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Association between CD36 gene variants, PROP taster status and oral fatty taste intensity perception among Malaysian obese and non-obese subjects

[1,3]Bahauddin, A. R., [1*]Karim, R., [1]Saari, N. and [2]Mohd Shariff, Z.

[1]Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43000, Serdang, Selangor, Malaysia
[2]Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43000 Serdang, Selangor, Malaysia
[3]Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Jln. UMS, 88400 Kota Kinabalu, Sabah, Malaysia

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Abstract

Genetic variation in taste is one of the factors that influence taste perception. This study is carried out to ascertain whether CD36 gene variants and PROP taster status are associated with fatty taste sensitivity and perception among lean and obese individuals. A total of 103 obese and 77 lean subjects with mean age of 25.78 ± 5.65 years who took part in the study were classified into PROP nontasters, medium tasters, or supertasters by using the PROP filter paper screening procedure. The suprathreshold sensitivity for linoleic acid solutions and intensity towards two food products ('*Bubur Chacha*' and mango pudding) with different fat content was assessed using the general Labeled Magnitude Scales. The subjects were genotyped for CD36 gene variants (Single Nucleotide Polymorphism (SNPs): rs1761667, rs152748 and rs1049673). It was observed that obese subjects were less sensitive toward fatty taste and gave a lower creaminess rating for the studied food products. Only one CD36 gene polymorphism i.e. rs1761677 and PROP taster status were associated with fat suprathreshold rating. Subjects with AA homozygous for rs1761667 and the supertaster perceived higher oiliness in linoleic acid solution. PROP supertaster significantly perceived higher creaminess in both the food products, but no association was observed between the creaminess rating and CD36 gene variant (rs1761667). All the CD36 gene variants and PROP taster status were not associated with obesity status. These findings indicated that even though the CD36 gene variant influences individuals' oral fat sensitivity, PROP taster status plays a more dominant role in fat taste perception among obese and non-obese individuals.

Introduction

Dietary fat is the most energy-dense macronutrient as it contains twice the energy per gram of carbohydrate and protein (Cotton *et al*., 2007). Overconsumption of this nutrient has been attributed to the rising prevalence of obesity ((Mela, 1996, 2001; Blundell and Finlayson, 2004; King, 2013). However, not all humans overconsumed fat even when it is readily available. In fact, there is a considerable variation in human fat preferences and consumption patterns (Drewnowski *et al*., 1992; Ledikwe *et al*., 2007). Although there are many factors that influence eating behavior, the sense of taste is the key role to the human daily dietary intake (Prinz, 2007; Stewart *et al*., 2010; Stewart *et al*., 2011a; Newman *et al*., 2013).

Numerous studies have revealed the involvement of gustatory or taste component in the oral fat perception of the animals (Gilbertson *et al*., 1997; Gilbertson, 1998; Gilbertson *et al*., 2005; Gaillard *et al*., 2008) and humans (Mattes, 2001, 2005, 2009; Poette *et al*., 2014). However, the taste variability are still observed within the population (Guido *et al*., 2016; Heinze *et al*., 2017). Obese individuals are less sensitive toward oro-sensory detection of dietary fat and are prone to consume more lipids and energy-densed food (Dando, 2015; Dramane *et al*., 2016; Stewart *et al*., 2011c). Meanwhile, growing evidence suggested that genetic variation on taste may explain the observed variability in taste perception and also the tendencies to consume higher or less total energy (Newman *et al*., 2013; Keast *et al*., 2014; Burgess *et al*., 2016). Understanding the genetic clustering

*Corresponding author.
Email:rosaz@upm.edu.my

of phenotypes on body composition will enrich our knowledge on the obesity development and prevention.

An increase in detection threshold for oral dietary lipids and weight gain in human are influenced by several CD36 gene variants. Keller *et al*. (2009) shed light on the association of CD36 gene polymorphism with orosensory detection of high-fat foods and obesity in African-American adults. They observed that the participants with AA genotype at rs1761667 perceived a greater creaminess, regardless of the fat content of the product. Following this, a study by Pepino *et al*. (2011) on CD36 SNP rs1761667 revealed several obese subjects with CD36 AA genotype exhibited a higher oral detection threshold for fat compared to the subjects with AG and GG genotypes. Similar findings were observed in other studies conducted on adult Tunisian women (Mrizak *et al*., 2015) and Algerian normal weight/obese children (Sayed *et al*., 2015) and teenagers (Daoudi *et al*., 2015). On contrary, Karmous *et al*. (2017) did not find any significant difference in the linoleic acid threshold among their Tunisian subjects based on the rs1761667 genotype stratified by obese and non-obese. Other studies found no significant association between rs1761677 of CD36 gene and fatty taste perception (Ong *et al*., 2017; Shen *et al*., 2016) but observed significant association with rs1527483 of CD36 gene (Ong *et al*., 2017).

The genetic ability to taste the bitterness of 6-n-propylthiouracil (PROP) is one of the best-known examples of taste variability. PROP taster status is often used as a general index of human oral chemosensory perception. A number of studies have suggested that PROP sensitivity is positively associated with fat perception and obesity (Tepper and Nurse, 1997; Nasser *et al*., 2001; Hayes and Duffy, 2007; Nachtsheim and Schlich, 2013; Shafaie *et al*., 2015). Hayes and Duffy (2007) found that subjects with higher intensity ratings towards the bitter-tasting substance PROP gave higher creaminess ratings to high fat food. It was also reported that the preference for food with varied fat contents is influenced by the PROP taster status. Anliker *et al*. (1991) noted that even though PROP tasters preferred whole milk significantly more than non-tasters, they showed lower preference for cheese. Tepper and Nurse (1997) found that medium tasters and supertasters could discriminate high fat salad dressings from low fat salad dressings, while the nontasters could not. On the other hand, Yackinous and Guinard (2001) discovered that the PROP taster status was not related to fat perception in a variety of foods (crisps, chocolate drink, vanilla pudding, and mashed potatoes). It is important to note that most of the studies did not considered body weight status as one of the variables along with PROP taster status in determining subjects' taste perception or food preference.

The role of CD36 gene variants and PROP taster status on individuals' fat perception and preferences seems to be dependent on obesity status (Melis *et al*., 2015; Karmous *et al*., 2017). However, to what extent the relationship is involved in fatty taste perception of population needs to be further clarified. To date, no study is available on the association between CD36 gene polymorphism and PROP taster status in the Asian population, particularly Malaysians. Therefore, the aim of this study is to assess whether or not the three common variants in CD36 gene and PROP taster status are associated with fatty taste perception among Malay obese and lean subjects.

Materials and methods

Subjects

One hundred and eighty participants from the Malay ethnic group with age ranging between 20 and 45 years were recruited for this study. Subjects were categorized based on their body mass index according to WHO/IOTF/IASO (2000). They comprised of 15 overweight subjects (BMI of 23 - 24.9), 88 obese subjects (BMI > 25) and 77 lean subjects (BMI of 18.5 to 22.9). However, for the purpose of this study the overweight and obese subjects were placed under one category. The merging of overweight and obese subjects into one category i.e. obese category is acceptable based on the report which stated that overweight subject has similar taste ability such as taste sensitivity (Stewart *et al*., 2010; Stewart *et al*., 2011b; Stewart and Keast, 2012).

