ANA PAULA BORONI MOREIRA

EFEITOS DO CONSUMO DE AMENDOIM COM DIFERENTES TEORES DE ÁCIDO GRAXO OLÉICO NA PERMEABILIDADE INTESTINAL E ENDOTOXEMIA EM HOMENS COM EXCESSO DE PESO

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência da Nutrição, para obtenção do título de *Doctor Scientiae*.

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APROVADA: 25 de setembro de 2013

Profª. Maria do Carmo Gouveia Peluzio

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Profª. Rita de Cássia Gonçalves Alfenas

(Orientadora)

Dedico aos meus amados pais, Vicente e Rita, e aos meus irmãos, Marco Antônio e Eduardo.

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BIOGRAFIA

Ana Paula Boroni Moreira nasceu em 21 de junho de 1980 no município de Ponte Nova, Minas Gerais.

Em março de 2000, iniciou o curso de graduação em Nutrição na Universidade Federal de Viçosa (MG), concluindo-o em julho de 2004. Foi bolsista de Iniciação Científica do PIBIC/CNPq durante o período de agosto de 2002 a julho de 2003, atuando em pesquisa sobre o avaliação de carotenóides em vegetais comercializados na microrregião de Viçosa, Minas Gerais.

Em agosto de 2004, ingressou no Curso de Pós-Graduação *Stricto Sensu* em Ciência da Nutrição da Universidade Federal de Viçosa (MG), atuando na linha de pesquisa 'Dietética e Qualidade de Alimentos', obtendo o título de mestre em julho de 2006.

Em outubro de 2006, ingressou na Universidade Presidente Antônio Carlos (UNIPAC) - *campus* Magnus (Barbacena) e *campus* VI (Juiz de Fora), onde permaneceu até novembro de 2007 e lecionou as disciplinas Avaliação Nutricional, Dietoterapia, Terapia Nutricional, Nutrição Materno-Infantil, Tópicos Especiais em Nutrição, Nutrição Experimental, Marketing em Alimentação e Supervisão de Estágio.

Entre novembro de 2007 a julho de 2009, atuou em prática clínica no Hospital Nossa Senhora das Dores no município de Ponte Nova, Minas Gerais.

Entre março de 2008 a fevereiro de 2009, realizou um curso de especialização (*Latu Sensu*) pelo Grupo de Apoio de Nutrição Enteral e Parenteral (GANEP) na área de nutrição clínica multiprofissional.

Em agosto de 2008, ingressou na Faculdade de Ciências Biológicas e da Saúde (FACISA/UNIVIÇOSA), onde permaneceu até fevereiro de 2010 e lecionou disciplinas relacionadas à área de Nutrição Cllínica.

Em março de 2010, ingressou na primeira turma de doutorado do Curso de Pós-Graduação *Stricto Sensu* em Ciência da Nutrição da Universidade Federal de Viçosa (MG) e pleiteou o título de doutora em setembro de 2013.

LISTA DE ABREVIATURAS

ALT: alanine aminotransferase

AP: alkaline phosphatase

AST: aspartate aminotransferase

ANOVA: analysis of variance

BMI: body mass index

CI: conicity index

CD14: co-receptor of toll-like receptor 4

CT: control group

CVP: conventional peanut group

DEXA: dual-energy X-ray absorptiometry

EU/ml: endotoxin units per milliliter

GGT: gamma-glutamyltransferase

HDL: high-density-lipoprotein

HOMA-B: homeostasis model assessment of beta cell function

HOMA-IR: homeostasis model assessment of insulin resistance

HOP: high-oleic peanut group

hsCRP: high-sensitivity C-reactive protein

IAC-505: high-oleic peanut developed by the Instituto Agronômico de Campinas

IAC-886: convencional peanut developed by the Instituto Agronômico de Campinas

IDF: International Diabetes Federation

IL: interleukin

iNOS: inducible nitric oxide synthase

IPAQ: International Physical Activity Questionnaire

IRS: insulin receptor substrate

LAL: Limulus Amebocyte Lysate

LBP: lipopolysaccharide-binding protein

LPS: lipopolysaccharide

L/M: lactulose/mannitol ratio

MAPK: mitogen-activated protein kinase

MCP-1: monocyte chemoattractant protein-1

NEFA: non-esterified fatty acids

NF-κB: factor nuclear kappa B

$_{pi}$AUC: positive incremental area under the curve

REE: resting energy expenditure

RMANOVA: repeated measures two-way ANOVA

SAD: sagital abdominal diameter

SEM: standard error of the mean

SI: sagittal index

TAG: triacylglycerols

TEE: total energy expenditure

TLR4: toll-like receptors 4

TNF-α: tumor necrosis factor alpha

VLDL: very-low-density-lipoprotein

%M: mannitol excretion

%L: lactulose excretion

SUMÁRIO

RESUMO

MOREIRA, Ana Paula Boroni, D.Sc., Universidade Federal de Viçosa, setembro de 2013. **Efeitos do consumo de amendoim com diferentes teores de ácido graxo oléico na permeabilidade intestinal e endotoxemia em homens com excesso de peso.** Orientadora: Rita de Cássia Gonçalves Alfenas. Coorientadoras: Maria do Carmo Gouveia Peluzio, Neuza Maria Brunoro Costa e Josefina Bressan.

O consumo de amendoim tem sido relacionado a inúmeros benefícios à saúde. A frequência de consumo de amendoim tem se associado inversamente ao índice de massa corporal (IMC) e risco de obesidade. O objetivo deste estudo foi avaliar os efeitos do consumo de uma dieta hipocalórica contendo ou não amendoim na permeabilidade intestinal e na concentração de lipopolissacarídeo (LPS) plasmático. Homens com excesso de peso (IMC entre 26-35 kg/m^2) e idade entre 18-50 anos foram aleatoriamente alocados em três grupos experimentais: amendoim convencional (AC), amendoim rico em ácido graxo oléico (AO) ou controle (CT). Foi prescrita uma dieta hipocalórica (-250 kcal/dia), que incluía (AC e AO) ou não (CT) uma porção diária de 56 g de amendoim torrado com pele. A intervenção dietética teve duração de quatro semanas consecutivas. No primeiro e no último dia de intervenção foi oferecida uma das três refeições teste compostas por um shake sabor morango mais uma porção de 56 g de amendoim convencional, amendoim rico em ácido graxo oléico ou biscoito. As refeições teste forneciam 25% da necessidade calórica de cada voluntário, avaliada por meio de calorimetria indireta. As referidas refeições continham 35% de carboidrato, 16% de proteína e 49% de lipídio. No primeiro e último dia de intervenção, foram avaliados os seguintes parâmentros: concentrações de LPS, triacilgliceróis, insulina e glicose em jejum e pós-prandiais (1, 2 e 3 h após consumo da refeição teste). Adicionalmente, em jejum foram determinados os seguintes parâmetros bioquímicos: proteína C reativa, aspartato aminotransferase (AST), alanina aminotransferase (ALT), gama-glutamil transferase (GGT), fosfatase alcalina, colesterol total, VLDL, LDL e HDL. Urina foi coletada em laboratório durante um período de 6 h após ingestão de uma solução contendo lactulose e manitol para verificar a permeabilidade intestinal. Medidas antropométricas (peso, circunferência da cintura e diâmetro abdominal sagital (DAS)) e de composição corporal também foram avaliadas antes e após a intervenção. Os resultados obtidos no estudo

estão apresentados em três artigos, sendo os dois primeiros com dados transversais e o terceiro com dados de intervenção. **Artigo 1:** ***Higher plasma lipopolysaccharide concentrations are associated with less favorable phenotype in men with similar BMI and total adiposity*** - Sessenta e sete homens, com IMC médio de 29.7 ± 0.3 kg/m^2 foram incluídos. Os voluntários com valores de LPS plasmático ≥ 0.9 EU/mL apresentaram maior DAS, percentual de gordura do tronco, percentual de gordura androide, massa de gordura androide, maiores concentrações de insulina e ALT e maiores índices *Homeostasis Model Assessment of Insulin Resistance* (HOMA-IR) e HOMA-Beta em relação aos homens com menor concentração de LPS. LPS se correlacionou positivamente com percentual de gordura do tronco, percentual de gordura androide, massa de gordura androide, insulina, AST, fosfatase alcalina, HOMA-IR e HOMA-B. Os resultados sugerem que uma maior concentração de LPS se associa a um fenótipo menos favorável, caracterizado por maior adiposidade central, menor sensibilidade à insulina e comprometimento da função das células beta pancreáticas. **Artigo 2:** ***A high-fat meal containing conventional or high-oleic peanuts is associated with delayed triglyceridemia and lower 3h postprandial lipopolysaccharide concentrations in overweight/obese men*** - Sessenta e cinco homens participantes foram alocados nos seguintes grupos: AC (n=21), AO (n=23) ou CT (n=21). Após a ingestão aguda das refeições teste, verificou-se: concentrações de LPS menores nos grupos AC (0.7 ± 0.5 EU/ml) e AO (1.0 ± 0.9 EU/ml) comparados ao grupo CT (1.6 ± 1.2 EU/ml) às 3 h pós-prandiais. Os níveis de triacilgliceróis e insulina aumentaram em todos os grupos. Entretanto, verificou-se que nos grupos AC e AO os triacilgliceróis só se elevaram a partir das 2 h pós-prandiais, enquanto no grupo CT o aumento ocorreu a partir de 1 h pós-prandial. Houve correlação positiva entre os níveis de LPS e de triacilgliceróis. Nos grupos AC e AO, os níveis de insulina avaliados às 3 h pós-prandiais foram semelhantes aos valores de jejum. Assim, o consumo agudo de amendoim atrasou o aumento de triacilgliceróis e favoreceu o retorno mais rápido da insulina para concentrações basais, especialmente no grupo AC. Os resultados sugerem que o consumo de amendoim convencional ou amendoim rico em ácido graxo oléico pode reduzir o risco de endotoxemia e de seus efeitos metabólicos. **Artigo 3:** ***Effect of a hypocaloric diet containing peanut cultivars differing in oleic acid content on intestinal permeability and***

endotoxemia - Cinquenta e cinco homens foram incluídos no estudo de intervenção, sendo alocados nos seguintes grupos AC (n=19), AO (n=18) ou CT (n=18). O consumo de dieta hipocalórica incluindo ou não cultivares de amendoim com diferentes teores de ácido graxo oléico não alterou a permeabilidade intestinal, as concentrações plasmáticas de LPS em jejum ou as respostas pós-prandiais de LPS após a ingestão de refeição hiperlipídica. Ao final da intervenção verificou-se a redução de medidas antropométricas e da massa de gordura corporal total, em todos os grupos. Houve redução da massa livre de gordura apenas no grupo CT. Os resultados sugerem que o consumo de dietas hipocalóricas com adição de amendoim contribui para a perda de peso e melhora na composição corporal. Estes efeitos não foram associados com alterações na permeabilidade intestinal ou na concentração de LPS plasmático.

ABSTRACT

MOREIRA, Ana Paula Boroni, D.Sc., Universidade Federal de Viçosa, September, 2013. **Effects of consumption of peanut with different oleic fatty acid content on intestinal permeability and endotoxemia in overweight and obese men.** Adviser: Rita de Cássia Gonçalves Alfenas. Co-Advisers: Maria do Carmo Gouveia Peluzio, Neuza Maria Brunoro Costa and Josefina Bressan.

Peanut consumption has been linked to numerous health benefits. An inverse association between the frequency of peanut consumption versus body mass index (BMI) and risk of obesity has been observed. The aim of this study was to evaluate the effects of the consumption of a hypocaloric diet with or without peanuts on intestinal permeability and on plasma lipopolysaccharide (LPS) concentration. Overweight and obese men (BMI between 26-35 kg/m^2) aged between 18-50 years were randomly assigned to one of the three experimental groups: conventional peanuts (CVP), high-oleic peanuts (HOP) or control (CT). It was prescribed a hypocaloric (-250 kcal/day) balanced-diet, which included (CVP and HOP) or not (CT) a portion of 56 g/day of roasted peanuts with skin. Dietary intervention lasted for four consecutive weeks. In the first and last day of the study one of the three test meals were offered. The test meals consisted of a strawberry shake and 56 g roasted conventional or high-oleic peanuts or biscuits. The test meals provided 25% of each participant´s daily energy requirement measured by indirect calorimetry and provided 35% of carbohydrate, 16% of protein, and 49% of fat. In the first and last day of the intervention, the concentrations of the following parameters were evaluated in fasting state and postprandial (1, 2, and 3 h after the consumption of the test meal) condition: LPS, triacylglycerols, glucose and insulin. In addition, high-sensitivity C-reactive protein (hsCRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase, total cholesterol, VLDL, LDL, and HDL were analyzed in the fasting state. Urine was collected in the laboratory over a period of 6 h after the ingestion of a solution containing the sugar probes lactulose and mannitol to verify intestinal permeability. Anthropometric data (weight, waist circumference, and sagital abdominal diameter (SAD)), and body composition were also assessed in the first and last day of the study. The results obtained in this study are presented in three articles. The first two articles reflect the results of cross-

sectional studies and the third article, the results of an intervention study. **Article 1:** ***Higher plasma lipopolysaccharide concentrations are associated with less favorable phenotype in men with similar BMI and total adiposity*** – Sixty-seven men with mean BMI of 29.7 ± 0.3 kg/m^2 were evaluated. Men with median plasma LPS ≥ 0.9 EU/mL presented higher SAD, trunk fat percentage, android fat percentage and mass, insulin and ALT concentrations, homeostasis model assessment of insulin resistance (HOMA-IR), and of beta cell function (HOMA-B) than those with lower plasma LPS. LPS correlated positively with trunk fat percentage, android fat percentage and mass, insulin, AST, ALT, alkaline phosphatase concentrations, HOMA-IR, and HOMA-B. Our results suggest that higher plasma LPS concentration is associated with a less favorable phenotype characterized by higher central adiposity, lower insulin sensitivity, and beta cell function impairment. **Article 2:** ***A high-fat meal containing conventional or high-oleic peanuts is associated with delayed triglyceridemia and lower 3h postprandial lipopolysaccharide concentrations in overweight/obese men*** - Sixty-five men were evaluated and assigned to one of the three experimental groups: conventional peanuts (CVP, n=21), high-oleic peanuts (HOP, n=23) or control (CT, n=21). After the consumption of the high-fat test meal LPS concentrations were lower in CVP (0.7 ± 0.5 EU/ml) and HOP (1.0 ± 0.9 EU/ml) groups compared to CT (1.6 ± 1.2 EU/ml) at 3 h postprandial. Triacylglycerols and insulin concentrations increased in all groups. However, triacylglycerols started to increase only after 2 h in CVP and HOP groups while in the CT group the increase was at 1 h postprandial. LPS correlated positively with triacylglycerols. Insulin returned to basal concentrations at 3 h only in CVP and HOP groups. The acute consumption of peanuts delayed the increase in triacylglycerols and favoured the quicker return of insulin to basal concentrations, especially in the CVP group. Our results suggest that the consumption of conventional or high-oleic peanuts may help to reduce the risk of endotoxemia and metabolic disorders. **Article 3:** ***Effect of a hypocaloric diet containing peanut cultivars differing in oleic acid content on intestinal permeability and endotoxemia*** – Fifty-five men participated in this clinical trial. The subjects were assigned to one of the three experimental groups: conventional peanuts (CVP, n=19), high-oleic peanuts (HOP, n=18) or control (CT, n=18). The consumption of hypocaloric diet associated with the consumption of both types of peanut

cultivars did not change intestinal permeability, plasma LPS concentrations in the fasting state or LPS postprandial concentration in responses to the high-fat meals. The proposed intervention promoted a reduction in anthropometric data, and total body fat mass in all groups. Only the CT group experienced a reduction in fat free mass. Our results suggest that the consumption of peanuts-enriched hypocaloric diets contributes to weight loss, and improves body composition. These effects were not associated with changes on intestinal permeability or plasma LPS concentrations.

1. INTRODUÇÃO GERAL

O lipopolisacarídeo (LPS) constitui um dos principais componentes da membrana externa de bactérias gram-negativas. A administração de pequenas doses dessa endotoxina na circulação pode induzir respostas imunes e ativar vias que levam à inflamação, inibindo a sinalização insulínica e promovendo ganho de peso (Cani et al., 2007; Manco et al., 2010). Estudos têm evidenciado que indivíduos obesos e diabéticos apresentam maiores concentrações de LPS na circulação (Creely et al., 2007; Basu et al., 2011; Pussinen et al., 2011; Harte et al., 2012). Mais recentemente, tem sido sugerido que a dieta pode desempenhar um papel importante no desenvolvimento da endotoxemia e entre os fatores dietéticos a ingestão excessiva de lipídio tem se destacado (Ghanim et al., 2010; Laugerette et al., 2011).

