Food & Function

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: D. M. U. P. Rocha, L. L. Lopes, A. Silva, L. L. de Oliveira, J. Bressan and H. H. M. Hermsdorff, *Food Funct.*, 2017, DOI: 10.1039/C7FO01139C.

ROYAL SOCIETY OF CHEMISTRY

rsc.li/food-function

View Article Online
DOI: 10.1039/C7FO01139C

Food & Function

Full paper

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

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Daniela M. U. P. Rocha,[a] Lílian L. Lopes,[a] Alessandra da Silva,[a] Leandro L. Oliveira,[b] Josefina Bressan[a] 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-aft 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-1R* [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.

[a] Department of Nutrition and Health, Universidade Federal de Viçosa, Viçosa, MG, Brazil.

[b] Department of General Biology, Universidade Federal de Viçosa, Viçosa, MG, Brazil.

† e-mail: helenhermana@ufv.br. Tel. (+55 31) 3899-1269. Fax: (+55 31) 3899-2541.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

View Article Online
DOI: 10.1039/C7FO01139C

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/m^2, 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 [26].

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 within 30 minutes. Blood samples were taken at fasting time as well as 2, 3 and 5 hours after the consumption of the test meals. During the postprandial assessment, subjects consumed nothing but water, kept their physical activity to a minimum and did not leave the LAMECC during the test meal day.

Table 1. Composition of test meals offered to study participants

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

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.

The subjects were served a lunch after the last blood sample was taken. Subjects were encouraged to maintain their regular physical activity and lifestyle throughout the duration of the study.

Test Meals

The study included two different groups: one group ingested an SFA high-fat meal consisted 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**).

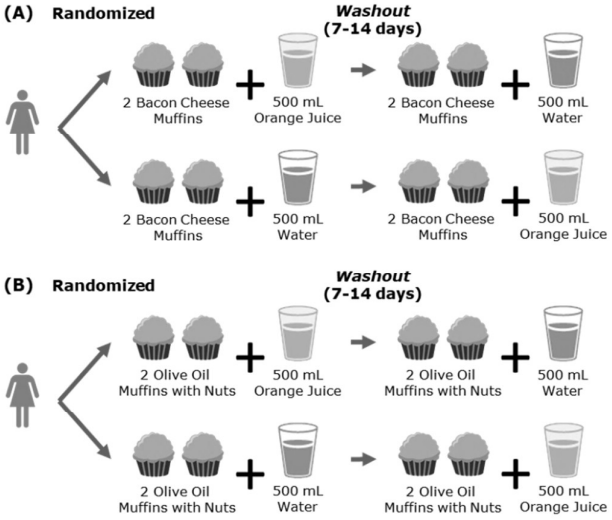


Figure 1. Schematic of study design.
(A) SFA high-fat meal group intervention: ≈1,000 kcal (14.6 E% from carbohydrate, 6.2 E% from protein, 79.2 E% from fat – 37.6 E% from SFA); (B) MUFA high-fat meal group intervention: ≈ 1,000 kcal (17.6 E% from carbohydrate, 4.2 E% from protein, 78.2 E% from fat – 56.3 E% from MUFA); E%: energy intake in percentage; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids.

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 drawn from the antecubital vein into EDTA Vacuette tubes via venipuncture (Vacuette ®, 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 FACSVerse™ 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).

View Article Online
DOI: 10.1039/C7FO01139C

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 overweight (≥25,0 kg / m2), and according to their percentage of body fat in normal (<30%) or excess body fat (≥30%). All statistical tests were performed considering the group or divided by the BMI classification and the percentage of body fat. Statistical analysis was conducted using SPSS 20 for Windows (SPSS, Inc., Chicago, IL, USA). The criterion for statistical significance was $\alpha \leq 0.05$ for all data analyses.

Results and Discussion

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 [17]. 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 [30]. Furthermore, (35% E) intake elicit NF-kB postprandial activation at 3h in healthy men SFA, but without changes observed for an MUFA (38% E) intake [31].