Subject recruitment was conducted through flyers posted around the campus of Universiti Putra Malaysia (UPM) and Serdang town area. Potential subjects were screened by a self-report questionnaire (including their BMI) prior to this study. The inclusion criteria are as follows: subject must be in a good health, not suffering any any chronic diseases, without any food allergies, currently not taking any medications that may interfere with the taste or olfactory perception, and are not pregnant or breastfeeding their children. They were excluded if they have eating restraint (score less than 13 for restrained scale) (Garner and Garfinkel, 1979) and under depression (score less than 50 for Zung self-rating depression scale) (Zung, 1965). Successful subjects were invited to attend two sensory sessions at the sensory laboratory of Faculty of Food Science

and Technology in UPM. A written informed consent was obtained from all subjects and the protocol was approved by the Human Research Ethics committee of UPM.

Study design

This exploratory study involved the use of comparison cross-sectional design. All subjects attended two sensory sessions, which took them approximately 30 to 40 min to complete. Prior to each sensory tasting session, the subjects' anthropometries measurement and DNA samples (buccal cell) were obtained.

Anthropometry measurement

The weight and height of the subjects were measured. Each subject's body weight was measured using a weighing scale (Omron HN-288, Kyoto, Japan) while the height was measured using a mechanical measuring tape (Seca 206, Hamburg, Germany).

Sensory test

Two type of sensory assesments, which are commonly used in chemosensory research, namely suprathreshold intensity rating and hedonic test were used. All tasting were done in individual booth to prevent discussion. The subjects were briefed and trained on the general usage of the general Labelled Magnitude Scale (gLMS) prior to the sensory test (Webb *et al*., 2015).

Taste-Stimuli

PROP and Natrium Chloride (Nacl) impregnated filter paper

The PROP and sodium chloride (NaCl) paper disk were prepared according to Zhao *et al*. (2003). A 50-mmol/l PROP solution was prepared by dissolving 8.5 g PROP powder in 1 L of boiling water (100°C). Prior to the treratment, several pieces of 1.5 cm diameter paper disks (10–12 pieces) were threaded together by using a string that was fitted with 2 cm tubing as a spacer. Then, the paper disks were soaked in the PROP solution for 30 s and the excess PROP solution was removed by shaking the string of paper disks before drying in an oven (Model UN30, Memmert, Schwabach, Germany) for 1 h at 121°C. Meanwhile, the NaCl paper disks were prepared similar to the preparation of the PROP disk by soaking the prepared disks for 30 s in a 1.0-mol/l NaCl solution at room temperature. All paper disks were stored separately in sealed plastic bags at room temperature until further usage.

Linoleic acid emulsion

Linoleic acid was used as a stimulus for the suprathreshold intensity rating. Five different concentrations of linoleic acid (Fluka Analytical Sigma-Aldrich®, St. Louis, MO) emulsion which include 0.04%, 0.50%, 1.00%, 2.10%, and 4.30% (v/v) were prepared, to represent a wide range of linoleic acid found in most food (Martinez-Ruiz *et al*, 2014). A 0.15% (w/v) xanthan gum (Sigma-Aldrich®, St. Louis, MO) and 0.18% (v/v) mineral oil (Spectrum Chemical® Gardena, CA) were added to produce perceptually identical textural attributes including viscosity and lubricity between fatty acid and control samples. All samples were mixed with 0.01% (w/v) EDTA (R&M Chemicals, Semenyih, Malaysia) to prevent oxidation. All the samples were prepared using distilled water and homogenized for 8 min at 18000 rpm (Heidolph homogenizer, Diax 900, Germany). The emulsions were placed in amber bottles that were covered with aluminium foil before being served to the panellists. Samples were prepared fresh on the testing day or at least 1hr prior to the tasting procedure. Control samples were prepared the same way, but without the addition of linoleic acid.

Food samples

Two food samples namely local creamy porridge or known as '*Bubur Chacha*' among the Malaysians and a mango pudding were used. Besides being commonly consumed desserts by the Malay ethnic population, these food products were chosen because their consistency can easily be controlled during the preparation. Different concentrations and type of fat sources were added to the food formulations. Coconut milk was used as the fat source for preparation of '*Bubur Chacha*', while evaporated milk was used for the mango pudding. Both fat sources were chosen as they were most commonly used ingredients in the preparation of Malay cuisines (Shahar *et al*., 2000; Rashid *et al*., 2011). Both food samples were prepared and were kept in a chiller (4±2oC) at least 24 h prior to sensory testing. Tables 1 (a) and (b) listed the ingredients for preparation of '*Bubur Chacha*' and mango pudding, respectively.

Five '*Bubur Chacha*' at different fat levels which were 7.0%, 8.0%, 10.0%, 13.0% and 17.0% (v/v) were prepared in this study. Variation of fat content in the products was achieved by changing the ratio of coconut milk to water in the final products accordingly. To ensure samples' consistency, '*Bubur Chacha*' was prepared in two separate parts. In the first part, 500 g of dried wheat dough strips were boiled for 5 min, drained to remove the cooking water followed by cooling to room temperature.

Table 1 The ingredients used in food samples preparation

(a) '*Bubur Chacha*'

Samples/ Formulation	Ingredients				Estimated Fat content (%) in product
	Coconut Milk (ml)	Water (ml)	Palm Sugar (g)	Wheat Dough strips (g)	
1	320	680	80.0	200.0	7.0
2	400	600	80.0	200.0	8.0
3	500	500	80.0	200.0	10.0
4	640	360	80.0	200.0	13.0
5	800	200	80.0	200.0	17.0

(b) Mango Pudding

Samples/ Formulation	Ingredients				Estimated Fat content (%) in product
	Evaporated Milk (ml)	Water (ml)	Instant Pudding powder (g)	Sugar (g)	
1	250	750	100.0	40.0	2.5
2	320	680	100.0	40.0	3.0
3	400	600	100.0	40.0	3.7
4	500	500	100.0	40.0	4.5
5	650	350	100.0	40.0	5.7

Meanwhile, in the second part, coconut milk gravy was prepared by boiling filtered water, coconut milk (Ayam Brand Sdn. Bhd., Malaysia), and palm sugar (SCS Food Manufacturing Sdn. Bhd., Malaysia) for 2 min at 100oC to allow the palm sugar to dissolve. Then, the cooked wheat dough strips (7 g) was added to the coconut milk gravy (40 ml) in a polypropylene plastic bowl (serving size of 2 oz), allowed to cool at room temperature and chilled in a chiller before testing.

Mango pudding preparation

Mango puddings with five different fat contents (2.5%, 3.5%, 3.75%, 4.5% and 5.7% v/v) were prepared. The fat content was varied by adding different proportion of evaporated milk (Fraser and Neave Limited, Singapore) to filtered water in the formulations (Table 1b). The mixture of filtered water and evaporated milk was allowed to boil at 100oC. Then, an instant mango flavoured pudding powder (Happy Grass Sdn. Bhd., Malaysia) and white granulated sugar (CSR Sdn. Bhd., Malaysia) were added to the mixture. The mixture was stirred for 2 min until all the ingredients were dissolved. Following this, 15 ml of the boiled mixtures were poured into individual ¾ oz cup and allowed to cool at room temperature before they were kept in a chiller and served to the panelists.

