard deviation
SNP	Single nucleotide polymorphisms
T2DM	Type 2 diabetes mellitus
TBC	Taste bud cells
TC	total cholesterol
TD	Transmembrane domain
TG	Triglycerides
TLR4	Toll-like receptor 4
TNF-α	Tumor necrosis factor alpha
TRC	Taste receptor cells
VFTD	Venus flytrap domain
VGCC	Voltage gated Ca ²⁺ channels
VGSC	Voltage-gated sodium channels
VLDL	Very low-density lipoprotein
WC	Waist circumference
WHO	World health organization
WHR	Waist-to-hip ratio
WHtR	Waist-to-height ratio

Preface

The present dissertation describes a possible relationship between "taste of fat" and obesity. The first part covers theoretical background. In the second part, main aims of the dissertation are determined. Parts three and four describe used methods and obtained results. The results are discussed in part six. Appendix contains author's publication related to the dissertation. This dissertation has been prepared under international joint supervision "Cotutelle" between Masaryk University and University of Burgundy - Franche-Comté.

I would like to express my profound gratitude to everybody without whom this work would not have been possible to accomplish. My deepest thanks belong to my supervisors, professor Naim Akhtar Khan and associated professor Omar Šerý, for the continuous support of my Ph.D. study. I am also grateful to all my colleagues from Brno and Dijon. They were not just colleagues but good friends. Finally, I would like to thank to my family and my beloved Agata for the everything they have done.

1. Literature Review

1.1 Obesity

1.1.1 Introduction

Obesity is a worldwide, multifactorial medical condition associated with number of serious health complications. Obesity is predominantly a consequence of an imbalance between energy expenditure and intake which results in accumulation of body fat¹. This medical condition is strongly associated with number of comorbidities, most significantly with type 2 diabetes mellitus (T2DM) where more than 80% of T2DM patients are considered obese or overweight²⁻⁴. Other health complication associated with obesity include metabolic syndrome⁵, dyslipidemia⁶, cardiovascular diseases^{7,8} respiratory complications⁹, otitis media¹⁰, Alzheimer's disease ^{11,12}, and many others.

1.1.2 History

Throughout human history, good energy storage made an important evolutionary advantage during food scarcity. Thus, corpulence was considered as a sign of good health, social status and wealth^{13,14}. For thousands of years, obesity has not been considered harmful. The ancient Greeks were the first who realized the danger of obesity. Greek physician Hippocrates (circa 460 – circa 370 BC) noticed that obesity led to infertility and early death¹⁵. The term “obesity” had not been known in English language until the 17th century, when English physician Tobias Venner (1577–1660) used this word in a medical context for the first time¹⁶. In the 18th century, impact of obesity on human health started to be appreciated^{13,17} but only in the middle of the 19th century obesity started to be considered as a possible cause of health complications¹⁷. First evidences showing increasing mortality caused by obesity were observe in the last century¹⁸. A rapid increase of the incidence of obesity in the second half of the 20th century led the World Health Organization (WHO) to an effort to raise awareness of this disease¹⁹. Nowadays, obesity is considered as one of the major health issues worldwide.

1.1.3 Socioeconomic Aspect

There is a significant economic aspect of obesity. In 2008, the annual cost of obesity was almost \$150 billion in USA alone. This is almost double than in 1998, when was the annual cost estimated at \$74 billion²⁰. The obesity is not just an economic burden, but also a burden for obese individuals. Finkelstein *et al.* showed that adult medical spending attributable to obesity is in obese individuals 41,5% greater than in normal-weight individuals²⁰. Moreover, obese individual face social stigma and potential discrimination which put these individuals at a disadvantage in the labor market²¹.

1.1.4 Epidemiology

1.1.4.1 Prevalence

In the 21st century, obesity has been considered as a worldwide pandemic. In 2013, Ng *et al.* published a vast epidemiological study showed the rapid increase of obesity and overweight worldwide. Between 1980 and 2013, a worldwide percentage of obese and overweight adults rose from 28,8% to 36,9% and from 29,8% to 38,0% in men and women, respectively²². World Health Organization currently indicates, using data from 2014, that the obesity affects globally over 600 million adults. This is more than double since 1980. According to WHO, the obesity is more abundant in women than in men (15% and 11% percent of the world's adult population, respectively). Obesity rate is not a problem only in the adult population. It is estimated that 41 million children under the age of 5 years suffer from overweight or obesity. This problem is most significant in Asia and Africa. In Asia, nearly half of the children under the age of 5 years were overweight and obese in 2014. In Africa, the prevalence of children overweight and obesity almost doubled from 5,4 to 10,6 between 1990 and 2014¹.

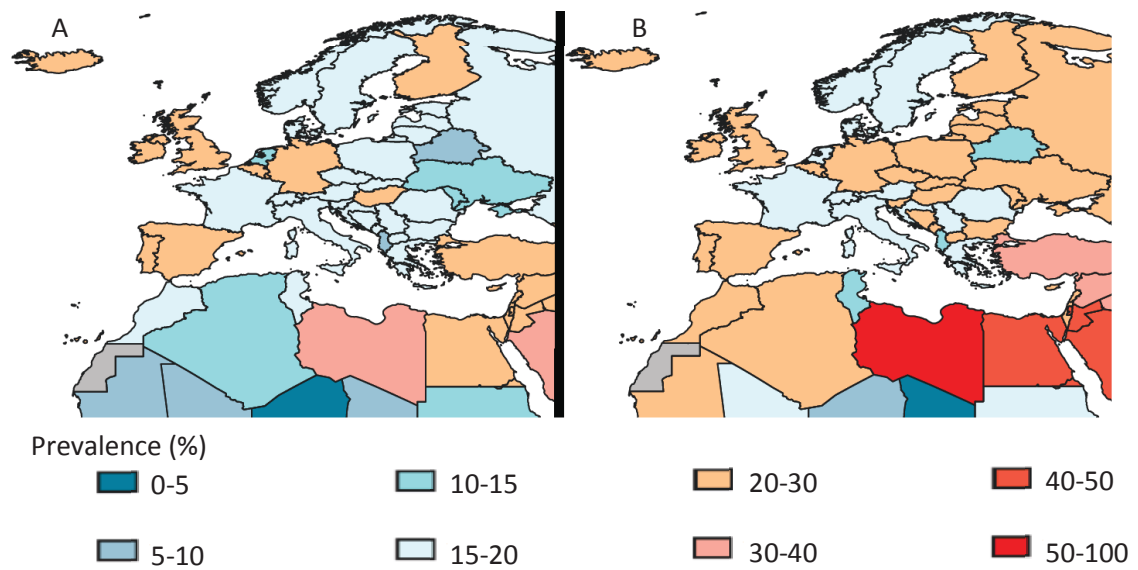


Figure 1: The prevalence of obesity (BMI ≥ 30 kg/m²) in Europe and North Africa, ages ≥ 20 years. The prevalence of obese men (A) is lower than obese women (B) (adapted from²²).

1.1.4.2 Mortality

Obesity greatly increase relative mortality risk²³. Regardless the gender, adult obesity is associated with reduced life expectancy. Individuals who were obese at the age of 40 years died around 6 years earlier than normal-weight individuals²⁴. Diminished life expectancy has been also observed in overweight persons where the overweight persons exhibited 3 years shorter life expectancy than normal-weight persons²⁴. Severely obese subjects die even 8 to 10 years earlier than controls²⁵. Prospective Studies Collaboration analysis showed that every 5 kg/m² increase in BMI was associated with a 30% increase in all-cause mortality²⁵. Recent European multicohort study confirmed this trend showing that excess BMI significantly shortens disease-free life expectancy²⁶.

1.1.5 Obesity Description

1.1.5.1 BMI

To understand and classify obesity, number of somatotypes indicators has been suggested. Out of used indicators, body mass index (BMI) is currently the most commonly used measure for assessing obesity in adults. BMI was developed by Belgian astronomer and statistician Alphonse Quetelet (1795-1844)¹³. Therefore, BMI is also known as “Quetelet Index”. It is calculated as weight in kilograms divided by the square of height in meters¹³. According to the BMI, we can classify whether is a certain person overweight ($\text{BMI} \geq 25 \text{ kg/m}^2$), or obese ($\text{BMI} \geq 30 \text{ kg/m}^2$)²⁷. BMI values are widely used to assess adiposity status in white, African American and Hispanic populations²⁸. This traditional classification is not, however, applicable in Asian populations where subjects with $\text{BMI} \geq 23 \text{ kg/m}^2$ are characterized as overweight and subjects with $\text{BMI} \geq 25 \text{ kg/m}^2$ are characterized as obese²⁹.

Table 1: The International Classification of adult underweight, overweight and obesity according to BMI²⁷.

<i>Classification</i>		<i>BMI (kg/m²)</i>
<i>Underweight</i>		< 18,50
<i>Normal range</i>		18,50 – 24,99
<i>Overweight</i>		25,00 – 29,99
<i>Obese</i>		$\geq 30,00$
	Obese class I	30,00 – 34,99
	Obese class II	35,00 – 39,99
	Obese class III	$\geq 40,00$

Although, BMI is not a perfect tool. It does not distinguish whether is the increase weight caused by the excess of muscles or lipid tissue³⁰. Nevertheless, it remains the most commonly used measure for assessing obesity in adults.

1.1.5.2 BMI Z-score

The standard classification of obesity based on BMI can be, however, use only in adult subjects. BMI values are not related to age and do not distinguish males and females. Thus, assessing obesity in children and adolescents must consider the children's age and gender³¹. The BMI z-score considers individual's gender and age and compares the BMI of the individual with standard BMI of her/his age group³². The resulting value is the standard deviation (SD) from the mean value. According to the SD value, the subject can be consider as severely thin ($SD < -3$), thin ($SD < -2$), normal weight ($SD -2$ to 1), overweight ($SD > 1$ and obese ($SD > 2$)³³.

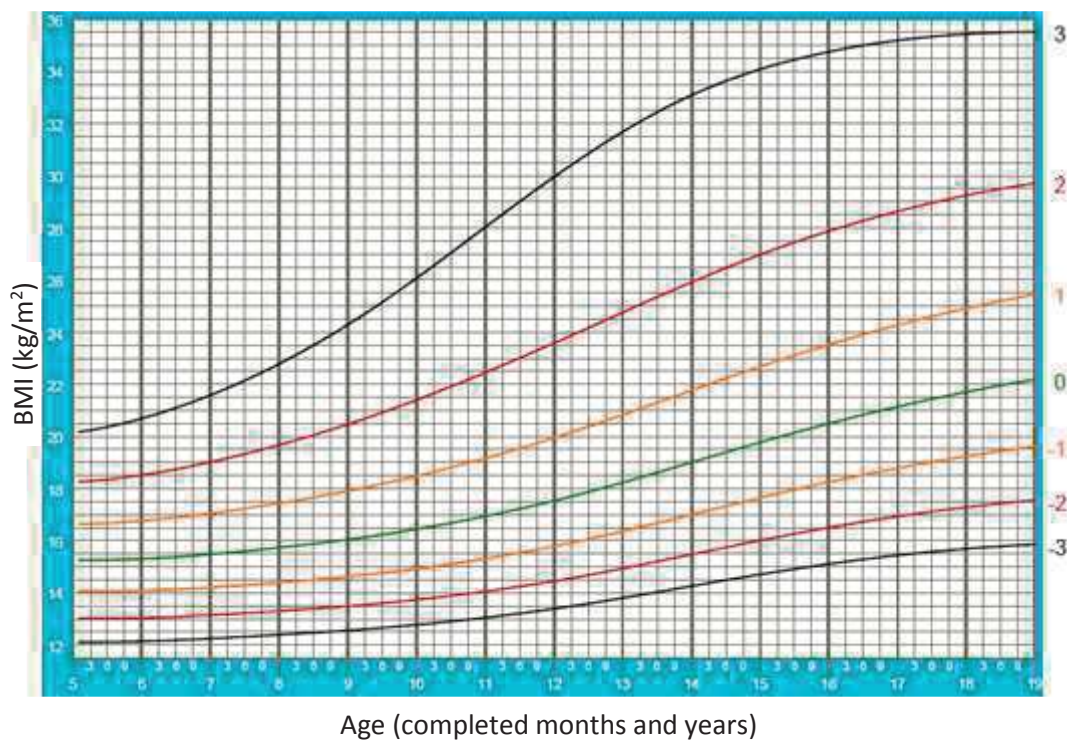


Figure 2: Boys chart according to BMI z-score. The optimal BMI is an area between yellow lines. Boys with BMI z-score > 1 are considered as overweight. BMI z-score > 2 (red line) indicates obese individual (adapted from³³).

Nevertheless, BMI or BMI z-score do not give us any indication of distribution of body fat. Central or abdominal fat increases the risk for metabolic and cardiovascular complications in adolescents, as in adults^{34,35}. Therefore, other adiposity indices, such as waist circumference, waist-to-hip ratio and waist-to-height ratio may be used.

1.1.5.3 Waist Circumference

Waist circumference (WC) is a highly sensitive and specific measure of upper body fat in young people and thus it is valuable for identifying overweight and obese adolescents at risk of developing metabolic complications. It has been shown, that WC is a better predictor for cardiovascular disease in children than BMI³⁶. Also, several other evidences indicate that WC combined with BMI predicts health risk better than does BM^{37,38}. According to the WHO, men and women are considered to have a high WC when the values exceed 102 and 88 cm, respectively³⁹.

1.1.5.4 Waist-to-Hip Ratio

Waist-to-hip ratio is another useful adiposity index. It is the ratio of the waist circumference divided by hip circumference. Previous studies have indicated a positive association between WHR and cardiovascular diseases^{40,41}. We consider men obese with WHR $\geq 0,9$. In women, WHR $\geq 0,85$ indicated obesity³⁹.

1.1.5.5 Waist-to-Height Ratio

Waist-to-height ratio (WHtR) is a dimensionless ratio of the waist circumference and height. Increased WHtR indicates higher risk of obesity-related cardiovascular diseases and correlates with abdominal obesity⁴². Moreover, Ashwell *et al.* showed that WHtR is more reliable tool for detecting cardiometabolic risk factors in both sexes compared to BMI and WC⁴³. For both genders, general cutoff of 0,5 has been suggested⁴⁴. However, more recent cutoff values depending on the risk we evaluate⁴³.

1.1.6 Risk Factors

Obesity is a multifactorial disorder and is often combination of many factors including lack of physical activity, inappropriate eating habits, socioeconomic factors, genetic and hormonal factors, abnormalities in the central nervous system, or psychological disorders. This chapter describes some of these factors.

1.1.6.1 Physical Activity

Sedentary lifestyle is well known risk factor associated with obesity⁴⁵. For this reason, regular physical activity is recommended to prevent weight gain⁴⁶. Sadly, lifestyle in most developed countries does not require the same physical activity that was necessary in the past⁴⁷. We may observe the same trend in children where regular sport activity helps to prevent development of childhood obesity^{48,49}.

1.1.6.2 Eating Habits

The 20th century brought affordable calorically dense food which greatly contributed to the rapid increase of obesity worldwide⁵⁰. Increase content of carbohydrates and fats in food has been associated high high body weight⁵¹. Diet high in vegetables, fruits, whole grains, nuts, and yogurt has the opposite effect⁵¹. In children and adolescents, consumption of sugar-sweetened beverages greatly contributes to obesity as well^{50,52}.

1.1.6.3 Socioeconomic Factors

Socioeconomic status is also one of the major aspects associated with obesity. In the first half of the 20th century, income was the major factor in development of obesity. Obese and overweight people often came from a high income social group⁵³. This causality is, however, no longer valid. In the present, we may observe that the obesity is more prevalent in individuals with low income rather than in those with high income^{54,55}.

1.1.6.4 Genetic Factors

Multifactorial etiology of obesity excludes a possibility of existence of single genetic marker which can determine a development of this medical condition. On the other hand, hereditary factors play an inconsiderable role in its development. Dozens of genes have been suggested to be associated with obesity^{56,57}. To date, the fat mass and obesity-associated (FTO) gene is considered the first unequivocal obesity-related gene⁵⁸. Previous studies repeatedly showed association between FTO variants and type 2 diabetes mellitus and obesity^{59–61}. Despite the advances in genetics, the full understanding of obesity genetics has not yet been reached.

1.1.6.5 Gut Bacteria

It is estimated that human body contains around 10^{14} microorganisms which coexist with the human body in symbiosis⁶². Besides skin, genitourinary and upper respiratory tracts, the lower gastrointestinal tract contains great number of the microorganisms^{63,64}. It has been shown that human microbiome plays an important role in many chronic diseases. Host-microbiome interactions are involved in the pathogenesis of obesity^{65,66}, diabetes, metabolic diseases^{67,68}, and cancer⁶². Furthermore, it has been shown that gut microbiota show altered lipid and carbohydrate metabolisms in obese subjects^{69,70}.

Typical examples of different microbiomes between obese and lean subjects are phyla *Bacteroidetes* and *Firmicutes*. Compared to normal weight subjects, gut microbiome of obese subjects shows higher concentration of *Firmicutes* and lower concentration of *Bacteroidetes*⁷¹. Moreover, high-fat diet induces an increase of *Firmicutes* and a reduction of *Bacteroidetes*.

1.2 Taste

1.2.1 Introduction

As strict heterotrophs, animals must obtain nutrients and energy from external organic molecules. Predicting the nutritional content of food before digestion allows the accurate selection of diet composition⁷². The primary role of taste is determination whether a potential food contains beneficial substances, such as ions for maintaining electrolyte balance and

macronutrients necessary as an energy source, or dangerous toxic molecules that should be avoided⁷³. Tongue is considered as main taste organ. However, tastants can be also perceived by different structures in oral cavity such as soft palate, epiglottis, throat, and larynx⁷⁴. For the long time, it was believed that individual parts of the tongue can detect only to one taste modality. Current finding, however, disproved this “taste map” concept⁷⁵. All the taste modalities are equally perceived on all part of the tongue⁷⁶.

The perception of these basic tastes is mediated by specialized taste receptor cells (TRCs) clustered in taste buds^{77,78}. It is generally accepted that humans can perceived five basic taste modalities: salty, sour, sweet, bitter and *umami*. However, last decades of intensive research of taste frontiers brought number of interesting finding. Previous studies showed that mammals’ TRCs can detect divalent ion, such as calcium⁷⁹, and, most importantly, dietary lipids.

1.2.2 Taste Papillae

Taste papillae are a nipple-like structures responsible for gustation. In mammals, three types of taste papillae, fungiform, circumvallate, and foliate, were identified⁸⁰. The most abundant papillae are fungiform papillae which are mucosal protrusions distributed in the anterior part of the tongue. The foliate papillae can be found in the lateral region. The biggest papillae, circumvallate papillae are positioned in central region of the posterior tongue⁸¹.

1.2.3 Taste Buds

Taste buds are the peripheral gustatory organs located predominantly in the oral epithelium. Most of the taste buds are located on the tongue epithelium, although their presence has been observed in other part of oral cavity such as soft palate, pharynx, and upper esophagus^{81,82}. The molecular recognition of tastants occurs at the apical tips of taste bud cells. At the apical part, specific taste cells are located. Each taste bud consists of 50-100 taste cells⁸³.

1.2.4 Taste Cells

1.2.4.1 Type I Cells

Type I cells are glial-like cells⁸⁴. In their cytoplasm, we can find many electron dense granules⁸⁵. Their role may be also a sensing of sodium⁸⁶.

1.2.4.2 Type II Cells

Type II are spindle shaped cells with short microvilli at the apical region⁸⁵. These cells express taste receptor and are associated with the taste of sweet, bitter and *umami* compounds⁸⁷. They express Phospholipase C β 2 (PLC β 2), a second messenger necessary for taste transduction⁸⁸.

1.2.4.3 Type III Cells

Type III cells are slender shaped and contain a single microvillus that protrudes into the taste pore⁸⁵. They are presynaptic cells associated with the taste of sour compounds⁸⁹.

1.2.4.4 Type IV Cells

Type IV cells (basal cells) are precursor cells. Their only known function is renewal of old taste cells⁹⁰.

1.2.5 Basic Taste Modalities

So far, five basic taste modalities have been generally recognized. Each of the taste modalities has its own specific function. The salty taste indicates the presence of sodium, which is important for the maintenance of the osmotic balance of the body. Sweet and *umami* tastes are perceived with pleasant feelings, which signals the presence of energy-rich nutrients. On the

other hand, the sour and bitter taste modalities are innately aversive and represents potentially harmful molecules such as acids or toxic compounds, respectively⁹¹.

1.2.5.1 Salty

Maintaining a stable concentration of inorganic cations is necessary for proper action potentials, muscle contraction, and numerous other body functions. However, excessive intake of salt, which is around 6 g/day according to the World Health Organization (WHO)⁹², may lead to serious health complications including stroke, gastric cancer, and hypertension^{93,94}. Salty taste is commonly think as the sensation of Na⁺. Other cations such as K⁺ and Li⁺ are also perceive by this taste modality but in lesser intensity than Na⁺⁹⁵. A perception of salty taste leads to both attractive and aversive reactions. The reaction is directly dependent on salt concentration where concentration lower than 100 mM is perceive as pleasant⁹⁵. Detection of salty taste is facilitating by epithelial sodium channel (ENaC)⁹⁶. ENaC is composed of three subunits (α , β and γ)⁹⁷ where the α subunit appears to be essential for sodium recognition^{97,98}.

1.2.5.2 Sour

The presence of sour molecules is detected by membrane ion channel receptor cells, which allow the direct entry of H⁺ ions⁹⁹. The detection of H⁺ ions is facilitated by transient receptor potential channels (PKD2L1 and PKD1L3)^{99,100}, hyperpolarization-activated cyclic nucleotide-gated channels (HCNs)¹⁰¹ and acid sensing ion channels¹⁰².

1.2.5.3 Sweet

In mammals, maintaining of glucose level within a narrow physiological range is necessary for proper physiological functions. Therefore, glucose intake, storage, mobilization, and breakdown are strictly regulated at different levels and multiple mechanisms of glucose sensing coexist⁷². Sugars are often equated with sweet stimuli but sweet tastants include a broad range of structurally distinct molecules generally referred as artificial sweeteners. The sweeteners have

very different structures from the traditional mono- and disaccharides that humans have evolved to detect as an energy source¹⁰³.

Sweet tastants are detected by type II taste receptor cells¹⁰⁴. The detection is mediated by the heterodimer formed of two GPCRs: namely, taste receptor type 1 member 2 (T1R2) and taste receptor type 1 member 3 (T1R3)¹⁰⁵. The T1Rs belong to class C of GPCR and are formed by an extracellular N-terminal domain, that is linked to a seven helical transmembrane domain (TD) at the C terminus via a cysteine-rich domain (CRD)¹⁰⁶, Figure 3). The extracellular N-terminal domain contains Venus flytrap domain (VFTD)¹⁰⁷ which is crucial for taste recognition.

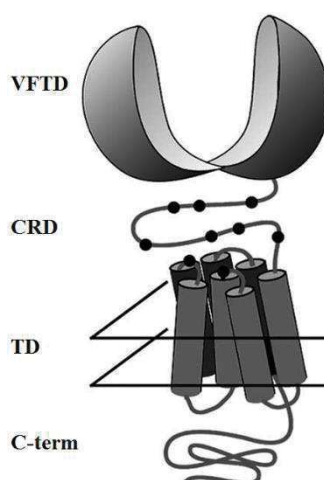


Figure 3: Schematic structure of T1Rs subunits. Each of the T1Rs receptors are constituted of N-terminal extracellular Venus flytrap domain (VFTD) attached to transmembrane domain (TD) via cysteine-rich domain (CRD) (adapted from¹⁰⁶).

Both T1R2 and T1R3 contain multiple binding sites, therefore is this heterodimer activated by various sweet tastants^{105,108,109}. Monosaccharides fructose and glucose and disaccharide sucrose interacts with Venus flytrap domain (VFTD) of the T1R2 and T1R3 subunit^{87,103,110–112}. This domain also contains binding sites for artificial and natural high-potency sweeteners like saccharin, sucralose, acesulfame K (AceK), and stevioside^{108–113}. Another high-potency sweeteners, aspartame and neotame, bind to the VFT domain of the T1R2 domain but not T1R3 domain^{111,114,115}.

1.2.5.4 Umami

In a similar manner to the sweet taste, a receptor for the *umami* taste is a heterodimer composed of two T1R family members (i.e. T1R1 and T1R3)¹⁰⁴. T1R1 and T1R3 receptors are highly affinitive to amino acid L-glutamate, but these receptors also show affinity to other L-amino acids. In contrast, D-amino acids do not serve as ligands¹¹⁶.

In the last decades, several studies reported that T1R3, the T1R subunit that involved in sweet and umami sensation, is also involved in “calcium taste” (i.e. Ca^{2+} oral sensitivity)⁷⁹.

1.2.5.5 Bitter

In direct contrast to sweet and *umami* tastes, the bitter taste is generally thought to indicate the presence of toxic molecules in food. In order to avoid consumption of such molecules, bitter taste evokes aversive reactions⁹¹. This hypothesis is, however, far from perfect. Numbers of bitter tasting molecules are known for their beneficial role for human body such as polyphenols from olive oil¹¹⁷ or red wine¹¹⁸.

Bitter tastants are detected by taste receptor type 2 (T2R) family which belong to GPCR¹⁰⁴. This family of GPCR consist of 25 different subunits^{119,120} in humans. T2Rs subunits can be activated by structurally very different molecules including toxic plant metabolites and synthetic compounds¹²¹. For instance, human TAS2R43 (hTAS2R43) is activated by aristocholic acid, hTAS2R1 is activated by sodium cyclamate, phenylthiocarbamide (PTC) is activated only by hTAS2R38, diphenidol activates 15 different hTAS2Rs¹²¹. In recent year, majority of human TAS2Rs (over 80%) have been deorphaned, which means that these receptors are sensitive to at least one bitter molecule^{121,122}.

1.2.5.6 Extraoral Taste Receptors

It has been shown that taste receptors are expressed in number of extraoral tissues. Sweet taste receptors have been found in pancreas and liver¹²³ or in testis¹²⁴. Furthermore, all three T1Rs taste receptors have been found in bladder¹²⁵ and brain¹²⁶. Some of the bitter taste receptors have been observer in gastrointestinal tract¹²⁷ or in the airways¹²⁸. Extraoral function of these

receptors depends on the tissue. In the airways for instance, the function of these receptors is protection against inhaled toxins and irritants via changing of respiratory function¹²⁹.

1.2.6 Taste Transduction

GPCRs-associated tastes (*i.e.* sweet, *umami* and bitter) share common signaling pathway. After activation of the specific receptor, tastant-binding T1R activates the heterotrimeric G-protein Gustducin. Gustducin activates phospholipase C $\beta 2$ (PLC $\beta 2$) which catalyzes hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂) to inositol trisphosphate (IP₃) and diacylglycerol (DAG). The increase of IP₃ concentration eventually leads to the increase of intracellular Ca²⁺ concentration^{130,131}. The high level of Ca²⁺ depolarizes the taste cell leading to the generation of action potentials via the voltage-gated sodium channels (VGSC) of cells. This action potential leads to the release of adenosine 5'-trisphosphate (ATP) through membrane depolarization-dependent channels which is detected by receptors of the taste axons which transmit information from the taste cells towards central nervous system (CNS)^{131,132}.

In case of sour and salty tastes, signal transduction is more straightforward. The signal is triggered by the ion channel activation induced depolarizations of the taste cells, which elicit action potentials that depolarize the taste cell leading to the generation of action potentials via VGSC¹³².

Depending on the taste papillae, nerve impulse is conveyed to CNS via three different pathways. The pathways are via chorda tympani nerve (fungiform papillae), glossopharyngeal nerve (circumvallate papillae), or chorda tympani and the glossopharyngeal nerve combined (foliate papillae)¹³³. In the CNS, the impulse reach part of gustatory apparatus¹³¹.

1.3 Fat taste

1.3.1 Introduction

Dietary lipids are known to be perceived by olfactory system (smell) and somatosensation (texture, oral irritation). Growing number of evidences pointed out a possible existence of the orosensory system which can detect the dietary lipids by its taste.

1.3.2 Dietary Lipids

Dietary lipids are one of the fundamental part of our diet and play a crucial role in human body. They serve as energy molecules and participate in human development and growth by supplying the essential fatty acids and fat-soluble vitamins (A, D, E, and K)¹³⁴. Dietary lipids deficiency causes many health conditions such as learning ability, growth retardation, and visual impairment¹³⁵. On the other hand, lipids overconsumption may cause other health difficulties such as obesity¹³⁶, diabetes¹³⁷, and cancer¹³⁸.

Out of the many types of lipids, polyunsaturated fatty acids (PUFAs), especially omega-3- fatty acids, are the most important for human health. Omega-3 fatty acids, including alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are known for their anti-inflammatory features. They are presented in the cell membranes, act as lipid mediators and are precursors for many metabolites. Moreover, they are important for fetal development and healthy aging. There are many researches which confirm their positive influence on prevention and treatment of some disorders.

1.3.3 Lipids Chemoreception in Taste Buds Cells

Growing number of studies have shown that dietary lipids are perceived not only by the olfactory and somatosensory systems¹³⁹, but also by its taste. So far, three different types of receptors have been proposed as candidates for the taste of fat. In this chapter, two of them will be briefly described. The last receptor, CD36, will be described in the separate chapter.

