UNIVERSIDADE FEDERAL DE VIÇOSA

RESPOSTA PÓS-PRANDIAL DE MARCADORES METABÓLICOS E INFLAMATÓRIOS AO CONSUMO DE GORDURA SATURADA E SUCO DE LARANJA EM MULHERES EUTRÓFICAS E COM EXCESSO DE PESO

Raquel Cristina Lopes Assis Coelho

Magister Scientiaie

VIÇOSA
MINAS GERAIS – BRASIL

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RAQUEL CRISTINA LOPES ASSIS COELHO

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Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência da Nutrição, para obtenção do título *Magister Scientiaie*

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(Coorientadora)

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(Orientadora)

À minha irmã, minha melhor amiga.

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ARTIGO 1 - ANTI-INFLAMMATORY PROPERTIES OF ORANGE JUICE:
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LISTA DE ABREVIATURA E SIGLAS

AGRP *Agouti related petid*e

ASP *Acylation stimulating protein*

BIA *Bioimpedance electrical analysis*

BMI	*Body mass index*
CRP	*C-reactive protein*
CVD	*Cardiovascular disease*
cm	*Centimeters*
CM	*Chylomicrons*
CMR	*Chylomicron remnants*
CS	*Complement system*
DBP	*Diastolic blood pressure*
DEXA	*Dual energy X-ray absorptiometry*
DM2	*Diabetes mellitus 2*
DNS	Departamento de Nutrição e Saúde
FA	*Fatty acids*
HDL	*High density lipoprotein*
HFM	*High fat meal*
HFM-OJ	*High-fat meal + 500 ml orange juice*
HFM-W	*High-fat meal + 500 ml water*
hs-CRP	*High-sensitivity C reactive protein*

HP	*Hip perimeter*
IL	*Interleukin*
IPAQ	Questionário Internacional de Atividade Física
JNK	*Janus Kinase*
Kg	*Kilograms*
Kg/m²	*Kilogram per square metre*
LAMECC	Laboratório de Metabolismo Energético e Composição Corporal
LDL	*Low density lipoprotein*
LPL	*Lipoprotein lipase*
MBR	*Metabolic basal rate*
Mets	*Metabolic syndrome*
mL	*Milliliters*
mmHg	Milímetros de mercúrio
NFκB	*Nuclear factor kappa B*
p	Nível de significância estatística
SFA	*Saturated fatty acids*
SD	*Standard deviation*
SE	*Standard error*

TC	*Total cholesterol*
TG	*Triglycerides*
TLR	*Toll-like receptors*
TNF-α	*Tumor necrosis factor alfa*
TRL	*Triglyceride-rich lipoproteins*
UFV	Universidade Federal de Viçosa
TCV	*Total caloric value*
VLDL	*Very low density lipoproteins*
WP	*Waistcircumference*
WHR	*Waist/hip ratio*
WHO	*World Health Organization*

RESUMO

COELHO, RCLA, M.Sc., **Resposta pós-prandial de marcadores metabólicos e inflamatórios ao consumo de gordura saturada e suco de laranja em mulheres eutróficas e com excesso de peso.** Universidade Federal de Viçosa, julho de 2013. Orientadora: Josefina Bressan. Co-orientadora: Helen Hermana Miranda Hermsdorff.

O presente trabalho teve como objetivo avaliar a resposta de marcadores metabólicos e inflamatórios no período pós-prandial após o consumo de uma refeição rica em gordura saturada, quando acompanhada de água ou suco de laranja em mulheres eutróficas e com excesso de peso. Trata-se de um estudo randomizado, controlado, cruzado, realizado no Laboratório de Metabolismo Energético e Composição Corporal do Departamento de Nutrição e Saúde da Universidade Federal de Viçosa, previamente aprovado pelo Comitê de Ética e Pesquisa com Seres Humanos da Universidade Federal de Viçosa (Of. Ref. nº 184/2011). Neste estudo, 36 mulheres aparentemente saudáveis (21 normopeso, 15 com excesso de peso) consumiram duas unidades de *muffin* de queijo e bacon ricas em ácidos graxos saturados (1010 kcal, 37,3% do conteúdo calórico em gordura saturada) acompanhados de 500 mL de água ou 500 mL de suco laranja, com um período de sete a 15 dias de *washout* entre as dietas. A avaliação antropométrica incluiu medidas de peso, altura e perímetros da cintura e do quadril. Para verificar o percentual de gordura corporal, utilizou-se a bioimpedância elétrica tetrapolar. Aferiu-se a pressão arterial utilizando o método auscultatório indireto com esfignomamômetro de mercúrio devidamente calibrado. Foram coletadas amostras de sangue em jejum, bem como duas, três e cinco horas após o consumo das refeições-testes. As concentrações de glicemia, colesterol total, colesterol HDL e LDL, triglicerídeos, ácido úrico, proteína C reativa e complemento C3 foram determinadas em todos os tempos mediante protocolo padronizado (em duplicata). Os principais efeitos analisados incluem: a bebida (água x suco de laranja), o tempo (jejum, duas, três e cinco horas após o consumo das dietas) e o grupo (eutróficas x excesso de peso). Os resultados apontam que houve alterações metabólicas e inflamatórias no período analisado, e essas alterações foram diferentes conforme o estado nutricional (eutrofia x excesso de peso) e a refeição teste consumida. As voluntárias com excesso de peso apresentaram maior perímetro da cintura e do quadril, relação cintura/quadril, percentual de gordura, pressão arterial sistólica, glicemia e uricemia no jejum, como esperado. Após o consumo das dietas, as voluntárias apresentaram maior glicemia quando consumiram a dieta acompanhada de suco de laranja. Não houve variações significativas no colesterol total e frações ao longo do tempo nem entre as dietas consumidas. Na refeição acompanhada de água, apenas as voluntárias obesas apresentaram elevação significante dos triglicerídeos na terceira hora após a ingestão ($p=0,01$). Quando a refeição foi acompanhada de suco de laranja, ambos os grupos apresentaram aumento significante das concentrações dos triglicerídeos na terceira hora

em relação ao jejum. Além disso, nas voluntárias obesas, esse aumento permaneceu significante na quinta hora pós-prandial (p=0,03). A resposta inflamatória foi caracterizada por maiores concentrações de complemento C3 nas voluntárias eutróficas após o consumo de suco de laranja (p=0,05). Em relação à proteína C reativa, não houve variação no tempo. Contudo, observou-se diferença na resposta das voluntárias obesas quando consumiram a dieta acompanhada de água ou suco de laranja. Conclui-se que mulheres com excesso de peso apresentaram uma lipemia no período pós-prandial diferente de mulheres eutróficas e que a adição de suco de laranja a uma dieta rica em gordura saturada contribuiu para a elevação dos triglicerídeos em eutróficas e o prolongamento da elevação dos triglicerídeos nas obesas.

ABSTRACT

COELHO, RCLA, M.Sc., Universidade Federal de Viçosa, July, 2013. **Postprandial response of metabolic and inflammatory markers to the consumption of saturated fat and orange juice in normal weight and overweight women.** Advisor: Josefina Bressan. Co-advisor: Helen Hermana Miranda Hermsdorff

This study aimed to evaluate the response of metabolic and inflammatory markers in the postprandial period after consumption of a high saturated fat meal, when accompanied by water or orange juice in normal weight and overweight women. This is a randomized, controlled, crossover, performed at the Laboratory of Energy Metabolism

and Body Composition in the Department of Nutrition and Health, Federal University of Viçosa, approved by the Ethics and Human Research of the Federal University of Viçosa (Of. Ref. No. 184/2011). In this study, 36 apparently healthy women (21 normal weight, 15 overweight / obese) consumed two units of cheese and bacon muffin, rich in saturated fatty acids (1010 kcal, 78% of the caloric content in fat) followed by 500 ml of water or 500 ml of orange juice, with a period of seven to 15 days washout between meal tests. Anthropometric measures included weight, height as well as waist and hip circumference. To check the percentage of body fat, we used the tetrapolar bioelectrical impedance. Blood pressure was measured using the auscultatory method with mercury sphygmomanometry properly calibrated. Blood samples were collected at fasting and two, three and five hours after consumption of meals-tests. The concentrations of glucose, total cholesterol, HDL and LDL cholesterol, triglycerides, uric acid, C-reactive protein and complement C3 were determined at all times by a standardized protocol (in duplicate). The main effects analyzed include: a drink (Water x Orange Juice), time (fasting, two, three and five hours after consumption of diets) and group (normal-weight x overweight/obese). The results show that there was metabolic and inflammatory changes in the postprandial period, and these changes were different depending on the nutritional status (lean x overweight / obesity) and meal test consumed. The overweight volunteers showed greater waist and hip circumferences, waist/hip ratio, body fat percentage, systolic blood pressure, fasting blood glucose and uricemia, as expected. After consumption of the meals, lean subjects had more glucose increment when consumed diet accompanied by orange juice. There were no significant changes in total cholesterol and fractions over time or between diets consumed. In meal followed by water only obese volunteers had a significant increase in triglycerides in the third hour after ingestion. When the meal was accompanied by orange juice, both groups showed significantly higher concentrations of triglycerides at the third time in relation to fasting. Furthermore, in obese volunteers, increase remained significant at fifth hour postprandial ($p=0.030$). The inflammatory response was characterized by higher concentrations of complement C3 in normal-weight volunteers after consumption of orange juice ($p=0.05$). Regarding, C-reactive protein did not change in the time. However, there were differences in the response of obese volunteers to consumed meal when accompanied by water or orange juice. In conclusion, women with overweight/obesity have a higher lipemia in the postprandial period than normal-weight women, and the addition of orange juice to a diet rich in saturated fat contributed to the

elevation of triglycerides in lean and longer rise triglycerides in the overweight/obese women.

INTRODUÇÃO GERAL

A lipemia pós-prandial refere-se às mudanças dinâmicas nos lipídeos e lipoproteínas séricos que ocorrem após uma refeição (KOLOVOU *et al.,* 2011). Essas mudanças são refletidas, principalmente, nas concentrações dos triglicerídeos (KOLOVOU *et al*., 2013). Dados recentes indicam que as concentrações dos triglicerídeos no período pós-prandial predizem mais fortemente o risco de doenças cardiovascular do que as concentrações de jejum. Acredita-se que a lipemia pós-prandial é mais comum e acentuada no paciente obeso (SARWAR *et al.,* 2010).

A hiperlipemia pós-prandial é um possível marcador precoce de anormalidades metabólicas e disfunção vascular não observado em jejum. Recentes resultados mostram que as alterações que ocorrem após uma única sobrecarga lipídica se relacionam com aumento de marcadores inflamatórios, sendo que tais alterações estão fortemente associadas à progressão da aterosclerose e aos eventos cardiovasculares (WIERZBICKI *et al.,* 2012). Essas alterações podem revelar um estado de intolerância às gorduras que já são detectadas em indivíduos aparentemente saudáveis, antes mesmo que anormalidades em jejum sejam percebidas (KLOP *et al*., 2012).

Nesse sentido, a hiperlipemia no período pós-prandial pode ativar leucócitos e aumentar a expressão de moléculas de adesão e migração leucocitária, além da secreção de citocinas pro-inflamatórias e ativação do sistema complemento (HERMSDORFF *et al.,* 2013). De acordo com o padrão alimentar seguido na atualidade, a maioria dos indivíduos está no estado pós-prandial a maior parte do dia (KOLOVOU *et al.,* 2013) . Dessa forma, a hiperlipemia e a resposta inflamatória desencadeadas após cada refeição podem ser gatilhos para a progressão da aterosclerose (KLOP *et al*., 2012).

Uma das dificuldades no estudo da lipemia pós-prandial é que a maioria das refeições é composta por outros macronutrientes além dos lipídeos, como os carboidratos. Isso significa que o metabolismo pós-prandial, resultante da digestão e absorção de vários nutrientes, é um processo altamente complexo, envolvendo numerosas potenciais interações (LAIRON *et al.,* 2011).

Por sua vez, as frutas e hortaliças, através de seu conteúdo em compostos bioativos, parecem modular mecanismos endógenos de defesa contra a resposta inflamatória (GHANIM *et al.*, 2010; MURSU *et al.*; 2008). Nesse contexto, alimentos com capacidade de reduzir marcadores inflamatórios se tornam uma estratégia atrativa na redução do risco cardiometabólico associado à obesidade (HERMSDORFF *et al*., 2011, HERMSDORFF *et al.,* 2010), e o suco de laranja apresenta grande potencial nesse sentido. Ao mesmo tempo, o suco de laranja é uma bebida calórica e com alto teor

de carboidrato, podendo, dessa forma, promover alterações no metabolismo pós-prandial (STOOKEY *et al.,* 2012)

O homem moderno vive no estado pós-prandial a maior parte do dia. Além disso, o padrão dietético ocidental é rico em gorduras e pobre em carboidratos integrais e fibras (BRESSAN, HERMSDORFF, 2008). Estas mudanças alimentares associaram-se a efeitos em longo prazo (obesidade, diabetes, dislipidemia, hipertensão arterial sistêmica e consequentemente mais doenças cardiovasculares) e efeitos agudos, os quais podem conferir risco cardiovascular extra (PELUSO *et al.,* 2012). O estado pós-prandial caracteriza-se por excursões glicêmicas, hipertrigliceridemia, aumento de marcadores inflamatórios e estresse oxidativo - fatores potencialmente aterogênicos - (SCHWARTZ; REAVEN, 2012) e que podem ser modificados pela associação do consumo de suco de frutas (PELUSO *et al.,* 2012; GHANIM *et al.,* 2010).