Ao mesmo tempo que o intestino é um reservatório de LPS, ele também atua como uma barreira à sua passagem para a corrente sanguínea. Assim, maiores concentrações de LPS na circulação podem sugerir a ocorrência de permeabilidade intestinal aumentada. Tem sido proposto que uma ingestão excessiva de lipídio pode promover a passagem de LPS pelo intestino por meio de dois mecanismos: permeabilidade intestinal aumentada (Cani et al., 2008; Cani & Delzenne, 2009; Suzuki & Hara, 2010) e excessiva formação de quilomícrons, capazes de incorporarem partículas de LPS em sua estrutura, com subsequente acesso ao sistema circulatório (Ghoshal et al., 2009; Laugerette et al., 2011; Clemente-Postigo et al., 2012). Além do conteúdo total de lipídio, o perfil de ácidos graxos da dieta também pode influenciar a endotoxemia. O ácido graxo oléico, por exemplo, estimulou em animais a formação de quilomicrons e consequentemente o aumento de LPS na circulação (Ghoshal et al., 2009). Por outro lado, o ácido oléico não alterou a função de barreira, uma vez que não modificou as *tight junctions in vitro* (Usami et al., 2001).

Dentro deste contexto, verifica-se a necessidade de se avaliar o efeito do consumo de alimentos ricos em ácido graxo oléico na permeabilidade intestinal e na concentração plasmática de LPS. O amendoim é um alimento energeticamente denso, rico em ácidos graxos insaturados, com destaque para o oléico. O amendoim convencional possui, em media, 50% de ácido graxo oléico em relação ao seu teor lipídico total enquanto o amendoim rico em ácido

graxo oléico possui, em media, 81%. Verifica-se ainda na semente, a presença de fibra alimentar, vitaminas antioxidantes, ácido fólico, magnésio, potássio, proteína vegetal, alto teor de arginina e compostos bioativos como, por exemplo, o resveratrol (Kris-Etherton et al., 1999; Griel et al., 2004; Ros et al., 2010).

Estudos clínicos apontam que a ingestão do amendoim tende a manter o peso corporal entre os consumidores, apesar do seu alto aporte calórico (Bes-Rastrollo et al., 2007; Flores-Mateo et al., 2013). O aumento da saciação/saciedade e do metabolismo basal, aumento da atividade antioxidante, melhoria na sensibilidade à insulina e redução de vários marcadores inflamatórios têm sido apontados como possíveis responsáveis por esse papel exercido pelo amendoim (Pérez-Jiménez et al., 2002; Alper & Mattes, 2003; Jiang et al., 2002; Mattes et al., 2008). É provável que o controle do peso corporal também esteja relacionado à menor bioacessibilidade dos lipídios do amendoim (Ellis et al., 2004; Flores-Mateo et al., 2013), o que pode ter implicações para a absorção de LPS.

Estudos sobre a permeabilidade intestinal e a endotoxemia metabólica em seres humanos são escassos na literatura. Considerando que ainda não foi publicado nenhum trabalho em que se avaliou o efeito do consumo do amendoim na integridade intestinal e na concentração plasmática de LPS, verifica-se a necessidade da condução de pesquisas para se avaliar os efeitos desta oleaginosa em tais parâmetros e daí na manifestação de doenças em humanos. Também são necessários estudos para se avaliar o efeito do consumo de amendoim rico em ácido graxo oléico em humanos, visando à identificação de estratégias dietéticas capazes de promover o aumento da integridade da mucosa intestinal e redução da endotoxemia, atenuando os possíveis efeitos deletérios do excesso de peso.

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2. OBJETIVOS

2.1. Objetivo Geral:

Avaliar os efeitos do consumo de amendoim convencional e de amendoim rico em ácido graxo oléico na integridade da mucosa intestinal e na endotoxemia metabólica em homens com excesso de peso.

2.2. Objetivos Específicos:

1) Avaliar a relação entre concentração plasmática de lipopolissacarídeo *versus* distribuição de gordura corporal e parâmetros bioquímicos indicativos de desordens metabólicas.

2) Avaliar o efeito agudo do consumo de amendoim convencional e de amendoim rico em ácido graxo oléico:

- Nos níveis de lipopolissacarídeo pós-prandiais;
- Nos níveis de triacilgliceróis pós-prandiais;
- Na glicemia e insulinemia pós-prandiais.

3) Avaliar os efeitos do consumo durante 4 semanas de amendoim convencional e de amendoim rico em ácido graxo oléico:

- Na glicemia e insulinemia de jejum e pós-prandiais;
- Na resistência insulínica;
- Nos níveis de lipopolissacarídeo em jejum e pós-prandial;
- Nos níveis de triacilgliceróis de jejum e pós-prandiais;
- No colesterol total, HDL, LDL e VLDL em jejum;
- Nos biomarcadores (proteína C reativa, aspartato aminotransferase, alanina aminotransferase, gama-glutamil transferase e fosfatase alcalina) em jejum;
- Na permeabilidade intestinal;
- Nos parâmetros antropométricos (peso, circunferência da cintura e diâmetro abdominal sagital);
- Na composição corporal (gordura corporal total e massa livre de gordura).

3. RESULTADOS

3.1. Artigo 1: Higher plasma lipopolysaccharide concentrations are associated with less favorable phenotype in men with similar BMI and total adiposity (Autores: Ana Paula Boroni Moreira, Raquel Duarte Moreira Alves, Tatiana Fiche Salles Teixeira, Viviane Silva Macedo, Richard Mattes, Josefina Bressan, Neuza Maria Brunoro Costa, Maria do Carmo Gouveia Peluzio, Rita de Cássia Gonçalves Alfenas).

ABSTRACT

Lipopolysaccharide (LPS) from the outer membrane of gram-negative bacteria might be an inflammation trigger in adipose tissue. It has been proposed recently that there is a link between adipose tissue distribution and blood LPS. However, the number of studies about this topic is scarce requiring further investigation in humans. This study explored the association between plasma LPS concentration and body fat distribution as well biochemical parameters that may indicate the occurrence of metabolic disorders. Sixty-seven young adult men (body mass index (BMI) 29.7 ± 0.3 kg/m^2) were evaluated. Anthropometry, body composition and body fat distribution, blood pressure, energy expenditure, physical activity level, dietary intake, and biochemical parameters were assessed. Men with median plasma LPS ≥ 0.9 EU/mL presented higher sagital abdominal diameter, trunk fat percentage, android fat percentage and mass, insulin and alanine aminotransferase concentrations, homeostasis model assessment of insulin resistance (HOMA-IR), and of beta cell function (HOMA-B) than those with lower plasma LPS. LPS correlated positively with trunk fat percentage, android fat percentage and mass, insulin, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase concentrations, HOMA-IR, and HOMA-B. Our results suggest that higher plasma LPS concentration is associated with a less favorable phenotype characterized by higher central adiposity, lower insulin sensitivity, and beta cell function impairment.

Key words: Lipopolysaccharide, abdominal obesity, insulin, homeostasis model assessment.

1. INTRODUCTION

Adipose tissue functions extend the storage of fat. The endocrine, paracrine and autocrine signals secreted by adipose tissue regulate the metabolism of other cells. Both the lack (lipoatrophy) and the excess of adipose tissue are detrimental to metabolic equilibrium, which is also dependent on the functionality of this tissue. It has been proposed that each person may have a threshold level of adiposity beyond which dysfunctionality occurs. In this case, integration of different biochemical pathways, especially lipogenesis, lypolisis and fatty acid oxidation are disturbed, with an impact on whole physiology (Vásquez-Vela et al., 2008).

Hyperglycemia, dyslipidemia, and hypertension are metabolic disorders that often occur together and characterize a phenotype frequently associated with obesity (Vásquez-Vela et al., 2008; Virtue & Vidal-Puig, 2008). However, obesity *per se* (body mass index (BMI) higher than 30 kg/m^2) is not necessarily associated with these metabolic disorders in clinical practice (Montague & O'Rahilly, 2000). It may require the co-occurrence of insulin resistance (Reaven, 1991; Shanik et al., 2008; Ding & Lund, 2011; Lupattelli et al., 2013).

Body fat distribution and adipocyte size rather than total adiposity are key physical characteristics that influence the functionality of adipose tissue and occurrence of insulin resistance. Visceral fat mass, in particular, is a strong and independent predictor of adverse health outcomes of obesity (Vásquez-Vela et al., 2008; Klöting et al., 2010; Ledoux et al., 2010). One of the mechanistic explanations is that expansion of visceral adipose tissue mass without adequate support of vascularization might lead to hypoxia, which in turn, activates signaling to recruit immune cells. Macrophage infiltration and subsequent increased expression of inflammatory molecules, such as tumor necrosis factor alpha (TNF-α), would impair the downstream signaling of the insulin receptor, creating resistance to insulin action (Klöting et al., 2010; Könner et al., 2011). Thus, inflammatory activation links insulin resistance and visceral adiposity (Brook et al., 2013; Trøseid et al., 2013).

The well-known involvement of inflammatory cytokines in insulin resistance has stimulated research aimed at identifying inflammatory triggers. Lipopolysaccharides (LPS), a molecule derived from the outer membrane of gram-negative bacteria, are potent triggers of inflammatory responses through interaction with toll-like receptors-4 (TLR4) (Könner et al., 2011).

LPS is capable of influencing adipogenesis. Chronic infusion of low-doses of LPS induces adiposity gain, as well as chronic inflammation, insulin resistance, hyperglycemia and dyslipidemia in mice (Cani et al., 2007). Later, Muccioli et al. (2010) reported that LPS may serve as a master switch to control adipose tissue metabolism *in vivo* and *ex vivo* through inhibition of cannabinoid-driven adipogenesis. There are also data showing that LPS suppress adipogenesis in 3T3-L1 preadipocytes (Wang et al., 2013). Thus, it remains poorly understood if LPS induces or inhibits adipogenesis and how this may affect metabolic control. LPS might trigger inflammation in adipose tissue since expression of TLR4 has been identified in adipose tissue and isolated adipocytes (Shi et al., 2006). In addition, LPS infusion has been shown to alter expression of inflammatory markers in subcutaneous adipose tissue and to cause insulin resistance in humans (Mehta et al., 2010).

The findings of higher blood LPS concentrations in obese and diabetics patients compared to apparently healthy/lean subjects (Creely et al., 2007; Miller et al., 2009; Devaraj et al., 2009; Pussinen et al., 2011; Liang et al., 2013; Trøseid et al., 2013) raises the possibility that adipose tissue functionality and expansion may be affected by LPS concentration. However, evidence of the relationship between adipose tissue distribution and circulating concentrations of LPS are emerging (Trøseid et al., 2013) and requires further investigation in humans.

Considering the trend to categorize obese subjects with similar body mass index (BMI) as ‘healthy’ or ‘unhealthy’ according to their biochemical characteristics (especially higher degree of insulin resistance) (Calori et al., 2011), we aimed to investigate whether subjects with a similar BMI and total adiposity would present a distinguishable phenotype based on plasma LPS concentrations.

2. EXPERIMENTAL METHODS

Subjects

Written advertisements and social network were used for recruitment. One hundred and fifty men were screened. The inclusion criteria were: BMI between 26-35 kg/m^2, age between 18-50 years, non-smoker, no food allergy, and ethanol consumption lower than 168g/week. Body weight changes over 3 kg, following weight loss diet, the use of drugs that affect the biochemical parameters evaluated in the study, and presence of acute or chronic diseases were the exclusion criteria. Sixty-seven young adult men were included in the study (mean age 27.1 ± 0.9 years).

The study was approved by the Ethical Committee in Human Research from Federal University of Viçosa, Brazil (protocol number 185/2011). All subjects signed a written informed consent. After an overnight fasting, the evaluations were performed using standardized protocols and environmental conditions.

Anthropometrics and body composition

Anthropometric data and body composition were assessed by a single trained technician. Neck circumference was measured at the middle point of the neck's height (Vasques et al., 2009). Waist circumference was measured at the midpoint between the iliac crest and the last rib. The sagital abdominal diameter (SAD) was measured with an abdominal caliper (Holtain Kahn Abdominal Caliper®). With the subject in the supine position waist circumference was at the same point. Hip circumference was measured at the largest point between the waist and thigh. Thigh circumference was measured at the midpoint between the inguinal crease and the proximal border of the patella. The subject remained standing, and with the right leg slightly bent (Vasques et al., 2009). The conicity index (CI) and the sagittal index (SI) were calculated according to Valdez et al. (1993) and Kahn et al. (1996), respectively.

$$CI = \text{waist circumference (cm)} / 0.109 \times \sqrt{\text{weight (kg)}} / \text{height (cm)}$$

$$SI = \text{SAD (cm)} / \text{thigh circumference (cm)}$$

Body fat distribution was measured by the dual-energy X-ray absorptiometry (DEXA) (Lunar Prodigy Advance DXA System, GE Lunar), which gives a report discriminating trunk, android, and gynoid adiposity. The trunk region includes the neck, chest, abdominal and pelvic areas. The android

region is the area between the ribs and the pelvis, and is totally enclosed by the trunk region. The gynoid region includes the hips and upper thighs, and overlaps both the leg and trunk regions.

Energy expenditure

Subjects were instructed to abstain from caffeine and alcohol consumption, to refrain from heavy physical activity, and to maintain a regular sleep-wake schedule (8 hours/night) during the 72-hours before test day. Respiratory gas exchange was measured over 30 minutes under fasting conditions by indirect calorimetry using a ventilated respiratory canopy (Deltatrac II, MBM-200; Datex Instrumentarium Corporation) in compliance with the manufacturer guidelines. Then, the resting energy expenditure (REE) was obtained. The subject's daily energy requirement was calculated by multiplying the measured REE by a physical activity factor (WHO, 2001). The physical activity factor was determined through the Portuguese version of International Physical activity Questionnaire (IPAQ) validated by Pardini et al. (2001).

Dietary intake assessment

Subjects provided three-day food records (two non-consecutive week days and one weekend day). A dietitian reviewed the food records with the subjects to check for errors or omissions. All the food records were analyzed by the same dietitian using software Dietpro 5.2i (Agromídia, Viçosa, Brazil).

Biochemical analysis

Blood samples were drawn from an antecubital vein after a 12-hour overnight fasting. Plasma-EDTA and serum were separated from blood through centrifugation (2.200 x g, 15 min, 4°C) and stored at -80ºC.

Serum glucose, total cholesterol, HDL, triacylglycerols, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase were analyzed through enzymatic colorimetric methods using commercial kits (Quibasa - Química Básica, Brazil) in a autoanalyzer (COBAS MIRA Plus; Roche Diagnostic Systems). The high-sensitivity C-reactive protein (hsCRP) was also analyzed by a commercial kit (Quibasa - Química Básica, Brazil) in an autoanalyzer by an immunotubidimetric assay.

LDL and VLDL concentrations were calculated according to the Friedewald et al. (1972) formula. Serum insulin concentrations were analyzed through electrochemiluminescence immunoassay (Elecsys-Modular E-170, Roche Diagnostics Systems). The homeostasis model assessment of insulin resistance (HOMA-IR) and homeostasis model assessment of beta cell function (HOMA-B) were calculated according to Matthews et al. (1985).

Plasma LPS concentrations were determined through a chromogenic method using a Limulus Amebocyte Lysate (LAL) commercial kit (Hycult Biotech, The Netherlands). Undiluted plasma samples were heated at 75ºC for 5 min to neutralize endotoxin inhibitors. Aliquots (50 µl) of plasma and standards were added to the pyrogen-free microplate. The LAL reagent (50 µl) was added in each well. After 30 min of incubation, absorbance was read at 405 nm (Multiskan Go, Thermo Scientific, USA). When the optical density of the standards 10 and 4 EU/ml differed by less than 10%, the reaction was interrupted by adding 50 µl of stop solution (acetic acid) and absorbance was read again. As absorbance is directly proportional to the concentration of endotoxin, a standard curve was used to calculate LPS concentration in the samples. The concentration of LPS was expressed as endotoxin units per milliliter (EU/ml).

Statistical analysis

Statistical analyses were performed using SAS, version 9.2 (SAS Institute Inc., Cary, NC, USA). Parametric and non-parametric tests were used based on results from Shapiro-Wilk tests of normality test. A 5% level of significance was adopted. Data are presented as mean ± standard error of the mean (SEM). To determine the influence of LPS, subjects were divided into two groups according to plasma LPS median values above/equal to 0.9 EU/ml and lower than 0.9 EU/ml. The Students t-test or Mann-Whitney test were used to compare the groups. Spearman correlation analyses were run to study the correlation between variables.