Table 2. Summary of characteristics of study participants

Characteristics	SFA high-fat meal (n=33)	MUFA high-fat meal (n=22)	p^*
Age (years)	27±0.8	27±0.7	0.661
BMI (kg/m^2)	25.96±0.67	23.41±0.47	0.010
Waist circunference (cm)	82.0±1.7	79.8±1.2	0.276
Hip circunference (cm)	98.6±1.1	98.8±0.8	0.460
Waist-Hip Ratio	0.83±0.01	0.81±0.01	0.107
Body fat (%)	30.6±0.8	30.1±0.9	0.736
SBP (mmHg)	106±1	107±1	0.676
DBP (mmHg)	67±0.9	67±1	0.829
IL-2 (pg/ml)	2.9±0.6	0.4±0.1	<0.001
IL-4 (pg/ml)	4.7±1.1	0.1±0.1	<0.001
IL-6 (pg/ml)	7.0±0.8	0.3±0.2	<0.001
IL-10 (pg/ml)	4.8±0.7	0.2±0.1	<0.001
IL-17A (pg/ml)	63.0±10.7	7.5±1.1	<0.001
IFN-γ (pg/ml)	21.0±5.1	0.66±0.2	<0.001
TNF-α (pg/ml)	6.5±1.4	0.2±0.1	<0.001
CRP (mg/L)	3.6±0.7	4.4±0.8	0.581

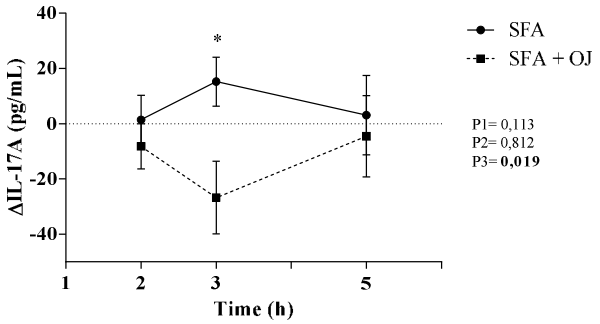
SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; IL: interleukin; TNF-α: tumor necrosis factor alpha; CRP: C-reactive protein. Data presented as mean ± SEM. * *p* values from Student t test or Mann-Whitney test, when appropriate.

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 [32,33]. 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 [34]. LPS is a natural TLR4 (toll-like receptor 4) ligand, therefore a potent stimulator of the inflammatory response [10]. 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) [10]. 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) [35]. Thus, olive oil phenolic compounds decrease the inflammatory response, possibly by reducing the NF-kB activation and postprandial plasma lipopolysaccharide concentrations [36]. 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

View Article Online
DOI: 10.1039/C7FO01139C

(**Graph 1**). IL-17 is a pro-inflammatory cytokine involved in various inflammatory diseases such as rheumatoid arthritis, psoriasis, Crohn's disease and multiple sclerosis [37]. In humans, obesity is associated with elevated concentrations of IL-17 [38]. Moreover, IL-17 contributed to promoting the subclinical atherogenesis in obese patients [39]. In experimental models, the pro-inflammatory and pro-atherogenic role of IL-17A in response to the consumption of a high-fat diet has already been evidenced by increased systemic and vascular inflammation, having an important role in the progression of atherosclerotic lesions [40,41]. Hence, the results from our study suggest that orange juice intake can reduce the postprandial inflammation induced by an SFA high-fat meal consumption, and possibly contributed to reducing the risk of subclinical atherosclerosis.



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; ΔIL-17A = IL-17A (T (h)) - IL-17A (T (0 h)); T0: baseline (12 hours fasting). Data are 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 cytokines concentrations, or reduced inflammation by reducing CRP concentrations, in healthy or overweight subjects [42,43]. Similarly, acute consumption of orange juice maintained constant the postprandial expression of NF-kB [44]. Moreover, postprandial studies have shown that high-fat meals have increased the concentration of LPS, the expression of SOCS3, TLR2, TLR4, NF-kB and 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 [47]. 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 [50].

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 [4]. 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.

