PAULA GUEDES COCATE

ASSOCIAÇÃO DA ATIVIDADE FÍSICA E DE COMPONENTES DA DIETA HABITUAL COM ESTRESSE OXIDATIVO E OUTROS FATORES DE RISCO CARDIOMETABÓLICO EM HOMENS DE MEIA-IDADE

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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LISTA DE ABREVIATURAS

8-iso-PGF2α: 8-iso-prostaglandin F2α
8-OHdG: 8-hydroxy-2´-deoxyguanosine
ANOVA: analysis of variance
BMI: body mass index
BP: blood pressure
CVD: cardiovascular disease
DCV: doenças cardiovasculares
DEXA: dual-energy X-ray absorptiometry
DM2: type 2 diabetes
DNA: deoxyribonucleic acid
FFA: free fatty acids
FFQ: food frequency questionnaire
FHL: frutas, hortaliças e legumes
FV: fruit and vegetable
GI: glycemic index
GL: glycemic load
HDL-c: high density lipoprotein cholesterol
HOMA-IR: homeostasis model assessment of insulin resistance
MetS: metabolic syndrome
MUFA: monounsaturated fatty acid
ox-LDL: oxidized low density lipoprotein
PUFA: polyunsaturated fatty acid
RM: red meat
SFA: saturated fatty acid
SM: síndrome metabólica
TBF: total body fat
TC: total cholesterol
TG/HDL-C: ratio between triglycerides and high density lipoprotein
TG: triglycerides
USDA: United State Department of Agriculture
WHO: World Health Organization
WM: white meat

RESUMO

COCATE, Paula Guedes, D.Sc., Universidade Federal de Viçosa, dezembro, 2013. **Associação da atividade física e de componentes da dieta habitual com estresse oxidativo e outros fatores de risco cardiometabólico em homens de meia-idade**. Orientador: Antônio José Natali. Coorientadores: Giana Zarbato Longo, Helen Hermana Miranda Hermsdorff, Maria do Carmo Gouveia Pelúzio, Rita de Cássia Gonçalvez Alfenas.

A síndrome metabólica (SM) e o estresse oxidativo têm sido reconhecidos como importantes fatores de risco cardiometabólico. Entre os principais fatores de risco comportamentais associados à SM e ao estresse oxidativo destacam-se o estilo de vida sedentário e o padrão alimentar não saudável. Dessa forma, este estudo transversal foi desenvolvido para avaliar a associação da atividade física habitual e de componentes da dieta habitual com fatores de risco cardiometabólico em homens de meia-idade. Dentre os 300 homens servidores da Universidade Federal de Viçosa-MG recrutados, 296 (idade 50,5 ± 3,5 anos e índice de massa corporal 25,8 ± 3,5 kg/m^2) completaram todas as etapas do estudo voluntariamente. A atividade física habitual foi avaliada pelo número de passos diários (média de sete dias consecutivos). Variáveis antropométricas (peso, estatura e perímetro da cintura) e clínicas (pressão arterial sistólica e diastólica), a ingestão alimentar habitual (questionário de frequência do consumo alimentar) e o estilo de vida foram avaliados por procedimentos validados. Marcadores metabólicos sanguíneos (perfil glicídico e lipídico), diagnóstico da SM, homeostase da resistência à insulina (HOMA-IR), razão triacilgliceróis e HDL-c e biomarcadores de estresse oxidativo também foram determinados apropriadamente. Verificou-se que a SM, indicadores de adiposidade corporal e resistência à insulina foram significativamente ($p< 0,05$) inferiores no grupo de indivíduos fisicamente ativos (≥ 10.000 passos/ dia) comparado ao grupo não fisicamente ativo (< 10.000 passos/ dia). Além disso, constatou-se que o número de passos foi negativamente associado com adiposidade corporal (ginóide, androide e total) e HOMA-IR, independentemente de fatores de confusão. Em contrapartida, a carga glicêmica da dieta habitual foi positivamente associada com fatores de risco cardiometabólico, como ácidos graxos livres, razão triacilgliceróis/HDL-c e o biomarcador de estresse oxidativo no DNA (8-hidróxi-2´-deoxiguanosina) entre os participantes fisicamente ativos. Os participantes incluídos no maior tercil de ingestão de carne vermelha (≥ 81,5 g/d) apresentaram maior ocorrência de SM,

maiores valores de resistência à insulina, de LDL oxidada e da razão triacilgliceróis/HDL-c, em comparação aos indivíduos incluídos no segundo tercil (56,0 a 81,5 g/d) e no primeiro tercil (< 56,0 g/d) de consumo de carne vermelha, independente de variáveis de confusão. Todavia, o maior consumo de frutas, hortaliças e legumes (≥ 341,1 g/d) associou-se negativamente com biomarcadores de oxidação em lipídeos (8-iso-prostaglandina F2α e LDL oxidada) e no DNA (8-hidróxi-2´-deoxiguanosina), após ajuste por variáveis de confusão. Estes resultados apoiam a conclusão de que atividade física habitual e componentes da dieta habitual estão associados com fatores de risco cardiometabólico em homens de meia-idade. De fato, nossos achados indicam que o maior número de passos diários, a maior ingestão de frutas, hortaliças e legumes, bem como, o menor consumo de carnes vermelhas e de dietas de alta carga glicêmica possam ser estratégias importantes na recomendação de hábitos de vida saudáveis para prevenção de fatores de risco cardiometabólico e o estresse oxidativo relacionado.

ABSTRACT

COCATE, Paula Guedes, D.Sc., Universidade Federal de Viçosa, December, 2013. **Association of physical activity and components of habitual diet with oxidative stress and other cardiometabolic risk factors in middle-aged men.** Adviser: Antônio José Natali. Co-adviser: Giana Zarbato Longo, Helen Hermana Miranda Hermsdorff, Maria do Carmo Gouveia Pelúzio, Rita de Cássia Gonçalvez Alfenas.

The metabolic syndrome (MetS) and oxidative stress have been recognized as important cardiometabolic risk factors. Among the major behavioral risk factors associated with MetS and oxidative stress the sedentary lifestyle and unhealthy dietary pattern are noteworthy. Thus, this cross-sectional study was designed to evaluate the association of habitual physical activity as well as some components of the usual dietary intake with cardiometabolic risk factors in middle-aged men. Among 300 male employees of the Federal University of Viçosa-MG who volunteered to take part in this study 296 (age 50.5 ± 3.5 years and body mass index 25.8 ± 3.5 kg/m^2) completed all phases of the study. The habitual physical activity was assessed by the number of steps walked per day (mean of seven consecutive days). Anthropometric (weight, height and waist circumference) and clinical parameters (blood pressure), the usual dietary intake (food frequency questionnaire) and lifestyle were assessed by validated procedures. Blood biochemical parameters (lipid and glycemic profile), diagnosis of MetS, homeostasis of insulin resistance (HOMA-IR), triacylglycerol and HDL-cholesterol ratio and oxidative stress biomarkers were also determined appropriately. It was found that MetS, adiposity indicators and insulin resistance were significantly ($p<0.05$) lower in the group of physically active (≥ 10,000 steps/day) compared to the group not physically active (< 10,000 steps/day). In addition, the number of steps was negatively associated with adiposity (gynoid, android and total) and insulin resistance, regardless the confounding factors. In contrast, the habitual dietary glycemic load was positively associated with cardiovascular risk factors such as free fatty acids, triacylglycerol/ HDL-c ratio and a biomarker of DNA oxidative stress (8-hydroxy-2'-deoxyguanosine) in physically active individuals. The analysis of meat consumption showed that participants included in the highest tertile of red meat intake (≥ 81.5 g/d) had a higher occurrence of MetS, higher insulin resistance,

oxidized LDL (lipid peroxidation biomarker) and triglyceride/HDL-c ratio, as compared to individuals included in the second (56.0 to 81.5 g/d) and first (< 56.0 g/d) tertiles of red meat consumption, independent of confounding factors. However, higher consumption of fruits and vegetables (≥ 341.1 g/d) was negatively associated with lipid (8-iso-prostaglandin F2α and oxidized LDL) and DNA (8-hydroxy-2'-deoxyguanosine) oxidation biomarkers after adjustment for confounding factors. These results support the conclusion that the habitual physical activity and some components of the usual dietary intake are associated with cardiometabolic risk factors in middle-aged men. In fact, these data indicate that a higher number of steps per day and fruits and vegetables intake as well as a lower consumption of red meat and diets with high glycemic load may be important strategies to recommend for a healthy lifestyle in order to prevent cardiometabolic risk factors and the related oxidative stress.

1. INTRODUÇÃO GERAL

A síndrome metabólica (SM) é caracterizada por um conjunto de anormalidades fisiológicas e metabólicas[1] e é reconhecida como significante fator de risco para diabetes mellitus (DM) tipo 2[2] e para o desenvolvimento e aumento da gravidade de doenças cardiovasculares (DCV) em geral[3].

A prevalência desta síndrome é cada vez mais frequente em países em desenvolvimento, atingindo mais de 35% entre os adultos mexicanos[4] e em torno de 30% da população adulta de um estado venezuelano[5]. No Brasil, um recente estudo com uma amostra representativa da população adulta residente na capital (Brasília) constatou prevalência de 32% de indivíduos com SM[6].

Os fatores que definem a SM incluem obesidade central, dislipidemia (elevadas concentrações sanguíneas de triacilgliceróis e baixas concentrações de lipoproteína de alta densidade colesterol), elevadas concentrações de glicemia e elevada pressão arterial[1]. Tais componentes estimulam o aumento da capacidade oxidante bem como a redução da capacidade antioxidante do organismo, criando um desequilíbrio redox que resulta no estresse oxidativo[3].

Dessa forma, o aumento das concentrações de radicais livres/espécies reativas manifestadas durante o estresse oxidativo favorece a oxidação de biomoléculas como lipídeos, ácido desoxirribonucleico (DNA) e/ou proteínas[7]. Nesse sentido, a oxidação de biomoléculas gera metabólitos específicos denominados biomarcadores de estresse oxidativo que são passíveis de avaliação e mensuração em fluídos biológicos[8].

A continuidade prolongada da oxidação dessas biomoléculas e os consequentes danos em suas funções biológicas[7] podem promover agravos das DCV[9]. Assim, durante a peroxidação do ácido araquidônico (lipídeo presente na membrana celular) ocorre a produção de F2-isoprostano, o qual tem sido positivamente associado com doença arterial coronariana[10] e DM tipo 2[11]. Adicionalmente, espécies reativas de oxigênio no interior dos vasos sanguíneos podem promover modificação oxidativa da molécula de LDL (*low-density lipoprotein*), gerando a LDL oxidada[12], que é diretamente associada com eventos ateroscleróticos[13]. Os danos oxidativos ao DNA podem ocorrer pela oxidação da deoxiguanosina e produzir o biomarcador 8-hidroxi-2´-deoxiguanosina (8-OHdG)[14], que também é considerado fator de risco para câncer, aterosclerose e DM[15].

O estilo de vida sedentário é um dos principais fatores de risco comportamentais associados à SM[16] e ao estresse oxidativo[17]. Em contrapartida, a prática regular de atividade física tem sido considerada como fator de proteção contra tais complicações metabólicas. A prática de atividade física relaciona-se negativamente com a SM[18] e com relevantes fatores de risco para DCV, inclusive com estresse oxidativo[19], assim como com melhoria da sensibilidade à insulina[20] e aumento da atividade da enzima lipase lipoprotéica que favorece o aumento das concentrações de lipoproteína de alta densidade colesterol e redução das concentrações de triacilgliceróis[21].

Tradicionalmente, estudos epidemiológicos nacionais envolvendo a relação do nível de atividade física com parâmetros metabólicos estimam a atividade física habitual por meio de questionários[22-24]. Dessa forma, para análise mais precisa dessa variável é interessante o uso de instrumentos objetivos, como os sensores de movimento. Nesse sentido, os pedômetros têm sido largamente empregados por serem instrumentos de baixo custo, não invasivo, de fácil interpretação[25]. Os mesmos têm sido confiáveis para uso em saúde pública[26], tendo como meta controlar o volume de atividade física habitual pela contagem do número de passos diários[27] o qual está diretamente relacionado com a caminhada, atividade física mais comumente praticada por adultos no Brasil[28] e em diversos países[29-31]. Adicionalmente, estudos atuais[32,33] tem constatado relação inversa entre número de passos diários e fatores de risco cardiometabólico, reforçando a relevância de estudos dessa natureza envolvendo indivíduos brasileiros.

Outro fator com relevante influência sobre a SM e estresse oxidativo é o comportamento alimentar. Nesse sentido, o papel do consumo de carboidrato como fator de risco para doenças crônicas tem recebido importante atenção pela comunidade científica. A carga glicêmica (CG) da dieta [índice glicêmico do alimento/refeição x seu carboidrato disponível][34] reflete a qualidade e quantidade do carboidrato consumido e a hiperglicemia/hiperinsulinemia causada pelo consumo de dietas com alta CG pode aumentar o risco para DCV[35,36] e para a produção de espécies reativas de oxigênio[37]. Adicionalmente, o aumento da CG da dieta tem sido associado com relevantes indicadores de riscos de DCV, como, obesidade central[38], resistência à insulina[39], dislipidemia (reduzidas concentrações da lipoproteína de alta densidade colesterol e

elevadas concentrações de triacilgliceróis)[40, 41] e com maiores concentrações de biomarcadores de estresse oxidativo[42].

Por sua vez, o padrão alimentar saudável inclui a elevada ingestão de frutas, hortaliças e legumes (FHL), exceto tubérculos (ex. batata-inglesa), que tem sido alvo de pesquisas atuais por relacionarem negativamente com SM[43] e com estresse oxidativo[44]. Dessa forma, o consumo adequado de FHL pode desempenhar um importante papel na prevenção de doenças cardiometabólicas em indivíduos de meia-idade[45]. De fato, em recente estudo envolvendo indivíduos brasileiros de ambos os gêneros de mediana idade o consumo adequado de frutas foi associado como fator de proteção contra SM[46]. A associação inversa do grupo alimentar FHL com doenças crônicas[47], fatores de risco cardiovascular[48] e estresse oxidativo[44, 49] pode ser explicada pelo potencial anti-inflamatório[50, 51] e efeitos antioxidantes[52-55] de seus componentes.

Em contrapartida, o padrão alimentar não saudável "ocidental", caracterizado pelo consumo habitual de panificados, doces, refrigerante, carne processada e carne vermelha tem sido relacionado positivamente com fatores de risco cardiometabólico[56]. Nesse sentido, o consumo excessivo de carne vermelha, relevante fonte de ácido graxo saturado[57] e de ferro[58], pode ser um potencial componente prejudicial do padrão alimentar brasileiro, pois associa-se com aumento da obesidade central[59], das concentrações séricas de triacilgliceróis[59], da incidência de DM tipo 2[60], do risco de SM[61] e da mortalidade por DCV[62]. Na Figura 1 estão ilustrados alguns importantes fatores comportamentais de risco e de proteção contra SM, estresse oxidativo e DCV.

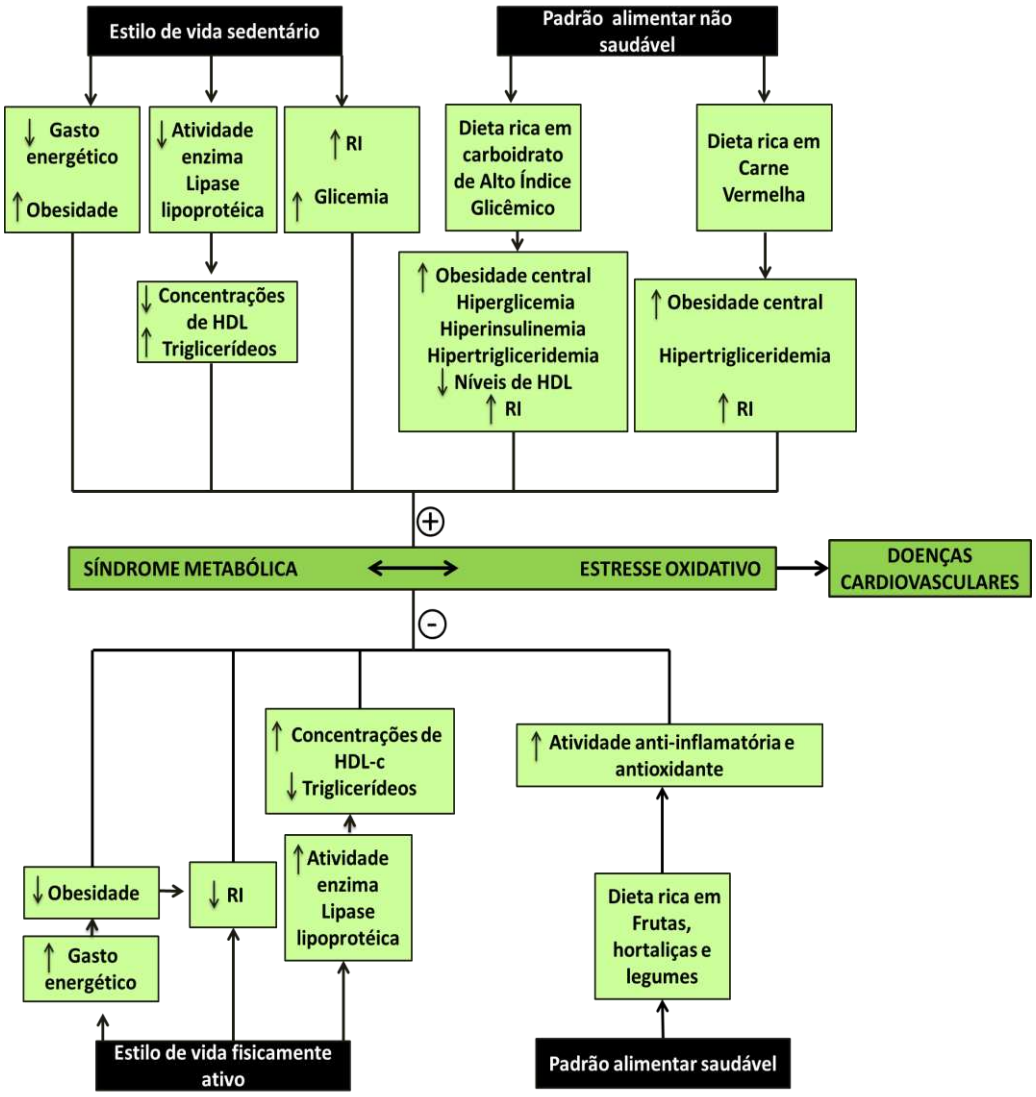

Figura 1: Fatores de risco e fatores de proteção contra síndrome metabólica, estresse oxidativo e doenças cardiovasculares. RI: resistência à insulina; +: fator de risco; - fator de proteção.

Apesar do descrito anteriormente, estudos sobre a relação entre a atividade física habitual estimada por número de passos, a carga glicêmica da dieta consumida, a ingestão de FHL e de carne vermelha com características clínicas, metabólicas e marcadores de estresse oxidativo na população nacional são ainda escassos.