3. RESULTS

General subjects' characteristics

Overall anthropometric, body composition, and biochemical parameters are shown in Table 1. From the total group (n=67), 61.2% (n=41) of volunteers were overweight and the others were obese. Furthermore, 94% (n=63) of volunteers had total body fat greater than 25%. The majority (95.5%, n=64) of volunteers had an abnormal waist circumference higher than 90 cm. Biochemical profile revealed that 47.8% (n=32) of men had HDL concentrations < 1.0 mmol/l; 34.3.8% (n=23) had triacylglycerols ≥ 1.7 mmol/l; and 20.9% (n=14) had high fasting blood glucose (≥ 5.55 mmol/l). In addition, 25.4% (n=17) had high blood pressure (systolic ≥ 130 and/or diastolic ≥ 85 mmHg). Metabolic syndrome occurred in 37.3% (n=25) of the subjects according to the International Diabetes Federation (IDF) criteria (Alberti et al., 2005).

Table 1. Anthropometric, body composition, and biochemical characteristics of the study participants (n=67)

	Mean ± SEM
BMI (kg/m^2)	29.7 ± 0.3
Waist circumference (cm)	101.4 ± 0.9
Body fat (%)	34.0 ± 0.6
Total fat mass (kg)	31.3 ± 0.9
Total fat free mass (kg)	63.5 ± 0.7
Total lean mass (kg)	60.0 ± 0.7
Glucose (mmol/l)	5.1 ± 0.1
Insulin (µU/ml)	9.7 ± 8.6
HOMA-IR	2.3 ± 0.2
HOMA-B	120.3 ± 7.9
TC (mmol/l)	4.9 ± 0.1
VLDL (mmol/)	0.7 ± 0.0
LDL (mmol/l)	3.1 ± 0.1
HDL (mmol/ll)	1.1 ± 0.0
TAG (mmol/l)	1.6 ± 0.1
Uric acid (µmol/l)	338.6 ± 11.9
AST (UI/l)	38.3 ± 2.1
ALT (UI/l)	26.5 ± 1.8
Alkaline phosphatase (UI/l)	55.8 ± 2.6
hsCRP (mg/dl)	1.5 ± 0.2
LPS (EU/ml)	1.3 ± 0.1

BMI: body mass index; HOMA-IR: homeostasis model assessment of insulin resistance; HOMA-B: homeostasis model assessment of beta cell function; TC: total cholesterol; TAG: triacylglycerols; AST: aspartate aminotransferase; ALT: alanine aminotransferase; hsCRP: high-sensitivity C-reactive protein; LPS: lipopolysaccharide.

Characterization of subjects below and above median LPS concentration

Plasma LPS was classified as above/equal or below the median (0.9 EU/ml) concentrations presented by the subjects. The subjects´ anthropometrics, body composition; clinical and biochemical characteristics; and energy intake, macronutrient, and dietary fiber consumption are shown in Tables 2, 3, and 4.

The group with higher LPS concentration had significantly higher SAD, trunk fat percentage, android fat percentage, android fat mass, and lower android fat free mass and android lean mass. They also had higher insulin, HOMA-IR, HOMA-B and ALT than the group with lower plasma LPS. The

energy intake, macronutrient, and fiber was similar between them (p>0.05). Blood pressure, physical activity level, and daily energy requirements were not significantly different between groups (data not shown).

Table 2. Anthropometric and body composition characteristics[1] of excessive body weight men according to plasma LPS concentration

	LPS < 0.9 EU/ml (n=33)	LPS ≥ 0.9 EU/ml (n=34)	P
Body weight (kg)	94.9 ± 2.0	93.5 ± 1.7	0.589
BMI (kg/m^2)	29.8 ± 0.4	29.7 ± 0.4	0.468
Neck (cm)	41.0 ± 0.4	40.8 ± 0.4	0.757
Waist circumference (cm)	101.3 ± 1.3	101.4 ± 1.3	0.960
SAD (cm)	22.5 ± 0.4	23.4 ± 0.4	0.045
Hip (cm)	108.8 ± 1.0	108.8 ± 0.8	0.981
Thigh (cm)	57.6 ± 0.6	57.5 ± 0.6	0.498
Waist-to-height	56.8 ± 0.7	57.3 ± 0.8	0.387
Waist-to-hip	0.9 ± 0.0	0.9 ± 0.0	0.947
Waist-to-thigh	1.8 ± 0.0	1.8 ± 0.0	0.382
CI	127.5 ± 0.8	128.2 ± 1.0	0.623
SI	0.4 ± 0.0	0.4 ± 0.0	0.056
Body composition (DEXA)			
Total body fat (%)	33.3 ± 0.9	34.7 ± 0.9	0.285
Total fat mass (kg)	31.0 ± 1.3	31.7 ± 1.2	0.687
Total fat free mass (kg)	64.6 ± 1.1	62.4 ± 1.0	0.132
Total lean mass (kg)	61.1 ± 1.0	58.9 ± 1.0	0.127
Trunk fat (%)	35.5 ± 1.1	38.8 ± 1.0	0.035
Trunk fat mass (kg)	15.5 ± 0.8	17.0 ± 0.8	0.179
Trunk fat free mass (kg)	28.3 ± 0.5	27.4 ± 0.5	0.222
Trunk lean mass (kg)	27.2 ± 0.5	26.3 ± 0.5	0.204
Gynoid fat (%)	39.4 ± 1.0	40.0 ± 0.9	0.661
Gynoid fat mass (kg)	5.3 ± 0.2	5.3 ± 0.2	0.864
Gynoid fat free mass (kg)	8.7 ± 0.2	8.3 ± 0.2	0.153
Gynoid lean mass (kg)	8.2 ± 0.2	7.9 ± 0.2	0.185
Android fat (%)	33.3 ± 1.3	38.4 ± 1.2	0.004
Android fat mass (kg)	2.1 ± 0.1	2.5 ± 0.1	0.043
Android fat free mass (kg)	4.3 ± 0.1	4.0 ± 0.1	0.035
Android lean mass (kg)	4.2 ± 0.1	3.9 ± 0.1	0.034

[1]Data are represented as mean ± standard error of the mean (SEM).

LPS: lipopolysaccharide; BMI: body mass index; SAD: sagittal abdominal diameter; CI: conicity index; SI: sagittal index. P value significant at p < 0.05.

Table 3. Clinical and biochemical characteristics[1] of excessive body weight men according to plasma LPS concentration

	LPS < 0.9 EU/ml (n=33)	LPS ≥ 0.9 EU/ml (n=34)	P
Glucose (mmol/l)	5.1 ± 0.1	5.2 ± 0.1	0.358
Insulin (µU/ml)	8.4 ± 0.9	10.9 ± 1.0	0.022
HOMA-IR	1.9 ± 0.2	2.3 ± 0.6	0.027
HOMA-B	106.1 ± 11.2	134.0 ± 10.8	0.017
TC (mmol/l)	4.6 ± 0.2	5.0 ± 0.2	0.128
VLDL (mg/dl)	0.6 ± 0.1	0.7 ± 0.0	0.056
LDL (mmol/l)	2.9 ± 0.2	3.2 ± 0.2	0.261
HDL (mmol/l)	1.1 ± 0.1	1.1 ± 0.0	0.221
TAG (mmol/l)	1.5 ± 0.2	1.8 ± 0.2	0.064
Uric acid (µmol/l)	338.6 ± 11.9	338.6 ± 11.9	0.356
AST (UI/l)	34.9 ± 2.1	41.6 ± 3.6	0.139
ALT (UI/l)	22.2 ± 2.2	30.7 ± 2.8	0.001
Alkaline phosphatase (UI/l)	56.0 ± 4.4	55.7 ± 3.0	0.217
hsCRP (mg/dl)	1.3 ± 0.2	1.6 ± 0.3	0.183

[1]Data are represented as mean ± standard error of the mean (SEM).

LPS: lipopolysaccharide; HOMA-IR: homeostasis model assessment of insulin resistance; HOMA-B: homeostasis model assessment of beta cell function; TC: total cholesterol; TAG: triacylglycerols; AST: aspartate aminotransferase; ALT: alanine aminotransferase; hsCRP: high-sensitivity C-reactive protein. P value significant at $p < 0.05$.

Table 4. Energy intake, macronutrient, and dietary fiber consumption[1] of excessive body weight men according to plasma LPS concentration

	LPS < 0.9 EU/ml (n=33)	LPS ≥ 0.9 EU/ml (n=34)	P
Energy (kcal/day)	2726.9 ± 137.6	2863.2 ± 95.1	0.118
Carbohydrate (%)	52.7 ± 1.1	51.8 ± 1.0	0.579
Protein (%)	16.7 ± 0.6	16.4 ± 0.4	0.741
Fat (%)	30.7 ± 1.0	31.7 ± 0.9	0.430
Dietary fiber (g/day)	27.6 ± 2.4	26.9 ± 1.3	0.224

[1]Data are represented as mean ± standard error of the mean (SEM). LPS: lipopolysaccharide.

Correlation between LPS concentration, fat distribution, and biochemical variables

Correlations were computed between LPS and the outcome variables. Significant associations are reported (Table 5). Plasma LPS concentration correlated positively with trunk fat percentage, android fat mass, and percentage. In addition, plasma LPS concentration correlated positively with serum insulin, AST, ALT, alkaline phosphatase, HOMA-IR, and HOMA-B. The other biochemical parameters as well as anthropometric, body composition, REE, and dietary intake data were not significantly correlated with LPS concentrations.

Table 5. Correlation coefficient between plasma LPS concentration, body composition, and biochemical variables

	LPS	
	r	p
Trunk fat (%)	0.26	0.033
Android fat mass (kg)	0.26	0.034
Android fat (%)	0.34	<0.004
Insulin (µU/ml)	0.28	0.021
HOMA-IR	0.27	0.029
HOMA-B	0.30	0.013
AST (UI/l)	0.26	0.030
ALT (UI/l)	0.41	<0.001
Alkaline phosphatase (UI/l)	0.25	0.048

Correlations were determined by Spearman's correlation test. LPS: lipopolysaccharide; HOMA-IR: homeostasis model assessment of insulin resistance; HOMA-B: homeostasis model assessment of beta cell function; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

4. DISCUSSION

The impact of total adiposity and of different locations of fat depots over metabolic abnormalities is difficult to characterize (Item & Konrad, 2012). However, considering subjects with similar BMI is still possible to identify those considered ‘metabolically healthy obese’ from the ‘at risk’ subjects. Visceral adipose tissue, degree insulin sensitivity, and expression of inflammatory markers are determinants for the distinction of these categories (Messier et al., 2010). Since LPS is involved in inflammatory activation and may influence intra-abdominal fat expansion (Lam et al., 2011) we investigated whether LPS concentrations could be used to discriminate the phenotype presented by obese individuals with similar BMI and total adiposity.

The data reveal that, despite having similar weight, BMI, waist circumference, and total body fat, subjects with higher plasma LPS concentrations presented a less favorable phenotype than subjects with lower LPS concentration. The ‘less favorable phenotype’ was basically characterized by higher android and trunk adiposity, higher fasting insulin, HOMA-IR and HOMA-B, and ALT.

Traditionally, waist circumference and BMI are indices that reflect excess abdominal and overall adiposity, respectively, and are usually considered good predictors of cardiometabolic risk factors (Abbasi et al., 2013). In the present study, these indices were similar between subjects with slightly different phenotypes, especially regarding insulin resistance markers. Contradictory results regarding the association of these indices with LPS concentration have been previously reported (Miller et al., 2009; Clemente-Postigo et al., 2012). We showed that SAD, an unusual indicator of abdominal obesity, distinguished subjects with different concentrations of plasma LPS. It has been claimed that SAD measured in the supine position reflects mainly visceral adipose tissue (Vasques et al., 2009). As previously mentioned, regional adipose tissue distribution is a relevant physical characteristic to be considered in clinical evaluation of an individual´s metabolic risk, especially because visceral (or intra-abdominal) fat depots are functionally and metabolically different from subcutaneous depots. The visceral depot is considered more hazardous and associated with metabolic alterations including insulin resistance (Despres & Lemieux, 2006; Cornier et al., 2011). The present findings showing that plasma LPS concentrations were not associated with total body fat, but were positively

associated with android and trunk fat (central region) reinforces the possible influence of LPS on fat distribution. Unfortunately, the use of BMI, waist circumference, SAD and measurement of adiposity using DEXA does not allow proper discrimination between subcutaneous and visceral adipose tissue at the central location.

Nevertheless, Trøseid et al. (2013) presented evidences that plasma LPS concentrations were more strongly correlated with intra-abdominal fat than with subcutaneous fat volumes. The mechanisms underlying this association are unclear. The cross-sectional nature of studies, including ours, does not allow determination of whether visceral depots increase due to excessive energy intake or to higher gut-derived LPS, and also whether a higher degree of insulin resistance arises before or after visceral adiposity accumulation. Since subjects from the present study had similar energy and macronutrient intakes, it is possible that higher LPS concentrations influence the accumulation of central fat and insulin resistance. A current hypothesis holds that translocation of gut-derived molecules to adipose tissue localized in close proximity to the gut, such as mesenteric fat, would trigger macrophage infiltration and inflammation, which in turn would stimulate expansion of this visceral depot (Lam et al., 2011; Trøseid et al., 2013). The downstream signaling of the insulin receptor can be impaired by inflammatory signals, which can be directly induced by LPS stimulation (Shanik et al., 2008; Ding & Lund, 2011). Mesenteric fat expresses higher concentrations of proinflammatory chemokines than other sites of adipose tissue in obese mice (Yu et al., 2006). It is also still unclear if inflammatory activation occurs before or after specific adipose tissue expansion and insulin resistance establishment.

Interestingly, subjects with higher LPS concentrations had simultaneously higher central fat, fasting insulin, HOMA-IR and HOMA-B. In addition, a positive correlation between plasma LPS and markers of insulin resistance was observed. This finding is in agreement with other studies reporting a relationship between plasma LPS and biomarkers of insulin signaling (Creely et al., 2007; Miller et al., 2009; Liang et al., 2013). The visceral localization and hypertrophy of intra-abdominal adipocytes are often related to the development of systemic insulin resistance through higher delivery of fatty acids to ectopic sites such as liver, and muscles. The induced lipotoxicity in these sites would in turn impair proper insulin signaling (Despres & Lemieux,

2006). The pathophysiological sequence of events that leads to insulin resistance considering regional fat distribution and an interactive influence of LPS remains to be established in humans.

Of note, we were expecting that the prevalence of metabolic syndrome would be higher in the group with higher LPS concentration. However, the prevalence did not differ (data not shown) between groups. As the volunteers are young adults, it is possible that the less favorable phenotype associated with higher LPS concentrations would increase the prevalence of metabolic syndrome in the long term. Longitudinal studies would be of great interest to test this hypothesis.

Finally, we verified that AST, ALT, and alkaline phosphatase enzymes were positively associated with plasma LPS, even though the mean values observed remained within normal ranges. AST and ALT are markers of liver injury (Lee et al., 2007). Since liver is responsible for circulating LPS clearance, higher LPS concentrations may negatively influence hepatic cells and increase the release of hepatic enzymes (Tuin et al., 2006; Manco et al., 2010). The infusion of LPS in animals increases ALT and AST blood concentrations (Coimbra et al., 2005). The correlation between alkaline phosphatase and LPS may be explained by the function of this enzyme in the dephosphorylation of the LPS, reducing by 100 times the toxicity of lipid A (Schromm et al., 1998; Bates et al., 2007).

In conclusion, our data indicate that for a similar body size and total fatness there are overweight/obese men with a less favorable phenotype predicted by LPS concentrations. It is characterized by higher central adiposity, lower insulin sensitivity, and beta cell function. Our study design does not permit establishment of causality between LPS, central obesity, and insulin resistance. However, it corroborates the view that fat distribution, in particular android/trunk fat, is a useful clinical marker to identify overweight/obese subjects who are potentially at increased risk for metabolic abnormalities in the long term. The potential role of LPS on adipose tissue distribution and expansion, and how this may impact insulin sensitivity needs further investigation.