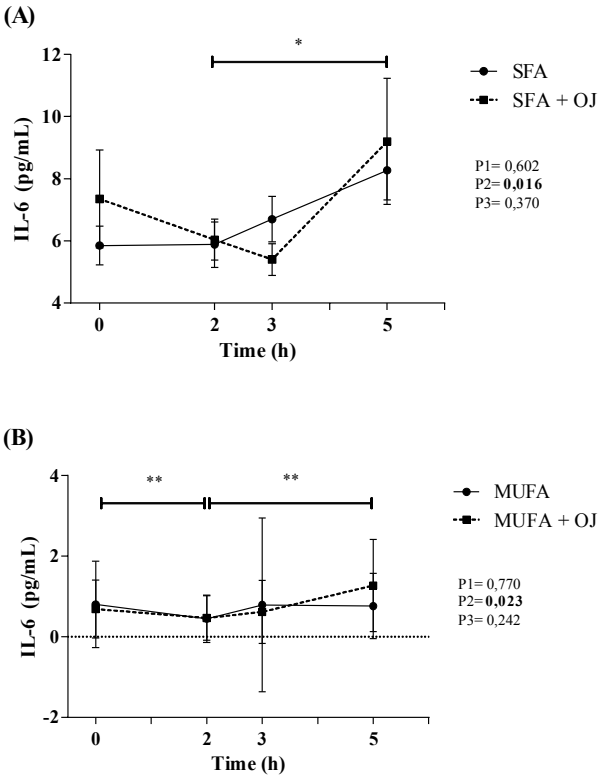
AUC	SFA (n=33)	SFA+OJ (n=33)	MUFA (n=22)	MUFA+OJ (n=22)	p *
IL-2	10.9±1.0 [a,c]	9.3±1.1 [a]	4.1±1.2 [b]	5.1±1.2 [b]	**<0.001**
IL-4	12.7±1.4 [a]	10.4±1.6 [a,b]	4.7±1.8 [b,c]	4.8±1.8 [b]	**<0.001**
IL-6	28.2±1.7 [a]	25.8±1.8 [a]	11.4±2.1 [b]	11.8±2.1 [b]	**<0.001**
IL-10	17.4±1.4 [a]	14.8±1.5 [a]	6.1±1.8 [b]	6.5±1.8 [b]	**<0.001**
IL-17A	228.8±20.4 [a]	179.7±22.0 [a,b]	112.0± 25.1 [b,c]	115.4±25.2 [b]	**<0.001**
IFN-γ	54.2±6.8 [a]	42.4±7.3 [a]	29.3±8.3 [a]	30.1±8.3 [a]	0.069
TNF-α	17.7±1,5 [a]	16.0±1.7 [a]	5.7±1.9 [b]	5.8±1.9 [b]	**<0.001**
CRP	18.4± 0.8 [a]	17.0±0.7 [a]	18.6±0.9 [a]	19.3±0.9 [a]	0.812

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

View Article Online
DOI: 10.1039/C7FO01139C

Food & Function

Full paper



Graph 2. IL-6 plasma concentration after an SFA (A) or MUFA (B) high-fat meal intake, accompanied by water or orange juice.
(A) SFA high-fat meal intervention; (B) MUFA high-fat meal intervention; 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. Data are expressed as mean ± SE. Two-way ANOVA repeated measures. P1: diet effect. P2: effect of time. P3: interaction diet * time. * p = 0.049 for 2h vs. 5h; ** p = 0.009 for 0h vs. 2h and 2h vs. 5h.

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 [55]. 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 [6]. 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 [56], 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 [55], 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/m^2) or overweight (≥25,0 kg/m^2), 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 weigh-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.

Acknowledgements

We would like to acknowledge the laboratories, Laboratório de Metabolismo Energético e Composição Corporal (LAMECC) and Laboratório de Análises Clínicas (LAC), at the Universidade Federal de Viçosa, for providing the facilities to conduct the study and the equipment required to the analysis performed. Also, we thank the Nucleus of Microscopy and Microanalysis (NMM - http://www.nmm.ufv.br/), at the Universidade Federal de Viçosa, for providing the equipment for experiments involving flow cytometry. We are also grateful for the voluntary participation of the participants in this study.

Financial Support

This study was made possible by funding from Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Foundation provided a research grant to DMUP Rocha and LL Lopes. HHM Hermsdorff and J Bressan are CNPq fellows.

