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#### Associations between fat taste sensitivity, nutritional intakes, body mass index and papillae density in healthy Algerian women: A cross-sectional study

#### Asociaciones entre la sensibilidad al sabor de las grasas, la ingesta nutricional, el índice de masa corporal y la densidad de las papilas en mujeres argelinas sanas : Un estudio transversal

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## ABSTRACT

**Introduction:** This study aimed to evaluate the link between nutritional intake, fat taste sensitivity, papillae density, and body mass index (BMI) in the Algerian women population.

**Methods:** This work is a cross-sectional study; 140 women were recruited. Weight, height, and waist circumference were measured. Detection thresholds for oleic acid (OA) were determined according to three alternative forced choice method. Based on the cumulative distribution of minimum detection thresholds, participants were classified into hyposensitive  $\geq 3$  mM and hypersensitive  $< 3$  mM. Food intakes were recorded using 24-hour diaries on three different days. Fatty food consumption frequencies were studied using a frequency consumption survey. Papillae density was determined by tongue photography. Statistical analysis was performed with SPSS software version 25.

**Results:** Our results indicated that 50 % of our population was overweight including 27 % of obesity. Average OA detection thresholds were greater in overweight and obese subjects compared to normal-weight subjects ( $p < 0.001$ ). A positive correlation was found between OA detection thresholds and BMI and waist circumference ( $p < 0.001$ ). Hyposensitive subjects consumed more energy and fat intake compared to hypersensitive subjects. Papillae density was inversely associated with BMI and OA detection thresholds.

**Conclusions:** The study confirms the association between fat taste sensitivity and weight status in Algerian women. Overweight and/or obese participants were the least sensitive to OA compared to normal-weight participants. Hyposensitive subjects had higher intakes of energy and lipids compared to hypersensitive subjects. They also expressed a lower number of fungiform papillae. Overweight participants also had a lower number of fungiform papillae compared to normal-weight participants. A better understanding of the links between the detection of dietary lipids and energy/fat intakes in obese subjects may lead to new nutritional strategies to limit the risk of obesity.

**Keywords:** Fat, Sensitivity, Taste, Detection, BMI, Papillae density.

## RESUMEN

**Introducción.** Este estudio tuvo como objetivo evaluar el vínculo entre la ingesta nutricional, la sensibilidad a las grasas, la densidad de las papilas y el índice de masa corporal (IMC) en la población de mujeres argelinas.

**Metodología.** Este trabajo es un estudio transversal, se reclutaron 140 mujeres. Se midieron el peso, la talla y la circunferencia de la cintura. Los umbrales de detección de ácido oleico (OA) se determinaron según tres métodos alternativos de elección forzada. Los participantes se clasificaron en hiposensibles  $\geq 3$  mM e hipersensibles  $< 3$  mM. La ingesta de alimentos se registró mediante diarios de 24 horas durante tres días. El consumo de grasas se estudió mediante una encuesta de frecuencia de consumo. La densidad de las papilas se determinó mediante fotografía de la lengua. El análisis estadístico se realizó con SPSS.

**Resultados.** El 50 % de la población tenía sobrepeso, incluido el 27 % de la obesidad. Los umbrales de detección de OA fueron mayores en sujetos con sobrepeso en comparación con sujetos con peso normal ( $p < 0,001$ ). Se encontró una correlación positiva entre los umbrales de detección de OA y el IMC ( $p < 0,001$ ). Los sujetos hiposensibles consumieron más energía y grasas en comparación con los sujetos hipersensibles. La densidad de las papilas se asoció inversamente con los umbrales de detección del IMC y la OA.

**Conclusión.** Los participantes obesos fueron los menos sensibles a la OA. Los sujetos hiposensibles tuvieron mayores ingestas de energía y lípidos en comparación con los sujetos hipersensibles. También expresaron un menor número de papilas fungiformes. Una mejor comprensión de los vínculos entre la detección de grasa y la ingesta de nutrientes en sujetos obesos puede conducir a nuevas estrategias nutricionales para limitar el riesgo de obesidad.

