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# A Practical Standardized Fat Challenge for the Oral Fat Tolerance Test (OFTT) in Men and Women

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## HIGHLIGHTS

- The oral fat tolerance test (OFTT) assesses postprandial hypertriglyceridemia.
- OFTT meal formulation needs standardization for data comparison among studies.
- The proposed meal was prepared with ingredients sold in any market.
- It increased postprandial TG, had good acceptability, and kept participants satiety.

**Abstract:** Postprandial lipemia (PPL) is associated with cardiovascular diseases, and it is assessed by the oral fat tolerance test (OFTT), which measures circulating triacylglycerol (TG). The objective is to propose an OFTT meal and to evaluate men's and women's perceptions, attitudes, sensory acceptability, and satiety. After overnight fasting, blood was collected (n=105), participants ingested the OFTT meal (75.4g lipids, 25.2g carbohydrates, and 10.8g protein/ 822.6 Kcal), and a new blood sample was collected 4h later. Fasting TG was 125.7±92.0 mg/dL (mean±SD) for men and 108.9±52.6 mg/dL for women. It increased by 97.7% and 86.7%, respectively, 4h after meal ingestion (P<0.0001), with no change in glucose. Participants felt satiated during the test. The meal was considered easy to eat and better tolerated by men. The median overall sensory acceptability was 7.0 [9-point hedonic scale]. The greatest fullness was seen in the first hour (magnitude satiety scale) and higher for women (P<0.01). The fatty meal proposed by the current study is adequate for the OFTT since it increased blood TG after 4h without hypoglycemia, it was easy to prepare, to eat, it kept participants satiated, and it displayed good perception, attitudes, and sensory acceptability.

**Keywords:** postprandial lipemia; oral fat tolerance test; cardiovascular disease; triacylglycerol.

## INTRODUCTION

Zilversmit firstly proposed the atherogenic potential of triacylglycerol (TG) [1], and now it is considered an independent risk factor for cardiovascular diseases [2-5]. Postprandial TG has been associated with coronary artery disease risk [6], ischemia [7], myocardial infarction, and death [8]. Literature also shows that postprandial TG would be a better predictor of atherosclerotic arterial disease than fasting TG [9]. Postprandial TG is influenced by age, gender, genetics, physical inactivity, and eating habits [10-12], and postprandial hypertriglyceridemia is commonly found in obese and insulin-resistant individuals [13].

Although we spend most of our day in the fed state and releasing lipoproteins into the bloodstream continuously [4], cardiovascular risk assessment is traditionally evaluated by the fasting lipid profile. Due to the emerging relevance of postprandial hypertriglyceridemia as a cardiovascular risk factor, an Expert Panel group of scientists and clinicians provided a series of recommendations for non-fasting TG evaluation in 2011, which was recently revisited in 2019 [14, 15]. Clinical guidelines now mention the assessment of postprandial lipemia in the primary prevention of cardiovascular diseases, such as the joint consensus from the European Federation of Clinical Chemistry and Laboratory Medicine [16], the Canadian Cardiovascular Society Guideline for the management of dyslipidemia [17], the National Institute for Health and Care Excellence from the UK [18], the American Heart Association [19] and the Brazilians Societies of Clinical Analysis, Cardiology, and Clinical Pathology and Laboratory Medicine [20].

Over the past 30 years, the postprandial TG response has been examined in humans [21]. Non-fasting TG should be assessed by a standardized oral fat tolerance test (OFTT) in which a fat meal is provided in the fasting state, and blood TG measured some hours later. According to Kolovou and coauthors [15], the OFTT meal should provide 75 g fat, 25 g carbohydrate, and 10 g protein, be ingested after eight hours fast, and TG measured four hours after meal intake. The meal should be easy to prepare, contain mixtures of saturated and unsaturated fatty acids, and have commercially available whipped cream cheese or cream cheese [about 250 g] and 15 g sugar [15].

LIPOTESTmeal (D. GENOMERES Advanced Medical Research, Athens, Greece) is the only OFTT meal available in the market to date to help standardize the OFTT protocol for clinical trials, which may limit its acquisition by researchers worldwide. Another limitation is that published meal formulation is highly variable, from hamburgers [22], cheese patties [9], and mixes of ice cream and milk cream [23]. Finally, reference cutoff values are still under debate, although the relevant contribution of Kolovou and coauthors [15]. These issues partially justify why the OFTT is not routinely performed in the clinic setting.

Despite these limitations, studying postprandial lipemia is of utmost importance, and there is a need to standardize meal preparation. Moreover, to know if the test meal is well tolerated by patients and does not lead to hypoglycemia over the test. Therefore, our goal is to propose a fatty meal for the OFTT and evaluate men's and women's perception, attitudes, sensory acceptability, and satiety during the four hours of the test.

