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Food & Function

Orange juice modulates proinflammatory cytokines after a high-fat saturated meal consumption

Daniela M. U. P. Rocha,ã Lilian L. Lopes,ã Alessandra da Silva,ã Leandro L. Oliveira,ã Josefina Bressanã and Helen H. M. Hermsdorff a†

We aimed to evaluate the postprandial secretion of inflammatory markers induced by an SFA or MUFA high-fat meal consumption and whether orange juice intake could modulate this induction. This study included 55 healthy women (aged 20 to 40 years): 33 participants received an SFA high-fat meal (≈1.000 kcal, 37.6% of energy intake (E) from SFA) and 22 participants received an MUFA high-fat meal (≈1.000 kcal, 56.3% E from MUFA). Both interventions were accompanied by 500 ml of orange juice (test) or water (control). Plasma concentrations of inflammatory cytokines (IL-2, IL-4, IL-6, IL-10, IL-17A, IFN-γ, TNF-α) and CRP were determined before (fasting) and 2, 3 and 5 hours after the test meal intake. SFA high-fat meal induced a significant increase in AUC values (for TNF-α, IL-12, IL-10, IL-6 and IL-2 adjusted for baseline concentrations), in comparison with MUFA high-fat meal intervention. The results were independent of the drink which accompanied the meal (water or orange juice). Both IL-4 and IL-17A AUC values were significantly increased after an SFA high-fat meal intake, accompanied by water, but not by orange juice. In addition, these values were higher in relation to MUFA high-fat meal interventions. Also, IL-17A significantly increased at 3h after an SFA high-fat meal intake accompanied by water, but not by orange juice. Overall, our outcomes indicate an anti-inflammatory effect of MUFA compared to SFA high-fat meal intake, while orange juice intake was able to mitigate the subclinical increase of postprandial inflammation, induced by SFA high-fat meal consumption, for a particular biomarker (IL-17A).

Introduction

Inflammation is a physiological response to tissue injury or infectious agent in order to eliminate the irritant and accelerate tissue regeneration. 1,2 In this process, several inflammatory mediators are released, including cell adhesion molecules, cytokines, chemokines, among others inflammatory agents. 3 To maintain homeostatic balance, a controlled inflammatory response, with the activation of inflammation followed by phase resolution and repair is required. On the other hand, a chronic, excessive or inappropriate inflammatory response leads to an imbalance in homeostasis and promotes an inflammatory pathological condition. 1 In this regard, the chronic and subclinical inflammation has been proposed as a link between adiposity and various clinical disorders, such as metabolic syndrome, diabetes and atherosclerosis. 4,5

In turn, studies have suggested that high-fat consumption can induce acute episodes of immune activation. 6-8 Indeed, fatty acids play an important role in regulating immune and inflammatory responses. 9-11 The consumption of saturated fatty acids (SFA) have an influence on the pro-atherogenic and inflammatory processes and is associated with high concentrations of C-reactive protein (CRP). 12,13 Besides, monounsaturated fatty acids (MUFA) have been associated with cardiovascular morbidity and mortality reduction and improved lipid profile. 14-16 Moreover, MUFA can modulate postprandial inflammatory response to an anti-inflammatory profile, particularly when compared to SFA consumption. 17,18

In addition, fruits and vegetables are important sources of vitamins, minerals, fiber, as well as bioactive compounds. The consumption of these foods is related to the improvement of oxidative stress, by reducing the oxidized LDL and increasing the antioxidant capacity. 19,20 In addition, fruits and vegetables also have good anti-inflammatory capacity through reduction of CRP and homocysteine concentrations and reduced expression of proinflammatory genes such as IL-6, TNF-α and IL-1β. 21,22 In this context, orange juice is the most popular among fruit juices and is encouraged by its nutritional composition: source of vitamin C, flavonoids and bioactive components. 23,24 Moreover, orange juice is able to modulate the inflammatory response due to its anti-inflammatory properties. 24,25

Therefore, the aim of this study was to evaluate the postprandial secretion of inflammatory markers induced by an SFA or MUFA high-fat meal consumption and whether orange juice intake could modulate this induction. We hypothesized that the intake of high-fat meal induces an increase in the inflammatory marks, which could be reduced by the consumption of orange juice.

