Orange juice with a high-fat meal prolongs postprandial lipemia in apparently healthy overweight/obese women

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ABSTRACT
Objective: We investigated the postprandial response of lipid markers to a high-fat meal (HFM) with two different beverages in apparently healthy normal-weight and overweight/obese women.

Subjects and methods: This crossover, randomized study enrolled 36 women, of whom 21 had normal weight (body mass index [BMI] 22 ± 1.8 kg/m²) and 15 had overweight/obesity (BMI 31 ± 3.7 kg/m²). In two different test days, the participants ingested a HFM (37% of energy as saturated fat) with 500 mL of water (HFM-W) or 500 mL of orange juice (HFM-OJ). Blood samples were collected at baseline (12-hour fasting), and at 2, 3, and 5 hours postprandial. The analysis included fasting and postprandial total cholesterol, HDL-c, LDL-c, triglycerides (TG), uric acid, and complement C3. Brazilian Clinical Trials Registry (ReBEC); Primary Identification Number: RBR-2h3wjn (www.ensaiosclinicos.gov.br).

Results: TG levels increased at 3 hours with HFM-OJ in normal-weight women (p = 0.01) and returned to normal levels at 5h. TG increased at 3 hours with HFM-W (p = 0.01) and HFM-OJ (p = 0.02), and remained high at 5 hours (p = 0.03) in overweight/obese women. Complement C3 remained unchanged, but showed different responses between meals (p = 0.01 for positive incremental area under the curve [pIAUC] HFM-OJ vs. HFM-W, respectively).

Conclusions: In apparently healthy overweight/obese women compared with normal-weight ones, the concomitant intake of orange juice with a HFM prolonged postprandial lipemia but had no effect on postprandial complement C3 concentrations.

Keywords
Orange juice; high-fat meal; postprandial lipemia; obesity

INTRODUCTION
Postprandial lipemia (PPL) refers to the dynamic changes in serum lipids and lipoproteins that occur after a fat load or meal. These responses are reflected mainly in changes in plasma triglycerides (TG). TG-rich lipoproteins and their remnants are known as risk predictors of coronary heart disease (1-2). Indeed, PPL has gained interest since recent reports have demonstrated that increases in postprandial TG levels are possibly even stronger independent predictors of cardiovascular diseases than fasting TG (3). Moreover, complement C3 has been positively associated with obesity, insulin resistance, metabolic syndrome features, and fasting and postprandial TG (4).

In this sense, the Western dietary pattern, characterized by a consumption of high-energy density diets and refined foods, contributes to a positive energy balance leading to weight gain and obesity and may trigger low-grade systemic inflammation and metabolic syndrome abnormalities (5,6). Western individuals remain in a postprandial state for most of the day (7). Consequently, repeated acute dietary stressors induced by a high-fat meal (HFM) could trigger a large increase of most risk factors for cardiovascular diseases related to obesity, such as increased circulating cholesterol, TG, and glucose (8). In turn, fruit intake has been associated with an improvement in lipid profile and reduction in concentrations of inflammatory and oxidative stress markers (9,10). We have previously published a review of studies assessing the anti-inflammatory properties of orange juice (OJ), which appears to mediate the plasma levels and gene expression of factors involved in metabolic and inflammatory responses in postprandial and chronic (≥ 7 consecutive days) periods (11).
In the present study, we investigated the postprandial response of lipid markers to a HFM with two different beverages (water and OJ) in apparently healthy normal-weight and overweight/obese women.

SUBJECTS AND METHODS

Subjects
Recruitment was conducted through the university website, posters, and active search in clinical and medical service centers. In total, 74 women were recruited and 45 were enrolled in the study. Six women failed to complete the study claiming lack of time and three presented technical problems in blood collection. The participants comprised normal-weight (n = 21) and overweight/obese (n = 15) women. They were apparently healthy with no recent acute or chronic inflammatory diseases and/or use of anti-inflammatory or immunosuppressive drugs and steroids. They were non-smokers and, to be enrolled, could not be pregnant or nursing. Subjects were excluded if they had any past or present cardiovascular disease, diagnosed diabetes or any inflammatory condition, or used medications known to affect the study outcomes. Approval for the study was obtained from the Ethics Committee for Human Research at Universidade Federal de Viçosa (Of. Ref. N° 184/2011) and all procedures involving human subjects complied with the Declaration of Helsinki, as revised in 2000. All participants signed a written informed consent form. The study is registered at the Brazilian Clinical Trials Registry (ReBEC; www.ensaiosclinicos.gov.br) with the primary identification number RBR-2h3wjn.