## MATERIAL AND METHODS

### Study design and participants

The current investigation is analytical, observational, descriptive cohort study with prospective temporality (ReBEC RBR-10x4hys8), conducted following the Declaration of Helsinki, and approved by the Research Ethics Committee of Instituto de Saude de Nova Friburgo, Universidade Federal Fluminense (CAAE: 49864015.2.0000.56.26). All participants provided signed informed consent. They were recruited from May to December 2018 in the Sociedade União Beneficente Humanitária dos Operários (Humanitária), where the university project of extension *Health care promotion: nutrition and cardiovascular risk in the population of Nova Friburgo* is developed (SigProj 292611.1598.196015.02022018). Inclusion criteria were men and women aged 18 years or older. Exclusion criteria were individuals with known genetic disturbance of lipid metabolism, dietary restriction (energy or macronutrient) until two months before the study, veganism, alcohol consumption, athletes, and in use of drugs that reduce blood lipids.

### Test meal

The OFTT nutrient composition was based on Kolovou and coauthors [14]. When the study was designed, Kolovou and coauthors [15] revisited recommendation was not published. The OFTT meal resembled a creamy chocolate milkshake and consisted of 290 g milk cream, 25 g cocoa powder, 24 g dry nonfat milk, and 11 g sugar (Table 1). Cold water (50 mL) was added to make the meal less thick. Ingredients were mixed in a blender immediately before serving. The OFTT meal was presented in a 500 mL plastic cup with a straw (21 cm height x 10 mm diameter). Nutrient composition per serving was 75.4 g total lipids (48.4

g saturated lipids), 25.2 g carbohydrates, 10.8 g protein, total energy 822.6 Kcal, and the final volume of about 500 mL.

**Table 1.** Oral fat tolerance test (OFTT) meal composition

<b>Composition per serving (395 g of ready product)</b>							
	<b>Ingredients</b>				<b>Energy</b>		
	<b>Milk cream (g)</b>	<b>Cocoa powder (g)</b>	<b>Dry nonfat milk (g)</b>	<b>Sugar (g)</b>	<b>Weight<sup>#</sup> (g)</b>	<b>Energy (Kcal)</b>	<b>Energy (%)</b>
OFTT meal	290.0	25.0	24.0	11.0	395.0	822.6	100.0
<b>Nutrition facts *</b>							
Protein	0.0	4.9	5.9	0.0	10.8	43.2	5.3
CHO	0.0	5.3	8.9	11.0	25.2	100.8	12.2
<b>Lipids</b>							
Total	65.7	3.4	6.3	0.0	75.4	678.6	82.5
SFA	42.5	2.0	3.9	0.0	48.4	435.6	53.0
<b>Fatty acids composition **</b>							
<b>Lipids</b>							
Total	68.7	3.4	6.3	-	78.4	705.6	-
SFA	34.2	2.0	3.8	-	40.0	360.0	-
MUFA	14.8	1.1	1.7	-	17.6	158.4	-
PUFA	1.3	0.1	0.1	-	1.5	13.5	-
TFA	1.8	-	0.2	-	2.0	18.0	-
Chol, mg	192.9	-	20.1	-	213.0	-	-

<sup>#</sup> The oral fat tolerance test (OFTT) meal should consist of 75 g fat, 25 g carbohydrates, and 10 g proteins [14, 15].

\* Nutrition facts were obtained from the food label. Fifty milliliters of water were added to make the meal less thick.

\*\* The fatty acid composition was obtained from the Brazilian Food Composition Table [24] since it was absent from the label. Food code and description: C0010G – Cream milk (canned, UHT, 25% fat) (average of several brands); C0090K – Cocoa, dry powder, without sugar; C0039G – Milk, cow, whole, powder; C0007K – Sugar powered (mean of the several samples).

Abbreviations: CHO, carbohydrate. SFA, saturated fatty acids. MUFA, monounsaturated fatty acids. PUFA, polyunsaturated fatty acids. TFA, Trans-fatty acids. Chol, cholesterol.

## Oral fat tolerance test

The OFTT was performed according to Kolovou and coauthors [14]. Participants were oriented not to ingest fatty foods, drink alcoholic beverages, or perform extenuating physical exercises three days before the test [15]. After overnight fasting (8 h), a venous blood sample was collected from veins located at the upper limb's anterior face (v. middle cubital, v. cephalic, or v. basilica). Participants ingested the OFTT meal, and four hours later a new venous blood sample was collected on the contralateral arm. Over the four hours after meal ingestion, participants were kept in the Humanitaria's facility to avoid energy expenditure by walking, and they were not allowed to eat or drink, except for water. The amount of meal ingested and the time spent eating was recorded. They were also asked if they had the habit of having breakfast in the morning.