**Palabras clave.** Grasa, Sensibilidad, Gusto, Detección, IMC, Densidad de papilas.

## INTRODUCTION

Obesity has become a real global epidemic. More than 1.9 billion adults around the world are overweight, and over 650 million are obese [1]. The rising prevalence of obesity affects both developed and developing countries. In Algeria, obesity indicators are increasingly alarming and the situation is worrying especially among women. A recent study of 7450 adults stated that obesity affected 30 % of women, while overweight affected 52 % of them [2]

Though several factors are responsible for the rising incidence of obesity, high fat intake remains one of the most important ones [3]. Humans are severely attracted to fatty foods. Repeated exposure to palatable foods increases hedonic pleasure and has been associated with overconsumption of energy intake, leading to obesity [4,5]. However, it remains difficult to assess what part of this attraction is specifically related to taste perception [6]. Fat

detection would likely combine with other modalities like olfaction and texture to form the full sensory perception of fat [7]. It is essential in the recognition and preferential consumption of fatty foods, which are very dense in energy and are involved in spontaneous lipid preference [8,9]. Thus, the alteration of the orosensory perception system may affect the quality as well as the quantity of food intake and lead to weight gain and obesity.

In North African populations, studies in this area of research were conducted mostly to evaluate orosensory perception of fat and single nucleotide polymorphism in the CD36 gene [10,11,12]. In Algeria, to our knowledge, this is the first study aimed to assess the relation between fat taste perception, nutritional intake, and body mass index (BMI) in the Algerian women population. We also studied the possible link between papillae density and fat sensitivity.

## **METHODS**

### *Population*

The study was conducted in the adult female population. Participants were recruited through a call for participation that was launched at the university of Constantine 1. From the initially 200 recruited subjects, only 140 were selected. Participants were aged from 18 to 50 years old. Eligibility criteria were as follows: participants must not have any history of chronic pathology; should not be under any medical treatment affecting taste perception; must be weight-stable in the last six months and should not be smokers. A written consent was obtained from all the subjects and the study was performed according to the principles established by the Declaration of Helsinki and institutional guidelines.

### *Anthropometric assessment*

Weight, height, and waist circumference were measured without shoes and in light clothing. Measurements were progressed according to the recommendations of the World Health Organization (WHO) using the same calibrated material (SECA). BMI was calculated as body weight (in kg) divided by height (in m<sup>2</sup>). Overweight and obesity were defined as a BMI  $\geq$  25 and BMI  $\geq$  30 respectively. The characteristics of the population are shown in Table 1.

### *OA detection threshold determination*

Oleic acid (OA) was used as a marker to determine sensitivity to fat [8]. Detection thresholds were determined according to three alternative forced choice method (AFC), at different

ascending concentrations of OA (0.018, 0.18, 0.37, 0.75, 1.5, 3, 6, and 12 mmol). The solutions were prepared in distilled water containing Arabic gum (5 %, w/v) and EDTA (0.01 %, w/v). Control samples contained only Arabic gum (5 %, w/v) and EDTA (0.01 %, w/v). All chemicals were purchased from Sigma-Aldrich. The emulsions were homogenized for 5 min with a homogenizer (ULTRA TURRAX, IKA T18 digital, Allemagne).

Participants were advised to arrive in a fasting state. The test procedure started with the lowest concentration. Each set of concentration had three solutions: two control samples and one odd sample with OA. Solutions were presented in opaque containers of 4 ml. Participants were instructed to taste, one by one, the three solutions without knowing the nature of the tested molecule. They kept each sample in their mouths for a few seconds and then spit it out. They rinsed their mouth with distilled water between each concentration. We increased the concentration of OA when a single incorrect response was given. The procedure was terminated when the subject correctly identified the “odd” sample three successive times and that concentration was defined as the subject’s detection threshold.

#### *Sensitivity classification*

Our participants were classified into hyposensitive and hypersensitive according to the cumulative distribution of minimum detection thresholds for OA. More than half of the participants detected OA at a concentration  $\geq 3$  mM. Thus, this concentration was used to determine the groups of sensitivity (hypersensitive  $< 3$  mM and hyposensitive  $\geq 3$  mM). In the literature, the same concentration was used to divide the groups of sensitivity [8–13].