1.3.3.1 DRK Channels

Delayed Rectifying K⁺ (DRK) channels are integrated within the apical membrane of lingual taste cells, which allows the flow of K⁺ into the extracellular space. These channels have been found in rat fungiform taste buds¹⁴⁰. Gilbertson *et al.* showed, for the first time, that these channels are inhibited by polyunsaturated fatty acids (PUFAs) in rat TBC¹⁴¹. The inhibition of the channels causes the depolarization of the taste cell which leads to opening of voltage gated Ca²⁺ channels (VGCC) that, eventually, sends signal about the taste to the CNS¹⁴². Out of all candidate DRK channels, KCNA5 (also known as kv1.5) appears to be the most promising one. It is expressed in rat fungiform taste buds and is highly sensitive to fatty acids¹⁴⁰. Another evidence supporting the DRK channel hypothesis is that obesity-prone and obesity-resistant rodents exhibit different expression of DRK channels^{140,143}. Nevertheless, presence of DRK channels in human taste cells have not been observed yet.

1.3.3.2 GPCRs

G protein-coupled receptor (GPCRs) superfamily is involved in many cellular processes including tastants recognition. Three different taste modalities (*i.e.* sweet, *umami*, bitter) are mediated through GPCRs from T1R and T2R families (discussed in chapters 1.2.5.3-5). Therefore, dietary fat oral recognition via GPCRs appears quite promising.

In 2005, Hirasawa *et al.* orphanized GPR120¹⁴⁴ and since that, GPR120 is the most promising candidate. It has been observed that GPR120 (also known as Free Fatty Acid Receptor 4, FFAR4) is activated by several MCFAs (medium-chain fatty acids) and LCFA (long-chain fatty acids)¹⁴⁵. GPR120 is expressed in several cell types including adipose, pancreatic or intestinal cells^{144,146,147}. Rodent studies reported GPR120 expression in all gustatory papillae¹⁴⁸, in type II taste cells in particular¹⁴⁹. In mice, GPR120 knock-out led to diminished preferences linoleic acid and oleic acid. Moreover, the taste nerve response to several fatty acids was reduced comparing to wild-type mice while other taste were not altered¹⁴⁹. In humans, mRNA of GPR120 was found in circumvallate, fungiform papillae and non-gustatory epithelia¹⁵⁰.

GPR40 is also another promising taste receptor. Despite similarity in ligand specificity, human GPR40 shares only 10% amino acid identity with human GPR120¹⁵¹. GPR40 (also called Free Fatty Acid Receptor, FFAR1) is also activated by MCFA and LCFA¹⁵². GPR40 knock-out mice also

exhibit reduced preferences for linoleic and oleic acid compared to wild-type mice¹⁴⁹. In mice, GPR40 is expressed in type I taste cells of foliate papillae and in small number of fungiform papillae¹⁴⁹. However, no expression of this receptor has been found in taste buds in rats¹⁴⁸ and in human¹⁵⁰. GPR40 is, however, expressed in pancreas, liver, skeletal muscle, and many other tissues¹⁵³. Therefore, he may act as extraoral fatty acid receptor.

Besides GPR120 and GPR40, other GPCRs show affinity to fatty acid. GPR84 shows affinity to medium-chain fatty acid (carbon chain length of 9-14) Capric acid (C10:0)¹⁵⁴. Also, GPR41 (FFAR3) and GPR43 (FFAR2) detect short-chain fatty (such as propionate) acids and were found in adipose and immune cells, respectively¹⁵⁵. They detect fatty acids produced by endogenous bacterial flor¹⁵⁶. Their role in oral fat sensing has not been observed.

1.4 CD36

Cluster of Differentiation 36 (CD36), also known as Fatty Acid Translocase (FAT), is a glycoprotein, which belongs to the class B scavenger receptor family¹⁴³. CD36 was originally isolated from membrane of human platelets¹⁵⁷ and to date is known to be expressed in various tissues such as endothelial cells¹⁵⁸, adipocytes¹⁵⁹, cardiomyocytes¹⁶⁰, and many others. Interestingly, expression of CD36 has been observed in TBC¹⁶¹.

1.4.1 CD36 Ligands

CD36 binds various types of molecules including proteins such as thrombospondin¹⁶², collagens type I and IV^{157,163}, apoptotic cells^{164,165}, or erythrocytes infected by *Plasmodium falciparum*¹⁶⁶. For studying of taste and obesity, ability of CD36 to bind a broad spectrum of lipids is the most important. CD36 is known to bind various long-chain fatty acids¹⁵⁹, native lipoproteins HDL, LDL, and VLDL¹⁶⁷, acetylated LDL (AcLDL)¹⁶⁸, oxidized low density lipoprotein (OxLDL)¹⁶⁹ and oxidized phospholipids¹⁷⁰.

1.4.2 Function

CD36 has been shown to have multiple roles as a class B scavenger receptor in a variety of cell types. In vascular cells, CD36 activation by thrombospondin-1 triggers downstream signaling via caspase and MAP (mitogen-activated protein) kinase-dependent pathway with increased apoptosis¹⁷¹. Furthermore, it has been shown that CD36 binds to apoptotic bodies and promotes phagocytosis and clearance¹⁷¹. CD36 is also an important member of pro-inflammatory response. Upon bacterial infection, CD36 binds microbial diacylglycerides and stimulates a pro-inflammatory TNF- α response. Moreover, CD36 acts as a co-receptor to TLR4-TLR6, which amplify the pro-inflammatory signaling in response to OxLDL¹⁷². A growing number of evidences show that CD36 also plays via its lipid-binding ability an important role in obesity. This issue will be addressed in 1.4.6.

1.4.4 Structure

Human *CD36* is located on chromosome 7q21.11^{172,173} and spans around 32 kb. *CD36* contains 15 exons. Exons 1, 2, and 15 are non-coding. Exons 3 and 14 encode the *N*- and *C*-terminal ends of the CD36 protein, respectively¹⁷⁴. Like other receptors of the class B scavenger receptor family, CD36 consists of one large extracellular domain and is docked in the membrane by *N*- and *C*-terminal domains¹⁷². CD36 includes two transmembrane domains spanning residues 7-34 (Exon 3) and 440-466 (Exon14), two short cytoplasmic tails at both the *N*,- and *C*-terminal ends (extending residues 1-6 (Exon3) and 467-472 (Exon 14), respectively), and a large highly glycosylated extracellular domain comprising residues 35-439 (Exon 3-14)^{166,174}. Human^{172,175}, mouse¹⁷² and rat¹⁷⁶ CD36 is composed of 472 amino acids. Mice and rats CD36 share percentage of identity with human CD36 (83% and 86%, respectively)¹⁷⁷. Estimated molecular weight of these sequences is approximately 53 kDa. However, a large number of post-translational modifications causes resulting molecular weight of 88 kDa¹⁷⁸.

1.4.4.1 Post-Translational Modifications

Different post-translation modifications in CD36 have been observed or predicted (Figure 4). Out of these modifications, *N*-linked glycosylation are the most abundant ones. Several

asparagine residues in extracellular loop have been shown to be glycosylated¹⁷⁹. The extracellular loop also contains several acetylated lysine residues¹⁸⁰, three disulfide bridges¹⁸¹, and three phosphorylations at Thr-92¹⁶³, Tyr-62 and Thr-323¹⁸⁰. Each of the two intracellular domains contain two palmitoylation (*N*-terminal: Cys-3 and Cys-7, C-terminal: Cys-464 and Cys-466)¹⁸². The C-terminal is also ubiquitinated at Lys-469 and Lys-472¹⁸³. The role of these glycosylation is essential for trafficking of CD36 to the cell membrane but not for ligand recognition¹⁷⁹. Smith *et. al* observed that the C-terminal ubiquitination is upregulated by LCFA and inhibited by insulin¹⁸³.

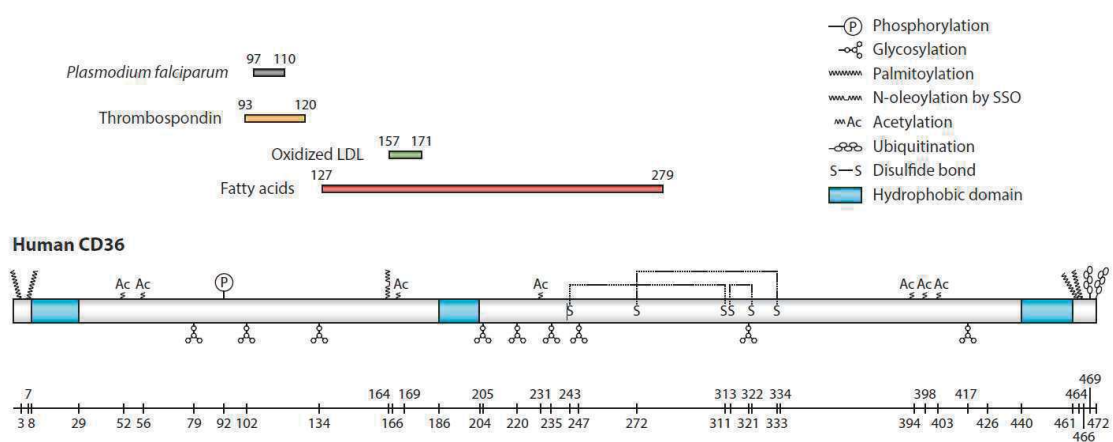


Figure 4: Schematic visualization of CD36 binding domain with predicted post-translational modifications and predicted binding sites for specific ligands (adapted from¹⁸⁴).

1.4.5 CD36 as Taste Receptor

Expression of CD36 have observed in mice¹⁸⁵ and rats¹⁸⁶ TBC. In mice, CD36 is predominantly localized at the apical side of TBC lining the taste pore. CD36 is predominantly expressed in mice circumvallate papillae and in lesser extent in foliate and fungiform papillae¹⁸⁵. In human, the presence of CD36 has been documented in foliate and circumvallate papillae^{161,187,188}. In the TBC, CD36 is coexpressed with α -gustducin¹⁸⁵ which indicates that that CD36 may be expressed in type II taste cells. Another evidence was brought using CD36 knock-out mice. During double-choice preference tests, knock-out of CD36 let to complete suppression of spontaneous

preference for lipid solutions in mice^{185,189}. Likewise, CD36 inactivation led to impaired preference of LCFAs without any change in sweet and bitter taste in rats¹⁸⁵.

1.4.6 CD36 and Obesity

Relationship between obesity and peripheral taste system has been shown both in rodents and humans. In rodents, obese rats and mice appeared to be less sensitive to oil in diet compared to lean controls. This means that spontaneous preference for dietary fat appears in higher concentration in obese rodents than in controls¹⁹⁰. This difference is also apparent in cellular response. Ca²⁺ response to fatty acids was significantly diminished in obese mice compared to lean mice. One of the explanations is that CD36 density in the lipid rafts of TBC is significantly reduced obese mice. This may also explain why the obese mice exhibited impaired detection of low concentrations of lipids properly during mentioned behavioral tests¹⁶¹.

1.4.6.7 CD36 Genetic Variations

Several *CD36* polymorphisms have been studied for their possible association with obesity and obesity-related diseases. Liu *et al.*⁸⁰ showed that there are at least 11 polymorphisms associated with type II diabetes mellitus¹⁹¹, metabolic syndrome¹⁹², or reduced fat oxidation rate¹⁹³ in various populations. Also, some of the *CD36* SNPs have been showed to be associated with obesity or BMI in European^{194,195} and African-American¹⁹⁶ populations. Nevertheless, data from European meta-analysis showed that there is no clear relationship between *CD36* polymorphisms and obesity¹⁹⁷.

So far, two *CD36* SNPs have been related to lipid taste perception. Pepino *et al.* showed, for the first time, that A-allele of *CD36* SNP rs1761667 is related to lipid taste perception¹⁹⁸. Follow-up studies have found similar results in African-American¹⁹⁹ Italian²⁰⁰ populations. Besides rs1761667, T-allele of *CD36* SNP rs1527483 was associated with different perceived ratings of fat content in food in African-American¹⁹⁹ and Malaysian²⁰¹ populations.

2 Aim of the Dissertation

The overall aim of the dissertation was to observe a relationship between orosensory fatty acid perception and obesity or selected adiposity parameters in children, adolescents and adults from different populations. Furthermore, we analyzed the most promising *CD36* genetic polymorphisms. We aim to assess how can be the polymorphisms in this lipid taste receptors related to orosensory detection threshold and obesity. Secondly, we assessed to bitter tastes oral sensitivity to observe a possible cross-talk between the two taste modalities. The last aim of the dissertation was an assessment of dietary habits and preferences of studied individuals.

3 Material and methods

3.1 Participants

The studies were carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association.

3.1.1. Algerian Population

We conducted two case-control studies on Algerian population. The first study group was composed of young school children. The children were between 7 and 8 years old. The second study group was composed of Algerian adolescents. The both study groups were composed of boys and girls and we recruited them from Constantine district in Algeria.

The participants with any history of a chronic condition such as cardiovascular disease, diabetes, liver or kidney disease were excluded from the study. A written consent was obtained from parents of all participants. The probands and their parents were assured about the confidentiality of the project and informed about the purpose, protocol and potential risks of the study. All personal data, such as names and dates of birth, were erased from the database. The study protocol was approved by the research council of the University of Constantine¹.

3.1.2 Tunisian Population

In Tunisian population, we conducted one cohort study and one case-control study on adult subjects. The cohort study group included obese Tunisian women recruited from the group of patients who visited the gynecology outpatient department of Farhat Hached University Hospital in Sousse, Tunisia. The women were between 38 and 43 years old. The case-control study included normal-weight and obese Tunisian adult, both male and female, age around 35 years. The subjects were recruited from the outdoor patient department of National Institute of Nutrition (Tunis, Tunisia), National School of Veterinary Medicine (Tunis, Tunisia) and Regional Hospital of Mateur (Tunis, Tunisia).

Medical records were screened by specialist clinicians. The exclusion criteria were smoking, diabetes, breastfeeding, pregnancy-related complications, chronic illness such as hypertension or any other inflammatory pathology, any autoimmune disease, any lipid-lowering medication,

recent weight loss, dieting and the use of any medications known to affect taste (such as birth control pills). All the included participants had normal glucose tolerance test and electrocardiogram. Moreover, the women from the cohort study could not have any history of gestational diabetes.

The studies were approved by Farhat Hached Hospital Committee for Research on Human Subjects (Tunisia) and the Research Council of National Institute of Nutrition (Tunis, Tunisia, respectively. Informed written consent was obtained from all the subjects. The present experimental protocol conforms to the relevant ethical guidelines for human research.

3.1.3 Czech Population

We recruited young adult with various BMI from South Moravian Region, Czech Republic. The group was formed of male and female Caucasians. The subjects agreed with all procedures and signed a written informed consent form. The study was approved by The Ethical Committee of Masaryk University in Brno, Czech Republic.

Exclusion criteria were history of medical conditions such as hypertension or diabetes, significant weight change in past three months, any medications known to affect appetite, body weight and taste, regular smoking (more than one pack per week), pregnancy and lactation. The subjects did not follow any caloric or dietary restriction diet (such as veganism or gluten-free diet) and were informed about the aim of the study protocol and potential risks of the study.

3.2 Anthropometric Parameter

We measured following anthropometric parameters: weight and height (all study groups), waist circumference (Algerian children and Czech adults), hip circumference (Czech adults). The parameters were measured without shoes and in light clothing or unclothed. Waist and hips circumferences were measured in standing position on the narrowest diameter between xiphoid process and the iliac crest and at the widest part over the greater trochanters, respectively.

From weight and height, BMI ($\text{weight}/\text{height}^2$) was calculated. Due to the young age of the Algerian participants, BMI z-score was used. In the Czech study, where waist and hip circumferences were obtained, we determined waist-to-hip-ratio ($\text{WHR} = \text{waist}$

circumference/hips circumference) and waist-to height ratio (WHtR = waist circumference/height).

3.3 Determination of Blood Parameters

3.3.1 Algerian Adolescents

From the Algerian adolescents, fasting venous blood was collected in heparinized tubes. The serum and the plasma were isolated by centrifugation (1 000 g, 20 min). The concentrations of fasting glucose, total cholesterol (TC), and triglycerides (TG) were measured by Biochemical analyzer XL 200 (ErbaLachema, Mannheim, Germany). Low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels were determined by cholesterol oxidase method (BioSystems, Barcelona, Spain). Insulin concentrations were measured by ELISA (RayBio, Norcross, GA, USA).

3.3.2 Tunisian Population

We collected fasting blood in the both Tunisian studies. We collected serum and plasma (using EDTA treated tubes). The serum and the plasma were prepared by centrifugation (1 000 g, 20 min). Serum was aliquoted and frozen at -80 °C and was immediately used for glucose determination. We determined these blood parameters: fasting plasma glucose concentration, concentration of plasma glycosylated hemoglobin (HbA1C), serum insulin concentration, TC, TG, free cholesterol (in Tunisian cohort study only), LDL-C, and HDL-C. Other biochemical parameters, such as urea, creatinine and C-reactive protein were analyzed by routine standard techniques using an automated Synchron CX7 Clinical System (Beckman Coulter, Brea, CA, USA). In Tunisian obese women, we determined IL-6 and TNF- α serum concentration. Moreover, we measured concentration of ALAT (alanine aminotransferase), AST (aspartate transaminase) in the Tunisian case-control study.

To observe a difference in some of the determined blood parameters (*i.e.* TC, TG and insulin), we collected the venous blood samples again 1 h after the LA tasting session in Tunisian case-control study

3.4 Taste Sensitivity Analysis

3.4.1 Fat Taste

Taste sensitivity to fatty acids was determined using the alternative-forced choice (AFC) method^{202,203}. For this method, ascending concentration of fatty acid (*e.i.* 0,018, 0,18, 0,37, 0,75, 1,5, 3, 6 and 12 mmol/L) was mixed with solution containing Arabic gum and EDTA (both from Sigma Aldrich, St. Louis, MO, USA). The Arabic gum was used to minimize textural cues of the different concentrations of the fatty acid. EDTA was used to prevent oxidation of the fatty acid. The samples were homogenized using sonication for 4–5 min at 4 °C in an ice bath, stored at 4°C in the absence of light and used within 48 hours. Control samples were prepared without the addition of the fatty acid in the same manner. Oleic acid (Tunisian cohort study, Algerian case-control studies) and linoleic acid (Tunisian case-control study, Czech cohort study) were chosen for the tests. Concentration of EDTA was 0,01% (w/v) and concentration of Arabic gum was either 0,01 % (Tunisian and Algerian studies) or 5 % (Czech population).

In the day before the test, the participants were asked to avoid smoking and consumption of alcoholic beverages and hot spicy meals (such as chilly, garlic or horseradish). The participants were called on a stipulated date and advised to arrive early in the morning without having eaten breakfast (fasting state) or to fast 2 hours prior to their test. Also, consumption of sweetened beverages was not allowed 2 hours before the test, the participants could drink only non-sparkling water. One hour before testing, the subjects were asked to refrain from eating, drinking or consuming oral irritants (gum, mouthwash).

During the test, the subjects were presented with three samples per set, two control samples (without the fatty acid) and one “odd” sample containing the fatty acid in ascending order of concentration from the lowest (0,018 mmol/L) to the highest (12 mmol/L). The samples were served at the room temperature. The participants kept each sample in their mouths and they were not allowed to drink the solution, rather they had to spit out it after several seconds. To mask olfactory cues, participants wore a nose clip. Visual cues were masked using a blindfold or the testing session was conducted under red light.

After each set, the subjects were asked to identify the odd sample which differed from the other two. If they identify the sample correctly, they were presented with three more samples at the same fatty acid concentration. If they failed, they were presented with another set with higher fatty acid concentration.

In the Tunisian and Algerian studies, we used an approach described by Pepino *et al.*¹⁹⁸. In this approach, we decreased the concentration of the fatty acid after two correct responses in the row and increased the concentration after each incorrect response. The procedure was terminated when four changes of direction (reversals) occurred and they met the following two criteria. First, there could not be more than two dilution steps between two successive reversals. Second, the series of reversals could not form an ascending pattern. The threshold concentration was calculated as mean of log values for the last four reversals.

In Czech population study, approach used by Steward *et al.*²⁰² was used. The test continued until the subject identified the odd sample at a given concentration three consecutive times. If the subject failed to identify the fatty acid at the given concentrations, he was classified as “non-tasters”.

3.4.2 Bitter Taste

In the Tunisian case-control study, we invited lipid-tasters to participate to the additional session regarding the bitter taste. As bitter tastant, 6-n-propylthiouracil (Sigma Aldrich, St. Louis, MO, USA) dissolved in deionized water was used. We used method performed by Bartoshuk *et al.*²⁰⁴. However, we did not correlate the PROP arbitrary perceived responses to NaCl-evoked intensity ratings as it seemed to us very subjective. Hence, we measured the detection thresholds for PROP (0,0001 - 3,2 $\mu\text{mol/L}$) as using the similar procedure as used in the linoleic acid test.

3.5 Genetic Analysis

3.5.1 Polymorphisms Genotyping

Genomic DNA (gDNA) was extracted from venous blood or flocced saliva swabs. We analyzed three polymorphisms in *CD36* gene. In the Algerian and Tunisian studies, rs1761667 polymorphism was analyzed. In the Czech cohort study, rs1527483 and rs3212018 polymorphisms were analyzed. Furthermore, we analyzed 2 SNPs of T2R38 (*i.e.* rs1726866 rs10246939) in Tunisian case-control study. The selected SNPs were analyzed using restriction fragment-length polymorphism (RFLP) method followed by agarose gel electrophoresis. Analysis of insertion/deletion polymorphism rs3212018 was analyzed using the similar method without

the endonuclease digestion step. Sequences of used primers are shown in Table 2. Amplification was initiated by denaturation (3 min at 95°C). After the denaturation, DNA was subjected to further amplification (denaturation for 30 s at 95°C, annealing for 30 s at different temperatures and extension for 30 s at 72°C). The amplification programs were identical for all the polymorphisms except annealing temperatures. The annealing temperatures are shown in Table 1. After 35 cycles, the PCR terminated by final extension for 5 min at 72°C.

Table 2: The list of analyzed polymorphisms, their sequences and annealing temperatures.

SNP	Gene	Variation	Primer sequence		Annealing temp.
rs1761667	CD36	A/G	F	5' CAAAATCACAATCTATTCAAGACCA 3'	56°C
			R	5' TTTTGGGAGAAATTCTGAAGA G 3'	
rs1527483	CD36	C/T	F	5' GCCAATTAGAATCACTTCATAAACC 3'	56°C
			R	5' TGATGGATTAAACCCAAATGAA 3'	
rs3212018	CD36	16 bp del.	F	5' TCTGGGAGAAATGAGATAAAAGATG 3'	56°C
			R	5' GCAGCAATCCTGGTCTTATG 3'	
rs1726866	TAS2R38	Ala262Val	F	5' GGAAGGCACATGAGGACAAT 3'	62°C
			R	5' ATGCCTGAGATCAGGATGG 3'	
rs10246939	TAS2R38	Val296Ile	F	5' TGTTCCTTCATCTCTGTGC 3'	62°C
			R	5' TGTGGTCGGCTCTTACCTTC 3'	

After the PCR, the amplicons were digested by specific restriction enzyme (Thermo Fisher Scientific, Waltham, MA, USA). Each restriction enzyme was chosen to be allele-specific. Used enzymes, their incubation temperatures and products of the digestions are displayed in Table 3.

The products of the digestion were separated agarose gel electrophoresis 2% (w/v) stained by ethidium bromide. The products were visualized under UV light.

Table 3: The list of used restriction enzymes, their incubation temperatures and the size of the restriction products.

SNP	Enzyme	Temp. (°C)	Allele	Product length (bp)
rs1761667	<i>HhaI</i>	37	A	190
			G	138, 52
rs1527483	<i>TaqI</i>	65	C	37, 70, 129
			T	107, 129
rs3212018	-	-	Ins	182
			Del	166
rs1726866	<i>BseXI</i>	65	Ala	122, 75, 19
			Val	197, 19
rs10246939	<i>FokI</i>	37	Val	194
			Ile	107, 87

3.5.2 CD36 Sequencing

In the Czech cohort study, we selected four ($n = 4$) subjects without an ability to detect linoleic acid (non-tasters) and the same number of subjects with the lowest linoleic acid detection threshold (supertaster). We sequenced exons 5 and 6, which encode hydrophobic pocket, a part of extracellular domain of CD36 which is responsible for fatty acids binding¹⁸⁰. The selected region included amino acids 95 – 143 (exon 5) and 144 – 203 (exon 6).

The selected exons were amplified using standard PCR. Primers for exon 5 span the exon and 31 bp and 110 bp before and a behind the exon, respectively. Primers for exon 6 span the exon and 122 bp and 170 bp before and a behind the exon, respectively. Sequences of used primers and temperatures profile are shown in Table 4.

The amplicons were purified using Shrimp Alkaline Phosphatase (SAP) and Exonuclease I (Thermo Fisher Scientific, USA). The mixture was incubated at 37 °C for 15 min and 85 °C for 15 min (enzyme inactivation), followed by sequencing with BigDye® Terminator v.3.1 (Thermo Fisher Scientific, USA). As sequencing primers, the forward PCR primers were used. The amplicons were then purified using EDTA/ethanol precipitation, re-suspended in 10 µl of Hi-Di

Formamide (Thermo Fisher Scientific, USA), and sequenced using automated ABI 3130 Genetic Analyzer (Thermo Fisher Scientific, USA). The resulting sequences were analyzed in MEGA 7 software²⁰⁵ and NC_000007.14 sequence from Nucleotide NCBI database was used for alignment.

Table 4: Primes sequences and a temperature profile used for sequencing of *CD36* exons 5 and 6.

Exon	Primers	Temperature profile				Product length (bp)
5	5' AAATGTTTTGAATTTGTTTACTGCT 3'	40 x 94 °C/54 °C/72 °C				296 bp
	5' CGTTTTGATAAAAATGGAAAAACA 3'	94°C	°C	72°C	10°C	
6	5' TGGCAGGATCTGGCAGTAA 3'	3 min	30 s/60 s/30 s	5 min	∞	492 bp
	5' TTCCCAACTAGGAAAGCTGAA 3'					

3.6 Questionnaires

3.6.1 Algerian Children

Using simple questionnaire, we collected the information about habitual preferred eating patterns in the Algerian children. The questionnaire was composed of the food products which are usually consumed in Algeria and served at breakfast, lunch and dinner. We asked the question “what do you eat preferably in the week among the listed food items” and we noted responses. In fact, we determined “preferred food pattern” of the children and tried to correlate the same. The food pattern was also, sometimes, cross-checked with the mothers.

3.6.2 Czech Adults

In the Czech cohort study, a modified version of the validated self-administered questionnaire, Food Craving Inventory²⁰⁶ was used. This questionnaire contained 37 items is designed to measure the frequency of cravings for specific food. These 37 items were divided into four categories: high fats, sweets, carbohydrates/starches and fast-food fats. The participants had to answer, on a scale from 1 “never” to 5 “always / almost every day”, how often did they crave

each item in the last month. The present study was focused only on dietary lipids. Therefore, we used only the food items which were categorized as “high fats” and “fast-food fats”. The food items from these categories are commonly consumed in both American and Czech diet, so we removed only one item from “high fats” group. Hence, we removed item “corn bread” which is very uncommon in Czech diet. We did not include all 37 items in the questionnaire, thus could not perform the same statistical analysis mentioned by White *et al.*²⁰⁶. We calculated the mean craving for each food group (*i.e.* high fats and fast-food fats) and for both the groups together.

The participants were also asked whether they consumed alcoholic beverages and what was their average weekly consumption. Based on their answers, average alcohol consumption (in units per week) was calculated.

3.7 Statistical Analysis

For statistical analysis, Statistica software (Statsoft, Tulsa, OK, USA) was used. P value $\leq 0,05$ was considered statistically significant. The data are displayed as mean \pm SD. In the performed case-control studies, we divided the subjects into two groups (*i.e.* obese and controls). The decisive criterion was BMI or BMI z-score (in children and adolescents). We considered adults with BMI $\geq 30,0$ kg/m² and children or adolescents with BMI z-score ≥ 2 as obese.

To assess relationships between rs1761667 and measured parameters, we consider each genotype separately. Due to the low minor allele frequency of rs1527483 and rs3212018, we combined the subjects with minor homozygous genotypes and those with heterozygous genotypes (which means TT + CC for rs1527483 and DD + ID for rs3212018). This approach was also used in some calculations regarding rs1761667.

We used following statistical tests: Shapiro-Wilks test to observe data distribution. Student's t -test, Mann-Whitney U test, one-way ANOVA and Kruskal–Wallis ANOVA for determination between study groups. Pearson's correlation coefficient and Spearman rank correlation to observe a correlation between measured parameters. Two tailed Fisher's exact test for comparison the genotype distributions. Chi-square (χ^2) was used to assess the Hardy-Weinberg equilibrium and differences in habitual preferred eating patterns in Algerian children. Dunn's method was used for all multiple comparisons among genotypes.

4. Results

4.1 Characteristics of the Participants

4.1.1 Algerian Children

The study included 116 children. The average age of the children was $8,10 \pm 0,53$ years. Out of them, 59 children were considered as controls (lean) with average BMI z-score = $-0,14 \pm 0,60$ and 57 were consider as obese with average BMI z-score = $2,53 \pm 0,49$ ($p < 0,01$). The study included equal number of boys and girls (57 and 59, respectively). Obese children were more than 3 cm higher than lean controls ($p < 0,01$). The description of the population is displayed in Table 5.