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3.2. Artigo 2: A high-fat meal containing conventional or high-oleic peanuts is associated with delayed triglyceridemia and lower 3h postprandial lipopolysaccharide concentrations in overweight/obese men

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ABSTRACT

Lipopolysaccharide (LPS) translocation from the gut into the circulatory system is favoured by high-fat consumption favoring the occurrence of metabolic disorders such as insulin resistance. Consumption of high-fat foods may not enhance LPS absorption if they present other nutritional advantages such as favourable fatty acid and dietary fiber profile. The effect of the acute consumption of a high-fat meal (49% of calories from fat) containing conventional or high-oleic peanuts on the postprandial LPS concentrations and its relationship with lipemia and insulinemia in overweight and obese men was evaluated. The test meal consisted of a shake containing conventional peanuts (CVP, n=21), high-oleic peanuts (HOP, n=23) or control biscuits (CT, n=21). Blood samples were collected in the fasting state and 1, 2, and 3 h postprandial. LPS, insulin, lipids, and glucose concentrations were assessed. LPS concentrations were lower in CVP (0.7 (SED 0.5) EU/ml) and HOP (1.0 (SED 0.9) EU/ml) groups compared to CT (1.6 (SED 1.2) EU/ml) at 3 h postprandial. TAG and insulin concentrations increased in all groups. TAG started to increase only after 2 h in CVP and HOP groups. LPS correlated positively with TAG. Insulin returned to basal concentrations at 3 h only in CVP and HOP groups. The acute consumption of peanuts delayed the increase in TAG and favoured the quicker return of insulin to basal concentrations, especially in the CVP group. Our results suggest that the consumption of conventional or high-oleic peanuts may help to reduce the risk of endotoxemia and metabolic disorders.

Key words: Peanuts, oleic acid, lipopolysaccharide, insulin.

1. INTRODUCTION

Lipopolysaccharide (LPS), also known as endotoxin, is the major component of the outer membrane of gram-negative bacteria. LPS activates immune cells and inflammatory pathways (Moreira & Alfenas, 2012). Chronic and systemic exposure to slightly increased LPS concentrations may induce subclinical chronic inflammation. Consequently, insulin signalling can be inhibited leading to insulin resistance. Higher blood LPS concentrations have been observed in obese and diabetics compared to healthy subjects (Creely et al., 2007; Thuy et al., 2008; Devaraj et al., 2009; Pussinen et al., 2011), suggesting an association between LPS concentrations and metabolic disorders such as insulin resistance.

The gut is a substantive source of LPS. Its translocation from the gut into the circulatory system is favoured by high-fat consumption (Erridge et al., 2007; Amar et al., 2008; Ghanim et al., 2009; Laugerette et al., 2011a; Moreira et al., 2012a). LPS has a lipidic region, known as lipid A (Raetz & Whitfield, 2002) that can be incorporated into chylomicrons during fat digestion and absorption (Vreugdenhil et al., 2003; Ghoshal et al., 2009). During its passage to the bloodstream, chylomicrons can accumulate in the intercellular space, which may impair intestinal barrier function and favour LPS translocation (Tso & Balint, 1986; Kvietys et al., 1991; Shen et al., 2009; Salim & Soderholm, 2011). Thus, the regular consumption of high-fat diets can lead to excessive and prolonged chylomicronemia, which in turn may increase exposure to extra-hepatic LPS (Ghoshal et al., 2009).

The amount of dietary fat as well as its fatty acid composition can affect chylomicron secretion, lipemia, and postprandial endotoxemia (Laugerette et al., 2011a). Oleic acid induces chylomicron formation and increases LPS concentrations in parallel with higher expression of TNF-α in mesenteric lymph nodes in mice (Ghoshal et al., 2009). This observation suggests that oleic acid consumption could increase LPS absorption. Alternatively, oleic acid consumption is associated with an improvement in insulin sensitivity (Ryan et al., 2000), increase in fatty acid oxidation (Lim et al., 2013) and prevention of endothelium reticulum stress, inflammation and insulin resistance in skeletal muscle cells (Salvadó et al., 2013). The discrepancy in the results of these studies (Ryan et al., 2000; Ghoshal et al., 2009; Lim et al., 2013; Salvadó et al.,

2013) indicates that there is a need to clarify the effect of the consumption of oleic acid rich foods on LPS concentrations and other biochemical parameters.

Dietary factors such as phytochemicals (Ghanim et al., 2009; Ghanim et al., 2010; Deopurkar et al., 2010) and probiotics/prebiotics (Cani et al., 2007; De Bandt et al., 2011) are associated with lower endotoxemia, besides leading to a better metabolic profile and lower inflammatory markers, suggesting that the LPS concentration reduction could be a target in chronic diseases management. Studies evaluating the influence of food and/or specific nutrients in blood LPS concentrations can help to identify nutritional strategies to ameliorate postprandial endotoxemia and its metabolic complications.

Consumption of high-fat foods may not necessarily enhance endotoxin absorption if they present other nutritional advantages such as favourable fatty acid and fiber profile. Although peanuts have a high-fat content, its consumption has been associated with health benefits, including reduction of the risk for cardiovascular disease and type 2 diabetes (Bes-Rastrollo et al., 2009; Ghadimi et al., 2010; Mattes & Dreher, 2010; Reis et al., 2012). This has been attributed to its content of mostly unsaturated fatty acids, protein, dietary fiber, an array of vitamins and minerals, and bioactive substances (Bansode et al., 2012). Regular consumption of peanuts may reduce lipemia despite its high-fat content (Alper & Mattes, 2003). The reduced lipemia induced by peanuts may be associated with lower endotoxemia.

Therefore, the aims of the study were to evaluate the impact of acute consumption of two peanut cultivars differing in monounsaturated fatty acids (conventional and high-oleic peanut) content on postprandial endotoxemia in overweight and obese men and to determine its influence on postprandial lipemia and insulinemia.

2. EXPERIMENTAL METHODS

Subjects

Written advertisements and social networks were used for recruitment. One hundred and fifty men interested were screened. The inclusion criteria were: BMI between 26-35 kg/m^2, age range 18-50 years old, non-smoker, no food allergy and ethanol consumption lower than 168g/week. Weight changes over 3 kg, following weight loss diet, the use of drugs that affect the biochemical parameters evaluated in the study, recent diagnosis of acute or chronic

diseases other than obesity, and eating disorders were considered exclusion criteria. Sixty-five men were included in the study. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethical Committee in Human Research from Federal University of Viçosa, Brazil (protocol number 185/2011). All subjects provided written informed consent.

Study design

Sixty five men were randomized into three experimental groups: conventional peanuts (CVP, n=21), high-oleic peanuts (HOP, n=23), or control group (CT, n=21). Subjects were instructed not to consume alcohol, to refrain from heavy physical activity and to maintain a regular sleep-wake schedule (8 hours/night) during the three days prior to the experimental day. In addition, a standardized meal was given to the volunteers to be consumed the night before the test. This meal consisted of instant noodles (Nissin®), parmesan cheese (Santa Amália Alimentos®), and 200 ml of fruit juice (Sucos Tial®). It provided 731 kcal, 119 g of carbohydrate, 14 g of protein, and 23 g of fat. Volunteers attended the laboratory after a 10 h fast for all evaluations.

Anthropometrics, body composition and energy expenditure

Body weight, height, waist circumference, body composition and resting energy expenditure (REE) were assessed in the fasting state. Body fatness was measured through bioimpedance (model TBF-300, Tanita Corp., Arlington Heights, IL, USA). REE was measured for 30 min by indirect calorimetry (Deltatrac II, MBM-200; Datex-Engstrom Instrumentarium Corporation, Helsinki, Finland) following the protocol described by Alves et al. (2013).

Test meal

After initial evaluations in the fasting state, volunteers received the test meal. It was consumed within 15 min and consisted of a strawberry shake and 56 g of control biscuits or roasted conventional (IAC-886) or high-oleic (IAC-505) peanuts with skins.

The total energy provided to each subject corresponded to 25% of the individual estimated total energy expenditure (TEE). TEE was calculated by multiplying the measured REE and a physical activity factor (WHO, 2001). The

physical activity factor was determined through the International Physical activity Questionnaire (IPAQ) (Pardini et al., 2001). The meal provided similar volume and macronutrient contribution (35% carbohydrates, 16% proteins, and 49% fat). For the total group, this corresponded to a mean of 38.2 g of fat and 702 kcal. The minimum and maximum quantity of fat provided was 29.9 g and 50 g respectively, but it always represented 49% of the total energy of the meal.

The shake was prepared with powder for preparation of a strawberry flavored drink (Nesquik®, Nestlé), whole milk powder (Indústria de Milho Anchieta Ltda), soybean oil (Leve®, IMCOPA S/A.), whey protein isolate (Bem Vital®), water, and ice. The nutrient composition of both types of peanuts was analyzed according to validated methods (AOAC, 1997; AOAC, 2003) (Table 1). The control biscuits were developed to provide similar quantities of macronutrients, fiber, and energy density to the mean composition of peanuts. The composition of the biscuits was also analyzed (AOAC, 1997; AOAC, 2003). The ingredients used to prepare the biscuits were: eggs, whey protein isolate (Bem Vital®), whole wheat flour (Vitao Alimentos Integrais), margarine (Sadia S/A.), hydrogenated vegetable shortening (Vida Alimentos Ltda), soybean oil (Leve®, IMCOPA S/A.), dietary fiber supplement (Bem Vital®), sesame seed (Yoki Alimentos S/A.), wheat bran (Granum Alimentos Integrais), salt (Cisne®), and powder yeast (Yoki Alimentos S/A). The fatty acid composition of peanuts and biscuits are shown in Table 2. The fatty acid methyl esters were determined by gas chromatography (Shimadzu, Japan) according to Folch et al. (1957) and Hartman & Lago (1973).

Table 1. Nutritional composition of 56 g of the conventional and high-oleic peanuts

	Conventional peanuts IAC-886	**High-oleic peanuts IAC-505**
Available carbohydrate (g)	8.6	7.3
Protein (g)	16.8	16.3
Fat (g)	24.0	24.7
Energy (kcal)	317.6	316.7
Dietary fiber (g)	5.0	5.5
Soluble (g)	0.2	0.7
Insoluble (g)	4.8	4.8

Table 2. Fatty acids composition (%) of the conventional peanuts, high-oleic peanuts and control biscuits (Mean values and standard deviation)

Fatty acid	Conventional Peanuts IAC 886	High-oleic Peanuts IAC 505	Control biscuits
Lauric acid (C12:0)	-	-	0.43 ± 0.19
Palmitic acid (C16:0)	8.78 ± 0.07	5.23 ± 0.14	12.76 ± 0.75
Heptadecanoic acid (C17:0)	0.46 ± 0.00	0.18 ± 0.00	0.27 ± 0.00
Stearic acid (C18:0)	2.14 ± 0.06	2.08 ± 0.07	8.08 ± 0.36
Elaidic acid (C18:1n9t)	-	-	7.11 ± 0.29
Oleic acid (C18:1n9)	50.96 ± 0.47	81.47 ± 1.03	35.16 ± 0.49
Linolelaidic acid (C18:2n6t)	-	-	0.96 ± 0.03
Linoleic acid (C18:2n6)	31.93 ± 0.21	3.87 ± 0.03	32.48 ± 1.19
Arachidic acid (C20:0)	0.82 ± 0.03	1.19 ± 0.02	0.53 ± 0.02
Gamma-Linolenic acid (C18:3n6)	-	-	0.16 ± 0.00
Eicosenoic acid (C20:1n9)	0.82 ± 0.03	1.45 ± 0.02	1.06 ± 0.07
Alpha-Linolenic acid (C18:3n3)	0.28 ± 0.03	0.44 ± 0.03	1.44 ± 0.16
Behenic acid (C22:0)	2.59 ± 0.19	2.68 ± 0.17	-
Erucic acid (C22:1n9)	-	0.17 ± 0.02	-
Lignoceric acid (C24:0)	1.46 ± 0.05	1.65 ± 0.00	-
Total SFA	16.25 ± 0.40	13.01 ± 0.40	22.07 ± 1.31
Total MUFA	51.78 ± 0.50	83.09 ± 1.06	36.21 ± 0.56
Total PUFA	32.21 ± 0.23	4.30 ± 0.06	34.08 ± 1.35
Total Trans	-	-	8.07 ± 0.32

(-): not detected.

Biochemical analysis

A catheter was introduced into an antecubital vein of the volunteers after REE measurement. Blood was collected in the fasting state and after 1, 2, and 3 h postprandially. Plasma-EDTA and serum were separated from blood through centrifugation (2.200 x g, 15 min, 4°C) and stored at -80ºC.

Serum glucose, total cholesterol, HDL, TAG, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT) and alkaline phosphatase were analyzed through enzymatic colorimetric methods using commercial kits (Quibasa - Química Básica, Brazil) in a autoanalyzer (COBAS MIRA Plus; Roche Diagnostic Systems). High-sensitivity C-reactive protein (hsCRP) was also analyzed by a

commercial kit (Quibasa - Química Básica, Brazil) in an autoanalyzer by an immunotubidimetric assay.

LDL and VLDL concentrations were calculated according to Friedewald et al. (1972). Serum insulin concentrations were analyzed through electrochemiluminescence immunoassay (Elecsys-Modular E-170, Roche Diagnostics Systems). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to Matthews et al. (1985).

Fasting and postprandial plasma LPS concentrations were determined through a chromogenic method using a Limulus Amebocyte Lysate (LAL) commercial kit (Hycult Biotech, The Netherlands). Undiluted plasma samples were heated at 75ºC for 5 min to neutralize endotoxin inhibitors. Aliquots (50 µl) of plasma and standards were added to the pyrogen-free microplate. The LAL reagent (50 µl) was added in each well. After 30 min of incubation, absorbance was read at 405 nm (Multiskan Go, Thermo Scientific, USA). When the optical density of the standards 10 and 4 EU/ml differed by less than 10%, the reaction was interrupted by adding 50 µl of stop solution (acetic acid) and absorbance was read again. As absorbance is directly proportional to the concentration of endotoxin, a standard curve was used to calculate LPS concentration in the samples. The concentration of LPS was expressed as endotoxin units per milliliter (EU/ml).

Statistical analysis

Statistical analyses were performed with SAS software, version 9.2 (SAS Institute, Cary, NC, USA). Parametric and non-parametric tests were used based on normality testing (Shapiro-Wilk) and variance homogeneity (Levene) tests. A 5% level of significance was adopted. Data are represented as mean and standard error of the difference between means (SED) and delta (Δ = postprandial value – fasting value). Anthropometric, body composition, energy expenditure, and biochemical fasting variables from the three groups were compared using ANOVA with *post hoc* Tukey´s test or using Kruskal-Wallis with *post hoc* Dunn´s test as appropriate. Repeated measures two-way ANOVA (RMANOVA) tests were used to assess the differences between groups over time for LPS, glucose, insulin, and TAG, considering the treatment and time as repeated factors. The *post-hoc* Tukey-Kramer´s test was used when significance was detected by multiple comparisons. Spearman correlation test

was used to evaluate the associations between variables. After data analysis, the statistical power of the comparisons was calculated and values superior to 99% were found, confirming that the number of volunteers was sufficient to ensure the statistical power needed.

3. RESULTS

Baseline characterization

Energy expenditure, anthropometrics, body composition, and biochemical fasting variables are described in Table 3. All measured indices were similar among groups, except for the insulin concentration and HOMA-IR, which were higher in HOP compared to the CVP group.

Table 3. Characterization of total energy expenditure, anthropometric, body composition, and biochemical variables from experimental groups in fasted state (Mean values and standard error of the difference between means)

	CVP (n = 21)	HOP (n = 23)	CT (n = 21)
Age (years)	27.2 ± 1.4	27.7 ± 1.8	26.1 ± 1.4
TEE (kcal/day)	3057 ± 64	3048 ± 46.7	3047 ± 78.9
BMI (kg/m^2)	29.5 ± 0.4	30.2 ± 0.5	29.9 ± 0.6
Waist circumference (cm)	100.5 ± 1.3	102.2 ± 1.6	102.5 ± 2.1
Body fat (%)	25.7 ± 0.8	27.7 ± 0.8	26.7 ± 1.0
Uric acid (µmol/l)	309.3 ± 17.8	339.0 ± 5.9	333.1 ± 11.9
hsCRP (mg/dl)	1.6 ± 0.3	1.7 ± 0.3	1.0 ± 0.1
AST (UI/l)	39.4 ± 3.1	37.0 ± 4.4	35.9 ± 3.5
ALT (UI/l)	22.3 ± 1.4	26.0 ± 2.8	26.8 ± 4.4
GGT (UI/l)	35.7 ± 4.2	39.4 ± 5.3	34.7 ± 4.9
Alkaline phosphatase (UI/l)	56.8 ± 4.5	59.5 ± 5.9	49.1 ± 2.1
Glucose (mmol/l)	5.0 ± 0.1	5.1 ± 0.1	5.1 ± 0.1
Insulin (µU/ml)	7.3 ± 0.8^a	11.1 ± 1.0^b	9.1 ± 1.5^{ab}
HOMA-IR	1.6 ± 0.2^a	2.5 ± 0.2^b	2.1 ± 0.3^{ab}
TC (mmol/l)	5.0 ± 0.3	4.9 ± 0.2	4.6 ± 0.2
VLDL (mmol/l)	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
LDL (mmol/l)	3.3 ± 0.3	3.1 ± 0.2	3.0 ± 0.2
HDL (mmol/l)	1.2 ± 0.1	1.0 ± 0.01	1.0 ± 0.1
TAG (mmol/l)	1.5 ± 0.2	1.6 ± 0.1	1.3 ± 0.1
LPS (EU/ml)	1.1 ± 0.2	1.4 ± 0.2	1.4 ± 0.3

Different letters in the same line indicate statistical difference ($p \leq 0.05$) between groups (ANOVA or Kruskal-Wallis). Groups: CVP: conventional peanuts; HOP: high-oleic peanuts; CT: control. TEE: Total Energy Expenditure; hsCRP: high-sensitivity C-reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyltransferase; HOMA-IR: Homeostasis model assessment of insulin resistance; TC: total cholesterol; LPS: lipopolysaccharide.