Por isso, justifica-se a necessidade de estudos sobre as modificações metabólicas e inflamatórias decorrentes do período pós-prandial. Entender como indivíduos aparentemente saudáveis respondem a uma refeição comumente consumida em nosso meio é importante no desenvolvimento de estratégias de prevenção de doenças relacionadas aos hábitos alimentares.

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ARTIGO 1.

ANTI-INFLAMMATORY PROPERTIES OF ORANGE JUICE: POSSIBLE FAVORABLE MOLECULAR AND METABOLIC EFFECTS

Propriedades antiinflamatórias do suco de laranja: possíveis efeitos benéficos moleculares e metabólicos

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Resumo: O estado inflamatório de baixo grau tem sido reconhecido como o elo entre a adiposidade e o risco de doenças metabólicas crônicas. Concentrações aumentadas de marcadores inflamatórios, tais como interleucinas e fator de necrose tumoral alfa, foram encontrados em indivíduos obesos. Por sua vez, a dieta pode influenciar positiva ou negativamente o risco de doenças crônicas, possivelmente pela modulação do estado inflamatório. Nesse contexto, o consumo do suco de laranja pode desempenhar um papel na modulação das concentrações de marcadores inflamatórios, através do seu conteúdo em compostos bioativos, como os flavonoides hesperidina e naringenina. De acordo com essa revisão, o consumo de suco de laranja aparenta modular a resposta inflamatória, tanto no nível plasmático como de expressão gênica, no período pós-prandial ou no uso crônico (mais de sete dias consecutivos). Os achados sugerem que o suco de laranja pode ser uma ferramenta dietética na prevenção e tratamento de doenças crônicas, embora mais estudos sejam necessários para elucidar os mecanismos fisiológicos e moleculares envolvidos.

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ORIGINAL PAPER

Anti-inflammatory Properties of Orange Juice: Possible Favorable Molecular and Metabolic Effects

Raquel Cristina Lopes Assis Coelho · Helen Hermana M. Hermsdorff · Josefina Bressan

Abstract The low-grade inflammation has been recognized as the link between adiposity and the risk of chronic metabolic disorders. Thus, increased concentrations of inflammatory markers, such as interleukins and tumor necrosis factor alpha have been found in obese individuals. In turn, diet can positively or negatively influence on the risk of chronic metabolic diseases by modulating the inflammatory status. In this context, orange juice consumption can play a role in modulation of inflammatory markers through bioactive compounds, such as the flavonoids (hesperidin, naringenin). According to this review, orange juice appears to mediate the inflammatory response in plasma level and gene expression, and in postprandial and chronic (≥7 consecutive days) periods. The current findings suggest that orange juice could be a dietary feature for prevention and treatment of chronic diseases, although more studies are necessary to evaluate the physiological and molecular mechanisms involved.

Keywords Orange juice · Hesperidin · Naringenin · Inflammation · Gene expression · Nutrigenomic

Abbreviations

BP	Blood pressure
CVD	Cardiovascular disease
CD-14	Cluster differentiation 14
CRP	C-reactive protein
COX2	Cyclooxygenase-2
DNA	Deoxyribonucleic acid
DBP	Diastolic blood pressure
FFA	Free fatty acids
GLUT4	Glucose transporter type 4
GST	Glutathione S-transferase
HFHC	High-carbohydrate
IL1R1	IL-1 receptor 1
Iκβ	Inhibitor κβ
ICAM-1	Intercellular adhesion molecule-1
IL	Interleukin
JNK	Janus kinase
LPB	Lipopolysaccharide binding protein
LPS	Lipopolysaccharide
MMP-9	Matrix metalloproteinase-9
METs	Metabolic syndrome
MCP-1	Monocyte chemoattractant protein-1
NRF2	NF-E2-related factor 2
NO	Nitric oxide
NF-κβ	Nuclear factor kappa-B
OJ	Orange juice
PBMC	Peripheral blood mononuclear cells
PAI-1	Plasminogen activator inhibitor-1
QR	Quinone reductase
ROS	Reactive oxygen species
SOCS3	Suppresser of cytokine signaling-3
TLR	Toll-like receptors
TNFα	Tumor necrosis factor alpha

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Introduction

The low-grade chronic inflammatory status has been recognized as the link between adiposity and risk of chronic metabolic disorders such as metabolic syndrome (MetS) and cardiovascular disease (CVD) [1]. Several studies demonstrated increased expression of nuclear transcription factors such as nuclear factor kappa-B (NF-κB), interleukin (IL), and tumor necrosis factor alpha (TNFα) in obeses [2, 3]. These changes

are involved in an increased production of proinflammatory molecules and pro-atherogenic, such as C-reactive protein (CRP) and adhesion molecules. Peripheral blood mononuclear cells (PBMC) play an important role in this complex process, regulating the gene expression of proinflammatory molecules [4]. In turn, dietary patterns or specific dietary factors have reduced the risk of chronic metabolic diseases [5], modulating low grade inflammatory status [6, 7]. Fruit intake has been associated with a reduction in the concentrations of inflammation and oxidative stress markers, indicating a potential effect in the prevention of metabolic disorders and CVD [1, 8, 9]. In fact, daily consumption equal to or greater than 100 ml/day of natural juices (no sugar) was negatively associated with gene expression in PBMC of TNFα, IL-6 and IL-1 receptor 1 (IL-1R1) [1]. These results have suggested a beneficial effect of fruit and vegetables on inflammatory status.

Citrus juices, especially orange juice (OJ), have been recommended by many health professionals as a healthy source of calories, and their consumption is associated with improved lipid profile [10, 11]. Also, OJ is a rich source of vitamin C and flavonoids, bioactive compounds with a potential effect on the inflammatory response [12]. Thus, this juice has been focused very current researches, but the new findings regard to OJ consumption and inflammatory response have not still been summarized and discussed altogether.

This review aimed to briefly describe the main markers of inflammatory status, identify current scientific evidence on the effects of OJ intake on these inflammatory markers in plasma concentrations and gene expression, and discuss the bioactive components of OJ potentially involved in these effects.

Search Strategy

This study is a literature review of scientific articles containing: (i) basic research on the effects of the main bioactive components of OJ, (ii) information on markers of inflammatory state, (iii) clinical studies about the consumption of OJ and its effects on inflammatory markers, (iv) clinical studies about other effects of OJ.

This literature review was conducted in major health databases: Medline, Lilacs, PubMed and SciELO. The following keywords were used in the systematic search: "orange juice", "hesperidin," "naringenin" paired with "inflammation", "inflammatory", "gene expression", "nutrigenomic". The titles and abstracts of all studies identified by the search on electronic platforms were screened. The full texts of potentially relevant studies were read to note the inclusion criteria. Full papers were obtained from journals available on the website of the CAPES Foundation (Ministry of Health, Brazil). We excluded those studies with OJ intake combined with other juices. The period considered for inclusion of articles was from 2000 to 2012. Figure 1 details the search strategy of randomized clinical trials described in Table 1.

Inflammatory Markers

Among the molecules involved in the proinflammatory status we included: cytokines, such as TNFα, IL-1, IL-6, an acute phase protein, CRP as well as Toll-like receptors (TLR) and transcription nuclear factors, especially NFκB, an enzyme (cyclooxygenase-2—COX2), and the lipopolysaccharides (LPS). These molecules, with different functions in the inflammatory process, appear to be modulated by orange juice consumption or by its bioactive compounds [1, 12].

Tumor Necrosis Factor Alpha

TNFα is a proinflammatory cytokine that is expressed significantly in adipose tissue as well as in leukocytes, endothelial and muscle cells [13]. Elevated concentrations of TNFα in obese individuals are involved in the insulin resistance through inhibition of insulin signaling [14], with a reduction in translocation of glucose transporter type 4 (GLUT4). In addition, TNFα is involved in endothelial deregulation by stimulating the migration of monocytes and macrophages, and inducing adhesion molecules expression for activation of NFκB [15].

Interleukin 6

IL-6 is also a proinflammatory cytokine secreted by macrophages and adipocytes, these are responsible for 30 % of its secretion [16]. Several studies indicate that its concentration is strongly associated with increased adiposity such as waist circumference, visceral fat, and total body fat. IL-6 is a major inductor of acute hepatic response by stimulating the production of proteins such as CRP, fibrinogen, haptoglobin and amyloid protein A [17, 18], besides it induces the secretion of plasminogen activator inhibitor-1 (PAI-1); all of them proteins involved in inflammation [19].

Interleukin 1

The IL-1 family is composed of two subunits, IL1α and IL1β, both potent mediators of inflammation which are secreted by PBMC, such as macrophages and lymphocytes B. IL1α and IL1β have similar biological functions through the activation of IL1R1, stimulating transcription factors related to immune response, such as NFκB, janus kinase (JNK) and protein kinase regulatory P38 [20]. In obese

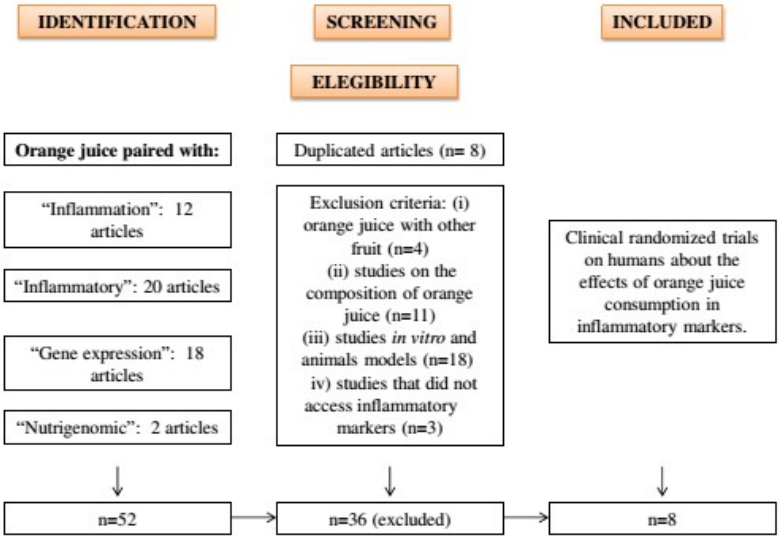

Fig. 1 Flow diagram of the review

patients the expression of genes encoding the subunits of IL-1 and IL1R1 is increased in adipose tissue and PBMC [21].

C-Reactive Protein

CRP is an acute phase protein produced in the liver, whose expression is mediated by cytokines, especially IL-1 and IL-6 [22]. Their concentrations are increased in obesity, associated with abdominal adiposity. High CRP levels are associated with increased risk of future cardiovascular events among apparently healthy individuals [23, 24]. In fact, consistent results from well-conducted prospective studies in initially healthy persons have shown a strong and independent association between the circulating CRP concentrations and cardiovascular end points, including acute myocardial infarction, stroke and progression of peripheral arterial occlusive disease [25, 26].

Nuclear Transcription Factor κB

NFκB has been studied by many researchers as the most important transcription factor involved in the inflammatory regulation genes [27]. An increase in NF–κB binding is associated with an increase in TNFα, CRP and IL-6 expression [28]. NFκB is the general name for a family of factors with five members: RELA (p65), c-Rel, RelB, NFκB1 (p50) and NFκB2 (p52). The most abundant complex and also more studied is the p65/p50. When inactive, NFκB is disabled by the action of the inhibitor κB (IκB). It links to NFκB, staying sequestered in the cytoplasm [29]. In turn, the activation of NFκB is given by extracellular stimuli including TNFα and IL-1 [30] as well as viral products, bacterial components, free fatty acids (FFA) and some nutrients [31]. The action of these agents results in the translocation of NFκB to the nucleus where it stimulates the expression of target genes. NFκB gene expression is increased in PBMC in obeses and in subjects with high visceral fat [2–4], with consequent increment in the expression of proinflammatory genes regulated by this transcription factor.

Toll-Like Receptors

TLR-2 is a specific receptor for lipopeptides and peptidoglycans from gram-positive bacteria [32], and TLR-4 is the specific receptor for LPS or endotoxin from gram-negative bacteria [33]. TLR-4 was also shown to play an important role in the pathogenesis of atherosclerosis [34], diet-induced obesity, and the related insulin resistance [35], whereas TLR-2 was shown to be involved in ischemia-reperfusion-induced myocardial injury [36]. Studies have shown that there was a significant increase in plasma concentrations of endotoxin and an increase in TLR-4 and TLR-2 expression in PBMC after the intake of a high-fat, high-carbohydrate (HFHC) meal [35, 37]. This increase could contribute to and prolong the inflammatory response that follows a meal.