Nesse contexto, considerando que: 1) a prevalência de SM é elevada em países em desenvolvimento[4-6]; 2) as DCV são responsáveis por aproximadamente 20% do total de mortes entre brasileiros acima de 30 anos[63]; 3) a prática regular da caminhada pode promover efeitos benéficos contra fatores de risco cardiometabólico[64]; 4) dieta padrão da sociedade moderna, caracterizada pelo elevado consumo de carboidrato de alto índice glicêmico[65] e

de carne vermelha[62] pode ser um importante fator de risco cardimetabólico; e 5) o consumo nacional de FHL é aproximadamente 90% menor[66] do que a quantidade diária recomendada pela WHO (400 g/dia)[47], julga-se relevante investigar a relação desses fatores comportamentais com marcadores de estresse oxidativo e outros fatores de risco cardiometabólico envolvendo indivíduos brasileiros de mediana idade.

1.2. Objetivos

1.2.1. Objetivo geral

Investigar a associação da atividade física habitual e de componentes da dieta habitual com SM, biomarcadores de estresse oxidativo e outros fatores de risco cardiometabólico em homens de meia-idade.

1.2.2. Objetivos específicos

1) Avaliar a relação do número de passos com fatores de risco cardiometabólico em brasileiros de meia-idade;
2) Verificar a associação da carga glicêmica da dieta habitual com marcadores de estresse oxidativo e outros indicadores de risco cardiovascular em homens fisicamente ativos de meia-idade;
3) Avaliar a relação entre a ingestão de frutas, hortaliças e legumes e concentrações de biomarcadores de estresse oxidativo em homens de meia-idade;
4) Verificar a associação do consumo de carne vermelha com síndrome metabólica e biomarcador de peroxidação lipídica em brasileiros de meia-idade.

1.3. Procedimentos metodológicos gerais

O presente estudo possui delineamento transversal e foi desenvolvido no período de março a dezembro de 2011, na cidade de Viçosa-MG, Brasil. A população de referência foi constituída por homens com idade entre 40 e 59 anos, servidores da Universidade Federal de Viçosa (UFV).

Para o cálculo do tamanho amostral foram considerados dados da prevalência de indivíduos fisicamente ativos no tempo de lazer na cidade de Belo Horizonte-MG (16,0%)[67] bem como da prevalência estimada de SM em homens brasileiros de meia-idade (24,3%)[68], associados ao número total de servidores da UFV (1.744), intervalo de confiança de 95% e erro amostral inferior a 4,5 pontos percentuais. Dessa forma, o número amostral mínimo correspondeu a 273 e 293 indivíduos, respectivamente. Os participantes foram selecionados por amostragem sistemática.

Foram excluídos do estudo indivíduos que autodeclararam apresentar: alterações de peso corporal ≥ 3 kg, alteração do nível de atividade física e dos hábitos alimentares nos três meses anteriores ao início do estudo; doenças tireoidianas, insuficiências cardíacas, doenças cerebrovasculares, doenças infecciosas, doenças inflamatórias, doenças do trato gastrintestinal, doenças hepáticas, doenças renais crônicas e/ou história de litíase renal, câncer nos dez anos anteriores, doenças de transtorno alimentar (bulimia e anorexia) e alergias alimentares. Indivíduos que usavam suplementos vitamínicos, diuréticos ou medicamento que afeta o consumo alimentar e metabolismo de nutrientes, usuários de prótese de membros e que eram atletas de elite também não foram selecionados.

O estudo atende à resolução do Ministério da Saúde 196/96 de pesquisa envolvendo seres humanos e foi aprovado pelo Comitê de Ética em Pesquisa com Seres Humanos da UFV (Of. Ref. n. 069/2010/CEPH) (Anexo 1). Os voluntários que concordaram participar da pesquisa assinaram o termo de consentimento livre e esclarecido (Anexo 3).

Após assinatura do termo de consentimento, os servidores participaram da segunda etapa, que consistiu na estimativa da atividade física habitual (número de passos diários) por meio do uso do pedômetro (média de 7 dias consecutivos). Em seguida, na terceira etapa, foram realizadas medidas antropométricas, de composição corporal, aferição da pressão arterial, bem

como, exames bioquímicos sanguíneos (perfil lipídico, perfil glicídico e um marcador de estresse oxidativo plasmático) e urinários (marcadores de estresse oxidativo) que estão detalhados na metodologia dos artigos científicos apresentados a seguir. Nesse mesmo dia foi aplicado um questionário de frequência do consumo alimentar para estimativa da ingestão alimentar habitual dos participantes. Após o cumprimento das etapas mencionadas os resultados das avaliações antropométricas, da composição corporal e dos exames bioquímicos foram apresentados aos voluntários e indicavam a busca de um profissional especializado caso houvesse alguma alteração nos parâmetros analisados segundo classificações validadas e condizentes com padrão “adequado” de saúde de homens da meia-idade (Figura 2).

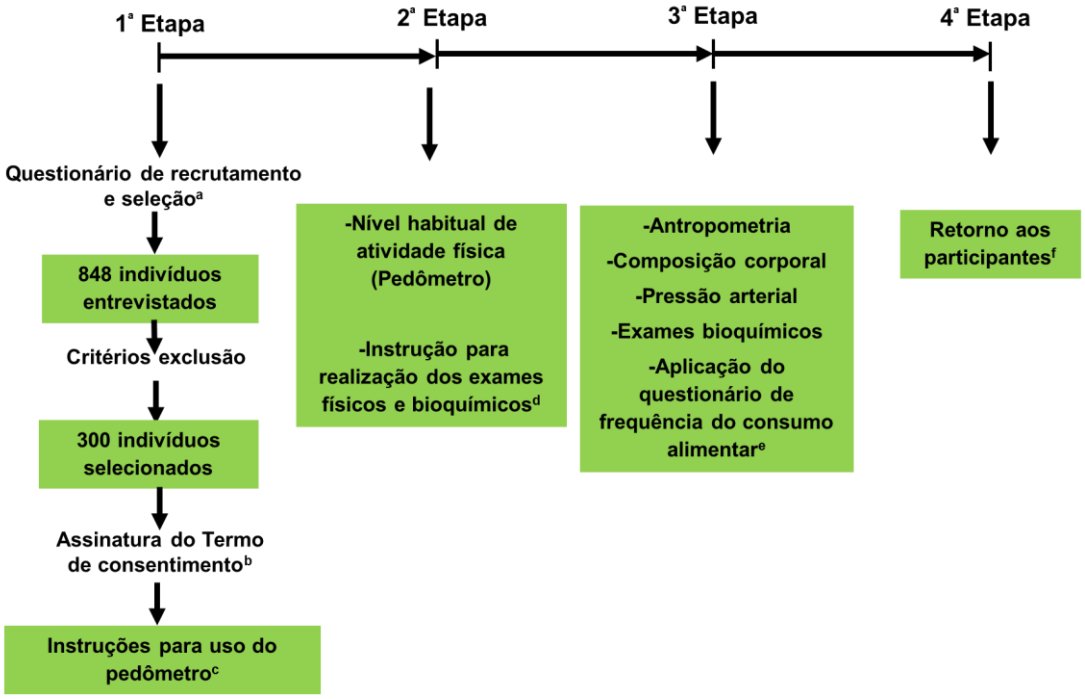

Figura 2: Recrutamento dos participantes e etapas realizadas ao longo do estudo. [a]: Anexo 2; [b]: Anexo 3; [c]: Anexo 4; [d]: Anexo 5; [e]: Anexo 8; [f]: Anexo 9.

Os parâmetros físicos e bioquímicos, dados do nível de atividade física e do consumo alimentar habitual foram utilizados para a elaboração dos artigos científicos conforme representados na Figura 3.

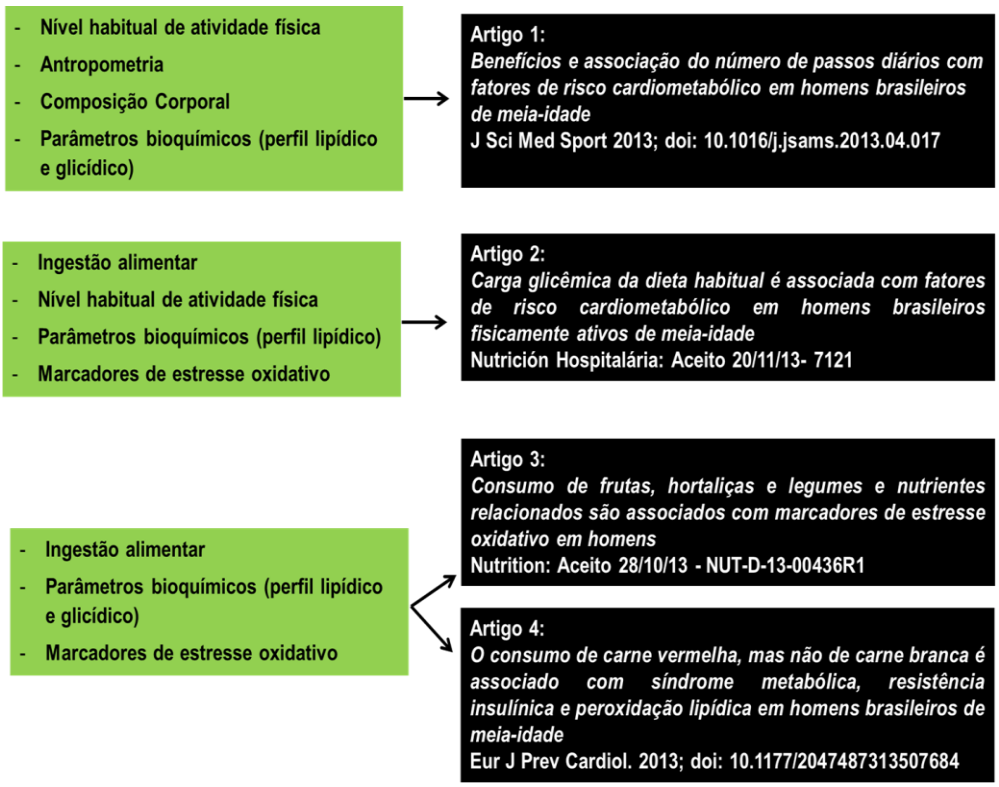

Figura 3: Principais dados utilizados para a elaboração dos artigos científicos

A seguir, os artigos científicos acima mencionados (Figura 3) estão apresentados com detalhamento das análises dos dados coletados.

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2.1. ARTIGO 1

BENEFITS AND RELATIONSHIP OF STEPS WALKED PER DAY TO CARDIOMETABOLIC RISK FACTOR IN BRAZILIAN MIDDLE-AGED MEN

Benefícios e associação do número de passos diários com fatores de risco cardiometabólico em homens brasileiros de meia-idade

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RESUMO

Objetivo: Avaliar os benefícios e a relação do número de passos diários com os fatores de risco cardiometabólico: indicadores de adiposidade, resistência à insulina e síndrome metabólica (SM) em homens aparentemente saudáveis de meia-idade. *Desenho:* Transversal. *Método:* Foram estudados 299 homens com idade de 50 ± 5 anos. O número de passos diários foi mensurado por pedômetro. Os indicadores de adiposidade corporal (circunferência da cintura, gordura corporal total, gordura androide e ginóide), concentrações séricas de insulina, glicose e triacilgliceróis, razão triacilgliceróis/lipoproteína de alta densidade colesterol (TG/HDL-c), homeostase da resistência à insulina (HOMA-IR) foram determinados. Os participantes foram alocados em grupos de risco que refletiam diferentes números de passos diários (média de 7 dias consecutivos): grupo 1: < 10.000 e grupo 2 ≥ 10.000. A relação entre as variáveis foi mensurada por regressão linear múltipla e pela correlação de Pearson conforme apropriado ($p < 0,05$). *Resultados:* Os fatores de risco cardiometabólico foram menores ($p < 0,05$) no grupo 2 em comparação ao grupo 1. O número de passos diários foi fator preditivo negativo para a gordura corporal total, gordura na região androide e na ginóide, independentemente da idade, cargo de trabalho e razão TG/HDL-c, bem como, para HOMA-IR

independentemente da idade, cargo de trabalho, razão TG/HDL-c, prevalência de sobrepeso/obesidade e gordura androide. Adicionalmente, houve correlação negativa do número de passos com gordura corporal total, gordura na região androide e ginóide e HOMA-IR. *Conclusão:* Homens brasileiros de meia-idade que realizam mais de 10.000 passos diários têm melhores condições cardiometabólica do que aqueles que caminham menos do que 10.000 passos diários. O número de passos/dia é inversamente relacionado com indicadores de obesidade total e regional e com resistência à insulina.

Palavras chave: Atividade física; Envelhecimento; Saúde; Adiposidade; Resistência à insulina; Síndrome metabólica.

ABSTRACT

Objectives: We evaluated the benefits and relationship of the number of steps per day to the cardiometabolic risk factors: adiposity indicators; insulin resistance; and metabolic syndrome (MetS) in apparently healthy Brazilian middle-aged men. *Design:* Cross-sectional. *Methods:* Apparently healthy men (age: 50 ± 5 years; n = 299) were studied. The number of steps per day was measured by pedometer. The adiposity indicators (waist circumference, total body fat, android and gynoid body fat), serum insulin, glucose and triglycerides, triglycerides/high-density lipoprotein cholesterol (TG/HDL-c) ratio, homeostasis model assessment of insulin resistance (HOMA-IR) and MetS were assessed. Subjects were placed in groups to reflect different levels of steps per day (average of 7 consecutive days): Group 1 < 10,000 and Group 2 ≥ 10,000. Relationships among variables were measured by multiple linear regressions and the Spearman correlation coefficient as appropriate ($p < 0.05$). *Results:* The cardiometabolic risk factors were lower ($p < 0.05$) in Group 2 than in Group 1. The number of steps per day was a negative predictive factor for total body fat, android and gynoid body fat independent of age, working position and triglycerides/HDL-c ratio as well as for HOMA-IR independent of age, working position, android fat, overweight/obesity prevalence, and triglycerides/HDL-c ratio. Moreover, there was a negative correlation between the number of steps and total body fat, android and gynoid body fat and HOMA-IR. *Conclusions:* Brazilian middle-aged men performing more than 10,000 steps per day have better cardiometabolic conditions than those walking fewer than 10,000 steps.

The number of steps per day is inversely related to the indicators of total and regional adiposity and insulin resistance.

Keywords: Physical activity; Aging; Health; Adiposity; Insulin resistance; Metabolic syndrome.

1. Introduction

The prevalence of overweight and obesity has increased at an alarming rate in Brazil, following what appears to be a global trend in contemporary society.[1] The last Household Budget Survey 2008–2009 held by the Brazilian Institute of Geography and Statistics in partnership with Ministry of Health evaluated approximately 188 thousand subjects from all over the Brazilian territory of urban and rural areas, ranging from children to seniors, and found that 50.1% and 12.4% of adult men were overweight or obese,respectively.[2] Obesity is often associated with the metabolic syndrome (MetS) and is closely related to major public health problems in the world, such as cardiovascular disease and diabetes mellitus type 2.[3]

The sedentary lifestyle of modern society contributes to lower energy expenditure and increases body fat, which may lead to insulin resistance and MetS.[4] In contrast, regular physical activity is related to improved insulin sensitivity, reduced risk for metabolic syndrome and cardiovascular diseases.[4–6] Population studies in Brazil have traditionally used questionnaires[7] to estimate the level of physical activity. However, we believe that the use of more objective instruments, such as movement sensors, can promote a more precise analysis of the physical activity levels. Pedometers-measured step counts are directly related to walking, a commonly practiced physical activity by adults in Brazil,[8] and pedometers are considered a reliable tool in public health studies.[9] Despite the use of this instrument in several epidemiological studies,[5,10] there is a lack of evidence relating the number of steps per day to clinical and metabolic characteristics in the Brazilian population. Therefore, the aim this study was to assess the benefits and relationship of the number of steps per day to the cardiometabolic risk factors: adiposity indicators; insulin resistance; and MetS in apparently healthy Brazilian middle-aged men. In this sense, we hypothesize that there is an inverse relationship between daily steps and cardiometabolic risk factors.

2. Methods

This cross-sectional study was carried out between March and December 2011 with men aged between 40 and 59 years, who were staff members of the Federal University of Viçosa (UFV), Viçosa city, Brazil. The sample size was calculated by: the total number of male staff members with ages between 40 and 59 years (1744 individuals), confidence level of 95%, 16% expected prevalence of physically active individuals during leisure time[11] and 4% sampling error, resulting in 273 participants as a minimum of sample size required. Ten percent was added in the recruitment to the total sample to offset losses and confounding variables. The Epi Info software, version 6.04, for cross-sectional studies was used to estimate sample size.

Participants were recruited by systematic sampling and replaced if they did not meet the inclusion criteria. We excluded those individuals who self-declared the following: body weight alterations greater than 3 kg in the three months preceding the study, altered levels of physical activity and eating habits in the three months preceding the study, thyroid diseases, heart failure, cerebrovascular diseases, infectious diseases, inflammatory diseases, diseases of the gastrointestinal tract, liver disease, chronic kidney disease and/or a history of kidney stones, cancer in the previous ten years, eating disorders (anorexia and bulimia) and food allergies. Individuals using diuretics or drugs that alter food intake and/or the metabolism of nutrients, pacemaker and/or prosthetic limbs users, and elite athletes were also excluded. The study is in accordance with the resolution 196/96 from the Ministry of Health regarding research involving human subjects and was approved by the institutional Human Research Ethics Committee (reference n^{o}.069/2010). Informed consent was obtained from all participants and those selected received the results of all tests performed for free.

The selected participants were instructed how to use a digital pedometer, and answered a questionnaire about lifestyle covariates. Two weeks later blood sample collection, anthropometric and body composition measures, and hemodynamic data were obtained after a 12-h fast.

The digital pedometer (Digiwalker SW-200, Yamax Corporation, Tokyo, Japan) was used to monitor the number of steps per day during a typical week,

including the weekend (eight continuous days). Participants were instructed to position the pedometer above the iliac crest in the midline of the right thigh, attached to their waist-band during all waking. In addition, they were advised to perform their routine activities and remove the pedometer only when lying down, riding a bicycle or motorcycle, bathing or practicing water activities. The participants were told that the pedometer should be placed back in the waistband immediately after these activities. They were also instructed to record the number of steps shown on the pedometer on a log given at the end of each day before lying down to sleep. On the next day after waking up, participants were told to reset the pedometer and verify if the display showed zero before placed back on the waist band.

To calculate the number of steps per day, the value obtained in the first day was excluded to minimize physical activity behavior changes due to monitoring. The average was obtained from the next 7 days.[12] The number of 10,000 steps/day was considered an adequate cut-off point, since it was associated with health-related parameters as well as it was proposed to classify individuals asactive.[9] Thus, the participants were placed in groups to reflect different reflect different levels of steps per day and were categorized as Group 1: <10,000 steps and Group 2: ≥10,000 steps. Such methodological procedure of dividing participants into groups of risk has been used in cross-sectional studies previously.[13,14]

The staff members who participated in this study occupied technical administrative positions, classified as levels A–E, or professors. To evaluate how lifestyle and occupation influenced the level of physical activity they were grouped according to their education level and positions: Group ABC was composed of technical and administrative staff, levels A–C, with an education level up to high school. Group DE was composed of technical and administrative staff, levels D, E and professors. Participants were also asked about smoking and categorized as smokers and nonsmokers.