Peanut consumption was associated with lower LPS concentrations at 3 h postprandial

Fasting plasma LPS concentrations were similar between the experimental groups. Compared to fasting concentrations, the increase in postprandial LPS was not significant within each experimental group. During the postprandial period, no difference in LPS concentrations was observed between experimental groups at the 1 and 2 h time points. However, in the last assessed postprandial period (3 h) the CT group had significantly higher LPS concentrations (1.6 ± 1.2 EU/mL) compared to the CVP (0.7 ± 0.5 EU/ml; p=0.002) and HOP (1.0 ± 0.9 EU/ml; p=0.039) groups (Figure 1).

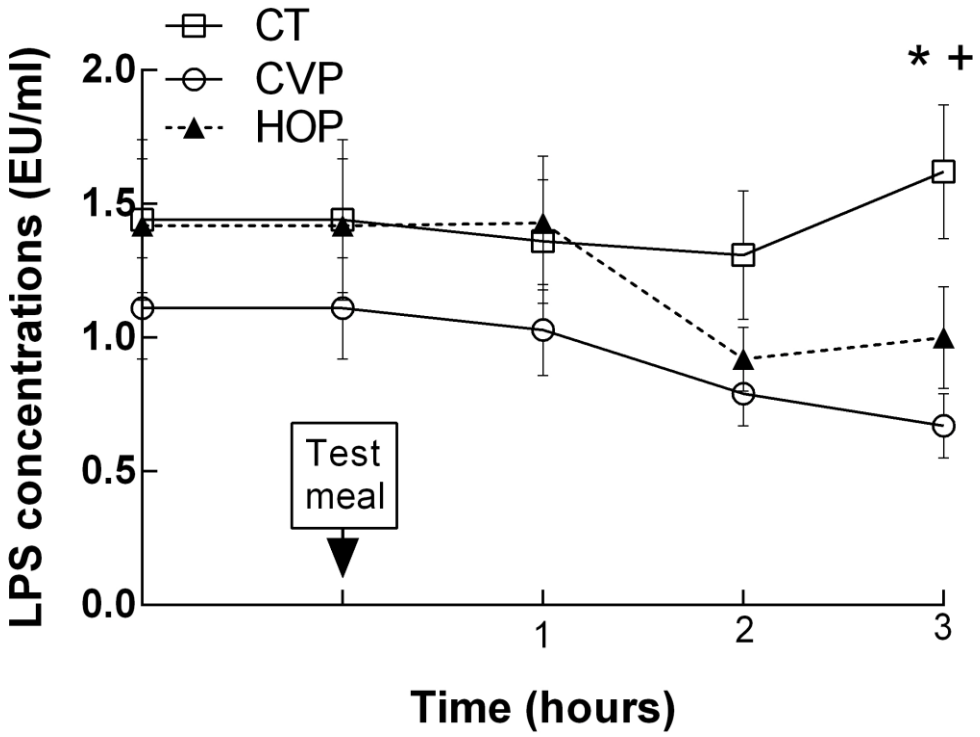

Figure 1: Mean (± SED) plasma lipopolysaccharide (LPS) concentration at fasting and after the consumption of the high-fat test meal. Experimental groups: conventional peanuts (CVP), high-oleic peanuts (HOP), and control biscuit (CT). Postprandial LPS concentrations did not differ from basal levels (p>0.05, RMANOVA) in all groups. Symbols represent statistical difference (p≤0.05) in LPS levels at 3h (2-*factor* RMANOVA) comparing: CVP *vs* CT$^{(*)}$, HOP *vs* CT$^{(+)}$, and CVP *vs* HOP$^{(\#)}$.

Peanut consumption delayed postprandial TAG increase

After meal consumption, all groups had increased serum TAG concentrations compared to the fasting state. This increase was significant at 1 h postprandial in the CT group (19.3%) and remained significantly higher at 2 and 3 h (37.1% and 36.1%, respectively). However, in the peanut groups, the TAG increase was delayed, starting only after 2 and 3 h. In the CVP and HOP groups, the increases were, 15.5% and 18.5% (2 h) and 24.7% and 31.5% (3 h) (Figure 2), respectively.

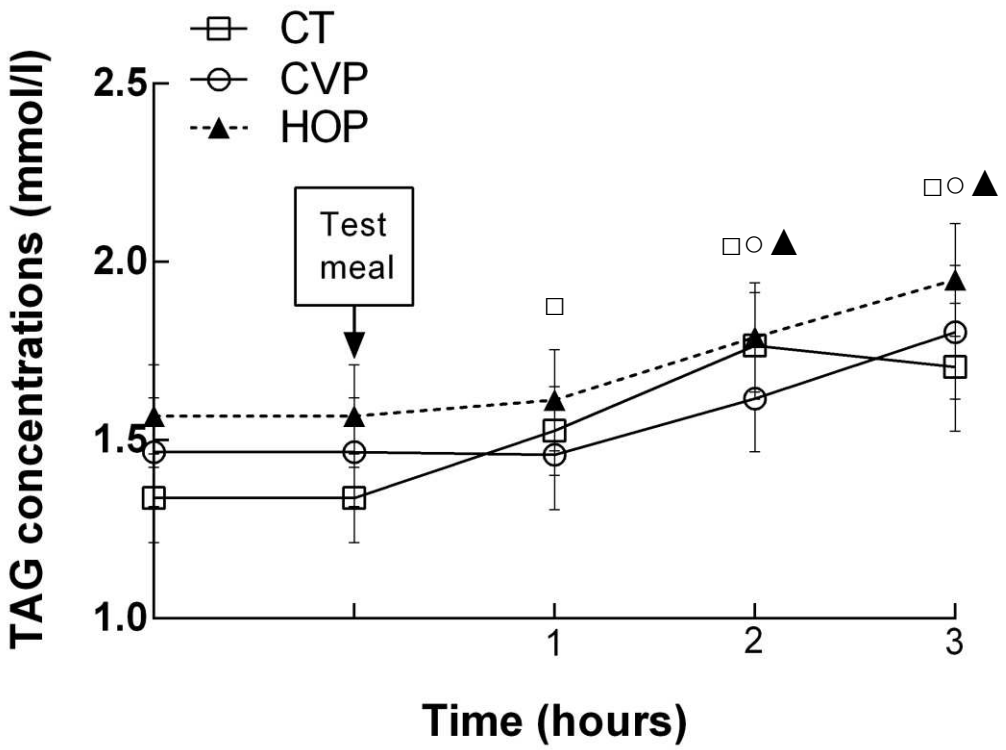

Figure 2: Mean (± SED) serum TAG at fasting and after the consumption of the high-fat meal. Experimental groups: conventional peanuts (CVP), high-oleic peanuts (HOP), and control biscuit (CT). Geometric symbols represent statistical significance ($p \leq 0.05$) for the comparison between fasting and postprandial values within each experimental group: CVP$^{(○)}$, HOP$^{(▲)}$, CT$^{(□)}$ (RMANOVA). No difference was observed ($p > 0.05$, 2-*factor* RMANOVA) for comparisons between CVP *or* HOP *vs* CT, and CVP *vs* HOP for each time point.

Peanut consumption was associated with lower postprandial insulinemia

Glucose concentrations did not increase after the high-fat meal consumption in any group (Figure 3A). The highest concentrations of insulin were observed 1 h postprandial: 2.5, 2.9 and 3.3 times higher for the CVP, HOP, and CT groups, respectively, compared to fasting concentrations (Figure 3B). At 2 h, insulinemia remained higher compared to fasting concentrations in all groups. However, in the third hour only in CT group the insulinemia remained higher compared to fasting state. The CVP group had lower insulinemia at all time points compared to the CT group. In addition, insulinemia was also lower in the CVP compared to the HOP group at 1 and 2 hours.

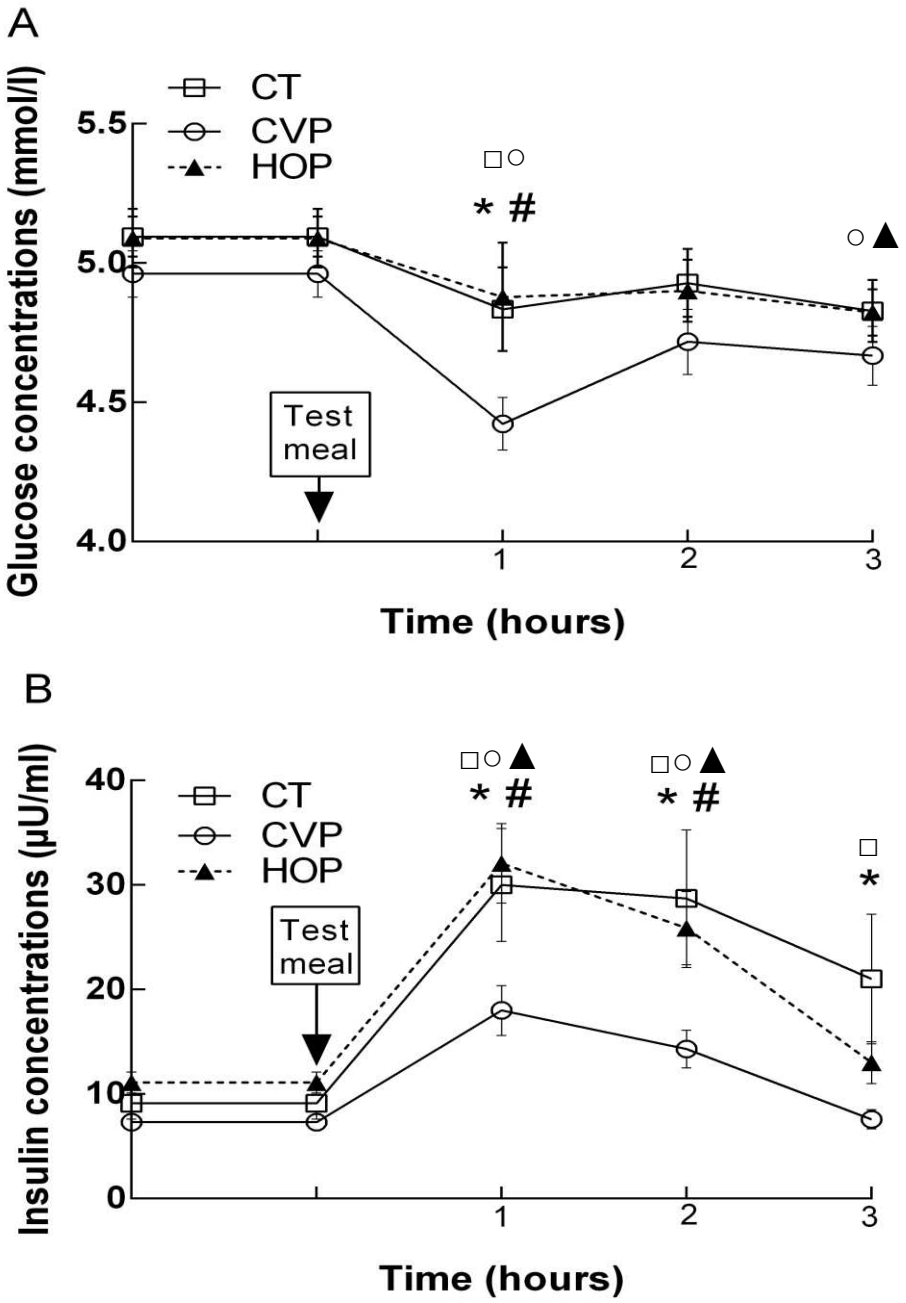

Figure 3: Mean (± SED) serum glucose (A) and serum insulin (B) concentrations at fasting and after the consumption of a high-fat meal. Experimental groups: conventional peanuts (CVP), high-oleic peanuts (HOP), and control biscuit (CT). Adjustments on p values for insulin were done by Tukey-Kramer's multiple group comparison procedures. Geometric symbols represent statistical significance ($p \leq 0.05$) for comparison between fasting and postprandial values for within experimental group: CVP$^{(\circ)}$, HOP$^{(\blacktriangle)}$, CT$^{(\square)}$ (RMANOVA). Comparisons between groups (2-*factor* RMANOVA) are shown by other symbols when significance was detected ($p \leq 0.05$): CVP *vs* CT$^{(*)}$, HOP *vs* CT$^{(+)}$, and CVP *vs* HOP$^{(\#)}$.

Fasting and postprandial TAG positively correlated with LPS concentrations

Fasting plasma LPS concentrations were positively correlated with fasting TAG concentrations. Additionally, the change of LPS concentration was positively correlated with for the change of TAG in the same time points (Table 4). There was a trend for a positive correlation between fasting concentrations of LPS and of insulin (r = 0.23, p=0.067). Anthropometric and body composition variables (BMI, waist circumference and body fat) did not correlate with LPS concentration (data not shown).

Table 4. Spearman correlation coefficients between LPS, glucose, insulin and TAG concentrations at fasting and after high-fat meal consumption (delta)

	Fasting LPS	Δ LPS 1 h	Δ LPS 2 h	Δ LPS 3 h
Fasting glucose	0.13	-	-	-
HOMA-IR	0.24	-	-	-
Fasting insulin	0.23	-	-	-
Insulin 1 h	-	-0.08	-	-
Insulin 2 h	-	-0.06	-0.19	-
Insulin 3 h	-	-0.12	-0.10	-0.16
Fasting TAG	0.27[a]	-	-	-
Δ TAG 1 h	-	0.26[a]	0.27[a]	0.35[a]
Δ TAG 2 h	-	-	0.25[a]	0.35[a]
Δ TAG 3 h	-	-	-	0.26[a]

Delta (Δ): postprandial value – fasting value; LPS: lipopolisccharide; HOMA-IR: *Homeostasis model assessment of insulin resistance*.

[a] $p \leq 0.05$.

4. DISCUSSION

The consumption of high-fat diets has been associated with increased LPS concentrations (Erridge et al., 2007; Ghanim et al., 2009; Ghanim et al., 2010; Laugerette et al., 2011b). There are evidences that plasma endotoxemia is not necessarily associated with dietary fat content, but rather with fat quality (Laugerette et al., 2012). To our knowledge, we observed for the first time the postprandial endotoxemia induced by the acute consumption of high-fat meal containing peanut cultivars with different levels of oleic acid.

In the present study, contrary to previous findings (Erridge et al., 2007; Ghanim et al., 2009; Ghanim et al., 2010; Laugerette et al., 2011b), our high-fat meal did not induce a significant increase in LPS concentrations in all test meals compared to fasting state, at least until 3h postprandial. In the last postprandial time point (3h), the control group started to show a trend for LPS increase, but it was not significant, indicating that for a given percentage of dietary fat consumption, other factors influence LPS increase. This is the first study in which the quantity of calories and fat of the test meal was not fixed for all subjects. Instead, it was based on the principle that each individual has a particular nutritional need according to their metabolic rate. The previous studies tested fixed quantities of fat and showed significant increase in LPS concentrations compared to baseline. The LPS peaks occurred in different postprandial time points (range 30 - 300 min) (Erridge et al., 2007; Ghanim et al., 2009; Ghanim et al., 2010; Laugerette et al., 2011b). A possible explanation could be the differences in the food items of the meal, since the net amount of fat and its percentage contribution of calories (range 33 g - 51 g of fat and 33% - 50% of the total calories of the meal) was close to ours (average consumption of 38 g of fat and 49% of the total calories of the meal).

A difference between groups in LPS concentrations was observed at 3 h postprandial, with peanut groups showing lower levels. Although the meal composition was very similar between groups (regarding total macronutrients and fiber), the fatty acid profile and the food structure (biscuits *vs* peanuts) differed. The different fatty acid composition and other dietary components in peanuts may have contributed to this result, confirming also in humans that overall meal composition is more important than its net fat content in determining postprandial LPS increase. The inclusion of orange juice to a high-fat meal may have prevented an increase in postprandial LPS (Ghanim et al.,

2010). This effect was suggested to be a consequence of the flavonoids content in the fruit juice. Flavonoids are able to bind and reduce the re-absorption of bile acids and sterols in the intestine causing a hypolipidaemic response (Sudheesh et al., 1997). Peanuts also have the flavonoids, resveratrol, and fiber (Lou et al., 2001; Francisco & Resurreccion, 2008; Bansode et al., 2012) that might exert a hypolipidaemic effect.