#### *Dietary intake evaluation*

Participants had to report their food intakes while maintaining their normal eating patterns during three days of the week (2 weekdays and 1 weekend). A 24-hour dietary recall was filled, in which all the consumed foods and drinks were recorded with the exact amounts (in grams), or using measuring utensils or common serving sizes. The brand, seasoning, and cooking method of foods were also reported. The French food composition table (CIQUAL2017) was used to assess the nutritional composition of foods. For traditional meals, local work data were used. The ingested amounts were converted into grams by using the reference manual SUIVIMAX.

#### *Consumption frequencies of fatty foods*

The consumption frequencies of fatty foods were collected by answers to multiple-choice questions: per day, per week, per month, or never consumed. Answers were converted into monthly consumption frequencies. Foods were selected according to their high-fat content and then classified into sweet fat (10), salty fat (16), pure fat (2), and total fat.

#### *Fungiform papillae assessment*

The fungiform papillae density was determined by tongue photography [17]. The procedure consisted of coloring the front part of the tongue with blue food coloring. A circle of 6 mm diameter filter paper was placed on the blue part after brief drying. Ensuring the confidentiality of the participant, three photos were taken with a NIKON Coolpix p900 brand digital camera with a resolution of 4608 x 3456 (fig 1). Fungiform papillae were counted according to specific criteria and had higher structures with lighter color [14].

#### *Statistical analysis*

Statistical analyses were conducted using SPSS software (IBM Corporation, version 25, SPSS Inc, Chicago, USA). Data in the tables were presented as means (standard deviation) (SD). For mean values, the significance of measured parameters between the study groups (sensitivity and weight categories) was determined by one-way ANOVA with a post hoc tukey-test. The analysis of covariance (ANCOVA) was used to evaluate the differences in nutrient intakes between the sensitivity groups while controlling the effect of BMI, a potential confounder for overall energy and fat intake. The adjusted values were only used for comparison between adjusted and non-adjusted values. The Chi2 test was used for comparison of percentages of the subject's sensitivity in weight categories. The correlation between fat detection thresholds and obesity indicators was performed with Spearman's rank correlation test. Non-parametric tests were used in each test. P values < 0.05 were considered statistically significant.

## **RESULTS**

#### *Subject characteristics*

Full data were obtained from 140 female subjects. The average age of the population was 22.64 (2.24) years. There were no significant variation based on age groups. According to BMI, 50 % of the population was overweight with 27.14 % of obesity (table 1).

**Table 1.** Characteristics of population

Parameters	Normal weight (n=70)		Overweight* (n=70)		Obese** (n=38)	
	Mean	SD	Mean	SD	Mean	SD
Age (year)	21.61	3.21	23.68	6.56	25.69	8.15
Weight (kg)	56.52	6.36	81.53	13.02	90.42	10.98
Height (m)	1.62	0.05	1.63	0.06	1.63	0.06
BMI (kg/m <sup>2</sup> )	21.44	1.87	30.55	4.85	34.00	4.21
Waist circumference (cm)	74.61	5.54	93.68	12.14	101.51	10.54
Glucose blood level (g/l)	0.89	0.09	0.90	0.10	0.92	0.08
Systolic blood pressure (mmHg)	105.85	10.40	112.5	10.97	116.35	11.20
Diastolic blood pressure (mmHg)	61.60	9.85	67.73	9.75	69.78	8.67