The participants were instructed not to perform physical activities of medium or high intensity, not to ingest alcohol 48 h prior and to fast for 12 h prior to the anthropometric, blood pressure and body composition measurements, and blood sample harvesting.

Body weight and height were determined using a digital scale with stadiometer (2096PP, Toledo, São Bernardo do Campo, SP, Brazil). Body mass

index (BMI) was calculated as weight (kg) divided by height squared (m^2). We adopted a cut-off of 25.0–29.9 kg/m^2 for overweight and ≥ 30 kg/m^2 for obesity classification by BMI.[15] Waist circumference was measured considering the midpoint between the last rib and the top of the iliac ridge as suggested by the World Health Organization,[16] using a flexible, and inelastic tape measure (TR4010, Sanny, São Bernardo do Campo, SP, Brazil). Waist circumference was measured in triplicate and the average value was considered for data analysis.[17]

Total body scanning with a dual energy X-ray absorptiometry (DXA) (LUNAR, GE, Encore software version 13:31, Madison, WI, USA) was used to assess total body fat percentage. Total body fat was considered the total adiposity indicator. We adopted a cut-off of 21% of total body fat for overweight classification.[18] The percentage of fat in the android and the gynoid regions were determined by using the "region of interest" program, according to the manufacturer's instructions. Waist circumference and android body fat were considered abdominal adiposity indicators. Blood pressure was measured and classified following the guidelines of the VI Brazilian Guidelines on Hypertension.[19] It was used an automatic inflation blood pressure monitor (BP3AA1-1, G-Tech, Onbo Electronic Co., Schenzen, China), properly calibrated and registered in ANVISA (n°. 80275310004).

Blood samples were collected from the anti-cubital vein and the serum was separated by centrifugation at 2225 × g for 15 min, at room temperature (2–3 Sigma, Sigma Laborzentrifuzen, Osterodeam Harz, Germany).

Blood glucose was measured by the glucose oxidase method using the Cobas Mira Plus equipment (Roche Diagnostics, GmbH, Montclair, NJ, USA). Insulin was measured by electrochemiluminescence using the Modular Analytics (E170, Roche Diagnostics, GmbH, Mannheim, Germany).

Serum total cholesterol, high-density lipoprotein cholesterol (HDL-c) and triglycerides were determined by an enzymatic colorimetric method using Cobas Mira Plus (Roche Diagnostics GmbH, Montclair, NJ, USA). The atherogenic index was calculated by ratio between triglycerides and HDL-c.[20]

Insulin resistance was estimated by homeostasis model assessment of insulin resistance (HOMA-IR) calculated by the product of fasting insulin (U/mL) × fasting glucose (mmol/L)/22.5.[21] The cut-off value used for the insulin resistance diagnosis was 2.71.[22]

Metabolic syndrome is characterized by an aggregation of relevant risk factors for cardiovascular disease, such as abdominal obesity, dyslipidemia, high blood pressure and high fasting blood glucose.[3] Metabolic syndrome, was diagnosed by using the ethnic value for Central America and South America: waist circumference ≥ 90 cm (specific value for men)[23] with two or more of the following alterations: systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg, fasting HDL-c < 40 mg/dL, fasting glucose ≥ 100 mg/dL and fasting triacylglycerol ≥ 150 mg/dL.[3]

The Skewness and Kurtosis test was used to evaluate the normality of the assessed data set. Data were exposed by descriptive statistics consisting of mean, standard deviation, median deviation and interquartile range for continuous variables and frequency for categorical variables.

The Student's t test was used for independent samples and its nonparametric equivalent, the Mann–Whitney test, was used for the differences between mean values per group. The Chi-square test compared proportions and analyzed the associations between categorical variables.

To examine the adjusted effects of the number of steps in relation to the variables, multiple linear regressions were used considering the body composition variables obtained from DXA and all metabolic variables (glucose, total cholesterol, HDL-c, triglycerides, ratio triglycerides/HDL-c and HOMA-IR) as dependent variables and the number of steps as the main independent variable. Those models were controlled by variables defined as confounding factors. Among the tested regression models only those in which the number of steps was statistically significant ($p < 0.05$) are presented. The Spearman correlation coefficient was used to investigate the correlation of the number of steps with total and regional body fat, adiposity and insulin resistance ($p < 0.05$). Data processing and analysis were performed by using the STATA software, version 9.1.

3. Results

Nearly eight hundred forty members of staff were interviewed and 300 of them meet the inclusion criteria and were selected to take part in the present study. One participant was excluded from data analysis as he did not complete all phases of the study. Overall, this study included 299 participants.

Anthropometric measures, body composition, clinical, metabolic and lifestyle characteristics are shown in Table 1, according to the category of the subjects as to the number of steps/day (<10,000 steps vs. ≥10,000 steps). All adiposity indicators and overweight prevalence from BMI data were significantly higher in participants from the Group <10,000 steps compared to the Group ≥10,000 steps. Among the clinical and metabolic parameters, higher values of insulin, triglycerides, HOMA-IR and triglycerides/HDL-c ratio were found in Group <10,000 steps compared to Group ≥10,000 steps (Table 1). A tendency ($p = 0.07$) for higher values of HDL-c in Group ≥10,000 steps compared to Group <10,000 steps was observed. Despite that, there were no statistical differences between groups for blood pressure, obesity, blood glucose and total cholesterol.

No statistical differences were found between the groups for prevalence of smoking. However, a higher frequency of individuals from the ≥10,000 steps group was found to be in the occupational levels A, B and C as compared with the <10,000 steps group (Table 1).

Table 1

Characteristics of the participants, presented by the number of steps per day.

Variables	<10,000 steps group[a] (n=123)	≥10,000 steps group[a] (n=176)	*p*-Value[b]
Age (years)	51 (46-54)	52 (47-54)	0.419
Steps per day (steps)	7,458 (1,984)	13,561 (2,779)[c]	< 0.001
Waist circumference (cm)	91.3 (85.1-97.1)	88.5 (81.0-95.7)[c]	0.038
BMI (kg/m^2)	25.5 (23.9-28.3)	24.9 (22.7-27.6)[c]	0.040
Overweight by BMI (%)	49.6	36.5[c]	0.024
Obesity by BMI (%)	12.2	12.9	0.852
Fat in the region gynoid (%)	29.0 (26.1-33.8)	25.8 (19.9-30.8)[c]	< 0.001
Fat in the region android (%)	28.8 (23.0-35.8)	24.2 (14.9-32.4)[c]	< 0.001
Total body fat (%)	25.1 (0.5)	20.8 (0.6)[c]	< 0.001
Overweight/obesity by TBF(%)	79.7	54.5[c]	< 0.001
Systolic BP (mm Hg)	123 (115-134)	126 (117-131)	0.777
Diastolic BP (mm Hg)	82 (10)	80 (9)	0.100
Glucose (mg/dL)	88.0 (83.0-95.0)	89.0 (83.0-95.0)	0.870
Insulin (μUI/mL)	6.5 (4.1-8.9)	4.4 (3.0-7.0)[c]	< 0.001
HOMA-IR	1.43 (0.94-2.01)	0.99 (0.65-1.57)[c]	< 0.001
Total cholesterol (mg/dL)	211.0 (188.0-241.0)	211.5 (183.0-241.0)	0.720
HDL-c (mg/dL)	43.0 (37.0-50.0)	45.5 (39.0-54.5)	0.070
Triglycerides (mg/dL)	138.0 (97.0-190.0)	105.5 (75.0-144.5)[c]	0.001
Triglycerides/HDL-c	3.2 (1.8-4.8)	2.3 (1.5-3.6)[c]	< 0.001
Smoker (%)	11.4	14.8	0.340
ABC staff position (%)	48.0	80.7[c]	< 0.001
DE professor position (%)	52.0	19.3[c]	< 0.001

[a] Data are mean (SD), median (interquartile range) and frequency for normally distributed, non-normally distributed and dichotomous variables, respectively.

[b] *p*-Value from t-test, Mann-Whitney test and Chi-squared, as appropriate to each variable.

[c] Statistical difference from < 10,000 steps group. n: number of individuals; ABC position: technical-administrative level classification A, B and C; BMI: body mass index; BP: blood pressure; DE professor position: technical-administrative level classification D and E and professors; TBF: total body fat; HDL-c: high density lipoprotein-cholesterol; HOMA-IR: homeostasis model assessment of insulin resistance.

Multiple linear regression models were applied for evaluation of the relationship between the number of daily steps and total and regional adiposity. Interestingly, the number of steps was a negative predictor ($p < 0.05$) for total body fat, android body fat and gynoid body fat (Table 2), independent of age, working position and triglycerides/HDL-c ratio. Concerning the metabolic markers, the number of steps was also negatively associated with HOMA-IR, a recognized insulin resistance indicator (Table 2), independently of age, working position, android fat, overweight/obesity prevalence, and triglycerides/HDL-c ratio.

Table 2

Multiple linear regression models with the number of steps per day as the main independent variable.

Dependent variables	β	CI 95%	R^2	*p*-Value
Total body fat (%)[a]	-0.000303	-0.0005; -0.0001	0.20	0.003[c]
Android fat (%)[a]	-0.000306	-0.0006;-0.0001	0.19	0.041[c]
Gynoid fat (%)[a]	-0.000340	-0.0005; -0.0001	0.18	0.001[c]
HOMA-IR[b]	-0.00002	-0.00005; -0.0000002	0.39	0.034[c]

[a] Models adjusted for age, position (ABC or DE professor) and triglycerides/HDL-c ratio.
[b] Model adjusted for age (years), position (ABC or DE professor), fat in the region android (%), prevalence of overweight/obesity (BMI ≥ 25 kg/m^2) and triglycerides/HDL-c ratio. CI: confidence interval; HOMA-IR: homeostasis model assessment of insulin resistance.
[c] Significant relationship.

The number of steps was negatively and significantly (Chi-square test) associated with HOMA-IR ($p = 0.004$) and MetS ($p = 0.02$). Thus, there was a higher occurrence of insulin resistance and metabolic syndrome in Group <10,000 steps (HOMA-IR = 62.5% and MetS = 55.6%) as compared to Group ≥10,000 steps (HOMA-IR = 37.5% and MetS = 44.4%). Moreover, there was a negative correlation between the number of steps and the values of total body fat ($r = -0.25$, $p = 0.001$), android body fat ($r = -0.20$, $p < 0.001$), gynoid body fat ($r = -0.26$, $p = 0.001$) and HOMA-IR ($r = -0.20$, $p = 0.01$).

4. Discussion

The findings of this cross-sectional study confirm our hypothesis that there is an inverse relationship between the number of steps walked per day and cardiometabolic risk factors. We showed lower values for the adiposity indicators (waist circumference, total body fat, android body fat and gynoid body fat) and lower overweight (BMI data) prevalence in participants from the ≥10,000 steps group as compared to those from the <10,000 steps group, as well as a negative relationship of the numbers of steps per day to total body fat, android body fat and gynoid body fat.

Our data show that the number of steps/day was a negative predictive factor for total body fat, android body fat and gynoid body fat. In agreement with these results, another cross-sectional study showed an inverse correlation between the number of daily steps and adiposity in African-American middle-aged women.[10] In addition, negative associations between number of steps and BMI and central obesity after adjusted for age, ethnicity, sex, insulin use and number of oral hypoglycemic agents were found in individuals with diabetes mellitus.[24]

Our findings together with those from the above mentioned studies indicate that the higher the number of steps per day the lower the body adiposity. It suggests that the increase in the number of steps per day would result in reduction of body fat. In fact, an increase of daily steps was associated with reduced BMI and abdominal fat in diabetic patients.[24] It is also suggested that there is a dose–response relationship between aerobic exercise, such as brisk walking, and visceral fat reduction in obese subjects with-out metabolic-related disorders.[25] In this context, it is noteworthy that current recommendations are made for 150 min of moderate-intensity physical activity each week or for walking 10,000 steps per day for adults to achieve health benefits.[9,26]

Another important finding of the present study was the lower occurrence of insulin resistance and lower values of HOMA-IR in participants who performed more than 10,000 steps per day, compared to participants who practiced fewer than 10,000 steps. In fact, the number of steps was also a negative predictor of insulin resistance assessed by HOMA-IR regardless of age, position, android body fat, overweight/obesity prevalence and the ratio total triglycerides/HDL-c. A previous cohort study has found that an increase of 2000 steps/day by sedentary individuals, 5 years after baseline, was related to higher insulin

sensitivity, compared with those who practiced a lower number of steps/day.[5] Thus, previous studies as well as the present findings suggest an association between increased physical activity and increased insulin sensitivity/decreased insulin resistance. We also observed an inverse relationship between the number of steps per day and MetS. There was a negative correlation between both variables as a function of the lowest frequency of MetS diagnosis in those who practiced ≥10,000 steps per day. Additionally participants in this group had higher levels of HDL-c and lower triglycerides and waist circumference, three components of MetS.

In this sense, the improvement of metabolic syndrome components through the practice of physical exercise is partly related to the reduction of abdominal adiposity. This may occur due to a significant increase in the release of lipolysis-stimulating hormones, which increases blood concentrations of free fatty acids that can be captured and used as an energy source by skeletal muscles.[27] Moreover, physical exercise can increase the activity of lipoprotein lipase which leads to reduced concentrations of triacylglycerol and possibly to increased levels of HDL-c.[28] Interestingly, the increase in lipoprotein lipase activity can also be obtained by the accumulation of low-intensity physical activity throughout the day,[29] and this type of exercise has been negatively associated with waist circumference, an indicator of central adiposity and metabolic syndrome risk scores independent of the time spent on moderate- and high-intensity physical activities.[30]

Despite the inverse relationship between the number of steps walked per day and cardiometabolic risk factors observed here, there were no statistical differences between the ≥10,000 steps and <10,000 steps groups for blood pressure, blood glucose and total cholesterol. Although moderate walking is thought to promote risk reductions for hypertension, hypercholesterolemia and diabetes mellitus,[6] in the present study those individuals who self-declared hypertensive, diabetic or under treatment for dyslipidemia were not included. Therefore, such absences of differences were expected.

Finally, our study has some limitations. First, cross-sectional studies may present bias of selection and reverse causality as well as residual confounders. However, we applied a representative sample from population (Federal University of Viçosa¸ staff members), used calibrated and validated tools, and performed linear regression models, controlled by potential confounding

variables; all methods for contributing to internal validity of study. And second, pedometers are limited as walking measurement devices because they capture movement only of the lower body in the vertical plane and cannot distinguish the intensity of walking, load carriage as well as between walking on different surfaces or gradients. Thus, our results should be seen as the number of steps only.

5. Conclusion

We conclude that: (a) apparently healthy Brazilian middle-aged men performing ≥10,000 steps per day have better cardiometabolic conditions than those walking <10,000 steps; and (b) the number of steps per day is inversely related to the indicators of total and regional adiposity, insulin resistance and metabolic syndrome in these individuals.

Practical implications

- The practice of accumulating ≥10,000 steps per day can be a useful indicator of protection against insulin resistance and metabolic syndrome in apparently healthy Brazilian middle-aged men.
- The higher the number of steps per day the lower the total body fat, android and gynoid fat and insulin resistance in apparently healthy Brazilian middle-aged men.
- Apparently healthy middle-aged men with low levels of physical activity should be encouraged to increase the number of steps per day to achieve cardiometabolic benefits.

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2.2. ARTIGO 2

HABITUAL DIETARY GLYCEMIC LOAD IS ASSOCIATED WITH CARDIOMETABOLIC RISK FACTORS IN PHYSICALLY ACTIVE BRAZILIAN MIDDLE-AGED MEN

Carga glicêmica da dieta habitual é associada com fatores de risco cardiometabólico em homens brasileiros fisicamente ativos de meia-idade

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RESUMO

Introdução: Os efeitos da carga glicêmica da dieta sobre os fatores de risco cardiometabólico em indivíduos fisicamente ativos não são completamente conhecidos.

Objetivo: Neste estudo transversal a associação da carga glicêmica da dieta habitual com fatores de risco cardiometabólico em homens brasileiros fisicamente ativos foi avaliada.

Materiais e Métodos: Cento e setenta e seis indivíduos (Idade: 50,6 ± 5,0 anos, índice de massa corporal: 25,5 ± 3,6 kg/m^2) foram avaliados. Dados antropométricos, características de estilo de vida, resistência à insulina (HOMA-IR), biomarcadores de estresse oxidativo (8-iso-prostaglandina F2α; 8-iso-PGF2α e 8-hidroxi-2-deoxiguanosina; 8-OHdG) e perfil lipídico foram determinados. A ingestão alimentar foi estimada por meio de um questionário de frequência do consumo alimentar.

Resultados: A carga glicêmica da dieta foi positivamente associada com concentrações de ácidos graxos livres (ß=0,311, r^2=0,13, valor de p=0,034) e razão triacilgliceróis/HDL-c (ß=0,598, r^2=0,19, valor de p=0,028) independente

de fatores intervenientes (obesidade central, consumo de carne vermelha, idade e ingestão calórica). O biomarcador de estresse oxidativo, 8-OHdG, também foi associado com a carga glicêmica da dieta habitual (ß=0,432, r^2=0,11, valor de p=0,004), independente dos fatores intervenientes previamente mencionados associado com as variáveis: consumo excessivo de álcool, ingestão de ferro e tabagismo.

Conclusão: A carga glicêmica da dieta habitual foi positivamente associada com perfil lipídico (ácido graxo livre e razão triacilgliceróis/HDL-c) e concentrações de 8-OHdG (marcador de estresse oxidativo). Esses resultados indicam um potencial prejuízo da dieta com maior carga glicêmica para fatores de risco cardiometabólico em homens de meia-idade, mesmo sendo fisicamente ativos.

Palavras chave: *Hábito alimentar. Carboidrato. Triacilgliceróis. Lipoproteína de alta densidade. Estresse oxidativo.*

ABSTRACT

Introduction: The effects of dietary glycemic load (GL) on cardiometabolic risk factors in physically active subjects are not completely known.

Objective: This cross-sectional study assessed the association of habitual dietary GL with cardiometabolic risk factors in physically active Brazilian middle-aged men.

Methods: One-hundred seventy-six subjects (Age: 50.6 ± 5.0 years, BMI: 25.5 ± 3.6 kg/m^2) were evaluated. Anthropometry, lifestyle features, insulin resistance, oxidative stress biomarkers (8-iso-prostaglandin F2α; 8-iso-PGF2α and 8-hydroxydeoxyguanosine; 8-OHdG) and lipid profile were assessed. Dietary intake was estimated through a quantitative food frequency questionnaire.

Results: The dietary GL was positively associated with free fatty acid concentrations (ß=0.311, r^2=0.13, P-value=0.034) and triglycerides/HDL cholesterol ratio (ß=0.598, r^2=0.19, P-value=0.028) regardless of confounding factors (central obesity, red meat consumption, age and energy intake). The oxidative stress biomarker, 8-OHdG, also was associated with habitual dietary GL (ß=0.432, r^2=0.11, P-value=0.004), regardless of previous confounding factors plus excessive alcohol consumption, iron intake and current smoking status.