Food chewing, digestion and absorption may also interfere with LPS absorption. Poor bioaccessibility of lipid and nutrients, has been demonstrated in humans after the consumption of whole peanuts compared to peanut butter, oil, and flour (Traoret et al., 2008). This may partly explain the delayed increase in postprandial TAG observed after the consumption of both peanut cultivars and also their lower LPS concentrations at 3 h. The positive correlation between LPS and fasting/postprandial TAG indicates how linked might be the digestibility of food and subsequent availability of fat droplets in determining postprandial endotoxemia. Fasting TAG is reportedly a predictor of basal concentrations of LPS (Clemente-Postigo et al., 2012). Further, subjects with higher postprandial hypertriglyceridemia have a significant increase in LPS after fat overload. Components of peanuts that block a TAG rise could therefore block a LPS rise with a high-fat meal.

The higher oleic acid content of the peanut cultivar IAC-505 does not seem to increase LPS absorption. Oleic acid was initially suggested by Ghoshal et al. (2009) to induce chylomicron formation in mice and increase LPS translocation compared to the short chain butyric acid. However, in another study with pigs, olive oil, another rich source of oleic fatty acid, reduced LPS transport in the intestinal epithelium compared to coconut oil, high in saturated fatty acids. Fish oil, has high omega-3 fatty acids content and also reduces LPS transport and plasma endotoxemia compared to coconut oil (Mani et al., 2013). This suggests that higher saturated fatty acid content may augment, while unsaturated fatty acids moderate LPS absorption through the intestinal epithelium.

One of the major concerns of increasing blood LPS concentration is its promotion of insulin resistance (Hotamisligil, 2003; Moreira et al., 2012b). The interaction of LPS with Toll-like receptors (TLR4) activates pathways involving NF-κB and mitogen-activated protein kinase (MAPK) (Song et al., 2006; Cani & Delzenne, 2011). This could lead to expression of genes that codify proteins

involved in inflammatory responses such as TNF-α, IL-6, inducible nitric oxide synthase (iNOS) and monocyte chemoattractant protein-1 (MCP-1). These molecules are able to induce phosphorylation of insulin receptor substrate (IRS) in serine residues. This action exerts a negative effect over insulin signalling, leading to insulin resistance and/or hyperinsulinemia, which are frequently observed in metabolic diseases such as obesity and diabetes (Gao et al., 2002; White, 2002; Hotamisligil, 2003; Moreira et al., 2012b). However, in this trial, fasting or postprandial insulin did not correlate significantly with fasting and postprandial LPS concentrations. However, we observed a lower postprandial insulin response after conventional peanut consumption. Continuous pancreatic stimulation and repeated postprandial excursions of insulin are promoted by Western-type diet consumption. It is hypothesized that excessive insulin stimulation may predispose to insulin resistance, β-cell dysfunction, and ultimately type 2 diabetes. Decreased postprandial insulin and glucose responses are common therapeutic targets that may be achieved through diets high in fiber and whole-grain cereal products (Juntunen et al., 2003). The different insulin responses observed for the peanut groups may be explained by the fatty acid profile of the peanuts. Insulin secretion is primarily induced by glucose but, in cell culture, the saturated fatty acid palmitate (Warnotte et al., 1994) as well as oleic acid (Fujiwara et al., 2005) act synergistically to increase insulin release induced by glucose. In men, acute NEFA elevation (90 min) induces insulin resistance, which was precisely countered by an induced increase in insulin release. Similarly to acute tests, chronic (48h) NEFA elevation also promotes insulin resistance. However, it may block the β-cell compensatory insulin release (Carpentier et al., 1999). Within this context, we hypothesize that the lower insulin response induced by peanut (especially conventional) consumption could be beneficial in the long term.

In summary, acute peanut consumption within a high-fat meal delays the postprandial TAG increase and does not contribute to postprandial endotoxemia. The digestibility of a meal might be an important factor influencing endotoxemia even when high-fat load is provided and further studies should address this topic. Conventional peanut consumption was associated with decreased postprandial insulinemia, which might be beneficial for saving β-cell function, independently of the influence on LPS concentrations. High-oleic peanuts do not potentiate LPS absorption, but the higher insulin response

compared to conventional peanuts might indicate that its consumption is less advantageous. The consumption of conventional or high-oleic peanuts, may aid reducing the risk of endotoxemia and metabolic disorders.

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3.3. Artigo 3: Effect of a hypocaloric diet containing peanut cultivars differing in oleic acid content on intestinal permeability and endotoxemia

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Abstract

The aim of this study was to assess the effects of a hypocaloric diet containing peanuts cultivars differing in oleic acid content (conventional or high-oleic peanuts) on intestinal permeability, plasma lipopolysaccharide (LPS) and biochemical parameters in overweight and obese men. In addition, we investigated if short-term induced weight loss could change the LPS postprandial response to high-fat test meals containing peanuts. A group of fifty-five men participated in this 4-weeks randomized clinical trial. The subjects were randomly assigned to one of the three experimental groups: conventional peanuts (CVP, n=19), high-oleic peanuts (HOP, n=18) or control (CT, n=18). It was prescribed a hypocaloric (-250 kcal/day) balanced-diet, which included (CVP and HOP) or not (CT) a portion of 56 g/day of peanuts. Intestinal permeability, anthropometry and body composition were assessed at baseline and at the end of the trial. Blood samples were collected in the fasting state and 1, 2, and 3 h postprandial. The consumption of hypocaloric diet associated with the consumption of both types of peanut cultivars did not change intestinal permeability, plasma concentrations of fasting LPS or LPS postprandial responses to the high-fat meals. Energy restriction was efficient to promote a reduction in body weight, waist circumference, sagittal abdominal diameter, and total body fat mass in all groups. Only the CT group experienced a reduction in fat free mass. Our results suggest that the consumption of peanuts-enriched hypocaloric diets may contribute to weight loss, and to improve body composition and some biochemical parameters.

Key words: Peanuts, oleic acid, lipopolysaccharide, intestinal permeability.

1. INTRODUCTION

Lipopolysaccharides (LPS) are molecules derived from the outer membrane of gram-negative bacteria, whose chronic, low-dose, intravenous infusion has been shown to trigger weight gain, obesity and insulin resistance (Cani et al., 2007). The results of several studies have demonstrated that blood LPS concentrations are, in fact, higher in obese and diabetic subjects (Creely et al., 2007; Basu et al., 2011; Pussinen et al., 2011; Harte et al., 2012). High-fat diets can also increase LPS in the circulation, besides inducing obesity and insulin resistance (Cani et al., 2007; Laugerette et al., 2012). This indicates that changes in dietary fat consumption could affect LPS concentration, which may be a target for metabolic improvements.

Although gut is a huge source of LPS, it also acts as a barrier to its passage. Thus, higher circulating LPS concentrations may imply disruption of the barrier function and higher absorption through enterocytes. Fat overload can influence LPS passage from the gut by increasing intestinal permeability (Cani et al., 2008; Cani & Delzenne, 2009; Suzuki & Hara, 2010) and inducing chylomicrons formation, which incorporates LPS particles inside its structure, with subsequent absorption (Ghoshal et al., 2009; Laugerette et al., 2011; Clemente-Postigo et al., 2012). The postprandial increase in LPS is followed by acute increases in inflammatory and oxidative stress markers (Ghanim et al., 2009; Ghanim et al., 2010; Laugerette et al., 2011), indicating that continuous intake of high-fat meals may have detrimental effects to health.

LPS plasma increase and the consequences of this increment are affected by dietary fatty acid profile (Ghoshal et al., 2009; Moreira et al., 2012; Laugerette et al., 2012). Oleic acid, for example, stimulated LPS increase through induction of chylomicron formation in mice (Ghoshal et al., 2009). However, it may not disrupt barrier function since it did not change tight junctions permeability *in vitro* (Usami et al., 2001). This suggests that food fatty acid profile may lead to different effects on intestinal permeability and blood LPS concentrations. Measurements of intestinal permeability in overweight/obese subjects (Brigdarnelo et al., 2010; Teixeira et al., 2012) are poorly explored, especially after an intervention for weight loss, being an interesting field of research.

On the other hand, high-fat foods may be included in diets when its fatty acid profile and overall composition is favorable. In particular, peanuts are

nutrient-dense foods with complex matrix, rich in unsaturated fatty acids and other bioactive compounds, such as L-arginine, fiber, minerals, tocopherols, phytosterols, and polyphenols (Ros et al., 2010). This unique composition may favor body weight management and lower body fat accumulation (Reis et al., 2012; Flores-Mateo et al., 2013; Moreno et al., 2013). These effects have been associated with peanuts satiation property (Reis et al., 2012), greater thermogenic effect of unsaturated fatty acids (Casas-Agustench et al., 2009), which are also preferably oxidized compared to saturated fatty acids (Piers et al. 2002). In addition, the fat contained within walled cellular structures in peanuts contributes to a small degree of fat malabsorption (Ellis et al., 2004; Flores-Mateo et al., 2013), which may have implications for LPS absorption. Higher oleic acid content peanuts have been cultivated. Its nutritional benefits, as well as its effects on intestinal permeability and LPS concentrations have not been investigated yet. Preliminary results of a study conducted by our group showed that compared to a control high-fat meal the acute consumption of conventional or high-oleic peanuts was associated with delayed triglyceridemia and lower 3 h postprandial LPS concentrations. These results suggest that regardless of its high-fat content peanuts consumption may lead to potential heath benefits in the long term basis.

Because higher concentrations of LPS are usually associated with weight gain and higher body weight, we assessed the effect of the consumption during 4-weeks of peanut cultivars with different oleic acid content included in a normal fat content hypocaloric diet on intestinal permeability, plasma LPS and other biochemical parameters in the fasting state in overweight and obese men. In addition, we investigated if short-term induced weight loss changes the LPS postprandial concentration in responses to a peanut high-fat test meal.

2. EXPERIMENTAL METHODS

Subjects and study design

Subjects were recruited through written advertisements and social networks. One hundred and fifty men were screened. The inclusion criteria were: body mass index (BMI) between 26-35 kg/m^2, age range 18-50 years old, non-smoker, no food allergy, and ethanol consumption lower than 168g/week. Subjects showing weight changes over 3 kg in the previous 3 months, following

weight loss diet, using drugs that affect the biochemical parameters evaluated in the study, with recent diagnosis of acute or chronic diseases other than obesity, and eating disorders were excluded.

This was an interventional, randomized, clinical trial of 4-weeks duration. Subjects were randomly assigned to one of the three experimental groups: conventional peanuts (CVP), high-oleic peanuts (HOP) or control (CT). Seventy six subjects were initially included; however, 69 completed the study. Yet, only 55 subjects were included in the final analysis: CVP (n=19), HOP (n=18) and CT (n=18). The reasons for exclusions were non-compliance to protocol, low adherence to the diet or technical problems at final assessments.

All the evaluations were performed in the first and last day of the study, after 10 hours of overnight fast. The subjects remained in the laboratory for 6.5 hours for this purpose. A standardized meal was given to the volunteers to be consumed the night before the first and last day of evaluations. This meal consisted of instant noodles (Nissin®), parmesan cheese (Santa Amália Alimentos®), and 200 ml of fruit juice (Sucos Tial®). It provided 731 kcal, 119 g of carbohydrate, 14 g of protein, and 23 g of fat. Subjects were also instructed not to consume alcohol. A list of foods containing mannitol and lactulose was given to the subjects, and they were instructed to exclude them from their diets. Participants also refrained from heavy physical activity and to maintain a regular sleep-wake schedule (8 hours/night) during the three days prior to the assessments. Subjects were asked to come the laboratory weekly for dietary counseling and to maintain the habitual level of physical activity.

The study protocol was previously approved by the Ethical Committee in Human Research from Federal University of Viçosa, Brazil (protocol number 185/2011). All subjects provided written informed consent.

Dietary intervention

Hypocaloric diets (restriction of 250 kcal/day to induce approximately 1 kg of weight loss during the intervention period) were individually prescribed, considering the total energy expenditure (TEE) of each subject. TEE was calculated by multiplying the measured resting energy expenditure (REE) and the physical activity factor (WHO, 2001). REE was measured over 30 minutes under fasting conditions through indirect calorimetry, using a ventilated respiratory canopy (Deltatrac II, MBM-200; Datex-Engstrom Instrumentarium

Corporation, Helsinki, Finland) in full compliance with the manufacturer guidelines. The physical activity factor was determined using the International Physical activity Questionnaire (IPAQ) (Pardini et al., 2001).

The prescribed diets contained 55% carbohydrate, 15% protein and 30% fat (IOM, 2005). The total caloric prescription was distributed in 5-6 meals a day.

Dietary intervention was implemented in a free-living condition. Thus, all subjects received nutritional advice and a one-day plan menu containing foods according to their preferences. They also received and were instructed on how to use a substitution food list. This list contained food items assigned into categories according to their macronutrient composition, allowing subjects to plan their own menus and choose foods for their meals based on their one-day dietary prescription.

Seven servings containing 56 g of roasted peanuts with skin were provided weekly to subjects from CVP (IAC-886) and HOP (IAC-505) groups. They were instructed to consume one serving of peanuts daily, at the preferred day-time and at once. The caloric value of the peanut serving was considered in the prescribed diet. The diet prescribed to CT group had the same level of caloric restriction, and subjects were instructed not to consume peanuts.

Anthropometry and body composition

Body weight, height, waist circumference, sagital abdominal diameter (SAD), body composition and REE were assessed in the fasting state. The SAD was measured with an abdominal caliper (Holtain Kahn Abdominal Caliper®). The subject laid in the supine position and the measurement was carried out at the same point of waist circumference, which reflects mainly visceral adipose tissue (Vasques et al., 2009). Body fatness was measured through bioimpedance (model TBF-300, Tanita Corp., Arlington Heights, IL, USA).

Dietary intake assessment

Subjects provided 3-day food records (two non-consecutive week days and one weekend day), filled out on the week before the baseline assessments and at the fourth week of the intervention period. A dietitian reviewed the food records with the subjects to check for errors or omissions. All the food records

were analyzed by the same dietitian using software Dietpro 5.2i (Agromídia, Viçosa, Brazil).

Biochemical analysis

The blood samples were collected from the antecubital vein in fasting and postprandial (1, 2 and 3 h after the intake of a high-fat test meal) states. Plasma-EDTA and serum were separated from blood through centrifugation (2.200 x g, 15 min, 4°C) and stored at -80ºC. Fasting serum glucose, total cholesterol, HDL, triacylglycerols, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase were analyzed through enzymatic colorimetric method using commercial kits (Quibasa - Química Básica, Brazil) in an autoanalyzer (COBAS MIRA Plus; Roche Diagnostic Systems). The high-sensitivity C-reactive protein (hsCRP) was also analyzed by a commercial kit (Quibasa - Química Básica, Brazil) in an autoanalyzer by an immunotubidimetric assay. LDL and VLDL concentrations were calculated according to Friedewald´s et al. (1972) formula. Serum insulin concentrations were analyzed through electrochemiluminescence immunoassay (Elecsys-Modular E-170, Roche Diagnostics Systems). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to Matthews et al. (1985). Glucose, insulin and triacylglycerols were also analyzed in the postprandial period using the same methods.

Plasma lipopolysaccharides

Fasting and postprandial (1, 2 and 3 h after the intake of a high-fat test meal) plasma LPS concentrations were determined through a chromogenic method using Limulus Amebocyte Lysate (LAL) commercial kit (Hycult Biotech, The Netherlands). Undiluted plasma samples were heated at 75ºC for 5 min to neutralize endotoxin inhibitors. Aliquots (50 µl) of plasma and standards were added to the pyrogen-free microplate. The LAL reagent (50 µl) was added in each well. After 30 min of incubation, absorbance was read at 405 nm (Multiskan Go, Thermo Scientific, USA). When the optical density of the standards 10 and 4 EU/ml differed by less than 10%, the reaction was interrupted by adding 50 µl of stop solution (acetic acid) and absorbance was read again. As absorbance is directly proportional to the concentration of endotoxin, a standard curve was used to calculate LPS concentration in the

samples. The LPS concentration was expressed as endotoxin units per milliliter (EU/ml).