## Test meal acceptability

Participants answered two questionnaires immediately before the second blood sample collection. The first one asked about their perceptions and attitudes toward the OFTT meal (Supplemental Table 1), by seven points Likert-type scale ranging from 1 to 7, in which 1 strongly disagrees, 4 is neither agreed nor disagreed, and 7 strongly agrees. The second questionnaire evaluated sensory acceptability (Supplemental Table 2). It consisted of a nine-point hedonic scale with a visual analogue scale combined with Likert-type scale ranging from 1 to 9, in which 1 dislikes extremely, 5 is neither liked nor disliked, and 9 is likes extremely [25]. Visual presentation, taste, temperature, texture, smell, and volume were the sensory aspects evaluated.

## Satiety

Participants rated their feelings of fullness or hunger through a 15 mm labeled magnitude satiety (LMS) scale [26]. Fullness and hunger were recorded in a line scale marked with 11 descriptors ranged from "greatest imaginable hunger" (-7.0 cm), "neither hungry nor full" (0.0 cm), to "greatest imaginable fullness" (7.0 cm) [28]. They were asked to record their satiety/hunger before meal ingestion and then every hour until

the fourth hour. The scale markings were enumerated by their distance from the mid-point on the line scale, and this score was plotted against time to generate the postprandial satiety response curve. The area under the curve (AUC) of postprandial satiety was calculated using the trapezoid rule [27].

### Biochemical assay

Blood samples were sent to the Laboratorio Multiusuário de Pesquisa Biomedica (Instituto de Saude de Nova Friburgo, Universidade Federal Fluminense). Blood serum was obtained after centrifugation at 3,500 g for 10 min and kept at -20 °C until the next morning when biochemical assays were performed for TG (TGML-0707, ELITech Clinical, Systems SAS, France) and glucose (GPSL-0500, ELITech Clinical, Systems SAS, France) by enzymatic colorimetric assays.

### Statistical analysis

The Kolmogorov-Smirnov normality test was applied. Table 1 shows data as n (%) tested by the Chi-squared test, or data as mean  $\pm$  standard deviation tested by either Student t test or Mann-Whitney test. TG and glucose were tested by Wilcoxon matched-pairs test and two-way ANOVA (factor 1: sex [men/women]; factor 2: feeding state [fasting/postprandial]). Ordinal data (Likert-type) are shown as median and 95% confidence interval and were tested by the Mann-Whitney test. Finally, sex difference in postprandial satiety AUC was assessed by the Mann-Whitney test. The analyzes were performed in GraphPad Prism v.8.0.2, and  $P < 0.05$  was considered significant.

## RESULTS

### Study population and feeding behavior

The study included data from 105 participants. As shown in Table 2, the sample consisted of 56 men aged  $40.9 \pm 12.4$  years and 49 women aged  $39.6 \pm 12.5$  years. The average fasting time was  $11\text{h}02 \pm 1\text{h}39$ . Women spent twice the time eating the OFTT meal than men ( $P = 0.0001$ ), but we saw no difference in the amount of food consumed ( $P = 0.71$ ). Most participants usually have breakfast in the morning, with no difference between sex ( $P = 0.54$ ). Seventy seven percent of participants ingested 100% of the meal, and the remaining participants consumed about 60%, with no difference between sex.