Conclusions: The dietary GL was positively associated with lipid profile (free fatty acid levels and triglycerides/HDL cholesterol ratio) and oxidative stress biomarker (8-OHdG). These results indicate potential harmfulness of diet with higher GL to cardiometabolic risk factors in middle-aged men, even in physically active individuals.

Keywords: *Food habits. Carbohydrate. Triglycerides. High-density lipoproteins. Oxidative stress.*

Abbreviations

8-iso-PGF2α: 8-iso-prostaglandin F2α

8-OHdG: 8-hydroxydeoxyguanosine

BMI: body mass index

CVD: Cardiovascular diseases

DM2: type 2 diabetes

FFA: Free fatty acids

FFQ: food frequency questionnaire

GI: glycemic index

GL: glycemic load

HDL-C: high density lipoprotein

HOMA-IR: Homeostatic model of assessment of insulin resistance.

NADH: Reduced nicotinamide adenine dinucleotide

NAD+: Oxidized nicotinamide adenine dinucleotide

TG/HDL-C: ratio between triglycerides and high density lipoprotein

TG: triglycerides

Introduction

Cardiovascular diseases (CVDs) are the leading cause of mortality worldwide. The World Health Organization estimated that approximately 30% of deaths from CVDs occurred in the world in 2008 and that this rate will reach higher proportions in 2030.[1] The occurrence of these diseases is highly influenced by environmental factors, particularly, the quality of food intake[2] and the level of physical activity.[3]

The role of carbohydrate intake as a risk factor for manifestation of chronic diseases has received important attention in the scientific community. The dietary glycemic load (GL), obtained by multiplying the glycemic index (GI) of a food/meal by its available carbohydrate content,[4] reflects the quality and the amount of the consumed carbohydrate. The consumption of high-GL diets leads to postprandial hyperglycemia/hyperinsulinemia, increasing the risk for CVDs.[5] Moreover, the increase of dietary GL has been associated with CVDs risk factors, such as reduced levels of high density lipoprotein (HDL-C), high concentrations of triglycerides[6-8] and higher levels of oxidative stress marker.[9] Thus the dietary pattern adopted by the modern society, characterized by high consumption of carbohydrate-rich foods and high-GI diet[10] may increase the risk for CVDs in the population.

Most of the previous studies associating dietary GI/GL with cardiovascular risk factors were typically conducted in women,[8, 11] type 2 diabetes (DM2)[12] and obese subjects.[13] On the other hand, the relationship between dietary GL and cardiometabolic risk factors in physically active individuals, to our knowledge, has not been clarified yet. Taking into consideration that regular physical activity has beneficial effects against CVDs[14] and that aging is a potent cardiovascular risk factor[1] this cross-sectional study was designed to assess the association of habitual dietary GL with cardiometabolic risk factors in physically active middle-aged men.

Materials and methods

Study population

One-hundred seventy-six (176) men aged between 40 and 59 years participated in this study. The staff members of Federal University of Viçosa, Brazil were recruited by systematic sampling using interview as previously described[15]. The following exclusion criteria were considered: body weight changes greater than 3 kg in the three months preceding the beginning of the study, thyroid diseases, heart failure, cerebrovascular diseases, infectious diseases, inflammatory diseases, gastrointestinal tract diseases, liver disease, chronic kidney disease and/or history of kidney stones, cancer in the previous ten years, eating disorders (anorexia and bulimia), food allergies, changes in the level of physical activity and in the eating habit in the three months preceding the study. Subjects using vitamin supplements, diuretics or drugs that affect food intake and/or the metabolism of nutrients, pacemaker and/or prosthetic limbs users, elite athletes and subjects who were not physically active (number of steps < 10,000)[16] were also excluded.

The study was conducted according to the Declaration of Helsinki guidelines and all procedures involving human subjects were approved by the Ethics Committee in Human Research of the Federal University of Viçosa (Reference nº 069/2010). Written informed consent was obtained from all participants of the study.

Dietary intake assessment

A food frequency questionnaire (FFQ), developed for the Brazilian population, was used to assess the usual dietary intake of the participants.[17] Daily food consumption was estimated as frequency x portion x size for each consumed food item. Nutrient intake was assessed using the software Dietpro® version 5.5i (AS Systems, Viçosa, Brazil), using mainly two Brazilian nutritional composition tables or an international composition table when the needed nutritional information was not described in these tables as previously described.[18]

The GI values for most foods listed on the FFQ were obtained from the University of Sydney GI data base website.[19] The GI of foods not listed in that database was estimated considering the GI of foods that had similar nutritional

composition and methods of preparation. Dietary GI and GL were calculated using the formulas described by Levitan *et al.*:[11] [Dietary GI = $\sum_{foods}$ Carbohydrate (g) in a serving of food × Frequency of consumed food × *GI* ⁄ $\sum_{foods}$ Carbohydrate (g) in a serving of food × Frequency of consumed food; Dietary GL = $\sum_{foods}$ Carbohydrate (g) in a serving of food × Frequency of consumed food × GI⁄100]

It should be noted, however, that we considered only the available carbohydrate for these calculations.

Anthropometric and body composition assessments

Body weight, height and waist circumference were taken using standard measurement procedures, as previously described.[15] Body mass index was calculated as weight (kg) divided by height squared (m^2).The cut-off used for central obesity was waist circumference ≥ 94 cm.[20]

Total body fat percentage was determined by total body scanning with a dual energy X-ray absorptiometry (GE/Lunar, Madison, WI, USA; enCORE software version 13.31).

Lifestyle co-variables

The participants were asked about their current smoking status (yes/no) and habitual quantity/frequency of alcohol consumption. Excessive alcohol consumption was defined as a daily ingestion above 21 units per week.[21] So, in the statistical analysis, were considered excessive alcohol consumer (yes) or no alcohol consumer (no).

The habitual physical activity was estimated by the mean number of daily steps (7 consecutive days) measured by Digi-Walker SW-200 pedometer (Yamax Corporation, Tokyo, Japan), according to the instructions previously described.[15] A minimum of 10,000 steps/day was considered the cut-off point to classify individuals as active.[16]

Sample collection and analysis

Blood samples were collected from the antecubital vein after 12-hour overnight fasting. The serum was separated from whole blood by centrifugation at 2.225 *g* for 15 min at room temperature (2-3 Sigma, Sigma Laborzentrifuzen,

Osterodeam Harz, Germany) and was immediately frozen at -80° C until analysis.

Glucose, insulin and the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index were assessed by protocol previously described.[15]

Free fatty acids (FFA) were determined by a kinetic spectrophotometry method using the kit EnzyChrom Free Fatty Acid Assay (Bioassay Systems, Hayward, CA). Serum HDL-C and triglyceride (TG) were determined by the enzymatic colorimetric method (Cobas Mira Plus - Roche Diagnostics GmbH, Montclair, NJ, USA). The atherogenic index was calculated by the ratio between triglycerides and HDL-C (TG/HDL-C ratio).[22]

The urine samples were collected after 12-hour overnight fasting and frozen at -80° C until analysis. The concentrations of 8-iso-prostaglandin F2α (8-iso-PGF2α) (Oxford, MI, USA) and 8-hydroxydeoxyguanosine (8-OHdG) (Cayman, MI, USA) were determined by competitive ELISA according to manufacturer's instructions. Although Cayman's kit recognizes the 8-OHdG from DNA, the ELISA values are always higher than LC/MS inasmuch as this method also detects 8-hydroxyguanosine and 8-hydroxyguanine from either DNA or RNA. The values of urinary 8-iso-PGF2α and 8-OHdG were normalized by milligrams of urinary creatinine which was measured by a kinetic colorimetric method using a Bioclin commercial kit (Cobas Mira Plus - Roche Diagnostics GmbH, Montclair, NJ, USA).

Statistical analysis

Normal distribution of data was assessed by the Shapiro-Wilk test. Non-normally distributed variables were log-transformed before statistical analysis. The nutrients, dietary GI, and dietary GL were energy adjusted using the residuals method as previously applied.[11, 23] The comparison of nutrients consumption among lower or higher dietary GL (estimated by median) was performed by t test. Such methodological procedure of dividing participants into groups of risk has been used in epidemiologic study previously.[8]

To evaluate the associations of dietary GL, available carbohydrate and dietary GI with FFA, TG/HDL-c ratio we used linear multiple regression controlled by the occurrence of central obesity (waist circumference ≥ 94 cm), red meat consumption (g/d), age (years) and energy intake (kcal/d). To evaluate the associations of dietary GL, available carbohydrate and dietary GI with 8-iso-

PGF2α and 8-OHdG we used linear multiple regression controlled by previous confounding factors plus others important confounding factors on oxidative stress such as excessive alcohol consumption (yes/no), daily iron intake (mg/d) and current smoking status (yes/no).

In addition, the Spearman correlation coefficient was used to investigate the correlation of HOMA-IR with FFA and with TG/HDL as well as of the dietary GL with available carbohydrate and with dietary GI.

Data processing and analysis were performed using the software STATA version 9.1 (Stata Corp., College Station, TX, USA). The P-value <0.05 was considered as statistically significant.

Results

Anthropometric, clinical and lifestyle characteristics of the participants are shown in Table 1. The mean BMI was equivalent to 25.5 kg/m^2 and the number of steps per day corresponded to 13,591. The central obesity occurrence (waist circumference ≥ 94 cm) was 31.2%.

Table 1. Anthropometric, clinical and lifestyle characteristics of participants

Variables	Values[a]
Age (years)	50.6 ± 5.0
Body mass index (kg/m^2)	25.5 ± 3.6
Waist Circumference (cm)	89.1 ± 9.8
Total body fat (%)	21.0 ± 7.5
HDL-C (mg/dL)	47.9 ± 12.6
HOMA-IR	1.29 ± 1.09
Triglycerides (mg/dL)	127.7 ± 86.0
TG/HDL-C ratio	3.0 ± 2.8
Free fatty acid (mmol/L)	0.78 ± 0.30
8-OHdG (ng/mg creatinine)	8.7 ± 3.1
8-iso-PGF2α (ng/mg creatinine)	1.53 ± 1.22
Central obesity n (%)	55 (31.2)
Excessive alcohol consumption n (%)	29 (16.5)
Steps numbers per day	13,591 ± 2,798
Smoker n (%)	26 (14.8)

Abbreviation: HDL-C, high density lipoprotein; TG/HDL-C ratio, ratio between triglycerides and high density lipoprotein; 8-iso-PGF2α, 8-iso-prostaglandin F2α; 8-OHdG, 8-hydroxy-2´-deoxyguanosine. [a] Values are mean ± SD of 176 individuals for continuous variables; and number (frequency) for categorical variables.

Regarding the dietary habits, according to the median of dietary GL consumed by participants, those subjects who consumed a diet with lower GL (<105.2 units) also consumed a lower GI diet, lower amount of carbohydrate, as well as higher amount of protein, fat, red meat and iron than those who consumed a diet with higher GL (≥ 105.2 units) (Table 2).

Table 2. Food and nutrient consumption, according to median energy-adjusted glycemic load intake

	Lower Glycemic Load <105.2 units	Higher Glycemic Load ≥105.2 units	P-value[a]
Energy intake (kcal/d)	1,421 ± 455.5	1,518 ± 496.7	0.174
Glycemic Index	62.8 ± 3.7	67.8 ± 6.1*	<0.001
Carbohydrate (g/d)	176.2 ± 23.4	220.6 ± 25.3*	<0.001
Protein (g/d)	73.8 ± 12.3	60.9 ± 9.6*	<0.001
Fat (g/d)	50.7 ± 9.1	36.6 ± 10.0*	<0.001
Fiber (g/d)	21.9 ± 5.7	23.7 ± 6.5	0.063
Iron (mg/d)	7.3 ± 1.2	6.8 ± 1.2*	0.002
Red meat (g/d)	82.1 ± 39.7	58.5 ± 22.0*	<0.001

Values are mean ± SD of 176 individuals. [a] P-value from Student t-test. *denotes statistical difference from diet with lower load glycemic.

Multiple linear regression models were applied to assess the relationship of dietary GL, dietary GI and available carbohydrate with blood lipid profile (FFA levels and TG/HDL-C ratio) and level of oxidative stress markers. Interestingly, TG/HDL-C ratio and levels of FFA and of a marker of oxidative DNA damage, 8-OHdG were positively associated with dietary GL, regardless of interfering variables (Table 3). Moreover, there was a positive association of TG/HDL-C ratio and 8-OHdG levels with the dietary available carbohydrate, regardless of interfering variables. However, there were no associations between dietary GI and the evaluated variables (Table 3).

Table 3. Multiple linear regression models with glycemic load, available carbohydrate and glycemic index as main independent variable

Dependent variables	Main independent variable	ß	CI 95%	R^2	P-value
Free fatty acid (mmol/L)[a]	Glycemic load	0.311	0.02354 /0.5992	0.13	0.034*
	Available carbohydrate	0.328	-0.003749 /0.09513	0.12	0.078
	Glycemic index	0.473	-0.1368 /1.08449	0.11	0.127
TG/HDL-C ratio[a]	Glycemic load	0.598	0.06646 /1.13069	0.19	0.028*
	Available carbohydrate	0.747	0.0730 /1.4223	0.19	0.030*
	Glycemic index	0.591	-0.54334 /1.7256	0.17	0.305
8-iso-PGF2α (ng/mg creatinine)[b]	Glycemic load	0.407	-0.312702 /1.12710	0.12	0.266
	Available carbohydrate	0.293	-0.615624 /1.202685	0.12	0.525
	Glycemic index	0.964	-0.5336651 /2.46290	0.12	0.205
8-OHdG (ng/mg creatinine)[b]	Glycemic load	0.432	0.1382791/0.726494	0.11	0.004*
	Available carbohydrate	0.614	0.244211/0.98540	0.12	0.001*
	Glycemic index	0.222	-0.4102/0.85448	0.03	0.489

Abbreviation: ß, beta coefficient; CI, confidence interval; TG/HDL-C ratio, ratio between triglycerides and high density lipoprotein; 8-iso-PGF2α, 8-iso-prostaglandin F2α; 8-OHdG, 8-hydroxy-2´-deoxyguanosine. [a] P-value from the linear regression model adjusted for occurrence of central obesity (waist circumference ≥ 94 cm), red meat intake (g/d), age and energy intake (kcal/d). [b] P-value from the linear regression model adjusted for occurrence of central obesity (waist circumference ≥ 94 cm), red meat intake (g/d), age (years), energy intake (kcal/d), excessive alcohol consumption (yes/no), iron intake (mg/d) and current smoking status (yes/no). *denotes significant relationship.

Finally, was verified a positive correlation of blood lipid profile (TG/HDL-C ratio and FFA concentrations) with HOMA-IR (Figure 1) as well as of dietary GL with available carbohydrate and dietary GI (Figure 2).

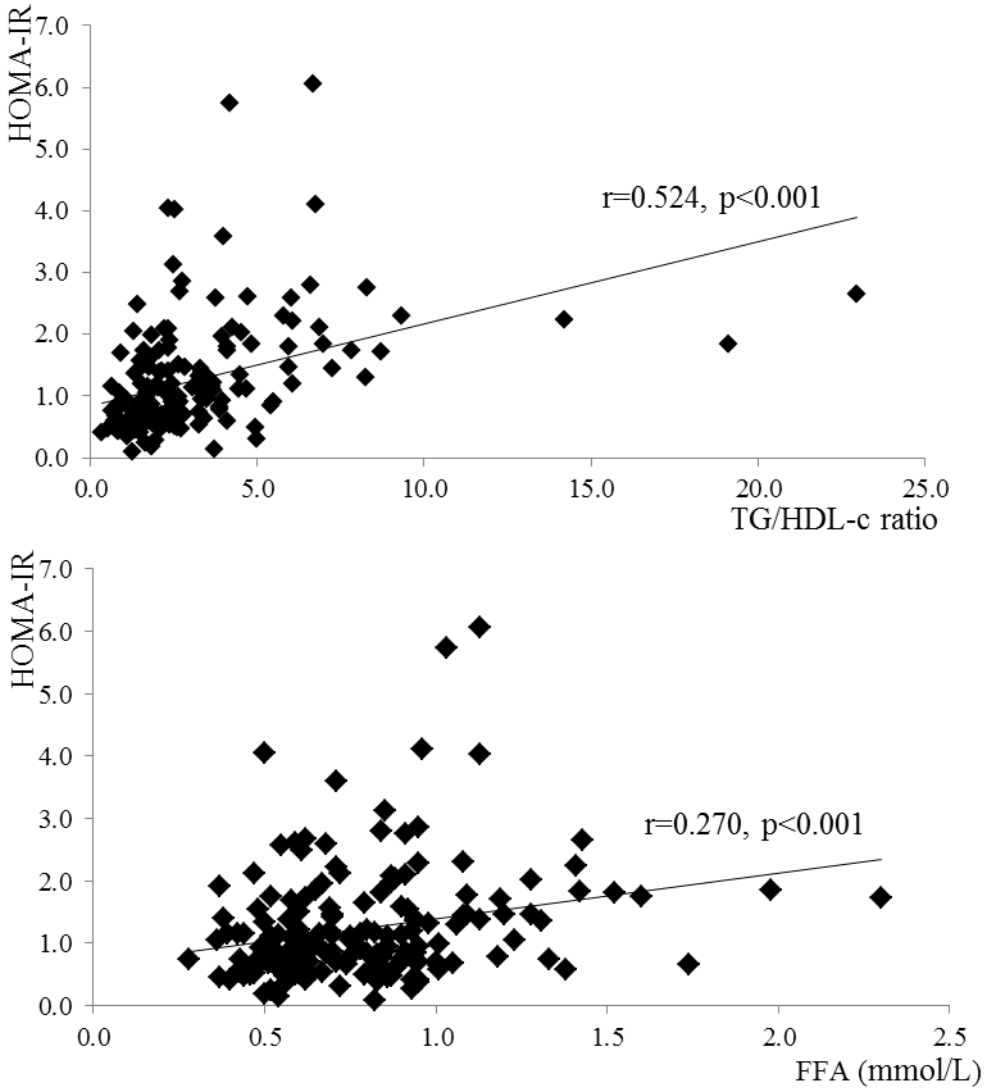

Figure 1. Spearman´s correlations coefficients (R) for HOMA-IR and lipid profile (TG/HDL-c ratio, ratio between triglycerides and high density lipoprotein; FFA, free fatty acids concentrations)