Tasting procedures

PROP taster status determination

The bitterness of the PROP was rated by the subjects by using the general Labeled Magnitude Scale (gLMS). The gLMS ranges from 0, which represents no sensation, to 170 mm, which represents the strongest imaginable sensation of any kind with the intermediate levels at 2.38 mm (barely detectable), 10.2 mm (weak), 28.5 mm (moderate), 59.5 mm (strong), and 86.7 mm (very strong). Before the scale was used, subjects were given an orientation to the gLMS so that they are confident to use the scale (Choi, 2015). During tasting, subjects were asked to place the PROP paper disk on the tip of their tongue, moisten the disk with saliva and waited for 30 s before proceeded with their evaluation. A ruler was used to measure the subject's response to the gLMS. Those who rated the intensity of the PROP disk in between 20 mm and 100 mm on the gLMS were classified as medium taster, subjects rated less than 20 mm were classified as nontasters, and subjects rated more than 100 mm were a supertaster. Meanwhile, NaCl ratings were used to help classify taster classification when subjects gave a borderline rating to PROP (Zhao *et al*., 2003).

Oiliness rating

Subjects were presented with five samples of linoleic emulsions in 1 oz. plastic cups labelled with random-digit numbers. Samples were tasted, spat, and

scored for perceived intensity using a 170 mm gLMS. Oiliness attribute was measured instead of other descriptors such as "pungent", "bitter", or "fattiness" when identifying the flavor for linoleic acid (Majid and Lavinson, 2008). Subjects were instructed to eat a plain biscuit to cleanse their palate and also to rinse their mouth with water until no aftertaste remained before proceeding to taste another sample. Also, subjects were required to rest for 1 min before tasting the next sample to avoid fatigue. Subjects were asked to put on a nose clip to avoid any olfaction cues or bias.

Creaminess Rating

Subjects were asked to taste the food samples, namely '*Bubur Chacha*' and mango pudding and they were instructed to evaluate the intensity of creaminess of both the products on a separate 170 mm gLMS sheet. Subjects rinsed their mouth with water and were instructed to take a 1 min rest before tasting the sample from left to right. Subjects were allowed to taste the following set of sample after 5 mins of elapse time. All samples were coded with three-digit random numbers and were arranged in a randomized order to avoid bias. In this session, all procedures were conducted under red light to mask colour differences between samples. However, subjects did not wear nose clip to stimulate actual eating experience (Keller *et al*., 2009).

DNA collection and genotyping

Disposable cytobrush (Medical Packaging Corporation, USA) was used to collect buccal cell specimens from the left and right cheeks of all subjects and kept in a labelled envelope at room temperature before the DNA extraction procedures were carried out using a standard commercial kits (Analytic Jena innuPREP DNA mini kit, Germany). The extraction procedures were conducted according to the manufacturer's protocols. The quality of the extracted genomic DNA was evaluated by means of gel electrophoresis whilst the quantity and purity of the extracted genomic DNA were determined by using the NanoPhotometer (Implen P300, USA). All the CD36 variants (rs1761667, rs1527483, rs1049673) were genotyped by using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR–RFLP) based on Banerjee *et al*. (2010) with a slight modification. PCR was performed in 20 µl reaction volume containing 10 pmol forward and reverse primer, 10 µl PCR buffer (including prime TaqTM DNA polymerase, Tris-HCl, KCl, MgCl2, enzyme stabilizer, sediment, loading dye, and 0.5 mM of each dATP, dCTP, dGTP, dTTP) (Bioline, USA) and 100 ng DNA template in thermal cycler (Biosystem Veriti 96, USA). The PCR products were digested with the respective restriction enzyme to genotyped all the CD36 variants in this study. About 1 µl of Novel juice (GeneDirex, Korea), a fluorescent agent, was mixed to 4 µl digested products before being electrophoresed on 3% agarose gel or 5% Super Fine Resolution (SFR) agarose based on the amplicon size. The procedures ended up with a UV light visualization. Details of the primer sequences, annealing temperature, Restriction Ensumes with product sizes and type of agarose gel used for each CD36 variant are summarized in Table 2.

Table 2 Primer sequences, PCR conditions, amplicon sizes, restriction enzymes with product sizes and agarose gel used

SNPs	Primer Sequence	Anneling Temp. (0C)	Product Size (bp)	Restriction Enzyme / allele sizes	Type of agarose gel
rs1761667	F : 5'- CAAAATCACAATCTATTCAAGACCA -3' R : 5'- TTTTGGGAGAAATTCTGAAGAG -3'	610C	190	Hha I GG 138, 52 GA 190, 138, 52 AA 190	3% agarose gel
rs1527483	F : 5'- CGCTACAACAATTTTATAGATTTTGAC -3' R : 5'- TGAAATAAAAATAATCTTGTCGATGA -3'	620C	252	Taq I CC 160,70,22 CT 230,160,70,22 TT 230,22	5% SFR gel
rs1049673	F : 5′- ACGCTTGGCATCTTCAGAATGCT-3′ R : 5′-TGAACCCCTGCTCAAGAAACAGAGT -3′	600C	465	Mnll GG 331, 134 GC 331, 265, 134, 66 CC 265, 134, 66	3% agarose gel

Statistical analysis

Statistical analysis was carried out using SPSS version 21.0 (IBM Incorporated, New York, USA). Normality test was used to verify any missing values and the data were screened for data outliers according to Hansen *et al*. (2006). Continuous data were log-transformed to achieve normal distribution, where necessary. Chi-square analysis was used to measure the association between PROP taster status and CD36 gene polymorphism with the BMI status. Two-way Repeated Analysis of Covariate (ANCOVA), controlling the age and sex was used to test the effect of BMI status towards oral fatty suprathreshold rating and creaminess ratings. A similar analysis was used to analyse the effect of PROP taster status or CD36 gene variants on sensory measures of obese and lean subjects. Gender and age were treated as covariate in all analyses. Pairwise comparisons were evaluated with a LSD post-hoc test to determine the differences between PROP taster status and CD36 gene polymorphism among obese and lean subjects where necessary. All continuous data were presented in means ± standard deviation (S.D.). A *p*-value of 0.05 or lower is reported as a significant difference.

Results

Subjects characteristics

A total of 202 subjects were involved in the initial stage of this study. However, only 180 subjects; comprising of 103 obese subjects (33 males, 70 females) and 77 lean subjects (21 males, 56 females) were included in the statistical analysis. Data from the remaining subjects were not included due to either being outliers or having some missing values. The means age of the subject were 25.78 ± 5.65 years. The BMI range in the current study was 19 to 45.5 kg/m^2 with the means of 27.56 ± 6.74 kg/m^2.

Overall oral fatty sensitivity and creaminess rating between obese and non-obese subjects

As shown in Figures 1a and 1b, intensity rating for oiliness (linoleic acid) and creaminess of '*Bubur Chacha*' and mango pudding were higher in lean subjects compared to obese subjects. Regardless of concentration used, generally the obese group showed a significantly ($p \leq 0.05$) lower ratings for oiliness of linoleic acid solution and creaminess of mango pudding. However, no significant difference ($p > 0.05$) was discovered for creaminess rating of '*Bubur Chacha*'.