**Table 2.** Participants and feeding behavior

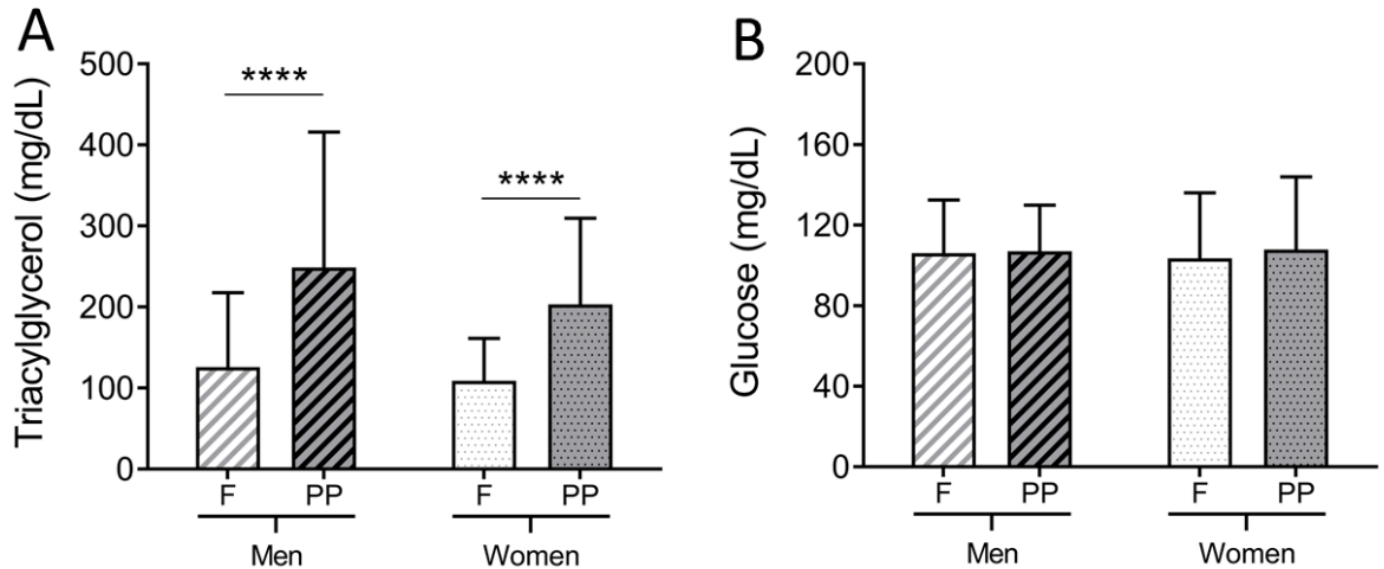
Parameter	Total	Men	Women	P
n, %	105	56 (53.3%)	49 (46.7%)	---
Age, years	$40.3 \pm 12.4$	$40.9 \pm 12.4$	$39.6 \pm 12.5$	0.58 <sup>1</sup>
Fasting, h/min	$10\text{h}54 \pm 1\text{h}43$	$10\text{h}48 \pm 1\text{h}47$	$11\text{h}02 \pm 1\text{h}39$	0.39 <sup>2</sup>
Usually have breakfast in the morning, n (%)				
Yes	80 (76.2%)	44 (78.6%)	36 (73.5%)	0.54 <sup>3</sup>
No	25 (23.8%)	12 (21.4%)	13 (26.5%)	
Time spent eating, min	$8.7 \pm 7.8$	$5.6 \pm 3.8$	$12.1 \pm 9.6$	0.0001 <sup>2</sup>
Ingested all the meal, n (%)				
Yes	81 (77.1%)	44 (78.6%)	37 (75.5%)	0.71 <sup>3</sup>
No	24 (22.9%)	12 (21.4%)	12 (24.5%)	
If no, volume ingested, %	$60.7 \pm 11.7$	$61.6 \pm 12.8$	$59.9 \pm 11.1$	0.73 <sup>1</sup>

Data are shown as n (%) or mean  $\pm$  standard deviation. Percentages refer to the total n of the column (not row). Comparison between sex is shown in the last column, and the p-value is indicated. Statistical tests:

<sup>1</sup>Student t test. <sup>2</sup>Mann-Whitney test. <sup>3</sup>Chi-squared test.

## TG and glucose response to the OFTT meal

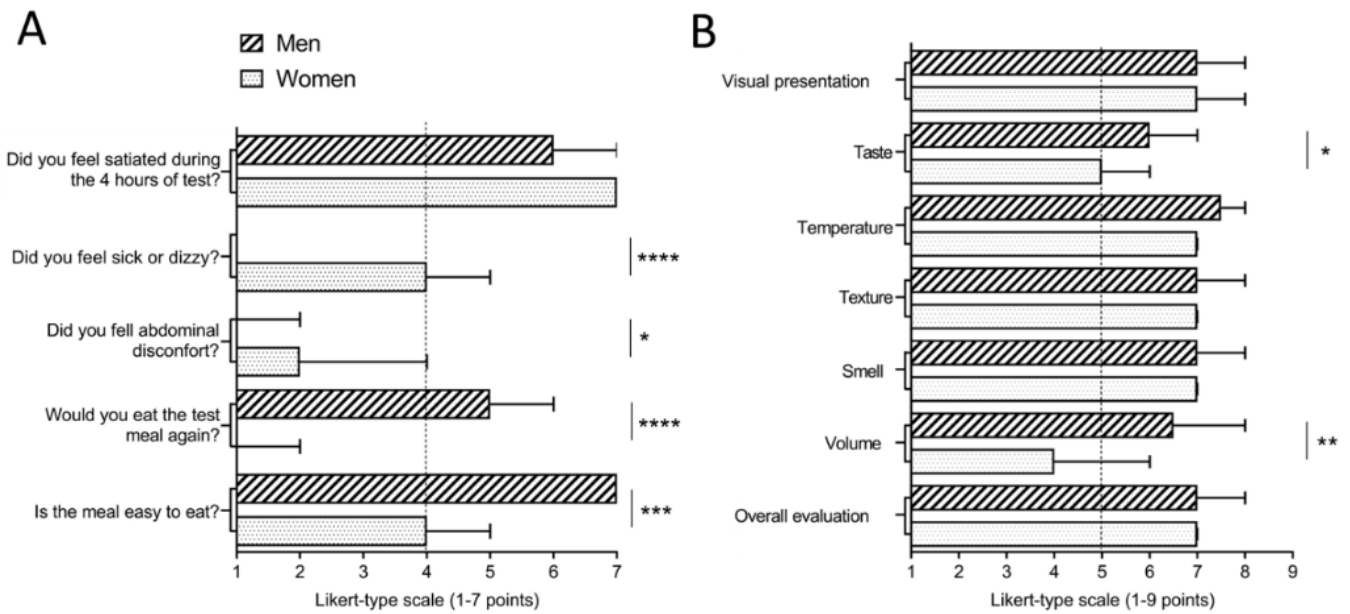
TG and glucose were assessed after overnight fasting and four hours after OFTT meal ingestion (Figure 1). We removed three participants from this analysis because they did not have the second blood sample. Baseline TG was  $125.7 \pm 92.0$  mg/dL in men and  $108.9 \pm 52.6$  mg/dL in women (Figure 1A). The test meal successfully increased serum TG in men (97.7 %,  $P < 0.0001$ ) and women (86.7 %,  $P < 0.0001$ ) when fasting TG and postprandial TG were compared (Wilcoxon matched-pairs test). TG fluctuation was due to the feeding state (18.2 % of total variation,  $P < 0.0001$ , two-way ANOVA), but not sex (1.5 % of total variation,  $P = 0.055$ , two-way ANOVA). Since participants were subject to overnight fasting and the meal had low carbohydrate content, a major concern was that they had an episode of hypoglycemia over the four hours of the test. As shown in Figure 1B, it was not true because glucose did not change for both sexes for four hours (men  $P = 0.89$  and women  $P = 0.28$ , Wilcoxon matched-pairs test).