Cyclooxygenase-2

Cyclooxygenase (COX) is an enzyme responsible for the prostanoids production. The three main groups of prostanoids —prostaglandins, prostacyclins, and thromboxanes—are all involved in the inflammatory response [38]. COX-1 is known to be present in most tissues. In the gastrointestinal tract, COX-1 maintains the normal lining of the stomach. This enzyme is also involved in kidney and platelet function [39]. In turn, COX-2 is primarily present at sites of inflammation. While

Table 1 Characteristics of included trials

Authors (year)	Subjects	BMI (kg/m^2)	Study design	Intervention	Intervention lenght	Orange juice	Inflammatory markers	Substrate
Sanchez-Moreno et al. (2003) [54]	n=12 M/F: 6/6 20–32 years	22.2±1.6 Healthy	Intervention	500 ml OJ/day	14 days	Freshly squeezed orange juice was obtained from orange fruits purchased in a local supermarket. 8.5 mg flavanones/ 100 mL	RCP	Plasma
Devaraj et al. (2006) [24]	n=72 M/F: 31/41 19–74 years	24±6 Healthy	Intervention Parallel	480 ml/day OJ Sterol Bev or Placebo Bev	8 weeks	Plant sterol with the targeted particle size distribution suspended in a reduced-calorie OJ beverage	RCP	Plasma
Ghanim et al. (2007) [62]	n=28 M/F: NA 20–40 years	20–25 Healthy	Post prandial Parallel	300 kcal OJ/meal	Acute	OJ obtained from a local supermarket, used portions \of the juice from 0.5- or 1-gal packages for multiple experiments	NF-kβ	RNA from PBMC
Deopurkar et al. (2010) [64]	n=48 M/F: NA 25–47 years	21.5–24.4 Healthy	Post prandial Parallel	300 kcal OJ/meal	Acute	Packages of recently produced "not from concentrate" Florida orange juice. Each package, once opened, was discarded after a single experiment	NF-kB, IL-1β, TNF-α, SOCS3, TLR-4	RNA from PBMC
Ghanim et al. (2010) [63]	n=30 M/F: NA 20–40 years	20–25 Healthy	Post prandial Parallel	300 kcal OJ + 900 kcal HFHC meal	Acute	Same as Deopurkar et al. (2010).	TLR-2, TLR-4, MMP-9	RNA from PBMC
Devaraj et al. (2011) [68]	n=144 M/F: 62/82 19–74 years	24±6 Healthy	Intervention Parallel	480 ml/day OJ Sterol Bev or Placebo Bev	8 weeks	Same as Devaraj et al. (2006)	IL-1, IL-6, IL-10, IL-8, PAI-1	Plasma
Milenkovic et al. (2011) [53]	n=24 M 50–65 years	27±0.3 Subjects ranged from normal to mildly hyperlipidemic, and two-thirds of the subjects were normotensive	Intervention Crossover	500 ml orange juice, 500 ml control drink plus hesperidin or 500 ml control drink and placebo	Three 4-week periods	Orange juice from concentrate was provided by the Florida Department of Citrus (Lake Alfred, FL, USA) and its hesperidin content was 292 mg/500 ml	NF-kB, IκB, genes that are potentially implicated in the processes of inflammation	RNA from PBMC

Table 1 (continued)

Authors (year)	Subjects	BMI (kg/m^2)	Study design	Intervention	Intervention lenght	Orange juice	Inflammatory markers	Substrate
Buscemi et al. (2012) [72]	n= 19 M/F: 10/9 18–70 years	32.1±4.9 BMI >28 + presence of 2 diagnostic criteria of the MetS	Intervention Crossover	500 mL ROJ/day (250 mL ROJ twice daily) or 500 ml placebo/ day (250 ml placebo twice daily)	two periods of 7±1 days	The orange juice was obtained from three red orange varieties stored at 220 °C in aliquots of 500 ml. Narirutin: 43 mg/l Hesperidin: 319 mg/l	PCR, IL-6, TNFα	Plasma

OJ orange juice, *HFHC* high-fat high-carbohydrate, *SOCS3* suppresser of cytokine signaling-3, *TLR-4* toll-like receptor-4, *TLR-2* toll-like receptor-2, *MMP-9* matrix metalloproteinase-9, *IkB* inhibitor of NFkB, *PBMC* peripheral blood mononuclear cell

both COX-1 and COX-2 convert arachidonic acid to prostaglandin, resulting in pain and inflammation, their other functions make undesirable the inhibition of COX-1 [38] and the activation of COX-2, respectively [39].

Lipopolysaccharides

LPS are large molecules consisting of a lipid and a polysaccharide joined by a covalent bond; they are found in the external membrane of Gram-negative bacteria, act as endotoxins and elicit strong immune responses in animals [40]. The LPS-induced cell activation involves the participation of several proteins: lipopolysaccharide binding protein (LPB), a protein produced in the liver, the co-receptor cluster differentiation 14 (CD-14) [41] and TLR-4 [34, 35]. High-fat, high-carbohydrate (HFHC) meals are known to induce oxidative and inflammatory stress, an increase in plasma endotoxin concentrations, and an increase in the expression of LPB [34]. Although the activation of TLR-4 can activate nuclear translocation of several transcription factors, the main cascades induce the translocation of transcription factor NFkB and subsequent expression of its target genes [42].

Bioactive Compounds in Orange Juice

Flavanones

Flavonoids are important micronutrients present in the human diet, and in the past decade an increasing number of studies regarding the positive effects on human health of these natural compounds have been reported [43, 44]. Good sources of these compounds are citrus fruit juices. Orange juice contains mainly the flavanones hesperidin (hesperetin-7-rutinoside) and narirutin (naringenin-7-rutinoside) [45]. The hesperidin and narirutin ingested with the food are metabolized by human intestinal bacterial microflora to the aglycones hesperetin and naringenin [46], respectively.

Hesperidin

Hesperidin represents about 90 % of OJ flavanones, the remainder being understood by narirutin [46]. Flavanones are among the flavonoids compounds that have the highest bioavailability. Recently, cardioprotective and anti-inflammatory effects were assigned to various flavonoids [44, 45]. The anti-inflammatory and antioxidant effects of hesperidin occur by several mechanisms, including: (i) reduction of expression of intercellular adhesion molecule-1 (ICAM-1), contributing to inhibition of the adhesion of monocytes to endothelial cells

[47]; (ii) suppression of gene expression of several proinflammatory cytokines (TNF-α, IL-1β, IL-6) [48, 49]; (iii) increased expression of NF-E2-related factor 2 (NRF2), a key regulator of the expression of enzymes such as glutathione S-transferase (GST) and quinone reductase (QR) with potent antioxidant activity [50]. NRF2 is currently known for its important role in protecting deoxyribonucleic acid (DNA) against oxidative stress [50]; (iv) increased production of nitric oxide (NO) synthase and consequent improvement in endothelial function [51]; (v) inhibition of janus kinase (JNK), a group of proteins activated by various types of environmental stress and cytokines, and consequent decreased production of metalloproteinases [52]; (vi) inhibits the generation of reactive oxygen species (ROS) [52].

Some of hesperidin effects on expression of inflammatory genes described *in vitro* were also observed in humans [53]. However, the effect of the consumption of OJ (500 ml) seemed to be larger than that obtained by hesperidin intake in capsules form. Nevertheless, 53 % of genes modulated by OJ also constitute potential molecular targets of hesperidin, suggesting that hesperidin may play an important role in the genomic effects of this beverage.

Moreover, changes in leukocyte gene expression mediated by hesperidin were observed in fasted subjects while hesperidin metabolites were no longer present in blood circulation. This persistent effect at the genomic level could be regarded as an adaptation of leukocytes to continuous high-flavanone exposure [53].

In addition, other compounds from OJ, such as vitamin C, could contribute to the anti-inflammatory effects. In fact, vitamin C concentrations after intervention with OJ have been inversely related to the concentrations of 8-epi-prostaglandin-F2α, an oxidative stress marker [54].

Naringenin

Naringenin is a flavonoid derived from plant foods, present in OJ, but in lower concentrations than hesperidin [46]. Various anti-inflammatory properties are attributed to this compound such as: (i) inhibition of inflammatory response induced by LPS, through inhibition of the phosphorylation of serines 67 and 73 in transcription factor proto-oncogene-encoded AP-1 in macrophages [55]; (ii) inhibition of proinflammatory enzyme COX-2 by inhibiting the inflammatory response induced by LPS [56]; (iii) inhibition of cytokines secretion by CD4 [57]; (iv) inhibition of NFκB binding and consequent reduction in the expression of cytokines stimulated by this transcription factor [58]. DNA damage caused by ifosfamide, an antineoplasic compound, in various types of mouse cells has been inhibited with the administration of naringenin [59].

However, further studies are not available on the effects of isolated naringenin in humans.

Bioavailability

The biological activity of flavanones is modulated by variables such as absorption rate, intermediate metabolites and tissue distribution [60]. Hesperidin and naringenin absorption occurs in distal intestine. In humans, hesperidin metabolites were identified in plasma as glucuronides of hesperidin [61]. Association with a meal can modify the accessibility of these compounds, leading to a lower absorption of flavanones. Twenty-four hours after the ingestion of OJ, urinary elimination was almost complete (98 %) and metabolites were not found in plasma [62]. However, the sources of flavonones are richer than most of other sources of flavonoids, which mean flavonones may represent an important part of the pool of polyphenols in plasma [61].

Effects of Orange Juice on Inflammatory Status: Studies in Humans

We included eight randomized controlled trials that assessed the effects of OJ consumption on inflammatory markers in individuals without established cardiovascular or metabolic disease. These studies measured concentrations of inflammatory markers and/or expression of genes related to inflammatory response after consumption of OJ, in postprandial period or after an intervention with consumption for at least seven days. The majority of trials were carried out in the United States from 2003 to 2011 (Table 1). Main inflammatory markers analyzed were: NF-κB, CRP, IL-1, IL-6, TNFα and TLR. One study examined the expression of genes encoding proteins involved in adhesion, chemotaxis and infiltration in PBMC [53].

Postprandial Studies

Acute effects of OJ consumption on inflammatory markers were evaluated in postprandial studies. In the study by Ghanim et al. (2007) [62], four groups (10 subjects each) received a drink of 300 kcal in the form of glucose (75 g), fructose (75 g), OJ or water sweetened with saccharin (control group). Consumption of OJ did not induce a postprandial inflammatory response (as NF-kB activity), compared to ingestion of 75 g of glucose. The increased activity of NF-kB in PBMC was associated with higher expression of TNFα and metalloproteinases. Although neither group had a significant change in concentrations of CRP over the 3 h postprandial, there was a fall 1 h after ingestion of OJ.

HFHC meals trigger postprandial inflammatory response and endotoxemia. In fact, after a HFHC meal, there was a significant increase in plasma concentrations of endotoxin, associated with an increased expression of receptors TLR-2 and TLR-4 in PBMC [37]. This increase may contribute to prolong the inflammatory response triggered after such a

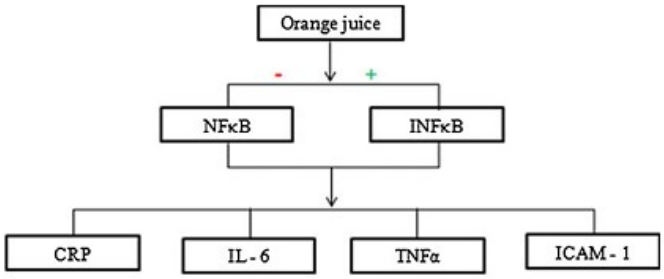

Fig. 2 Potential mechanisms for anti-inflammatory effects of orange juice intake. *NF-kB* nuclear factor kappa-β; *Iκβ* inhibitor κβ; *CRP* C-reative protein; *IL-6* interleukin 6; *TNFα* Tumor necrosis factor alpha; *ICAM-1* intercellular adhesion molecule-1

meal. Interestingly, Ghanim et al. [63] observed that OJ neutralized proinflammatory effects of a HFHC meal in healthy subjects, by a lower expression of TLR. These findings were replicated by Deopurkar et al. [64]. In this study, indexes of inflammation including NFκB binding and the expression of TNFα and IL-1 in PBMC increased significantly after glucose and cream intake, but TLR-4 expression and plasma LPS concentrations increased only after cream intake. The intake of OJ or water did not induce any change in any of the indexes measured. These results suggest that nutritional choices, such as fruit juices, could minimize postprandial oxidative stress and inflammatory response.

Besides, in healthy, middle-aged, moderately overweight men, OJ increased endothelium-dependent microvascular reactivity postprandially [65]. Since endothelial function is one of the parameters altered in low grade inflammatory status, improved endothelial reactivity could be associated with lower progression of proinflammatory process, as suggested by other authors [66, 67].

Intervention Studies

The consumption of OJ has been able to alter inflammatory markers already in postprandial period. Likewise, regular consumption is also accompanied by effects on biomarkers of the inflammatory status.