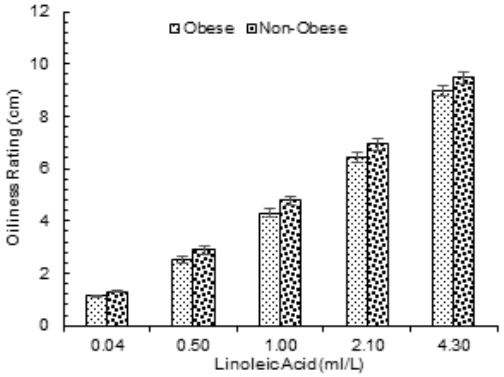

Figure 1(a) Mean suprathreshold rating for oiliness of five different concentrations of linoleic acid solutions in obese and lean subjects (*p≤0.05)

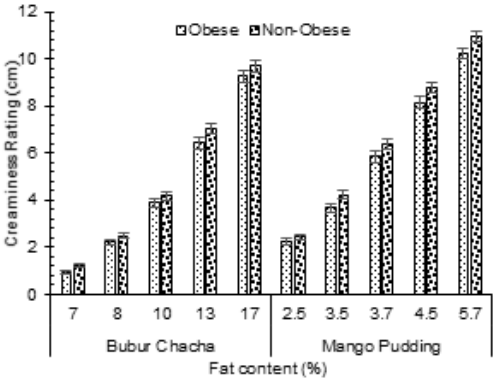

Figure 1(b) Mean intensity rating for creaminess of different food samples with the increasing of fat content in obese and lean subjects (*p≤0.05).

Error bar represents standard error of the mean (SEM)

Effect of PROP taster status and variant of CD36 genes towards oral fatty taste suprathreshold

Subjects' distribution based on the CD36 gene polymorphism and PROP taster status is shown in Table 3 (a). There was no significant association between obesity status and each CD36 gene polymorphism or PROP taster status ($p > 0.05$). In addition, no association was observed between PROP taster groups and the distribution of CD36 gene variant at rs1761677 in both obese and lean subjects ($p > 0.05$). In term of suprathreshold rating, the addition of linoleic acid into the food sample increased subjects' oiliness rating of the product [$F(4, 172) = 6.136$, $p < 0.001$). There was a significant main effect between fat suprathreshold rating and PROP taster status in both the obese [$F(1, 98) = 8.677$, $p < 0.001$] and lean [$F(1, 72) = 7.314$, $p = 0.001$] subjects. Supertasters showed a significantly higher rating for oiliness compared to non-taster subjects in both BMI groups ($p \leq 0.05$). With regard to CD36 gene variant, only the variation rs1761667 showed a significant association with oiliness rating in obese [F(1,

Table 3 (a) Distribution of genotypes and PROP taster status in non-obese and obese subjects

CD36 gene SNPs/ PROP Taster Status	Non-obese (n=77)	Obese (n=103)
CD36 (rs1761667)		
Alleles (%)		
A	72 (39.13)	78 (44.31)
G	112 (60.87)	98(55.68)
Genotype (%)		
AA	10 (10.9)	14 (15.9)
GA	43 (56.8)	59 (56.8)
GG	24 (27.3)	30 (27.3)
HWE (χ^2); p value	3.2002 ; 0.074	2.013 ; 0.156
BMI x rs1761667 (χ^2) ; p value	0.526	
CD36 (rs1527483)		
Alleles (%)		
T	54 (29.4)	54 (30.7)
C	130 (70.6)	122 (69.3)
Genotype (%)		
TT	7 (9.8)	12 (11.4)
CT	30 (39.1)	40 (38.6)
CC	40 (51.1)	51(50.0)
HWE (X^2); p Value	0.293 ; 0.588	0.740 ; 0.389
BMI x rs1761667 (χ^2) ; p value	0.432	
CD36 (rs1049673)		
Alleles (%)		
C	106 (57.6)	97 (55.1)
G	78 (42.4)	79 (44.9)
Genotype (%)		
GG	22 (28.3)	24 (22.7)
GC	48 (58.7)	63 (64.8)
CC	7 (13.0)	16 (12.5)
HWE (X^2); p Value	3.744; 0.053	8.410; 0.004
BMI x rs1049673 (χ^2) ; p value	0.664	
PROP Taster Status		
Supertaster	38 (43.5)	40 (41.2)
Medium Taster	26 (38.0)	36 (30.7)
Non taster	13 (18.5)	27 (26.1)
BMI x PROP Status ; p value	0.388	

Table 3 (b) The association between gene polymorphisms and PROP taster status with suprathreshold fat taste sensitivity

Genotypes or PROP taster Status	Linoleic Acid Concentration (%)										p^a	p^b	p^c
	Non-obese					Obese							
	0.04	0.50	1.00	2.10	4.30	0.04	0.50	1.00	2.10	4.30			
CD36 - rs1761667													
Genotype													
AA	1.475 ± 0.14	3.377 ± 0.40	4.936 ± 0.45	7.949 ±0.57	10.930 ± 0.50	1.292 ± 0.11	2.975 ± 0.34	5.397 ± 0.47	7.739 ± 0.58	10.241 ± 0.58	p < 0.001	p = 0.032	p = 0.049
GA	1.338 ± 0.06	2.875 ± 0.18	4.834 ± 0.22	6.919 ± 0.27	9.636 ± 0.24	1.292 ± 0.12	2.586 ± 0.16	4.156 ± 0.23	6.350 ± 0.28	8.972 ± 0.28			
GG	1.210 ± 0.09	2.616 ± 0.25	4.575 ± 0.29	6.518 ± 0.36	8.739 ± 0.32	1.292 ± 0.13	2.279 ± 0.23	4.074 ± 0.32	5.952 ± 0.39	8.289 ± 0.40			
CD36 - rs152783													
Genotype													
TT	1.372 ± 0.08	2.977 ± 0.22	5.056 ± 0.26	7.376 ± 0.33	9.829 ± 0.31	1.111 ± 0.07	2.379 ± 0.20	4.083 ± 0.28	6.077 ± 0.34	8.551 ± 0.34	p < 0.001	p = 0.197	p = 0.273
CT	1.428 ± 0.17	2.787 ± 0.46	4.849 ± 0.54	7.006 ± 0.69	9.937 ± 0.65	1.050 ± 0.12	2.433 ± 0.36	4.008 ± 0.51	6.082 ± 0.62	8.304 ± 0.63			
CC	1.253 ± 0.07	2.787 ± 0.20	4.536 ± 0.23	6.578 ± 0.29	9.223 ± 0.27	1.148 ± 0.06	2.711 ± 0.17	4.54 ± 0.25	6.775 ± 0.30	9.406 ± 0.30			
CD36 - rs1049673													
Genotype													
GG	1.373 ± 0.06	2.966 ± 0.18	4.809 ± 0.21	7.058 ± 0.26	9.615 ± 0.25	1.141 ± 0.05	2.448 ± 0.16	4.251 ± 0.22	6.390 ± 0.27	8.869 ± 0.28	p < 0.001	p = 0.878	p = 0.685
GC	1.199 ± 0.09	2.581 ± 0.26	4.644 ± 0.31	6.613 ± 0.40	9.483 ± 0.37	1.008 ± 0.08	2.756 ± 0.26	4.532 ± 0..36	6.533 ± 0.45	9.131 ± 0.46			
CC	1.288 ± 0.17	3.003 ± 0.46	4.865 ± 0.55	7.024 ± 070	9.033 ± 0.65	1.218 ± 0.10	2.642 ± 0.31	4.151 ± 0.44	6.389 ± 0.55	8.967 ± 0.56			
PROP taster status													
Supertaster	1.394 ± 0.06	2.946 ± 0.19	4.827 ± 0.23	7.214 ± 0.28	10.02 ± 0.25	1.252 ± 0.06	2.964 ± 0.19	4.980 ± 0.23	7.131± 0.28	9.778 ± 0.25	p < 0.001	p = 0.001	p = 0.001
Medium Taster	1.441 ± 0.08	3.024 ± 0.24	5.081 ± 0.27	7.273 ± 0.34	9.685 ± 0.30	1.159 ± 0.08	2.489 ± 0.24	4.353 ± 0.27	6.317 ± 0.34	8.897 ± 0.34			
Non taster	0.836 ± 0.11	2.277 ± 0.33	3.961 ± 0.39	5.401 ± 0.48	7.752 0/43	0.88 ± 0.11	2.017 ± 0.33	3.220 ± 0.39	5.516 ± 0.48	7.777 ± 0.43			