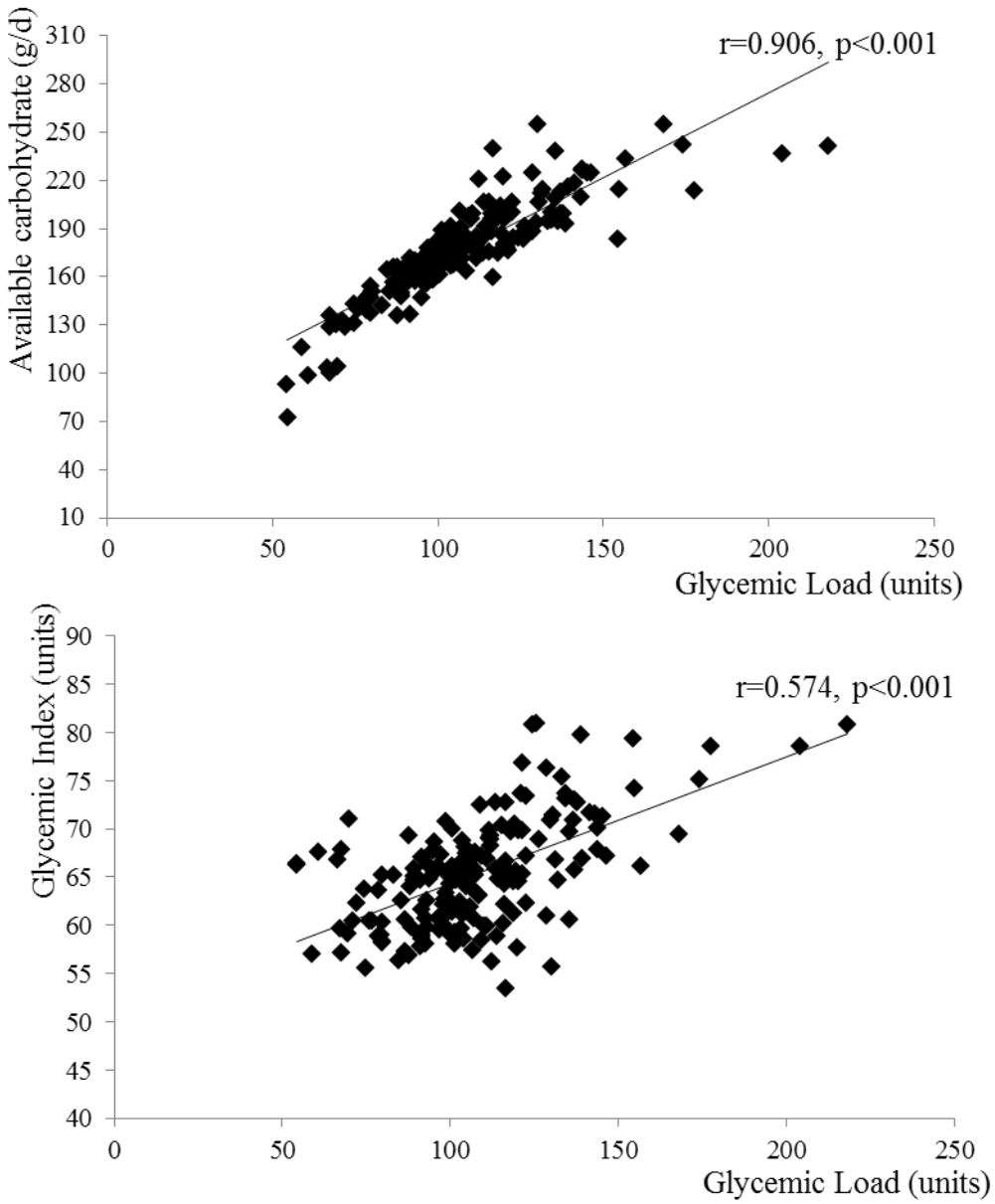

Figure 2. Spearman´s correlations coefficients (R) for dietary glycemic load and available carbohydrate and dietary glycemic index.

Discussion

This cross-sectional study was carried out to evaluate the association of habitual dietary GL with cardiometabolic risk factors in physically active Brazilian middle-aged men.

An important finding was the positive association of dietary GL with the TG/HDL-C ratio in this population, after adjustment for the confounding variables. The increased levels of TG and the reduced levels of HDL-C promoted by the consumption of high-carbohydrate diets may be related to decreased clearance of TG-rich particles. The clearance of TG occurs through the action of lipoprotein lipase (LPL) both in the TG stored in adipose tissue or skeletal muscle. In the skeletal muscle LPL is also involved in TG oxidation. However, the competition between chylomicron from enterocytes and VLDL-C particles (from liver) by LPL leads to its accumulation in plasma, which stimulates cholesterol ester transfer from HDL-C and LDL-C to those TG-rich lipoproteins. Subsequently, the content of TG from HDL-C particles could be hydrolyzed by hepatic lipase forming small particles of such lipoprotein that are rapidly removed via circulation.[24]

Similar results were reported in the Health Workers Cohort Study[7] with men and women aged 20 to 70 years and by other[11] in women (≥ 45 years) without diabetes/DCVs showing a positive association of dietary GL with TG concentrations and a negative association with HDL-C levels. In contrast, a randomized clinical trial demonstrated that despite the reduction in dietary GL, no association of this change with improvements in TG and HDL-C levels was found in middle-aged men and women.[23] It is possible that the results reported by Lin *et al.*[23] reflects the impact of the food intake under-reporting on GL classification and on its association with risk factors.

Hypertriglyceridemia induced by high-carbohydrate diets can also be triggered by insulin resistance due to stimulation of lipolysis and consequently an endogenous overproduction of FFA and VLDL-C.[25] Moreover, the glucose and insulin levels increases considerably in the period immediately after consumption of high GI[26]/GL[5] diet while in the period 4 to 6 hours post-prandial there is glycaemia reduction and possibility hypoglycemia, which leads to increased release of counter-regulatory hormones, and consequently the increase in glycaemia and FFA levels.[27] In fact our results shows a positive

association of FFA levels with dietary GL, as well as, a positive correlation of insulin resistance (HOMA-IR) with FFA levels and with TG/HDL-C ratio.

The inverse relationship between HDL-C and CVDs risk is well established inasmuch as HDL-C exerts antioxidant, anticoagulant, antiplatelet and antiatherogenic functions.[28] Moreover, high concentrations of FFA[29] and TG/HDL-C ratio[30] may favor increased of CVDs risk. Thus, our results indicate that the increase in dietary GL is a possible risk factor for CVDs, even in physically active individuals.

The practice of walking (metabolic equivalent hours per day walk) was associated with lower CVDs risk in a cohort study that included the participation of men and women.[14] In addition, daily walking can attenuate the fasting TG levels and remnants particles in response to high-carbohydrate intake,[31] and the number of steps walked per day have been inversely associated with TG levels and TG/HDL-C ratio.[15] However, dietary GL was not assessed by Koutsari *et al.*[31] and in other studies[14, 15] the association of physical activity with dietary intake was not investigated. Thus, it is still not yet clear whether the number of steps/walking may actually attenuate the deleterious effects of dietary high GL in cardiometabolic risk factors manifestation.

Postprandial hyperglycemia also has been related to oxidative stress,[32] possibly due to increased production of free radicals by the non-enzimatic glycation; auto-oxidation of glucose; and intracellular activation of the polyol pathway, which produces an imbalance in the NADH/NAD+ ratio.[33] In this context, another important result obtained in this study was the positive association of dietary GL and urinary 8-OHdG an oxidative DNA damage marker.

Besides postprandial hyperglycemia, oxidative stress may be a consequence of a reduction in the antioxidant activity.[34] However, since HDL-C has antioxidant function[35] and a negative association of dietary GL with this lipoprotein was demonstrated in the present study, we performed complementary analysis and observed a negative correlation between 8-OHdG and HDL-C (R=-0.153, P-value= 0.04). This outcome indicates that the pro-oxidant effect of dietary GL could also be related to reduce HDL-C levels.

Our results showed no association between dietary GL and the lipid peroxidation marker, 8-iso-PGF2α. In concert, a crossover study there were no significant changes in the fasting urinary isoprostane levels in young men (29.4

± 4.4 years) after the consumption of high or low GI.[36] Another cross-sectional study reported that plasma malondialdehyde levels, but not isoprostane, increased linearly with the increase in GL in healthy men and women (46.7 ± 13.5 years).[9] In view of these results, investigations are needed to further test the relationship between dietary GL and changes in levels of 8-iso-PGF2α.

It is noteworthy that in the present study, we observed a strong correlation of dietary GL with available carbohydrate consumption and a moderate correlation between dietary GI and dietary GL. Similar results were obtained in the Women's Health Study conducted in postmenopausal women without diagnosis of CVDs or cancer[11] and in another study conducted among healthy men and women (18-75 years).[9] These outcomes suggest that the available carbohydrate but not the dietary GI explains better the statistical results obtained for dietary GL.

Some limitations of the current study should be noted. First, since this is a cross-sectional, the results showed here must be cautiously considered as we cannot assure that the observed associations show a cause/effect relationship, although we controlled potential interfering variables. Second, further studies involving larger numbers of physically active individuals and/or with longitudinal design should be conducted before considering the application of these results at the population level.

In summary, the data presented in this cross-sectional study, showed that dietary GL was positively associated with blood lipid profile (TG/HDL-C ratio and FFA levels) and with oxidative stress marker (8-OHdG) in physically active men. These results indicate the potentially harmful influence of the consumption of higher GL diets on cardiometabolic risk factors, even in physically active subjects.

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2.3. ARTIGO 3

FRUIT AND VEGETABLE INTAKE AND RELATED-NUTRIENTS ARE ASSOCIATED WITH OXIDATIVE STRESS MARKERS IN MIDDLE-AGED MEN

Consumo de frutas, hortaliças e legumes e nutrientes relacionados são associados com marcadores de estresse oxidativo em homens de meia-idade

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Classificação Qualis Nutrição: A1

RESUMO

Objetivo: O objetivo deste estudo transversal foi avaliar a potencial relação entre o consumo de frutas, hortaliças e legumes (FHL) e marcadores de estresse oxidativo, com ênfase no conteúdo de vitamina C, fibra e magnésio**.** *Métodos e procedimentos:* O estudo foi conduzido com 296 indivíduos saudáveis, com idade de 50,5 ± 5,0 anos e índice de massa corporal de 25,8 ± 3,5 kg/m^2. Biomarcadores urinários e sanguíneos, dados de ingestão alimentar, antropométricos, pressão arterial, bem como, dados de estilo de vida foram avaliados por procedimentos validados. Os marcadores de estresse oxidativo selecionados foram: lipoproteína de baixa densidade oxidada plasmática (LDL oxidada), 8-iso-prostaglandina F2α (8-iso-PGF2α) e 8-hydroxy-2´-deoxyguanosine (8-OHdG) urinários. *Resultados:* Os indivíduos incluídos no maior tercil de consumo de FHL (≥ 341,1 g/d) apresentaram menores concentrações de LDL oxidada, 8-iso-PGF2α and 8-OHdG (P de tendência <

0,05), independente de fatores intervenientes. Adicionalmente, as concentrações de LDL oxidada foram negativamente associadas com a ingestão de fibra do grupo de FHL independente de fatores intervenientes. As concentrações de LDL oxidada e 8-OHdG tenderam ser menores no maior tercil de consumo de magnésio (P de tendência = 0,06) e vitamina C (P de tendência = 0,05) do grupo de FHL. Ademais, as concentrações de 8-iso-PGF2α foram menores nos indivíduos alocados no maior tercil de ingestão de fibra (≥ 6,5 g/d, P de tendência = 0,034), vitamina C (≥ 98,0 mg/d, P de tendência = 0,007) e magnésio (≥ 48,9 mg/d, P de tendência = 0,018) do grupo FHL. *Conclusão:* Maior ingestão de FHL foi independentemente associada com redução de LDL oxidada, 8-OHdG e 8-iso-PGF2α em homens de meia-idade. O conteúdo de fibra, vitamina C e magnésio das FHL parece contribuir nesta relação benéfica.

Palavras chave: Frutas, hortaliças e legumes; Vitamina C; Magnésio; Fibra; LDL oxidada; 8-iso-prostaglandina F2α; 8-hidroxi-2'-deoxiguanosine

ABSTRACT

Objective: The aim of this cross-sectional study was to assess the potential relationships between fruit and vegetable (FV) intake and oxidative stress markers in middle-aged men, with emphasis on vitamin C, fiber and magnesium contents. *Research Methods & Procedures:* The study was conducted with 296 healthy subjects, with 50.5 ± 5.0 years of age and BMI of 25.8 ± 3.5 kg/m2. Dietary intake, anthropometry, blood pressure, lifestyle features and blood and urine biochemical data were assessed with validated procedures. The oxidative stress markers selected were: plasma oxidized low density lipoprotein (ox-LDL); urinary 8-iso-prostaglandin F2α (8-iso-PGF2α) and 8-hydroxy-2´-deoxyguanosine (8-OHdG). *Results:* The subjects included in the highest tertile of FV intake (≥ 341.1 g/d) showed lower concentrations of ox-LDL, 8-iso-PGF2α and 8-OHdG (P for trend<0.05), regardless of confusion factors. The concentrations of ox-LDL was negatively associated with fiber from the FV intake (P for trend<0.050) regardless of confusion factors. The ox-LDL and 8-OHdG concentrations tended to be lower in the higher tertile of magnesium (P for trend=0.060) and vitamin C from FV intake (P for trend=0.05), respectively. In addition, the concentrations of 8-iso-PGF2α were lower in subjects in the

highest tertile of fiber (≥ 6.5 g/d, P for trend=0.034), vitamin C (≥ 98.0 mg/d, P for trend=0.007) and magnesium (≥ 48.9 mg/d, P for trend=0.018) from the group FV intake. *Conclusion(s):* Greater FV intake was independently associated with reduced ox-LDL, 8-OHdG and 8-iso-PGF2α in middle-aged men. Fiber, vitamin C and magnesium from FV seem to contribute to this beneficial relationship.

Keywords: Fruit and vegetable; Vitamin C; Magnesium; Fiber; Oxidized LDL; 8-iso-prostaglandin F2α; 8-hydroxy-2´-deoxyguanosine

1. Introduction

Free radicals when produced in adequate proportion have relevant biological functions such as gene activation and participation in body's defense mechanisms against infections [1]. However when the production of free radicals and/or reactive species exceeds the antioxidant defense, it may favor the oxidation of biomolecules such as lipids, proteins and deoxyribonucleic acid (DNA) resulting in cell damage and loss of its biological functions [1]. In this sense, the oxidative stress may play a decisive role in the pathogenesis and progression of several chronic diseases, including cardiovascular disease [2] and cancer [3].

During lipid oxidation, the peroxidation of the arachidonic acid (lipid present in body cell membranes) produces F2 isoprostane. Increase in this biomarker has been positively related to coronary artery disease [4], diabetes mellitus [5] and other diseases [6]. Reactive oxygen species within blood vessels can also promote oxidative modification of the LDL molecule generating oxidized LDL (ox-LDL) [7], which is directly related to atherosclerotic events [8]. Moreover, oxidative damage to DNA may occur by the oxidation of deoxyguanosine, producing the 8-hydroxy-2´-deoxyguanosine (8-OHdG) [9], which is considered a risk factor for cancer, atherosclerosis and diabetes mellitus [10].

In their turn, eating habits can significantly influence the development of most chronic diseases, affecting the health of individuals throughout life. Thus, a healthy diet including fruits and vegetables (FV) can play an important role in the prevention of these diseases in middle-aged individuals [11]. It has been reported that the daily intake of adequate amounts of FV is inversely associated with chronic disease [12], cardiovascular risk factors [13] and oxidative stress [14, 15] probably due to their potential anti-inflammatory [16, 17] and antioxidant effects [18-21]. However, studies investigating the relationship of FV intake with lipid and/or DNA oxidation biomarkers in middle-aged men are still scarce [22-24], especially considering the dietary habits of the Brazilians whose FV intake is nearly 90% lower [25] than that recommended by the WHO [12].

Fruits and vegetables are source of nutritional components with high antioxidant capacity such as carotenoids [26] and polyphenols [27, 28]. In addition, this food-group is an important source of vitamin C, fiber and/or magnesium, nutrients that have been negatively associated with oxidative stress events [29-33]. Despite that, the relationship of nutrients derived from FV, such as magnesium, vitamin C and fiber with oxidative stress markers has not been clarified yet.

Therefore, the aim of this cross-sectional study was to assess the relationship between FV intake and the concentrations of ox-LDL, 8-iso-PGF2α and 8-OHdG in middle-aged men, with emphasis on vitamin C, fiber and magnesium contents.

2. Materials and methods

Participants

This cross-sectional study was carried out between March and December 2011. The sample size was calculated [34] considering the total number of male staff at Federal University of Viçosa (UFV), Viçosa city, Brazil in February 2011, aged between 40 and 59 years (1,744 individuals), confidence level of 95%, 24.4% expected prevalence of metabolic syndrome (metabolic condition highly related with oxidative stress [35]) in Brazilian middle-aged men [36] and 4.5% sampling error, resulting in 293 participants as a minimum of sample size required.

Participants were recruited by systematic sampling. We excluded those individuals who self-declared the following: body weight alterations greater than 3kg in the three months preceding the study, altered levels of physical activity and eating habits in the three months preceding the study, thyroid diseases, heart failure, cerebrovascular diseases, infectious diseases, inflammatory diseases, diseases of the gastrointestinal tract, liver disease, chronic kidney disease and/or a history of kidney stones, cancer in the previous ten years, eating disorders (anorexia and bulimia) and food allergies. Individuals using vitamin supplements and those using diuretics or drugs that alter food intake and/or the metabolism of nutrients, pacemaker and/or prosthetic limb users and elite athletes were also excluded.

We interviewed 848 men and 548 were eliminated by the exclusion criteria. Of 300 individuals selected, four did not answer the food frequency questionnaire, so the final sample comprised 296 individuals.

Each participant signed a written informed consent, which was approved by the Ethics Committee in Human Research of the Federal University of Viçosa (Reference nº 069/2010) in accordance with principles of the Declaration of Helsinki.

Information about lifestyle factors including work position, current smoking status and alcohol consumption was collected using a questionnaire. The criteria for classification of work position were previously described [37], while excessive alcohol consumptions were defined as intake above 21 units per week [38].

The habitual physical activity was estimated by the mean number of daily steps (7 consecutive days) measured by Digi-Walker SW-200 pedometer (Yamax Corporation, Tokyo, Japan), according to the instructions previously described [37].

A quantitative food frequency questionnaire (FFQ) with 105 food-items, validated for the Brazilian population [39] was used to assess the usual dietary intake of the participants during the previous six months. Daily food consumption was estimated as frequency x portion x size for each consumed food item. The intake of each nutrient, such as fiber, vitamin C, magnesium and others were assessed using the software Dietpro® version 5.5i (AS Systems, Viçosa, Brazil), using mainly two Brazilian nutritional composition tables [40, 41]. When the needed nutritional information was not observed in these tables the USDA table [42] was used.

The FV intake assessed from the data in the FFQ included the evaluation of 8 fruits (only fresh): orange, banana, apple, papaya, watermelon/melon, pear and other fruits (grapes and pineapple) and 10 vegetables (fresh or cooked): lettuce, watercress/kale/spinach (dark green leaves), cabbage, cauliflower/broccoli, carrot/pumpkin, tomatoes, beets, chayote/zucchini, okra and cucumber. Juice intake was not considered in this study due to the joint determination of FFQ for sugar-sweetened and unsweetened juices.

Anthropometric and biomedical data collection

Anthropometric determinations such as weigh, height, and waist circumference were taken using standard measurement procedures, as previously described [37]. Body mass index was calculated as weight (kg) divided by height squared (m^2). Total body fat percentage was determined by total body scanning with a dual energy X-ray absorptiometry (GE/Lunar, Madison, WI; enCORE software version 13.31) while the percentage of fat in the android region was determined using the "region of interest" program, according to the manufacturer's instructions. Systolic and diastolic blood pressures were measured following VI Brazilian Guidelines on Hypertension [43].