**Figure 1.** Fasting (F) and postprandial (PP) response to the oral fat tolerance test (OFTT) meal in men and women. (a) Triacylglycerol was elevated four hours after meal ingestion; (b) Glucose remained unchanged after four hours. Data are shown as mean  $\pm$  standard deviation. Wilcoxon matched-pairs test, \*\*\*\* $p < 0.0001$ .  $n = 102$  (men = 55, women  $n = 47$ ).

## OFTT meal perception, attitudes, and sensory acceptability

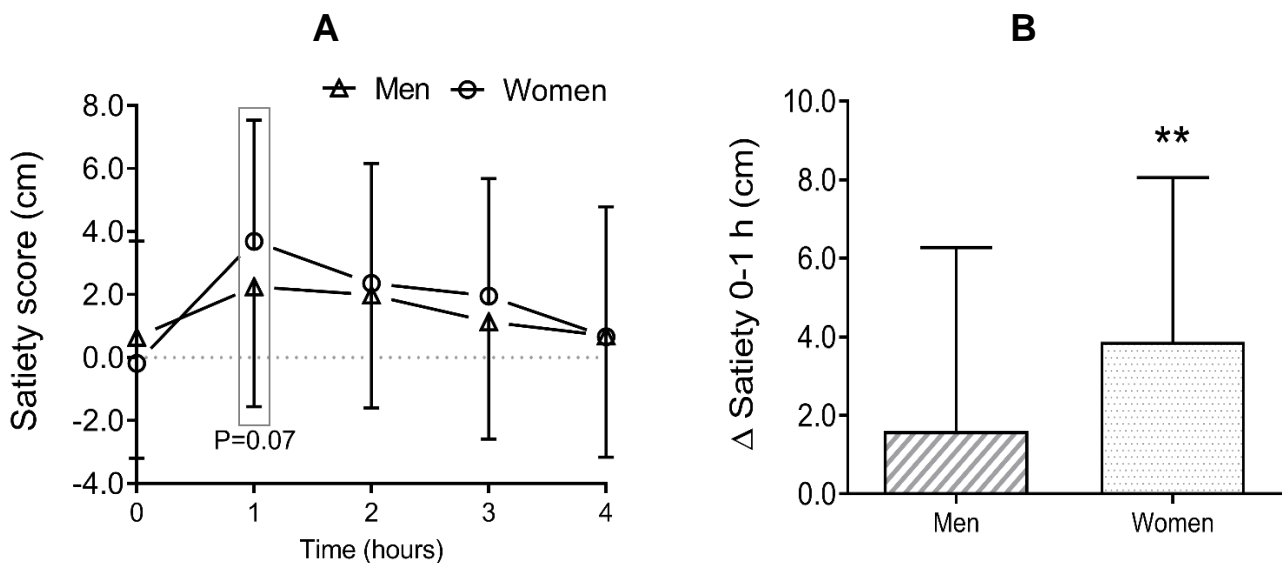
We assessed the OFTT meal's perception, attitudes, and sensory acceptability before the second blood sampling (Figure 2). Both men and women agreed they felt satiated over the four hours (Figure 2A). Men disagreed to felt sick or dizzy (score 1.0 [1.0-1.0]), whereas women neither agreed nor disagreed with this statement (score 4.0 [2.0-5.0],  $P < 0.0001$  vs. men). Men partially agreed they would eat the OFTT meal again (score 5.0 [4.0-6.0]) and strongly agreed that it was easy to eat (score 7.0 [6.0-7.0]). On the other hand, women strongly disagreed that they would eat the OFTT meal again (score 1.0 [1.0-2.0],  $P < 0.0001$  vs. men), and they neither agreed nor disagreed that it was difficult to eat (score 4.0 [2.0-5.0],  $P = 0.0002$  vs. men). Both men and women strongly disagreed they felt abdominal discomfort or pain throughout the four hours. Sensory perception was similar between men and women, except for taste and volume (Figure 2B). Overall, the median for visual presentation, temperature, texture, smell, and overall evaluation was "like moderately" (score 7.0), and women neither liked nor disliked taste (score 5.0 [3.0-6.0]) and meal volume (score 4.0 [3.0-6.0]).