Data are expressed as mean ± SE

p^a p -value by repeated measures ANOVA for the diffference among the fat added in the samples (within subjects)

p^b p-value by repeated measures ANOVA for main effect genotype /phenotype among obese subjects; adjusted for age and sex

p^c p-value by repeated measures ANOVA for main effect genotype /phenotype among non-obese subjects; adjusted for age and sex

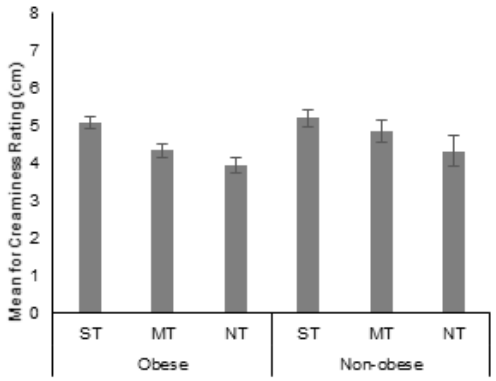

Figure 2(a) Mean creaminess rating of '*Bubur Chacha*' in PROP taster status stratified by BMI status

Error bars are SEM. **ST-Supertaster, MT-Medium Taster, NT-Non-taster

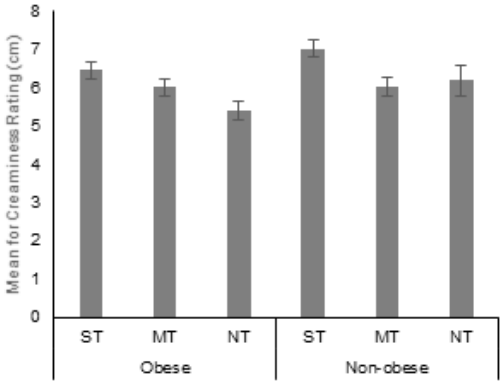

Figure 2(b) Mean creaminess rating of mango pudding between PROP taster status stratified by BMI status. Different letters indicate a significant difference at $p \leq 0.05$.

98)=3.581, p=0.032) and lean subjects [1, 72=2.922; p=0.049] (Table 3b). Pairwise comparison analysis revealed that the carrier of G allele for rs1761667 had a significantly (p<0.05) lower fatty taste sensitivity than the respective minor allele homozygote AA in obese and lean subjects.

Influence of PROP taster status and variant of CD36 genes on oral fatty taste acceptance in food model

As mentioned earlier, only PROP taster status and rs1761667 of CD36 gene had a significant association with fatty suprathreshold rating. This indicated that both variables play a significant role in fatty taste sensitivity among the subjects of this study. Thus, only these two variables were analyzed further analyzed to determine their effect on subject' s creaminess rating in two food models, namely, '*Bubur Chacha*' and mango pudding.

Overall, AA genotype of CD36 gene variant (rs1761667) perceived higher creaminess rating compared to G allele carriers. However, no significant association between creaminess rating of both food products and rs1761667 polymorphism of CD36 gene was found in obese and lean subjects (p>0.05). In contrast, the PROP taster status was associated with creaminess rating in both obese and lean subjects ($p \leq 0.05$). As illustrated in Figure 2 (a) – (b), supertaster and medium taster individuals indicated a higher creaminess rating compared to non-tasters from both BMI groups ($p \leq 0.05$).

Discussion

The present study focused on oral fat suprathreshold sensitivity and fat taste perception in obese and lean subjects. The relationship between CD36 gene polymorphism and PROP taster status towards fat taste perception was also examined. The findings demonstrated that obese individuals had lower sensitivity towards fatty taste compared to non-obese subjects. In fact, significant differences were detected in the fat suprathreshold rating between obese and non-obese subjects. Meanwhile, PROP taster status plays a dominant role in determining fat taste perception among obese and non-obese subjects compared to the CD36 gene polymorphisms.

Generally, obese subjects showed a lower oral fat sensitivity compared to lean subjects in this present study. This is supported by various studies which reported that the increase in body weight reduced fatty taste sensitivity, hence could lead to overconsumption of high-fat foods and weight gain (Keast *et al*., 2016). In addition, BMI has been reported to be associated with fat taste sensitivity (Stewart *et al*., 2010, 2011b, 2011c; Stewart and Keast, 2012; Martinez-Ruiz *et al*., 2014). However, some researchers found no differences on the oral fatty taste threshold between obese and lean individuals (Chevrot *et al*., 2014; Tucker *et al.*, 2014; 2015). The cause of the discrepancies among the findings remained uncertain. However, the methodology for measuring taste sensitivity (threshold vs. suprathreshold) used might lead to variation in findings. Webb and colleagues (2015) revealed that the detection and recognition threshold are closely linked together but they were seldom found to correlate with the suprathreshold intensity rating. Additionally, the taste thresholds have been reported as poor predictors to the real world perception while the suprathreshold comparisons are more practical for comparing groups differences (Bartoshuk *et al*., 2006). Furthermore it was also observed that most of the studies do not take into account the taste genetic variation which may attribute to the taste differences within the groups in their studies.

This study hypothesized that the differences in oral fatty taste sensitivity and perception among individuals might be contributed by by CD36 gene variation and PROP taster status. As expected, our results showed that orosensory perception of fatty acid (linoleic acid) is associated with the PROP taster status and variant rs1761677 of CD36 gene among the subjects. However, contrary to majority of literatures (Pepino *et al*., 2012; Daoudi *et al*., 2015; Melis *et al*., 2015; Mrizak *et al*., 2015; Sayed *et al*., 2015), the data observed in this study demonstrated that homozygous individuals for A allele showed a higher sensitivity towards linoleic acid compared to homozygous G allele. A similar study by Keller *et al*. (2009) supported our findings. The reason for the discrepancies are not known at this stage, but it could be due to differences in the genetic background of the population (Pioltine *et al*., 2016). Furthermore, this study observed that Minor Allele Frequency (MAFs) for rs1761677 of CD36 gene in our cohort was higher (0.42) compared to other population. Data from HapMap project showed that MAF for other population ranged from 0.17 (Bengali from Bangladesh) to 0.47 (Toscani in Italy) (NCBI, 2017). Interestingly, the findings from studies with higher MAFs demonstrated a similar result with the present work (Keller *et al*., 2009; Ong *et al*., 2017). Keller *et al*. (2009) in their study on African-Caucasian population obtained MAF of 0.23, whilst Ong *et al*. (2017) observed a MAF value of 0.30 in their study on Malaysian population with Chinese as the major ethnic group. Future studies on other population including cross-cultural studies, particularly on gene expression are needed to determine a definitive answer.

Out of 180 subjects who participated in this study, only 22.3% subjects were classified as non- taster. Our finding is slightly higher compared to the ratio in our neighbouring countries of which the Philippines was only 12% (Villarino *et al*., 2009) and Thailand was only 9.5% for the Thai people (Boobphanirojana *et al*., 1970). It was predicted that the variation of PROP blindness among the population ranged from 3% in the Africa region, 6%–23% in China, 40% in India and 30% among the Caucasian populations (Guo *et al*., 2001). However, the ratio excluded the weight status among their samples. Our study only focused on obese and non-obese subjects but not on the group overall BMI.