Study design
The dietary intervention followed a randomized crossover design, with a washout period of at least 7 days between the test days. Two days prior to each test day, the subjects followed a low-antioxidant diet (washout) by avoiding olive and fish oils, fresh fruits and vegetables, tea, coffee, fruit juices, and wine. The subjects were then randomly assigned to either a HFM plus 500 mL of water (HFM-W) or a HFM plus 500 mL OJ (HFM-OJ) group. On the morning of each test day, the participants arrived at the laboratory at 7:00-7:30 am. Body weight, height, and blood pressure were measured, and a fasting blood sample was taken before the test meal. Body weight and height were measured using standard procedures according to previously described protocols (12). Body mass index (BMI) was calculated using the equation: BMI = weight (kg)/height² (m²). The percentage of body fat was estimated by bioelectrical impedance analysis (Biodynamics 310e, Chicago, USA) using standard protocols (12). Blood pressure was measured using a standard mercury sphygmomanometer, with the patient in the seated position.

The test meals were served at 7:30-8:00 am and consumed within 30 min. Postprandial blood samples were obtained at 2, 3, and 5 hours after the beginning of the test meals. The subjects remained in the laboratory and were not allowed to consume any additional foods or beverages, except for water.

Composition of the meals
The HFM consisted of two muffins with bacon and cheese (90g each), providing 1010 kcal, with 78% of energy as fat (37% as saturated fat), 16% as carbohydrates, and 6% as protein. The muffins were accompanied by 500 mL of water (HFM-W) or OJ (HFM-OJ) in different test meal days. Concentrated integral sugar-free OJ (100% OJ) was provided by Fast Fruit® in 1-liter packages; an amount of 500 mL provides 215 kcal from 50g of carbohydrates (information provided by the manufacturer). The juice package was opened at the time of the consumption.

Assessment of metabolic markers
Fasting and postprandial blood samples were collected in EDTA tubes using a 21G butterfly needle and were centrifuged immediately at 2,200 x g at 5°C for 15 min. The plasma was then separated and stored at -80°C. The analyses were performed in the semiautomatic analyzer BS200 (Mindray, Nanshan, China). Plasma concentrations of TG, total cholesterol, HDL-c, LDL-c, uric acid, and glucose were measured using colorimetric enzymatic commercial kits (Bioclin, Belo Horizonte, Brazil). Plasma concentrations of complement C3 were measured by turbidimetric methods using commercially available kits (Bioclin, Belo Horizonte, Brazil).

Statistical analysis
The results are presented as mean ± standard deviation (SD). Age, BMI, body weight and composition, and plasma baseline metabolic biomarkers were compared between groups using Student’s t test or Mann-Whitney test, as appropriate. As the groups (normal-weight and overweight/obese) differed in age, the analyses were
adjusted for this variable (Tukey-Kramer test). A mixed
model using a three-way repeated-measures ANOVA
was applied to test the differences between test meals
throughout the test day for postprandial metabolic and
inflammatory variables with test meals, groups, and time
as repeated factors. Post hoc testing was performed using
the Tukey-Kramer test. The statistical analyses were
performed using the SAS statistical package (version 9.2;
SAS Institute Inc, Cary, NC, USA). The rejection level
of significance used was 5%. The incremental area under
the curve (piAUC) was calculated using GraphPad
Prism (Version 5; GraphPad Software Inc., USA). Power
analysis, calculated with the analyst procedure of SAS,
indicated that a sample of 15 subjects per group would
allow the detection of a treatment effect accounting for
5% of the within-subject variance in TG with more than
99% of power at the 5% level of probability.

RESULTS

Baseline

A total of 36 women completed the study and served
as controls for themselves. Baseline values of weight,
BMI, body fat, glucose, HDL-c, and uric acid differed
between groups (normal weight vs. overweight/obese),
as presented in Table 1.