A venous blood sample was taken after 12-hour overnight fast for measuring glucose, insulin, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides, free fat acid (FFA) and ox-LDL. Glucose was measured by the glucose oxidase method (Cobas Mira Plus, Roche Diagnostics, GmbH, Montclair, NJ, USA) and insulin by electrochemiluminescence using the Modular Analytics (E170, Roche Diagnostics, GmbH, Mannheim, Germany). Insulin resistance was estimated by the Index of Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) which was obtained using the formula described by Matthews et al. [44]. Then, serum TC, HDL-C and triglycerides were determined by the enzymatic colorimetric method (Cobas Mira Plus - Roche Diagnostics GmbH, Montclair, NJ, USA). FFA concentrations were determined by the kinetic spectrophotometry method using the kit EnzyChromFree Fatty AcidAssay (Bioassay Systems, Hayward, CA). The metabolic syndrome was diagnosed by Alberti et al. criteria [45]. Finally, plasma concentrations of ox-LDL were determined by a commercially available ELISA kit (Mercodia, Uppsala, Sweden) based on the direct sandwich technique.

Urinary biomarkers of oxidative stress

Urine was collected in sterile tubes (after 12-hour overnight fasting) for measuring the oxidative stress biomarkers, 8-iso-PGF2α and 8-OHdG.

Competitive ELISA was used to determine urinary concentrations of 8-iso-PGF2α (Oxford, MI, USA) and 8-OHdG (Cayman, MI, USA). The analyses were performed according to manufacturer's instructions. Although the Cayman's kit recognizes the 8-OHdG from DNA, the ELISA values are always higher than LC/MS inasmuch as this method also detects 8-hydroxyguanosine and 8-hydroxyguanine from either DNA or RNA. The values of urinary 8-iso-PGF2α and 8-OHdG were normalized by milligrams of urinary creatinine, measured by a kinetic colorimetric method, using a Bioclin commercial kit (Cobas Mira Plus, Roche Diagnostics GmbH, Montclair, NJ, USA).

Statistical methods

Normal distribution of the data was determined by the Shapiro-Wilk test. Non-normally distributed variables were log-transformed before statistical analysis. To evaluate the association between FV intake, oxidative stress and other variables, the participants were categorized into tertiles based on this food-group consumption adjusted by energy daily intake using the residual method. The quantiles cutoff criteria have been previously applied [16, 29] and are based on a valid and reliable method to assign two or more groups of risk in nutritional epidemiology studies [46]. A comparison between the three groups was performed by ANOVA followed by a Bonferroni post hoc test. A chi-square test for linear trend was used to compare proportions between FV intake and categorical variables.

Linear trends were assessed by assigning the average value to each tertile of FV intake and modeling those values as a continuous variable. Initially, it was used a model controlled by android fat (%), habitual physical activity, work position, excessive alcohol consumption, daily caloric intake (kcal/day), FFA concentrations (mmol/l) and HOMA-IR. Then, it was developed another model controlled by the same variables associated with the covariate "current smoker". The same procedures were performed to assess the relationship between dietary fiber, vitamin C and magnesium (from FV intake) and markers of oxidative stress. A stepwise multiple regression analysis was also used to

identify the key foods (FV food-group) consumed by the participants. Calorie consumption outliers were defined by dispersing interquartile according Vittinghoff et al. [47]. Outliers were excluded (five individuals with caloric intake above the upper limit ≥2.640 kcal/d) followed by all statistical analyzes previously described. The results maintained the same trend with all subjects included. Data processing and analysis were performed using the software STATA version 9.1 (Stata Corp., College Station, TX, USA), considering $P<0.05$.

3. Results

Anthropometric, clinical and lifestyle characteristics were assessed according to tertiles of FV intake (Table 1). The diastolic blood pressure was higher (P=0.040) in the first than third tertile. There were lower percentages of participants with metabolic syndrome (P=0.027), individuals in technical-administrative positions A, B and C (P=0.002) and current smokers in the highest tertiles (P=0.003).

Table 1: Anthropometric, clinical and lifestyle characteristics, according to tertiles (T) of energy-adjusted fruit and vegetable consumption (g/d)

	T1 <200.2 n=98	T2 200.2-341.1 n=98	T3 ≥341.1 n=100	*P* value*
Age (years)	52 (47-54)	52 (46-54)	52 (47-54)	1.000
BMI (kg/m^2)	25.7 (23.0-28.0)	25.2 (23.0-28.4)	25.1 (23.8-27.5)	0.914
WC (cm)	91.5 (83.2-97.7)	89.9 (82.7-97.0)	88.5 (83.5-94.9)	0.893
TBF (%)	23.8 (16.4-28.8)	22.3 (17.8-27.9)	23.7 (19.2-26.9)	0.845
Android fat (%)	27.4 (18.4-35.1)	25.6 (18.1-35.8)	27.7 (21.7-33.5)	0.400
SBP (mmHg)	127.2 (116-135)	124.2 (115-130)	125.7 (116-132)	0.090
DBP (mmHg)	81(76-86)†	80 (73-87)	80 (72-85)	0.040
MetS (%)	33.6	20.4	20.0	0.027
FFA (mmol/l)	0.80 (0.6-1.1)	0.70 (0.6-0.9)	0.75 (0.5-0.9)	0.104
TC (mg/dL)	216.5 (187-244)	210.0 (192-236)	207.0 (172-241)	0.266
HDL-C (mg/dl)	43 (37-53)	46 (40-55)	44 (37-51)	0.120
TG (mg/dl)	114.0 (84-172)	116.5 (80-160)	114.5 (81-161)	0.485
Glucose (mg/dl)	87.5 (82.0-95.0)	88.0 (83.0-95.0)	89.0 (83.0-95.0)	0.831
Insulin (µUI/ml)	5.3 (3.3-8.5)	5.0 (3.1-7.8)	5.4 (3.5-8.7)	0.664
HOMA-IR	1.1 (0.7-1.9)	1.1 (0.7-1.6)	1.2 (0.8-1.9)	0.651
HPA (Steps/day)	10,808 (8,693-13,100)	10,797 (8,309-14,079)	10,501 (8,384-13,924)	0.716
Excessive alcohol consumption (%)	33.3	36.8	29.8	0.666
Work position ABC (%)	78.6	62.2	58.0	0.002
Current smoker (%)	21.4	12.2	7.0	0.003

Data are median (25th-75th quartile) or (%). BMI, Body mass index; WC, Waist circumference; TBF, total body fat; SBP, systolic blood pressure; DBP, diastolic blood pressure; MetS, metabolic syndrome; FFA, free fat acid; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; TG, Triglycerides; HOMA-IR, homeostasis model assessment of insulin resistance; HPA, habitual physical activity; Work position ABC, technical-administrative positions A, B or C *P-value from one-factor ANOVA test or X^2 test for continuous or categorical variables, respectively. †Significantly different from T3 (P<0.05, from the Bonferroni post-hoc test)

It was observed an estimate of inadequacy for FV intake of 76.4% compared to WHO´s recommendation (at least 400.0 g/d) [12].

The most commonly consumed vegetables were lettuce, carrot/pumpkin and tomato, which together explained 85.4% of the variability in this food-group, while orange and apple were important items that together explained 84.3% of total variability in fruit intake, other consumed vegetable and fruit together

explained only 14.6% and 15.7% of the total variability in intake of these food-groups, respectively.

The assessment of dietary habits in relation to tertiles of FV intake showed that the consumption of monounsaturated fatty acids (*P*=0.005), saturated fatty acids (*P*=0.010) and cholesterol (*P*=0.031) were lower, whereas fiber and magnesium intakes were higher (*P*<0.001) in the third tertile compared with the first and second tertiles. In addition, carbohydrate intake was higher (*P*=0.015) and lipid intake was lower (*P*=0.020) in subjects allocated in the third tertile compared with the first tertile. Finally, the vitamin C intake was higher (*P*<0.001) in the highest tertile compared with the other tertiles (Table 2).

Table 2: Food and nutrient consumption, according to tertiles (T) of energy-adjusted fruit and vegetable consumption (g/d)

	T1 <200.2 n=98	T2 200.2-341.1 n=98	T3 ≥341.1 n=100	*P* value*
Energy intake (Kcal)	1,354.7 (1,009.8-1,777.4)	1,373.2 (1,092.5-1,737.6)	1,365.1 (1,098.4-1,727.3)	0.986
CH (g/d)	172.3 (128.2-224.3)†	181.6 (144.7-227.9)	196.3 (156.4-240.8)	0.015
Protein (g/d)	65.8 (54.5-85.5)	65.4 (54.4-83.2)	64.2 (54.3-80.1)	0.729
Lipids (g/d)	44.7 (31.0-59.8)†	42.8 (32.9-54.8)	35.9 (28.4-49.3)	0.020
MUFA (g/d)	14.3 (10.3-19.4)†	13.9 (10.5-18.5)‡	12.2 (8.8-15.8)	0.005
PUFA (g/d)	6.4 (4.7-9.5)	6.5 (4.9-8.7)	5.9 (4.4-7.7)	0.179
SFA (g/d)	15.4 (10.3-19.8)†	15.3 (11.0-19.6)‡	12.6 (9.7-16.5)	0.010
Cholesterol (mg/d)	207.9 (154.0-294.6)†	212.6 (165.2-270.9)‡	180.5 (131.2-243.4)	0.031
Fiber (g/d)	18.1 (15.2-22.0)†	20.8 (15.8-25.3)‡	24.0 (19.0-27.7)	<0.001
Mg (mg/d)	159.8 (119.5-189.7)†	169.9 (138.9-209.6)‡	185.5 (158.9-215.3)	<0.001
Vitamin C (mg/d)	35.0 (23.4-48.7)¶	74.9 (56.4-110.4)‡	134.8 (96.4-197.2)	<0.001

Data are median (25th-75th quartile). CH, carbohydrate; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; Mg, Magnesium. **P*-value from one-factor ANOVA test. †Significantly different from T3 (*P*<0.05, from the Bonferroni post-hoc test). ‡Significantly different from T3 (*P*<0.05, from the Bonferroni post-hoc test). ¶Significantly different from T2 and T3 (*P*<0.05, from the Bonferroni post-hoc test)

Considering the oxidative stress markers, subjects in the highest tertile of FV intake showed lower values of ox-LDL (*P* for trend=0.050), urinary 8-iso-PGF2α (*P* for trend =0.003) and 8-OHdG (*P* for trend=0.028) regardless of confounding variables. The inclusion of the covariate "current smoker" (yes/no) in the linear regression model attenuated the statistical significance in relation to oxidative stress markers (Table 3).

Table 3: Oxidative stress markers with respect to tertiles (T) of energy-adjusted fruit and vegetable consumption

	ox-LDL (U/l)	8-iso-PGF2α (ng/mg Crn)	8-OHdG (ng/mg Crn)
Energy-adjusted fruit intake (g/d)			
T1 (<134.1)	56.8 (45.8-65.5)	1.21 (0.9-1.9)	8.5 (6.3-11.3)
T2 (134.1-248.0)	55.9 (42.9-66.6)	1.14 (0.8-1.6)	7.9 (5.8-9.9)
T3 (≥248.0)	55.9 (42.3-63.2)	1.10 (0.6-1.6)	7.4 (5.9-9.9)
P for trend†	0.090	0.040	0.192
P for trend‡	0.107	0.120	0.250
Energy-adjusted vegetable intake (g/d)			
T1 (<47.4)	57.3 (48.2-68.9)	1.34 (0.9-1.9)	8.7 (6.9-11.6)
T2 (47.4-84.1)	57.4 (43.9-66.6)	1.01 (0.6-1.6)	8.2 (6.1-10.3)
T3 (≥84.1)	52.6 (42.2-61.0)	1.18 (0.8-1.6)	6.7 (5.7-9.0)
P for trend†	0.006	0.103	<0.001
P for trend‡	0.006	0.098	<0.001
Energy-adjusted fruit and vegetable intake (g/d)			
T1 (<200.2)	57.1 (45.2-66.6)	1.29 (0.9-2.1)	8.6 (6.5-11.6)
T2 (200.2-341.1)	56.4 (44.9-64.8)	1.16 (0.8-1.7)	7.9 (5.9-9.8)
T3 (≥341.1)	54.4 (41.4-62.3)	1.08 (0.7-1.5)	7.1 (5.7-9.6)
P for trend†	0.050	0.003	0.028
P for trend‡	0.062	0.013	0.040

Data are median (25th-75th quartile). ox-LDL, oxidized low density lipoprotein; Crn, creatinine; 8-iso-PGF2α, 8-iso-prostaglandin F2α; 8-OHdG, 8-hydroxy-2′-deoxyguanosine †*P* for trend from the linear regression model, adjusted for android fat, habitual physical activity, work position, excessive alcohol consumption, energy intake, free fat acid and HOMA-IR. ‡*P* for trend from the linear regression model, adjusted for same variables above plus current smoker.

Sample categorization into tertiles based on the content of fiber, vitamin C and magnesium from FV, showed that ox-LDL concentrations were negatively associated with fiber (*P* for trend=0.013) and magnesium (tendency, *P* for trend=0.06). The urinary values of 8-iso-PGF2α also were inversely associated

with the content of fiber (*P* for trend=0.034), vitamin C (*P* for trend=0.007) and magnesium (*P* for trend=0.018) from FV. Lower urinary concentrations of 8-OHdG were found in subjects with higher vitamin C intake from FV (*P* for trend=0.050), regardless of the adjustment covariates. There was attenuation of statistical significance between oxidative stress markers and nutrient intake from FV after addition of the covariate "current smoker" in the linear regression model (Table 4).

Table 4: Oxidative stress markers with respect to tertiles (T) of energy-adjusted dietary fiber, vitamin C and magnesium from fruit and vegetable intake

	ox-LDL (U/l)	8-iso-PGF2α (ng/mg Crn)	8-OHdG (ng/mg Crn)
Energy-adjusted fiber from fruit and vegetable intake (g/d)			
T1 (<4.0)	57.2 (45.8-69.9)	1.20 (0.9-1.8)	8.6 (6.5-11.8)
T2 (4.0-6.5)	56.8 (45.0-64.8)	1.19 (0.8-1.8)	7.8 (5.7-9.6)
T3 (≥6.5)	50.3 (41.9-61.9)	1.10 (0.6-1.6)	7.3 (5.7-9.9)
P for trend†	0.013	0.034	0.100
P for trend‡	0.016	0.085	0.140
Energy-adjusted vitamin C from fruit and vegetable intake (mg/d)			
T1 (<56.8)	57.3 (45.8-66.8)	1.15 (0.8-1.7)	8.3 (6.4-11.1)
T2 (56.8-98.0)	53.9 (43.2-66.3)	1.30 (0.8-2.1)	8.2 (5.8-10.6)
T3 (≥98.0)	55.8 (43.3-62.3)	1.00 (0.6-1.3)	7.1 (5.7-9.0)
P for trend†	0.201	0.007	0.050
P for trend‡	0.230	0.019	0.070
Energy-adjusted magnesium from fruit and vegetable intake (mg/d)			
T1 (<30.4)	57.4 (45.2-69.7)	1.30 (1.0-2.1)	8.4 (6.6-11.5)
T2 (30.4-48.9)	56.2 (43.6-65.8)	1.10 (0.8-1.7)	7.9 (5.7-10.1)
T3 (≥48.9)	52.3 (42.5-61.9)	1.00 (0.6-1.6)	7.4 (5.7-9.6)
P for trend†	0.060	0.018	0.136
P for trend‡	0.070	0.048	0.178

Data are median (25th-75th quartile). ox-LDL, oxidized low density lipoprotein; Crn, creatinine; 8-iso-PGF2α, 8-iso-prostaglandin F2α; 8-OHdG, 8-hydroxy-2´-deoxyguanosine.†*P* for trend from the linear regression model, adjusted for android fat, habitual physical activity, work position, excessive alcohol consumption, energy intake and free fat acid. ‡*P* for trend from the linear regression model, adjusted for same variables above plus current smoker.

4. Discussion

The first relevant finding of this study was the inverse association between FV consumption and the assessed biomarkers of oxidative stress (8-iso-PGF2α, 8-OHdG and ox-LDL) in middle-aged men. Similarly, in a cross-sectional study involving young adults, there was a negative association between FV intake and concentrations of ox-LDL [29]. Another study with adults showed that a diet rich in FV was also inversely associated with the values of 8-iso-PGF2α in both transversal and longitudinal analysis [15]. Additionally, intervention studies with diets rich in FV (9.2 and 12.0 portions/day) showed a reduction in the concentrations of urinary 8-iso-PGF2α [14] and 8-OHdG [48].

In this study, vegetable intake showed greater negative association with ox-LDL and 8-OHdG levels than fruit intake. In fact, the most consumed vegetables (lettuce, carrot/pumpkin and tomato) have higher fiber content than the most consumed fruits (orange and apple) [40], thus fiber consumption from FV has been inversely associated with ox-LDL [29]. In this context, an intervention study with apparently healthy men found that a higher consumption of vegetables (200 g/d) for four weeks was associated with decreased levels of substances involved in the pathways of ox-LDL [49]. Moreover, a cross-sectional study with healthy subjects demonstrated an inverse association of urinary 8-OHdG with polyphenol intake from vegetable, but not from fruit [27].

Interestingly, in the present study, 70% of participants included in the third tertile of FV (participants with lower oxidative stress levels) consumed 400 g/d or more of FV which is in concert with the daily recommendation by the WHO [12]. These outcomes reinforce the benefits of adequate consumption of FV, including, against an important risk factor for chronic diseases, the oxidative stress.

Another relevant finding of this study was that subjects allocated in the highest tertile of vitamin C intake (from FV) presented lower concentrations of urinary 8-iso-PGF2α and 8-OHdG. Vitamin C is an important reduction agent that converts the reactive species of oxygen and nitrogen into harmless species acting as an *in vivo* antioxidant [50, 51]. The increased FV consumption has been associated with the increased intake of vitamin C, which is related to higher antioxidant capacity and reduced oxidative stress [29, 32]. In fact, the

consumption of a diet rich in vitamin C (500 ml of gazpacho), during 14 days, reduced the concentrations of 8-iso-PGF2α in healthy subjects [32].

We observed that the fiber intake of the FV group was associated with lower concentrations of lipid peroxidation biomarkers (ox-LDL and 8-iso-PGF2α) in this study. A previous cross-sectional study with adult men and women also reported that higher fiber consumption from FV was associated with lower plasma concentrations of ox-LDL [29]. In this context, diets rich in fiber reduced lipid peroxidation in obese adult mice when compared to animals fed a standard diet [52]. The action of certain fibers on the sequestration of bile salts and/or the partial blockage of the enterohepatic cycle that avoids the recycling of bile acids and increases the expression of the cholesterol enzyme 7α hydroxylase [53] may help to explain the reduction of the circulating LDL cholesterol molecules susceptible to oxidation.