Interestingly, we observed that the association between the variation at rs1761677 and PROP taster status towards fatty perception was not modified by the BMI status. Therefore, both variables have a direct effect on the fatty sensitivity in both obese and non-obese individuals. Surprisingly, our findings differ from previous studies whereby reduced taste sensitivity was observed only among the obese subjects, particularly due to the decreased in CD36 gene expression by A-allele (Pepino *et al*., 2012; Mrizak *et al*., 2015; Sayed *et al*., 2015). To the best of our knowledge, there have been very limited studies that compared the influence of genotype on taste perception among obese and non-obese subjects. The study by Daoudi *et al*. (2015) revealed that A-allele frequency was higher among obese children but no association was recorded between the variation of rs1761677 of CD36 gene and oleic acid (OA) threshold. Meanwhile, Sayed *et al*. (2015) showed that OA detection threshold in A-allele was higher than in G-allele but only among obese children. However, both studies were conducted among younger age group (aged 8–14 years), which could lead to the discrepancies in the findings. The reason could be explained by the fact that as human fungiform papillae only attained its full size at the age of 8–10 years and the circumvallate papillae continue to grow until the age of 15–16 years (Temple *et al*., 2002). Furthermore, Laugrette *et al*. (2005) had demonstrated that CD36 mRNA is expressed 9 times higher in circumvallate papillae compared to fungiform papillae.

A similar pattern was observed in the variation of PROP taster status whereby the supertaster has a higher oral fatty sensitivity in both obese and non-obese individuals. Research on the influence of PROP taster status on obesity development and fatty taste perception among subjects had been done since 1960s (Beckett *et al*., 2014). It was postulated that the non-tasters were more likely to be endomorphs (heavier) because they required more fatty taste stimulus and signal to elicit satiety response (Drewnowski *et al*., 1985; Newman *et al*., 2013). In the present study, our data failed to show a similar pattern but the findings supported the hypothesis that genetic ability to detect PROP bitterness influenced the individuals' fatty taste sensitivity. Our finding also revealed that obese individuals for all PROP taster status variant had reduced their oral fatty taste sensitivity but failed to achieve a significant level. This result suggested that fatty taste sensitivity among obese and lean individuals is also influenced by genetic taste variation rather than only by diet (Stewart and Keast, 2012; Newman *et al*., 2013) and weight regulation (Drewnowski *et al*., 1985, 1991). Besides, this finding could also shed some light on the inconsistent findings regarding fat taste perception between obese and non-obese subjects from the previous studies reported by Cox *et al*. (2015) However, further

studies are required to confirm this hypothesis.

Notably, our results did not observe any significant association between CD36 gene variation and subject's creaminess rating of food samples used in this study. However, a consistent pattern on the subjects' rating was observed whereby AA genotype individuals had higher creaminess rating compared to other genotype groups. The absence of CD36 gene influence on subjects' creaminess rating is not surprising even though some studies showed different findings. In this study, creaminess was used as the measured attribute because it was commonly used in milk-based food products (Drewnowski *et al*., 1989; Hayes and Duffy, 2007; Hayes and Duffy, 2008; Shen *et al*., 2016). Creaminess is a complex sensory characteristic that consists of both the flavor and textural components. Since fatty acids are ligands for CD36 gene, the differences in creaminess ratings between different genotype groups were observed to be due differences in their ability to detect the amount of free fatty acids in the samples. In contrast, the portion of fatty acid in fatty food is low, thus, the textural and flavors attributes became more pronounced during rating process. This is in agreement with a study by Shen *et al*. (2017) who demonstrated that the liking rating of ice cream was attributed by the tactile sensory cues such as mouthfeel, powdery, and greasy compared to the genetic variation of CD36 and CA6 gene. It could be suggested that the genetic variation of CD36 gene may be more pronounced in the fatty stimulus detection (sensitivity) of simple food system but not in complex food model because probably other mechanism such as external sensory cues (e.g. texture and flavor) might be involved in the latter.

Since this study was focused on the influence of genotypical and phenotypical variation of fatty taste perception, it is important to take into account the sample's characteristic (carrier and type of fat) used. Instead of using aqueous solution, two complex foods that are high in fat and are commonly consumed daily (coconut milk and evaporated milk) were used in this study. This is to simulate real eating experience as people would not generally choose to consume prototypical laboratory tastants outside of laboratory settings. However, the variation of carriers, type of fat, and state of products (either solid, or liquid) could lead to different outcomes from one study to another (Heinze *et al*., 2015). Indeed, our data demonstrated that there are some differences in subject's responses on the studied food products. For instance, the sensory qualities were considerably easier to evaluate in '*Bubur Chacha*' compared to mango pudding. This could due to mechanical properties such as texture, tenderness, and firmness of the tested product which could also influence the fat taste perception. Apart from that, fatty acid composition of both fat sources may affect the perceived product's fatty taste and flavor component among the subjects (Chen *et al*., 2004; Mattes, 2009; Heinze *et al*., 2015). It was noted that both fat sources used in this study contained different fatty acid compositions which could also explain the absence of association between CD36 variant (rs1761667) and creaminess rating among the subjects (Mattes, 2009).

It is important to emphasize that our data revealed that the effect of PROP taster status was dominant and consistent compared to the variation of CD36 gene throughout this study. This finding is comparable with recent observation made by Melis *et al*. (2015) who found that capability to detect oleic acid was directly associated with PROP responsiveness but not on CD36 variant (rs1761677). Furthermore, they also found that non-taster had lower distribution of taste papillae, which cause the lower oleic acid detection. Several studies also indicated that supertaster has a higher density of fungiform on their tongue that could be reflected in their taste and tactile sensitivity towards food products (Hayes and Duffy, 2007; Hayes and Duffy, 2008; Bakke and Vickers, 2011). A previous study by Tepper and Nurse (1997) found that the PROP supertasters and medium-tasters were able to differentiate the fat content of 40% and 10% in the salad dressing, unlike the nontasters. Moreover, the same study also suggested the nontasters showed a higher hedonic preference for the 40% fat salad dressing despite failing to discriminate the difference in fat content between the two samples (Tepper and Nurse, 1997). It could be suggested that the high papillae distribution among supertaster may explain the higher detection of fatty components in food due to higher tactile sense among them (Hayes *et al*. 2007, 2008). On the other hand, the density of taste papillae could explain the availability of taste receptor but could not be directly correlated with their function due to the gene variances and expression (Melis *et al*., 2015; Priego *et al*., 2015).

Limitations of this study should be noted. First, the present study only focused on healthy young adults among the Malay population. Thus, our findings could not be generalized to other groups (e.g. elderly subjects, other etnicities). Second, these findings may not be applicable to complex foods as other food systems may have different oral cues of fat content (Drewnowski *et al*., 1989). Third, the CD36 gene is highly polymorphic, containing SNPs, insertions and deletions, and duplications. Therefore, other SNPs in CD36 that have high linkage disequilibrium (LD)

with rs1761667 could also be associated with oral fat perception and obesity. Lastly, a larger sample size should be considered in the future study as the number of panellists in this study was relatively small.