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Food & Function

Full paper

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Materials and Methods

Subjects

Subjects were recruited through internet ad, flyers and advertisements within the Universidade Federal de Viçosa. Inclusion criteria were woman, age between 20-40 y, BMI between 18.5-24.9 kg/m², nonathletic, nonalcoholic, non-smoker, had no history of drug or alcohol abuse/addiction, had no recent digestive, hepatic, renal, cardiovascular, thyroid or inflammatory diseases, no diagnosis of cancer in the previous year, and stable body weight (5% of the usual weight) for at least 3 months before the study. Subjects were excluded if they were allergic to food components present in the test meals, had any medical conditions or received any medication that could interfere with the outcomes of the study, were pregnant, breastfeeding or menopause. The protocol of the present study was approved by the local ethic at Universidade Federal de Viçosa (Of. Ref. N° 184/2011 and 542.585/2014). Also, this work is registered with the Brazilian Registry of Clinical Trials (ReBEC: trials RBR-2h3wjn and RBR-66jx7j). Written informed consent was obtained from all subjects, according to the general recommendations of the Declaration of Helsinki.

Study Design

Two parallel interventions were designed as an acute (postprandial), controlled, randomized cross-over interventions conducted in healthy women. Two days prior to the test meal, the volunteers were advised to restricted consumption of foods such as fruits and vegetables (sources of vitamins, minerals and phenolic compounds), alcohol and caffeine. On the test meal days, the subjects arrived at Laboratory of energy metabolism and body composition (LAMECC) at Universidade Federal de Viçosa after an overnight fast (12h). Anthropometric measurements (body weight, height, waist and hip circumference) and body composition (body fat percentage) analyzed with tetrapolar bioimpedance (InBody, model Y230, Bispace., Ltd) were taken before each test meal to evaluate any variation. The test meal was served and ingested (crossover). The second group ingested an MUFA high-fat meal: ≈1,000 kcal (14.6 E% from carbohydrate, 6.2 E% from protein, 78.2 E% from fat – 56.3 E% from MUFA); E%: energy intake in percentage; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids.

Table 1. Composition of test meals offered to study participants

<table>
<thead>
<tr>
<th></th>
<th>SFA high-fat meal</th>
<th>MUFA high-fat meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (Kcal)</td>
<td>1008 E% 1008</td>
<td>1008 E% 1008</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>14.6 36.7</td>
<td>17.6 44.2</td>
</tr>
<tr>
<td>Protein</td>
<td>6.2 15.6</td>
<td>4.2 10.7</td>
</tr>
<tr>
<td>Fat</td>
<td>79.2 88.7</td>
<td>78.2 87.6</td>
</tr>
<tr>
<td>SFA</td>
<td>37.6 42.1</td>
<td>12.0 13.4</td>
</tr>
<tr>
<td>MUFA</td>
<td>30.8 34.5</td>
<td>56.3 63.1</td>
</tr>
<tr>
<td>C18:1</td>
<td>13.0</td>
<td>59.0</td>
</tr>
<tr>
<td>PUFA</td>
<td>5.4 6.0</td>
<td>8.5 9.5</td>
</tr>
<tr>
<td>ALA</td>
<td>4.3</td>
<td>1.6</td>
</tr>
<tr>
<td>LA</td>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>EPA</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>DHA</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

E%: energy percentage; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; ALA: alpha-Linolenic acid; LA: linoleic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

Blood sampling and analysis

Blood samples were taken at baseline, 2, 3 and 5 hours after the consumption of the test meals. All samples were taken from the antecubital vein into EDTA Vacutube tubes via venipuncture (Vacutec®, K3E K3EDTA, Greiner BIO-one, UK). After centrifugation at 3,500 rpm at 4º C for 15 minutes, plasma was collected and immediately stored at -80º C until assayed. All analyses were performed at the end of both interventions. Flow cytometry analysis was performed by using a BD FACSVersé™ flow cytometer (BD Biosciences, EUA). Plasma concentrations of the cytokines interleukin-17A (IL-17A), interleukin-10 (IL-10), interleukin-6 (IL-6), interleukin-4 (IL-4), interleukin-2 (IL-2) c (TNF-α), and interferon gamma (IFN-γ) were measured using commercial kit (Cytometric Bead Array CBA Human Th1/Th2/Th17 Kit, BD Biosciences, EUA) according to the manufacturers’ instructions. The data were analyzed using the FCAP Array Software v3.0 (BD Biosciences, EUA).

