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

Ines Mrizak1, Omar Šery2,3, Jiří Plesník3,4, Amel Arfa1, Mariem Fekih1,5, Ali Bouslema1, Monia Zaouali1, Zouhair Tabka1 and Naim Akhtar Khan4*

1Department of Physiology and Functional Exploration, Farhat Hached University Hospital, Sousse, Tunisia
2Institute of Animal Physiology and Genetics, Academy of Science, Veverí 97, 602 00 Brno, Czech Republic
3Laboratory of Neurobiology and Molecular Psychiatry, Laboratory of Molecular Physiology, Department of Biochemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic
4Physiologie de la Nutrition et Toxicologie, UMR U866 INSERM/Université de Bourgogne/Agro-Sup, 6, Boulevard Gabriel, Dijon 21000, France
5Department of Biochemistry, Sahloul University Hospital, Sousse, Tunisia

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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 mortality1,2. The aetiology of obesity is multifactorial, and genetic inheritance and behavioural/environmental causes are considered to be the main factors3. 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 fat4. Humans and rodents can detect long-chain fatty acids in their diets as gustatory cues4–9. Some recent studies have shown that obese subjects exhibit a high preference for dietary lipids as compared to lean subjects10,11, 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-sensory detection of fat10,11.

Linguial 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 fat6,12–15. The lingual lipid receptors bind to long-chain fatty acids, which are released by lingual lipases in the buccal cavity12–15. 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 epithelium16. Moreover, Scalfani et al17 have shown that CD36 is directly involved in early fat detection, whereas GPR120 plays a role in the post-ingestive regulation of fat preference18.

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 subjects19. Keller et al20 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 al19 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.

* Corresponding author: Professor N. A. Khan, fax +33 3 80 39 63 30, email naim.khan@u-bourgogne.fr
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-sensory 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 man 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 gynecology 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 gynecology 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>
<thead>
<tr>
<th>Parameters</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>Height (cm)</td>
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<td>6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34·6</td>
<td>4·2</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>73·3</td>
<td>48·7</td>
</tr>
<tr>
<td>Age (years)</td>
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<td>11·4</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
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</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>3·39</td>
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</tr>
<tr>
<td>Creatinine (μmol/l)</td>
<td>60·39</td>
<td>14·58</td>
</tr>
<tr>
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<tr>
<td>TAG (mmol/l)</td>
<td>1·77</td>
<td>1·06</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
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</tr>
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<td>LDL (mmol/l)</td>
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<td>1·01</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5·60</td>
<td>1·22</td>
</tr>
<tr>
<td>ApoA1 (g/l)</td>
<td>2·04</td>
<td>0·74</td>
</tr>
<tr>
<td>ApoB (g/l)</td>
<td>1·26</td>
<td>1·13</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>78·76</td>
<td>67·64</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>7·8</td>
<td>7·8</td>
</tr>
</tbody>
</table>

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 (1000g 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 (Immunootech; Beckman Coulter, Inc.) with a detection limit of 0·5 μIU/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. (19). 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 (0.01 %, 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 48h of preparation. Control samples were prepared in the same way but without added oil.

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 contained oleic acid in a solution that also included acacia gum (0.01 %). 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 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 ACC AGA-3' and 5'-TTT TGG GAG AAA TTC TGA AGA G-3'; IL-6: 5'-AAT TTT CCC CCT AGT TGT GTC TTT C-3' and 5'-AGA ATG AGC TCT 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 Ncol (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 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 241 and 180 bp (G allele) and an unrestricted fragment of 204 bp (C allele) were obtained. Two fragments of CD36 (138 and 528 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 χ² 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).
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 3-5 times lower than those of subjects with the AA genotype (95% CI of relative risk 2.50, 4.43; 95% CI of OR 9.96, 19.9). 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 knock (CD36<sup>-/-</sup>) had lower CD36 protein expression and a lower oral fat detection threshold than wild type animals (CD36<sup>+/+</sup>). The CD36 knock (CD36<sup>-/-</sup>) failed to exhibit a spontaneous preference for fat.

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

CD36, cluster of differentiation 36. |
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. (51) 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 Rač 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...
from obesity and metabolic syndrome\textsuperscript{(51)}. The SNP of these cytokines have been suggested to predispose for obesity\textsuperscript{(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 \textit{et al.}\textsuperscript{(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\textsuperscript{(54)}. With regards to TNF-\(\alpha\), we noticed a positive relationship between circulating creatinine concentrations and the TNF-\(\alpha\) GG genotype, which indicates that the present subjects might be at risk for renal complications. We also observed a significant association between IL-6 and TNF-\(\alpha\) 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\textsuperscript{(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 \textit{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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