View Article Online
DOI: 10.1039/C7FO01139C

References

1. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008;454(7203):428–35.
2. Carraro JCC, Hermsdorff HHM, Puchau B, Zulet MÁ, Milagro FI, Bressan J, et al. Interleukin-6 is a better metabolic biomarker than interleukin-18 in young healthy adults. J Physiol Biochem. 2015 Sep 27;71(3):527–35.
3. Kvietys PR, Granger DN. Role of reactive oxygen and nitrogen species in the vascular responses to inflammation. Free Radic Biol Med. 2012 Feb;52(3):556–92.
4. Hermsdorff HHM, Puchau B, Zulet MAÁ, Martínez JA. Association of Body Fat Distribution with Proinflammatory Gene Expression in Peripheral Blood Mononuclear Cells from Young Adult Subjects. Omi A J Integr Biol. 2010 Jun;14(3):297–307.
5. Hermsdorff HHM, Zulet MÁ, Puchau B, Martínez JA. Central adiposity rather than total adiposity measurements are specifically involved in the inflammatory status from healthy young adults. Inflammation. 2011 Jun 14;34(3):161–70.
6. Herieka M, Erridge C. High-fat meal induced postprandial inflammation. Mol Nutr Food Res. 2014;58(1):136–46.
7. de Vries M a., Klop B, Eskes S a., van der Loos TLJM, Klessens-Godfroy FJM, Wiebolt J, et al. The postprandial situation as a pro-inflammatory condition. Clin e Investig en Arterioscler. 2014;26(4):184–92.
8. Poppitt SD, Keogh GF, Lithander FE, Wang Y, Mulvey TB, Chan YK, et al. Postprandial response of adiponectin, interleukin-6, tumor necrosis factor-α, and C-reactive protein to a high-fat dietary load. Nutrition. 2008;24(4):322–9.
9. Teng K-T, Chang C-Y, Chang LF, Nesaretnam K. Modulation of obesity-induced inflammation by dietary fats: mechanisms and clinical evidence. Nutr J. 2014;13(1):12.
10. Rocha DM, Caldas AP, Oliveira LL, Bressan J, Hermsdorff HH. Saturated fatty acids trigger TLR4-mediated inflammatory response. Atherosclerosis. 2016 Jan;244:211–5.
11. Coelho OGL, da Silva BP, Rocha DMUP, Lopes LL, Alfenas R de CG. Polyunsaturated fatty acids and type 2 diabetes: impact on the glycemic control mechanism. Crit Rev Food Sci Nutr [Internet]. 2016 Feb 6;8398(February):00–00. Available from: http://www.tandfonline.com/doi/full/10.1080/10408398.2015.1130016
12. Santos S, Oliveira A, Lopes C. Systematic review of saturated fatty acids on inflammation and circulating levels of adipokines. Nutr Res. 2013 Sep;33(9):687–95.
13. Santos S, Oliveira A, Casal S, Lopes C. Saturated fatty acids intake in relation to C-reactive protein, adiponectin, and leptin: a population-based study. Nutrition. 2013 Jun;29(6):892–7.
14. Estruch R, Ros E, Salas-Salvadó J, Covas M-I, Corella D, Arós F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med. 2013;368(14):1279–90.
15. Widmer RJ, Flammer AJ, Lerman LO, Lerman A. The Mediterranean Diet, its Components, and Cardiovascular Disease. Am J Med. 2015;128(3):229–38.
16. Solá R, Fitó M, Estruch R, Salas-Salvadó J, Corella D, de La Torre R, et al. Effect of a traditional Mediterranean diet on apolipoproteins B, A-I, and their ratio: a randomized, controlled trial. Atherosclerosis. 2011 Sep;218(1):174–80.