Sánchez-Moreno et al. [54] found that drinking two glasses of OJ (500 ml/day) reduced the concentrations of CRP, prostaglandin E, uric acid and 8-epiPGF2α, inflammatory and oxidative stress markers. In another study [68], the consumption of OJ has failed to affect plasma inflammatory markers or PAI-1 activity in healthy volunteers, but the addition of plant sterols to OJ has resulted in a significant attenuation of plasma IL-1β and IL-6 concentrations, potent inducers of CRP synthesis. In healthy overweight men, the consumption of OJ or purified hesperidin for 4 weeks significantly decreased diastolic blood pressure (DBP) in healthy subjects [65]. In this study OJ consumption did not affect serum concentrations of inflammatory markers, but affected blood leukocyte gene expression,

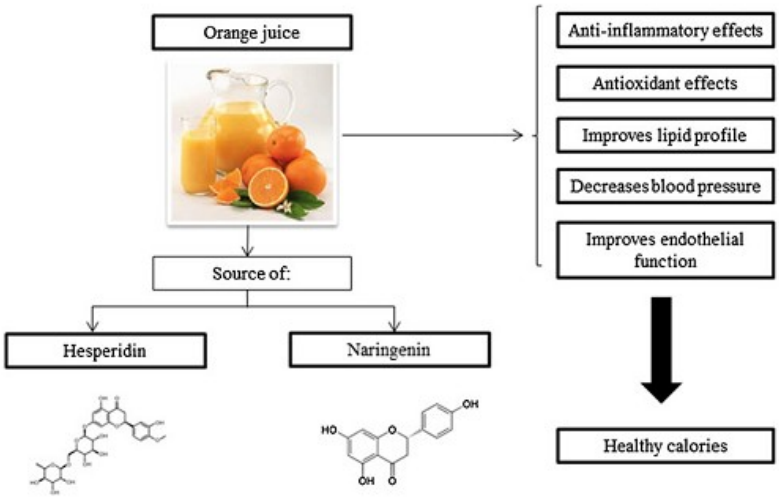

Fig. 3 Main effects of orange juice on cardiovascular risk

characterized by an anti-inflammatory profile [53]. Orange juice consumption has been associated with reduced expression of numerous proinflammatory genes, such as: (i) CCL26, expressed on monocytes and regulated by IL-4 [69]; (ii) CX3CR1, also expressed on monocytes and involved in the recruitment of these cells [70]; (iii) monocyte chemoattractant protein-1 (MCP-1), a strong chemoattractant involved in monocyte/macrophage migration and infiltration [71]. Microarray analysis has also revealed that OJ may upregulate the expression of IκB, which could, in turn, inhibit NF-kB activity, and subsequently downregulate the expression of genes encoding chemokines [53]. Recently, Buscemi et al. [72] found reduced concentrations of CRP, IL-6 and TNFα in non-diabetics subjects with increased cardiovascular risk after one week of red-OJ consumption. Endothelial function, which was measured as flow-mediated dilation, significantly improved in these subjects.

Thus, the intake of orange juice was able to neutralize the oxidative and inflammatory stress caused by the HFHC meal and the associated increases in plasma endotoxin concentrations and the expression of TLR4, the receptor for endotoxin, and TLR2. This pathway regulation may explain at least in part the anti-inflammatory effect of OJ intake (Fig. 2).

Other Benefits of Orange Juice

A growing number of epidemiologic studies have consistently shown a protective effect of polyphenol-rich foods against cardiovascular diseases [43–45]. These evidences have been supported by findings from numerous studies conducted in animal models, using normally isolated flavonoids [49–54]. OJ has been reported to exert beneficial effects on some intermediate risk factors for CVD, such as low density lipoprotein (LDL) cholesterol, blood pressure (BP), and endothelial function [73]. The consumption of citrus fruits has been associated with a lower risk of acute coronary events and stroke [74]. From clinical data, citrus juice consumption reduces oxidative DNA damage in blood cells and improves plasma concentrations of markers of oxidative stress. In addition, the consumption of citrus juices improves lipid profile in even in non dyslipidemics subjects [11]. According to data from National Health and Nutrition Examination Survey, 2003–2006, OJ consumption is associated with better diet quality, improved nutrient adequacy and decreased risk for obesity. Consumers (210 mL/day) had a lower mean body mass index, total cholesterol levels and low density lipoprotein-cholesterol levels. Finally, compared to non-consumers (50 mL/day) of 100 % OJ, consumers were 21 % less likely to be obese and male consumers were 36 % less likely to have metabolic syndrome [75]. Figure 3 summarizes the main effects of OJ on cardiovascular risk.

Concluding Remarks

Inflammation plays a pivotal role in several chronic diseases, including cardiovascular and metabolic diseases. Dietary choices which lower inflammatory biomarkers would be an attractive strategy to reduce risk for cardiovascular diseases.

In turn, the history of biomedical interest in orange juice is recent, since its association with protective mechanisms began in the 90s. Despite so, these less than two decades have witnessed the materialization of a huge amount of literature detailing the role of OJ and its bioactive compounds in different protective pathways. In this review, we found relevant evidences of potential role of OJ in the prevention of inflammation and oxidative stress related to chronic diseases.

However, despite evidence that OJ beneficially modulates the inflammatory status, some issues still remain unclear and more studies are necessary.

In summary, we described scientific evidences regarding to favorable changes in inflammatory markers and cardiovascular risk factors after the consumption of OJ in healthy subjects. The flavanones hesperidin and naringenin appears be involved in observed effects. The findings presented in this review suggest that moderate consumption of OJ should be encouraged to help individuals meet the daily recommendation for fruit intake and as a component of a healthy diet.

Conflict of Interest The authors declare that they have no conflict of interest.

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ARTIGO 2.

ORANGE JUICE PROLONGED POSTPRANDIAL LIPEMIA IN APPARENTLY HEALTHY OVERWEIGHT WOMEN

Lipemia pós-prandial prolongada com suco de laranja em mulheres aparentemente saudáveis com excesso de peso

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A ser submetido à Lipids

Fator de Impacto (2012): 2.557

Classificação Qualis Nutrição: A2

Resumo:

Introdução: A lipemia pós-prandial ganhou interesse porque aparenta ser um preditor independente para o risco de aterosclerose mais forte do que as concentrações de triglicerídeos de jejum. Nosso objetivo foi investigar a resposta metabólica e inflamatória no período pós-prandial após o consumo de uma refeição rica em gordura saturada acompanhada de duas bebidas diferentes em mulheres aparentemente saudáveis de peso normal e sobrepeso/obesidade.

Metodologia: Nesse estudo controlado cruzado, trinta e seis mulheres aparentemente saudáveis de peso normal (n=21, IMC 22±1,8 kg/m^2) e com sobrepeso/obesidade (n=15, IMC=31±3,7 kg/m^2) ingeriram uma refeição rica em gordura saturada (78% das calorias provenientes de gorduras) acompanhada de 500 mL de água ou suco de laranja. Amostras de sangue foram coletadas após jejum de 12h e 2, 3 e 5 horas após o consumo das refeições. Foram medidas as concentrações plasmáticas no jejum e no pós-prandial de glicose, colesterol total e frações, ácido úrico e de marcadores inflamatórios (proteína C reativa e complemento C3). Os principais efeitos e interações foram analisados: tempo, grupos (peso normal x sobrepeso/obesidade) e bebida (água x suco de laranja).

Resultados: A resposta glicêmica foi maior na dieta com suco de laranja (p=0,030) em todas as participantes. Houve aumento dos triglicerídeos em relação ao jejum após 3 horas do consumo da dieta com suco de laranja nas voluntárias eutróficas (p=0,010), com retorno para as concentrações basais na quinta hora. Os triglicerídeos aumentaram na terceira hora com ambas as dietas nas voluntárias com sobrepeso/obesidade e

permaneceram elevados na quinta hora apenas com a dieta associada a suco de laranja (p=0,030). A proteína C reativa e complemento C3 não modificaram suas concentrações ao longo do período pós-prandial, mas foram diferentes entre as dietas (p=0,010 e p=0,040, respectivamente).
Conclusão: As mudanças metabólicas e inflamatórias ocorridas no período pós-prandial em resposta a uma refeição rica em gordura foram condicionadas pelo estado nutricional (eutrofia x sobrepeso/obesidade) e bebida consumida (água x suco de laranja).

Palavras-chave: Período pós-prandial, gordura saturada, obesidade, suco de laranja

ABSTRACT

Introduction: Postprandial lipemia has gained interest because it appears to be a stronger independent predictor of the risk for atherosclerosis, compared to fasting triglycerides (TG). We investigated the postprandial metabolic and inflammatory response to a high saturated fat meal (HFM) and two different beverages in apparently healthy normal-weight and overweight/obese women.

Methods: In this crossover study, thirty-six apparently healthy normal-weight (n=21, BMI 22±1.8 kg/m^2) and overweight/obese (n=15, BMI 31±3.7 kg/m^2) women ingested two HFM (37% of energy as saturated fat), accompanying of 500 ml of water (HFM-W) or 500 ml of orange juice (HFM-OJ). Blood samples were collected at baseline (12-h fasting), 2, 3, and 5 hours postprandial. Fasting and postprandial glucose, total cholesterol, HDL-c, LDL-c, TG, uric acid and inflammatory markers (C reactive protein, complement C3) were assessed. The main effects and the interactions between them were analyzed: time, groups (normal weight versus overweight/obese) and meals (HFM-W vs. HFM-OJ).

Results: Glycemic response was higher in HFM-OJ (p=0.030) in all participant)TG raised at 3-h only with HFM-OJ in normal-weight women (p=0.010) and returned to normal levels at 5h. TG increased at 3h with HFM-W (p=0.010) and HFM-OJ (p=0.020), and remained high at 5h (p=0.03) only in HFM-OJ in overweight women. C3 and CRP did not change throughout time, but were different between meals (p=0.010 and p=0.040, respectively).

Conclusion: Metabolic and inflammatory changes in response to intake of a HFM, in apparently healthy women, was conditioned the nutritional status (normal-weight vs. overweight/obese) and consumed beverage (water vs. orange juice).

Keywords: Postprandial period, saturated fatty acids, obesity, orange juice

INTRODUCTION

Postprandial lipemia (PPL) refers to the dynamic changes in serum lipids and lipoproteins that occur after a fat load or a meal. These changes are reflected mainly in changes in plasma triglycerides (TG) (1). Plasma TG is known to be a surrogate for

TG-rich lipoproteins (TRL) and is present in chylomicrons (CM), very low density lipoproteins (VLDL) and their remnants [2]. TRL and their remnants are significantly increased in the postprandial period, being known as risk predictor of coronary heart disease (CHD) [1-3], independent of the total cholesterol, LDL-c or HDL-c concentrations. In this sense, PPL has gained interest, since recent reports have demonstrated that no fasting TG are possibly even stronger independent predictors of cardiovascular disease (CVD) than fasting TG [4,5].

In turn, Western dietary pattern, characterized by high energy density diet and refined foods, may lead to the development of a positive energy balance, weight gain, obesity, and eventually to be a key promoter of low-grade systemic inflammation [6-9] and metabolic syndrome abnormalities. People in the Western world are in postprandial state for most of the day [10]. Consequently, repeated dietary acute stressors induced by high fat meal (HFM) could trigger a large increase in most of the risk factors for CVD associated with obesity, such as cholesterol, TG and glucose [11,12].

In fact,

PPL is evident after a fat meal containing >30 g fat and the rise in plasma TG is dose dependent up to about 80 g [13]. Since the average content of Western style meals is 20–40 g fat and 3-4 meals/day are typically consumed, it can be concluded that postprandial lipemia is likely to be present for 18 h/day in the Western population [14]. The most pronounced lipemia is caused by a meal containing saturated fatty acids (SFA) [4, 8]. Postprandially, when TG and glucose rise, neutrophil counts increase with concomitant production of pro-inflammatory cytokines, oxidative stress and activation of complement system [15]. Furthermore, TG and glucose are able to induce leukocyte activation, as has been shown *in vitro* and *ex vivo* in hypertriglyceridemic patients [16,17].

Moreover, fruit intake has been associated with an improvement in lipid profile and reduction in inflammatory markers concentrations [18,19]. We have recently reviewed anti-inflammatory properties of orange juice [20], which appears to mediate the inflammatory and metabolic response in plasma level and gene expression, in postprandial and chronic (≥7 consecutive days) periods [20].

Our aim was to investigate the postprandial metabolic and inflammatory response following a high fat meal (HFM) and two different beverages 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. 74 women were recruited. 22 did not meet the inclusion criteria and 7 were not interested in participating. 45 women started the study. 6 did not completed claiming lack of time and 3 had problems in obtaining venous access for blood collection.

Participants to the study were normal-weight and overweight/obese women.

Participants were apparently healthy with no recent acute or chronic inflammatory disease, not using anti-inflammatory and immunosuppressive drugs and steroids, non-smokers and they could not be pregnant or nursing were included in the study. The decision to choose healthy subjects was made in order to determine whether a single meal would have a pro or anti-inflammatory effect on the general population. Subjects were excluded if they had any past or present cardiovascular disease, diagnosed diabetes or inflammatory condition, or were taking medications known to affect inflammation.