Furthermore, the higher intake of magnesium from FV was associated with lower lipid peroxidation (ox-LDL and 8-iso-PGF2α). In this context, an experimental study found higher antioxidant biomarkers levels and lower lipid peroxidation biomarkers levels in rats that consumed higher and lower quantities of magnesium, respectively [31]. Thus, the magnesium deficiency (which can be caused by its low intake [31]) may contribute to the release of inflammatory cytokines along with the excessive production of free radical and promotion of oxidative stress. Moreover, the inflammation provoked by magnesium deficiency could promote pro-atherogenic changes in lipoprotein metabolism, thrombosis, endothelial dysfunction and other metabolic disorders [54]. Therefore, we suggest the high consumption of FV could contribute to minimize the oxidative stress.

The statistical attenuation observed between oxidative stress biomarkers and food variables evaluated after adding “current smoker” as covariate in the models, could be explained by higher values of 8-iso-PGF2α (2.29±1.69 *vs* 1.36±1.47 ng/mg creatinine, *P*-value<0.001) and 8-OHdG (9.54±4.29 *vs* 8.25±2.90 ng/mg creatinine, *P*-value=0.080) found among smokers compared to nonsmokers in the present study. In fact, the oxidative stress was found high among smokers [55], probably because the cigarette smoke contains potent oxidants, such as acrolein, hydroxyl radical and organic radicals [56]. Thus, the smoking habit may have a negative effect on oxidative stress even in subjects consuming foods with antioxidant capacity like FV.

Finally, this study showed the importance of the consumption of FV, close (341.1 g/d to 399 g/d) or similar (≥ 400 g/d) to that recommended by the WHO, to reach a lower oxidative stress status, which has been recognized as a risk condition to cardiovascular diseases [2] and cancer [3]. Furthermore, the selection of FV as source of vitamin C, fiber and magnesium, also could help minimizing the oxidative stress. In this context, the implementation of public policies and adoption of multiple nutritional education actions to stimulate the high FV intake in middle-aged men should be encouraged among Brazilians.

The limitations of the study relate firstly to the cross-sectional nature of the investigation, for which we cannot prove the reported associations are causal. However, we controlled as much as possible potential confounding variables. Second, we were unable to distinguish between the cooking techniques used to prepare FV, which might influence the bioavailability of bioactive compounds, including vitamin C [57]. However FV consumption analyses from FFQ have been related with oxidative stress markers in previous cross-sectional studies [29, 30]. Further studies involving analysis of others contents of FV with potential antioxidant capacity (e.g. polyphenols and carotenoids) may clarify the role of FV on the redox status.

Conclusion

Fruits and vegetables intake were inversely associated with oxidative stress markers in middle-aged men, and the contents of vitamin C, fiber and magnesium from this food-group seem to contribute to their beneficial effects on the redox balance.

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2.4. ARTIGO 4

RED BUT NOT WHITE MEAT CONSUMPTION IS ASSOCIATED WITH METABOLIC SYNDROME, INSULIN RESISTANCE AND LIPID PEROXIDATION IN BRAZILIAN MIDDLE-AGED MEN

O consumo de carne vermelha, mas não de carne branca é associado com síndrome metabólica, resistência à insulina e peroxidação lipídica em homens brasileiros de meia-idade

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RESUMO

Introdução: A influência da dieta na síndrome metabólica e estresse oxidativo não é completamente conhecida.

Desenho: Neste estudo transversal foi avaliada a associação do consumo de carne vermelha e branca com síndrome metabólica, resistência à insulina e peroxidação lipídica em homens brasileiros de meia-idade.

Método: Um total de 296 indivíduos (idade: 50,5 ± 5,0 anos, índice de massa corporal: 25,8 ± 3,5 kg/m^2) foram avaliados. Dados antropométricos, características do estilo de vida, parâmetros bioquímicos sanguíneos, diagnóstico da síndrome metabólica, homeostase da resistência à insulina, um biomarcador de peroxidação lipídica (lipoproteína de baixa intensidade oxidada) e razão triacilgliceróis:lipoproteína de alta densidade colesterol foram determinados. A ingestão alimentar foi estimada por um questionário de frequência do consumo alimentar.

Resultados: Os participantes incluídos no maior tercil de carne vermelha (≥81,5 g/d) e de ácido graxo saturado proveniente do consumo de carne vermelha (≥4,3 g/d) tiveram maior ocorrência de obesidade central (aproximadamente 60%, p<0,01), hipertrigliceridemia (aproximadamente 43%, p<0,01) e síndrome metabólica (35%, p<0,01). Eles também apresentaram maiores valores de homeostase da resistência à insulina, lipoproteína de baixa densidade oxidada e razão triacilgliceróis:lipoproteína de alta densidade colesterol independente de fatores intervenientes. Não foi verificada associação do maior tercil de carne branca (≥ 39,4 g/d) e de ácido graxo saturado advindo do consumo de carne branca (≥1,0 g/d) com os parâmetros avaliados (p>0,05).
Conclusões: O consumo de carne vermelha foi transversalmente associado com ocorrência de obesidade central, hipertrigliceridemia e síndrome metabólica, bem como, com maior valor de homeostase da resistência à insulina, lipoproteína de baixa densidade oxidada e razão triacilgliceróis:lipoproteína de alta densidade colesterol. O conteúdo de gordura saturada proveniente do consumo de carne vermelha parece ser um fator que contribui para essa relação, todavia o consumo de carne branca não foi associado com síndrome metabólica e com os biomarcadores avaliados.
Palavras chave: Carne, obesidade abdominal, síndrome metabólica, lipoproteína de baixa densidade oxidada

ABSTRACT

Background: The influence of diet on metabolic syndrome and oxidative stress are not completely known.
Design: This cross-sectional study assessed the association of red meat and white meat consumption with metabolic syndrome, insulin resistance and lipid peroxidation in Brazilian middle-aged men.
Methods: A total of 296 subjects (age: 50.5 ± 5.0 years, body mass index: 25.8 ± 3.5 kg/m^2) were evaluated. Anthropometry, lifestyle features, blood biochemical parameters, diagnosis of metabolic syndrome, homeostatic model assessment for insulin resistance, a lipid peroxidation marker (oxidized low-density lipoprotein) and triglycerides:high-density lipoprotein cholesterol ratio were assessed. Dietary intake was estimated by a food frequency questionnaire.

Results: The subjects included in the highest tertile red meat (≥81.5 g/d) and saturated fatty acid from red meat consumption (≥4.3 g/d) had higher occurrence of central obesity (nearly 60%, $p<0.01$), hypertriglycaeridemia (nearly 43%, $p<0.01$) and metabolic syndrome (35%, $p<0.01$). They also had higher values of homeostatic model assessment for insulin resistance, oxidized low-density lipoprotein, and triglycerides:high-density lipoprotein cholesterol ratio, regardless of interfering factors. There were no associations of highest white meat tertile (≥ 39.4 g/d) and saturated fatty acid from white meat (≥1.0 g/d) consumption with the assessed parameters ($p>0.05$).

Conclusions: Red meat consumption was cross-sectionally associated with the occurrence of central obesity, hypertriglyceridemia, and metabolic syndrome as well as with higher homeostatic model assessment for insulin resistance, oxidized low-density lipoprotein concentrations and triglycerides:high-density lipoprotein cholesterol ratio. The content of saturated fatty acid from red meat consumption may be a factor that contributed to this relationship, while white meat consumption was not associated with metabolic syndrome and the assessed biomarkers.

Keywords: Meat, abdominal obesity, metabolic syndrome, oxidized LDL

Introduction

Metabolic syndrome (MetS) is characterized by an aggregation of metabolic abnormalities such as central obesity, high blood pressure, high fasting blood glucose and dyslipidaemias, which are considered relevant risk factors for cardiovascular diseases.[1] Moreover, oxidative stress is a state where the production of free radicals and/or reactive species exceeds the antioxidant defence favoring the oxidation of biomolecules such as lipids, resulting in loss of its biological functions[2] and aggravation of cardiovascular diseases.[3]

Among the behavioral risk factors associated with MetS, oxidative stress and/or cardiovascular disease is an unhealthy dietary pattern.[4–6] In this context, two major prospective cohort studies (The Health Professionals Follow-up Study and The Nurses' Health Study) stated that high red meat (RM) consumption increased the risk for cardiovascular mortality and all-causes mortality.[7]

The last Household Budget Survey 2008–2009[8] held by the Brazilian Institute of Geography and Statistics showed a reduction in carbohydrate and an increase in fat and protein consumption, especially of animal source protein. Beef was considered one of the foods with the highest average consumption per capita[8] and this food group (meat) contributed mostly to saturated fatty acid (SFA) intake[9], which has been associated with increased adiposity, inflammation and insulin resistance (IR).[10]

In this sense, the high contribution of RM to daily energy consumption could be a potential harmful component of the Brazilian dietary pattern.[11] However, the number of studies concerning the relationships of white meat (WM) and/or RM consumption with the occurrence of MetS[11–14] and IR[15,16] is still modest. Moreover, the relationships of RM and WM consumption with lipid peroxidation, to our knowledge, have yet to be clarified.

Thus, in this cross-sectional study we assessed the potential associations between RM and WM consumption and MetS, IR and lipid peroxidation in Brazilian middle-aged men.

Methods

Study population

This cross-sectional study was carried out between March and December 2011. The sample size was calculated 17 considering the total number of male staff at the Federal University of Viçosa, Viçosa, Brazil in February 2011, aged between 40 and 59 years (1744 individuals), a confidence level of 95%, an expected MetS prevalence of 24.4% in Brazilian middle-aged men[18] and 4.5% sampling error, resulting in 293 participants required.

Participants were recruited by systematic sampling. We excluded those individuals who self-declared the following: body weight alterations greater than 3 kg in the 3 months preceding the study; altered levels of physical activity and eating habits in the 3 months preceding the study; thyroid diseases; heart failure; cerebrovascular diseases; infectious diseases; inflammatory diseases; diseases of the gastrointestinal tract; liver disease; chronic kidney disease and/or a history of kidney stones; cancer in the previous 10 years; eating disorders (anorexia and bulimia); food allergies. Individuals using vitamin supplements and those using diuretics or drugs that alter food intake and/or the metabolism of nutrients, pacemaker and/or prosthetic limbs users and elite athletes were also excluded.

We interviewed 848 men and 548 were eliminated by the exclusion criteria.[19] Of 300 selected, four did not answer the food frequency questionnaire (FFQ), so the final sample comprised 296 individuals.

The study was conducted according to the Declaration of Helsinki guidelines and all procedures involving human subjects were approved by the Ethics Committee in Human Research of the Federal University of Viçosa (Reference n^{o}069/2010). Written informed consent was obtained from all subjects.

Dietary intake assessment

A FFQ, validated for the Brazilian population, was used to assess the usual dietary intake of the participants.[20] Daily food consumption was estimated as frequency x portion x size for each consumed food item. Nutrient intake was assessed using the software Dietpro® version 5.5i (AS Systems, Viçosa, Brazil), using mainly two Brazilian nutritional composition tables.[21, 22] When the required

nutritional information was not observed in these tables, the USDA table[23] was used.

The meat consumption assessed from data in the FFQ included 12 food-items: lean beef; high-fat beef; ground beef; lean pork; high-fat pork; bacon/pork rinds; poultry with skin; skinless poultry; fish; sausage; ham; hamburger. In the present study we considered for the RM group the consumption of lean beef, high-fat beef, ground beef, lean pork, high-fat pork and bacon/pork rinds. For the WM group the intake of poultry with skin, skinless poultry and fish was considered. The consumption of sausage, ham and hamburger was not considered in the statistical analysis due to the fact the FFQ did not discriminate the use of RM or WM in the production of these foods.

Blood pressure, anthropometric and body composition assessments

Systolic and diastolic blood pressures were measured following VI Brazilian Guidelines on Hypertension,[24] while anthropometric determinations such as weight, height and waist circumference were taken using standard measurement procedures, as previously described.[19]

Body mass index was calculated as weight (kg) divided by height squared (m^2). Total body fat percentage was determined by total body scanning with a dual energy X-ray absorptiometry (enCORE software version 13.31; GE/Lunar, Madison, WI, USA).

Lifestyle co-variables

The participants were asked about their current smoking status and alcohol consumption (yes/no). High alcohol consumption was also defined as a daily ingestion above 21 units per week.[25] Habitual physical activity was estimated by the mean number of daily steps (7 consecutive days)[26,27] measured by a Digi-Walker SW-200 pedometer (Yamax Corporation, Tokyo, Japan), according to the instructions previously described.[19]

Sample collection and analysis

Blood samples were collected from the antecubital vein after 12-h overnight fasting. Serum concentrations of glucose, insulin, high-density lipoprotein (HDL-c) and triglycerides were measured by standard methods as previously described.[19] IR was estimated by the Index of Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) using the Matthews et al. equation[28] and the atherogenic index was calculated by the ratio between triglycerides and HDL-c.[29] MetS was diagnosed by Alberti et al. criteria.[1]

Finally, plasma oxidized low-density lipoprotein (ox-LDL) concentrations were determined by a commercially available enzyme-linked immunosorbent assay kit from Mercodia (Uppsala, Sweden).

Statistical analysis

Data distribution was determined by the Shapiro–Wilk test. Non-normally distributed variables were log transformed before statistical analysis. To evaluate the associations among consumption of meats and SFA with MetS occurrence, metabolic and lipid peroxidation markers, the participants were categorized into tertiles based on food-group consumption, which was adjusted by daily energy intake using the residual method. A comparison of nutrient consumption and lifestyle co-variables among tertiles of RM intake was performed by analysis of variance followed by Bonferroni's post-hoc test or by chi-square test for linear trend according to continuous and categorical variables, respectively.

The prevalence ratio was determined by Poisson regression with a confidence interval of 95% to assess the associations among MetS and tertiles RM, SFA from RM, WM and SFA from WM consumption. The chi-square test for linear trend was used to compare proportions among food-group consumption and MetS and its components.

Linear trends were assessed by assigning the average value to each tertile of RM, SFA from RM, WM and SFA from WM consumption, modeling those values as a continuous variable to assess its association with HOMA-IR, ox-LDL concentrations and triglycerides:HDL-c ratio. Multivariate regression models were controlled by confounding variables.

Calorie consumption outliers were defined by dispersing interquartile according to Vittinghoff et al.[30] Outliers were excluded (five individuals with caloric intake ≥ 2,640 kcal/d) followed by all statistical analyses previously described. After that the results maintained the same trend and statistical significance, where the results include all study participants. Data processing and analysis were performed using the software STATA version 9.1 (Stata Corp, College Station, TX, USA), considering *p*-values < 0.05 as statistically significant.

Results

Anthropometric and clinical characteristics of study participants are shown in Table 1. The occurrences of MetS and central obesity in the study sample were 24.7% and 47.3%, respectively.

Table 1. Anthropometric and clinical characteristics of participants (n=296)

Variables	Values
Age (years)	50.5 ± 5.0
Body mass index (kg/m^2)	25.8 ± 3.5
Total body fat (%)	22.7 ± 7.2
HOMA-IR	1.4 ± 1.1
HDL-c (mg/dL)	46.9 ± 12.7
Triglycerides (mg/dL)	142.4 ± 95.1
ox-LDL (U/L)	55.6 ± 16.8
Central obesity n (%)	140 (47.3)
Metabolic syndrome n (%)	73 (24.7)

HOMA-IR, homeostasis model assessment of insulin resistance; HDL-c, high density lipoprotein; ox-LDL, oxidized low density lipoprotein; Values are mean ± SD or n (%).

Regarding dietary habits, protein, total fat, monounsaturated fatty acid, SFA and cholesterol intakes were higher in the third tertile of RM consumption compared with the second and first tertiles. Sausage, ham and hamburger consumption and iron intake were higher and fibre consumption was lower in subjects included in the third tertile compared to those in the first tertile of RM consumption (Table 2). Moreover, regarding the lifestyle co-variable, there were no statistical differences of current smoking status (number of smokers) and of

habitual physical activity (steps number/d) according to tertile RM intake (Table 2).

Table 2. Food, nutrient consumption and lifestyle characteristics according to tertiles (T) of energy-adjusted red meat intake

	T1 < 56.0 g/d (n=98)	T2 56.0 – 81.5 g/d (n=98)	T3 ≥ 81.5 g/d (n=100)	*p*-value
White meat (g/d)	36.9 ± 28.5	37.2 ±23.0	37.7 ± 26.2	0.564
Sausage, hamburger and ham (g/d)	11.6 ± 10.7[a]	14.9 ± 14.9	17.9 ± 14.1	0.005*
Energy (kcal/d)	1463.2 ± 443.5	1429.4 ± 475.7	1475.7 ± 555.4	0.749
Protein (g/d)	59.9 ± 10.2[b]	67.4 ± 8.0[c]	79.5 ± 13.2	<0.001*
Carbohydrate (g/d)	208.7 ± 28.2[b]	199.5 ± 26.6[c]	175.6 ± 31.8	<0.001*
Fat (g/d)	42.4 ± 11.7[a]	43.2 ± 10.6[c]	48.4 ± 10.4	<0.001*
SFA (g/d)	14.3 ± 5.2[a]	14.7 ± 4.0[c]	16.9 ± 4.6	<0.001*
PUFA (g/d)	6.9 ± 2.5	6.9 ± 2.3	7.5 ± 2.6	0.268
MUFA (g/d)	12.7 ± 3.6[b]	14.1 ± 3.6[c]	17.1 ± 4.2	<0.001*
Cholesterol (g/d)	189.5 ± 89.9[b]	211.9 ± 85.3[c]	261.1 ± 88.4	<0.001*
Fiber (g/d)	23.6 ± 6.3[a]	21.8 ± 5.3	20.4 ± 6.3	<0.001*
Sodium (mg/d)	1,320.7± 559.7	1,449.5 ± 634.1	1,301.4 ± 527.2	0.090
Iron (mg/d)	7.0 ± 1.7[a]	7.1 ± 1.2	7.3 ± 1.2	0.030*
Alcohol (g/d)	7.3 ±13.8[b]	16.0 ±18.6	16.9 ± 19.4	<0.001*
Habitual physical activity (steps numbers/day)	11,586 ± 4,052	11,138 ± 3,388	10,587 ± 4,168	0.200
Current smoker n (%)	10.0 (25.0)	15.0 (37.5)	15.0 (37.5)	0.326

SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; Values are mean ± SD or n (%); [a]Significantly different from T3 (*p-value<0.05, post-hoc Bonferroni test); [b]Significantly different from T2 and T3 (*p-value<0.05, post-hoc Bonferroni test); [c]Significantly different from T3 (*p-value<0.05, post-hoc Bonferroni test).

Interestingly, there was higher MetS occurrence in those subjects included in the highest tertiles of RM and of SFA from RM consumption compared to those in the first tertile, regardless of interfering factors (Table 3). In addition, central obesity occurrence was also higher in the third tertile compared with the first tertiles of RM and SFA from RM consumption (first tertile: 39.8%, 40.8%; second tertile: 41.8%, 39.8%; third tertile: 60.0%, 61.0% respectively, p<0.01). Similar findings were seen with the hypertriglyceridemia (first tertile: 23.5%, 22.4%; second tertile: 23.5%, 26.5%; third tertile: 43.0%,

44.0% respectively, p<0.01). There were no significant associations of MetS and its components with WM and SFA from WM consumption (p>0.05).