Table 5: Characteristics of the Algerian children.

Parameters	Controls		Obese	
	mean	SD	mean	SD
Age (years)	8,00	$\pm 0,51$	8,20	$\pm 0,51$
BMI z-score	-0,14	$\pm 0,60$	2,53	$\pm 0,49^{**}$
Waist circumference (cm)	55,25	$\pm 2,42$	69,23	$\pm 7,13^{**}$

****** $p < 0,01$ between controls and obese

4.1.2 Algerian Adolescents

Out of total number of adolescents ($n = 165$) included in the study, 83 were considers as obese (females = 39, males = 44) with average BMI z-score = $2,67 \pm 0,29$ and 82 as lean controls (females = 37, males = 45) with average BMI z-score $0,03 \pm 0,00$. There was no significant age difference between obese and lean controls. The average age of the adolescents was $13,90 \pm 1,10$ years. Concentration of fasting glucose was normal in both obese and control subjects, though the obese had significantly higher glycemia than the control individuals ($p < 0,05$). Also, total cholesterol (TC) concentration was within normal range in the both groups, but elevated in obese participants ($p < 0,05$). Compared to obese individuals, lean participants exhibited higher HDL-C concentration ($p < 0,01$). The obese adolescents had significantly higher LDL-C concentration compared to the controls ($p < 0,01$). Levels of triglycerides (TG) and insulin were also significantly higher in the obese group ($p < 0,01$ for both parameters). Moreover, level of

insulin positively correlated with fasting glucose concentration ($p < 0,05$). HOMA index was also higher in obese participants than that in lean ones ($p < 0,01$). As expected, TC and TG, HDL-C and LDL-C levels positively correlated with each other ($p < 0,01$, $p < 0,04$, $p < 0,01$, respectively). Also, TG concentration positively correlated with LDL-C ($p < 0,01$) and negatively with HDL-C levels ($p < 0,01$). Regarding the above-mentioned parameters, no difference between boys and girls was observed. The characteristics of the Algerian adolescents are displayed in Table 6.

Table 6: Characteristics of the Algerian adolescents.

Parameters	Controls		Obese	
	mean	SD	mean	SD
Age (years)	13,92	± 2,08	14,01	± 1,73
BMI z-score	0,03	± 0,59	2,67	± 0,45**
Glycemia (mmol/L)	4,41	± 0,54	4,76	± 0,46*
TC (mmol/L)	3,04	± 0,72	3,39	± 0,64*
LDL-C (mmol/L)	1,64	± 0,63	2,00	± 0,55**
HDL-C (mmol/L)	1,08	± 0,27	0,91	± 0,18**
TG (mmol/L)	0,74	± 0,36	1,04	± 0,46**
Insulin (pmol/L)	45,98	± 6,25	54,38	± 20,23**
HOMA index	1,29	± 0,27	1,70	± 1,09**

* $p < 0,05$ ** $p < 0,01$ between controls and obese. Abbreviations: TC (total cholesterol), LDL-C (low-density lipoprotein cholesterol), HDL-C (high-density lipoprotein cholesterol), TG (triglycerides), HOMA (homeostasis model assessment).

4.1.3 Tunisian Cohort Study

This cohort study included 203 obese Tunisian women with average BMI = $34,6 \pm 4,2$ kg/m². The average age of the women was $38,4 \pm 11,4$ years. The characteristics of the women are shown in Table 7. Compared to control values^{207,208}, serum TG, interleukin 6 (IL-6) and Tumor necrosis factor alpha (TNF- α) concentrations were higher in this sample. Measured levels of fasting glucose, urea, creatinine, cholesterol, TG, HDL-C, LDL-C, glycated hemoglobin (HbA1c), apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), insulin and C-reactive protein (CRP) were within normal range for obese women.

Table 7: Characteristics of the obese Tunisian women included in the cohort study.

Parameters	mean	SD
Age (years)	38,4 ±	11,4
BMI (kg/m ²)	34,6 ±	4,2
TNF-α (pg/ml)	131,4 ±	165,5
IL-6 (pg/ml)	73,3 ±	48,7
Glucose (mmol/l)	5,97 ±	1,84
Urea (mmol/l)	3,39 ±	1,58
Creatinine (mmol/l)	0,39 ±	14,58
Cholesterol (mmol/l)	5,08 ±	1,29
TG (mmol/l)	1,77 ±	1,06
HDL-C (mmol/l)	1,14 ±	0,31
LDL-C (mmol/l)	3,04 ±	1,01
HbA1c (%)	5,6 ±	1,22
ApoA1 (g/l)	2,04 ±	0,74
ApoB (g/l)	1,26 ±	1,13
Insulin (pmol/l)	78,76 ±	67,64
CRP (mg/l)	7,8 ±	7,8

Abbreviations: TNF-α (Tumor necrosis factor alpha), IL-6 (interleukin 6), LDL-C (low-density lipoprotein cholesterol), TG (triglycerides), HDL-C (high-density lipoprotein cholesterol), HbA1c (glycated hemoglobin), ApoA1 (apolipoprotein A1), ApoB (apolipoprotein B), CRP (C-reactive protein).

4.1.4 Tunisian Case-Control Study

We recruited 52 obese ($34,29 \pm 5,31 \text{ kg/m}^2$) and 52 control ($23,22 \pm 1,44 \text{ kg/m}^2$, $p < 0,01$) Tunisian adults. The study included male ($n = 33$) and female ($n = 71$) participants. The characteristics of the obese and control subjects are shown in Table 8.

Both controls and obese exhibited normal blood glucose level, though the obese group exhibited significantly higher glycemia level than the control group ($p < 0,01$). Also, insulin level was elevated in the obese group as compared to the control group ($p < 0,01$). Both transaminase enzymes (*i.e.* ALAT, ASAT) were higher in the obese subjects ($p < 0,01$, $p < 0,05$, respectively). No significant difference was observed in LDL-C and HDL-C levels between the two groups. Likewise, there was no difference in uric acid, creatinine, CRP, and HbA1C values between control and obese subjects.

Table 8: Characteristics of control and obese Tunisian adults included in the Tunisian case-control study.

Parameters	Controls		Obese	
	mean	SD	mean	SD
Age (years)	35,30	± 4,10	35,00	± 5,43
Weight (kg)	67,65	± 8,21	95,24	± 16,32**
Height (m)	1,70	± 0,08	1,67	± 0,09*
BMI (kg/m ²)	23,22	± 1,44	34,29	± 5,31**
Glycemia (mmol/L)	4,65	± 0,76	5,23	± 0,78**
Insulin (pmol/L)	6,52	± 3,93	26,57	± 13,81**
ALAT (U/l)	16,26	± 8,42	22,25	± 11,97**
ASAT (U/l)	19,32	± 4,73	21,77	± 6,64*
LDL-C (mmol/L)	2,64	± 0,64	2,57	± 0,72
HDL-C (mmol/L)	1,25	± 0,41	1,11	± 0,32
Uric acid (μmol/L)	254,59	± 89,28	271,82	± 90,08
Creatinine (μmol/L)	68,27	± 34,67	62,91	± 16,7
CRP (mg/L)	4,89	± 2,35	4,91	± 3,41
HbA1c (%)	5,59	± 0,44	5,74	± 0,58

* $p < 0,05$ ** $p < 0,01$ between controls and obese. Abbreviations: ALAT (Alanine transaminase), ASAT (Aspartate transaminase), LDL-C (low-density lipoprotein cholesterol), TG (triglycerides), HDL-C (high-density lipoprotein cholesterol), CRP (C-reactive protein), HbA1c (glycated hemoglobin).

4.1.5 Czech Cohort Study

Czech cohort study included 116 participants with various BMI from 15,93 kg/m² to 39,52 kg/m². The study included males ($n = 43$) and female ($n = 73$). Table 9 shows characteristics of the Czech population. Out of the subjects, 12 of them were considered as obese (BMI > 30 kg/m², 6 males, 6 females). The average age in the group was $21,84 \pm 0,22$ years. The male group has higher BMI, waist-to-hip-ratio (WHR), waist-to-height ratio (WHtR) and waist circumference (WC) than the female group. As expected, we noticed a strong positive association in all four measured anthropometric parameters ($p < 0,01$).

Table 9: Characteristics of the study Czech adult population.

Parameters	mean	SD
Age (years)	21,84 ±	2,38
BMI (kg/m ²)	23,34 ±	4,28
WC (cm)	76,88 ±	10,05
WHR (cm/cm)	0,79 ±	0,08
WHtR (cm/cm)	0,44 ±	0,05
LA (mmol/L)	1,47 ±	2,75

Abbreviations: WC (waist circumference), WHR (waist-to-hip-ratio), WHtR (waist-to-height ratio), LA (linoleic acid oral detection threshold).

4.2 Taste Sensitivity

4.2.1 Fat Taste and Obesity

We noticed statistically significant difference in oleic acid oral detection threshold between obese and lean subjects in both Algerian case-control studies (Figure 5). Studied obese children exhibited significantly lower detection threshold ($0,34 \pm 0,93$ mmol/L) than the control children ($2,14 \pm 2,97$ mmol/L, $p < 0,01$). In addition, we reported positive correlation between waist circumference and the detection threshold in these children (Figure 6, $p < 0,01$). We did not find any correlation between BMI z-score and the detection threshold.

The same trend was observed in the Algerian adolescents (Figure 5). The detection threshold in obese adolescents was almost twofold higher ($2,57 \pm 2,95$ mmol/L) than in controls ($1,33 \pm 2,96$ mmol/L, $p < 0,01$). We also observed direct correlation between BMI z-score and the oleic acid detection threshold in this population ($p < 0,01$). Furthermore, if we divide the participants based on oral detection thresholds, into three categories: high tasters (0,018 mmol/L), middle tasters (from 0,18 to 1,5 mmol/L), and low tasters (from 3 to 12 mmol/L), we observe a significant relationship between the corpulence and oleic acid detection threshold ($p < 0,01$). The relationship between oleic acid detection threshold and thee taster categories shows Figure 7. No difference in oral detection threshold between genders was observed.

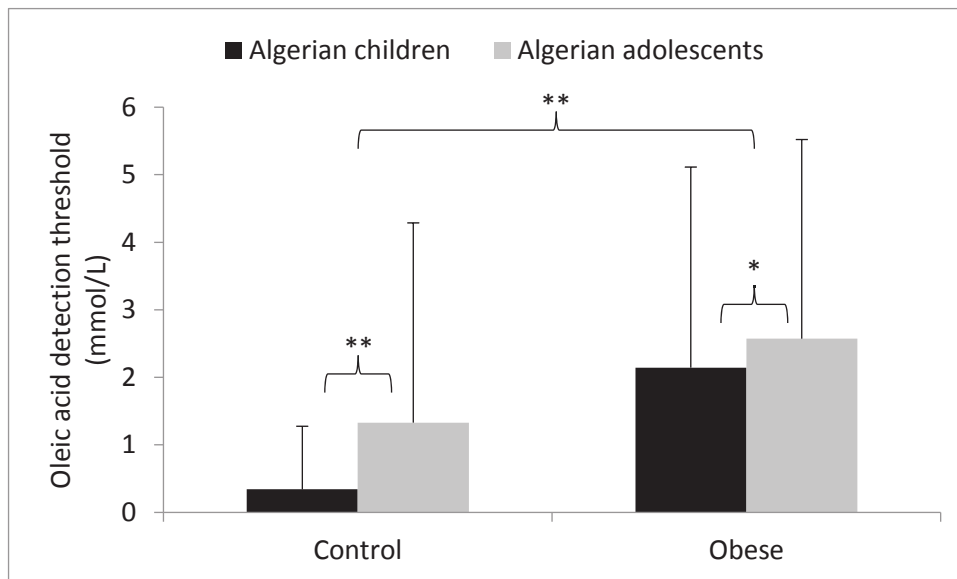


Figure 5: Oleic acid detection threshold in Algerian children (black) and adolescents (grey). We observed significantly higher detection threshold in both age groups. Moreover, the control adolescents were less sensitive to oleic acid than the control children ($p = 0,04$). This difference is even greater in the obese ($p < 0,01$). * $p < 0,05$; ** $p < 0,01$

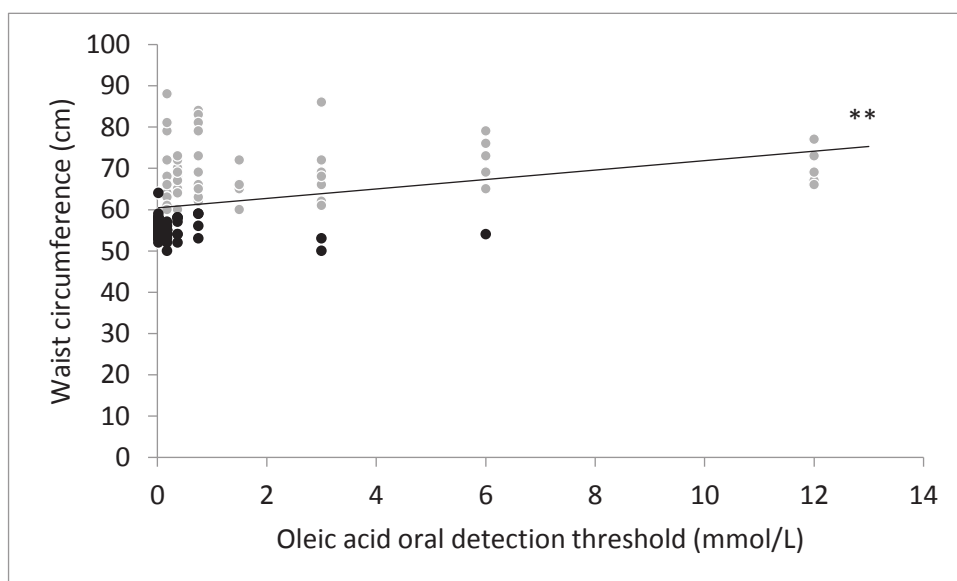


Figure 6: Correlation between waist circumference and oleic acid detection threshold in the Algerian lean (black) and obese (grey) children. ** $p < 0,01$

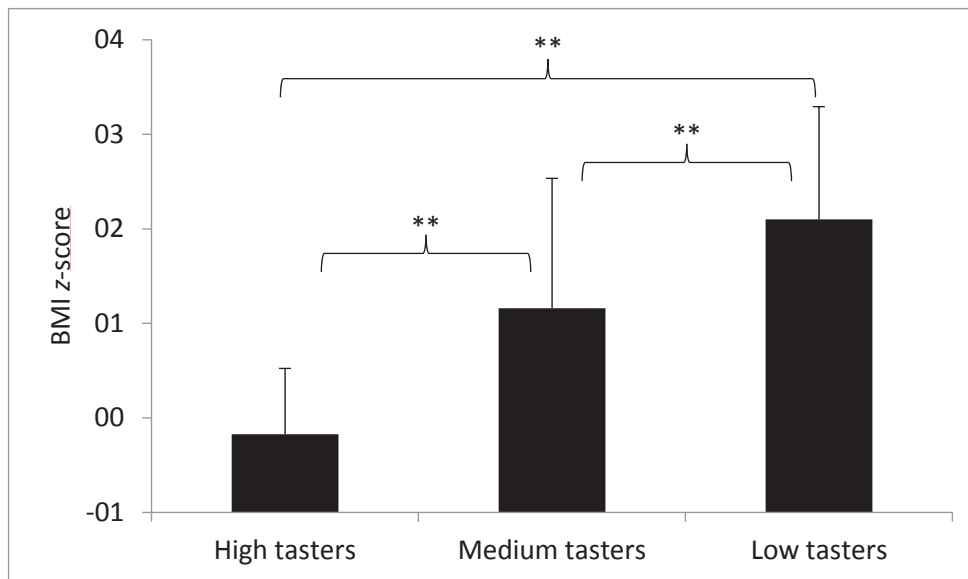


Figure 7: Average BMI z-score in each of three taster categories. “High tasters” group consist of control subjects only ($n = 8$). The most common group, “Medium tasters” contained both controls ($n = 60$) and obese ($n = 45$) subjects. The “Low tasters” group contained 52 subjects (11 controls, 41 obese). * $p < 0,05$

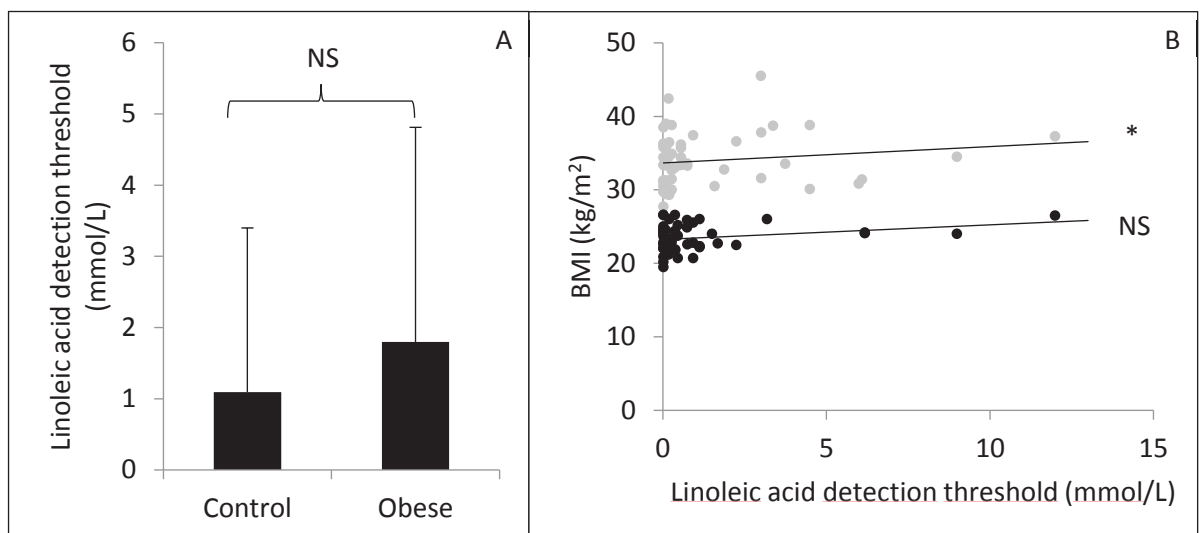


Figure 8: Difference of linoleic acid detection threshold between controls and obese Tunisian adults(A). Correlation between BMI and linoleic acid detection threshold in obese (grey) and control (black) groups (B). * $p < 0,05$; NS = insignificant difference

In the Tunisian case-control study, obese subjects exhibited slightly higher detection threshold ($1,80 \pm 3,01$ mmol/L) than lean controls ($1,09 \pm 2,30$, Figure 8a). However, this difference was

too small to be statistically significant ($p = 0,18$). Moreover, we observed a positive association between BMI and the detection threshold in obese subjects ($p = 0,037$), but not in control subjects (Figure 8b).

In the Czech cohort study, we also found a significant relationship between linoleic acid detection threshold and measured anthropometric parameters (Figure 9). There was the positive correlation between LA detection threshold and BMI ($p = 0,047$), waist circumference ($p = 0,004$), waist-to-hip-ratio ($p = 0,016$), and waist-to-height ratio ($p = 0,025$). The male subjects had more than two-fold lower sensitivity (higher detection threshold) than female subjects ($p < 0,01$).

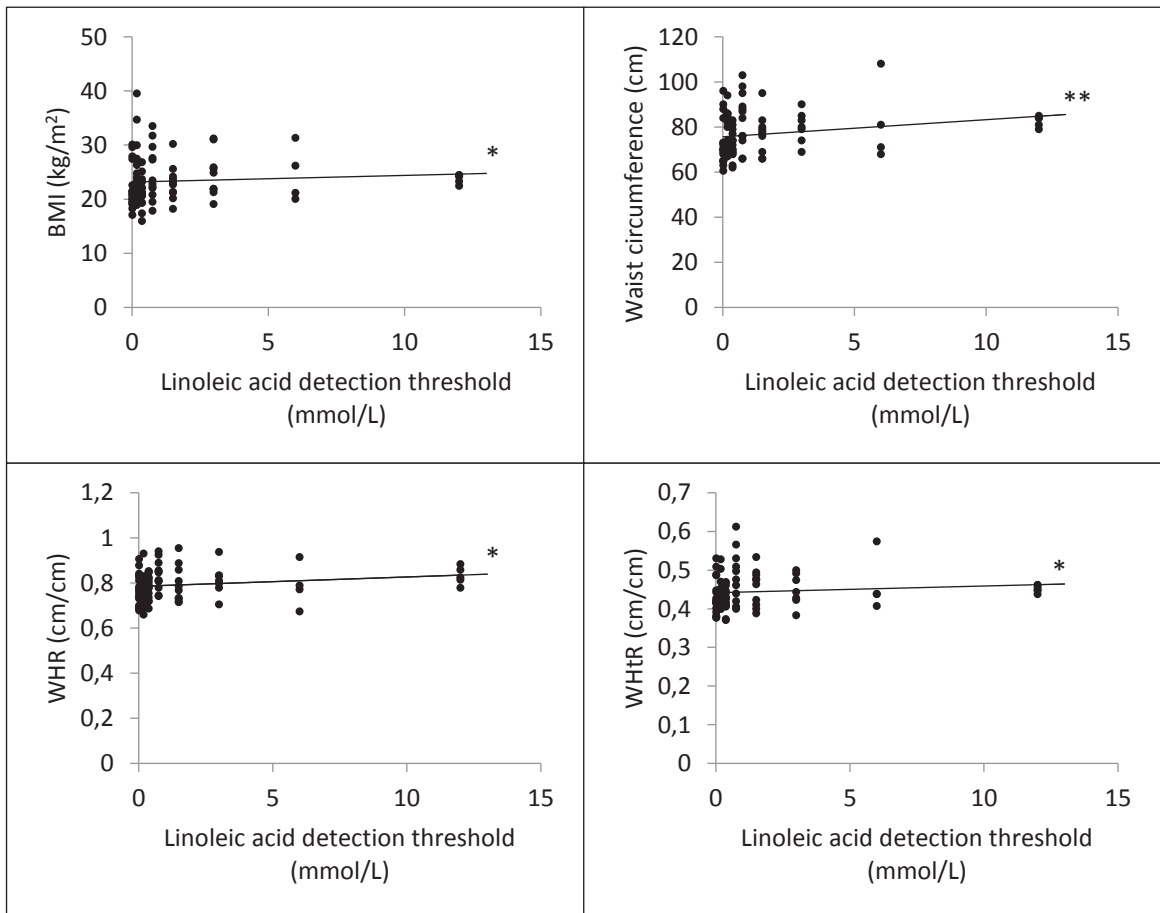


Figure 9: Relationship between BMI, WC, WHR, WHtR, and linoleic acid detection threshold in Czech adults. * $p < 0,05$; ** $p < 0,01$

4.2.2 PROP

We noticed that obese Tunisian subjects exhibit higher PROP detection threshold than control subjects ($0,40 \pm 0,34 \mu\text{mol/L}$ and $0,18 \pm 0,298 \mu\text{mol/L}$, respectively, $p < 0,01$). Also, PROP detection threshold positively correlated with BMI in both control ($p = 0,03$) and obese ($p < 0,01$) groups. The results of the PROP detection threshold are shown in Figure 10. Moreover, we observe strong positive correlation between LA and PROP detection threshold in the obese participants ($p < 0,01$, Figure 11).

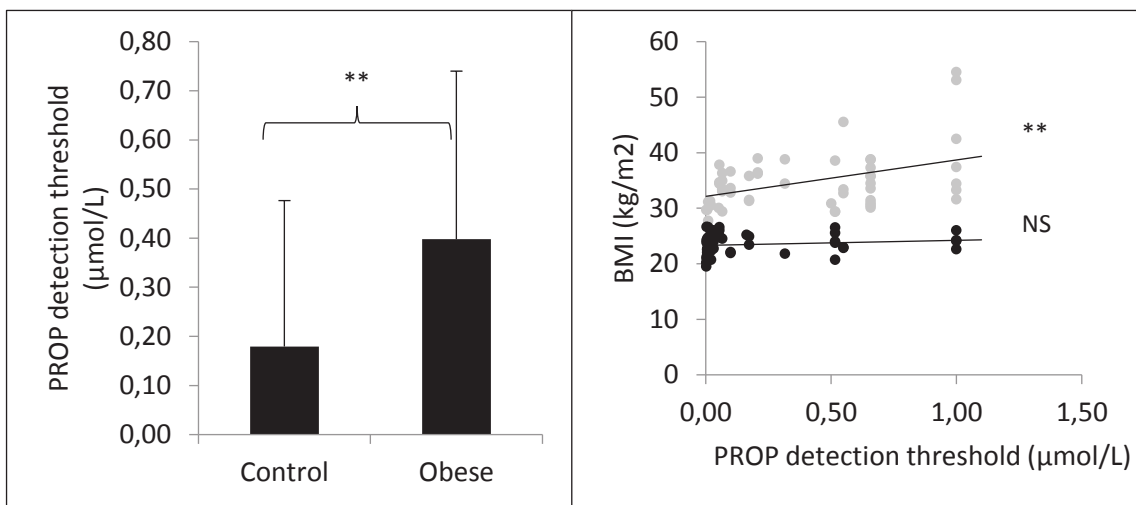


Figure 10: Orosensory detection thresholds of PROP in obese and control subjects (A). Correlation between BMI and orosensory detection thresholds of PROP in obese (grey) and control (black) groups (B). * $p < 0,05$; ** $p < 0,01$, NS = insignificant difference.

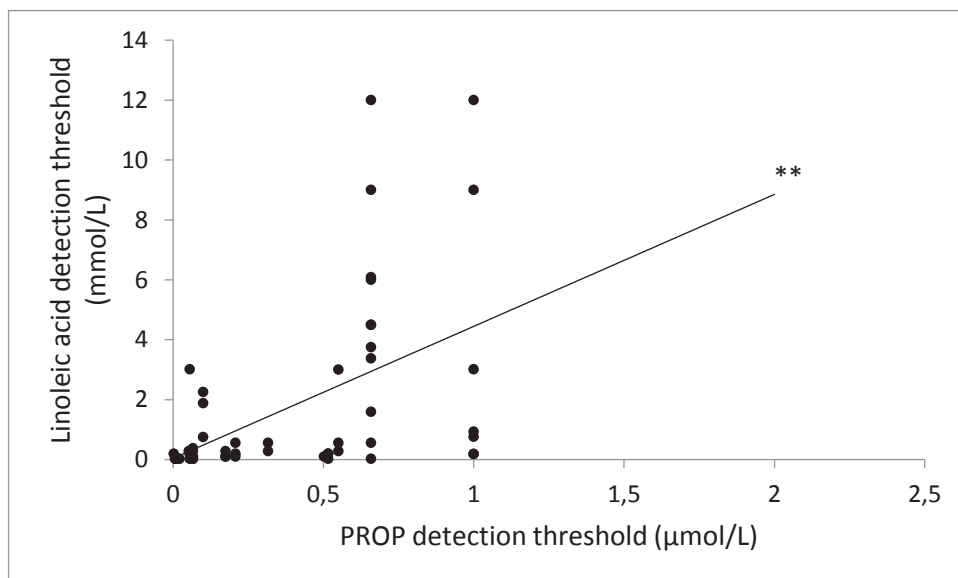


Figure 11: Relationship between detection thresholds of linoleic acid and PROP. ** $p < 0,01$.

4.2.3 Cholesterol and Triglyceride Levels after Tasting Session

One hour after the linoleic acid tasting session, we observed a noteworthy increase of total cholesterol (TC) level in the obese subject ($p < 0,01$), though the control group exhibited the opposite trend ($p < 0,05$). In case of triglyceride level, we noticed the opposite trend. The tasting session cause a decrease of triglyceride level in obese and increase in control subjects ($p < 0,01$). The changes of the total cholesterol and the triglycerides are shown in Figure 12.

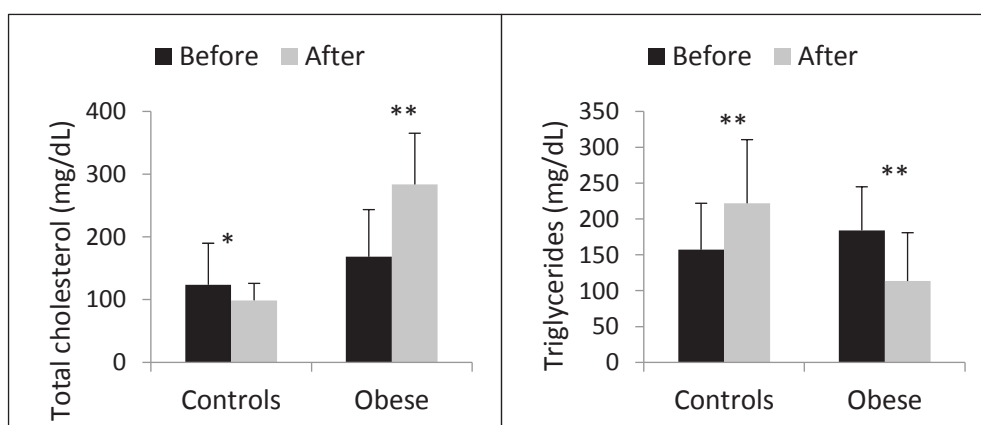


Figure 12: The effect of fatty acid-tasting on total cholesterol and triglyceride concentration in Tunisian control and obese adults. * $p < 0,05$; ** $p < 0,01$.