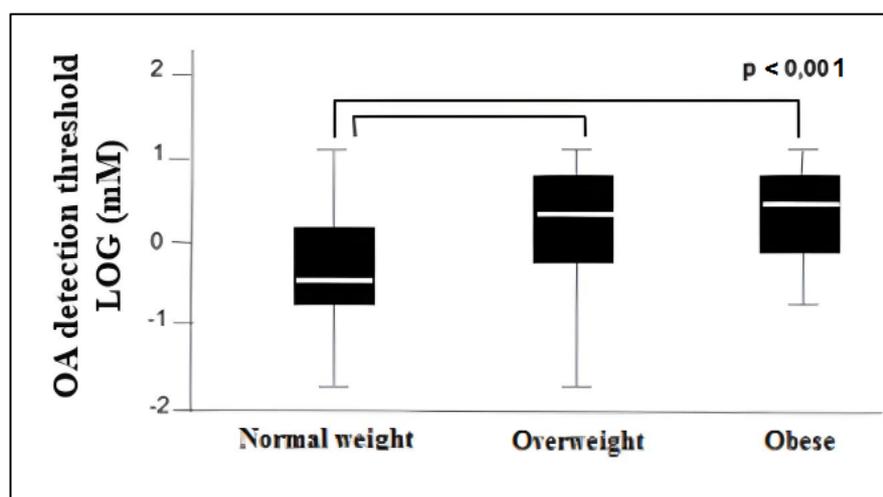
BMI: Body Mass Index

\*Overweight including obesity (BMI ≥ 25), \*\*obese : BMI ≥ 30, normal weight : BMI < 25

SD: standard deviation

### *Orosensory detection of oleic acid*

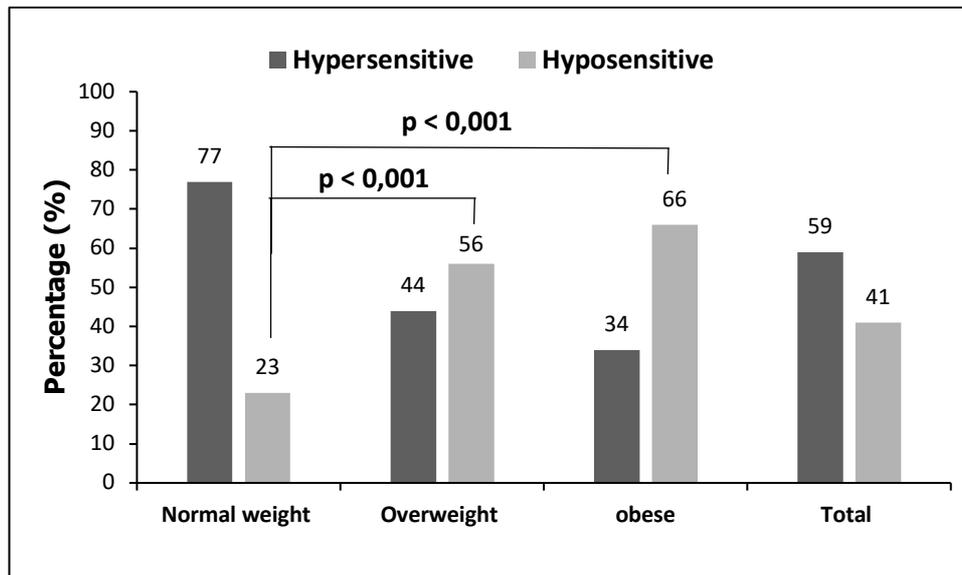
Our results indicated that 59 % of subjects were hypersensitive to OA and 41 % of them were hyposensitive. A statistically significant difference was found in mean detection thresholds between the weight categories ( $p < 0.001$ ) (fig 1).



**Figure 1.** Average OA detection thresholds according to weight status. Overweight and obese participants had greater mean OA detection thresholds compared to normal-weight participants. Post hoc tukey-test was used after ANOVA with  $p < 0.05$ .

Fat sensitivity was also inversely associated with BMI. Obese subjects were more hyposensitive to fat compared to overweight and normal-weight subjects ( $p < 0.001$ ) (fig 2).

Furthermore, a positive correlation was found between OA detection thresholds and obesity indicators: BMI (Rho = 0.45,  $p < 0.001$ ) and waist circumference (Rho = 0.46,  $p < 0.001$ ).



**Figure 2.** Fat sensitivity distribution according to weight status. Overweight and obese participants were more hyposensitive to OA compared to normal-weight participants. The Chi2 test was used to compare percentages in the hyposensitive category: obese/normal weight subjects (66% vs 23%) and overweight / normal-weight subjects (56% vs 23%) ( $p < 0.05$ )

### *Dietary intake*

There were significant differences between hypo and hyper-sensitive participants in energy and nutrient intakes (fat, carbohydrates, proteins, and saturated fats) ( $p < 0.05$ ). Hyposensitive subjects consumed more energy, fat, carbohydrates, and proteins than hypersensitive subjects (table 2).