Intestinal permeability

The subjects were asked to eliminate any eventual residual urine upon arrival to the laboratory. An isomolar solution (200 ml) containing the sugar probes lactulose (7.6 g, Colonac®) and mannitol (2.0 g, Synth®) was given orally. Then, urine was collected over a period of 6 h. Two hours after the solution had been ingested the subjects received a high-fat test meal. At the end of the 6 h, the whole volume of urine was measured and an aliquot of 50 ml was taken, to which 12 mg of thimerosal (Synth®) was added to prevent bacterial growth. The urine samples were stored at -20ºC.

Two milliliters of the stored urine samples were centrifuged (10.000 rpm, 10 min, 4º C) and filtered through a micropore membrane (0.22 µl, Millipore, USA). Lactulose and mannitol were quantified through high-performance liquid chromatography (Shimadzu® system, model SPD-10A VP) using refractive index detector - RID 6A. The volume of filtered urine injected was 20 µl. Mobile phase was composed of 5mM sulfuric acid in water, used at a predetermined flow rate of 0.8 ml/min, providing a pressure of 45 kgf into the column BIORAD (30 cm x 7.9 mm), which was heated to 80ºC. Under these conditions, lactulose and mannitol standards had retention times of 6.3 and 8.0 min, respectively. Standards curves were used to determine the concentration of sugar probes in urine samples. The net amount of sugar probes excreted was calculated multiplying the determined concentration of each sugar probe in the urine by the total volume of urine collected over 6 h. Then, the dose of sugar probes administered was used to calculate the percentage of lactulose (%L) and mannitol (%M) doses that were excreted in the urine. These results were used to calculate Lactulose/Mannitol ratio (L/M).

High-fat test meal

In the first and last day of study, subjects were given one of the high-fat test meals, two hours after the consumption of sugar probes. Its caloric content corresponded to 25% of each individual´s TEE and provided 35% of carbohydrate, 16% of protein, and 49% of fat. Thus, the net amount of calories and fat varied between subjects, according to their physiological needs.

The meal was consumed within 15 min and consisted of a strawberry shake and 56 g of control biscuit or roasted conventional or high-oleic peanuts with skin, according to the experimental group that the individual was allocated. The subjects received the same volume of liquid. The control biscuit was developed to provide similar quantities of macronutrients, fiber, and energy density to the mean composition of peanuts. The ingredients used to prepare the biscuit were: eggs, whey protein isolate (Bem Vital®), whole wheat flour (Vitao Alimentos Integrais), margarine (Sadia S/A.), hydrogenated vegetable shortening (Vida Alimentos Ltda), soybean oil (Leve®, IMCOPA S/A.), dietary fiber supplement (Bem Vital®), sesame seed (Yoki Alimentos S/A.), wheat bran (Granum Alimentos Integrais), salt (Cisne®), and powder yeast (Yoki Alimentos S/A). The nutrient composition of both types of peanuts was analyzed according to validated methods (AOAC, 1997, AOAC, 2003). The fatty acid methyl esters content was determined by gas chromatography (Shimadzu, Japan) according to Folch et al. (1957) and Hartman & Lago (1973). The macronutrients and fatty acids composition of peanuts and biscuit are shown in supplementary material.

Statistical analysis

Statistical analyses were performed with SAS software, version 9.2 (SAS Institute, Cary, NC, USA). Parametric and non-parametric tests were used based on normality (Shapiro-Wilk) and variance homogeneity (Levene) tests. A 5% level of significance was adopted. Data are represented as mean and standard error of the difference between means (SED). The results of postprandial LPS, triacylglycerols, insulin, and glucose were expressed as the positive incremental area under the curve ($_{pi}$AUC), calculated using GraphPad Prism, version 6 (GraphPad software Inc). Anthropometric, body composition, dietary intake, energy expenditure, and biochemical variables, as well as changes (Δ=final–baseline) from the three groups were compared using ANOVA with *post hoc* Tukey´s test or using Kruskal-Wallis with *post hoc* Dunn´s test as appropriate. Changes in all variables were compared within each group by using the paired t-test or the Wilcoxon test, as appropriate. The pairwise tests were performed to compare the caloric restriction prescribed and 4-week caloric intake. After data analysis, the statistical power of the comparisons was calculated and values superior to 99% were found, confirming that the number of volunteers was sufficient to ensure the statistical power needed.

3. RESULTS

Subjects and baseline characteristics

At baseline, energy expenditure, anthropometric, body composition, and biochemical variables did not differ between groups, except for the insulin concentrations and HOMA-IR, which were higher in HOP group in comparison to CVP. In addition, L/M ratio was lower in CT than in the peanuts groups. However, fasting plasma LPS concentrations did not differ between groups (Table 1).

The dietary data from the food record filled the week before baseline assessments showed that the habitual dietary intake were not different between groups (data not shown).

Table 1. Characterization of total energy expenditure, anthropometric, body composition, biochemical, and intestinal permeability parameters[1] presented by the subjects at baseline according to study group

	CVP (n=19)	HOP (n=18)	CT (n=18)	P
Age (years)	28.5 ± 1.7	27.8 ± 2.1	27.1 ± 1.9	0.28
TEE (kcal/day)	3037.8 ± 84.8	3082.4 ± 55.3	3026.3 ± 79.1	0.86
BMI (kg/m^2)	29.4 ± 0.5	30.3 ± 0.6	29.5 ± 0.6	0.62
Waist circumference (cm)	100.7 ± 1.4	102.5 ± 2.0	101.8 ± 2.3	0.79
SAD (cm)	22.6 ± 0.4	23.5 ± 0.6	23.1 ± 0.7	0.48
Body fat (%)	25.9 ± 0.9	27.7 ± 1.0	26.0 ± 0.9	0.34
Fat mass (kg)	24.1 ± 1.2	26.9 ± 1.5	24.7 ± 1.6	0.48
Fat free mass (kg)	68.3 ± 1.5	69.4 ± 1.6	69.2 ± 1.7	0.88
hsCRP (mg/dl)	1.5 ± 0.3	1.7 ± 0.3	1.4 ± 0.4	0.34
AST (UI/l)	38.4 ± 3.1	33.2 ± 2.6	34.0 ± 3.3	0.36
ALT (UI/l)	23.9 ± 2.3	23.8 ± 2.5	25.2 ± 3.7	0.90
GGT (UI/l)	35.7 ± 4.4	37.9 ± 6.3	36.7 ± 5.3	0.98
AP (UI/l)	58.0 ± 5.0	63.3 ± 7.4	50.4 ± 2.5	0.40
Glucose (mmol/l)	5.1 ± 0.1	5.1 ± 0.1	5.2 ± 0.1	0.68
Insulin (µU/ml)	8.1 ± 0.9^a	11.7 ± 1.1^b	8.7 ± 1.4^{ab}	0.02
HOMA-IR	1.9 ± 0.2^a	2.7 ± 0.3^b	2.0 ± 0.3^{ab}	0.03
TC (mmol/l)	5.0 ± 0.3	4.8 ± 0.3	4.9 ± 0.3	0.88
VLDL (mmol/l)	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.75
LDL (mmol/l)	3.2 ± 0.3	2.9 ± 0.2	3.2 ± 0.2	0.57
HDL (mmol/l)	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.0	0.36
TAG (mmol/l)	1.6 ± 0.2	1.8 ± 0.2	1.6 ± 0.1	0.75
LPS (EU/ml)	1.1 ± 0.2	1.2 ± 0.3	1.4 ± 0.3	0.99
$_{pi}$AUC LPS	1.0 ± 0.3	1.0 ± 0.3	1.5 ± 0.4	0.82
$_{pi}$AUC TAG	48.0 ± 11.8	57.0 ± 13.4	84.3 ± 16.7	0.23
$_{pi}$AUC insulin	20.1 ± 3.2	37.1 ± 6.8	48.7 ± 13.3	0.16
$_{pi}$AUC glucose	1.7 ± 0.8	6.6 ± 2.3	3.8 ± 1.6	0.06
%L	0.55 ± 0.11	0.42 ± 0.05	0.36 ± 0.04	0.43
%M	17.31 ± 3.72	13.78 ± 1.76	13.93 ± 1.67	0.90
L/M	0.033 ± 0.002^a	0.032 ± 0.002^a	0.027 ± 0.001^b	0.04

[1]Data are represented as mean ± standard error of the mean (SEM). Different letters in the same line indicate statistical difference ($p<0.05$) between groups (ANOVA or Kruskal-Wallis). Groups: CVP: conventional peanuts; HOP: high-oleic peanuts; CT: control; TEE: Total Energy Expenditure; BMI: body mass index; SAD: sagittal abdominal diameter; hsCRP: high-sensitivity C-reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyltransferase; AP: alkaline phosphatase; HOMA-IR: Homeostasis model assessment of insulin resistance; TC: total cholesterol; piAUC: positive incremental area under the curve; LPS: lipopolysaccharide; TAG: triacylglycerols; %L: lactuose excretion; %M: mannitol excretion; L/M: lactulose/mannitol ratio.

Dietary intake from the fourth week of intervention

Although the dietary restriction prescribed was similar in all groups (-250 kcal or equivalent to an average of 8.2% of TEE), data from dietary records obtained on the fourth week showed that subjects of CT group experimented a higher energy restriction (27.4 ± 4.6%) than did CVP and HOP groups (14.9 ± 5.8% and 14.0 ± 5.8%, respectively) ($p<0.05$). Table 2 summarizes dietary intake from the fourth week of intervention.

Table 2. Consumption of energy, macronutrients (g and percentage of total energy intake), and fiber[1] on the fourth week of the study according to the experimental group

	CVP (n=14)	HOP (n=14)	CT (n=11)	P
Energy (kcal/day)	2539.2 ± 160.8	2642.7 ± 184.1	2252.0 ± 176.6	0.30
Carbohydrate (g)	312.4 ± 20.9	305.9 ± 22.1	300.5 ± 22.4	0.93
Carbohydrate (%)	49.3 ± 1.2^{ab}	46.7 ± 2.1^{a}	53.8 ± 1.0^{b}	<0.01
Protein (g)	100.4 ± 7.4	121.0 ± 6.7	98.4 ± 7.7	0.06
Protein (%)	15.7 ± 0.5^{a}	18.6 ± 0.7^{b}	17.7 ± 0.8^{ab}	<0.01
Fat (g)	98.6 ± 7.0^{ab}	103.9 ± 13.5^{a}	72.9 ± 7.3^{b}	0.04
Fat (%)	34.9 ± 1.2^{a}	34.6 ± 1.9^{a}	28.5 ± 1.1^{b}	<0.01
SFA (g)	28.1 ± 2.3	30.6 ± 4.9	24.5 ± 2.2	0.68
SFA (%)	10.1 ± 0.8	9.9 ± 0.5	9.8 ± 0.6	0.95
MUFA (g)	29.0 ± 2.5^{a}	42.4 ± 6.0^{b}	18.1 ± 2.0^{a}	<0.01
MUFA (%)	10.3 ± 0.6^{a}	14.2 ± 1.0^{b}	7.3 ± 0.5^{c}	<0.01
PUFA (g)	19.1 ± 1.9^{a}	14.1 ± 1.3^{a}	11.7 ± 1.8^{b}	0.01
PUFA (%)	6.6 ± 0.4^{a}	4.8 ± 0.3^{b}	4.5 ± 0.5^{b}	<0.01
Dietary fiber (g/day)	24.9 ± 2.2	30.9 ± 2.3	27.7 ± 3.2	0.23

[1]Data are represented as mean ± standard error of the mean (SEM). Different letters in the same line indicate statistical difference ($p<0.05$) between groups (ANOVA or Kruskal-Wallis). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Anthropometric, body composition and fasting biochemical variables

In comparison to baseline, the intervention significantly decreased body weight, BMI, waist circumference, and SAD similarly in all groups (Table 3). The percentage of weight loss was similar between groups and corresponded to 1.9 ± 0.4% in HOP, 1.6 ± 0.4% in CVP, and 2.1 ± 0.4% in CT (p=0.66), while changes in body composition slightly differed. Reduction of body fat percentage was significant only in HOP group, while it tended to decrease in CVP group and did not change in the CT group. However, total body fat mass significantly reduced in all groups. Only the CT group showed a decrease in total fat free mass, while HOP and CVP did not. According to the IPAQ filled out in at final assessment day, subjects did not change their physical activity level, and no difference between groups was verified (data not shown).

There was a significant reduction in liver enzyme AST, ALT and GGT in CVP subjects, and only in GGT in HOP group compared to baseline. Fasting glucose significantly increased in HOP subjects in comparison to baseline, but remained within the normal range. In CT group, total cholesterol, LDL and HDL decreased significantly after the study period. Triacylglycerols significantly decreased in HOP and there was a tendency for reduction in CVP group. Changes in biochemical variables were not different between groups except for the AST (CVP *vs* CT) and triacylglycerols concentrations (HOP *vs* CT) (Table 3).

Table 3. Changes in anthropometric and body composition variables[1] after 4-weeks of dietary intervention

	CVP (n=19)	P*	HOP (n=18)	P*	CT (n=18)	P*	P**
Body weight (kg)	-1.4 ± 0.3	<0.01	-1.7 ± 0.4	<0.01	-2.1 ± 0.4	<0.01	0.48
BMI (kg/m^2)	-0.5 ± 0.1	<0.01	-0.6 ± 0.1	<0.01	-0.6 ± 0.1	<0.01	0.60
Waist circumference (cm)	-2.0 ± 0.5	<0.01	-1.7 ± 0.4	<0.01	-1.8 ± 0.3	<0.01	0.89
SAD (cm)	-0.7 ± 0.2	<0.01	-0.7 ± 0.2	<0.01	-0.8 ± 0.1	<0.01	0.62
Body fat (%)	-0.5 ± 0.2	0.05	-1.3 ± 0.3	<0.01	-0.6 ± 0.4	0.11	0.65
Fat mass (kg)	-0.8 ± 0.2	<0.01	-1.7 ± 0.4	<0.01	-1.1 ± 0.4	0.02	0.25
Fat free mass (kg)	-0.6 ± 0.3	0.09	-0.1 ± 0.3	0.78	-1.0 ± 0.3	0.01	0.94
hsCRP (mg/dl)	-0.2 ± 0.2	0.38	0.0 ± 0.3	0.30	0.8 ± 0.4	0.42	0.16
AST (UI/l)	-6.5 ± 2.6^{a}	0.03	-0.8 ± 3.0^{ab}	0.80	4.6 ± 3.3^{b}	0.19	0.04
ALT (UI/l)	-4.9 ± 1.6	0.01	-0.9 ± 2.1	0.66	1.4 ± 2.0	0.49	0.10
GGT (UI/l)	-5.2 ± 1.9	0.01	-9.3 ± 4.2	0.02	-4.1 ± 2.7	0.14	0.52
AP (UI/l)	0.2 ± 1.8	0.93	-3.3 ± 2.7	0.31	2.1 ± 1.9	0.30	0.21
Insulin (µU/ml)	0.0 ± 0.6	0.47	-1.2 ± 0.9	0.21	1.4 ± 1.6	0.39	0.15
Glucose (mmol/l)	0.2 ± 0.1	0.17	0.2 ± 0.1	0.01	0.1 ± 0.1	0.17	0.79
HOMA-IR	0.0 ± 0.2	0.65	-0.1 ± 0.2	0.59	0.4 ± 0.4	0.33	0.17
TC (mmol/l)	0.0 ± 0.2	0.96	-0.3 ± 0.2	0.16	-0.4 ± 0.2	0.02	0.25
VLDL (mmol/l)	-0.1 ± 0.1	0.08	-0.2 ± 0.1	0.02	0.1 ± 0.1	0.46	0.05
LDL (mmol/l)	0.1 ± 0.1	0.45	0.0 ± 0.2	0.95	-0.4 ± 0.1	0.03	0.13
HDL (mmol/l)	-0.1 ± 0.0	0.05	0.0 ± 0.0	0.17	-0.1 ± 0.0	0.02	0.69
TAG (mmol/l)	-0.1 ± 0.1^{ab}	0.08	-0.2 ± 0.1^{a}	0.02	0.1 ± 0.1^{b}	0.22	0.02
LPS (EU/ml)	-0.2 ± 0.2	0.47	-0.2 ± 0.3	0.99	0.0 ± 0.5	0.92	0.99

[1]Data are represented as mean ± standard error of the mean (SEM). *P value column refer to difference between baseline and final assessments within group (paired t-test or Wilcoxon test). **P value column refer to differences between groups (ANOVA or Kruskal-Wallis). Different letters in the same line indicate statistical difference between groups ($p<0.05$). Groups: CVP: conventional peanuts; HOP: high-oleic peanuts; CT: control; BMI: body mass index; SAD: sagittal abdominal diameter; hsCRP: high-sensitivity C-reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyltransferase; AP: alkaline phosphatase; HOMA-IR: Homeostasis model assessment of insulin resistance; TC: total cholesterol; TAG: triacylglycerols; LPS: lipopolysaccharide.