4.3 Genetic Analysis

4.3.1 CD36 Polymorphisms

Tables 10 and 11 show genotype and allelic frequencies of studied CD36 polymorphisms in the selected populations. Regarding rs1761667, G-allele is the minor allele in both Algerian and Tunisian populations. In Czech populations, T-allele of rs1527483 and deletion allele of rs3212018 were considered as minor with allelic frequencies 9,1 % and 18,5 %, respectively. The studied polymorphisms did not deviate from Hardy-Weinberg equilibrium.

Table 10: Genotype and allelic frequencies of CD36 polymorphism rs1761667 in Algerian and Tunisian populations.

Sample	Genotype	Allele	Total number		Obese		Control		p value
			n	%	n	%	n	%	
Algerian children	AA		34	29	22	39	12	20	
	AG		58	50	26	46	32	54	
	GG		24	21	9	16	15	25	
	AG+GG		82	71	35	61	47	80	0,041
		A	126	54	70	61	56	47	0,036
		G	106	46	44	39	62	53	
Algerian adolescents	AA		65	39	35	42	30	37	
	AG		76	46	43	52	33	40	
	GG		24	15	5	6	19	23	
	AG+GG		100	61	48	58	52	63	0,525
		A	206	62	113	68	93	57	0,041
		G	124	38	53	32	71	43	
Tunisian adults	AA		35	34	24	46	11	21	
	AG		56	54	22	42	34	65	
	GG		13	13	6	12	7	14	
	AG+GG		69	66	28	54	41	79	0,012
		A	126	61	70	67	56	54	0,065
		G	82	39	34	33	48	46	
Tunisian obese cohort	AA		59	29					
	AG		102	50					
	GG		42	21					
	AG+GG		144	71					
		A	220	54					
		G	186	46					

Table 11: Genotype and allelic frequencies of *CD36* polymorphisms rs1527483 and rs3212018 in Czech population.

Polymorphism	Variation	Genotype	Allele	Total number	
				<i>n</i>	%
rs1527483	C/T	CC		97	83.6
		CT		17	14.7
		TT		2	1.7
		CT+TT		19	16.4
			T	211	90.9
			C	21	9.1
rs3212018	16 bp del	ins/ins		77	66.4
		ins/del		35	30.2
		del/del		4	3.5
		ins/ins+del/del		39	33.7
			ins	189	81.5
			del	43	18.5

4.3.1.1 Rs1761667 and Obesity

We noticed significantly higher rs1761667 A-allele frequency in obese Algerian children compared with controls, whereas the G-allele was more common in the control group ($p = 0,036$). This suggests that subjects with A-allele has higher risk for obesity (odds ratio = 1,76, 95% confidence interval of odds ratio = 1,04–2,97, risk ratio= 1,29; confidence interval = 1,02–1,64) than individuals with G-allele. Likewise, we observed higher A-allele frequency in obese Algerian adolescents compared to lean controls ($p = 0,041$, odd ratio = 1,63; 95% confidence interval of odd ratio = 1,04–2,55, risk ratio = 1,20; 95% confidence interval of risk ratio = 1,01–1,42). However, we did not find any significant difference of BMI z-score and rs1761667 genotypes.

In the Tunisian case-control study, we did not find a statistically significant difference of allelic distribution between obese and control subjects. Using dominant model, we found a higher AA genotype frequency of rs1761667 in obese subjects compared to controls ($p = 0,012$).

4.3.1.2 Rs1761667 and Fatty Acid Detection Threshold

Obese Algerian children with AA genotype exhibited significantly higher oleic acid oral detection threshold compared to obese children with GG genotype (Figure 13A, $p < 0,05$). Interestingly, we did not observe any difference in the difference in the control group. In the Algerian adolescents, no significant association between rs1761667 and oleic acid detection threshold was observed (Figure 13B).

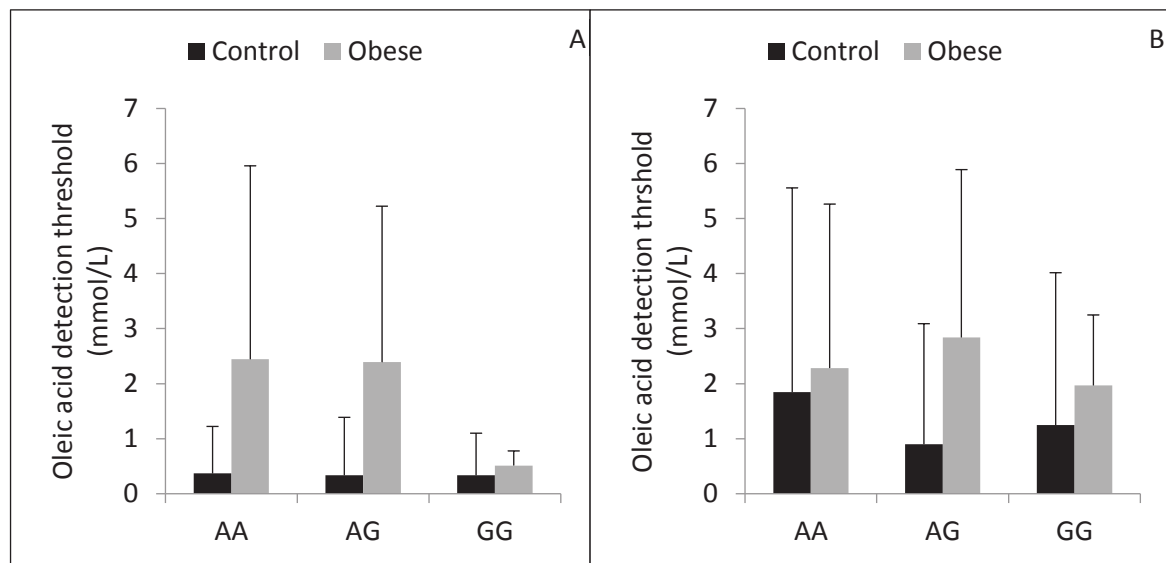


Figure 13: Oleic acid detection threshold in Algerian children (A) and adolescents (B). We noticed, that obese children with AA genotype exhibit significantly higher detection threshold compared to obese children with GG genotype (Figure 9A, $p < 0,05$).

In the Tunisian cohort study, we noticed that subjects with GG genotype of rs1761667 exhibit significantly higher oleic acid oral sensitivity (lower detection threshold) than individuals with AA genotype (Figure 14, $p < 0,01$). We did not observe a statistically significant difference in the taste detection thresholds of subjects with the AG or the AA (or GG) genotypes. On the other hand, we failed to replicate these results in the Tunisian case-control study. There was no difference of linoleic acid detection threshold and rs1761667 genotype neither in obese nor in control group.

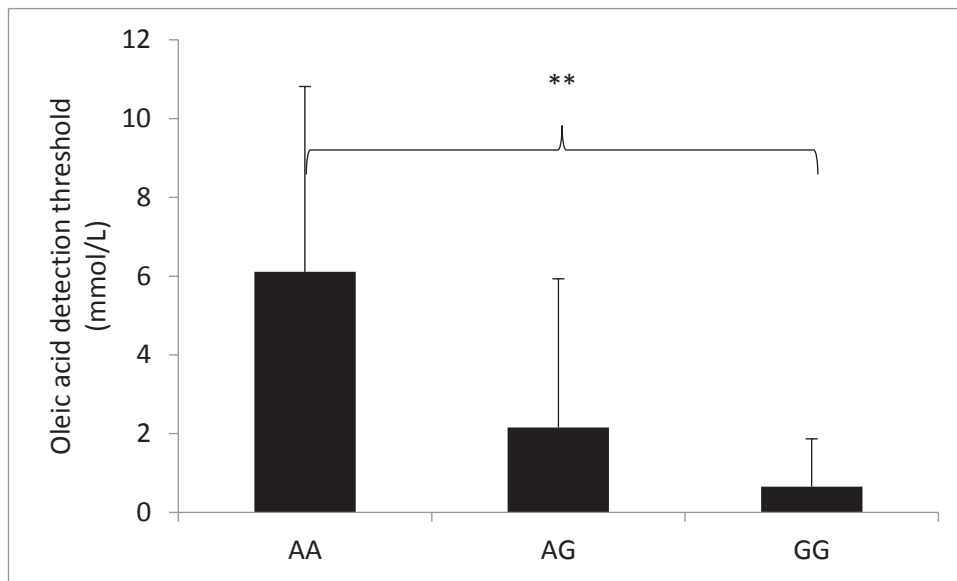


Figure 14: Oleic acid detection threshold in Tunisian cohort study. We observed statistically significant difference between the three groups. The difference between the AG and GG genotypes was not statistically significant. ** $p < 0,01$.

4.3.1.4 Rs1527483 and Corpulence

Figure 15 shows the relationship between measured anthropometric parameters and rs1527483 genotypes. In the Czech cohort study, we noticed that persons with CT/TT had higher BMI, waist circumference and waist-to-height ratio (WHtR) than the participants with CC genotype (p values were 0,011; 0,005 and 0,010, respectively). This difference was not observed in waist-to-hip-ratio (WHR) ($p = 0,077$). Furthermore, the subjects with CT/TT genotypes exhibited higher detection threshold for linoleic acid than participants with CC genotype ($p = 0,037$, Figure 16).

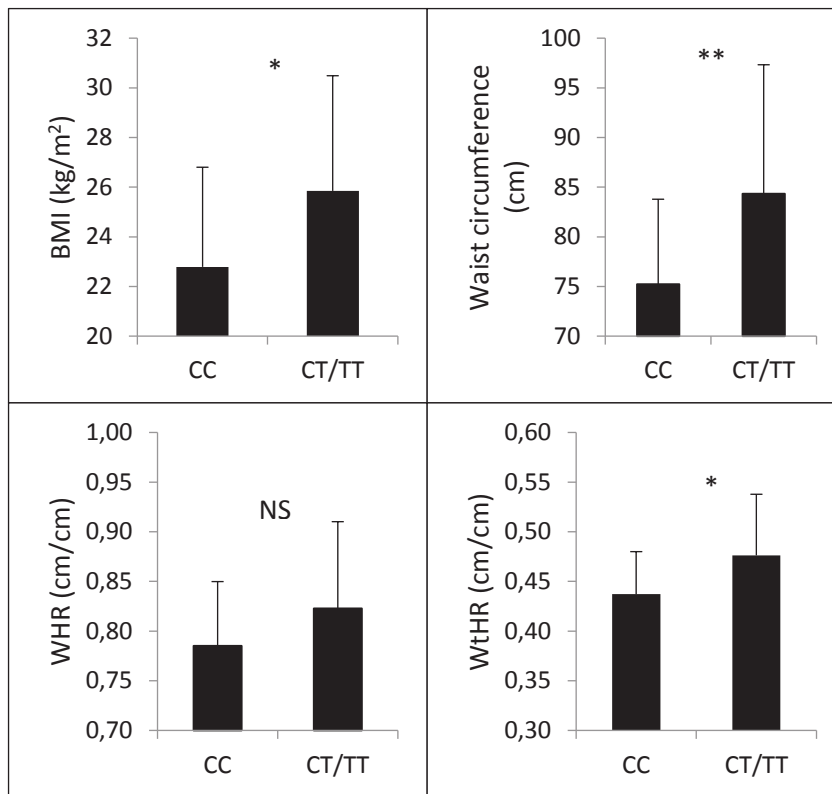


Figure 15: Association between measured anthropometric parameters and rs1527483 genotype in the Czech cohort study. Out of measured anthropometric parameters, BMI, waist circumference and waist-to-height ratio (WtHR) differences were related to rs1527483 genotypes. There was no relationship between waist-to-hip ratio (WHR) and rs1527483 genotype. * $p < 0,05$; ** $p < 0,01$; NS = insignificant difference.

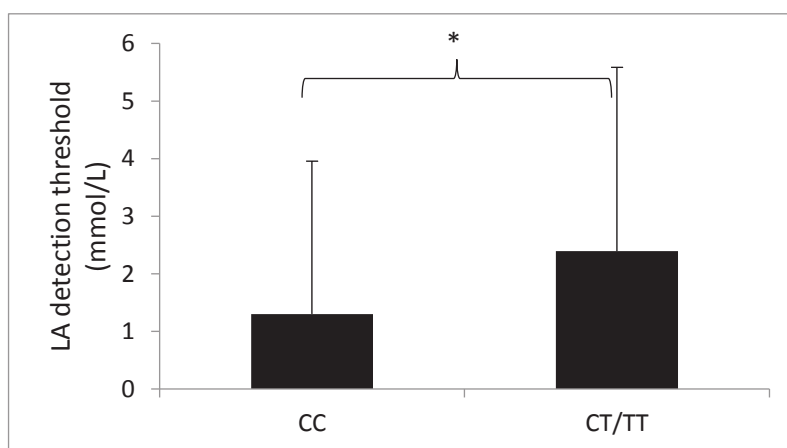


Figure 16: Relationship between rs1527483 genotypes and linoleic acid (LA) detection threshold in Czech adults. Individuals with CC genotype exhibited significantly higher orosensory sensitivity to LA than individuals with CT and TT genotypes. * $p < 0,05$

4.3.2 CD36 and Fatty Acid Non-Tasters

Regardless *CD36* genotypes, we observed that fatty acid non-tasters are equally distributed in all studies populations. There was no significant relationship between non-tasters and any of the *CD36* polymorphism.

The selected *CD36* exons 5 and 6 corresponded with sequences in online database. We sequenced base pairs 80,661,027 - 80,661,322 (exon 5) and base pairs 80,662,855 - 80,663,346 (exon 6) on chromosome 7. In the analyzed DNA sequences of 4 non-tasters and 4 supertasters, we did not find any polymorphism. We can state that the cause of altered dietary lipids oral perception is not related to mutations in exons 5 and 6 of *CD36* gene in our study.

4.3.3 T2R28 SNPs

Table 12 shows genotype and allelic frequencies of studied T2R38 polymorphisms in obese and control Tunisian adults. Using dominant model, we observed that AA genotype (which encodes alanine) of rs1726866 was more frequent in the obese Tunisian group ($p = 0,017$). We did not find any significant association between rs10246939 SNP and obesity. Combination of rs1726866 and rs10246939 formed 9 different haplotype combinations. However, none of them differed between obese and control groups. Furthermore, we observed strong linkage disequilibrium (LD) between rs1726866 and rs10246939 ($D' = 0,863$, $r^2 = 0,616$). Compared to other two genotypes of rs10246939, the controls with the VV genotype have significantly lower LA detection threshold ($p = 0,042$) than obese individuals. The individuals with AA genotype (alanine) of rs1726866 had significantly higher BMI than the individuals with AV and VV genotypes ($p < 0,01$). The genetic frequencies studied SNPs did not deviate from Hardy-Weinberg equilibrium.

Table 12: Genotype and allelic frequencies of rs1726866 and rs10246939 in Tunisian obese and lean adults.

SNP	Genotype	Amino acid	Total number		Obese		Control		<i>p</i> value
			<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	
rs1726866	CC	AA	23	22	17	33	6	12	0,017
	CT	AV	43	41	15	29	28	54	
	TT	VV	38	37	20	39	18	35	
	CT+TT	AV+VV	81	78	35	67	46	89	
rs10246939	AA	II	28	27	14	27	14	27	NS
	AG	IV	53	51	23	44	30	58	
	GG	VV	23	22	15	29	8	15	
	AA+AG	II+IV	81	78	37	71	44	85	
rs1726866 and rs10246939 haplotype	CCAA	AAII	1	1	1	2	0	0	NS
	CTAA	AVII	2	2	0	0	2	4	NS
	TTAA	VVII	25	24	13	25	12	23	NS
	CCAG	AAIV	2	2	2	4	0	0	NS
	CTAG	AVIV	40	38	15	29	25	48	NS
	TTAG	VVIV	11	11	6	12	5	10	NS
	CCGG	AAVV	20	19	14	27	6	12	NS
	CTGG	AVVV	1	1	0	0	1	2	NS
	TTGG	VVVV	2	2	1	2	1	2	NS

4.4 Nutrition Questionnaires

In Algerian children, we compare habitual food pattern between control and obese children, we noticed a statistically significant difference between these two groups ($p < 0,01$), as far as the consumption of four food products (pasta, chips, chocolates and bread) and three food products (wafer, bread and candy) is concerned (Table 13). In addition, we noticed that AA genotype significantly predisposed these children to prefer palatable food products, that is, pasta, chips, chocolates and bread ($p = 0,04$) (Table 14).

Table 13: Difference of preferred food patter between Algerian obese and control children.

Food contents	Controls	Obese	Total
Pasta, chips, chocolates and bread	4	44	48
Wafer, bread and candy	23	13	36
Candy, bread and cheese	32	0	32

Table 14: Relationship between CD36 polymorphism rs1761667 and preferred food patten in Algerian children.

Food contents	Rs1761667 genotype		
	AA	AG	GG
Pasta, chips, chocolates and bread	20	20	8
Wafer, bread and candy	9	21	6
Candy, bread and cheese	5	17	10

In the Czech cohort study, we observed statistically significant correlation between measured anthropometric parameters and craving for “High fats” food items (Table 15). The subjects with BMI, waist circumference, waist-to-hip ratio and waist-to-height ratio tend to have more frequent craving for fatty food (p values respectively 0,026; 0,007; 0,011; 0,044). In addition, the subject with frequent craving for “High-fats” food items have higher linoleic acid detection threshold ($p = 0,033$). We did not observe any of these trends with the “Fast-food fats” items. Interestingly, we found association between consumption of alcohol and the craving for “Fast-food fats” food items ($p = 0,021$).

Table 15: Correlation between FCI food items categories and measured anthropometric parameters, linoleic acid detection threshold and weekly alcohol consumption in the Czech adults.

Parameter	Food item group					
	Fast-food fats		High fats		Total fats	
	R	p	R	p	R	p
BMI (kg/m ²)	0.163	NS	0.225	0.026	0.211	0.037
WC (cm)	0.151	NS	0.277	0.007	0.238	0.022
WHR (cm/cm)	0.162	NS	0.263	0.011	0.239	0.021
WHtR (cm/cm)	0.161	NS	0.212	0.044	0.201	0.056
LA (mmol/L)	0.173	NS	0.214	0.033	0.187	0.062
WAC (unit/week)	0.224	0.021	0.182	0.06	0.21	0.03

Abbreviations: WC (waist circumference), WHR (waist-to-hip-ratio), WHtR (waist-to-height ratio), LA (linoleic acid oral detection threshold), WAC (weekly alcohol consumption). NS = insignificant correlation.

5 Discussion

Obesity remains one of the most important global health issues nowadays. Multifactorial ethology of this disease requires complete understanding of all factors which might have any effect on this worldwide pandemic. Inappropriate diet is one of key factors. Fat overconsumption is the main cause of positive energetic balance that eventually leads to accumulation of body fat and obesity²⁰⁹. Humans, or mammals in general, perceive dietary lipids as palatable part of daily diet. Are mammals able to detect dietary lipids? Is there any relationship between hypothetical “taste of fats” and obesity?

It is generally accepted, that dietary lipids are detected by the olfactory and somatosensory systems¹³⁹. However, last decades brought various evidences supporting the hypothesis that mammals can detect dietary lipids via gustatory system. Moreover, the ability to detect the dietary lipids varies amongst individuals. This difference may play an important role in obesity. Therefore, we conducted the present studies on different populations.

In Algerian adolescents and Tunisian adults, we have determined differences of blood parameters between obese and lean controls. In Algerian adolescents, we noticed higher glycemia, LDL-C, triglycerides, and insulin concentrations in obese individuals than the lean ones. It has been already shown that the Brazilian obese teenagers suffer from high blood concentrations of LDL-C, glucose and insulin²¹⁰. Elsewhere, it has been observed that the prevalence of hyperinsulinemia and hypertriglyceridemia was significantly higher in severely obese children and adolescents, compared to the less obese persons²¹¹. Moreover, adolescents from the obese group exhibited low HDL-C concentrations. Indeed, it has been previously reported that low HDL is associated with high BMI and waist circumference²¹². Also, Jiang *et al.*²¹³ have shown that insulin concentration positively correlated with serum triglyceride, and negatively with HDL-C levels all age group obese children including 12–17 years old individuals. The differences might have adverse consequences for cardiovascular diseases in adulthood in these adolescents. Moreover, we observed a high HOMA index, an indicator of insulin-resistance (IR) which is directly associated with aggravation of obesity²¹⁴ in the obese adolescents.

Obese Tunisian women exhibit a normal biochemical profile, marked with normal uric acid, creatinine, LDL-C, HDL-C and CRP concentrations. Nevertheless, they suffer from hyperinsulinemia which is associated with a mild increase of blood glucose concentrations. The glucose concentration seems under metabolic control as the HbA1c concentrations were not altered in these individuals. However, these women had higher levels of blood triglycerides, IL-

6 and TNF- α compared to normal range^{207,208}. Dyslipidemia is one of the most common obesity comorbidity²¹⁵ and the high level of blood triglycerides is one of the risk factor for atherosclerosis. IL-6 and TNF- α are pro-inflammatory cytokines often associated with obesity. High IL-6 level is one of the symptom of obesity and its high concentration in obese has been already shown elsewhere^{216–218}.

In the Tunisian case-control study, we did not observe any abnormality in the measured blood parameters. Only the obese subjects suffer from hyperinsulinemia associated with a mild increase of fasting glucose concentration. Hyperinsulinemia is a common result of obesity. Long-lasting hyperinsulinemia may result to insulin resistance²¹⁹. Furthermore, obese individuals exhibited higher ALAT and ASAT levels than lean controls. These results are consistent with previous findings. High aminotransferase levels in obese individuals have been previously shown in adults²²⁰, adolescents²²¹ and children²²². Obesity is associated with a spectrum of liver abnormalities, collectively called of nonalcoholic fatty liver disease (NAFLD). High transaminase concentration is one of the NAFLD indicators²²³. Also, prevalence of NAFLD increases with increasing BMI²²⁴. Nevertheless, transaminase concentrations of the Tunisian adults were in normal range. Therefore, we cannot state that these individuals may have suffered from NAFLD.

One of the key objectives of the dissertation was assessment whether obese subjects exhibit altered orosensory perception for dietary lipids. In the first study conducted on Algerian children, we observed that obese children exhibited significantly higher orosensory detection threshold than lean controls. We obtained the similar results in the second study where Algerian adolescents were recruited. However, there results from Tunisian case-control study did not show the same results. These results are consistent with studies conducted in French²²⁵ and Australian²²⁶ populations where the obese subjects exhibited high orosensory detection threshold for fatty acids. The different orosensory perception in obese children and adolescents has been also observed in the other taste modalities. Obese children and adolescents have a significantly lower ability to identify the correct taste modalities. The difference has been observed in salty, *umami* and bitter tastes²²⁷. This difference was also observed in young adults where obese individuals exhibited a significantly higher taste threshold for salty taste compared controls²²⁸.

When we consider respective differences from the performed case-control studies, we may observe a certain trend. The most significant difference of detection threshold was observed in children. This difference was observed in the adolescents too, but in the lesser extent. And finally, difference between obese and lean adults was not statistically significant. This trend may

have several explanations. Compared to adolescence and adulthood, childhood obesity is affected by fewer environmental factors such as chronic stress^{229,230} or sedentary lifestyle⁴⁵. Individuals are exposed to these factors throughout their lives, however their impact on obesity increases over time. Furthermore, we noticed that Algerian children have significantly lower orosensory detection threshold (higher sensitivity) than the Algerian adolescents. To date, no study elucidate how are dietary lipids perceived in different age groups. However, Segovia *et al.* already reported that male children are more sensitive to sweet tastant sucrose than male adults²³¹. According the results, Segovia *et al.* believe that this difference is caused by higher taste pore density in children than in adults²³¹.

The difference of the altered detection threshold in obese individuals may have another explanation. Obesity is greatly influenced by the individual's diet. Previous studies on animal models have showed that diet-induced obese (DIO) rodents are less sensitive to oil in diet than lean controls¹⁹⁰. Similar results were obtained in human volunteers. Stewart *et al.* showed that lean subjects exhibited significantly decreased oral sensitivity to oleic acid after diet rich in dietary fats (high-fat diet) and increased oral sensitivity after low-fat diet²³². Moreover, Brennan *et al.* noticed that obese subjects show enhanced gastrointestinal sensitivity to fat after acute dietary restrictions²³³. From our data, we cannot clearly state whether the low orosensory sensitivity to dietary lipids in obese is a consequence or a cause of obesity. Nevertheless, when we consider quoted publications it seems likely that low orosensory sensitivity is rather a consequence of high body weight than the cause of obesity.

In the present studies, we observed that orosensory detection threshold for dietary lipids often correlated with anthropometric parameters. Waist circumference of Algerian children positively correlated to oleic acid detection threshold. Likewise, BMI z-score of Algerian adolescents positively correlated with oleic acid threshold. In the Tunisian case-control study, the detection threshold correlated with BMI of obese individual but not with BMI of lean controls. And finally, selected anthropometric parameters correlated with linoleic acid detection threshold in the Czech cohort study. In the Czech study, we decided to use several different anthropometric parameters to eliminate any misinterpretation of BMI values³⁰. Nevertheless, the selected parameters were in strong correlation. According to these results, it seems that corpulence, not obesity alone, is the factor responsible for difference in orosensory detection threshold. Indeed, we did not find any correlation between the detection threshold and obesity indicators (such as CRP, IL-6 or TNF- α) besides BMI, BMI z-score, WC, WHR, and WHtR.

We also observed that Tunisian obese individuals exhibited lower orosensory sensitivity (higher detection threshold) for PROP. Moreover, PROP detection threshold significantly correlated with BMI of the obese individual but not with BMI of the lean controls. PROP is a typical bitter molecule but it is also considered marker for general taste sensitivity²³⁴. This result supports the theory that obese subjects have attenuated orosensory detection ability. As above mentioned, obese children and adolescents exhibited significantly diminished ability to identify the correct taste modalities, bitter taste included²²⁷. Similarly, obese children from another study also exhibited a low ability to identify presented tastants²³⁵. Nevertheless, their detection ability was improved after weight loss. Moreover, obese subjects were showed to possess low intensity ratings for bitter vegetables, the source of vitamins and minerals²³⁶.

In the Tunisian case-control study, we noticed interesting strong correlation between PROP and LA oral detection thresholds. Previously, it has been proposed that alteration of fatty acid detection threshold might be associated with alteration of bitter taste modality²³⁷. Furthermore, PROP tasters exhibited high taste intensity rating for linoleic acid than PROP non-tasters²³⁸. This finding supports the hypothesis that orosensory sensitivities of each taste modalities are related to each other (as mentioned before).

In the Tunisian adults, we observed noteworthy differences of total cholesterol and triglycerides concentration after the taste test. These results partly corroborate with finding of Chevrot *et al.*²²⁵. Chevrot *et al.* have reported significant increase of triglyceride concentrations after the oral fatty acid stimulation. In our study, the triglyceride increase has been observed only in lean subjects, not in obese. This rapid decrease of triglycerides and increase of total cholesterol in obese subjects might be due to a defect in the vagal reflex loop, tongue-brain-intestine. To date, no other evidence about this phenomenon has been published. Therefore, more studies focused on endocrinological response to orosensory stimulation is required.

In this thesis, we have been focused on *CD36* polymorphisms. *CD36* appears to be the most promising taste receptor for dietary lipids. So far, the most studied polymorphism is rs1761667.

This SNP has been related to several health complications such as coronary artery disease²³⁹ or ischemic stroke²⁴⁰. Our findings suggest that this SNP may be also related to obesity. We observed that A-allele was more abundant in obese Algerian children and Adolescents than in the lean controls. The same analysis was performed in Tunisian case-control study, but the differences were only statistically marginal. Furthermore, A-allele of this SNP is related to increase orosensory detection threshold in Algerian children and Tunisian obese women. However, there we did not observe any difference in Algerian adolescents and in Tunisian case-

control study. The reason for this failure in Algerian adolescents might be the young age of the participants. It has been shown that circumvallate papillae, which are the papillae with highest expression of *CD36*^{161,187,188}, continue to grow until the age of 15-16 years²⁴¹. Circumvallate papillae express nine-times more *CD36* mRNA than fungiform papillae¹⁸⁵.

Rs1761667 has been, for the first, associated with altered orosensory lipid perception by Pepino *et al.*¹⁹⁸ and later confirmed in African-American¹⁹⁶ and Italian²⁰⁰ populations. Nevertheless, influence of rs1761667 on obesity and orosensory lipid perception remains controversial. Recent Malaysian study did not find any relationship between rs1761667 and lipid perception or obesity (rs1761667). Also, study of Pioltine *et al.*²⁴² did not find any relationship between rs1761667 and obesity. Surprisingly, this study showed that A-allele of rs1761667 is associated with a decreased intake of fat in obese subjects. This is rather an opposite effect that we would expect.

In the Czech study, we analyzed other *CD36* polymorphisms, rs1527483 and rs3212018. Regarding rs3212018, we did not observe any significant relationship with orosensory threshold nor with anthropometric parameters. Rs3212018 has been previously associated with waist circumference and obesity¹⁹⁶. However, a comprehensive meta-analysis published by Choquet *et al.*¹⁹⁷ failed to obtain the similar results. Deletion of rs3212018 had very low frequency among populations. Thus, assessment of rs3212018 relevance in obesity remains difficult.