Following adjustment for BMI, differences in fat and protein intakes between hyper and hyposensitive subjects were no longer significant. However, total energy and carbohydrate intake were still significant ( $p < 0.05$ ).

### *Consumption frequencies of fatty foods*

Hyposensitive subjects expressed higher consumption of pure fat ( $p = 0.001$ ) and total fat ( $p = 0.007$ ). They consumed more butter ( $p = 0.001$ ), more olive oil ( $p = 0.04$ ), and more salty-fat food compared to hypersensitive subjects ( $p = 0.007$ ) (table 3).

**Table 2.** Energy and macronutrient contribution of hyper and hyposensitive subjects

Nutritional intakes	Hypersensitive (n = 83)		Hyposensitive (n = 57)		p*	Adjusted p value BMI
	Mean	SEM	Mean	SEM		
Energy (kcal/day)	1360.85	470.98	1804.88	747.06	0.001	0.02
Fat intake (g/day)	47.06	22.05	61.10	33.97	0.007	0.17
Fat energy (kcal/day)	423.51	198.43	549.92	304.09		
Carbohydrates intake (g/day)	173.50	58.27	242.90	104.58	0.001	0.002
Carbohydrates energy (kcal/jour)	693.99	233.06	971.59	418.30		
Protein intake (g/day)	54.78	22.51	68.73	27.25	0.003	0.09
Protein energy (kcal/jour)	220.56	89.85	274.91	109.02		
Saturated fats (g/day)	17.68	8.13	22.81	11.60	0.005	0.11
Monounsaturated fats (g/day)	14.80	10.06	18.72	12.97	0.06	0.52
Polyunsaturated fats (g/day)	11.64	50.36	8.23	6.66	0.63	0.75
Insaturated fats (g/day)	26.44	50.96	26.95	18.87	0.94	0.90

Hypersensitive < 3 mM and hyposensitive ≥ 3 mM

Nutrient intakes were quantified from a 3 days diet records

Significant differences were found in mean values of total energy and nutrients intakes (fat, carbohydrates, proteins and saturated-fats) between hyposensitive and hypersensitive participants (p < 0.05). Adjusted p values for BMI were determined using one way ANCOVA test

#### *Fungiform papillae assessment*

The results concerned 86 subjects. The rest of the participants did not agree to be photographed. Obese subjects expressed lower fungiform papillae density compared to overweight and normal-weight subjects (p = 0.001) (table 4). Moreover, hypersensitive subjects had higher fungiform papillae density compared to hyposensitive subjects (p = 0.001).

**Table 3.** Average frequencies of fatty foods consumption according to sensitivity

Food	Hypersensitive (n = 83)	Hyposensitive (n = 57)	p values
Pure fat	14.86 (15.16)	24.68 (20.55)	<b>0.001</b>
Butter	3.40 (4.76)	8.47 (10.34)	<b>0.001</b>
Olive oil	10.87 (13.70)	16.21 (16.87)	<b>0.04</b>
Salty fat	102.80 (58.44)	132.91 (71.31)	<b>0.007</b>
Mayonnaise	5.16 (8.43)	8.61 (10.04)	<b>0.03</b>
Mustard	2.07 (4.59)	1.89 (3.72)	0.809
Salad dressing	20.80 (16.38)	20.73 (16.61)	0.982
Pizza	5.43 (5.51)	7.74 (5.78)	<b>0.02</b>
Burgers	2.82 (4.49)	4.28 (4.50)	0.061