The study included two different groups: one group ingested an SFA high-fat meal consisting of a muffin mainly of bacon (72 g) and cheese (36 g), accompanying of 500 ml of water or orange juice (crossover). The second group ingested an MUFA high-fat meal consisted of a muffin mainly of olive oil (73 mL) and nuts (30 g), accompanying of 500 ml of water or orange juice (crossover). The chemical composition was determined based on composition tables 27–29. Both high-fat meals provide ≈1,000 kcal (Table 1). The two visits of each group were performed in a randomized order (https://www.random.org/), with nearly 2-weeks washout period (Figure 1).

Figure 1. Schematic of study design.

(A) SFA high-fat meal group intervention: ≈1,000 kcal (14.6 E% from carbohydrate, 6.2 E% from protein, 79.2 E% from fat – 37.6 E% from SFA); (B) MUFA high-fat meal group intervention: ≈1,000 kcal (17.6 E% from carbohydrate, 4.2 E% from protein, 78.2 E% from fat – 56.3 E% from MUFA); E%: energy intake in percentage; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids.
Plasma C-reactive protein (CRP) were measured by a high sensitivity immunoturbidimetric assay (kit CRP, Beckman Coulter, Inc.) according to the manufacturers’ instructions, by a third-party laboratory.

Statistical Analysis

Data are expressed as mean ± SEM. The Student t test or Mann-Whitney test, as appropriate, were used to compare differences in mean baseline characteristics of the study participants who consumed SFA high-fat meal from those who consumed MUFA high-fat meals. Differences in plasma cytokine and CRP concentrations to the two isocaloric meals were analyzed by two-way ANOVA with repeated measures, followed by the post hoc Bonferroni’s multiple comparisons test. In this analysis, we studied the statistical effects of the time alone (within-subject effect), the effect of the meal independently of the time (between-subject effect) and the interaction of both factors (meal x time) which is indicative of the magnitude of the postprandial response in each meal. The same test was conducted to delta (final–baseline) in plasma cytokines and CRP concentrations. These tests were applied separately for each group (SFA high-fat meal and MUFA high-fat meal). The cytokines and CRP area under the curve (AUC) was calculated for cytokines from 0 to 5 hours, using the trapezoid rule in GraphPad Prism 5 software (GraphPad, La Jolla, CA, USA). Paired t test was employed to analyze differences in the cytokines and CRP’s AUC between SFA high-fat meals and MUFA high-fat meals, separately. For efficacy and safety analyses, ANCOVA adjusting for baseline as a covariate was used to assess differences in cytokines and CRP’s AUC between SFA and MUFA high-fat meals comparisons.

The subjects were classified according to their BMI in normal (<25.0 kg/m²) and CRP’s AUC between SFA and MUFA high-fat meals comparisons. Thirty-three participants completed the SFA high-fat meal intervention, and 22 volunteers participated in the MUFA high-fat meal intervention. Subject characteristics are presented in Table 2. Inflammatory cytokines (baseline) was different between the groups. Participants of the SFA high-fat meal intervention had a higher cytokine concentrations and BMI, compared to those from the MUFA high-fat meal intervention. However, the mean CRP concentration was the same for both groups. Participant’s baseline characteristics had no change, during the study.

The intake of an SFA high-fat meal induced a significant increase in AUC values (adjusted for baseline concentrations), in comparison with MUFA intervention, for the inflammatory cytokines TNF-α, IL-12, IL-10, IL-6 and IL-2. Results were independent of the drink which accompanied the meal (500 mL of water or orange juice). Previous interventional studies have supported the proinflammatory effect of SFA compared to MUFA. In a study with SFA (35% energy (E)) and MUFA intake (36% E), despite having a TNF-α plasma concentrations unchanged, its expression increased at 3 hours after the SFA intake. In another study, plasma concentrations of TNF-α and its expression increased after 12 weeks of SFA (16% E) or MUFA (20% E) interventions. However, TNF-α expression was higher when the SFA diet was consumed. This may be related to postprandial reduced NF-kB and increased IκB-α expressions, after the MUFA diet intake. Furthermore, (35% E) intake elicited NF-kB postprandial activation at 3h in healthy men SFA, but without changes observed for an MUFA (38% E) intake.