Approval for the study was obtained from the Ethics Committee for Human Research of Federal University of Viçosa (Of. Ref. Nº 184/2011) and all procedures involving human subjects complied with the Declaration of Helsinki as revised in 2000.

Study design

The dietary intervention followed a randomized crossover design, with at least a 07-day washout period between meal test days **(Figure 1).** For two days prior to each test-day, the subjects followed a low antioxidant diet (washout) by avoiding all olive and fish oils, fresh fruits and vegetables, tea, coffee, fruit juices and wine.

Subjects were randomly assigned to either: the high-fat meal plus 500 ml water (HFM-W) or the high-fat meal plus 500 ml orange juice (HFM-OJ).

On the test day, after an overnight fast, anthropometric measures were taken and venous blood samples were collected before the test meal (fasting state) as well as 2, 3 and 5 hours after meal consumption (2h, 3h and 5h). Subjects remained in the laboratory and were not allowed to consume any additional foods or beverages except water (150 mL) in the postprandial period.

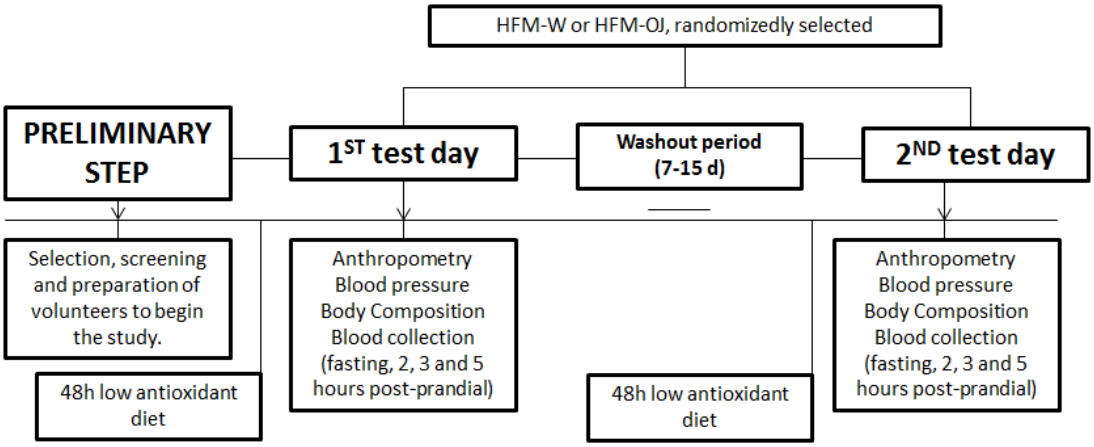

Figure 1. Study design.
HFM-W: High-fat meal plus water; HFM-OJ: high-fat meal plus orange juice.

Meals composition

The chosen meals represented a very popular meal habitually consumed by the general western population and consisted of muffins with bacon and cheese (2 units, 90g each) with 500 mL of water (HFM-W) or orange juice (HFM-OJ) **(Table 1, Supplementary Figure 1).** The composition of the meals used in the study was close to that of the meals eaten at home or in fast-food restaurants, providing 1010 kcal, with 78% of energy as fat (37% as saturated fat), 16% as carbohydrates and 6% as protein.

Concentrated no sugar added orange juice was provided by Fast Fruit®, in 1L package. 500 mL provided 215 kcal (50g carbohydrates). The juice package was opened at the time of the consumption, and if not all the juice was used, it was discarded.

Table 1. High-fat meal and beverages.

	HFM-W	HFM-OJ
Energy (kcal)	1010	1225
Protein (g)	14,5	14,5
Carbohydrates (g)	40,6	91
Fat (g)	87,6	87,6
SFA (g)	42,8	42,8
Vitamin C (mg)	0,4	150

Anthropometric assessment

Height and weight were measured with the subjects wearing no shoes and in light clothes. The subjects were weighed on electronic scales (Tanita®, precision of 100 grams).

Hip and waist perimeters were measured twice with a tape measure by the same investigator in order to avoid interpersonal differences. If the two measurements differed by more than 0.5 cm, a third measurement was taken. If two measurements were similar – the mean was calculated, if not the mean was calculated with the 3rd measurement and with the one closest to it. Waist/hip ratio was calculated.

Body mass index was calculated using the equation: BMI=weight (kg)/ height^2 (m). Percent body fat was estimated by bioelectrical impedance analysis (Biodynamics 310e, Chicago, USA).

Blood pressure was measured in the seated position using a standard mercury sphygmomanometer.

Metabolic and inflammatory markers assessment

Blood was collected in EDTA-tubes and centrifuged immediately at 1300 x *g* at 5ºC for 15 min, and then plasma was separated and stored at –80ºC. Analyzes were performed in the semi-automatic analyzer BS200 (Bioclin, Belo Horizonte, Brasil). Plasma concentrations of TG, total cholesterol (TC), HDL-c, LDL-c, uric acid and glucose were measured using colorimetric enzimatic assays (Bioclin, Belo Horizonte, Brasil). Plasma high sensitive C reactive protein (hs-CRP) and complement C3 were measured using commercially available kits (Bioclin, Belo Horizonte, Brasil), using immunoturbidimetry and turbidimetric methods, respectively.

STATISTICAL ANALYSIS

The incremental area under the curve ($_{pi}$AUC) was calculated using GraphPad Prism (Version 5; GraphPad software Inc., PAIS). The statistical analyses were performed by using the procedures of the SAS statistical package (Version 9.2; SAS Institute Inc, Cary, NC, USA). The variable distribution was evaluated by Shapiro-Wilk tests. The rejection level of significance used was 5%. Results were presented as mean ± standard error of the mean (SEM).

Age, BMI, anthropometric, body composition and plasma baseline metabolic and inflammatory biomarkers were compared between groups using *t* test or Mann-Whitney test, as appropriate. Two-way repeated-measures ANOVA were applied to test the differences between groups (normal-weight x overweight/obese) throughout the test day for the $_{pi}$AUC of postprandial variables with meal tests (HFM-W x HFM-OJ) and time (baseline, 2, 3 and 5 h postprandial) as repeated factors. Post-hoc testing was performed using Tukey-Kramer test.

A mixed model using the three-way repeated-measures ANOVA were 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 Tukey-Kramer test.

Power analysis of the analyses was also calculated by using the analyst procedures of SAS statistical package. It indicated that a sample of 15 per group would permit detection of a treatment effect that accounted for 5% of the within-subject variance in TG and glucose with more than 99% of power at the 5% level of probability.

RESULTS

Baseline

The study was completed by 36 women, who served as controls of themselves, with a mean age of 24±4 years and a mean BMI of 22.01±1.82 kg/m^2 for normal-weight and 31±8 years and 31.19±3.71 kg/m^2 for overweight/ obese volunteers. BMI, body fat percentage, waist and hip circumferences, waist/hip ratio, systolic blood pressure (SBP)**,** glucose and uric acid were greater in overweight/obese, compared to lean participants **(Table 2).**

Table 2. Baseline characteristics of the participants.

	Normal-weight women	Overweight/Obese women	P value
Participants (n)	21	15	
Age (y)	24±4	31±8	0.022
Weight (kg)	58±5	81.4±13	<.001
Height (m)	1.62±0.05	1.61±0.06	0.579

BMI (kg/m^2)	22±1.8	31.1±3.7	<.001
Body fat (%)	25.8±3.2	37±3.2	<.001
Lean mass (kg)	43.2±4.7	50.9±6.6	0.001
MBR (kcal)	1300.1±98	1550±201.6	0.007
Waist perimeter (cm)	72.5±4.9	94.8±10.3	<.001
Hip perimeter (cm)	92.8±6.7	105.4±6.6	<.001
Waist/hip ratio	0.77±0.05	0.89±0.05	<.001
SBP (mmHg)	103.3±7.2	110.6±8.8	0.024
DBP (mmHg)	64.8±6.7	69.4±8.7	0.103
Glucose (mg/dL)	88.2±6.5	97.9±7	0.004
TC (mg/dL)	168.5±31.6	168.21±26.4	0.793
HDL-c (mg/dL)	67.2±17.2	50.8±7.1	0.005
LDL-c (mg/dl	81.4±24	87±16.5	0.672
TG (mg/dL)	96.8±32.8	136.3±65.8	0.176
Uric acid (mg/dL)	3.7±0.7	4.4±0.6	0.004
hs-CRP (mg/L)	3.7±5.6	8.2±11.9	0.207
C3 (mg/dL)	137.5±29.3	142.9±25.9	0.436

Values are means ± SD. BMI: body mass index; MBR: metabolic basal rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; TG: triglycerides; hs-CRP: high-sensitivity C reactive protein. P Value column refers to a comparison between groups using t test or Mann-Whitney.

Metabolic and inflammatory posptrandial responses

The main effects that were analyzed included: meal test (HFM-W x HFM-OJ), the time (before and 2, 3 and 5 hours after meal consumption) and nutritional state of the participants (normal-weight x overweight/obese).

Regarding the metabolic response, no glucose peak was observed over time. The difference between groups that already existed at baseline remained throughout the analyzed postprandial period. Plasma glucose piAUC was significantly higher after HFM-OJ ($p = 0.030$).

Total cholesterol did not vary over time or even between diets. In relation to HDL-c, the difference between groups in fasting ($p<0.010$) remained throughout the postprandial period. There were no significant variations in this variable over time. LDL-c also remained stable at all hours postprandial and showed no significant variations between groups.

After HFM-W consumption, TG tended to increase in lean volunteers at the third hour postprandial relative to fasting ($p = 0.070$). However, this increase at the third hour was significant when they consumed HFM-OJ ($p = 0.010$), with return to baseline at 5h ($p = 0.99$).

Overweight/obese increased TG in relation to fasting at third postprandial hour even after the HFM-W (p = 0.010) and the HFM-OJ (p = 0.020). Furthermore, the increment in TG compared to fasting remained at 5h after consumption of HFM-OJ in overweight/obese volunteers (p = 0.030) **(Figure 3).**

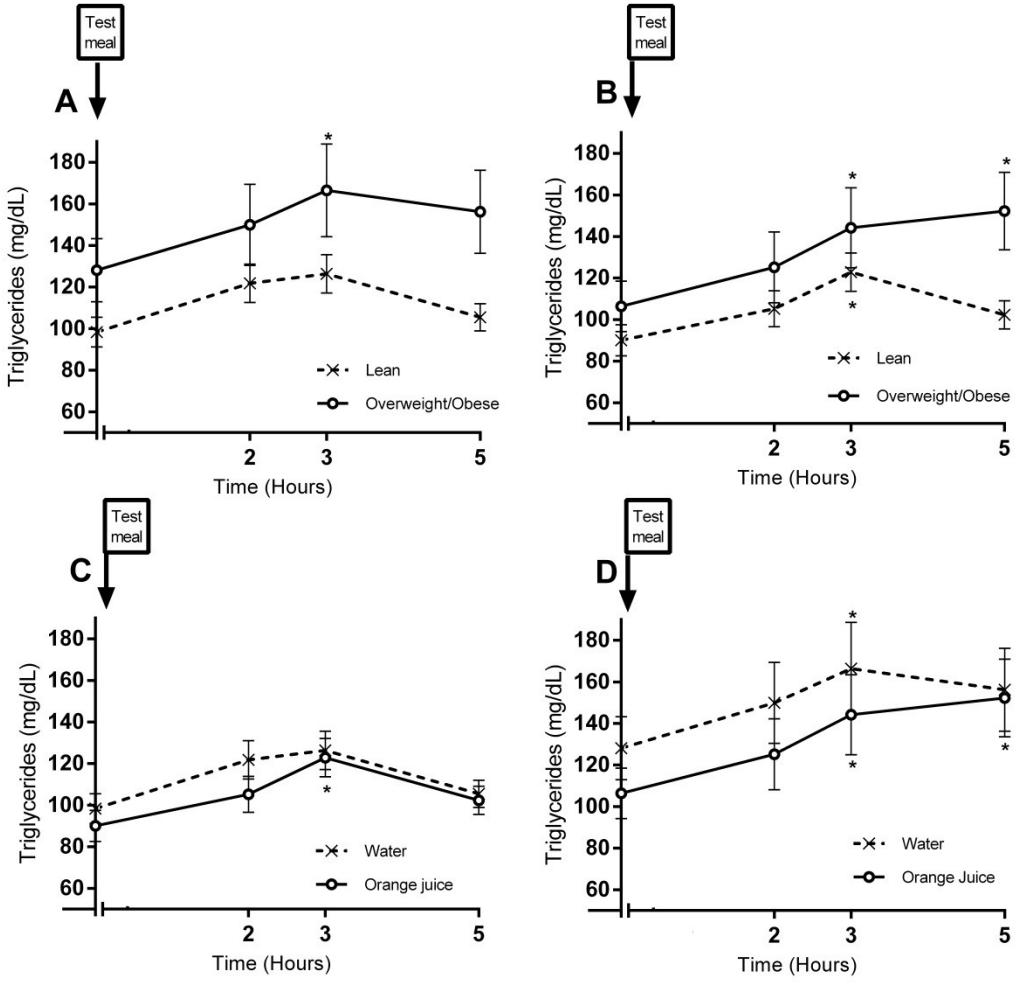

Figure 2. Line plots showing the changes (as mean ± standard errors) in plasma triglycerides after HFM-W **(A) and** HFM-OJ **(B)**; normal weight in HFM-W and HFM-OJ **(C)** and overweight in HFM-W and HFM-OJ **(D).** Baseline levels are presented in **Table 2**. Mixed model using three way Repeated Measure ANOVA followed by Tukey-Kramer post-hoc analysis: *p<0.05 single time point versus before meal intake.