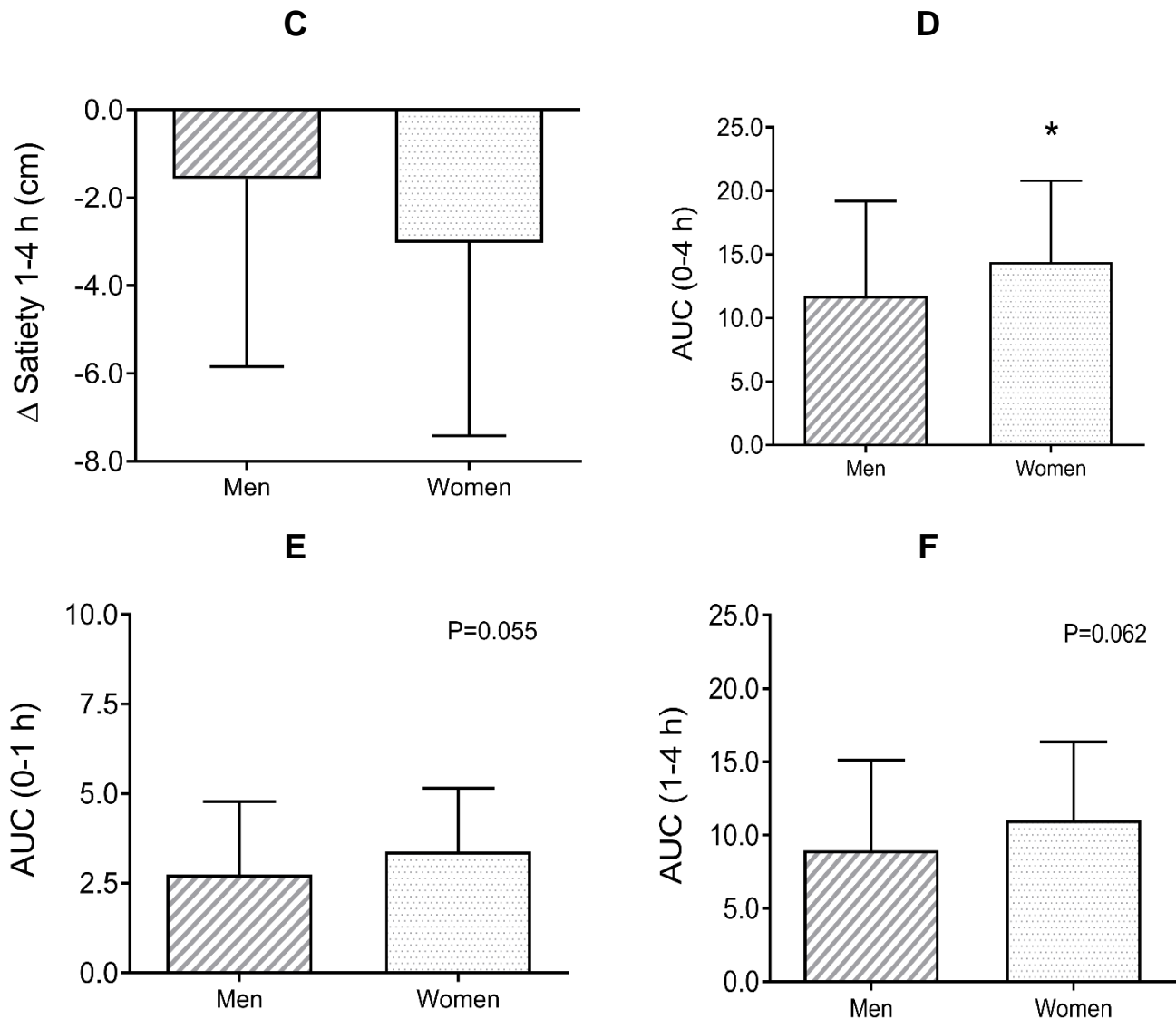


**Figure 2.** Perception, attitudes, and sensory acceptability of the oral fat tolerance test (OFTT) meal. (a) Perception and attitudes were evaluated by five questions and a 7-point Likert-type scale ranging from 1 (strongly disagree) to 7 (strongly agree). Four is the center (neither agree nor disagree, dashed line). (b) Sensory acceptability was evaluated by seven questions and a 9-point hedonic Likert-type scale, where 1 is strongly dislike, 5 is neutral (dashed line), and 9 is strongly like. Data are shown as median and 95% confidence interval. Mann-Whitney test, \* $p < 0.05$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .  $n = 105$  (men = 56, women  $n = 49$ ).