17. Jiménez-Gómez Y, López-Miranda J, Blanco-Colio LM, Marín C, Pérez-Martínez P, Ruano J, et al. Olive oil and walnut breakfasts reduce the postprandial inflammatory response in mononuclear cells compared with a butter breakfast in healthy men. Atherosclerosis. 2009 Jun;204(2):e70–6.
18. Camargo A, Delgado-Lista J, Garcia-Rios A, Cruz-Teno C, Yubero-Serrano EM, Perez-Martinez P, et al. Expression of proinflammatory, proatherogenic genes is reduced by the Mediterranean diet in elderly people. Br J Nutr. 2012 Aug 15;108(3):500–8.
19. Hermsdorff HHM, Barbosa KBF, Volp ACP, Puchau B, Bressan J, Zulet MÁ, et al. Vitamin C and fibre consumption from fruits and vegetables improves oxidative stress markers in healthy young adults. Br J Nutr. 2012;107(8):1119–27.
20. Cocate PG, Natali AJ, de Oliveira A, Longo GZ, Alfenas RDCG, Peluzio MDCG, et al. Fruit and vegetable intake and related nutrients are associated with oxidative stress markers in middle-aged men. Nutrition. 2013 Oct 30;1–6.
21. Hermsdorff HHM, Zulet MA, Puchau B, Martínez JA. Fruit and vegetable consumption and proinflammatory gene expression from peripheral blood mononuclear cells in young adults: a translational study. Nutr Metab (Lond). 2010;7:42.
22. Hermsdorff HHM, Zulet MÁ, Abete I, Martínez JA. A legume-based hypocaloric diet reduces proinflammatory status and improves metabolic features in overweight/obese subjects. Eur J Nutr. 2011 Feb 25;50(1):61–9.
23. O'Neil CE, Nicklas T a, Rampersaud GC, Fulgoni VL. 100% orange juice consumption is associated with better diet quality, improved nutrient adequacy, decreased risk for obesity, and improved biomarkers of health in adults: National Health and Nutrition Examination Survey, 2003-2006. Nutr J. 2012;11:107.
24. Coelho RCLA, Hermsdorff HHM, Bressan J. Anti-inflammatory Properties of Orange Juice: Possible Favorable Molecular and Metabolic Effects. Plant Foods Hum Nutr. 2013;68(1):1–10.
25. Ghanim H, Sia CL, Upadhyay M, Korzeniewski K, Viswanathan P, Abuaysheh S, et al. Orange juice neutralizes the proinflammatory effect of a high-fat, high-carbohydrate meal and prevents endotoxin increase and toll-like receptor expression. Am J Clin Nutr. 2010;91(4):940–9.
26. World Medical Association, Review C, Communication S, Principles G. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013 Nov 27;310(20):2191–4.
27. NEPA-UNICAMP. Brazilian Table of Food Composition (TACO). Campinas; 2011.
28. PHILIPPI S. Tabela de composição de alimentos: suporte para decisão nutricional. 2 ed. São Paulo; 2002.
29. USDA. National Nutrient Database for Standard Reference Release 28. National Agricultural Library; 2016.
30. Cruz-Teno C, Pérez-Martínez P, Delgado-Lista J, Yubero-Serrano EM, García-Ríos A, Marín C, et al. Dietary fat modifies the postprandial inflammatory state in subjects with metabolic syndrome: the LIPGENE study. Mol Nutr