Normal-weight volunteers have not significant increase (p = 0.110) in plasma uric acid fast at 2h. Then, plasma uricemia decreased at 3h and 5h, the last significant compared to 2h (p =0.002 and p =0.050 in HFM-W and HFM-OJ, respectively). At 5h

postprandial, there was a trend towards a difference between the groups of normal weight and obese volunteers (p =0.060).

The inflammatory response following the meals was characterized by differences between groups for complement C3 in HFM-OJ, with piAUC higher in normal-weight group (p=0.010). There were also difference between diets, with a higher C3 in lean women when them consumed HFM-OJ (p=0.05) **(Figure 3).** There was no significant variation over time. In CRP, we observed an interaction diet-group, with a difference when overweight/obese women consumed HFM-W or HFM-OJ (p = 0.040). Plasma concentrations of CRP did not change after the consumption of both meals.

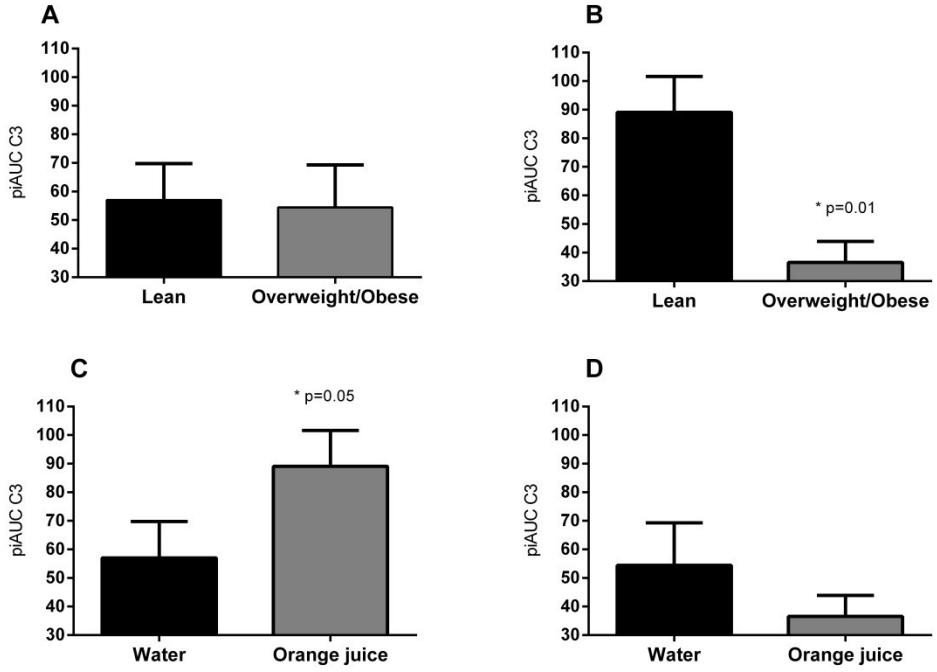

Figure 3. piAUC for complement C3 in HFM-W **(A)**; HFM-OJ **(B)**; normal- weight in HFM-W and HFM-OJ **(C)** and overweight in HFM-W and HFM-OJ **(D).** P-values from two way repeated measure ANOVA followed by Tukey-Kramer post-hoc analysis.

DISCUSSION

The first relevant outcome of this study was that there is a TG increase in relation to fasting when a HFM is consumed (87,7 g of fat) cursed with a clear PPL.

Most people in Western countries consume fat-containing meals at regular 4- to 5-h intervals and, frequently, beverages. Following the consumption of a typical

fat-containing meal (30–60 g of fat), circulating TG show a pronounced increase (i.e., postprandial lipemia) after 1 h and can remain high for 5–8 h [21]. In addition, the postprandial state is elicited by fat-containing meal intake and digestion, and is a dynamic, non-steady state condition, with rapid remodeling of lipoproteins [22]. Recent epidemiological studies have clearly evidenced the predictive relationship existing between the extent of postprandial hypertriglyceridemia and the relative risk for cardiovascular events [23-25].

This study also showed a different PPL in lean and overweight/obese women. Normal weight women had TG increased only in HFM-OJ while overweight/obese volunteers had a TG rise in both diets. In fact, the amplitude and the duration of PPL are related to the meal composition and the physiopathological condition of the subjects, including obesity [21]. PPL in overweight/obese women disclosed a lipid intolerance state that could not be detected in apparently healthy subjects in fasting.

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We also showed differences in metabolic and inflammatory response when orange juice is added to a HFM. Other clinical studies have established that postprandial lipemia is influenced by the amount and type of dietary fat present in the test meal, as well as other dietary components including fiber and carbohydrate [26,27].

Most daily meals are mixed meals made of various food stuffs that provide numerous nutrients, including lipids and digestible carbohydrates [28]. This means that postprandial metabolism resulting from the digestion and absorption of available nutrients is a highly complex process involving numerous potential interactions. This is reinforced by the fact that current diets are especially rich in fats and readily available carbohydrates and are poor in dietary fibers [29].

In addition, oOther studies have shown that the amount or nature of carbohydrate in a meal alter postprandial lipid metabolism (30,31). 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 (32,33). However, data obtained after addition of glucose (50, 100g) tothigh-fat meals have not provided reproducible findings in healthy subjects (34). In this study, addition of 50 g of carbohydrate prolonged PPL only in overweight women and enhanced lipemic response in lean volunteers.

By design, the total energy intake of the meal paired with orange juice was 215 kcal higher than the meal paired with drinking water. Orange juice addition more than doubled the carbohydrate content of the meal (40.7g for HFM-W and 91g for HFM-OJ), without contribute for fat content of these meals. Given its fructose content, orange

juice may have altered PPL increasing hepatic fat synthesis [35]. Other study has shown that addition of orange juice to a meal with 12g of fat limited fat oxidation in postprandial period [36]. These results suggest that reduced fat oxidation might mediate effects of caloric beverages on weight gain, independent of energy excess. In adults, reduced fat oxidation predicts weight gain, independent of metabolic rate [37, 38].

In both diets, TG increased occurred at three hours postprandial in obese. This result is expected according to physiology of fat digestion and absorption [1-5]. Overweight/obese women showed a prolonged TG enhance when consumed HFM-OJ, with higher TG levels at 5h postprandial. An enhanced TG rise postprandially has been reported in patients with obesity [39]. Studies show that obese individuals have prolonged elevations in postprandial lipemia and an exacerbated inflammatory response to high fat meals [40].

In obese humans, fasting plasma lipids can be normal but postprandial lipid metabolism is abnormal with an accumulation of triglyceride-rich remnant lipoproteins. In addition, their CM remnant catabolism was markedly decreased when compared with lean [41,42]. The decreased clearance of CM remnants in obese subjects may be explained by competition between CM remnants and the increased hepatic production of VLDL for clearance by low density lipoprotein receptors.

The postprandial inflammation after a HFM is established [43]. However, how another macronutrients, bioactive compounds and nutritional status influence in this inflammatory response remains controversy [44].

In summary, this study demonstrated that metabolic and inflammatory changes occurred within a few hours after the ingestion of a HFM in apparently healthy women. Overweight women showed an impaired lipid metabolism in postprandial period compared with lean women. The intake of orange juice in combination with a HFM prolonged the PPL in overweight women and was accompanied by higher inflammatory markers in normal-weight volunteers. These results reinforce the idea that postprandial metabolism is conditioned the nutritional status (normal-weight vs. overweight/obese) and consumed beverage (water vs. OJ).

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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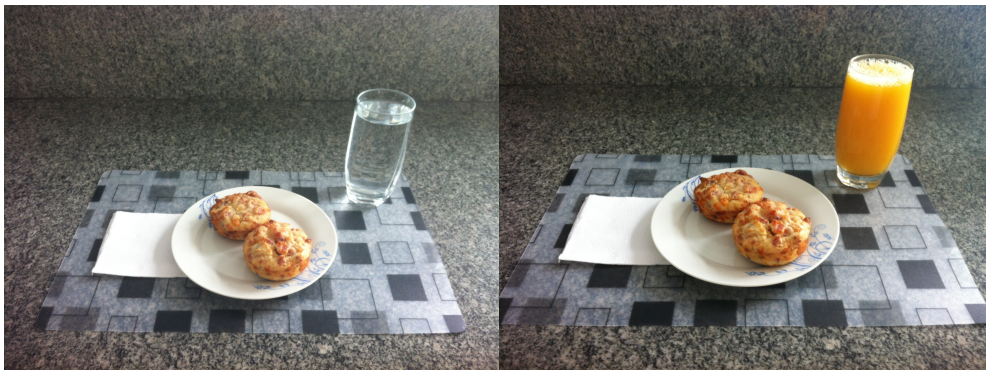
Supplementary Figure 1: High fat meal and beverages



CONCLUSÕES

O conjunto de resultados obtidos no presente trabalho nos permite concluir que:

- As voluntárias com excesso de peso apresentaram, além do maior IMC, maior percentual de gordura, perímetros da cintura e do quadril, relação cintura/quadril e pressão arterial sistólica do que as voluntárias eutróficas. As voluntárias com sobrepeso/obesidade também apresentaram maior glicemia e uricemia no jejum, além de menores concentrações de HDL.
- A ingestão de uma refeição rica em gordura desencadeou uma importante lipemia pós-prandial.
- A resposta lipêmica, caracterizada pelo aumento dos triglicerídeos, foi mais importante na terceira hora pós-prandial.
- A lipemia pós-prandial foi influenciada pelo estado nutricional: as voluntárias com sobrepeso/obesidade apresentaram um aumento dos triglicerídeos em ambas as refeições testadas, enquanto nas voluntárias eutróficas esse aumento ocorreu apenas na dieta acompanhada de suco de laranja.
- A duração da lipemia pós-prandial foi influenciada pela bebida, visto que a adição do suco de laranja prolongou o aumento dos triglicerídeos até a quinta hora pós-prandial nas voluntárias com excesso de peso.
- A resposta inflamatória no período pós-prandial foi influenciada pela dieta e pelo estado nutricional. As maiores concentrações de marcadores inflamatórios foram encontradas nas voluntárias eutróficas quando consumiram a refeição rica em gordura acompanhada de suco de laranja.O
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CONSIDERAÇÕES FINAIS

No contexto do fenômeno mundial de mudanças no padrão alimentar, algumas questões permanecem não resolvidas na ciência da nutrição. Uma delas diz respeito ao papel das gorduras e carboidratos da dieta na saúde e na doença.

A alta ingestão de gorduras, exacerbando a lipemia pós-prandial, já está estabelecida como um conhecido fator de risco cardiovascular. De modo geral, recomenda-se a ingestão de carboidratos digestíveis e não digestíveis, e restrição do consumo de açúcares. Entretanto, para recomendações dietéticas mais específicas e conclusivas, é necessário um entendimento mais detalhado de como os carboidratos e gorduras da dieta interagem e modulam vias metabólicas no estado pós-prandial.

Foi observado, nesse trabalho, que a adição de 50g de carboidrato em uma refeição rica em gordura saturada exacerbou e prolongou a lipemia, principalmente em mulheres obesas. Dessa forma, sugere-se que as interações entre carboidratos e lipídios no estado pós-prandial resultem tanto de efeitos agudos (composição da dieta, biodisponibilidade dos nutrientes) e de efeitos crônicos, tais como a obesidade.

Portanto, o estado pós-prandial caracteriza-se por excursão glicêmica, hipertrigliceridemia e aumento de marcadores inflamatórios, todos fatores potencialmente determinantes de aterogênese.

Considerando que o homem moderno vive em estado pós-prandial a maior parte do dia, intervenções dietéticas que determinem redução das lipoproteínas remanescentes ricas em triglicerídeos devem ser pesquisadas, visando recomendações nutricionais adequadas.

Nosso conhecimento científico sobre o assunto é ainda limitado, e mais pesquisas são ainda necessárias para melhor entendimento dos processos que ocorrem durante o processamento de uma refeição.

ANEXOS

ANEXO 1

UNIVERSIDADE FEDERAL DE VIÇOSA
CENTRO DE CIÊNCIAS BIOLÓGICAS
DEPARTAMENTO DE NUTRIÇÃO E SAÚDE

Nutrição

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DE VIÇOSA
COMITÊ DE ÉTICA EM PESQUISA COM SERES HUMANOS
Campus Universitário - Viçosa, MG - 36570-000 - Telefone: (31) 3899-1269

Of. Ref. Nº 184/2011/Comitê de Ética

Viçosa, 16 de dezembro de 2011.