<b>Sandwiches</b>	5.18 (5.68)	7.02 (6.39)	0.076
<b>French fries</b>	9.66 (7.97)	13.60 (10.26)	<b>0.01</b>
<b>Cheese</b>	7.53 (9.41)	15.33 (15.46)	<b>0.001</b>
<b>Mahdjouba</b>	2.63 (3.29)	3.81 (3.57)	<b>0.046</b>
<b>Bourak</b>	2.11 (2.48)	3.75 (4.48)	<b>0.006</b>
<b>Potato chips</b>	5.70 (8.87)	8.41 (12.52)	0.137
<b>Fried chicken</b>	7.82 (9.96)	10.44 (12.32)	0.168
<b>Canned tuna</b>	5.30 (6.23)	6.42 (4.90)	0.257
<b>Sausages</b>	2.46 (3.96)	2.79 (3.61)	0.621
<b>Salted nuts</b>	7.71 (10.96)	8.91 (12.02)	0.544
<b>Salted sunflower seeds</b>	5.18 (8.51)	8.91 (15.04)	0.065
<b>Sweet fat</b>	86.36 (56.31)	104.02 (64.13)	0.09
<b>Pastries</b>	11.83 (13.41)	18.81 (17.79)	<b>0.009</b>
<b>Wafers</b>	8.98 (9.57)	10.42 (12.36)	0.438
<b>Cookies</b>	13.07 (12.48)	13.61 (14.96)	0.816
<b>Madeleine</b>	8.58 (9.27)	9.75 (11.37)	0.506
<b>Pastry croissant</b>	12.54 (11.13)	14.47 (11.75)	0.335
<b>Deserts (custard, chocolate mousse, crepes)</b>	10.46 (9.90)	12.30 (10.28)	0.289
<b>Chocolate</b>	12.16 (11.12)	14.67 (12.64)	0.217
<b>Spread chocolate</b>	7.13 (13.57)	6.42 (11.48)	0.746
<b>Donuts</b>	2.28 (3.43)	2.75 (3.36)	0.423
<b>Bradj / makroud</b>	1.37 (1.93)	1.82 (3.79)	0.361
<b>Total fat</b>	226.70 (119.41)	289.02 (146.57)	<b>0.007</b>

Hypersensitive < 3 mM and hyposensitive ≥ 3 mM

Values presents mean monthly consumption food frequencies (SD)

Differences were detected via independent samples t-test, p < 0.05

\* Total fat: includes all foods

**Table 4.** Papillae density according to weight status and sensitivity

Papillae density	Mean (SD)	Minimum	Maximum	p value
<b>Weight status</b>				
Normal weight (n=48)	20.97 (6.82)	8	39	<b>0.001</b>
Overweight (n=38)	13.81 (6.51)	5	34	
Obeses (n=15)	10.40 (4.34)	5	19	
Total (n=86)	17.71 (7.50)	5	39	
<b>Sensitivity</b>				
Hypersensitive (n=54)	20.06 (7.23)	7	39	<b>0.001</b>
Hyposensitive (n=32)	13.75 (6.27)	5	27	

Hypersensitive < 3 mM and hyposensitive ≥ 3 mM

Comparisons of mean values between weight categories and sensitivity categories were determined using one way ANOVA test and t-test respectively, p < 0.05

## DISCUSSION

Participants who were hyposensitive to OA had expressed a decreased ability to detect fat, consumed more energy and more fat in their diet. They had higher consumption frequencies of fatty foods. They also expressed higher BMI and waist circumference values than hypersensitive subjects. These data suggest a possible role of fat detection thresholds in the general consumption of energy/fat and obesity.

The present mean fat detection thresholds were close to that reported by other authors in the literature [15,16]. In Algeria, two studies in both children and adolescents presented mean thresholds that were close to our results [10,12]. In the literature, the reported mean detection threshold in humans for OA was 2.2 mM and individuals were classified based on that as hypersensitive and hyposensitive [19,17]. Based on these reports and other studies the concentration of 3 mM was used in this study [13,18].

The distribution of detection thresholds by weight status showed a large individual variation among participants. This finding has been reported by other studies [19,20]. Our results indicated that overweight and obese subjects had the highest mean detection thresholds compared to normal-weight subjects ( $p < 0.001$ ). Moreover, they expressed lesser sensitivity to OA compared to normal-weight subjects (66 % vs 23 %,  $p < 0.001$ ). We also found a positive correlation between detection thresholds and obesity indicators ( $p < 0.01$ ). Previous studies found a similar correlation [21]. These results confirm the association between fat sensitivity and weight status. A lot of studies reported a positive correlation between BMI and fat detection thresholds [15,16,22,23]. In North Africa, the same correlation was found in Algerian, Moroccan, and Tunisian populations, respectively [11,24]. Some authors explained this association by the fact that a reduced ability to detect fat can lead to a lower probability of rejection of aversive unesterified fatty acids which can induce higher consumption of lipids [23]. Others suggested that a high-fat diet may lead in the long term to a habituation of higher stimulus to generate a positive oral response leading to a greater food intake [16].