Table 1. Baseline characteristics of the participants

<table>
<thead>
<tr>
<th></th>
<th>Normal-weight women</th>
<th>Overweight/Obese women</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants (n)</td>
<td>21</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>24 ± 4</td>
<td>31 ± 8</td>
<td>0.022</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>58 ± 5</td>
<td>81.4 ± 13</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22 ± 1.8</td>
<td>31.1 ± 3.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>25.8 ± 3.2</td>
<td>37 ± 3.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>103.3 ± 7.2</td>
<td>110.6 ± 8.8</td>
<td>0.200</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>64.8 ± 6.7</td>
<td>69.4 ± 8.7</td>
<td>0.103</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>88.2 ± 6.5</td>
<td>97.9 ± 7</td>
<td>0.004</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>168.5 ± 31.6</td>
<td>168.21 ± 26.4</td>
<td>0.793</td>
</tr>
<tr>
<td>HDL-c (mg/dL)</td>
<td>67.2 ± 17.2</td>
<td>50.8 ± 7.1</td>
<td>0.005</td>
</tr>
<tr>
<td>LDL-c (mg/dL)</td>
<td>81.4 ± 24</td>
<td>87 ± 16.5</td>
<td>0.672</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>96.8 ± 32.8</td>
<td>136.3 ± 65.8</td>
<td>0.176</td>
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<tr>
<td>Uric acid (mg/dL)</td>
<td>3.7 ± 0.7</td>
<td>4.4 ± 0.6</td>
<td>0.004</td>
</tr>
<tr>
<td>C3 (mg/dL)</td>
<td>137.5 ± 29.3</td>
<td>142.9 ± 25.9</td>
<td>0.436</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation (SD).
BMI: body mass index; MBR: basal metabolic rate; SBP: systolic blood pressure; DBP: diastolic
blood pressure; TC: total cholesterol; TG: triglycerides.

RESULTS

Metabolic postprandial response

After consumption of the HFM-W meal, TG levels
tended to increase in normal-weight volunteers at
3 hours postprandial relative to fasting (p = 0.07).
However, this increase at the third hour was only
significant when these participants consumed the
HFM-OJ meal (p = 0.01). At 5 hours, there was no
difference between baseline and postprandial TG in
normal-weight women (p = 0.99). Overweight/obese
women presented increased TG in relation to fasting
at 3 hours postprandial both after the HFM-W (p =
0.01) and HFM-OJ (p = 0.02) meals. Furthermore,
the increase in TG compared with fasting remained
at 5 hours after consumption of the HFM-OJ meal in
overweight/obese volunteers (p = 0.03) (Figure 1).

At 5 hours postprandial, there was a trend
between the groups of normal-weight and overweight/obese
(p = 0.06). Complement C3 presented a significantly
higher piAUC (p = 0.03) in the normal-weight
group.

Total cholesterol, HDL-c, and LDL-c did not vary
statistically over time or between diets.

DISCUSSION

In this study, PPL occurred 3 hours after the ingestion
of a HFM. Studies have shown that circulating TG
presents a pronounced increase (i.e., PPL) 1 hour after
consumption of a typical fat-containing meal (30–60g
of fat) and may remain high for 5–8 hours (13,14).
Recent epidemiological studies have clearly evidenced
the predictive relationship between the extent of
postprandial hypertriglyceridemia and a relative risk of
cardiovascular events (15,16). This finding is relevant
concerning the analyses of lipids as biomarkers in
chronic diseases.

The relevant finding of this study was that
overweight/obese women consuming a HFM-OJ meal
had a prolonged TG increase, with higher TG levels at 5
hours postprandial. Peairs and cols. reported increased
postprandial TG in obese patients (17). The first point of
the discussion is the difference in PPL between normal-
weight and overweight/obese women. Normal-weight
women presented a TG increase after both the HFM-
OJ meal, while overweight/obese volunteers had a TG
increase after both meals. In fact, the amplitude and
duration of the PPL are related to the meal composition
and the physiopathological condition of the subjects,
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including obesity (18,19). PPL in overweight/obese women disclosed a lipid intolerance state that could not be detected in fasting. In obese humans, fasting plasma lipids can be normal but postprandial lipid metabolism is abnormal with an accumulation of TG-rich remnant lipoproteins. In addition, the catabolism of their chylomicrons (CM) remnants was markedly decreased when compared with lean women (20). The decreased clearance of CM remnants in overweight/obese subjects may be explained by a competition between these remnants and the increased hepatic production of VLDL for clearance by low-density lipoprotein receptors.