### Satiety over the four hours of OFTT

Figure 3 shows the participants' satiety after eating the OFTT meal. Two men and two women were not considered for this analysis because of missing data. The satiety curve peak appeared at one hour and had a trend to be higher in women than men ( $P = 0.07$ , Figure 3A). On the other hand, the postprandial satiety AUC was higher in women than men (+23 %,  $P = 0.04$ , Figure 3D). Since satiety displayed a peak at one hour, and it diminished over time until the fourth hour, we decided to split the curve into two-time ranges: from 0 h to 1 h (Figure 3B-E) and 1 h to 4 h (Figure 3C-F). Satiety variation ( $\Delta$ ) during the first hour proved to be higher in women compared to men (+142 %,  $P = 0.005$ , Figure 3B), but similar between sex until the last hour ( $P = 0.12$ , Figure 3C). The AUC for each time interval was similar between sex (Figure 3E-F). Overall, we noticed some statistical differences between men and women, but data within each group had a high coefficient of variation and standard deviation, resulting in  $p$ -values close to 0.05.





**Figure 3.** Postprandial satiety response. (a) Satiety was assessed every hour over four hours after participants had ingested the oral fat tolerance test (OFTT) meal. Since satiety displayed a peak at 1 hour, data was subsequently divided into two time-intervals, from 0 to 1 hour (B and E) and 1 to 4 hours (C and F). (B) satiety variation in the first hour. (C) Satiety variation during the last three hours. (D) Area under the curve (AUC) of data presented in A. (E) AUC for graph A considering the first hour solely. (F) AUC for the last three hours of the OFTT test, based on graph A. Data are expressed as mean ± standard deviation. Mann-Whitney test, \*P<0.05, \*\*P<0.01. n = 101 (men = 54, women n= 47).

## DISCUSSION

Postprandial hypertriglyceridemia is an emerging risk factor for cardiovascular disease [2, 10]. A call to standardize the OFTT was proposed ten years ago [14], but few research groups adhered to it, as shown in a recent meta-analysis where only 18% of randomized controlled trials (n=61) published up to September 2018 meet the recommendation of 75 g of fat [28]. It makes comparison across different studies a challenge and maybe why Kolovou and coauthors [15] considered that studying postprandial lipemia with an OFTT meal still has some difficulties, mainly due to the type, structure, and amount of fat used across studies. They also acknowledge that the OFTT is primarily used for research until the relationship between TG and cardiovascular risk clarifies since much evidence in the literature is still controversial [15].

Our biggest concern was to prepare a meal that was stimulating enough to be eaten. After searching for culinary recipes, we adapted the ingredients of a chocolate milkshake to get close enough to the amount of lipids, carbohydrates, and proteins recommended by Kolovou and coauthors [14, 15]. Our goal was successfully achieved since participants spontaneously reported that the OFTT meal resembled a milkshake from fast food restaurants (e.g., McDonald's). Some participants also said that the OFTT meal was too sweet, while others reported it was too bitter. We believe that this perception was associated with their habit of eating chocolate because participants that used to eat dark chocolate — which has a high percentage of cocoa —

did not qualify the OFTT meal as bitter. Unfortunately, we could not find other studies reporting OFTT meal perception and acceptability to compare with our investigation.

Gastrointestinal pain is a concern highlighted by Lee and coauthors [28] when conducting clinical trials with 75 g fat. In our study, men strongly disagree with feeling abdominal discomfort, sick, or dizzy during the four hours of the test, but women reported feeling sick, dizzy, and experiencing abdominal discomfort. According to Dhillon and coauthors [29], when food sensory profile is linked to a negative experience (e.g., gastric distress), the stimulus is likely to be avoided, which may justify why women strongly agreed they would not eat the OFTT meal again. Additionally, female gastric transit is slower than men in solid and liquid diets [30, 31]. Thus the difference in women's satiety can be explained by the rate of gastric emptying being slower than men's [32]. It would help develop an OFTT meal with lower fat content to diminish abdominal discomfort, especially in women, but it may not elicit the postprandial TG response required for the test. Lee and coauthors [28] show that the lack of response to the fat challenge in some studies is likely due to fat content since their meta-analysis found studies reporting from 7.8 g to 100 g fat.