Conclusion

In conclusion, our results showed that CD36 rs1761677 SNP and PROP taster status influenced the individuals' fatty taste sensitivity among obese and non-obese subjects. However, PROP taster status plays a dominant role in determining oral fatty taste perception as the effect of this variable was consistent throughout simple and complex sample used in this study. Nevertheless, all the three CD36 gene variants and PROP taster status did not associated with obesity status. Thus, it can be stated that our findings support the hypothesis that genetic variation on taste, particularly PROP taster status influences human fatty taste perception but not the obesity status. Besides, this present study also raises the possibility of other external sensory cues such as texture, viscosity, and state of a product in determining fat preference among the subjects. Future studies are needed to ascertain the role of tactiles properties, taste genetic variation, and gene expression in determining fatty food perception among individuals.

References

Anliker, J. A., Bartoshuk, L., Ferris, A. M. and Hooks, L. D. 1991. Children's food preferences and genetic sensitivity to the bitter taste of 6-n-propylthiouracil (PROP). The American Journal of Clinical Nutrition54(2): 316-320.

Bakke, A. and Vickers, Z. 2011. Effects of bitterness, roughness, PROP taster status, and fungiform papillae density on bread acceptance. Food Quality and Preference 22(4): 317–325.

Beckett, E. L., Martin, C., Yates, Z., Veysey, M., Duesing, K. and Lucock, M. 2014. Bitter Taste Genetics - the relationship to tasting, liking, consumption and health. Food & Function 5: 3040–3054.

Blundell, J. E. and Finlayson, G. 2004. Is susceptibility to weight gain characterized by homeostatic or hedonic risk factors for overconsumption? Physiology and Behavior 82(1): 21–25.

Boobphanirojana, *P*., Chetanasilpin, M., Saengudom, C. and Flatz, G. 1970. Phenylthiocarbamide taste thresholds in the population of Thailand. Humangenetik 10(4): 329-334.

Burgess, B., Rao, S. P. and Tepper, B. J. 2016. Changes in liking for sweet and fatty foods following weight loss in women are related to prop phenotype but not to diet. Obesity 24(9): 1867–1873.

Chen, S., Bobe, G., Zimmerman, S., Hammond, E. G., Luhman, C. M., Boylston, T. D., … and Beitz, D. C. 2004. Physical and sensory properties of dairy products from cows with various milk fatty acid compositions. Journal of Agricultural and Food Chemistry 52(11): 3422–3428.

Chevrot, M., Passilly-Degrace, P., Ancel, D., Bernard, A., Enderli, G., Gomes, M., … and Besnard, P. 2014. Obesity interferes with the orosensory detection of long-chain fatty acids in humans. The American Journal of Clinical Nutrition 99(5): 975-983.

Choi, S. E. 2015. Taste Preference Evaluation: The First Step towards a Successful Weight Loss Strategy. J Obes Weight Loss Therapy 5: e117.

Dando, R. 2015. The plasticity of taste function links the appetitive taste of fats with obesity. Chemosensory perception 8(2): 53-60.

Daoudi, H., Plesník, J., Sayed, A., Šerý, O., Rouabah, A., Rouabah, L. and Khan, N. A. 2015. Oral Fat Sensing and CD36 Gene Polymorphism in Algerian Lean and Obese Teenagers. Nutrients 7(11): 9096–9104.

Dramane, G., Gnanka, S. and Ahyi, V. 2016. Marked differences of fat taste sensitivity between obese and lean subjects. International Journal of Nutrition and Metabolism 8(1): 1–6.

Drewnowski, A., Brunzell, J. D., Sande, K., Iverius, P. H. and Greenwood, M. R. 1985. Sweet tooth reconsidered: Taste responsiveness in human obesity. Physiology and Behavior 35(4): 617–622.

Drewnowski, A., Eileen, E., Lipsky, C., Stellar, E. and Greenwood, M. R. 1989. Sugar and Fat : Sensory and Hedonic Evaluation of Liquid and Solid Foods. Physiol Behav 45(16): 177–183.

Drewnowski, A., Kurth, C. L. and Rahaim, J. E. 1991. Taste preferences in human obesity : environmental and familial factors. The American Journal of Clinical Nutrition 54: 635–541.

Drewnowski, A., Saari, J., Kurth, C. and Holden-wiltse, J. 1992. Food Preferences Fats in Human Obesity : Carbohydrates Versus. Appetite 18(3): 207–221.

Garner, D. M. and Garfinkel, P. E. 1980. Socio-cultural factors in the development of anorexia nervosa. Psychological Medicine 10(4): 647-656.

Guido, D., Perna, S., Carrai, M., Barale, R., Grassi, M. and Rondanelli, M. 2016. Multidimensional evaluation of endogenous and health factors affecting food preferences, taste and smell perception. Journal of Nutrition, Health and Aging 20(10): 971-981.

Hayes, J. E. and Duffy, V. B. 2008. Oral sensory phenotype identifies level of sugar and fat required for maximal liking. Physiol Behav. 95(1-2): 77-87.

Hayes, J. E. and Duffy, V. B. 2007. Revisiting sugar-fat mixtures: Sweetness and creaminess vary with phenotypic markers of oral sensation. Chemical Senses 32(3): 225–236.

Heinze, J. M., Preissl, H., Fritsche, A. and Frank, S. 2015. Controversies in fat perception. Physiology & Behavior 152: 479-493.

Heinze, J. M., Costanzo, A., Baselier, I., Fritsche, A. Lidolt, M., Hinrichs, J., … and Keast, R. 2017. Oil Perception – Detection thresholds for varying fatty stimuli and inter-individual differences. Chemical Senses 42(7): 585–592.

Karmous, I., Plesník, J., Khan, A. S., Šerý, O., Abid, A., Mankai, A., … and Khan, N. A. 2017. Orosensory detection of bitter in fat-taster healthy and obese participants: Genetic polymorphism of CD36 and TAS2R38. Clinical Nutrition 37(1): 313-320.

Keller, K. L., Liang, L. C., Sakimura, J., May, D., van Belle, C., Breen, C., … and Chung, W. K. 2009. Common Variants in the CD36 Gene Are Associated With Oral Fat Perception, Fat Preferences, and Obesity in African Americans. Obesity 20(5): 1066–1073.

King, B. M. 2013. The modern obesity epidemic, ancestral hunter- gatherers, and the sensory/reward control of food intake. American Psychologist 68(2): 88–91.

Laugerette, F., Passilly-degrace, P., Patris, B., Niot, I., Febbraio, M., Montmayeur, J. and Besnard, P. 2005. CD36 involvement in orosensory detection of dietary lipids, spontaneous fat preference, and digestive secretions. J Clin Invest. 115(11): 3177–3184.

Ledikwe, J. H., Ello-Martin, J., Pelkman, C. L., Birch, L. L., Mannino, M. L. and Rolls, B. J. 2007. A reliable, valid questionnaire indicates that preference for dietary fat declines when following a reduced-fat diet. Appetite 49(1): 74–83.

Majid, A. and Levinson, S. C. 2008. Language does provide support for basic tastes. Behavioral and Brain Sciences 31(1): 86-87.

Martínez-Ruiz, N. R., López-Díaz, J. A., Wall-Medrano, A., Jiménez-Castro, J. A. and Angulo, O. 2014. Oral fat perception is related with body mass index, preference and consumption of high-fat foods. Physiology & behavior 129: 36-42.

Mattes, R. D. 2001. The taste of fat elevates postprandial triacylglycerol. Physiology and Behavior 74(3): 343–348.