The same test was conducted to delta (final−baseline) in cytokines and CRP concentrations to the two isocaloric meals were analyzed by two-way ANOVA with repeated measures, followed by the post hoc Bonferroni’s multiple comparisons test. In this analysis, we studied the statistical effects of the time alone (within-subject effect), the effect of the meal independently of the time (between-subject effect) and the interaction of both factors (meal x time) which is indicative of the magnitude of the postprandial response in each meal. The same test was conducted to delta (final–baseline) in cytokines and CRP concentrations. These tests were applied separately for each group (SFA high-fat meal and MUFA high-fat meal). The cytokines and CRP area under the curve (AUC) was calculated for cytokines from 0 to 5 hours, using the trapezoid rule in GraphPad Prism 5 software (GraphPad, La Jolla, CA, USA). Paired t test was employed to analyze differences in the cytokines and CRP’s AUC between SFA and MUFA high-fat meals comparisons. Thirty-three participants completed the SFA high-fat meal intervention, and 22 volunteers participated in the MUFA high-fat meal intervention. Subject characteristics are presented in Table 2. Inflammatory cytokines (baseline) was different between the groups. Participants of the SFA high-fat meal intervention had a higher cytokine concentrations and BMI, compared to those from the MUFA high-fat meal intervention. However, the mean CRP concentration was the same for both groups. Participant’s baseline characteristics had no change, during the study.

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High-fat meals induce a proinflammatory postprandial phase evidenced by the stimulation of postprandial lipemia, which is associated with the activation of leukocytes, and also the upregulation of inflammatory genes in endothelial cells. A proposed mechanism to explain postprandial inflammation is the metabolic endotoxemia caused by increased bacterial endotoxin (lipopolysaccharide - LPS) found in large amounts in the human gut. The LPS seems to be transported from the intestine to the circulation after a high-fat meal intake. LPS is a natural TLR4 (toll-like receptor 4) ligand, therefore a potent stimulator of the inflammatory response. Also, SFA can act as non-microbial TLR4 agonist and modulate TLR4-induced inflammatory response, and promote the expression of proinflammatory transcript factors such as factor nuclear kappa B (NF-kB), which plays a crucial role in the induction of inflammatory mediators (cytokines, chemokines, or costimulatory molecules). On the other hand, olive oil consumption due to its polyphenol content can suppress the expression of several pro-inflammatory genes (IFN-γ, Rho GTPase-activating protein15 (ARHGAP15), and IL-7R) . Thus, olive oil phenolic compounds decrease the inflammatory response, possibly by reducing the NF-kB activation and postprandial plasma lipopolysaccharide concentrations. Thus, olive oil bioactive components (polyphenols and carotenoids) seem to play an important role in inflammation.

Interestingly, AUC values for IL-17A and IL-4 were also increased significantly after SFA high-fat meal accompanied with water only, in comparison to the MUFA high-fat meal interventions. However, when accompanied by orange juice, both meals showed same AUC values for these proinflammatory cytokines (Table 3). In addition, our study, apparently for the first time, showed a change in IL-17A concentration, was significantly increased at 3h after an SFA high-fat meal intake accompanied by water, but not by orange juice.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>SFA high-fat meal (n=33)</th>
<th>MUFA high-fat meal (n=22)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27±0.8</td>
<td>27±0.7</td>
<td>0.661</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.96±0.67</td>
<td>23.41±0.47</td>
<td>0.010</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>82.0±1.7</td>
<td>79.8±1.2</td>
<td>0.276</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>98.6±1.1</td>
<td>98.8±0.8</td>
<td>0.460</td>
</tr>
<tr>
<td>Waist-Hip Ratio</td>
<td>0.83±0.01</td>
<td>0.81±0.01</td>
<td>0.107</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>30.6±0.8</td>
<td>30.1±0.9</td>
<td>0.736</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>106±5</td>
<td>107±1</td>
<td>0.676</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>67±1.9</td>
<td>67±1</td>
<td>0.229</td>
</tr>
<tr>
<td>IL-2 (pg/ml)</td>
<td>2.9±0.6</td>
<td>0.4±0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-4 (pg/ml)</td>
<td>4.7±1.1</td>
<td>0.1±0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>7.0±0.8</td>
<td>0.3±0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>4.8±0.7</td>
<td>0.2±0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-17A (pg/ml)</td>
<td>63.0±10.7</td>
<td>7.5±1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IFN-γ (pg/ml)</td>
<td>21.0±5.1</td>
<td>0.6±0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>6.5±1.4</td>
<td>0.2±0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>3.6±0.7</td>
<td>4.4±0.8</td>
<td>0.581</td>
</tr>
</tbody>
</table>