T-allele of rs1527483 has been previously associated with high BMI and percentage of body fat (BF%)²⁴³. In our study, we did not measure the BF%, however BMI and other used adiposity parameters could be sufficient substitution for BF%²⁴⁴. We observed that individuals with CC genotype of rs1527483 have lower orosensory detection threshold for linoleic acid than subjects with CT/TT genotypes. So far, there is no study that shows relationship between orosensory detection and this. So far, these studies study relationship of rs1527483 with orosensory lipid perception. Two studies conducted on African-American and Asian populations reported that this SNP is associated with oral fat perception of lipids^{196,201}. However, study conducted on Italian population did not, however, confirm this finding²⁰⁰. Apart from differences between study populations, the discrepancy between these results may be caused by two major factors: 1) While the studies of Keller *et al.* and Ong *et al.* included 317 and 313 subjects, respectively, the study of Melis *et al.* was performed only on 64 subjects (with only 7 subjects with CT genotype and none with TT genotype). Despite the small sample group, the results of Melis *et al.* suggest a possible, but not statistically significant, influence of rs1527483 in bitter taste perception. In this study, T-allele of this SNP was presented rather in subjects with decreased

PROP sensitivity. 2) During paper screening test used by Melis *et al.*, a paper disc impregnated with oleic acid was placed on the center of the tongue which means that there was no contact with circumvallate papillae located on posterior region of the tongue. On the contrary, testing method used by our group and Keller *et al.* use the whole surface of the oral cavity for oral sensitivity test which means the used fatty acid can reach the whole tasting area. So far, we do not know a level of CD36 expression in different taste papillae, nevertheless CD36 expression in circumvallate papillae and foliate papillae is 9-fold and 3,5-fold higher than in fungiform papillae in mice, respectively²⁴⁵.

Rs1761667 has been shown to associated with low expression of CD36 in platelets and monocytes²⁴⁶. Whether this SNP has the same effect on TRC remains unknown. Regarding rs1527483, it remains unknown this SNP may influence CD36 function. Rs1527483 is intronic polymorphism. Therefore, regulatory role of this SNP can be considered²⁴⁷.

In each performed study, we noticed that some of the subjects are not able to identify the fatty acids at any given concentration. These subjects were defined as non-tasters. It is interesting that we identified the non-tasters throughout all age groups, populations and in both genders. Used statistical analysis did not reveal any significant influence on this phenomenon. One explanation for this might be an existence of mutation within CD36 gene. Based on the results of Kuda *et al.*¹⁸⁰, we searched for a mutation within the exons known to encode the hydrophobic pocket of CD36. This pocket is responsible recognition of most of CD36 ligands, LCFA included. However, our results did not reveal any mutation in the region. So far, no clear cause of fatty acid insensitivity has been presented. Therefore, this phenomenon deserves further deeper study. Another explanation might be an existence of more pathways of oral lipid sensing. In human foliated and circumvallate papillae, two different fatty acid receptors, CD36 and GPR120, have been detected²⁰⁹. According to Sclafani *et al.*, CD36 is more important in fatty acid detection than GPR120²⁴⁸. Indeed, experiments performed on human taste cells revealed that CD36 is more sensitive to fatty acids than GPR120¹⁶¹. Also, exposition of linoleic acid to CD36 triggers its degradation from the lipid raft, whereas GPR120 is still presents. If the same mechanism applies in human cells, dietary fat can be detected by its taste without the CD36 via GPR120.

We noticed significant correlation between obesity and palatable food pattern in Algerian children. Stewart *et al.* previously showed that subjects with high fat breakfast showed impaired fatty acid sensitivity²²⁶. In another study, Stewart *et al.* showed that study participants classified as hyposensitive to oleic acid consumed significantly more energy, fat, saturated fat, fatty foods

(butter, meat, dairy), had greater BMI and were lesser perceptive of small changes in the fat content of custard, compared with hypersensitive participants²³².

Dietary habits are one of the most important environmental factors in adiposity development. In the Czech study, we noticed that craving for dietary lipids is related to measured adiposity parameters. From the used questionnaire, we noticed that craving for “fatty” food rises with increasing BMI, waist circumference, waist-hip ratio, and waist-to-height ratio. The similar results were previously obtained by Chao *et al.* who showed that BMI positively correlated (using also FCI) with craving for high fats and fast-food fats²⁴⁹. The food items used in the FCI are rich in saturated and trans-unsaturated fatty acids. High consumption of saturated and trans-unsaturated fatty acids have been previously related with high BMI, obesity and T2DM^{250–252}. It has been shown that craving for food is related to individual’s diet. The individuals who follow low-fat diet exhibited reduced craving for high-fat foods²⁵³. In addition, subjects exhibited significant decrease of food craving after six months weight loss compared to controls²⁵⁴.

These differences may be caused by the attenuation of the hypofunctioning reward circuitry in individuals with high BMI. The striatum plays a crucial role in encoding reward from food intake. The food intake is associated with neurotransmitter dopamine²⁵⁵. Obese individuals have smaller density of striatal D2 receptor compared to lean individuals^{256–258}. After palatable food intake, obese persons exhibited attenuated activation of striatal dopamine target regions (*i.e.* caudate, putamen)²⁵⁹. Interestingly, striatum of obese individuals is more activated in response to pictures of food than in lean controls²⁶⁰.

Furthermore, we noticed that increased craving for the dietary lipids is associated with high oral detection threshold for linoleic acid. This finding agrees with findings that high consumption of dietary lipids causes decrease of oral lipid sensitivity both humans²⁰² and rodents²⁶¹. We did not analyze daily diet of the participants in the study. Yet, a few of the participants did not follow the necessary 2 hours fasting before the taste test and the resulting linoleic acid oral detection threshold was significantly higher than we expected (data not shown). We asked the participants to come again to perform the taste test one more time under the proper conditions. The divergent data were excluded from the final calculation.

6 Conclusion

The results from the five performed studies prove that high adiposity is closely related to orosensory lipid perception. Obese subject showed lower oral sensitivity to dietary lipids. Moreover, we observed increased craving for fatty food with growing weight. Furthermore, summary results from fatty taste and bitter taste show that low orosensory detection is also present in other taste modalities.

Comprehensive interpretation of *CD36* genetic polymorphisms remains complicated. Mechanism of taste is complex process. Determined orosensory detection threshold is an outcome of vast number of factor where these factors act together is different ratios. Therefore, we can state that genetic polymorphisms do not play the crucial role in taste but their effect is undeniable.

7 Bibliography

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8 Appendices

Publication Related to the Dissertation:

Appendix A

Mrizak I, Šerý O, **Plesník J**, et al. The A allele of cluster of differentiation 36 (CD36) SNP 1761667 associates with decreased lipid taste perception in obese Tunisian women. *Br J Nutr*. 2015;113(8):1330-7. doi: 10.1017/S0007114515000343.

Appendix B

Sayed A, Šerý O, **Plesník J**, et al. CD36 AA genotype is associated with decreased lipid taste perception in young obese, but not lean, children. *Int J Obes (Lond)*. 2015;39(6):920-4. doi: 10.1038/ijo.2015.20.

Appendix C

Daoudi H, **Plesník J**, Sayed A, et al. Oral Fat Sensing and CD36 Gene Polymorphism in Algerian Lean and Obese Teenagers. *Nutrients*. 2015;7(11):9096-104. doi: 10.3390/nu7115455.

Appendix D

Karmous I, **Plesník J**, Khan AS, et al. Orosensory detection of bitter in fat-taster healthy and obese participants: Genetic polymorphism of CD36 and TAS2R38. *Clin Nutr*. 2017 Jun 21. pii: S0261-5614(17)30215-7. doi: 10.1016/j.clnu.2017.06.004.

Appendix A

The A allele of Cluster of Differentiation 36 (CD36) SNP 1761667 Associates with Decreased Lipid Taste Perception in Obese Tunisian Women

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The A allele of cluster of differentiation 36 (CD36) SNP 1761667 associates with decreased lipid taste perception in obese Tunisian women

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Abstract

Recent studies have suggested that excessive intake of dietary fat is associated with obesity. Some obese subjects have been reported to exhibit high thresholds for the gustatory detection of lipids via lipid receptors, such as cluster of differentiation 36 (CD36). We studied lingual detection thresholds for emulsions containing oleic acid in obese Tunisian women (*n* 203) using a three-alternative forced choice (3-AFC) method. Genotyping of the *TNF-α* (rs1800629), *IL-6* (rs1800795) and *CD36* (rs1761667) genes was performed to associate with lipid taste perception thresholds. The *CD36* genotype distribution was as follows: GG (*n* 42), AG (*n* 102) and AA (*n* 59). Women with the *CD36* GG genotype exhibited oral detection thresholds for oleic acid that were more than three times lower than those with the *CD36* AA genotype. The present study confirms a high threshold of gustatory fat detection in obese women with the *CD36* AA genotype, but there is no significant association with the *IL-6* and *TNF-α* gene polymorphisms.

Key words: Obesity; Lipids; Taste; Genes

As the obesity epidemic continues, more subjects are getting fatter and are therefore at increased risk for metabolic complications, hypertension and cancer-related mortality^(1,2). The aetiology of obesity is multifactorial, and genetic inheritance and behavioural/environmental causes are considered to be the main factors⁽³⁾. Dietary fat is considered palatable to humans, and several factors, including its olfactory, visual and textural properties, have been proposed as playing a key role in the attractiveness of fat⁽⁴⁾. Humans and rodents can detect long-chain fatty acids in their diets as gustatory cues^(4–9). Some recent studies have shown that obese subjects exhibit a high preference for dietary lipids as compared to lean subjects^(10,11), which suggests that inappropriate lipid perception might influence obesity risk by impacting feeding behaviour. In fact, obesity is associated with a low sensitivity to the oro-sensorial detection of fat^(10,11).

Lingual cluster of differentiation 36 (CD36), like G protein-coupled receptor 120 (GPR120) and G protein-coupled receptor 40 (GPR40), has been shown to act as a lipid receptor

that is involved in a spontaneous preference for fat^(6,12–15). The lingual lipid receptors bind to long-chain fatty acids, which are released by lingual lipases in the buccal cavity^(12–15). We performed the present study on *CD36* SNP because CD36 is a high-affinity receptor, whereas GPR120 and GPR40 are low-affinity receptors. In addition, GPR40 could not be detected on human lingual epithelium⁽¹⁶⁾. Moreover, Sclafani *et al.*⁽¹⁷⁾ have shown that CD36 is directly involved in early fat detection, whereas GPR120 plays a role in the post-ingestive regulation of fat preference⁽¹⁸⁾.

Recent studies have shown that CD36 protein expression is influenced by *CD36* gene polymorphism, and it is related to the detection threshold of dietary lipids in obese subjects⁽¹⁹⁾. Keller *et al.*⁽²⁰⁾ reported that obese subjects with the *CD36* AA genotype (rs1761667) perceived more creaminess in salad as compared to those with the AG or GG genotypes. Pepino *et al.*⁽¹⁹⁾ reported that obese subjects with the AA genotype exhibited higher oral detection thresholds for fat than those with the AG and GG genotypes. These novel findings

Abbreviations: GPR120, G protein-coupled receptor 120; HbA1c, glycosylated Hb.

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are changing our view about the pathogenesis of obesity; however, future studies must be conducted to confirm these interesting findings, particularly in developing countries where obesity is quickly rising.

In chronic pathological conditions such as obesity, IL-6 plays a synergic role in inflammation^(21–23), because macrophages within adipose tissue might secrete IL-6⁽²²⁾. An association between the rs1800795 polymorphism of the *IL-6* gene and increased adiposity, inflammation and metabolic disturbances has been demonstrated^(24,25). In obesity, adipose tissues also secrete TNF- α abundantly⁽²⁶⁾, and rs1800629 polymorphism of the *TNF- α* gene⁽²⁷⁾ has been reported to be associated with obesity risk⁽²⁸⁾ as well as a high incidence of type 2 diabetes⁽²⁹⁾. Because obesity is marked by inflammation, the present study is also designed to explore the relationship between pro-inflammatory markers (IL-6, TNF- α and C-reactive protein) and the oro-sensorial detection of lipids in obese subjects.

There has recently been a rapid upsurge in overweight/obesity and obesity-related diseases in Tunisia, especially in women as compared to men^(30,31). This sex gap between women and men differs greatly according to environmental and socio-economic conditions⁽³¹⁾. Keeping in mind the aforementioned literature on lipid oral taste sensitivity and *CD36* SNP, we thought it would be worthwhile to investigate whether *CD36* SNP in obese Tunisian women is associated with decreased fat taste perception.

Materials and methods

Subjects

Inclusion criteria. Obese women (n 203) were recruited from the group of patients who visited the gynaecology outpatient department (OPD) of Farhat Hached University Hospital, Sousse (Tunisia), in 2012 and 2013 for a general health check-up. Medical records were screened by specialist clinicians. The studied women were between 38 and 43 years old. The women were asked to return to the gynaecology OPD when they were in their first week of menstruation, and they were given an appointment for a particular date so that blood sampling and an analysis of other parameters could be performed.

The exclusion criteria included smoking, diabetes, breast-feeding, pregnancy-related complications, a history of gestational diabetes, the use of oral contraception, chronic illness such as hypertension or any other inflammatory pathology, any autoimmune disease, any lipid-lowering medication, recent weight loss, dieting and the use of any medications known to affect taste. The inclusion criterion constituted a normal glucose tolerance test and electrocardiograms.

Anthropometrics. Body weight and height were measured in the morning while participants were unclothed and not wearing shoes. BMI was calculated as body weight (in kg) divided by height (in m²). Obesity was defined as a BMI of 30 kg/m² or higher, in accordance with the recommendations of WHO. The characteristics of the women are shown in Table 1.

Table 1. Clinical characteristics of obese Tunisian women (n 203) (Mean values and standard deviations)

Parameters	Mean	SD
Weight (kg)	86.4	16.3
Height (cm)	158	6
BMI (kg/m ²)	34.6	4.2
TNF- α (pg/ml)	131.4	165.5
IL-6 (pg/ml)	73.3	48.7
Age (years)	38.4	11.4
Glucose (mmol/l)	5.97	1.84
Urea (mmol/l)	3.39	1.58
Creatinine (μ mol/l)	60.39	14.58
Cholesterol (mmol/l)	5.08	1.29
TAG (mmol/l)	1.77	1.06
HDL (mmol/l)	1.14	0.31
LDL (mmol/l)	3.04	1.01
HbA1c (%)	5.60	1.22
ApoA1 (g/l)	2.04	0.74
ApoB (g/l)	1.26	1.13
Insulin (pmol/l)	78.76	67.64
CRP (mg/l)	7.8	7.8

HbA1c, glycosylated Hb; CRP, C-reactive protein.

Ethics

The present study was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association and was approved by Farhat Hached Hospital Committee for Research on Human Subjects (Tunisia). Informed written consent was obtained from all of the subjects. The present experimental protocol conforms to the relevant ethical guidelines for human research.

Blood samples

Fasting venous blood samples were collected from each woman to obtain plasma (EDTA tubes) and serum. Serum and plasma were prepared by centrifugation (1000 *g* at 20 min). Plasma was immediately used for glucose determination. Serum was aliquoted and frozen at -80°C for further analysis of blood parameters.

Determination of blood parameters

Serum TAG, total cholesterol and free cholesterol concentrations were determined using enzymatic methods, according to the manufacturer's instructions furnished with the kit (Boehringer). HDL-cholesterol was also measured by a kit (Boo Scientific). LDL-cholesterol concentrations were calculated according to Friedewald *et al.*⁽³²⁾. All biochemical parameters were analysed on a Synchron CX7 Clinical System (Beckman). Plasma fasting glucose was determined by the glucose oxidase method with a glucose analyser (Beckman Instruments). Plasma glycosylated Hb (HbA1c) concentrations were determined by isolab column chromatography⁽³³⁾. Insulin serum concentrations were determined using an Insulin IRMA kit (Immunotech; Beckman Coulter, Inc.) with a detection limit of 0.5 $\mu\text{IU/ml}$ (3.4725 pmol/l). The inter-assay CV was 3.3 and 4% for the 13 and 54 IU/ml (90.285 and 375.03 pmol/l) concentrations, respectively.



Serum concentrations of urea, creatinine and C-reactive protein were analysed by routine standard techniques using an automated Synchron CX7 Clinical System (Beckman). Serum concentrations of IL-6 and TNF- α were measured with ELISA kits (Immunotech).

Oleic acid sensitivity analysis

Taste emulsions containing food grade oleic acid (Sigma) were prepared according to Chalé-Rush *et al.*⁽³⁴⁾. EDTA (0.01%, w/v) was added to prevent fatty acid oxidation. The emulsions were sonicated for 4–5 min in a Labo-Modern sonicator at 4°C in an ice bath. Samples were stored in opaque polypropylene tubes and used for testing within 48 h of preparation. Control samples were prepared in the same way but without added oil.

The women were called on a stipulated date and advised to arrive early in the morning without having eaten breakfast (fasting state). The subjects were weighed, and a blood sample was drawn before the preference test to assess blood parameters. Taste preference tests for dietary lipids were performed by employing oleic acid at different ascending concentrations (0.018, 0.18, 0.37, 0.75, 1.5, 3, 6 and 12 mmol/l) by using a three-alternative forced choice (3-AFC) method⁽³⁵⁾. According to the 3-AFC method, the patients were instructed to taste, one by one, three solutions; two of the solutions contained a control substance (acacia gum, 0.01%), and the third one contained oleic acid in a solution that also included acacia gum (0.01%). The acacia gum was used to mimic the textural properties of oil in the control solution.

We increased the concentration of oleic acid in the test solution when a single incorrect response was given, and we decreased the quantity of this fatty acid after two correct responses, in accordance with the method described by Pepino *et al.*⁽¹⁹⁾. A reversal in the response was considered when the concentration sequence changed direction. The procedure was terminated when there were four reversals that met the following two criteria. First, there could not be more than two dilution steps between two successive reversals. Second, the series of reversals could not form an ascending pattern. The threshold concentration was calculated as mean of log values for the last four reversals. To avoid visual and olfactory cues, the testing session was conducted under red light and participants used nose clips. The women were not allowed to drink the solutions; rather, they had to spit out each solution after keeping it in their mouths for a few seconds. If they responded that they observed no difference in the taste sensation, we increased the concentration of oleic acid. If they were able to detect a difference, it meant they were capable of detecting the presence of 'fatty taste'.

Genotyping analyses

Genomic DNA was extracted from 5 ml of whole blood with the use of a commercially available DNA isolation kit (Wizard Genomic DNA purification kit; Promega Corporation) according to the manufacturer's protocol. Genotyping of

TNF- α – 308 G/A (rs1800629), IL-6 – 174 G/C (rs1800795) and CD36 A/G (rs1761667) was performed according to methods that have been previously used by our laboratory^(36,37). The PCR primers for the three genotypes were as follows: (TNF- α : 5'-AGG CAA TAG GTT TTG AGG GGC AT-3' and 5'-CGG GGA AAG AAT CAT TCA ACC AG-3'; CD36: 5'-CAA AAT CAC AAT CTA TTC AAG ACCA-3' and 5'-TTT TGG GAG AAA TTC TGA AGA G-3'; IL-6: 5'-ACT TTT CCC CCT AGT TGT GTC TTT C-3' and 5'-AGA ATG AGC CTC AGA CAT CTC CAG T-3'). PCR amplification reactions were performed in a Veriti thermal cycler (Life Technologies). After initial denaturation for 3 min at 95°C, DNA was subjected to further amplification as follows for TNF- α and IL-6: denaturation for 30 s at 95°C, annealing for 30 s at 66°C and extension for 30 s at 72°C. After thirty-five cycles, a final extension for 5 min at 72°C was used. Amplified DNA was digested by either endonuclease NcoI (TNF- α) or TaqI (IL-6) and further incubated at either 37°C for 16 h (TNF- α) or 65°C for 5 h (IL-6). For CD36, the conditions were as follows: denaturation for 30 s at 95°C, annealing for 30 s at 56°C and extension 30 s at 72°C. After forty cycles, a final extension for 5 min at 72°C was used. Amplified DNA was digested by HhaI at 37°C for 30 min. The digestion products were analysed by 2% (w/v) agarose gel electrophoresis (Elisabeth Pharmacon) containing ethidium bromide, and DNA fragments were visualised under UV light. The following fragments were detected for TNF- α : 264 bp (GG genotype), 264 and 284 bp (AG genotype) and 284 bp (AA genotype). For IL-6, two fragments of 24 and 180 bp (G allele) and an unrestricted fragment of 204 bp (C allele) were obtained. Two fragments of CD36 (138 and 52 bp) in the presence of the G allele were visualised, and an unrestricted fragment (A allele) had a length of 190 bp.

Statistical analysis

CSS Statistica software (StatSoft) was used for statistical analysis. An ANOVA was used for correlation of the different parameters and genotypes. A Kruskal–Wallis test was used for one-way analyses on ranks. The χ^2 test was used for the comparison of genotype frequencies. Fisher's exact test was used for the comparison of allelic frequencies. For correlation studies, we used Pearson's correlation coefficient method. Dunn's method was used for all pairwise multiple comparisons between the AA, AG and GG genotypes and the detection thresholds.

Results

Subject characteristics

Table 1 shows the anthropometric measures and concentrations of different blood parameters in the present cohort of obese Tunisian women (*n* 203). The values of glucose, insulin, urea, creatinine, cholesterol, HDL, LDL, HbA1c, apoA1, apoB, insulin and C-reactive protein were within normal ranges for obese women. Serum TAG, IL-6 and TNF- α concentrations were higher in the women as compared to previously reported control values^(38,39).

Table 2. Genotype frequencies in obese Tunisian women

Gene	SNP ID	Genotype	All subjects	Frequencies
<i>CD36</i>	rs1761667	GG/AG/AA	42/102/59	0.21/0.50/0.29
<i>TNF-α</i>	rs1800629	GG/GA/AA	140/56/7	0.69/0.27/0.04
<i>IL-6</i>	rs1800795	GG/GC/CC	146/47/10	0.72/0.23/0.05

CD36, cluster of differentiation 36.

CD36 genotype and oleic acid detection thresholds

Table 2 shows the genotype frequencies of three polymorphisms in the present cohort of obese women. Fig. 1 shows that the subjects with the GG genotype of the *CD36* gene had thresholds for oleic acid detection that were 3.3 times lower than those of subjects with the AA genotype (95% CI of relative risk 2.5032, 4.4298, OR 9.9615; 95% CI of OR 6.2101, 15.9793). We did not observe a statistically significant difference in the taste detection thresholds of subjects with the AG or the AA (or GG) genotypes. It is also noteworthy that some subjects, which have been termed non-tasters, could not detect fatty acid even at the highest concentration. There were a total of four non-tasters in the AA, AG and GG genotypes of *CD36* gene (Fig. 1).

Association between cholesterol, LDL and glycosylated Hb and CD36 polymorphism

Fig. 2(a) and (b) shows that cholesterol and LDL concentrations were significantly lower in subjects with the *CD36* GG and AG genotypes than in subjects with the AA genotype ($P < 0.01$). Interestingly, the women with the GG genotype exhibited higher HbA1c plasmatic concentrations than did those with the AA genotype ($P < 0.05$) (Fig. 2(c)).

CD36 genotypes and TNF-α and IL-6 concentrations

Serum concentrations of TNF-α was higher in women with the *CD36* AA genotype as compared to subjects with the GG genotype (Fig. 3(a)). Interestingly, serum IL-6 concentrations were lower in women with the AA and AG genotypes than they were in women with the GG genotype (Fig. 3(b)).

Association between TNF-α polymorphism and creatinine serum level and association between IL-6 polymorphism and IL-6 serum level

Fig. 4 shows that the women with the *IL-6* GG and *TNF-α* GG genotypes exhibited higher serum IL-6 and creatinine concentrations, respectively, than did those with the *IL-6* CC and *TNF-α* AA genotypes. Moreover, we did not observe a statistical association between the *TNF-α* SNP and serum TNF-α concentrations ($P > 0.05$).

Discussion

Evidence suggests that there might be a sixth taste modality that is devoted to the oro-gustatory perception of dietary lipids^(14,40). Hence, it seems imperative to explore and better

understand the mechanisms that underlie the oro-gustatory detection of dietary fat in order to help prevent and treat obesity^(5,41). A number of studies have suggested that lingual CD36, a glycoprotein that is highly expressed in circumvallate papillae, is implicated in the perception of dietary fat taste^(6,12–15). In the present study, we confirm that obese women with the *CD36* AA genotype (rs1761667) possess higher thresholds for lipid taste sensitivity than do those with GG genotypes.

Keller *et al.*⁽²⁰⁾ have provided preliminary evidence that CD36 is involved in human oral fat perception and the human attraction to added fats and oils in food. Pepino *et al.*⁽¹⁹⁾ have demonstrated that *CD36* gene polymorphism, which results in a decrease in the gene's expression, is responsible for an increase in the oral detection threshold of dietary lipids in obese subjects. Aside from the present study, no confirming or refuting report is available on this subject, particularly from developing countries where diets are rich in fat. The present data strongly suggested that the oro-sensorial perception of fat taste is altered in some obese subjects. Indeed, we showed that the A allele of *CD36* rs1761667 polymorphism in obese women, which was previously associated with decreased expression of the CD36 protein, is associated with a high oro-gustatory threshold detection for oleic acid. Conversely, the subjects with the G allele were more sensitive in their oleic acid lingual detection as compared to the subjects with the A allele. These data corroborate not only the clinical findings of Pepino *et al.*⁽¹⁹⁾ but also experimental data where an association between *CD36* gene expression and oral fat detection has been demonstrated⁽⁴²⁾. Mice with partial *CD36* gene knockout (*CD36*^{+/-}) had lower CD36 protein expression and a lower oral fat detection threshold than wild type animals (*CD36*^{+/+}). The *CD36* knockout (*CD36*^{-/-}) failed to exhibit a spontaneous preference for fat.

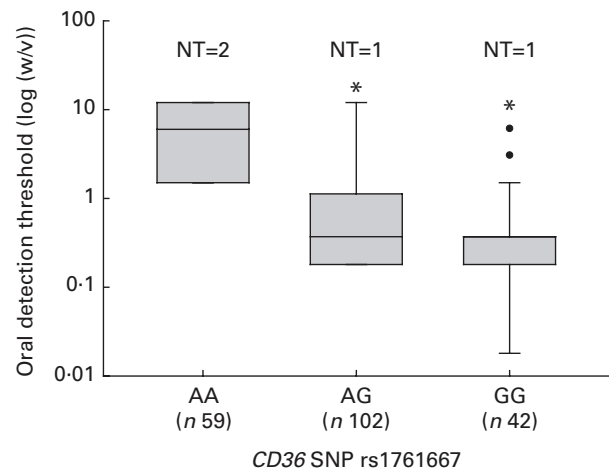


Fig. 1. Oleic acid detection thresholds in obese Tunisian women. The women ($n = 203$) had either the AA genotype ($n = 59$) or the GG ($n = 42$) or AG ($n = 102$) genotype of the cluster of differentiation 36 (*CD36*) gene. The figure shows the box plots of the medians, first and third quartiles, standard deviations and extreme values. The difference between the three groups was statistically significant ($P < 0.001$; Kruskal–Wallis test). * Median value was significantly different from that of the AA genotype ($P < 0.05$; one-way ANOVA). The difference between the AG and GG genotypes was not statistically significant. NT, non-tasters.

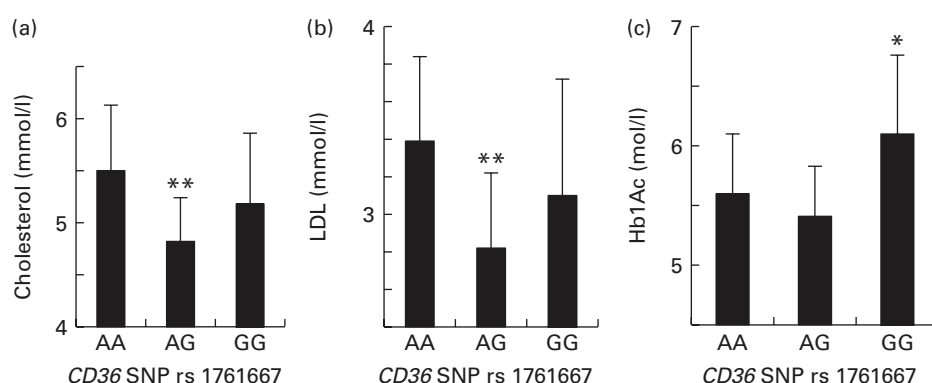


Fig. 2. Association between blood parameters and cluster of differentiation 36 (*CD36*) SNP in obese Tunisian women. Concentrations of cholesterol (a), LDL (b) and glycosylated Hb (HbA1c) (c) in women with the AA, AG or GG genotype of the *CD36* gene. Values are means, with standard deviations represented by vertical bars. Mean value was significantly different from that of the AA genotype: * $P < 0.05$, ** $P < 0.01$ (one-way ANOVA).