Prezada Professora:

Cientificamos V. Sª. de que o Comitê de Ética em Pesquisa com Seres Humanos, em sua 9ª Reunião de 2011, realizada nesta data, analisou e aprovou, sob o aspecto ético, o projeto intitulado *Resposta inflamatória frente ao consumo de componentes dietéticos específicos: um estudo nutrigenômico.*

Atenciosamente,

Professora Patrícia Aurélia Del Nero
Comitê de Ética em Pesquisa com Seres Humanos
Presidente

ANEXO 2

TERMO DE CONSENTIMENTO LIVRE ESCLARECIDO (Em duplicata)

Convidamos você a participar, voluntariamente, do estudo denominado "Resposta inflamatória frente ao consumo de componentes dietéticos específicos: um estudo nutrigenômico", cujo objetivo é conhecer a resposta inflamatória frente a componentes da dieta. Você deverá comparecer por três vezes no Laboratório de Metabolismo Energético e Composição Corporal (LAMECC), no Departamento de Nutrição e Saúde. Na primeira visita, será feita uma entrevista para completar um questionário e se medirá sua pressão arterial. Você deverá apresentar exames laboratoriais recentes (<3 meses) de colesterol total, triglicerídeos e glicemia de jejum. Caso não disponha desses exames, os mesmos poderão ser realizados no dia da entrevista. Você não terá nenhum gasto por sua participação nesse estudo. Na segunda e na terceira visita, será oferecido a você café da manhã, que deverá ser consumido no LAMECC. Será coletado sangue em jejum e após o consumo da refeição. A extração de sangue pode ser dolorosa e causar hematomas (roxo) no local da punção (picada) na dobra do cotovelo, como qualquer outra coleta de sangue que você possa ter feito no passado. Tanto a medida da pressão arterial, como a de peso, altura, circunferência da cintura, circunferência do qual e bioimpedância não causarão nenhum inconveniente ou tipo de risco. Você receberá os resultados de todos os exames realizados para que possa levá-los ao seu médico, quem decidirá, com essas informações, que medida tomar.

A decisão de participar desse estudo é completamente voluntária. Você poderá se recusar a participar ou sair do estudo a qualquer momento depois de dar o seu consentimento, e esta atitude não lhe trará prejuízos no futuro. Em qualquer momento, você poderá fazer perguntas sobre o estudo ou esclarecer dúvidas. Você poderá entrar em contato com Raquel C. L. Assis Coelho para esta finalidade através dos telefones: (31-3899-3388 / 31-8879-2910).

Os resultados de todas as medidas, pesquisa e exames realizados serão apresentados, comunicados e/ou publicados, sempre preservando sua confidencialidade e privacidade.

Ao assinar este documento, confirmo que me foi explicado o objetivo deste estudo, os procedimentos a que serei submetido, os riscos e os benefícios potenciais que eu possa experimentar, e os possíveis destinos dos resultados que serão obtidos neste estudo. As perguntas que foram feitas foram satisfatoriamente respondidas, li e compreendi este termo de consentimento, ficando em meu poder uma cópia do mesmo. Portanto, assino e dou meu consentimento para participar deste estudo.

Viçosa, _____ de _____ de 2012.

_____

Voluntário

_____

Pesquisador

_____

Profª. Dra. Helen Hermana M. Hermsdorff -

Coordenadora

ANEXO 3

UNIVERSIDADE FEDERAL DE VIÇOSA
CENTRO DE CIÊNCIAS BIOLÓGICAS
DEPARTAMENTO DE NUTRIÇÃO E SAÚDE

Data: _____/_____/_____

FICHA DE IDENTIFICAÇÃO PESSOAL (confidencial)

ID RECRUTAMENTO: _____ **ID ESTUDO**: ____

Nome:_____

Data nascimento: _____/_____/_____ Idade:_____ anos

Sexo: ( ) Feminino ( ) Masculino

Endereço:_____

Cidade: _____Estado:_____

CEP: _____

Email:_____

Telefones:_____

ANEXO 4

UNIVERSIDADE FEDERAL DE VIÇOSA
CENTRO DE CIÊNCIAS BIOLÓGICAS
DEPARTAMENTO DE NUTRIÇÃO E SAÚDE

Nutrição

HISTORIA CLÍNICA E HÁBITOS PESSOAIS

Data: _____/_____/_____

INICIAIS: _____ ID RECRUTAMENTO:_____ ID ESTUDO:____

Data de nascimento: ___/___/___

Sexo: ( ) masculino ( ) feminino

Idade:_____

Escolaridade:_____

Ocupação:_____

Tabagismo: ( ) fumante ( ) não fumante ( ) ex-fumante

Etilismo: ( ) não bebe ( ) <2x/semana ( )>2x/semana

Alteração peso últimos 03 meses: ( ) Sim ( ) Não Se sim, _____kg

História pessoal de: ( ) HAS

( ) DM

( ) Dislipidemia

( ) Doenças da tireóide

( ) Outras doenças: _____

Medicação em uso (medicamento, dose, duração do uso):

_____

_____

História pregressa:_____

História familiar de: ( ) HAS
( ) DM
( ) Dislipidemia
( ) Doenças da tireoide
( ) Outras doenças: _____

* Para preenchimento da história familiar, considerar pais e irmãos do voluntário.

História social: _____

História dietética:

Alergia a algum alimento: ( ) Sim ( ) Não

Se sim, qual (is)? _____

Vegetariano: ( ) Sim ( ) Não

Algum hábito alimentar específico? ( ) Sim ( ) Não

Observações :_____

Exame físico:

Peso: _____ kg Altura: _____ m IMC: _____ kg/m^2

PA: _____ mmHg

Exames bioquímicos (data: __/__/__):

Glicemia de jejum: _____ mg/dl

Colesterol total: _____ mg/dl HDL: _____ VLDL: _____mgdL LDL: _____ Triglicerídeos:_____mg/dl

ANEXO 5

UNIVERSIDADE FEDERAL DE VIÇOSA
CENTRO DE CIÊNCIAS BIOLÓGICAS
DEPARTAMENTO DE NUTRIÇÃO E SAÚDE

QUESTIONÁRIO INTERNACIONAL DE ATIVIDADE FÍSICA (IPAQ) – VERSÃO CURTA

Nome:_____

Data: _____/ _____ / _____ Idade : _____ Sexo: F ( ) M ( )

Nós estamos interessados em saber que tipos de atividade física as pessoas fazem como parte do seu dia a dia. As perguntas estão relacionadas ao tempo que você gasta fazendo atividade física na ÚLTIMA semana. As perguntas incluem as atividades que você faz no trabalho, para ir de um lugar a outro, por lazer, por esporte, por exercício ou como parte das suas atividades em casa ou no jardim. Suas respostas são MUITO importantes. Por favor, responda cada questão mesmo que considere que não seja ativo. Obrigado pela sua participação!

Para responder as questões lembre que:

- atividades físicas VIGOROSAS são aquelas que precisam de um grande esforço físico e que fazem respirar MUITO mais forte que o normal
- atividades físicas MODERADAS são aquelas que precisam de algum esforço físico e que fazem respirar UM POUCO mais forte que o normal

Para responder as perguntas pense somente nas atividades que você realiza por pelo menos 10 minutos contínuos de cada vez.

1a Em quantos dias da última semana você CAMINHOU por pelo menos 10 minutos contínuos em casa ou no trabalho, como forma de transporte para ir de um lugar para outro, por lazer, por prazer ou como forma de exercício?
Dias _____ por SEMANA ( ) Nenhum

1b Nos dias em que você caminhou por pelo menos 10 minutos contínuos quanto tempo no total você gastou caminhando por dia?
Horas: _____ Minutos: _____

2a. Em quantos dias da última semana, você realizou atividades MODERADAS por pelo menos 10 minutos contínuos, como por exemplo, pedalar leve na bicicleta, nadar, dançar, fazer ginástica aeróbica leve, jogar vôlei recreativo, carregar pesos leves, fazer serviços domésticos na casa, no quintal ou no jardim como varrer, aspirar, cuidar do jardim, ou qualquer atividade que fez aumentar moderadamente sua respiração ou batimentos do coração (POR FAVOR NÃO INCLUA CAMINHADA)
Dias _____ por SEMANA ( ) Nenhum

2b. Nos dias em que você fez essas atividades moderadas por pelo menos 10 minutos contínuos, quanto tempo no total você gastou fazendo essas atividades por dia?
Horas: _____ Minutos: _____

3a Em quantos dias da última semana, você realizou atividades VIGOROSAS por pelo menos 10 minutos contínuos, como por exemplo correr, fazer ginástica aeróbica, jogar futebol, pedalar rápido na bicicleta, jogar basquete, fazer serviços domésticos pesados em casa, no quintal ou cavoucar no jardim, carregar pesos elevados ou qualquer atividade que fez aumentar MUITO sua respiração ou batimentos do coração.
Dias _____ por SEMANA ( ) Nenhum

3b Nos dias em que você fez essas atividades vigorosas por pelo menos 10 minutos contínuos quanto tempo no total você gastou fazendo essas atividades por dia?
Horas: _____ Minutos: _____

Estas últimas questões são sobre o tempo que você permanece sentado todo dia, no trabalho, na escola ou faculdade, em casa e durante seu tempo livre. Isto inclui o tempo sentado estudando, sentado enquanto descansa, fazendo lição de casa visitando um amigo, lendo, sentado ou deitado assistindo TV. Não inclua o tempo gasto sentando durante o transporte em ônibus, trem, metrô ou carro.

4a. Quanto tempo no total você gasta sentado durante um dia de semana?
_____horas ____minutos

4b. Quanto tempo no total você gasta sentado durante em um dia de final de semana? _____horas ____minutos

ANEXO 6

UNIVERSIDADE FEDERAL DE VIÇOSA
CENTRO DE CIÊNCIAS BIOLÓGICAS
DEPARTAMENTO DE NUTRIÇÃO E SAÚDE

Nutrição

INSTRUÇÕES PARA A PARTICIPAÇÃO NO ESTUDO

NOS DIAS: ___/___/___ e ___/___/___

- Seguir a "dieta branca", conforme o modelo.
- Restringir o consumo de alimentos ricos em antioxidantes nas 48 horas anteriores à visita, como: todas as frutas e sucos de frutas; hortaliças (batata e mandioca podem ser consumidas), café; castanhas (nozes, amêndoas, castanha de caju); qualquer tipo de suplemento vitamínico, mineral ou fitoterápico; alimentos enriquecidos com ômega-3.

NO DIA ___/___/___ (ANTERIOR À VISITA)

- Jantar aproximadamente às _____h

PARA O DIA DA VISITA: ___/___/___

- Comparecer no Laboratório de Metabolismo Energético e Composição Corporal às 7:30h.
- Estar em jejum de 12h.
- Vestir roupas leves.
- Não realizar exercícios físicos intensos ou ingerir bebida alcóolica no dia anterior à visita.

ANEXO 7

UNIVERSIDADE FEDERAL DE VIÇOSA
CENTRO DE CIÊNCIAS BIOLÓGICAS
DEPARTAMENTO DE NUTRIÇÃO E SAÚDE

Para a realização de sua participação como voluntária no projeto "Resposta inflamatória frente ao consumo de componentes dietéticos específicos: um estudo nutrigenômico" será necessário que se siga uma "dieta branca", durante os dois dias (48 horas) antes da coleta de sangue, para não interferir nos resultados finais do estudo.

Essa "dieta branca" é pobre em carotenoides e polifenois. Desse modo, não será permitido o consumo, nos dois dias anteriores ao estudo, dos seguintes alimentos: café, verduras de cores fortes (cenoura, beterraba, brócolis, abóbora, tomate, couve, alface), achocolatado, chás todos os tipos de frutas, linhaça, manteiga, molho de tomate, mostarda, carne de boi, pão integral, cerveja.

Por outro lado, será permitido o consumo de carnes brancas, como frango sem pele, peito de peru, ovos e lombo de porco, queijos, batata, arroz, inhame, mandioca, macarrão, margarina, maionese, azeite de oliva, óleo de girassol, torradas, molho branco, iogurte natural ou coco (não pode conter pedaços de frutas).

Para facilitar o seguimento de tal "dieta branca", você receberá um plano alimentar para cada dia, de 1500 kcal/dia, adequado às suas recomendações nutricionais e aos alimentos que podem ser consumidos e suas quantidades. Em caso de que não consuma algum alimento incluído na dieta, o responsável será capaz de substituí-lo por outro.

Notas importantes

- Beba bastante água durante o dia, entre 6 a 8 copos por dia.
- A colação, o lanche e a ceia são intercambiáveis durante o mesmo dia.
- É muito importante que respeite as quantidades dos alimentos incluídos no plano alimentar, principalmente aqueles ricos em lipídios.

Em caso de dúvidas sobre o seguimento da dieta ou alimentos, você poderá entrar em contato com Renata Sena Gomide através dos telefones (31-88546207-oi/31-3891-5068).