Postprandial biochemical variables

The consumption of the high-fat test meals did not affect plasma LPS in all groups at the end of the intervention. The $_{pi}$AUC for LPS and triacylglycerol concentrations did not significantly change after weight loss (within group and between groups) (Figure 1A and B). Although changes in the $_{pi}$AUC for insulin concentration did not differ between groups, there was a significant increase in the CVP group compared to baseline. At the end of the intervention, $_{pi}$AUC for glucose decreased at the end of the intervention in HOP group and the changes differed between groups (HOP and CVP) (Figure 1C and D).

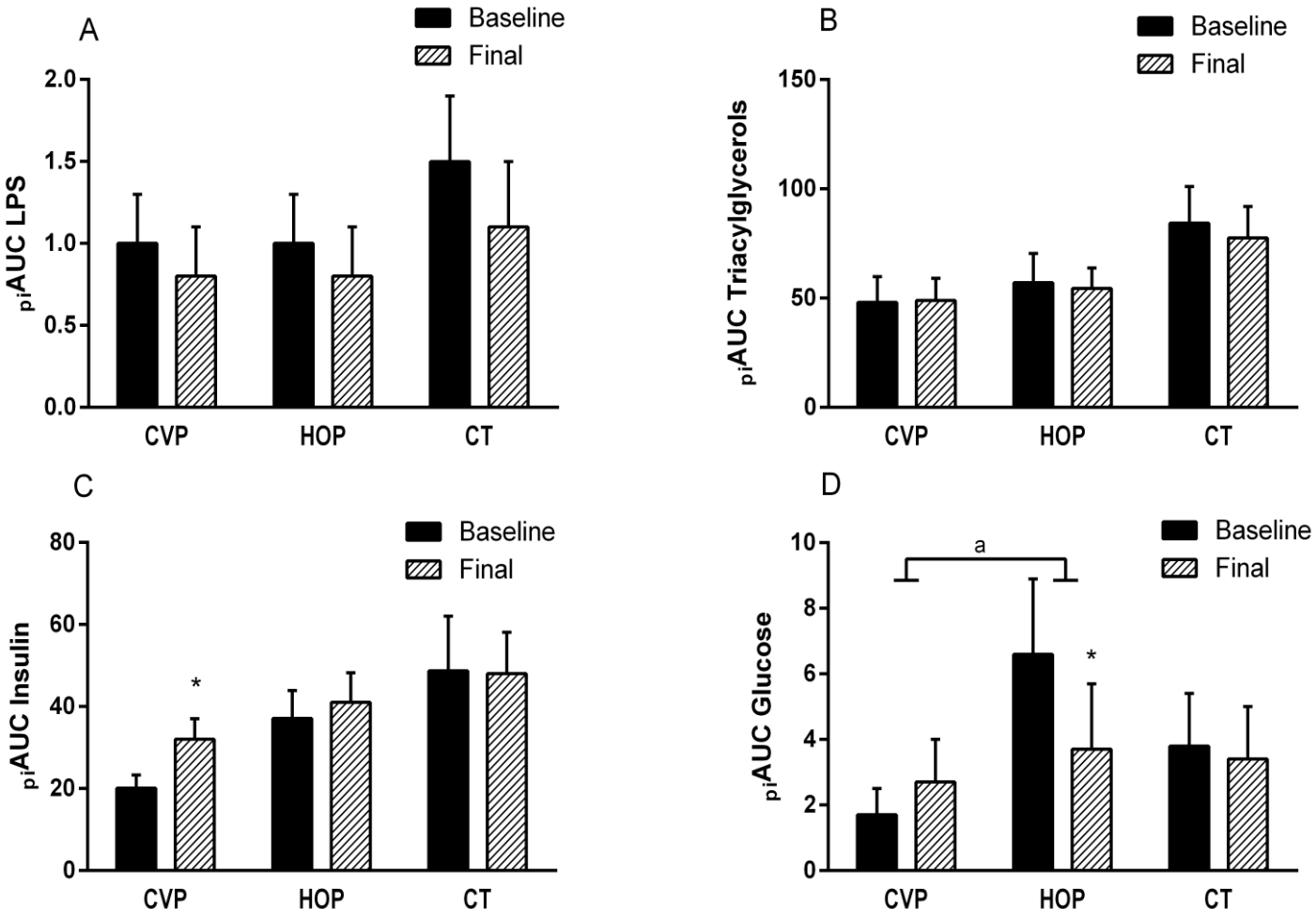

Figure 1: Mean (± standard error of the mean) area under the curve ($_{pi}$AUC) of lipopolysaccharide (LPS) (A); triacylglycerols (B); insulin (C); and glucose (D) concentration at baseline and after 4-weeks of dietary intervention. Experimental groups: conventional peanuts (CVP), high-oleic peanuts (HOP), and control (CT). *p<0.05 between baseline and after dietary intervention within the group (paired t-test or Wilcoxon test). The letter “a” indicates significant differences in changes between groups (ANOVA or Kruskal-Wallis).

Intestinal Permeability

Intestinal permeability did not differ between groups at the end of study. There were also no differences within each group comparing the end and baseline results of lactulose, mannitol, and L/M ratio (Table 4).

Table 4. Changes on intestinal permeability parameters[1] after 4-weeks of dietary intervention

	CVP (n=19)	P*	HOP (n=18)	P*	CT (n=18)	P*	P**
%L	0.02 ± 0.19	0.83	0.10 ± 0.07	0.18	0.14 ± 0.09	0.20	0.48
%M	-2.52 ± 4.69	0.89	2.94 ± 2.74	0.30	4.06 ± 2.72	0.26	0.40
L/M	0.0029 ± 0.0032	0.71	-0.0003 ± 0.0019	0.89	0.0006 ± 0.0013	0.66	0.11

[1]Data are represented as mean ± standard error of the mean (SEM). *P value column refer to difference between baseline and final assessments within group (paired t-test or Wilcoxon test). **P value column refer to differences between groups (ANOVA or Kruskal-Wallis). Groups: CVP: conventional peanuts; HOP: high-oleic peanuts; CT: control; %L: lactulose excretion; %M: mannitol excretion; L/M: lactulose/mannitol ratio.

4. DISCUSSION

Modulation of intestinal permeability and endotoxemia can help improve biochemical parameters, systemic and hepatic inflammation, and adiposity (van Dielen et al., 2004; Cani et al., 2008; Monte et al., 2012), since increased intestinal permeability and higher LPS concentrations have been associated with obesity and metabolic disturbances (Brun et al., 2007; Cani et al., 2008; Gummesson et al., 2011; Teixeira et al., 2012). The role of high-fat diet (Cani et al., 2008; Ghanim et al., 2009; Kim et al., 2013; Martinez-Medina et al., 2013) and the importance of fatty acids profile and the onset of the previously mentioned diseases (Usami et al., 2001; Aspenstrom-Fagerlund et al., 2007; Laugerette et al., 2012) stimulated us to investigate how a short-term 30% fat dietary intervention that included the consumption of peanuts differing in fatty acid content would affect intestinal permeability, plasma LPS concentrations and biochemical parameters in overweight and obese men under a slight caloric restriction. We hypothesized that this intervention would positively affect all these parameters.

The intervention was efficient to promote weight loss. However, the consumption of hypocaloric diet associated with the consumption of both types of peanut cultivars did not change intestinal permeability, plasma LPS concentrations or LPS postprandial responses to the high-fat meals in overweight and obese men.

An important aspect of our study design was not fixing the quantity of calories and fat of the test meals for all subjects, contrary to what was done in some of the previously published studies (Erridge et al., 2007; Laugerette et al., 2011; Clemente-Postigo et al., 2012). We consider this aspect a strong characteristic of the methodology if one considers that each person has a particular nutritional need. In addition, we used indirect calorimetry to estimate daily energy expenditure, which is more reliable than using prediction equations. The fixed quantities of fat given to subjects in previous studies were associated with significant increase in postprandial LPS concentrations compared to fasting condition (Erridge et al., 2007; Laugerette et al., 2011; Clemente-Postigo et al., 2012).

It is possible that significant changes in LPS or LPS markers (lipopolysaccharide-binding protein (LBP), toll-like receptor 4 (TLR4), and co-receptor of TLR4 (CD14)) may only happen after a greater weight loss and

when the lower body weight is maintained for a longer period of time (van Dielen et al., 2004; Monte et al., 2012). Monte et al. (2012) observed a positive correlation between changes in LPS and body weight. In the present study, weight loss was modest (average of 1.8 ± 0.2%) compared to the verified in other studies. We believe the weight loss occurred among our subjects was not enough to cause changes on intestinal permeability and plasma LPS concentration. Human studies about this topic are still scarce and of great interest. Since we did not include lean subjects in our study, we cannot say that the intestinal permeability and plasma LPS in obese subjects were higher than expected.

Despite the absent effect of the intervention on intestinal permeability and LPS, it is noteworthy highlighting beneficial traits obtained with hypocaloric restriction. Peanuts consumption reduced body fat mass and maintained fat free mass, which was significantly reduced in the CT group. Still, it is not possible to conclude that the maintenance of fat free mass is favored by peanuts consumption, since the control group showed higher caloric restriction in the last week of intervention. There were some biochemical improvements in comparison to baseline in peanuts groups. Conventional peanuts improved hepatic enzymes and tended to improve triacylglycerols. Higher oleic acid content improved only one of the hepatic enzymes (GGT) and reduced triacylglycerols. Decreased total cholesterol, LDL, and HDL concentrations were observed at the end of the study in the CT group. Subjects from peanuts groups showed no reduction in HDL, consistent with published data (Li et al., 2009).

Taken together, our results suggest that the consumption of peanuts-enriched hypocaloric diets contributes to weight loss and to improve body composition and some biochemical parameters. After the consumption of peanuts during 4 weeks, intestinal permeability and LPS concentration were not affected in fasting and postprandial. Further studies are needed to investigate the long-term effects of peanuts, combined with caloric restriction, on weight loss, intestinal permeability and endotoxemia.

5. SUPPLEMENTARY MATERIAL

Table 1. Nutritional composition of 56 g portion of the conventional and high-oleic peanuts

	Conventional peanuts IAC 886	**High-oleic peanuts IAC 505**
Available carbohydrate (g)	8.6	7.3
Protein (g)	16.8	16.3
Lipid (g)	24.0	24.7
Energy (kcal)	317.6	316.7
Dietary fiber (g)	5.0	5.5
Soluble (g)	0.2	0.7
Insoluble (g)	4.8	4.8

Table 2. Fatty acids composition (%) of the conventional peanuts, high-oleic peanuts and control biscuits

Fatty acid	Conventional Peanuts IAC 886	High-oleic Peanuts IAC 505	Control biscuits
Lauric acid (C12:0)	-	-	0.43 ± 0.19
Palmitic acid (C16:0)	8.78 ± 0.07	5.23 ± 0.14	12.76 ± 0.75
Heptadecanoic acid (C17:0)	0.46 ± 0.00	0.18 ± 0.00	0.27 ± 0.00
Stearic acid (C18:0)	2.14 ± 0.06	2.08 ± 0.07	8.08 ± 0.36
Elaidic acid (C18:1n9t)	-	-	7.11 ± 0.29
Oleic acid (C18:1n9)	50.96 ± 0.47	81.47 ± 1.03	35.16 ± 0.49
Linolelaidic acid (C18:2n6t)	-	-	0.96 ± 0.03
Linoleic acid (C18:2n6)	31.93 ± 0.21	3.87 ± 0.03	32.48 ± 1.19
Arachidic acid (C20:0)	0.82 ± 0.03	1.19 ± 0.02	0.53 ± 0.02
Gamma-Linolenic acid (C18:3n6)	-	-	0.16 ± 0.00
Eicosenoic acid (C20:1n9)	0.82 ± 0.03	1.45 ± 0.02	1.06 ± 0.07
Alpha-Linolenic acid (C18:3n3)	0.28 ± 0.03	0.44 ± 0.03	1.44 ± 0.16
Behenic acid (C22:0)	2.59 ± 0.19	2.68 ± 0.17	-
Erucic acid (C22:1n9)	-	0.17 ± 0.02	-
Lignoceric acid (C24:0)	1.46 ± 0.05	1.65 ± 0.00	-
Total SFA	16.25 ± 0.40	13.01 ± 0.40	22.07 ± 1.31
Total MUFA	51.78 ± 0.50	83.09 ± 1.06	36.21 ± 0.56
Total PUFA	32.21 ± 0.23	4.30 ± 0.06	34.08 ± 1.35
Total Trans	-	-	8.07 ± 0.32

Data are reported as means ± SD for 3 independent analyses. - = not detected.

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4. CONSIDERAÇÕES FINAIS

No presente estudo, verificou-se que a ocorrência de níveis mais elevados de lipopolissacarídeo (LPS) plasmático estão associados a um fenótipo menos favorável, caracterizado por maior adiposidade central, menor sensibilidade à insulina e prejuízo da função das células beta em indivíduos com sobrepeso e obesidade. O consumo de refeição hiperlipídica, contendo amendoim convencional ou amendoim rico em ácido graxo oléico retardou o aumento dos níveis de triacilgliceróis e resultou em menor concentração de LPS às 3 h pós-prandiais comparado ao grupo controle. Houve ainda retorno mais rápido da insulinemia para concentrações basais, especialmente nos indivíduos que consumiram o amendoim convencional. Ao associar dietas hipocalóricas ao consumo diário de amendoim convencional ou amendoim rico em ácido graxo oléico durante quatro semanas verificou-se que não houve alterações na permeabilidade intestinal, nas concentrações plasmáticas de LPS em jejum ou nas respostas pós-prandiais de LPS após refeição hiperlipídica contendo amendoim. A restrição calórica proposta foi eficiente para promover a redução de medidas antropométricas e da massa de gordura corporal total sem redução da massa livre de gordura nos grupos contendo amendoim. Os resultados indicam potenciais benefícios com a inclusão de amendoim convencional ou amendoim rico em ácido graxo oléico na dieta, além de redução no risco de endotoxemia e de seus feitos metabólicos. Entretanto, mais pesquisas são necessárias para investigar os efeitos a longo prazo do consumo de amendoim com diferentes teores de ácido oléico, combinando ou não a restrição calórica na permeabilidade intestinal e na endotoxemia.

5. ANEXOS

Anexo 1: Termo de Consentimento Livre e Esclarecido

Universidade Federal de Viçosa
Centro de Ciências Biológicas e da Saúde
Departamento de Nutrição e Saúde

Estou ciente de que:

1. Os procedimentos que serão adotados na pesquisa "Efeitos do consumo de amendoim na composição corporal, metabolismo energético, apetite, marcadores de inflamação e do estresse oxidativo e na microbiota e permeabilidade intestinal em obesos" consistem em: aplicação de questionários para obtenção de dados pessoais, ingestão alimentar e nível de atividade física; avaliações antropométricas (peso, altura, circunferência da cintura/quadril e composição corporal); de medida da pressão arterial; de exames de sangue (por punção digital e venosa) e de gasto energético; coleta de urina e fezes. O estudo completo terá duração de 4 semanas consecutivas, sendo que o voluntário seguirá durante este período uma dieta hipocalórica e receberá ou não uma porção de amendoim para ser consumida diariamente.
2. Como participante do estudo não serei submetido a nenhum tipo de intervenção que possa causar danos à minha saúde, visto que as condutas a serem adotadas objetivam a promoção da mesma e são respaldadas na literatura científica.
3. Estou ciente de que não terei nenhum tipo de vantagem econômica ou material por participar do estudo, além de poder abandonar a pesquisa em qualquer etapa do desenvolvimento, sem qualquer prejuízo.
4. Estou em conformidade que meus resultados obtidos estarão disponíveis para a agência financeira e para a equipe envolvida na pesquisa e poderão ser publicados com a finalidade de divulgação das informações científicas obtidas, sempre resguardando minha individualidade e identificação.

De posse de todas as informações necessárias, concordo em participar do projeto.

Data:___/___/____ _____

Voluntário

Profª Rita de Cássia G. Alfenas
Responsável pelo projeto

Profª Neuza Maria Brunoro Costa
Responsável pelo projeto

Ana Paula Boroni Moreira
Doutoranda

Raquel Duarte Moreira Alves
Doutoranda

Anexo 2: Aprovação pelo Comitê de Ética em Pesquisa com Seres Humanos da Universidade Federal de Viçosa (UFV)

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 185/2011/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 *Efeitos do consumo de amendoim na composição corporal, metabolismo energético, apetite, marcadores de inflamação e do estresse oxidativo e na microbiota e permeabilidade intestinal em obesos.*

Atenciosamente,

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

À Professora
Rita de Cássia Gonçalves Alfenas
Departamento de Nutrição e Saúde

/rhs.