A low detection threshold for fat in the present study may not have been caused by the low expression of α -gustducin, a marker of taste receptor cells. Indeed, alteration in *CD36* expression is not related to changes in α -gustducin expression⁽⁴²⁾. Moreover, α -gustducin knockout mice, like wild type animals, exhibited an unaltered preference for dietary fat⁽⁴³⁾. It is possible that other proteins which are likewise involved in fat taste detection, such as GPR120, might also participate in low-fat taste sensitivity⁽⁴⁴⁾. However, the *CD36* and GPR120 receptors seem to be differently regulated in lipid taste perception^(18,44).

In the present study, we also observed that some of the subjects failed to detect oleic acid in the emulsions. These subjects were defined as non-tasters, and they were also reported by Kamphuis *et al.*⁽⁹⁾. The mutation responsible for gustatory insensitivity to fatty acid in non-taster subjects deserves further in-depth study.

We performed the present study on Tunisian obese women (who probably eat an above-average amount of fatty food due to cultural customs in Tunisia), because it has been shown that some obese subjects had a low sensitivity to oleic acid^(35,45). Oral and gastrointestinal sensitivity to oleic acid are related to each other, and they are decreased in obese subjects⁽³⁵⁾. Nonetheless, the present association studies cannot distinguish whether the decreased sensitivity to fat in obese women is a cause or a consequence of obesity. However, Stewart *et al.*⁽¹¹⁾ have shown that oral sensitivity towards oleic acid in lean subjects is decreased with a high-fat diet and increased with a low-fat diet. Brennan *et al.*⁽⁴⁶⁾ have reported that acute dietary restriction in obese subjects enhances their gastrointestinal sensitivity to fat, and this is associated with an increased effect of fat on satiation. In addition, a high-fat diet has been shown to decrease the expression of *CD36* in mice⁽⁴²⁾.

We observed an association between the *CD36* AA genotype and high serum levels of cholesterol and LDL in obese women. Because the *CD36* A allele was previously associated with reduced expression of the *CD36* gene, it is possible that high blood lipid concentrations are the result of their curtailed uptake by adipocytes that also express *CD36*; in this case, it acts as a fatty acid transporter^(47,48). Moreover, *CD36* gene

polymorphisms have been significantly associated with high TAG concentrations among ethnic Chinese in Taiwan⁽⁴⁹⁾.

Interestingly, obese women with the *CD36* G allele had higher plasma HbA1c concentrations than women with the A allele. These observations corroborate the findings of Rać *et al.*⁽⁵⁰⁾, who have shown that the GG genotype was significantly associated with higher HbA1c concentrations as compared to the AA genotype of *CD36* in obese children.

We observed that obese women had high IL-6 and TNF- α serum concentrations. Homozygous women with the *CD36* AA genotype had higher TNF- α serum concentrations than did those with the GG or AG genotypes. Conversely, IL-6 serum concentrations were higher in women with the GG genotype than they were in women with the AA or AG genotypes. The importance of the association of high serum levels of TNF- α with the *CD36* AA genotype and the association of high serum levels of IL-6 with the *CD36* GG genotype is not well understood. These cytokines play a key role in the regulation of insulin sensitivity in subjects who are suffering

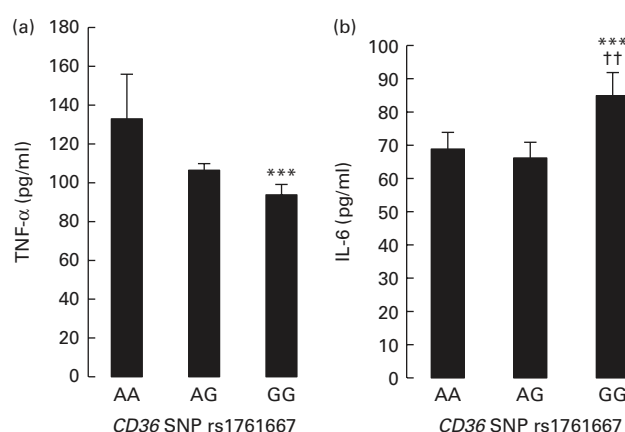


Fig. 3. Serum TNF- α (a) and IL-6 (b) concentrations in obese Tunisian women with the AA, AG or GG genotype of the cluster of differentiation 36 (*CD36*) gene. The serum concentrations of cytokines were determined as described in the Materials and Methods section of the present paper. Values are means, with standard deviations represented by vertical bars. ***Mean value was significantly different from that of the AA genotype ($P < 0.001$; one-way ANOVA). ††Mean value was significantly different from that of the AG genotype ($P < 0.01$; Fisher's exact test).

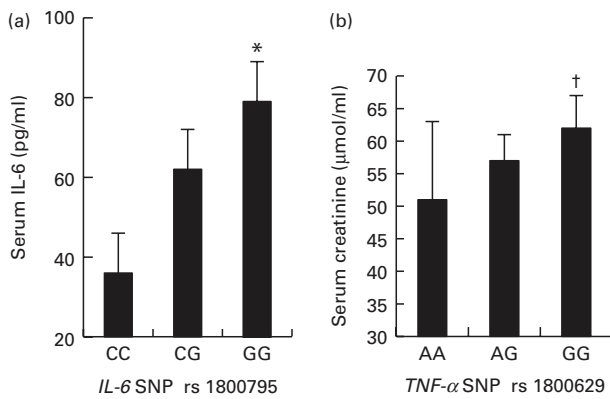


Fig. 4. Serum IL-6 (a) concentrations in obese Tunisian women with the IL-6 polymorphisms CC, CG or GG. Serum creatinine (b) concentrations in obese Tunisian women with the TNF- α polymorphisms AA, AG or GG. Values are means, with standard deviations represented by vertical bars. *Mean value was significantly different from that of the CC genotype ($P < 0.05$; Fisher's exact test). †Mean value was significantly different from that of the AA genotype ($P < 0.05$; one-way ANOVA).

from obesity and metabolic syndrome⁽⁵¹⁾. The SNP of these cytokines have been suggested to predispose for obesity⁽⁵²⁾. The homozygous subjects with the GG genotype exhibited high serum IL-6 concentrations. These observations are in close agreement with the results of Pereira *et al.*⁽⁵³⁾, who studied the association between the *IL-6* gene and plasma IL-6 concentrations in community-dwelling and institutionalised older women. Those authors reported that women with the *IL-6* GG genotype had high IL-6 serum concentrations. High serum IL-6 concentration in homozygous subjects with the GG genotype might take part in increased fat oxidation in response to fat load in obesity, as has been suggested elsewhere⁽⁵⁴⁾. With regards to TNF- α , we noticed a positive relationship between circulating creatinine concentrations and the TNF- α GG genotype, which indicates that the present subjects might be at risk for renal complications. Chang *et al.*⁽⁵⁵⁾ have shown that the G allele of the TNF- α gene was associated with high serum creatinine concentrations that increased the risk for contrast-induced nephropathy. We also observed a significant association between *IL-6* and TNF- α gene polymorphisms in obese women, which indicates that inflammatory status, as indicated by pro-inflammatory cytokines, is a key element of obesity in these women. Curtis & Singh⁽⁵⁶⁾ have likewise shown that the SNP of these two cytokines predispose for obesity.

Finally, we can state that a major value of the present study is that it validates the importance of a common *CD36* SNP rs1761667 in obese women. The present results must be confirmed by additional studies in other developing countries. It is also possible that in the present study, there might be an influence of female sex hormones on fat taste perception and other parameters. At this stage, it is difficult to determine whether oral fat perception sensitivity affects fat intake or body weight. Future studies are needed to answer these important questions. The stimulation of taste receptors, such as CD36, by synthetic fatty acid analogues within the oral cavity may provide a new target for obesity treatment.

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The authors' contributions are as follows: N. A. K. designed the research (project conception, development of the overall research plan and study oversight); I. M. conducted the research (hands-on conduct of the experiment and data collection); N. A. K. and O. S. wrote the manuscript; O. S. supervised the SNP research and statistical analysis; J. P. completed the technical part of the SNP analysis and participated in writing the manuscript; A. A., M. F., A. B. and M. Z. provided the facilities in the sample collections; N. A. K. and Z. T. supervised the study. All authors have read and approved the final content of the manuscript.

The authors declare no conflicts of interest.

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Appendix B

CD36 AA genotype is Associated with Decreased Lipid Taste Perception in Young Obese, but not Lean, Children

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Technical part of the SNP analysis

Statistical analysis

Participation in writing the manuscript

ORIGINAL ARTICLE

CD36 AA genotype is associated with decreased lipid taste perception in young obese, but not lean, children

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BACKGROUND/OBJECTIVE: Obesity is an alarming threat for all age groups, including children. Fat overconsumption is one of the factors that directly influences this pathology. Recent studies have suggested that a common variant in the CD36 gene, that is, single-nucleotide polymorphism (SNP) rs1761667-A allele, that reduces CD36 expression, associates with high oral fat detection thresholds in some obese subjects. The objective was to assess fatty acid sensitivity in relation to CD36 SNP in young lean and obese children.

SUBJECTS/METHODS: We studied lingual detection thresholds for emulsions, containing oleic acid, in Algerian children ($n=116$, age = 8 ± 0.5 years) who were divided into two groups: obese ($n=57$; body mass index (BMI) z-score = 2.513 ± 0.490) and lean children ($n=59$; BMI z-score = -0.138 ± 0.601) by alternative-forced choice method. To correlate the lipid taste perception thresholds with CD36 SNP, the children were genotyped for A/G SNP rs1761667 in 5'UTR region of CD36 by using PCR and restriction fragment length polymorphism.

RESULTS: We noticed significantly higher CD36 A-allele frequency ($P=0.036$) in young obese children compared with leans. CD36 A-allele was associated with higher lipid taste perception thresholds than G-allele in obese children, but not in lean controls. Moreover, waist circumference was positively correlated with reduced fat taste sensitivity in these children.

CONCLUSIONS: CD36 SNP A-allele, being present both in young lean and in obese children, is associated with high threshold for fatty acid taste sensitivity only in obese children.

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INTRODUCTION

Obesity is one of the biggest worldwide health problem with rising prevalence. Since 1980, the incidence of obesity is almost doubled with ~35% of adults being overweight and ~11% of adults being obese.¹ This rising trend of obesity is also observed in children. Worldwide prevalence of obesity among children rose from 4.2% in 1990 to 6.7% in 2010.² Obesity is considered as multifactorial and polygenic disease, and onset of this disease can be caused by various factors.¹ One of the reasons of the onset of obesity is altered energetic homeostasis, particularly caused by an increase in consumption of high caloric diet containing large amount of fat.¹

It is known that rodents and humans can detect long-chain fatty acids, present in their diet, as gustatory cue.^{3,4} The CD36, known as fatty acid translocase, and GPR120, a G protein-coupled receptor, have been shown to act as receptors in the tongue, involved in the detection of dietary fatty acids. Indeed, the mice lacking the expression of CD36 and GPR120 lose the spontaneous preference for solutions containing oily emulsions.^{3,5}

Some recent studies have shown that obese subjects exhibit a high intake of dietary lipids as compared with lean subjects,⁶ suggesting that altered lipid perception might influence obesity risk by impacting feeding behavior. Besides, obese subjects have been shown to exhibit lower oral sensitivity for a dietary fatty acid than lean subjects, and hypersensitivity to the taste of oleic acid (OA) (C18:1) was associated with decreased consumption of dietary fats and low BMI.^{6,7} However, the fatty acid taste sensitivity

may be modulated by environmental factors such as exposure to a high-fat diet.⁶

In humans, the CD36 gene polymorphism, resulting in its decreased expression, is responsible for an increase in the detection threshold for oral dietary lipids in some obese subjects.⁸ Keller *et al.*⁹ shed light on the association of CD36 gene polymorphism with oro-sensorial detection of high-fat foods and obesity in African-American adults. By employing a self-reported taste test, these investigators observed that participants with A/A genotype at rs1761667 had greater perceived creaminess, regardless to fat concentration of the salad dressings. Later on, Pepino *et al.*⁸ used CD36 single-nucleotide polymorphism (SNP) rs1761667 and showed that some of obese subjects with CD36 AA genotype exhibited higher oral detection thresholds for fat than subjects with AG and GG genotypes. These novel findings, available only in obese subjects, are changing our view on the understanding of pathogenesis of obesity. No comparative study on lean and obese subjects is available. It would be interesting to know whether CD36 SNP would have a role in fat detection in low-age group children. We, therefore, designed the present study to determine the role of CD36 rs1761667 SNP in gustatory perception of OA in lean and obese Algerian children.

MATERIALS AND METHODS

Subjects

We recruited one hundred sixteen ($n=116$) school children, aged from 7 to 8 years, from Constantine district in Algeria by employing a multi-stage

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cluster random sampling method. The medical check-up was routinely performed by physicians, nurses and physician assistants of Primary Health Care Centers. The children having any history of a chronic condition such as cardiovascular disease, diabetes, liver or kidney disease were excluded from the study. A written consent from parents was obtained for the participation of the children, and they were assured about the confidentiality of the project. The parents were informed about the purpose, protocol and potential risks of the study. All personal data related to names and dates of birth were erased from the database. The study protocol was approved by the research council of the University of Constantine1.

Body mass index criteria and questionnaire on eating food pattern

Body mass index (BMI=weight/(height)²) is generally used to assess overweight or obesity in adults where a BMI of $\geq 25 \text{ kg m}^{-2}$ but $< 30 \text{ kg m}^{-2}$ is generally defined as overweight and a BMI of $\geq 30 \text{ kg m}^{-2}$ as obese. However, in our study, we used the growth graphs, that is, z-scores, of WHO 2007 to determine the overweight and obesity in young children.

The children were also subjected to a questionnaire to collect the information about their habitual preferred eating patterns. The questionnaire contained the list of food products, generally consumed in Algeria and served at breakfast, lunch and dinner, with a standard serving meal size. We asked the question 'what do you eat preferably in the week among the listed food items' and the 'response' was noted. In fact, we determined 'preferred food pattern' of the children and tried to correlate the same. The food pattern was also, sometimes, cross-checked with the mothers.

OA taste sensitivity analysis

OA (18:1) oro-sensory test was performed as previously described by Stewart *et al.*¹⁰ We prepared emulsions containing food-grade (Sigma, St Louis, MO, USA) OA, as per protocol of Chalé-Rush *et al.*⁴ at various concentrations (0.018, 0.18, 0.37, 0.75, 1.5, 3, 6 and 12 mmol l⁻¹) in deionized water. The solutions contained EDTA (Merck, Darmstadt, Germany) at 0.01% (w/v) to prevent oxidation of OA. The emulsions were homogeneously stirred and sonicated at 4 °C in Labo-Modern sonicator. The samples were aliquoted in opaque polypropylene tubes and used within 24 h. The acacia gum (Sigma) at 0.01% (w/v), present in all the solutions, served as a control to mimic the textural properties of the oils in the control solution.

On the day of tasting, the children were called to come in a fasting state (without taking breakfast), anthropometric parameters were taken and they were subjected to undergo the OA taste sensitivity analysis at different ascending concentrations of OA (0.018, 0.18, 0.37, 0.75, 1.5, 3, 6 and 12 mmol l⁻¹) as per three alternative-forced choice method.^{10,11} The three alternative-forced choice method consists of tasting one-by-one, the three test solutions were two solutions contain a control substance and the third one contains OA. During performing the test, the children were subjected to detect an odd solution, containing OA in the ascending order, in one of the three test solutions unless a single correct response was obtained.^{8,10} The children were presented with two more sets of samples at the same concentration of OA, again, if the response was correct,

the concentration was defined as the detection threshold for OA. We confirmed it by presenting OA solution at a lower concentration and after the negative response; we further went up to the 'correctly' detected OA concentration. Hence, we obtained at least two reversals. The children were asked to rinse their mouth between each new set of samples. During the tasting sessions, conducted in the red light to hide visual cues, the children were advised to wear nose clips.

PCR genotyping

Genomic gDNA was extracted from flocked saliva swabs using automatic DNA extraction system Prepito (Chemagen, Baesweiler, Germany) based on magnetic particles separation. Polymorphism rs1761667 was genotyped using PCR and restriction fragment length polymorphism method. For 25 µl PCR, we used 100 ng of gDNA together with Kapa G2 fast mix (Kapa Biosystems, Wilmington, MA, USA), forward (5'-CAA AAT CAC AAT CTA TTC AAG ACCA-3') and reverse (5'-TTT TGG GAG AAA TTC TGA AGA G-3') primers. The amplified DNA was digested by *HhaI* restriction enzyme (Thermo Fisher Scientific, Waltham, MA, USA). Genotyping of rs1761667 was performed by electrophoresis through a 2% agarose gel where, in case of G-allele, two fragments were identified at the length of 138 and 52 bp. In case of A allele, we observed undigested 190 bp product.

Statistical analysis

For statistical analysis, the CSS Statistica 12 software (StatSoft, Tulsa, OK, USA) was used. We used Student's t-test for comparing heights of subjects. Allele frequencies were analyzed with Fisher's exact test which is used in the analysis of contingency tables. The Hardy-Weinberg equilibrium was assessed by chi-square (χ^2). The Hardy-Weinberg equilibrium is a principle stating that the genetic variation in a population will remain constant from one generation to the next in the absence of disturbing factors. When mating is random in a large population with no disruptive circumstances, the law predicts that both genotype and allele frequencies will remain constant because they are in equilibrium. Dunn's method was used for all multiple comparisons among genotypes and study groups. Dunn's test allows to highlight the difference using multiple and stepdown comparisons. We used Spearman's rank to observe correlation between OA sensitivity and physical parameters. Criterion for statistical significance was $P < 0.05$. Mann-Whitney test was used to compare different genotypes.

RESULTS

Characteristics of the sample population

Out of the total number of subjects ($n=116$) included in the study, 57 were considered as obese with average BMI z-score = 2.513 ± 0.490 and 59 as controls with average BMI z-score = -0.138 ± 0.601 ($F 1.114=51.000$; $P < 0.000001$). The average age of the children was 8 ± 0.5 years (Table 1). The number of boys and girls was 57 and 59, respectively. We also observed the difference in height between lean and obese children. Obese children were more than 3 cm higher than lean controls ($P < 0.0005$).

Table 1. CD36 genotypes in lean and obese children

Characteristics	Control			Obese		
	AA	AG	GG	AA	AG	GG
Age (years)	7.9 \pm 0.5	8 \pm 0.5	8 \pm 0.5	8.2 \pm 0.5	8.3 \pm 0.5	8.2 \pm 0.4
Gender						
Female	7	17	7	11	13	4
Male	5	15	8	11	13	5
BMI z-score	-0.19 \pm 0.74	-0.19 \pm 0.54	0.02 \pm 0.59	2.37 \pm 0.30	2.65 \pm 0.54	2.44 \pm 0.62
Waist (cm)	54.67 \pm 1.82	55.75 \pm 2.55	54.67 \pm 2.44	67.27 \pm 5.25	70.77 \pm 8.09	69.67 \pm 7.74
Number of children	12	32	15	22	26	9
Genotype Frequency	20.34	54.20	25.42	38.60	45.61	15.78

Abbreviation: BMI, body mass index. $\chi^2 P=0.081$; F test, $P=0.04$ (for genotype frequencies) and $P=0.036$ (for allele frequencies).

Genetic analysis

No deviation from the Hardy–Weinberg equilibrium ($P > 0.05$) was observed for the CD36 genotype in control and obese groups. We observed significant deviation in allelic distribution of rs1761667 between obese and control children. Table 1 shows that A allele was higher in obese subjects as compared with control group, whereas the G allele was more common ($P = 0.036$). This observation indicates increased risk for obesity in children with A allele (odds ratio = 1.76, 95% confidence interval = 1.04–2.94, risk ratio = 1.29; confidence interval = 1.01–1.16). Furthermore, we assessed influence of CD36 genotype on BMI in obese subjects and AG genotype was found to be related to 0.14 z-score BMI ($P > 0.05$). This effect was not observed in lean children. There was no significant differences between CD36 genotype frequencies and allele frequencies between male and female subjects ($P = 0.69$).

OA detection threshold

We observed significant difference in oral detection sensitivity for OA between obese and control children ($P < 0.01$).

Figure 1a shows that control subjects exhibit high OA oral sensitivity (low detection threshold). Obese children seem to have 40-fold decreased sensitivity (high detection threshold) for this fatty acid ($P < 0.000005$). Figure 1b shows that there was a positive correlation between waist size and increased oral detection threshold for OA in these children ($P = 0.00009$).

Then, we tried to assess influence of genotype on OA sensitivity in each group. In control group, we did not observe any significant difference (Figure 2a); however, in obese children (Figure 2b), OA detection threshold in A-allele children was higher than that in G-allele children ($P < 0.05$, Mann–Whitney test).

Correlation between CD36 genotypes and preferred food pattern

To compare food behavior and CD36 genotypes, we used Chi-square and F tests. Table 2 shows a correlation between different CD36 genotypes and food pattern. We observed that A allele significantly predisposed children to prefer palatable food products, that is, pasta, chips, chocolates and bread ($P < 0.04$). When we compare habitual food pattern between control and

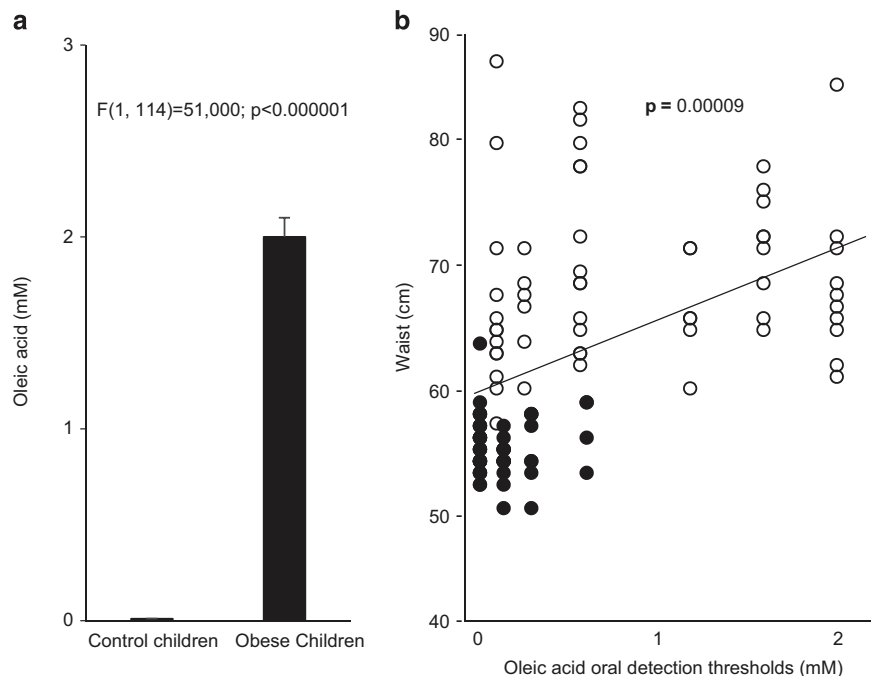


Figure 1. Relationship between BMI and fatty acid sensitivity in young leans and obese children. **(a)** The OA oro-sensorial detection in lean ($n = 59$) and obese children ($n = 57$) is shown. The results are means \pm s.d. **(b)** Spearman rank correlation between waist (cm) and OA oro-sensorial detection in all the children ($n = 116$) is shown. Open and closed circles show, respectively, obese and lean children distribution.

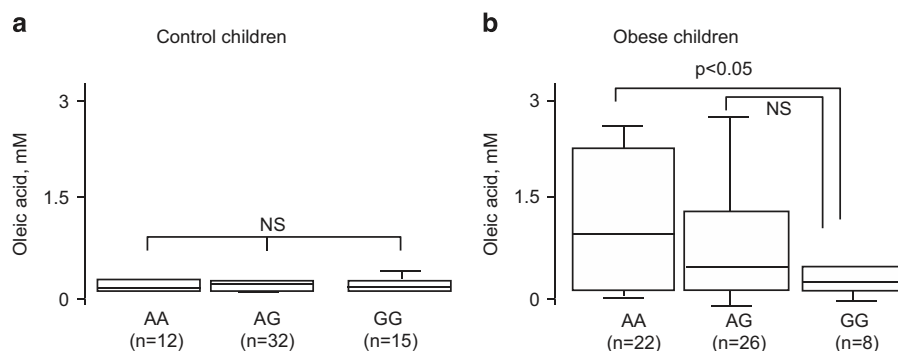


Figure 2. OA detection thresholds in young leans and obese children. The figure shows the box plots of the medians. No statistically significant difference was observed in lean children **(a)** for three genotypes. The difference between AA and GG groups was statistically significant only in obese subjects **(b)** according to Mann–Whitney test ($P < 0.05$). The difference between AG vs GG or AA genotypes is not statistically different in obese children. The results are medians \pm s.d. NS, insignificant differences.

Table 2. Relationship between preferred food pattern and CD36 genotypes

Food contents	AA genotype	AG genotype	GG genotype	Total (n)
Pasta, chips, chocolates and bread	20	20	8	48
Wafer, bread and candy	9	21	6	36
Candy, bread and cheese	5	17	10	32
Total (n)	34	58	24	116

$\chi^2 P = 0.078$; allele frequency difference $P < 0.04$.

Table 3. Relationship between preferred food pattern and obesity

Food contents	Control children	Obese children	Total (n)
Pasta, chips, chocolates and bread	4	44	48
Wafer, bread and candy	23	13	36
Candy, bread and cheese	32	0	32
Total (n)	57	59	116

$\chi^2 P < 0.000001$.

obese children, we observe a statistically highly significant difference between the two groups ($P < 0.001$), as far as the consumption of four food products (pasta, chips, chocolates and bread) and three food products (wafer, bread and candy) is concerned (Table 3).

DISCUSSION

It is becoming clear that the origin of obesity is multifactorial and eating behavior has a significant role in this disease. In recent years, compelling evidence have been accumulated on fat taste, suggesting that dietary lipids can be sensed by oro-gustatory system.^{3,4} The two lipido-receptors, GPR120 and CD36, have been detected in human foliate and circumvallate papillae.¹² The CD36 is high affinity receptor whereas GPR120 exhibit low affinity for fatty acids. The CD36 seems to have a role in fatty acid detection whereas GPR120 seems critically involved in post-prandial feeding behavior.¹³ The importance of CD36 has been exemplified in a recent study linking variants in CD36 gene with oral fat perception and ultimately intake of dietary fat.⁹ In the present study, we shed light on CD36 rs1761667 polymorphism and oro-sensorial detection threshold for a fatty acid in 7–8 years old lean and obese children.

We observed that there was a positive correlation between OA detection threshold and obesity. To our knowledge, this is the first report to show this relationship in 7–8 years old children, though Stewart *et al.*⁶ have shown the BMI was correlated with high thresholds detection of long-chain fatty acids in adult obese subjects. It is possible that high-fat diet in obese children may result in increased thresholds. We can presume that as a result of low sensitivity (high detection thresholds) to fatty acid, there would be excess fat intake in obese children, and high amounts of fats/fatty acids would be required to elicit a response within taste receptor cells, thus contributing to excess energy intake and perhaps increasing obesity. Nonetheless, it is interesting to mention that the detection thresholds for OA in our study are very low as compared with other studies,^{4,8,11} for instance, Stewart *et al.*¹¹ reported that lean and obese subjects had a threshold for OA, respectively, ~ 3 mM and ~ 7 mM.¹¹ The difference in thresholds may be due to the fact that we performed the present study on children and other studies were conducted on adult subjects. It is possible that the children might have lower detections thresholds for fatty acids than adults.

We studied association of SNP rs1761667 located in 5'UTR of CD36 gene with obesity and fatty acids oral sensitivity. We observed significantly higher A-allele frequency in obese children ($P = 0.036$) compared with that in control group. Though the lean children had A allele, they did not show any significant change in fatty acid sensitivity. However, obese children with A allele exhibited significantly higher detection threshold for fatty acid as compared with obese with G allele. Pepino *et al.*⁸ previously reported that A allele was associated with decreased OA oral sensitivity, although these studies were conducted only in obese adult subjects. In support of our observations, we would like to cite the data obtained on mice where CD36 gene deletion (CD36^{-/-}) resulted into failure to exhibit spontaneous preference for fat.¹⁴

It is interesting to note that there was a significant correlation between obesity and palatable food pattern in obese children. Whether daily intake of high energy food influences fatty acid detection thresholds remains not understood. However, Stewart *et al.*¹⁰ have shown that consumption of the high-fat diet significantly increased taste sensitivity thresholds to OA in lean subjects. Furthermore, after a period of fat restriction and attenuation of high-fat diet, the OA sensitivity was decreased, stressing that daily fat intake might be crucial in dietary fat detection. Similarly, subjects with high fat breakfast showed impaired fatty acid sensitivity.¹¹ In another study, Stewart *et al.*⁶ have classified subjects as hypo- or hyper-sensitive to OA detection, and they concluded that hyposensitive subjects consumed significantly more energy, fat, saturated fat, fatty foods (butter, meat, dairy), had greater BMI and were lesser perceptive of small changes in the fat content of custard, compared with hypersensitive subjects. In fact, we observed that CD36 A allele in obese children, associated with high threshold for fatty acid detection (see above), was significantly associated with food intake comprised of palatable ingredients, that is, pasta, chips, chocolates, wafer and candy. Whether other taste modalities are also altered in obese children as compared with control population remains to be studied in future, though there are some reports that taste sensitivity to monosodium glutamate is attenuated in obese women.¹⁵

The present study suggests that the CD36 genotype is not sufficient to impact oral fat sensitivity, because variations in CD36 did not affect fat taste perception in lean children. Further, it suggests that perhaps there is an interaction between CD36 SNP and diet, in such a way that the obese with AA phenotype, while consuming a high fat diet, would exhibit less sensitivity to fatty acids. Our study is not only a confirmatory report on the high fatty acid detection thresholds in obese subjects but also presents new insights in gene and fat taste interaction in young children. The results of our study present some limitations. Our study was conducted on a limited number of children and the observations must be reproduced in different young populations on a large number of subjects. Some studies should be performed on obese and young children, given a low or a high-fat diet for a short period to confirm the oral fatty acid detection sensitivity and BMI and preference of a palatable food.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Appendix C

Oral Fat Sensing and CD36 Gene Polymorphism in Algerian Lean and Obese Teenagers

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Technical part of the SNP analysis

Participation in writing the manuscript

Article

Oral Fat Sensing and *CD36* Gene Polymorphism in Algerian Lean and Obese Teenagers

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Abstract: Growing number of evidences have suggested that oral fat sensing, mediated by a glycoprotein CD36 (cluster of differentiation 36), plays a significant role in the development of obesity. Indeed, a decreased expression of CD36 in some obese subjects is associated with high dietary fat intake. In the present study, we examined whether an increase in body mass index (BMI) is associated with altered oleic acid lingual detection thresholds and blood lipid profile in young Algerian teenagers ($n = 165$). The obese teenagers ($n = 83$; 14.01 ± 0.19 years; BMI z-score 2.67 ± 0.29) exhibited higher lingual detection threshold for oleic acid than lean participants ($n = 82$, 13.92 ± 0.23 years; BMI z-score 0.03 ± 0.0001). We also studied the association between rs1761667 polymorphism of *CD36* gene and obesity. The AA and AG genotypes were more frequent in obese teenagers, whereas GG genotype was more common in lean participants. The A-allele frequency was higher in obese teenagers than that in lean children. We report that rs1761667 polymorphism of *CD36* gene and oro-gustatory thresholds for fat might play a significant role in the development of obesity in young teenagers.