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; IL: interleukin; TNF-α: tumor necrosis factor alpha; CRP: C-reactive protein. Data presented as mean ± SEM. p values from Student t test or Mann-Whitney test, when appropriate.
**Graph 1.** Change in IL-17A plasma concentration after an SFA high-fat meal intake, accompanied by water or orange juice. SFA: saturated fatty acids + 500 mL of water; SFA + OJ: saturated fatty acids + 500 mL of orange juice; MUFA: monounsaturated fatty acids + 500 mL of water; MUFA + OJ: monounsaturated fatty acids + 500 mL of orange juice; IL-17A: interleukin-17A; IL-6: interleukin-6; IL-12: interleukin-12; IL-17: interleukin-17; IFN-γ: Interferon-gamma; CRP: C-reactive protein. Data expressed as mean ± SE. Two-way ANOVA repeated measures. P1: diet effect, P2: effect of time, P3: interaction diet × time. *p = 0.04 (SFA vs. SFA + OJ at 3h).

In this sense, regular consumption of orange juice has been able to maintain unchanged pro and anti-inflammatory cytokine concentrations, or reduced inflammation by reducing CRP concentrations, in healthy or overweight subjects [37,42,44]. Similarly, acute consumption of orange juice maintained constant the postprandial expression of NF-kB [40,41]. Moreover, postprandial studies have shown that high-fat meals have increased the concentration of proinflammatory cytokines (IL-1β and TNF-α), but orange juice intake was able to suppress this proinflammatory effect [45,46].

Similarly, a high-fat meal accompanied by the intake of antioxidants, vitamin E (800 IU) and ascorbic acid (1.000 mg), has also suppressed the rise of inflammatory markers - proinflammatory cytokines (IL-6, TNF-α) and adhesion molecules (ICAM-1 and VCAM-1) in healthy subjects [37]. The suppressive effect on postprandial inflammation induced by high-fat meal is associated with the prevention of inflammatory cell activation [48].

The orange juice is a nutrient-dense beverage which provides substantial amounts of vitamins, including vitamin C, minerals and flavonoids [23,24]. The anti-inflammatory effects of orange juice are probably attributable to its antioxidants (ascorbic acid), and especially to its bioactive compounds, hesperidin and naringenin [24,49]. Indeed, the consumption of 500 mL of orange juice with high concentrations of polyphenols, in comparison to a normal concentration, improve metabolites related to oxidative stress and inflammation [30].

In this study, IL-6 increased significantly, between 2 and 5h, after an SFA and MUFA high-fat meals interventions (Graph 2 A and B). Also, IL-6 increased significantly, at 2h after an MUFA high-fat meal intake (Graph 2B). Previous SFA and MUFA high-fat interventional studies have also shown increased IL-6 plasma concentrations (at 4h postprandial), regardless the received meal, and IL-6 expression was associated or not to its increase [18,30]. However, in another study with SFA and MUFA interventions, IL-6 plasma concentrations remains unchanged in the postprandial period (3, 6, 9h) assessed [17]. In a review article, 73% of 30 high-fat meal trials found an increase of IL-6, with a postprandial peak between 2 and 4 hours [51]. The contradictory findings may be related to the postprandial assessed time, in some studies, which may have been insufficient to detect differences in the concentrations of the cytokine, while IL-6 gene expression could be increased after the high-fat meals in the same time. In fact, transcription and translation processes do not occur simultaneously [52]. Furthermore, various translation mechanisms and posttranslational may be involved in the production and secretion of cytokines [53], and there is confounding factors involved such as gender, age, physical activity, smoking and BMI [3]. This increase in IL-6 concentrations, regardless of diet, suggests a diurnal cycle of this cytokine [54].

**Table 3.** The area under the curve for plasma inflammatory markers in subjects after SFA or MUFA high-fat meal accompanied or not by orange juice.