The OFTT meal should be easy to prepare and made with commercially available ingredients like whipped cream or cream cheese (~250 g) and sugar (15 g) [15]. Our meal consisted of milk cream, cocoa powder, dry nonfat milk, sugar, and cold water, which are not expensive and can be easily bought in any local market worldwide. As mentioned earlier, the only standardized meal is the LIPOTEST<sup>meal</sup> (D. GENOMERES Advanced Medical Research, Athens, Greece), which can be expensive to buy worldwide, especially from Latin America. Therefore, our recipe may serve as an alternative. LIPOTEST<sup>meal</sup> consists of hydrogenated vegetable fat (palm and coconut oil), glucose syrup solids, milk proteins, sugar, emulsifiers, cocoa powder, and flavorings [33]. Of note, LIPOTEST<sup>meal</sup> has 832 Kcal per serving, and our meal has very similar energy content (823 Kcal).

The LIPOTEST<sup>meal</sup> is a 115 g sachet powder which should be mixed with 150 mL lukewarm water, mixed by 2-3 min with a hand-held mixer, and then refrigerated to become mousse. On the other hand, our preparation took from 3-5 min to weigh the ingredients and mix them in the kitchen blender to prepare two servings at once. After mixing, the meal was dispensed in a plastic cup and kept in the refrigerator until serving. We do not recommend that this preparation be kept for more than 40 min waiting to be served, because its appearance becomes less homogeneous, which can be a limitation for its acceptability. Another limitation is that we did not test its stability after long storage (e.g., 24 hours to be eaten the next day after prepared).

The OFTT meal should be a mixture of saturated and unsaturated fatty acids [15], but Kolovou and coauthors [15] did not recommend a specific fat source since the literature does not have enough evidence for such a recommendation. Our meal agrees with this recommendation since it is a mixture of fatty acids, mainly saturated (40 g) and monounsaturated (17.6 g) fatty acids, based on the Brazilian Food Composition Table [24], whereas LIPOTEST<sup>meal</sup> has 75 g of saturated fatty acids [33]. Further research should focus on defining the fatty acid composition of the OFTT meal because it may interfere with postprandial TG response, but data in the literature is still controversial. A meta-analysis by Monfort-Pires and coauthors [34] showed no difference over four hours between saturated and unsaturated fatty acids, but lower postprandial TG response to polyunsaturated and a trend to monounsaturated over 8 hours of the test. A recent meta-analysis by Lee and coauthors [28] showed that some fats exhibited a postprandial TG lowering effect than saturated fatty acids.

Satiety is associated with the inter-meal period by suppressing hunger and inhibiting further eating [35]. Stribitcaia and coauthors [35] demonstrated that food texture (e.g., solid or semi-solid foods) significantly decreases hunger than liquid food. Incremental increases in satiety are achieved by increasing the thicker gradation of dairy products (e.g., milk, yogurt, and custard) [36]. Moreover, high viscous food increases fullness and slows gastric emptiness compared to low viscous food [37]. In the present study, the meal used is dairy-based and resembled a fast-food milkshake, whereas the LIPOTEST<sup>meal</sup> is a mousse, and some studies also use sandwiches [38] and McDonald's meal [22] for the fat challenge. These data support that care must be taken when choosing OFTT meal ingredients and preparation because it will impact satiety and the postprandial TG response. Considering that participants have fasted overnight, and the OFTT meal is the only food they will eat for the next four hours, promoting satiety is essential to increase protocol adherence, considering its potential future use in the clinical setting.

A concern in making the OFTT clinically feasible is the extended stay in the laboratory. Tentolouris and coauthors [33] reported that 23 of 65 subjects (35%) recruited for their research did not accept because of either the long duration of the test or because they would need to visit the laboratory twice (they compared postprandial response between two visits). To solve this problem, Sciarrillo and coauthors [39] showed that allowing participants to leave between the baseline and the four hours follow-up did not significantly alter the



TG result. Adherence to fasting is also a challenge since 8 hours of fasting requires that participants had their last meal at 11h00 p.m. Some participants ate earlier than 11h00 p.m. because they did not eat so late at night and needed to be early in bed to get ready in the next morning at 7h00 a.m. for blood collection. Although participants had about 11 hours of fasting, we found satisfactory postprandial TG response with a similar increase magnitude as other studies using 8 hours of fasting or more prolonged periods [22, 33, 39, 40]. Moreover, we monitored blood glucose every hour (data not shown), and no hypoglycemia events were noticed, as previously demonstrated by others [9, 22, 34].

Overall, an ideal OFTT meal should be easy to eat, do not promote abdominal discomfort, and good sensory acceptability. The patient or study participant should be allowed to leave the laboratory and fast for more than 8 hours (8-10 h). Finally, the amount and type of fatty acids mixture should elevate postprandial TG in four hours and fat quantity enough to discriminate between subjects at cardiovascular risk from those that are not, without leading to hypoglycemia.

## CONCLUSION

The fatty meal proposed by the current study is adequate for the OFTT since it increases blood TG after four hours without hypoglycemia, it is easy to prepare, to eat, and it kept participants satiated. It displayed good perception, attitudes, and sensory acceptability by study participants.

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