Keywords: CD36; taste; obesity; adolescents; oleic acid

1. Introduction

During the last decades, obesity has become one of the major health issues for our civilization with its increasing prevalence in all age groups. According to WHO, there are more than 1.9 billion obese adults and 42 million overweight young children worldwide [1]. It is generally accepted that obesity is influenced by environmental and genetic factors [2]. However, one of the key factors is also an excess of fat in our diet which, associated with the lack of physical activity, leads to an increase in body mass index (BMI) [3,4]. Dietary fat provides more than twofold energy compared to proteins and carbohydrates, thus high consumption of lipids would worsen obesity and result into several pathologies like atherosclerosis, hypertension, and some other diseases [3,4].

Dietary fat is mainly perceived by its textural properties [5]. Nevertheless, growing evidences indicate the existence of another factor, *i.e.*, taste for fat, which could play a role in the attraction for dietary lipids [6]. There are two main long-chain fatty acid receptors, *i.e.*, CD36 and GPR120,

which play a role in the gustatory detection of lipids. The CD36 (also known as FAT, fatty acid translocase) belongs to the scavenger receptor family, and is known to bind to various ligands such as thrombospondin-1, oxidized low-density lipoproteins, growth hormone (GH)-releasing peptides and also dietary fatty acids [7]. The GPR120 belongs to the G-protein-coupled receptor (GPCR) family and is expressed in human and rodent taste bud cells [8]. Recent studies conducted on animal models and *in vitro* cell cultures showed possible alternative roles of GPR120 and CD36 in oral fat sensing. Hence, GPR120 seems to play a role in post-prandial regulation, whereas CD36 serves as a primary fat taste sensor in the lingual epithelium [6,9–11].

It has been previously shown that a single nucleotide polymorphism (SNP) rs1761667 of *CD36* gene, located in the 5' flanking exon 1A area [10], is associated with the decreased expression of CD36 protein [12]. This *CD36* gene polymorphism has been associated with some pathologies like coronary artery disease [10,13] and type 2 diabetes mellitus [14]. Besides, rs1761667 polymorphism has been shown to influence gustatory perception of dietary lipids in humans. The first evidence of the impact of rs1761667 polymorphism on oral fat sensing was reported by Pepino *et al.* [15] who showed that A-allele is associated with decreased oro-gustatory detection of oleic acid in some Afro-American obese subjects. We recently conducted a study on obese Tunisian women and showed that the participants with A-allele of rs1761667 polymorphism exhibited decreased oral sensitivity (high thresholds) to oleic acid [16]. In another study conducted on young Algerian children age seven to eight, we have observed higher A-allele frequency of rs1761667 polymorphism in obese children compared to leans [17]. As expected, the obese young children exhibited higher detection threshold for oleic acid than lean participants [17]. Moreover, in the recent study Melis *et al.* [18] have shown that high expression of *CD36* (influenced by rs1761667) may be the determining factor for oral detecting of dietary fat predominantly in subjects with the low density of taste papillae.

The early period of childhood and adolescence is critical for the development of obesity in the later stage of life. It has been shown that young obese teenagers, predominantly males, are unable to return to the normal healthy state [19]. Risk factors for childhood obesity include parental fatness, social status, birth weight, timing or rate of growth, physical activity, dietary factors, and other behavioral or psychological factors [20]. Childhood obesity has been shown to result into high central adiposity and high blood pressure including high carotid extra-medial thickness in adulthood [21]. Janssen *et al.* [22] have clearly shown that overweight and obesity during childhood are strong predictors of obesity and risk for coronary heart disease in young adults. Longitudinal studies have demonstrated that the transition from childhood to adulthood should be taken into account to build obesity prediction models [23]. Hence, it seems imperative to know better the predictive factors of childhood obesity to avoid the obesity-associated complications in adulthood.

As mentioned above, there seems a relationship between decreased oral fat sensing and *CD36* SNP in adult and young obese subjects; however, no such study is available in teenagers. We, therefore, conducted the present study to investigate the relationship between rs1761667 polymorphism of *CD36* gene, oral fatty acid detection thresholds in young lean and obese Algerian teenagers.

2. Experimental Section

2.1. Subjects

We recruited ($n = 165$) male and female adolescents from Constantine district in Algeria. All the participants belonged to Arab-Berber ethnicity. The study was conducted on a young population (Table 1). The exclusion criteria for participants were any history of a chronic pathology such as cardiovascular disease, diabetes, liver, or kidney disease. The smokers were also excluded from the study. A written consent was obtained from all participants and their parents, and they were assured about the confidentiality of the study. All personal data, such as names and dates of birth, were erased from the database.

Table 1. Characteristics of study groups and concentrations of blood parameters between controls and obese participants.

Parameters	Control Participants (<i>n</i> = 82)	Obese Participants (<i>n</i> = 83)
Age (years)	13.92 ± 0.23	14.01 ± 0.19
BMI z-score	0.03 ± 0.00	2.67 ± 0.29 **
Glycemia (mmol/L)	4.41 ± 0.06	4.76 ± 0.05 *
TC (mmol/L)	3.04 ± 0.08	3.39 ± 0.07 *
LDL-C (mmol/L)	1.64 ± 0.07	2.00 ± 0.06 **
HDL-C (mmol/L)	1.08 ± 0.03	0.91 ± 0.02 **
TG (mmol/L)	0.74 ± 0.04	1.04 ± 0.05 **
Insulin (pmol/L)	45.98 ± 0.69	54.38 ± 2.22 **
HOMA index	1.29 ± 0.03	1.70 ± 0.12 **

* $p < 0.05$, ** $p < 0.01$ between controls and obese. Abbreviations: TC (total cholesterol); LDL-C (low-density lipoprotein cholesterol); HDL-C (high-density lipoprotein cholesterol); TG (triglycerides); HOMA (homeostasis model assessment).

2.1.1. Ethics

The study was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association, and the research council of the University of Constantine-1 approved the study protocol (10 September 2014). Our experimental protocol conforms to the relevant ethical guidelines for human research.

2.2. BMI z-Score

The BMI of teenagers was calculated as per WHO guidelines and expressed as z-score [24]. The lean subjects had a BMI z-score below 1 and obese more than 2. To observe a clear difference between lean and obese groups, the subjects with BMI z-score between 1 and 2 were excluded from the study.

2.3. Determination of Fasting Blood Glucose and Lipids Parameters

Venous blood from all the subjects was collected in heparinized tubes. The concentrations of fasting glucose, total cholesterol (TC), and triglycerides (TG) were determined by Biochemical analyzer XL 200 (ErbaLachema, Mannheim, Germany). LDL and HDL cholesterol levels were measured by cholesterol oxidase method (BioSystems, Barcelona, Spain). Insulin concentrations were determined by ELISA (RayBio, Norcross, GA, USA).

2.4. Oleic Acid Sensitivity Analysis

The participants were called on a stipulated date and advised to come early in the morning without taking breakfast (fasting state). The subjects were weighed and a blood sample was drawn, before the sensitivity test, to assess blood parameters. We used the alternative-forced choice (AFC) method as described before [16,17]. Briefly, different concentrations of oleic acid, OA (0.018, 0.18, 0.37, 0.75, 1.5, 3, 6, and 12 mmol/L) were prepared and the teenagers were subjected to taste, one-by-one, the three solutions. One solution contained OA with acacia gum (0.01%) and the other two served as controls with 0.01% acacia gum only. The taste sessions were performed in an isolated chamber, close to the laboratory. Control samples were prepared in the same way but without added oil. We started with the lowest OA concentration, and the detection threshold was established when the subject identified twice the same solution containing OA. The participants were asked to use a nose clip to minimize olfaction cues during the test and to rinse the mouth between every tasting. The teenagers were not allowed to drink the solutions, rather they had to spit them out after keeping the solution in mouth for few seconds.

2.5. Genotyping Analysis

Genomic DNA (gDNA) was extracted from venous blood, using Wizard® Genomic DNA Purification Kit (Promega, USA). Rs1761667 polymorphism of CD36 gene was genotyped using PCR-RFLP. As per our method [16,17], the gDNA was amplified with Kapa mix, containing Taq polymerase (Kapa Biosystems, Wilmington, MA, USA) with forward and reverse primers (5'-CAA AAT CAC AAT CTA TTC AAG ACCA-3' and 5'-TTT TGG GAG AAA TTC TGA AGA G-3'). After amplification, the 190 bp PCR product was digested by *HhaI* endonuclease (Thermo Fisher Scientific, Waltham, MA, USA) which cleaves the product into two fragments of 138 bp and 52 bp if the G-allele is present, whereas in the presence of A-allele we observed undigested 190 bp product. The final products were separated and analyzed in 2% agarose gel electrophoresis, stained with ethidium bromide.

2.6. Statistical Analysis

Statistical analysis was conducted by Statistica 14 software (Statsoft, Tulsa, OK, USA). One-way ANOVA was used to compare the difference between parameters in the study groups. For correlation between various parameters, Spearman rank correlation was performed. Hardy-Weinberg equilibrium (HWE) was assessed by chi-square (χ^2) test. For the comparison of allelic and genotype frequencies between obese and control, Fisher exact test was used. All data in the tables and figures are presented as means \pm SEM, and $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Characteristics of the Participants

The teenager participants ($n = 165$) were divided into two groups: obese with a BMI z-score higher than 2 ($n = 83$ (females = 39, males = 44), z-score 2.67 ± 0.29) and leans with a BMI z-score below 1 ($n = 82$ (females = 37, males = 45), z-score 0.03 ± 0.0). The average age of the subjects was 13.9 ± 1.1 years.

3.2. Blood Parameters

Table 1 shows that both lean and obese young teenagers had fasting glucose concentrations within normal range, though the latter had slightly higher glycemia than the former ($p < 0.05$). Similarly, total cholesterol (TC) concentration was normal in both the groups, but obese participants had higher TC concentration than control children ($p < 0.05$). Lean participants had higher HDL-C concentration compared to obese teenagers ($p < 0.01$). Obese children had significantly elevated LDL-C concentration compared to lean ones ($p < 0.01$). Triglycerides (TG) concentration was higher in obese teenagers than that in lean participants ($p < 0.01$). Insulin concentration was also higher in obese teenagers than that in lean children ($p < 0.01$). We observed a positive association between total TC and TG, HDL-C and LDL-C levels ($p < 0.01$, $p < 0.04$, $p < 0.01$ respectively). TG concentration was positively correlated with LDL-C ($p < 0.01$) and negatively with HDL-C levels ($p < 0.01$). Fasting glucose concentration was positively correlated with insulin level ($p = 0.026$). HOMA index was also higher in obese participants than that in lean ones. No difference between boys and girls was observed as regards the above-mentioned parameters.

3.3. Oleic Acid Sensitivity

We observed statistically significant difference in oleic acid oral detection threshold between obese and lean adolescents (Figure 1). Obese subjects exhibited almost twofold OA detection threshold (2.57 ± 0.29 mmol/L, $p < 0.01$) than lean participants (1.33 ± 0.15 mmol/L). We noticed a positive correlation between BMI z-score and OA detection ($p < 0.01$). If we divide all the participants, on the basis of oral detection thresholds, into three categories: high tasters (between 0 to 0.018 mM),

middle tasters (between 0.18 and 1.5 mM), and low tasters (between 3 and 12 mM), we notice a relationship between BMI and fat taste thresholds ($p < 0.001$; Figure 2). We did not find any significant difference in the measured parameters between genders.

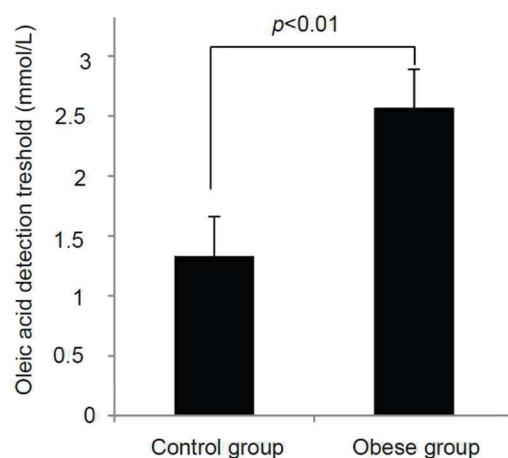


Figure 1. Relationship between BMI and oro-sensory detection of a fatty acid in young leans and obese children. The oleic acid detection thresholds were determined in lean ($n = 82$) and obese children ($n = 83$) as described in the Materials and Methods section. The results are means \pm SEM.

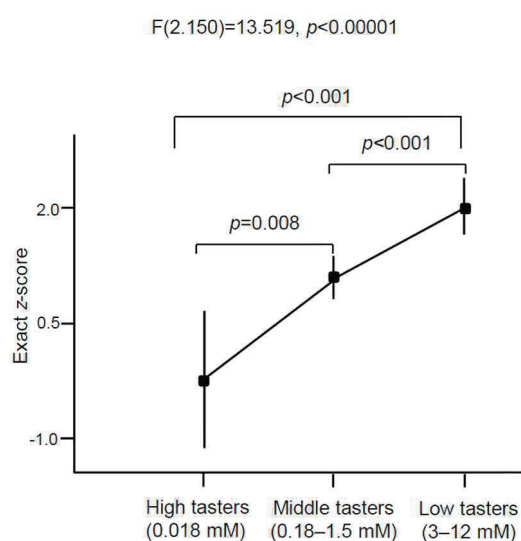


Figure 2. Fatty acid sensitivity in all young teenagers in relation to BMI. The lean and obese children ($n = 165$) were divided into three groups on the basis of oro-sensory detection of oleic acid as high, middle, and low tasters. “High tasters” group contained lean teenagers only ($n = 8$), most of the teenagers from the both groups (control, $n = 60$; obese, $n = 45$) belonged to the “Middle tasters” group and the “Low tasters” group consisted predominantly of obese participants ($n = 41$) and controls ($n = 11$). The results are means \pm SEM.

3.4. CD36 Genotyping

Figure 3 shows rs1761667 genotypes on agarose gel. We did not observe any deviation from Hardy-Weinberg equilibrium ($p > 0.05$) in lean and obese participants (Table 2) in genotype frequencies of rs1761667 polymorphism of CD36 gene. The frequencies of A-allele in lean and obese groups were 56.7% and 68.1%, respectively ($p = 0.041$, OR = 1.63; 95% CI of OR = 1.04–2.55). AA and AG genotypes are present predominantly in obese teenagers ($p = 0.008$; $p = 0.002$, respectively). Minor genotype was, on the other hand, present in the controls. We did not find any significant difference

between CD36 genotype and oleic acid oral sensitivity threshold. Similarly, we did not observe any significant difference between CD36 genotypes and BMI z-score neither in obese nor control teenagers ($p = 0.58$; $p = 0.41$, respectively). We did not find any significant difference between the genders.

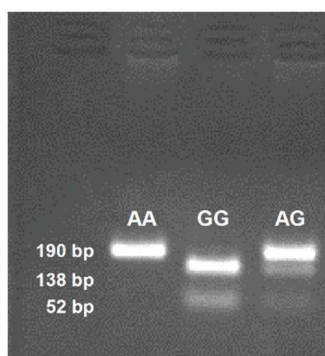


Figure 3. Rs1761667 genotypes separated on 2% agarose gel and stained with ethidium bromide. The blot shows one identical photograph from several reproduced ones.

Table 2. Genotype and allelic frequencies of CD36 rs1761667 between control and obese participants.

Parameters	Control Participants ($n = 82$)	Obese Participants ($n = 83$)	Statistical Calculations
HWE χ^2	2.67	3.05	NS
Alleles (%)			$p = 0.041$
A	93 (56.7)	113 (68.1)	OR = 1.63; 95% CI
G	71 (43.3)	53 (31.9)	RR = 1.28; 95% CI
Genotypes (%)			
AA	30 (36.6)	35 (42.2)	$p = 0.008$
AG	33 (40.2)	43 (51.8)	$p = 0.002$
GG	19 (23.2)	5 (6.0)	-

Abbreviations: HWE χ^2 (Hardy-Weinberg equilibrium χ^2); MAF (Minor allele frequency); OR (odd ratio); RR (relative risk).

4. Discussion

It has been previously shown that the subjects which are obese at a young age became severely obese in adulthood [25,26]. Excess of caloric intake, largely contributed by fat overconsumption, seems to be one of the factors implicated in this pathology [6]. Moreover, altered oro-gustatory perception of lipids has been associated with obesity [6]. It, therefore, seems mandatory to shed light on oral fat sensing that might take part in the regulation of feeding behavior in obese subjects.

As regards blood parameters, we observed higher glycemia, LDL-C, triglycerides, and insulin concentrations in obese children than the lean participants. It has been previously shown that the teenagers with a high degree of obesity exhibited high blood concentrations of LDL-C, glucose and insulin [27]. Similar results were also obtained in an American population, where the prevalence of hyperinsulinemia and hypertriglyceridemia was significantly higher in severely obese children and adolescents, compared to the less obese individuals [28]. We noticed low HDL-C concentrations in the obese group. Indeed, Ruel *et al.* [29] have reported that low HDL is associated with high BMI and waist circumference. Jiang *et al.* [30] have also shown that insulin levels were positively correlated with serum triglyceride, and negatively with HDL-C levels in all age group obese children including 12–17 years old participants. These investigators concluded that these changes in obese children might have adverse consequences for cardiovascular diseases in adulthood. Furthermore, in obese children, we also observed a high HOMA index, an indicator of insulin-resistance (IR) which is directly associated with the aggravation of obesity [31].

As regards the gustatory detection of lipids, we noticed that obese participants exhibited a significantly higher detection threshold (lower sensitivity) compared to lean participants. Whilst the “High tasters” group is composed mainly of controls, in “Low tasters” group we can find predominantly obese teenagers. Previous studies performed on Australian [32] and Tunisian [16] adults also showed that the obese subjects exhibited reduced oleic acid sensitivity. Low fatty acid oro-sensory detection in obesity has been attributed to low expression of CD36 protein in the mouse [33] or to AA genotype of rs1761667 polymorphism of CD36 in human beings [12,16,17]. However, we did not observe a relationship between CD36 gene AA genotype and fatty acid detection thresholds. The reason for this failure might be the less developed papillae which might not have expressed sufficiently the truncated CD36 protein, transduced by CD36 rs1761667 AA genotype [12], in the young Algerian children. In fact, it has been shown that fungiform papillae attain full size at the age of 8–10 years, and the circumvallate papillae, located in the posterior region, continue to grow until the age of 15–16 years [34]. This argument is pertinent as the circumvallate papillae have been shown to express nine-time higher CD36 mRNA than fungiform papillae [35]. Alternatively, it is also possible that a variant of GPR120, another lipido-receptor that is associated with obesity in a European study [36], might be involved in low oro-sensory sensitivity in obese Algerian teenagers; however, further studies are required to confirm this hypothesis. We also noted higher A-allele frequency compared to G-allele in our study, and this kind of distribution has been, so far, reported in Arabic populations, namely in Tunisia [16] and Algeria [17]. Interestingly, previous studies conducted on different populations, namely Caucasians [18], Indians [14], and Asians [13] showed a high frequency of G-allele.

Nonetheless, A-allele frequency of rs1761667 polymorphism of CD36 gene was higher in obese children than lean participants. A-allele was found to be associated with the intake of soda and French fries in obese children, suggesting that fat-containing products might influence, in the long-term, the fatty acid oro-sensory detection capacity. Our hypothesis is supported by the observations of Stewart *et al.* [37] who have reported that feeding a high-fat diet significantly increased oleic acid oral detection threshold in lean subjects. Similarly, feeding a high-fat diet in mice resulted in high oro-sensory threshold for linoleic acid [11].

Ours is the first study to show an association between high oro-sensory threshold for a fatty acid and obesity in 13–14 year old teenagers. These results might be confirmed in other young population with different cultural and eating habits. Though the obese participants had CD36 A-allele, it was not associated with high oro-detection threshold for the fatty acid. Besides, we cannot rule out an influence of altered levels of sex hormones in obese teenagers on fat taste perception and other parameters. It is also difficult to determine whether oral fat perception sensitivity affects fat intake or body weight regulation. Future studies are required to address these questions.

Author Contributions: The authors’ contributions are as follows: NAK designed research (project conception, development of overall research plan, and study oversight); HD conducted research (hands-on conduct of the experiments and data collection); AS helped oral testing of fatty acids; NAK wrote the manuscript; OS supervised the SNP research and statistical analysis; JP conducted technical part of the SNP analysis and participated in writing the manuscript; LR and AR provided the facilities in the sample collections; NAK and LR supervised the study. All authors have read and approved the final content of the manuscript.

Conflicts of Interest: Authors declare that they have no conflict of interest.

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Appendix D

Orosensory Detection of Bitter in Fat-Taster Healthy and Obese Participants: Genetic Polymorphism of CD36 and TAS2R38

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Orosensory detection of bitter in fat-taster healthy and obese participants: Genetic polymorphism of CD36 and TAS2R38

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SUMMARY

Background & aims: We assessed orosensory detection of a long-chain fatty acid, linoleic acid (LA), and a bitter taste marker, 6-*n*-propylthiouracil (PROP), and correlated lipid-taster subjects with PROP detection and polymorphism in genes encoding bitter and lipid taste receptors, respectively, TAS2R38 and CD36, in normal weight and obese subjects.

Design: The normal weight ($n = 52$, age = 35.3 ± 4.10 years, BMI = 23.22 ± 1.44 kg/m²) and obese ($n = 52$, age = 35.0 ± 5.43 years, BMI = 34.29 ± 5.31 kg/m²) participants were recruited to determine fat and bitter detection thresholds. The genomic DNA was used to determine single nucleotide polymorphism (SNP) of CD36 (rs1761667) and TAS2R38 (rs1726866 and rs10246939).

Results: The study included the participants who could detect LA, i.e., lipid-tasters. There was a positive correlation between BMI and detection thresholds for fat and bitter taste in normal weight and obese subjects. Obese participants showed a positive correlation between LA and PROP detection thresholds. PROP detection thresholds were higher for CD36 SNP (rs1761667) and TAS2R38 SNPs (rs1726866 and rs10246939) in obese participants compared to normal weight subjects. LA detection thresholds were not high for CD36 SNP (rs1761667) or TAS2R38 SNP (rs1726866 and rs10246939) in obese participants.

Conclusions: Orosensory detection thresholds for fat and bitter taste are associated with BMI, and CD36 and TAS2R38 genotypes are not always associated with taste phenotypes.

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1. Introduction

The increased incidence of obesity represents a critical issue worldwide [1], and the southern Mediterranean countries are increasingly affected by its epidemic. Since 1980, the prevalence of obesity has grown up rapidly in Tunisia [2]. Though there are

several factors implicated in this disease, the sense of taste plays an important role in the development of dietary habits [3,4]. Bitter taste has been associated with feeding behavior and obesity [3,5]. The TAS2R38 gene encodes bitter taste receptor [5,6] with two variants, i.e., rs1726866 and rs10246939, and the former has been reported to be associated with BMI in participants [5–8].

Obese individuals have been shown to be less sensitive to orosensory detection of dietary fat and, therefore, might eat more lipids [9,10]. Recent years have suggested that there might exist a sixth taste modality, i.e., the taste for fat [11]. Two main taste lipid receptors, i.e., CD36 and GPR120, have been shown to be involved in the preference for dietary lipids [12–15]. The ability to perceive dietary lipids via CD36 might be influenced by a common

Abbreviations: BMI, body weight index; LA, linoleic acid; PROP, 6-*n*-propylthiouracil; SNP, single nucleotide polymorphism.

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polymorphism rs1761667 [16]. Conversely, obese subjects with AA genotype of rs1761667 have been reported to exhibit higher oral detection thresholds for a long-chain fatty acid than those with AG and GG genotypes. These observations were, later on, confirmed by three studies, conducted on Algerian normal weight/obese children [17] and adults [18], and Tunisian obese women [19].

There seems a relationship between fat and bitter taste, and obesity. Tepper and Nurse [20] showed that bitter non-taster participants were unable to accurately distinguish fat content in high- and low-fat Italian salad dressings. Several studies have clearly suggested that there exists a higher preference for dietary fat in PROP non-taster than that in PROP-taster participants [6,20,21]. Consequently, the subjects who are unable to taste PROP are supposed to consume more dietary fat and, therefore, might develop obesity [22]. Melis et al. [23] have recently established a link between fat taste perception, CD36 polymorphism and PROP-tasting capacity. These investigators reported that the capacity to detect a fatty acid was directly associated with TAS2R38 or PROP responsiveness [23]. The PROP non-taster subjects belonged to AA genotype of CD36 rs1761667 [23]. These studies remain to be confirmed in other populations. No study is available on the correlation between bitter and fat taste perception and CD36 and TAS2R38 polymorphism in an African population.

Since fat intake is altered in obesity and fat taste perception is associated with PROP (bitter) status along with CD36 and TAS2R38 genetic polymorphism, the present study was designed to investigate these parameters in normal weight vs obese participants in a Tunisian population.

2. Methods

2.1. Participants

The normal weight ($n = 52$, age = 35.3 ± 4.10 years, male = 23, female = 29, BMI = 23.22 ± 1.44 kg/m²) and obese ($n = 52$, age = 35.0 ± 5.43 years, male = 10, female = 42, BMI = 34.29 ± 5.31 kg/m²) non-smoking and healthy Tunisian adults, both males ($n = 33$) and females ($n = 71$), were recruited from the outdoor patient department (OPD) of National Institute of Nutrition (Tunis, Tunisia), National School of Veterinary Medicine (Tunis, Tunisia) and Regional Hospital of Mateur (Tunis, Tunisia). Medical records were screened by specialist clinicians and dietitian nutritionist. The exclusion criteria were as follows: any history of recent weight loss, diabetes, pregnancy-related complications, chronic illness like hypertension or other inflammatory pathology or any autoimmune diseases. Breastfeeding or lipid lowering medication-using persons as well as the subjects using any medication, known to affect taste (such as birth control pills), were also excluded from the study. All the included participants had normal glucose tolerance test and electrocardiogram.

The study was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association, and the Research Council of National Institute of Nutrition (Tunis, Tunisia) approved the study protocol. Informed written consent was obtained from all the participants. Our experimental protocol conforms to the relevant ethical guidelines for human research. As a routine, the participants were also asked to fill in a nutrition questionnaire [24].

2.2. Linoleic acid sensitivity analysis

The participants were recruited in the fasting state between 7:30 and 8 AM in a room with a constant temperature of 25 ± 0.5 °C. Taste preference tests for dietary lipids were performed by employing linoleic acid (LA) at different ascending concentrations (i.e., 0.018, 0.18, 0.37, 0.75, 1.5, 3, 6 and 12 mmol/L) by “sip and spit”

technique and a three-alternative forced choice (3-AFC) method as we have explained elsewhere [9,17,18]. In brief, we prepared three solutions where two contained the acacia gum (0.01%, w/v), termed as control solutions, and the third one contained the same quantity of acacia gum and linoleic acid, termed as test solution. In an isolated room, the participants were proposed to taste, at random, the three solutions, one-by-one, and in case of a negative response (non-detection of the fatty acid), they were further proposed to taste another set of three solutions, where the third one contained the ascending/increased concentration of the fatty acid, and we continued the session, by increasing the concentration of the fatty acid, until they detected the presence of linoleic acid. Between each “sip and spit”, the participants were asked to rinse their mouth with distilled water. In order to check whether the detection threshold was correct, we proposed another set of three solutions, wherein the one contained descending/lower concentration of the fatty acid than the detected one. In case of a negative response, we further proposed the solution where the test solution contained higher concentration of linoleic acid, and if this response was correct, it was noted as linoleic acid detected threshold. To avoid olfactory and visual cues, the participants used nose clips and were blindfold. The subjects were not allowed to drink the solutions, rather they had to spit them out, after keeping the solution in mouth for a few seconds.