<table>
<thead>
<tr>
<th>AUC</th>
<th>SFA (n=33)</th>
<th>SFA+OJ (n=33)</th>
<th>MUFA (n=22)</th>
<th>MUFA+OJ (n=22)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>10.9±1.0</td>
<td>9.3±1.1 *</td>
<td>4.1±1.2 *</td>
<td>5.1±1.2 *</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-4</td>
<td>12.7±1.4</td>
<td>10.4±1.6 *</td>
<td>4.7±1.8 *</td>
<td>4.8±1.8 *</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-6</td>
<td>28.2±1.7</td>
<td>25.8±1.8 *</td>
<td>6.4±1.3 *</td>
<td>6.8±1.3 *</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-10</td>
<td>17.4±1.4</td>
<td>14.8±1.5 *</td>
<td>6.1±1.8 *</td>
<td>6.5±1.8 *</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-17A</td>
<td>228.8±20.4</td>
<td>179.7±22.0</td>
<td>112.0±25.1</td>
<td>115.4±25.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>54.2±6.8</td>
<td>42.4±7.3 *</td>
<td>29.3±8.3</td>
<td>30.1±8.3</td>
<td>0.069</td>
</tr>
<tr>
<td>TNF-α</td>
<td>17.7±1.5</td>
<td>16.0±1.7 *</td>
<td>5.7±1.9</td>
<td>5.8±1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP</td>
<td>18.4±0.8</td>
<td>17.0±0.7 *</td>
<td>18.6±0.9</td>
<td>19.3±0.9</td>
<td>0.812</td>
</tr>
</tbody>
</table>

AUC: area under the curve, calculated for cytokines (pg/mL) and CRP (mg/L) from 0 to 5 hours, and adjusted for baseline concentrations; SFA: saturated fatty acids + 500 mL of water; SFA + OJ: saturated fatty acids + 500 mL of orange juice; MUFA: monounsaturated fatty acids + 500 mL of water; MUFA + OJ: monounsaturated fatty acids + 500 mL of orange juice; IL: interleukin; TNF-α: tumor necrosis factor alpha; CRP: C-reactive protein. Data expressed in adjusted mean (for baseline concentrations) ± SE. Different letters represent statistical difference (p<0.05). *p = values from ANCOVA statistical test to one factor followed by the Post-hoc Bonferroni test.
systemic inflammation is an acute phase protein commonly used as a non-specific marker of inflammation not sufficient for detection in CRP modification. Furthermore, CRP is considered clinically significant in subclinical inflammation (<10 mg/L) related to obesity, are considered clinically significant. However, there is almost no detectable increase in CRP concentrations up to 5h post-stimulation, only about 24h after is observed its peak of production.

Moreover, CRP postprandial concentrations remain unchanged at the assessed time (2h, 3h, 5h), for both SFA and MUFA high-fat meals, also for the interaction of meals* time. Minor increment in baseline CRP concentrations, as occur in low-grade inflammation or subclinical inflammation (<10 mg/L) related to obesity, are considered clinically significant. However, there is almost no detectable increase in CRP concentrations up to 5h post-stimulation, only about 24h after is observed its peak of production.

Therefore, it is reasonable to believe that the absence of CRP change is because the postprandial assessed time (up to 5h) was not sufficient for detection in CRP modification. Furthermore, CRP is an acute phase protein commonly used as a non-specific marker of systemic inflammation, being produced in the liver induced by the cytokines IL-1, IL-6 and IL-17 in response to an inflammatory agent or tissue damage, supporting the hypothesis of lack of time to secretion stimulation. Thus, the modulation of circulating CRP by SFA and MUFA high-fat intake needs more investigation.

By splitting the groups according to weight-status in normal (<25.0 kg/m²) or overweight (≥25.0 kg/m²), and to body fat in normal (<30%) or excess body fat (≥30%), we did not observe statistically significant differences in the postprandial inflammatory response of the volunteers after any test meal. Therefore, the data were presented without considering the classifications described above. This outcome indicates that diet can modulate the postprandial inflammatory response regardless of weight-status or body fat composition.

Our study has some limitations, as noted baseline inflammatory markers were different among the participants of SFA and MUFA high-fat meals interventions. However, we were careful to compare the mean AUC adjusted for baseline, as a covariate, for these markers. In addition, we could not evaluate the effect of single nutrients in the meals offered. Because the meals resulted from a combination of foods, that besides their nutrients, are sources of vitamins and minerals, in addition to the bioactive compounds.

**Conclusions**

Taking together, our outcomes demonstrated that acute intake of different fatty acids (SFA or MUFA), are capable of subclinical postprandial inflammatory response, in healthy individuals regardless of weight-status. In addition, orange juice intake was able to mitigate the increment in postprandial inflammation, induced by SFA high-fat meal consumption, for a particular cytokine (IL-17A). Long-term studies are needed to assess the benefits of regular consumption of MUFA and orange juice, as well as the mechanisms involved.

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