The second point of the discussion is the role of OJ on PPL in normal-weight and overweight/obese women. Studies about the effect of fruit-derived antioxidant and fructose intake on fatty meal-induced metabolic changes have reported contradictory results. We observed an enhanced PPL when OJ was added to the HFM. Similar to our findings, Stanhope and cols. observed that diets rich in highly digestible carbohydrates can lead to higher levels of fasting plasma TG as a result of hepatic VLDL and CM remnant accumulation due to altered lipoprotein secretion and/or clearance (21). Cerletti and cols. found that the concomitant intake of OJ resulted in a reduced increase in plasma TG and reduction in total cholesterol (22). These findings altogether indicate that postprandial metabolism resulting from the digestion and absorption of available nutrients is a highly complex process involving numerous potential interactions. In addition, other studies have shown that the amount or type of carbohydrate in a meal alter the postprandial lipid metabolism (23). However, data obtained after addition of glucose (50 to 100g) to a HFM have not provided reproducible findings in healthy subjects (24). Thus, despite epidemiological data suggesting an inverse association between citrus fruits intake and cardiovascular disease risk, our understanding of the mechanisms by which flavonones potentially reduce this risk remains unclear.

As a limitation of the study, the total energy intake in the meal with OJ was 215 kcal higher than that in the meal with water. The addition of OJ increased the amount of carbohydrates in the meal (40.7g for HFM-W vs. 91g for HFM-OJ), without contributing to fat content. Given its fructose content, OJ may have altered the PPL by increasing hepatic fat synthesis (25).
However, Stookey and cols. have shown that the addition of OJ to a meal with 12g of fat limited fat oxidation in the postprandial period (26). These results suggest that reduced fat oxidation might mediate the effects of caloric beverages on weight gain, independent of energy excess. In adults, reduced fat oxidation predicts weight gain, independent of metabolic rate (27). Another point is the 48-hour washout diet applied before the test meal days. This low-antioxidant diet aimed to minimize the variability of the biochemical and inflammatory markers analyzed, which was important in view of the physiological approach in the current study. However, this diet could hamper the application of the results in free-living conditions.

Regarding complement C3, some studies have shown a correlation between fasting C3 and TG (28,29). However, we did not find correlations between fasting C3 and metabolic syndrome parameters, probably because of the small number of individuals assessed. Furthermore, Halkes and cols. have found plasma C3 levels significantly higher than fasting at 2, 4, and 6 hours after fat ingestion (50g/m² of fat) by normolipidemic subjects with coronary artery disease and healthy controls (30). We did not find a significant postprandial difference in plasma C3. Possible causes for this negative outcome are the different populations assessed, different fat overload, and the addition of OJ. Charlesworth and cols. also found no significant postprandial changes in C3 after a mixed meal, although their study lacked information about the amount of fat (31). Van Oostrom and cols., while studying the relationship of PPL with meal composition in healthy subjects, observed that the addition of glucose to a fat overload decreased PPL and prevented a fat-specific increase in C3 (32). For Schär and cols., OJ or hesperidin supplement did not acutely affect cardiovascular risk biomarkers (33). Because of existing controversial results concerning postprandial response of complement C3 to meal composition, more studies are necessary to investigate the biochemical and dietary factors related to variations in circulating C3 in healthy and obese people.

In conclusion, our study has shown that overweight/obese women have enhanced PPL when compared with normal-weight women, and that the increase in TG was more prolonged when these overweight/obese volunteers consumed a HFM with OJ. Since the findings of our present study and previously published ones are still controversial, more studies are necessary to clarify the role of OJ or citrus juice intake in the lipid metabolism and in the prevention of chronic diseases.

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