In the present study, we continued further investigations only on lipid-tasting participants and we excluded the lipid non-taster subjects who were only 7; hence, 3 subjects belonged to the control group (age = 35.67 ± 1.52 years, male = 1, female = 2) and 4 to the obese group (age = 37.25 ± 3.40 years, male = 1, female = 3). Moreover, these participants did not turn back to complete the sessions.

2.3. Bitter taste sensitivity analysis

The above-recruited lipid-taster subjects were further invited to participate to the additional session regarding the bitter taste, detected by 6-n-propylthiouracil (PROP), as per method of Bartoshuk et al. [25]. However, we did not correlate the PROP arbitrary perceived responses to NaCl-evoked intensity ratings as it seemed to us very subjective. Hence, we measured the detection thresholds for PROP (0.0000001–0.0032 mmol/L) as mentioned for LA.

2.4. Determination of blood parameters

Before performing the fatty acid sensitivity test, fasting venous blood was collected from all the participants. A plasma sample was immediately used for glucose determination. Serum was aliquoted and frozen at -80 °C for further analysis of blood parameters. Concentrations of total cholesterol (TC) and triglycerides (TG) were determined using enzymatic methods, according to the manufacturer's instructions furnished with the kit (DiaSys Diagnostic Systems GmbH, Holzheim, Germany). Glucose, ALAT (alanine aminotransferase), AST (aspartate transaminase), LDL and HDL cholesterol levels were measured by UniCel DxC 800 Synchron Clinical Systems (Beckman Coulter Inc., Brea, CA, USA). Plasma glycosylated hemoglobin (HbA1C) concentrations were determined by column chromatography (Isolab, Seattle, WA, USA). Insulin was determined by ELISA (RaybioTech, Inc, Norcross, GA, USA). Serum concentrations of urea, creatinine and C-reactive protein (CRP) were analyzed by routine standard techniques using an automated Synchron CX7 Clinical System (Beckman Coulter Inc., Brea, CA, USA). To observe a difference in some of the determined blood parameters (i.e., TC, TG and insulin), we collected the venous blood samples again 1 h after the LA tasting session.

2.5. SNP analyses

Genomic DNA was extracted from 100 μ l of whole blood, using Wizard® Genomic DNA Purification Kit (Promega, Fitchburg, WI, USA). For genotyping of rs1761667, we used the previously described method [17,18]. The TAS2R38 polymorphisms, rs10246939 (Val296Ile) and rs1726866 (Ala262Val), were analyzed using restriction fragment-length polymorphism (RFLP) method and agarose gel electrophoresis. For rs10246939, we used the forward primer, 5' TGTTCCTTCATCTCTGTGC 3' and a reverse primer, 5' TGTGGTCGGCTCTTACCTTC 3'. For rs1726866, the following primers were used: 5' GGAAGGCACATGAGGACAAT 3' as forward and 5' ATTGCTGAGATCAGGATGG 3' as reverse. The annealing temperature for both the reactions was 62 °C.

To determine rs10246939 genotype, we digested the PCR product with *FokI* restriction enzyme (Thermo Fisher Scientific, Waltham, MA, USA) at 37 °C for 1 h. In case of I-allele, two fragments (107 bp and 87 bp) were observed. In case of V-allele, an undigested product of 194 bp was observed. For the determination of rs1726866 genotype, *BseXI* restriction enzyme (Thermo Fisher Scientific, Waltham, MA, USA) was used. After digestion (65 °C for 1 h), we were able to observe either three (122 bp, 75 bp and 19 bp) or two (197 bp and 19 bp) fragments. Three fragments indicated presence of A-allele, whilst two fragments indicated presence of V-allele.

2.6. Statistical analysis

Statistical analyses were conducted using the Statistica 12 software (Statsoft, Tulsa, OK, USA). The data in the table and figures are presented as means \pm SD. The significance in measured parameters between study groups was determined by one-way ANOVA. To observe a correlation between BMI, PROP and LA taste sensitivity thresholds, Spearman rank correlation was used. To compare the genotype distribution, two tailed Fisher's exact test was used. For comparison of PROP detection threshold and any other parameters in individual genotypes, Mann–Whitney *U*-test was used. In this study, $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Characteristics of the participants

All participants ($n = 104$) were divided into two groups based on their BMI (kg/m^2): obese with BMI = $34.29 \pm 5.31 \text{ kg/m}^2$ ($n = 52$) and normal weights with a BMI = $23.22 \pm 1.44 \text{ kg/m}^2$ ($n = 52$, $p < 0.01$). Both the groups were of the same age (normal weights, 35.3 ± 4.10 years; obese, 35.0 ± 5.43 years, $p > 0.05$). Our study included male ($n = 33$) and female ($n = 71$) participants. Table 1 shows the anthropometric parameters, and concentrations of measured blood parameters, determined under fasting condition. The participants exhibited normal range blood glucose level, although it was significantly higher in the obese group than the control group ($p < 0.01$). Insulin level was also higher in the obese group as compared to the control group participants ($p < 0.01$). ALAT and ASAT were elevated in obese subjects ($p < 0.01$, $p < 0.05$, respectively). We did not observe any difference in LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) between the two groups. There was no alteration in uric acid, creatinine, CRP and HbA1c values between control and obese participants.

3.2. Orosensory detection of LA and PROP is altered in obese participants

We did not observe any difference in LA detection thresholds between obese and control groups (Fig. 1A). The average LA

Table 1

Characteristics of normal weight and obese participants.

Parameters	Normal weight (mean \pm SD) ($n = 52$)	Obese (mean \pm SD) ($n = 52$)
Age (years)	35.3 \pm 4.10	35.0 \pm 5.43
Weight (kg)	67.65 \pm 8.21	95.24 \pm 16.32**
Height (m)	1.70 \pm 0.08	1.67 \pm 0.09*
BMI (kg/m^2)	23.22 \pm 1.44	34.29 \pm 5.31**
Glycemia (mmol/L)	4.65 \pm 0.76	5.23 \pm 0.78**
Insulin (pmol/L)	6.52 \pm 3.93	26.57 \pm 13.81**
ALAT (U/l)	16.26 \pm 8.42	22.25 \pm 11.97**
ASAT (U/l)	19.32 \pm 4.73	21.77 \pm 6.64*
LDL-C (mmol/L)	2.64 \pm 0.64	2.57 \pm 0.72
HDL-C (mmol/L)	1.25 \pm 0.41	1.11 \pm 0.32
Uric acid (μ mol/L)	254.59 \pm 89.28	271.82 \pm 90.08
Creatinine (μ mol/L)	68.27 \pm 34.67	62.91 \pm 16.70
CRP (mg/L)	4.89 \pm 2.35	4.91 \pm 3.41
HbA1c (%)	5.59 \pm 0.44	5.74 \pm 0.58

Values are mean \pm SD. * $p < 0.05$. ** $p < 0.01$, according to one-way ANOVA.

detection thresholds were $1.09 \pm 2.30 \text{ mmol/L}$ and $1.80 \pm 3.01 \text{ mmol/L}$ ($p = 0.18$) for control and obese groups, respectively. Nonetheless, LA oral sensitivity was associated with BMI in obese participants ($p = 0.037$), but not in control subjects (Fig. 1B).

The obese subjects exhibited higher PROP detection threshold than normal weight subjects ($0.398 \pm 0.342 \mu\text{mol/L}$ and $0.179 \pm 0.297 \mu\text{mol/L}$, respectively, $p < 0.001$) (Fig. 1C). Figure 1D shows a positive correlation between BMI and PROP oral detection thresholds in both the groups. Interestingly, a positive correlation between LA and PROP detection threshold was observed in obese participants ($p < 0.001$) (Fig. 1E).

3.3. SNP analysis

Genotype frequencies in polymorphisms are shown in Table 2. For rs1761667, we compared the AA genotype with AG and GG genotypes, for rs1726866 we compared AA genotype with AV and VV genotypes, and for rs10246939, we compared II genotype with IV and VV genotypes. Using this dominant model, we found a higher AA genotype frequency of rs1761667 in obese subjects compared to normal weight ones ($p = 0.012$). Also, AA genotype (which encodes alanine) of rs1726866 was more abundant in the obese group ($p = 0.017$). There was no significant association between rs10246939 and obesity (Table 2). After haplotype calculation, 9 different haplotypes were observed, and none of them differed between obese and normal weight groups. We also observed strong linkage disequilibrium (LD) between rs1726866 and rs10246939 ($D' 0.863$, $r^2 0.616$).

After comparing PROP detection threshold in different genotype groups, we observed that obese participants of the three SNP (rs1761667, rs1726866 and rs10246939) always had higher detection thresholds than those in normal weight group (Fig. 2), though no significant correlation was noticed among different genotypes whether it was control or obese group (Table 2).

As far as the LA detection thresholds in different genotypes is concerned, only VV genotype of rs10246939, but no genotype of rs1761667 or rs1726866, in obese participants exhibited higher value than the control subjects (data not shown, $p = 0.03$). Compared to other two genotypes, the control individuals with the VV genotype also exhibited significantly lower LA detection threshold ($p = 0.042$).

The participants with AA genotype (homozygous for alanine) of rs1726866 had significantly higher BMI than the participants with other two genotypes ($p = 0.003$). Subjects with VV genotype

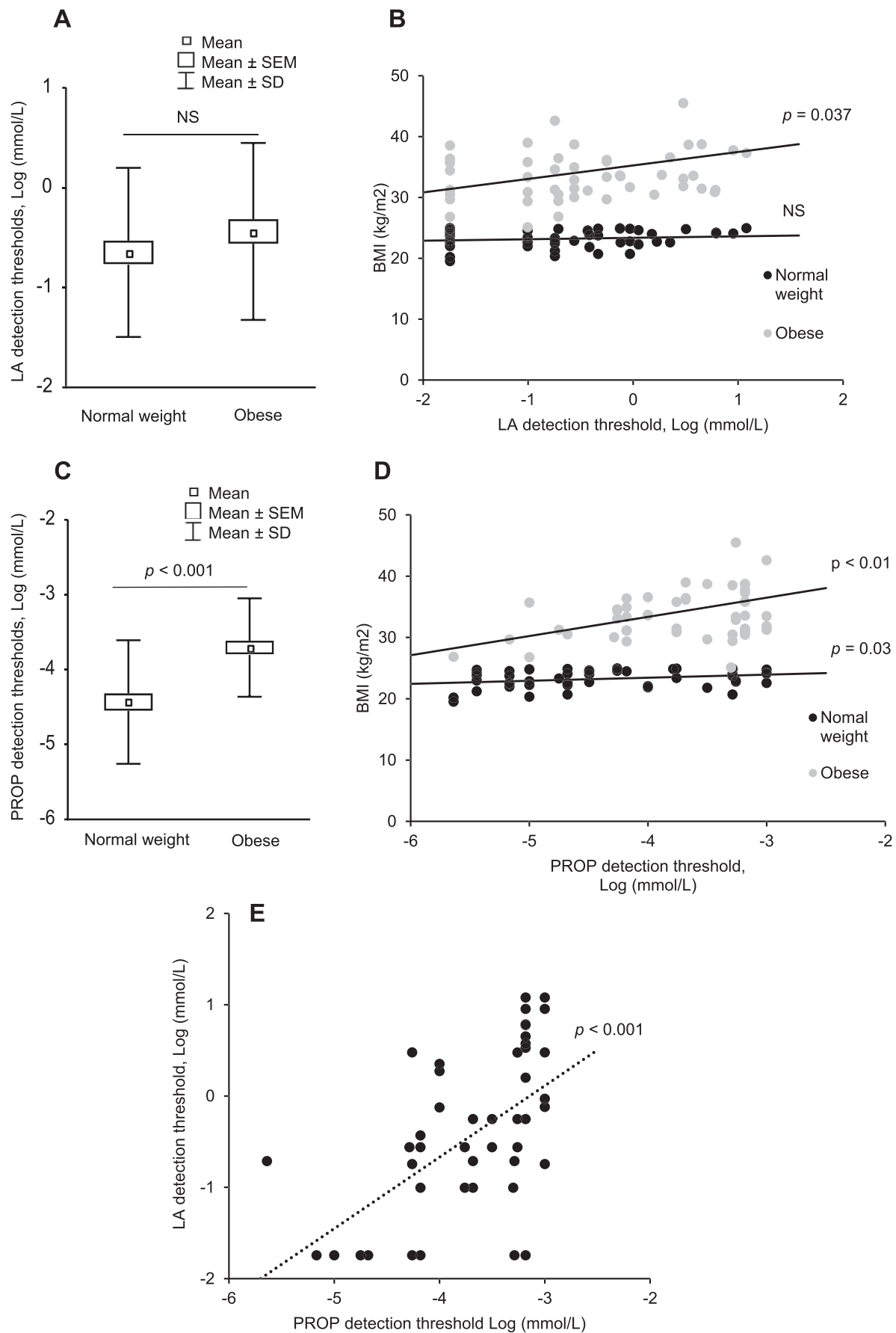


Table 2

Genotypes in normal weight and obese subjects.

a) Genotype distribution between control and obese subjects					
SNP	Genotype	Obese (<i>n</i> = 52)		Normal weight (<i>n</i> = 52)	
		<i>n</i>	%	<i>n</i>	%
rs1761667	AA	24	46.2	11	21.2
	AG	22	42.3	34	65.4
	GG	6	11.5	7	13.5
	AG + GG	28	53.8	41	78.8
rs1726866	AA	17	32.7	6	11.5
	AV	15	28.8	28	53.8
	VV	20	38.5	18	34.6
	AV + VV	35	67.3	46	88.5
rs10246939	VV	15	28.8	8	15.4
	IV	23	44.2	30	57.7
	II	14	26.9	14	26.9
	II + IV	37	71.2	44	84.6
rs1726866 and rs10246939 haplotype	AAII	1	1.9	0	0.0
	AVII	0	0.0	2	3.8
	VVII	13	25.0	12	23.1
	AAIV	2	3.8	0	0.0
	AVIV	15	28.8	25	48.1
	VVIV	6	11.5	5	9.6
	AAVV	14	26.9	6	11.5
	AVVV	0	0.0	1	1.9
	VVVV	1	1.9	1	1.9
b) PROP detection threshold (μmol/L) and genotype distribution					
SNP	Genotype	Normal weight (mean ± SD) (<i>n</i> = 52)		<i>p</i> Value	Obese (mean ± SD) (<i>n</i> = 52)
rs1761667	AA	0.073 ± 0.104			0.377 ± 0.366
	AG	0.232 ± 0.342		0.224	0.378 ± 0.328
	GG	0.093 ± 0.202		0.684	0.557 ± 0.300
rs1726866	AA	0.189 ± 0.398			0.431 ± 0.367
	AV	0.210 ± 0.306		0.557	0.229 ± 0.231
	VV	0.128 ± 0.255		0.714	0.496 ± 0.357
rs10246939	II	0.148 ± 0.286			0.501 ± 0.394
	IV	0.203 ± 0.296		0.659	0.301 ± 0.264
	VV	0.144 ± 0.347		0.562	0.451 ± 0.379

Values are mean ± SD. Fisher's exact (in **a**) and Mann–Whitney *U* test (in **b**) were used for statistical calculations. NS = insignificant differences.

rs10246939 had significantly higher BMI than the participants with IV and II genotypes (*p* = 0.013).

3.4. Linoleic acid-tasting session alters cholesterol and triglyceride levels

Figure 3 shows that LA tasting induced a notable increase in blood TC in the obese group (*p* < 0.01), though in the control group, the TC concentration was diminished after lipid tasting session (*p* < 0.05). Interestingly, the exact opposite trend was observed in case of blood TG level. The LA tasting session brought about a decrease in TG in obese subjects, whereas the TG concentrations were increased in control participants after the tasting round (*p* < 0.01).

4. Discussion

The obese subjects exhibit a normal biochemical profile, marked with normal uric acid, creatinine, LDL-C, HDL-C and CRP concentrations; however, they suffer from hyperinsulinemia, associated with a slight increase in blood glucose concentrations, which seems

under metabolic control as the HbA1c concentrations are not altered in these individuals.

We assessed the detection thresholds for linoleic acid (LA), a long-chain fatty acid, and PROP which is an indicator of bitter taste phenotype. There was no significant difference between two genders among the same groups of participants as far as taste sensitivity is concerned (not shown). We observed that there was a positive correlation between BMI and fatty acid/PROP lingual detection thresholds in obese, but not in control, subjects. Interestingly, there was a strong correlation between LA and PROP detection thresholds in these subjects. Our observations corroborate several reports that have shown decreased oral fatty acid detection capacity, *i.e.*, increased thresholds, in obese objects [10,16–19,26]. Our findings on the relationship between fatty acid and PROP detection thresholds in obese subjects are very interesting as it has been proposed that alterations in fatty acid detection might be related to a change in both bitter and fatty acid taste modalities [27]. Though PROP is a candidate of bitter taste marker, this agent has also been considered an example to detect a change, in general, in oral chemosensory perception since it is associated with the perception of a wide range of oral stimuli [6]. Our results

Fig. 1. Relationship between obesity and taste sensitivity in normal weight and obese participants. **A:** shows box plots of LA orosensory detection thresholds in normal weight (*n* = 52) and obese subjects (*n* = 52). **B:** shows Spearman rank correlation between BMI (kg/m²) and LA orosensory detection thresholds in all the participants (*n* = 104). **C:** shows the box plots of PROP orosensory detection thresholds in normal weight (*n* = 52) and obese subjects (*n* = 52). **D:** shows Spearman rank correlation between BMI (kg/m²) and PROP orosensory detection thresholds in all the participants (*n* = 104). **E:** shows PROP vs LA orosensory detection thresholds in obese subjects (*n* = 52). The results are means ± SD.

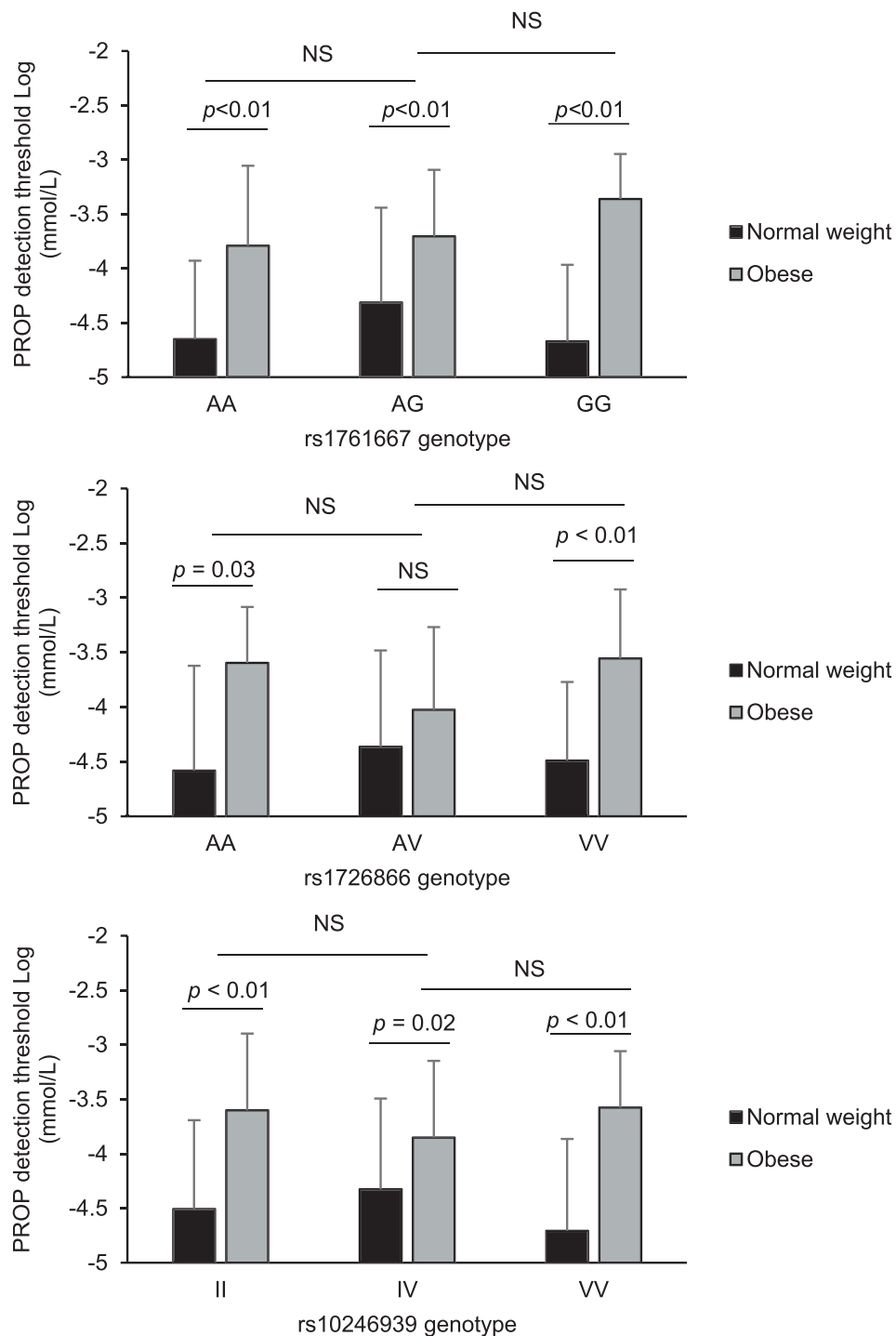


Fig. 2. Relationship between PROP detection thresholds and genetic polymorphism in normal weight ($n = 52$) and obese ($n = 52$) participants. The genotype analysis of *CD36* (rs1761667) and *TAS2R38* (rs1726866 and rs10246939) were performed as described in the Materials and Methods. Mann–Whitney *U* test was used for the analyses.

strengthen the link between PROP sensitivity and fat perception, as PROP tasters have been shown to exhibit high taste intensity ratings for linoleic acid, and vice versa [28]. Though high detection thresholds, *i.e.*, low sensitivity, for fat and bitter might be a contributing factor to obesity, it is important to mention that the family economic situations may also be determinant on the incidence of obesity, as demonstrated by Burd et al. [29]. These investigators very elegantly showed that PROP-non taster children

from unhealthy environment had higher BMI than PROP-non taster from healthy environment. The high thresholds for PROP in obese subjects, in our study, will also aggravate obesity as PROP less sensitive subjects have been shown to possess low intensity ratings for bitter vegetables [30], the source of vitamins and minerals.

Our study, surprisingly, failed to show a correlation between *CD36* SNP and high fatty acid detection thresholds in obese participants as observed in other studies from Algeria [17,18] and

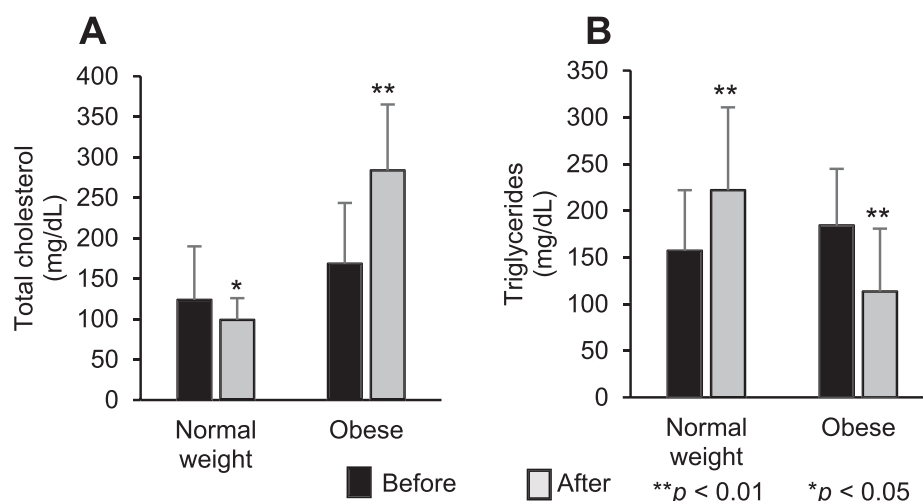


Fig. 3. The effect of fatty acid-tasting on cholesterol and triglyceride concentrations. The blood samples were collected before (fasting condition) and after 1 h of linoleic acid-tasting session. The concentrations of total cholesterol (TC) and triglycerides (TG) were determined as described in the Materials and Methods. One-way ANOVA was used for the analyses.

Tunisia [19]. The reasons for this contradictory observation are not understood. In these three studies, oleic acid, but not linoleic acid, was used for detection thresholds, and in Arabic countries, particularly in the Mediterranean countries, olive oil is consumed in high quantities. It is possible that these subjects have different thresholds for oleic and linoleic acids. Nonetheless, in a French study, a high detection threshold for linoleic acid was reported in obese subjects [26]. In our study, we also failed to confirm the observations of Melis et al. [23] on the association of GG genotype of CD36 rs1761667 with high sensitivity to oleic acid rather we noticed lower sensitivity (i.e., high thresholds) in obese subjects with GG genotype as compared to normal weight subjects. The lack of confirmation might be due to underlying differences in CD36 alleles across populations. Nonetheless, we notice that obese subjects had more AA genotype of rs1761667, compared to normal weights, and this observation is interesting as the expression of rs1761667-A allele results into reduced CD36 expression and reduced fatty acid sensitivity [16], and, consequently, these subjects might eat more fatty food as they will perceive them creamier due to the presence of triglycerides in the fat of daily food [27].

We were further interested in the association of PROP detection thresholds with CD36 and TAS2R38 genotypes in obesity. All the genotypes of rs1761667 (CD36) and rs1726866 and rs10246939 (TAS2R38), except AV genotype of rs1726866, were associated with higher PROP detection thresholds in obese subjects than normal weight participants. The participants with AA genotype of rs1726866 had significantly higher BMI than the subjects with AV and VV genotypes. High PROP thresholds in VV genotype of rs1726866 may also participate in obesity as V-allele of this genotype has been shown to be associated with high plasma leptin and increased disinhibition [8].

Interestingly, the obese participants with VV genotype of rs10246939 exhibited higher thresholds for LA than normal weight controls, and these VV genotype subjects had significantly higher BMI than the participants with IV and II genotypes. Also, the normal weight controls with VV genotype of rs10246939 showed significantly lower detection threshold than the control with II and IV genotypes.

Since we have observed that fatty acid-taster participants are PROP tasters, and the detection thresholds for both sapid molecules are high in obese subjects, the question arises whether fat and bitter taste might communicate with each other, if yes, how? TAS2R38 is coupled to a G-protein, whereas CD36 does not belong

to the GPCR family. Keller [7] proposed that TAS2R38 might be involved in the textural perception of fat, whereas CD36 might assure the chemosensory detection of fat. It is possible that there might be a downstream coupling between CD36 and TAS2R38 receptors at the cell signaling cascade. Besides, we have observed that some of CD36-positive human fungiform cells also express TAS2R38 in immunochemical studies (unpublished observations).

Our results on the TC and TG corroborate, in part, the findings of Chevrot et al. [26] and Mattes [32]. Chevrot et al. [26] have demonstrated that lean fatty acid-taster subjects, after an oral stimulation by a fatty acid, had higher TG levels than that before tasting session. However, in our study, we observed low TG levels, after lingual-stimulation by the fatty acid, in obese subjects, in contrary to Chevrot et al. [26]. This rapid decrease in TG and increase in TC in obese subjects might be due to a defect in the vagal reflex loop, tongue-brain-intestine [26]. This defect might not be due to the release of PYY or cholecystokinin as we did not observe any alternation in their blood concentrations after lipid-tasting session (results non-shown).

Our study has several limitations. We did not determine the number of tongue papillae as the PROP threshold is associated with taste bud cells in fungiform papillae [20,33], though some authors demonstrated no relationship between fungiform papillae density and variations in TAS2R38 genotype [21,34]. Moreover, we did not use the criterion of the magnitude of arbitrary responses between PROP and NaCl to classify the subjects as high, medium or low tasters because salt itself is a taste modality, and salt threshold may not match with the fatty acid thresholds. It is also possible that the “salt taste” might interfere with “fat taste”. Though we do not know how the two modalities interact with other, a recent report has beautifully shown that salt might promote passive over intake of fat [35]. Moreover, Burd et al. [29] also used the similar criteria to classify the children for bitter taste by proposing a solution containing PROP (0.56 mmol/L), without the use of NaCl. Menella et al. [36] also used the three concentrations of PROP without including NaCl to determine the bitter taste thresholds.

To sum up, we can state that our study clearly demonstrates that there exists a relationship between fat and bitter taste modalities and they might play a crucial role in obesity. We also show that TAS2R38 variants, rs1726866 and rs10246939 are associated with obesity. However, the cellular and molecular mechanisms along with the implication of endocrine or paracrine factors involved in their modulation during obesity remain to be studied in future.

Author contributions

IK collected all the data; IK, JP, ASK and OS analyzed the data; AM assisted with the experiments; A Ab, A Ao supervised the protocol and recruitment of participants; JP, NAK wrote the MS. NAK designed research (project conception, development of overall research plan, and study oversight); all authors have read and approved the contents of the article.

Conflict of interest

Authors declare that they have no conflict of interest.

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