Regarding dietary intake evaluation, our hypothesis suggests that a higher intake of energy and fat might be associated with decreased taste sensitivity. Our results showed that hyposensitive subjects had higher intakes of energy, lipids, and carbohydrates compared to hypersensitive subjects ( $p < 0.01$ ). Adjustment for BMI resulted in non-significant differences between groups of sensitivity regarding fat consumption. However, energy and carbohydrate

intake were still significant ( $p < 0.05$ ). The conclusions from this study were based on the unadjusted values for BMI. Fatty acids interact directly with taste receptors within the lingual epithelium, and the absolute value of fat consumed is the most biologically relevant [18]. Other studies have identified an association between sensitivity and consumption of fat [25]. Hyposensitive rodents were more likely to consume excess fat and gain weight more rapidly [25]. In humans, there was also significant evidence of the link between fat taste sensitivity and energy/fat intake [16,26]. Hypersensitive subjects consumed less energy/fats and had lower BMI compared to hyposensitive subjects [8]. These results suggest that dietary fat might modulate fat taste sensitivity. Increased fat intake causes a decrease in fat taste sensitivity with higher detection thresholds [26]. Altered detection of fat in the oral cavity and gastrointestinal tract may contribute to decreased satiety leading to excessive consumption of high fat and obesity [27]. In addition, dietary fats become nowadays a commonly consumed and easily accessible source of energy. Thus, the taste system of some individuals was adapted to higher lipid intake and became less sensitive [16].

Fungiform papillae vary from 5 to 60 per 6 mm diameter area [28]. The camera used is fundamental to obtaining appropriate results and may explain the variability between studies. Our study indicated that obesity was linked to lower papillae density. These results were consistent with previous studies [29]. Although the number of fungiform papillae is probably genetically programmed, there have been reports in obese subjects showing an association between a reduction in papillae density and dysfunction of taste sensitivity [29]. Our results also indicated that fungiform papillae density was significantly associated with fat detection. Taste buds contain the receptors responsible for the perception of fat [30]. Thus, theoretically, a higher number of fungiform papillae could improve fat sensitivity. However, it remains controversial and needs more research.

The present results need to be considered alongside several limitations. First, the 24-hour dietary recall does not accurately reflect the real and exact amounts of intake; it is proportional to the participant's declarations. Second, due to selection criteria, this work concerned only 140 women (with 27 % of obesity). Smoking was one of the determinant criteria that limited the recruitment of men. Moreover, there might be an influence of female sex hormones on fat taste perception and eating behavior. Finally, the subjects of this study

were in good health condition and their results may not reflect that of the general population. These aspects should be considered in future studies.

## CONCLUSIONS

Our study confirms the existence of an association between fat sensitivity and weight status in Algerian women. Overweight or obese subjects were more likely to express hyposensitivity to fat. In addition, a positive correlation was found between obesity indicators and OA detection thresholds. These results indicate that impaired lipid detection is possibly related to overweight. This work also highlighted a link between fat sensitivity and excessive consumption of fat, whether in terms of quantity or consumption frequencies. Hyposensitive subjects had higher intakes of energy and lipids. Lower fungiform papillae density was linked to both overweight and hyposensitive subjects. Although the association between papillae density and fat sensitivity is still unclear, it may present a new area of research to explain the overconsumption of fat.

Our study is the first in Algeria to elucidate the relationship between fat taste sensitivity, nutritional intake, papillae density, and obesity in adults. A better understanding of the links between those parameters especially between the orosensory detection of dietary lipids and energy/fat intakes in obese subjects may lead to new nutritional strategies aiming to limit the risk of obesity. These interventions would promote healthier eating behavior despite the actual food environment in the Algerian population.

## COMPETING INTERESTS

No potential conflict of interest relevant to this article was reported.

## AUTHORS' CONTRIBUTION

The authors are responsible for the research and have participated in the concept, design, analysis, and interpretation of the data, writing and correction of the manuscript.

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