Mattes, R. D. 2005. Fat taste and lipid metabolism in humans. Physiology & Behavior 86(5): 691–7.

Mattes, R. D. 2009. Oral detection of short-, medium-, and long-chain free fatty acids in humans. Chemical Senses 34(2): 145–150.

Mela, D. J. 1996. Eating behaviour, food preferences and dietary intake in relation to obesity and body-weight status. Proceedings of the Nutrition Society 55(3): 803-816.

Mela, D. J. 2001. Determinants of food choice: relationships with obesity and weight control. Obesity Research 9(Suppl 4): 249S-255S.

Melis, M., Sollai, G., Muroni, P., Crnjar, R. and Barbarossa, I. T. 2015. Associations between Orosensory Perception of Oleic Acid, the Common Single Nucleotide Polymorphisms (rs1761667 and rs1527483) in the CD36 Gene, and 6-n-Propylthiouracil (PROP) Tasting. Nutrients 7(3): 2068–2084.

Mrizak, I., Šerý, O., Plesnik, J., Arfa, A., Fekih, M., Bouslema, A., … and Khan, N. A. 2015. The A allele of cluster of differentiation 36 (CD36) SNP 1761667 associates with decreased lipid taste perception in obese Tunisian women. British Journal of Nutrition 113(8): 1330–1337.

Nachtsheim, R. and Schlich, E. 2013. The influence of 6-n-propylthiouracil bitterness, fungiform papilla count and saliva flow on the perception of pressure and fat. Food Quality and Preference 29(2): 137–145.

Nasser, J. A, Kissileff, H. R., Boozer, C. N., Chou, C. J. and Pi-Sunyer, F. X. 2001. PROP taster status and oral fatty acid perception. Eating Behaviors 2(3): 237–45.

National Centre for Biotechnology Information (NCBI). 2017. Reference SNP (refSNP) Cluster Report: rs1761667, 2016. Retrieved from website:https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/?gts=rs1761667

Newman, L., Haryono, R. and Keast, R. 2013. Functionality of fatty acid chemoreception: A potential factor in the development of obesity? Nutrients 5(4): 1287–1300.

Ong, H. H., Tan, Y. N. and Say, Y. H. 2017. Fatty acid translocase gene CD36 rs1527483 variant influences oral fat perception in Malaysian subjects. Physiology and Behavior 168: 128–137.

Pepino, M. Y., Love-Gregory, L., Klein, S. and Abumrad, N. A. 2012. The fatty acid translocase gene CD36 and lingual lipase influence oral sensitivity to fat in obese subjects. J Lipid Res. 53(3): 561-6.

Pioltine, M. B., de Melo, M. E., Santos, A., Machado, A. D., Fernandes, A. E., Fujiwara, C. T., … and Mancini, M. C. 2016. Genetic Variation in CD36 Is Associated with Decreased Fat and Sugar Intake in Obese Children and Adolescents. Journal of Nutrigenetics and Nutrigenomics 9(5-6): 300–305.

Poette, J., Mekoué, J., Neyraud, E., Berdeaux, O., Renault, A., Guichard, E., … and Feron, G. 2014. Fat sensitivity in humans: oleic acid detection threshold is linked to saliva composition and oral volume. Flavour and Fragrance Journal 29(1): 39–49.

Priego, T., Sánchez, J., Picó, C., Ahrens, W., De Henauw, S., Kourides, Y., … and IDEFICS and the I.Family consortia. 2015. TAS1R3 and UCN2 transcript levels in blood cells are associated with sugary and fatty food consumption in children. Journal of Clinical Endocrinology and Metabolism 100(9): 3556–3564.

Rashid, Z. M., Rahman, S. A., Kasim, Z. M., Mustapha, W. A. W., Halip, M. H. M., Arifin, Z. and Rauf, U. F. A. 2011. Nutritional status and physical activities among army trainees in public institutions of higher education in Malaysia. Food and Nutrition Sciences 2(6): 511-520..

Sayed, A., Šerý, O., Plesnik, J., Daoudi, H., Rouabah, A., Rouabah, L. and Khan, N. A. 2015. CD36 AA-genotype is associated with decreased lipid taste perception in young obese, but not lean, children A. International Journal of Obesity 39(6): 920–924.

Shafaie, Y., Hoffman, D. J. and Tepper, B. J. 2015. Consumption of a high-fat soup preload leads to differences in short-term energy and fat intake between PROP non-taster and super-taster women. Appetite 89: 196–202.

Shen, Y., Kennedy, O. B. and Methven, L. 2016. Exploring

the effects of genotypical and phenotypical variations in bitter taste sensitivity on perception, liking and intake of brassica vegetables in the UK. Food Quality and Preference 50: 71–81.

Stewart, J. E., Feinle-Bisset, C. and Keast, R. S. 2011a. Fatty acid detection during food consumption and digestion: Associations with ingestive behavior and obesity. Progress in Lipid Research 50(3): 225–33.

Stewart, J. E. and Keast, R. S. 2012. Recent fat intake modulates fat taste sensitivity in lean and overweight subjects. International Journal of Obesity 36(6): 834–842.

Stewart, J. E., Newman, L. P. and Keast, R. S. 2011b. Oral sensitivity to oleic acid is associated with fat intake and body mass index. Clinical Nutrition 30(6): 838–844.

Stewart, J. E., Seimon, R. V., Otto, B., Keast, R. S., Clifton, P. M. and Feinle-Bisset, C. 2011c. Marked differences in gustatory and gastrointestinal sensitivity to oleic acid between lean and obese men. American Journal of Clinical Nutrition 93(4): 703–711.

Temple, E. C., Hutchinson, I., Laing, D. G. and Jinks, A. L. 2002. Taste development: Differential growth rates of tongue regions in humans. Brain Res. Dev. Brain Res. 135: 65–70.

Tepper, B. J. and Nurse, R. J. 1997. Fat perception is related to PROP taster status. Physiology & Behavior 61(6): 949–54.

Tucker, R. M., Edlinger, C., Craig, B. A. and Mattes, R. D. 2014. Associations between BMI and fat taste sensitivity in humans. Chemical Senses 39(4): 349-357.

Tucker, R. M., Nuessle, T. M., Garneau, N. L., Smutzer, G. and Mattes, R. D. 2015. No difference in perceived intensity of linoleic acid in the oral cavity between obese and nonobese individuals. Chemical Senses 40(8): 557-563.

Webb, J., Bolhuis, D. P., Cicerale, S., Hayes, J. E. and Keast, R. 2015. The Relationships Between Common Measurements of Taste Function. Chemosensory Perception 8(1): 11–18.

WHO/IOTF/IASO. 2000. The Asia-Pacific Perspective: Redefining Obesity and its Treatment. Hong Kong: World Health Organization, International Obesity Task Force, International Association Association for the Study of Obesity.

Wijk R. A. and Prinz, J. F. 2007. Fatty versus creamy sensations for custard desserts, white sauces, and mayonnaises. Food Quality and Preference 18(4): 641–650.

Yackinous, C. and Guinard, J. X. 2001. Relation between PROP taster status and fat perception, touch, and olfaction. Physiology and Behavior 72(3): 427–437.

Zhao, L., Kirkmeyer, S. V. and Tepper, B. J. 2003. A paper screening test to assess genetic taste sensitivity to 6-n-propylthiouracil. Physiology & behavior 78(4): 625-63.

Zung, W. W. 1965. A self-rating depression scale. Archives of general psychiatry 12(1): 63-70.