View Article Online
DOI: 10.1039/C7FO01139C

Food Res. 2012 Jun;56(6):854–65.
31. Bellido C, Lopez-Miranda J, Blanco-Colio LM, Perez-Martinez P, Muriana FJ, Martin-Ventura JL, et al. Butter and walnuts, but not olive oil, elicit postprandial activation of nuclear transcription factor kappaB in peripheral blood mononuclear cells from healthy men. Am J Clin Nutr. 2004;80(6):1487–91.
32. Oostrom AJHHM Van, Rabelink TJ, Verseyden C, Sijmonsma TP, Plokker HWM, De Jaegere PPT, et al. Activation of leukocytes by postprandial lipemia in healthy volunteers. Atherosclerosis. 2004;177(1):175–82.
33. Alipour A, Elte JWF, van Zaanen HCT, Rietveld AP, Castro Cabezas M. Novel aspects of postprandial lipemia in relation to atherosclerosis. Atheroscler Suppl. 2008;9(2):39–44.
34. Erridge C, Attina T, Spickett CM, Webb DJ. A high-fat meal induces low-grade endotoxemia: Evidence of a novel mechanism of postprandial inflammation. Am J Clin Nutr. 2007;86(5):1286–92.
35. Konstantinidou V, Covas M-I, Munoz-Aguayo D, Khymenets O, de la Torre R, Saez G, et al. In vivo nutrigenomic effects of virgin olive oil polyphenols within the frame of the Mediterranean diet: a randomized controlled trial. FASEB J. 2010 Jul 1;24(7):2546–57.
36. Camargo A, Rangel-Zuñiga OA, Haro C, Meza-Miranda ER, Peña-Orihuela P, Meneses ME, et al. Olive oil phenolic compounds decrease the postprandial inflammatory response by reducing postprandial plasma lipopolysaccharide levels. Food Chem. 2014;162:161–71.
37. Miossec P. IL-17 and Th17 cells in human inflammatory diseases. Microbes Infect. 2009;11(5):625–30.
38. Ahmed M, Gaffen SL. IL-17 in obesity and adipogenesis. Cytokine Growth Factor Rev. 2010;21(6):449–53.
39. Tarantino G, Costantini S, Finelli C, Capone F, Guerriero E, La Sala N, et al. Is serum Interleukin-17 associated with early atherosclerosis in obese patients? J Transl Med. 2014;12:214.
40. Madhur MS, Funt SA, Li L, Vinh A, Chen W, Lob HE, et al. Role of interleukin 17 in inflammation, atherosclerosis, and vascular function in apolipoprotein e-deficient mice. Arterioscler Thromb Vasc Biol. 2011;31(7):1565–72.
41. Chen S, Shimada K, Zhang W, Huang G, Crother TR, Arditi M. IL-17A Is Proatherogenic in High-Fat Diet-Induced and Chlamydia pneumoniae Infection-Accelerated Atherosclerosis in Mice. J Immunol. 2010 Nov 1;185(9):5619–27.
42. Devaraj S, Jialal I, Rockwood J, Zak D. Effect of orange juice and beverage with phytosterols on cytokines and PAI-1 activity. Clin Nutr. 2011;30(5):668–71.
43. Dourado GKZS, Cesar TB. Investigation of cytokines, oxidative stress, metabolic, and inflammatory biomarkers after orange juice consumption by normal and overweight subjects. Food Nutr Res. 2015 Oct 20;59:1–8.
44. Ghanim H, Mohanty P, Pathak R, Chaudhuri A, Chang LS, Dandona P. Orange juice or fructose intake does not induce oxidative and inflammatory response. Diabetes Care. 2007;30(6):1406–11.
45. Deopurkar R, Ghanim H, Friedman J, Abuaysheh S, Sia CL, Mohanty P, et al. Differential effects of cream, glucose, and orange juice on inflammation, endotoxin, and the expression of toll-like receptor-4 and suppressor of cytokine signaling-3. Diabetes Care. 2010;33(5):991–7.
46. Ghanim H, Sia CL, Upadhyay M, Korzeniewski K, Viswanathan P, Abuaysheh S, et al. Orange juice neutralizes the proinflammatory effect of a high-fat, high-carbohydrate meal and prevents endotoxin increase and toll-like receptor expression. Am J Clin Nutr. 2010;91(4):940–9.
47. Nappo F, Esposito K, Cioffi M, Giugliano G, Molinari AM, Paolisso G, et al. Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. J Am Coll Cardiol. 2003;39(7):1145–50.
48. Cerletti C, Gianfagna F, Tamburrelli C, De Curtis A, D'Imperio M, Coletta W, et al. Orange juice intake during a fatty meal consumption reduces the postprandial low-grade inflammatory response in healthy subjects. Thromb Res. 2015 Feb;135(2):255–9.
49. Dourado GKZS, Ribeiro LCDA, Carlos IZ, César TB. Orange juice and hesperidin promote differential innate immune response in macrophages ex vivo. Int J Vitam Nutr Res. 2014;83(3):162–7.
50. Rangel-Huerta OD, Aguilera CM, Perez-de-la-Cruz A, Vallejo F, Tomas-Barberan F, Gil A, et al. A serum metabolomics-driven approach predicts orange juice consumption and its impact on oxidative stress and inflammation in subjects from the BIONAOS study. Mol Nutr Food Res. 2017 Feb;61(2):1600120.
51. Erridge C, Attina T, Spickett CM, Webb DJ. A high-fat meal induces low-grade endotoxemia: Evidence of a novel mechanism of postprandial inflammation. Am J Clin Nutr. 2007;86(5):1286–92.
52. Berg J, Tymoczko J, Stryer L. Eukaryotic Transcription and Translation Are Separated in Space and Time. In: Biochemistry. 5th editio. New York: W H Freeman; 2002.
53. Kelso A. Cytokines : Principles and prospects. 1998;300–17.
54. van Oostrom a JHHM, Sijmonsma TP, Verseyden C, Jansen EHJM, de Koning EJP, Rabelink TJ, et al. Postprandial recruitment of neutrophils may contribute to endothelial dysfunction. J Lipid Res. 2003;44(3):576–83.
55. Eklund CM. Proinflammatory cytokines in CRP baseline regulation. Adv Clin Chem. 2009;48(9):111–36.
56. Yousuf O, Mohanty BD, Martin SS, Joshi PH, Blaha MJ, Nasir K, et al. High-sensitivity C-reactive protein and cardiovascular disease: A resolute belief or an elusive link? J Am Coll Cardiol. 2013;62(5):397–408.