Dia 1

Refeição	**Alimento**	**Quantidade** **Medida caseira e g/ml**
Desjejum	Leite Semi-Desnatado Pão Francês Margarina	1 copo americano(150 ml) 1 unidade (50g) 1 ponta de faca cheia (4g)
Colação	Biscoito água e sal (cream crackers)	6 unidades(60g)
Almoço	Couve-Flor Cozida Arroz Cozido Bife de Frango Purê de Batata Água	1 colher de sopa cheia(25g) 2 colheres de servir cheia (90 g) 1 pedaço médio (100g) 2 colheres de sopa cheia(90g) 1 copo americano cheio(165ml)
Lanche da Tarde	Pão de Queijo	1 unidade média (20g)
Jantar	Pão de Forma Queijo Mussarela **OU** Peito de Peru	2 unidades (50g) 2 fatias pequena (40g) 2 fatias finas(34g)

	Margarina **OU** Maionese	1 ponta de faca cheia (4g) 1 colher de sopa rasa(19g)
Ceia	Biscoito água e sal (cream crackers)	5 unidades(50g)

Dia 2

Refeição	**Alimento**	**Quantidade** **Medida caseira e g/ml**
Desjejum	Leite Semi-Desnatado Pão Francês Requeijão Queijo Minas	1 copo americano(150 ml) 1 unidade (50g) 1 ponta de faca (14g) 2 fatias média(60g)
Colação	Pão de batata	1 unidade média (50g)
Almoço	Batata Cozida Peixe Grelhado Macarrão ao Alho e Óleo	1 colher de servir cheia(60g) 1 filé médio (115g) 2 colheres de servir cheia(100g)
Lanche da Tarde	Biscoito água e sal	6 unidades (60g)
Jantar	Sopa de Inhame com Peito de Frango Desfiado	1 unidade média (160g) 2 colheres de servir cheia (130g)
Ceia	Iogurte Natural	1 unidade(200g)

	Açúcar	1 colher de sopa nivelada(15g)

ANEXO 8

CADERNO DE REGISTRO DE DADOS

Projeto Suco_Lar

ID do paciente:
Iniciais:
ID Recrutamento:

Instruções para o preenchimento do caderno de coleta de dados:

- Cada registro deberá ser datado e assinado pelo pesquisador autorizado.
- Deverá completar todas e cada uma das quadrículas. Caso não se dispor de algum dado que é solicitado, deverá colocar ND (não disponível), NR (não realizado) ou DE (desconhecido), de acordo com o que corresponda.

- Utilize caneta de tinta preta ou azul para o preenchimento.
- As datas serão registradas no seguinte formato: DD-MM-AAAA
- Os erros devem se riscados com uma linha horizontal, escrevendo ao lado da correção. Não utilize nenhum tipo de corretivo líquido ou em fita.
- As datas que não estiverem de acordó com a sequência esperada deverão ser comprovadas e corrigidas, caso se tratar de um erro de transcrição.
- Os resultados incomuns ou valores laboratoriais que excedam os intervalos fixados deverão ser verificados e seu significado será anotado ao lado do dado.

Compromisso do pesquisador

Eu,_____
(nome e sobrenome do pesquisador), certifico que as informações contidas neste caderno de coleta de dados são um registro completo e preciso dos dados correspondentes a este paciente, que o estudo foi realizado de acordo com as diretrizes emitidas pelo protocolo e com os principios éticos da Declaração de Helsinki (52nd WMA Assembleia Geral, em Edimburgo, Escócia, Outubro de 2000) e que se obteve o consentimento do paciente para participar deste estudo.

Data: _____/_____/_____

Assinatura:_____

Cronograma de visitas

PARÂMETRO	INCLUSÃO	VISITA 1	VISITA 2
Cumprimento dos critérios de inclusão	**X**		
Consentimento informado	**X**		

História clínica	**X**		
Exame físico	**X**	**X**	**X**
Avaliação da atividade física	**X**		
Registro alimentar	**X**		
Peso	**X**	**X**	**X**
Altura	**X**	**X**	**X**
IMC	**X**	**X**	**X**
Circunferência da cintura		**X**	**X**
Circunferência do quadril		**X**	**X**
Pressão arterial	**X**	**X**	**X**
BIA		**X**	**X**
Refeição teste		**X**	**X**
Coleta de sangue		**X**	**X**
VAS		**X**	**X**

O X indica quando será realizado o teste com o paciente.

Critérios de inclusão

( ) SIM ( ) NÃO	Sexo feminino
( ) Sim ( ) Não	Idade entre 20 e 40 anos
( ) Sim ( ) Não	Não está em período gestacional, menopausa ou lactação
( ) Sim ( ) Não	Ausência de processo infeccioso
( ) Sim ( ) Não	Ausência de doenças inflamatórias, hormonais, cardíaca respiratória, renal, hepática ou gastrintestinal que afete digestão e absorção de nutrientes
( ) Sim ( ) Não	Ausência de uso de medicamentos que possam afetar metabolismo energético, glicídico ou lipídico
( ) Sim ( ) Não	Não fumante
( ) Sim ( ) Não	Não tem antecedentes de alcoolismo ou dependência de

	drogas
( ) Sim ( ) Não	Peso estável nos últimos 3 meses
( ) Sim ( ) Não	Não é atleta
( ) Sim ( ) Não	CT<240, TG<150, GJ<100, PA<130x85

Para que o voluntário possa ser incluído no estudo, todas as respostas aos critérios de inclusão devem ser "SIM".

Assinatura do consentimento livre informado (duas vias): ( ) Sim ( ) Não

Imprescindível assinar o consentimento informado para continuar o estudo.

Entrega dos registros alimentares e orientação para preenchimento:

( ) Sim ( ) Não

Preenchimento do IPAQ: ( ) Sim ( ) Não

Resultado do IPAQ:

( ) muito ativo ( ) irregularmente ativo ( ) sedentário

Para a próxima visita:

( ) SIM ( ) NÃO	DATA DA PRÓXIMA VISITA: ____/____/_____
( ) Sim ( ) Não	Pedir ao paciente para vir em jejum de 12 horas, entregar e repassar as demais orientações para a próxima visita.

Observações:

_____

_____

_____

ID:	**VISITA _____**
Data: ____/____/_____	**Hora: ___:___h**

DIETA: ( ) 1 ( ) 2

Seguiu a "dieta branca": ( ) Sim ( ) Não

Horário da última refeição: ___:___h

Jejum de 12 horas? ( ) Sim ( ) Não

Bexiga vazia? ( ) Sim ( ) Não

Menstruada? ( ) Sim ( ) Não

DUM: ___/___/___

Fez uso de medicamento dos últimos 7 dias? ( ) Sim ( ) Não

Se sim, qual medicamento e dose?

_____

Alguma alteração na última semana? ( ) Sim ( ) Não

Seguiu a orientação alimentar? ( ) Sim ( ) Não

Observações:

_____

_____

Medidas antropométricas

Peso (kg)		
Altura (m)		
IMC (kg/m^2)		
Perímetro cintura (cm)		
Perímetro quadril (cm)		
Relação C/Q		

Pressão arterial sistólica: _____mmHg Pressão arterial diastólica:_____mmHg

BIA

% gordura corporal	
Massa magra (kg)	
Taxa de metabolismo basal	

Coleta de sangue T0	( ) Sim	( ) Não	Hora: _____
Início refeição	( ) Sim	( ) Não	Hora: _____
Término refeição	( ) Sim	( ) Não	Hora: _____
Coleta de sangue T2	( ) Sim	( ) Não	Hora: _____
Coleta de sangue T3	( ) Sim	( ) Não	Hora: _____
Coleta de sangue T5	( ) Sim	( ) Não	Hora: _____

Observações:

_____

_____

_____

_____

_____

ANEXO 9

ANÁLISE SENSORIAL DO MUFFIN DE BACON E QUEIJO

Abaixo da pergunta, se encontra uma linha, que tem dois extremos, um representando o estado com pouco de fome e o outro extremo com muita fome. Para responder à pergunta, você deverá marcar um traço, podendo ser ao longo de toda a linha, de acordo com a sua sensação de fome.

Como está sua fome nesse momento?

Nem um pouco Extremamente

Por favor, avalie a amostra utilizando a escala abaixo para descrever o quanto você gostou ou desgostou do produto. Marque a posição da escala que melhor reflita seu julgamento.

CÓDIGO DA AMOSTRA: _____

( ) Gostei extremamente

( ) Gostei muito

( ) Gostei moderadamente

( ) Gostei ligeiramente

( ) Indiferente

( ) Desgostei ligeiramente

( ) Desgostei moderadamente

( ) Desgostei muito

( ) Desgostei extremamente

Comentários_____

_____

ANEXO 10

PREPARAÇÃO PRONTA

FICHA TÉCNICA
PREPARAÇÃO: Muffin de Bacon e Queijo **PORÇÕES: 5**

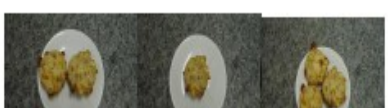
INGREDIENTES	PER CAPITA			QUANT. TOTAL (QT)	MEDIDA CASEIRA	CUSTO	
	PL	FC	PB			Kg ou L	QT
Farinha de Trigo	44	-	44	220g*	2 xícara cheia	1,96	0,43
Açúcar	4	-	4	20 g*	2 col de sopa n	1,81	0,04
Sal	0,48	-	0,48	2,4g*	½ col de chá ch	1,43	0,0
Fermento	1,2	-	1,2	6g*	1 col sopa rasa	17,90	0,14
Leite	44	-	44	220g*	1 xícara nivelada	2,17	0,47
Ovo	10,46	1,13	11,84	59,2g*	1 unidade	2,98**	0,25
Iogurte	9	-	9	45g*	2 col de sopa ch	10,82	0,53
Manteiga	7	-	7	35g*	4 col de sopa n	13,78	0,48
Bacon	71,6	1,06	76	380g*	2 xícaras cheia	18,14	6,89
Queijo	36	-	36	180g*	1 e 1/3 xícara n	16,38	2,95

*** Para realização da preparação os valores de QT, partiram-se das medidas caseiras.**

****O primeiro valor correspondente ao ovo, corresponde à dúzia de ovos, e não ao Kg, como proposto acima.**

TÉCNICA DE PREPARAÇÃO: Cortar as fatias de bacon em pedaços pequenos, fritar em uma frigideira ao fogo médio-alto e reserve. Em uma tigela média, misturar a farinha, açúcar, fermento e o sal. Em outra tigela, misturar o leite, iogurte, ovo e manteiga. Adicionar a mistura liquida sobre a mistura seca, não misturar muito. Adicionar o bacon e o queijo, misturar.
Untar a forma de muffin com manteiga.

Obs- o bacon também pode ser utilizado moído.

FOTO DOS INGREDIENTES EM MEDIDAS CASEIRAS	FOTO DAS MEDIDAS CASEIRAS	MEDIDA PADRÃO:
		2 muffins : 177g g OUTRAS MEDIDAS:

Ficha Técnica de Analise Química

Alimentos	PL	Ref	Carb (g)	Fibra (g)	Prot (g)	Lip (g)	Gor.sat. (g)	Colesterol	Sódio (mg)	Cálcio (mg)	Ferro (mg)	Vit. A (RE)	Vit C (mg)	Vit B12
Farinha de Trigo	44	taco	33,04	1,01	4,31	0,62	0,09	Na	0,44	7,92	0,44	0,00	0,00	0,00
Açúcar	4	philippi	4,00	0,00	0,00	0,00	0,00	0,00	0,00	0,04	0,00	0,00	0,00	0,00
Sal	0,48	philippi	0,00	0,00	0,00	0,00	0,00	0,00	186,04	0,12	0,00	0,00	0,00	0,00
Fermento	1,2	philippi	0,45	0,00	0,06	Tr	0,00	0,00	141,60	13,56	Tr	0,00	0,00	0,00
Leite	44	philippi	2,05	0,00	1,45	1,46	0,94	5,98	21,56	52,36	0,02	13,64	0,41	0,16
Ovo	10,46	Taco	0,17	NA	1,36	0,93	0,27	37,24	17,57	4,39	0,17	8,26	0,00	0,00
Iogurte	9	Taco	0,17	NA	0,37	0,27	0,16	1,26	4,68	12,87	Tr	2,07	0,00	0,00
Manteiga	7	taco	Tr	NA	0,03	6,02	3,61	14,98	0,28	0,28	0,00	64,68	Tr	Tr
Bacon	71,6	philippi	0,00	0,00	0,00	70,59	32,07	72,03	390,36	0,47	0,00	0,00	0,00	0,00
Queijo	36	philippi	0,80	0,00	6,98	7,78	4,75	28,22	134,28	186,12	0,06	86,76	0,00	0,23
Total	227,7		40,69	1,01	14,57	87,66	41,89	159,72	896,81	278,13	0,70	175,41	0,41	0,39
Conversão/Kcal			162,76		58,27	788,98	377,01							
Kcal	**1010**													

1