Table 3. Prevalence ratio (PR) and 95% confidence intervals (95%CI) of metabolic syndrome (MetS) according to tertiles (T) of energy-adjusted red meat, white meat and saturated fatty acid from read meat and white meat intake

	MetS n (%)[a]	Model 1 PR (95%CI)[b]	Model 2 PR (95%CI)[b]	Model 3 PR (95%CI)[b]
RM (g/d)				
T1: < 56.0	17 (17.3)	1.00	1.00	1.00
T2: 56.0-81.5	21 (21.4)	1.23 (0.65-2.34)	1.25 (0.66-2.38)	1.15 (1.06-3.44)
T3: ≥ 81.5	35 (35.0)	2.01 (1.13-3.60)*	2.00 (1.12-3.57)*	1.90 (1.06-3.44)*
p-value	0.004*	0.013*	0.015*	0.023*
SFA from RM (g/d)				
T1: < 2.7	19 (19.4)	1.00	1.00	1.00
T2: 2.7-4.3	19 (19.4)	1.00 (0.66-2.38)	1.02 (0.54-1.93)	0.95 (0.50-1.83)
T3: ≥ 4.3	35 (35.0)	1.80 (1.03-3.15)*	1.82 (1.04-3.18)*	1.79 (1.01-3.15)*
p-value	0.011*	0.028*	0.026*	0.033*
WM (g/d)				
T1: <24.0	23 (23.5)	1.00	1.00	1.00
T2: 24.0-39.4	23 (23.5)	1.00 (0.56-1.78)	0.94 (0.52-1.68)	0.95 (0.53-1.71)
T3: 39.4	27 (27.0)	1.15 (0.66-2.00)	1.14 (0.65-1.99)	1.12 (0.64-1.97)
p-value	0.564	0.616	0.625	0.682
SFA from WM (g/d)				
T1: < 0.6	23 (23.5)	1.00	1.00	1.00
T2: 0.6-1.0	22 (22.4)	0.95 (0.53-1.71)	0.91 (0.50-1.64)	0.86 (0.47-1.55)
T3: 1.0	28 (28.0)	1.19 (0.68-2.07)	1.14 (0.65-1.99)	1.10 (0.63-1.93)
p-value	0.460	0.519	0.609	0.712

Values are n (%) to MetS occurrence and prevalence ratio (95% CI) to Models 1, 2 and 3. Model 1, unadjusted model; Model 2, adjusted for age; Model 3, adjusted for age (years), habitual physical activity (steps number/d), smoking habit (yes/no), excessive alcohol intake (yes/no) and daily caloric intake (kcal); [a]*p*-value from chi-square for linear trend; [b]*p*-value from Poisson regression; *Significant prevalence ratio and p-value<0.05.

Moreover, HOMA-IR, ox-LDL concentrations and the triglycerides:HDL-c ratio were positively associated with RM and with SFA from RM consumption, regardless of interfering factors. However, HOMA-IR, ox-LDL levels and the triglycerides:HDL-c ratio were not associated with WM and SFA from WM consumption (Table 4).

Table 4. Homeostasis model assessment of insulin resistance (HOMA-IR), oxidized LDL (ox-LDL) and triglycerides: HDL-c ratio according to tertiles (T) of energy-adjusted red meat, white meat and saturated fatty acid from red meat and white meat intake.

		HOMA-IR	ox-LDL (U/L)	Triglycerides: HDL-c ratio
RM (g/d)	T1: < 56.0	1.3 ± 0.9	52.4 ± 14.7	3.1 ± 2.5
	T2: 56.0-81.5	1.4 ± 1.1	53.8 ± 16.3	3.0 ± 2.2
	T3: ≥ 81.5	1.6 ± 1.3	60.7 ± 18.2	4.1 ± 3.7
	P for trend[a]	0.039*	0.009*	0.029*
SFA from RM (g/d)	T1: < 2.7	1.3 ± 0.8	52.5 ± 15.2	3.0 ± 2.2
	T2: 2.7-4.3	1.3 ± 1.0	54.7 ± 16.3	3.0 ± 2.3
	T3: ≥ 4.3	1.7 ± 1.3	59.7 ±18.1	4.2 ± 3.8
	P for trend[a]	0.019*	0.049*	0.032*
WM (g/d)	T1: <24.0	1.4 ± 1.1	56.7 ± 18.1	3.6 ± 3.0
	T2: 24.0-39.4	1.4 ± 1.0	56.7 ± 16.6	3.5 ± 3.4
	T3: 39.4	1.5 ± 1.2	53.6 ± 15.6	3.1 ± 2.2
	P for trend[a]	0.580	0.212	0.154
SFA from WM (g/d)	T1: < 0.6	1.3 ± 1.0	56.2 ± 17.8	3.7 ± 3.4
	T2: 0.6-1.0	1.4 ± 1.1	55.8 ± 16.4	3.3 ± 2.6
	T3: 1.0	1.5 ± 1.2	55.0 ± 16.4	3.3 ± 2.6
	P for trend[a]	0.778	0.355	0.168

HDL-c, high density lipoprotein; Values are mean ± SD; [a]*p* for trend from the linear regression model, adjusted for age (years), total body fat (%), habitual physical activity (steps number/d), smoking habit (yes/no), fibre consumption (g/d), sausage, ham and hamburger consumption (g/d) and daily caloric intake (kcal/d); *Significant *p*-value<0.05.

Given the role of central fat accumulation on cardiometabolic risk, we replaced the total body fat by central obesity indicator (waist circumference ≥ 90 cm), as an adjustment variable, and thus the statistical significances for the associations of RM consumption with HOMA-IR (*p* for trend=0.448) and triglycerides:HDL-c ratio (*p* for trend=0.208), as well as of SFA from RM consumption with HOMA-IR (*p* for trend=0.169), ox-LDL concentrations (*p* for trend=0.092) and triglycerides:HDL-c ratio (*p* for trend=0.142) disappeared.

Discussion

The first important finding of the current study was the positive association of RM and SFA from RM consumption with the occurrence of MetS, central obesity and hypertriglyceridemia. The relationship between MetS and

RM consumption was also reported in a cohort study involving migrants and Japanese descendants living in Brazil, where the highest tertile of RM consumption (mean=144.2 g/d) was associated with a 4.7 times higher risk of MetS, regardless of interfering factors.[11] In turn, the *Prevención Dieta Mediterránea* trial study found that RM consumption was positively associated with a risk of central obesity and MetS incidence, having a tendency to influence RM consumption in hypertriglyceridemia.[12]

Regarding the positive associations of the occurrence of MetS, central obesity, and hypertriglyceridemia with SFA from RM consumption, it is important to highlight that the consumption of a diet with high amounts of this type of fatty acid favors the occurrence of hypertriglyceridemia by stimulating hepatic secretion of lipoproteins containing apoB-100[31] as well as a possible increase in weight gain by its lower thermogenic effect compared with unsaturated fat from vegetable sources.[32] In fact, RM is rich in SFA[33] and, accordingly, we verified a linear increase of SFA intake with an increment of RM consumption. Babio et al.[12] also verified this direct association between SFA and RM consumption, suggesting the relationship of this nutrient with central obesity. Although a recent study has proposed another RM compound (L-carnitine) as a risk factor for metabolic complications,[34] our results indicate the important role of SFA from increased RM consumption on the occurrence of metabolic disorders.

We also showed that RM and SFA from RM consumption were positive predictors of HOMA-IR. In a recent meta-analysis including nine prospective cohort studies, six of them verified a significant positive relationship between unprocessed RM consumption and incidence of type-2 diabetes mellitus (DM2).[35] In fact, RM provides significant amounts of SFA, particularly palmitic acid.[33] In excess, palmitate may inhibit the activation of insulin receptor substrate-1, fosfatidilinisitol-3-kinase or protein kinase B in adipocytes causing impaired IR, as well as reducing muscle cell insulin sensitivity by stimulating the secretion of inflammatory cytokines.[10]

Furthermore, RM is a relevant source of iron.[36] If 100 g of RM has roughly 1.1 mg of haeme iron,[37] our participants probably consumed 1.26 mg/d of haeme iron from RM (last tertile). In this context, prospective studies have shown that haeme iron consumption from RM, but not from poultry/fish, is positively associated with a higher incidence of MetS[38] and DM2.[39] In addition,

beta cells are susceptible to oxidative stress induced by iron and the deposition of this nutrient in these cells may lead to apoptosis and insulin deficiency.[40]

Interestingly, we found a positive association of RM and SFA from RM consumption with ox-LDL concentrations. It is possible that an excess of palmitate expands white adipose tissue, thus increasing the reactive oxygen species and the expression of inflammatory genes in human macrophages by a nuclear factor kappa b-dependent mechanism.[10] Inflammatory cytokine production may further stimulate the production of free radicals, causing the occurrence of oxidative stress.[41] Iron ions also induce the oxidation of polyunsaturated fatty acid in macrophages and this lipid peroxidation may lead to LDL oxidation.[42] In addition, since that increased formation of ox-LDL has been associated with endothelial dysfunction related to atheromatous plaque and atherosclerosis,[41] our findings suggest that RM intake could also be a possible risk factor for atherosclerosis. Reinforcing this hypothesis, we also observed positive associations between RM consumption and the triglycerides:HDL-c ratio, another potential indicator of atherogenesis.[29]

Moreover, no relationships of WM consumption with MetS occurrence and metabolic/oxidative stress markers were noted in this study. Our findings are in accordance with those from previous studies, in which no associations of poultry consumption with DM2[43] and MetS[11] were verified. All findings together reinforce the idea that, although a protective effect of WM consumption on metabolic and oxidative stress markers has not been proven, moderate consumption of WM could be an interesting dietary strategy to be adopted over the life course.

Since central obesity has an extremely important role in metabolic disorders[44,45] and oxidative stress,[46] it could affect the relationship between meat consumption and the variables studied here. The loss of statistical significance in the associations tested, by the inclusion of central obesity as an adjustment variable, corroborates this hypothesis.

The main limitation of the present study is its cross-sectional nature. Thus, the results shown here must be cautiously considered as we cannot guarantee that the observed associations show a cause/effect relationship, although we controlled potential interfering variables. Future studies involving the participation of women should be conducted before considering the application of these results at the population level.

In conclusion, RM consumption was cross-sectionally associated with the occurrence of MetS and its components, central obesity and hypertriglyceridemia as well as with higher values of HOMA-IR, triglyceride:HDL-c ratio, and ox-LDL in Brazilian middle-aged men. Its SFA content appears to contribute to the harmful effects. Additionally, WM consumption was not associated with MetS and with the assessed biomarkers, indicating that WM consumption is a more secure dietary strategy to be recommended in terms of healthy eating habits.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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3. CONCLUSÕES GERAIS

Os resultados desse estudo mostraram que homens brasileiros de meia-idade, aparentemente saudáveis e fisicamente ativos (≥ 10.000 passos diários) possuíram melhores condições cardiometabólicas em comparação com aqueles que não eram fisicamente ativos (< 10.000 passos diários) e que o maior número de passos realizados por esses homens foi inversamente associado com adiposidade corporal e resistência à insulina.

Adicionalmente, a carga glicêmica da dieta habitualmente ingerida pelos indivíduos fisicamente ativos (≥ 10.000 passos diários) apresentou-se positivamente associada com o perfil lipídico aterogênico (ácidos graxos livres e razão triacilgliceróis/HDL-c) e com um biomarcador de oxidação no DNA (8-OHdG) o que indica influência potencialmente prejudicial do consumo de dietas com maiores cargas glicêmicas mesmo em indivíduos fisicamente ativos.

O consumo de carne vermelha também se apresentou com resultados considerados prejudiciais em relação aos componentes cardiometabólicos, porém, incluindo tanto indivíduos fisicamente ativos como não ativos. Nesse sentido, esse grupo de carne associou-se positivamente com ocorrência de síndrome metabólica, resistência à insulina e LDL oxidada, sendo que seu conteúdo de ácidos graxos saturados pareceu ser um grande contribuinte para os referidos efeitos deletérios.

Contrariamente, a ingestão de fruta, hortaliças e legumes foi negativamente associada com marcadores de estresse oxidativo e o conteúdo de vitamina C, fibra e magnésio desse grupo alimentar pareceu ter auxiliado nos seus efeitos benéficos sobre o balanço “redox”.

Os resultados deste estudo, em conjunto, indicam que o maior número de passos diários, a maior ingestão de frutas, verduras e legumes, bem como, o menor consumo de carnes vermelhas e de dietas de alta carga glicêmica são estratégias importantes na recomendação de hábitos de vida saudáveis para proteção da população estudada contra fatores de risco cardiometabólico.

4. ANEXOS

Anexo 1: 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 069/2010/Comitê de Ética

Viçosa, 04 de junho de 2010.

Prezado Professor:

Cientificamos V.Sª. de que o Comitê de Ética em Pesquisa com Seres Humanos, em sua 4ª Reunião de 2010, realizada em 31-5-2010, analisou e aprovou, sob o aspecto ético, o projeto de pesquisa intitulado *Avaliação da associação entre índice glicêmico/carga glicêmica da dieta habitual e nível de atividade física com componentes da síndrome metabólica e estado oxidativo.*

Atenciosamente,

Professor Gilberto Paixão Rosado
Comitê de Ética em Pesquisa com Seres Humanos
Presidente

Professor
Antônio José Natali
DES

/rhs.

Anexo 2: Questionário para a seleção da população do estudo

I – IDENTIFICAÇÃO:

1. Data da entrevista: ____/____/____
2. Nome:_____
3. Endereço Residencial:_____
   Cargo de trabalho que ocupa na UFV:_____
4. Telefones:

Residência:_____ Celular:_____ Trabalho: _____

II – INFORMAÇÕES GERAIS:

5. Data de nascimento: _____
6. Você perdeu ou ganhou mais de 3 kg nos últimos 3 meses?
___ Sim ___ Não

7. Você já teve ou tem alguma das doenças abaixo especificadas?

DOENÇA	ESTADO ATUAL				
	Nunca	Data Provável do diagnóstico	Mau controlado	Bem controlado	Curado
a. Diabetes Mellitus					
b. Hipertireoidismo					
c. Hipotireiodismo					
d. Doenças Cardiovasculares					
e. Insuficiência Cardíaca					
f. Doença Cerebrovasculares					
g. Doenças Infecciosas					
h. Doenças Inflamatórias					
i. Doenças Intestinais					
j. Doenças Hepáticas					
k. Doença Renal Crônica					
l. Litíase Renal					
m. Câncer					
n. Alergia Alimentar					
o. Anorexia ou Bulimia					
p. Outra doença grave					

No caso da letra p., especifique: _____

8. Faz uso regular de algum medicamento?

□ Sim Qual? _____ □ Não

9. Usa marcapasso e/ou prótese de membros? □ Sim □ Não

10. Você fuma?

□ Sim – Há quanto tempo?____ Quantos cigarros por dia?____ □ Não

11. Você ingere bebidas alcoólicas?

□ Sim – Há quanto tempo?____ Tipo de bebida_____ Com que frequência em dias/semana?_____ Quantidade (copo/garrafa)?___ □ Não

12. Você é atleta (participa de competições esportivas profissionais)?

□ Sim □ Não

13. Você mudou o seu padrão de atividade física nos últimos 3 meses?

□ Sim □ Não

14. Você é vegetariano? □ Sim □ Não

15. Você mudou o seu hábito alimentar nos últimos 3 meses? □ Sim □ Não

16. Você tem algum familiar que apresenta (ou) glicose elevada ou Diabetes? □ Sim □ Não

Se sim. Qual o grau de parentesco?_____

17. Você tem algum familiar que apresenta (ou) pressão arterial elevada (Hipertensão)?

□ Sim □ Não Se sim. Qual o grau de parentesco?_____

18. Você têm algum familiar que apresenta(ou) câncer?

□ Sim □ Não Se sim. Qual o grau de parentesco?_____

19. Você consome alguma vitamina ou qualquer suplemento dietético?

□ Sim □ Não Se sim. Qual (is)?_____

Outros tratamentos (hormônios ou quimioterapia) □ Outra razão_____

☐ Apto

☐ Não Apto

Anexo 3: Termo de consentimento livre e esclarecido

1. Título do Estudo: Estado oxidativo segundo o nível de atividade física, carga glicêmica da dieta e componentes da síndrome metabólica em indivíduos da meia-idade

2. Local de Execução: Universidade Federal de Viçosa; Departamento de Nutrição e Saúde; Divisão de Saúde; Campus da UFV – CEP: 36.571-000 - Viçosa – MG - Fone: 31-899-2692

3. Nomes e Números de Telefones dos Investigadores:

Paula Guedes Cocate (estudante de doutorado) 31-3892-4034

Alessandro de Oliveira (estudante de doutorado) 31-3891-8323

Rita de Cássia Gonçalves Alfenas. Departamento de Nutrição e Saúde. Universidade Federal de Viçosa 31-3899-3740

Antônio José Natali. Departamento de Educação Física. Universidade Federal de Viçosa 31-3899-2766

Número telefônico 24-horas: 31-8701-7718

4. Objetivo do Estudo:

Avaliar o estado oxidativo e indicadores da síndrome metabólica segundo o nível de atividade física e carga glicêmica da dieta habitual de indivíduos da meia-idade

5. Critério de Inclusão dos Indivíduos: Eu poderei ser incluído no estudo se atender aos seguintes critérios: não estar enfermo, estar na faixa etária de 40 a 59 anos de idade, ser servidor da Universidade Federal de Viçosa, não ser atleta, não estar fazendo dieta para controle de peso, não ter modificado o peso corporal e nível de atividade física nos 3 meses anteriores ao estudo, não ser diabético tipo 1 e 2, não ter hipo ou hipertireoidismo, não ter doença cardiovascular, não ter doença renal, não ter doença hepática, não ter bulimia/anorexia e não ter câncer diagnosticado, não estar usando suplementos alimentares, bem como, medicamentos que afetem o metabolismo, a ingestão alimentar e diuréticos.

6. Critério de Exclusão dos Indivíduos: Eu não poderei ser incluído no estudo ou poderei ser excluído se não atender aos critérios de inclusão.

7. Descrição do Estudo: O estudo recrutará 300 voluntários. Inclui avaliação do nível de atividade física, da qualidade da dieta habitual, da antropometria (peso, estatura e circunferências da cintura), composição corporal (percentual de gordura corporal por meio do DEXA) e extração de amostra de sangue e urina para análise dos marcadores do estresse oxidativo, risco cardiovascular, obesidade e complicações associadas. Caso seja incluído no estudo, devo estar em jejum para coleta da amostra de sangue e urina.

8. Benefícios para o Indivíduo: Eu conhecerei meu nível de atividade física, a qualidade da minha dieta habitual, minha composição corporal, concentração de

colesterol total e frações, triacilgliceróis, glicemia, insulinemia, pressão arterial e marcadores do estresse oxidativo, risco cardiovascular, obesidade e complicações associadas. Estes dados permitirão saber em que condições de saúde me encontro.

9. Riscos para o Indivíduo: O estudo não oferece riscos. Os equipamentos e materiais usados para estes procedimentos serão estéreis e/ou descartáveis. Não serei submetido a nenhum tipo de intervenção que possa causar danos à minha saúde, visto que as condutas adequadas a serem adotadas objetivam a promoção de mesma e são respaldadas na literatura científica.

10. Alternativas para a Participação no Estudo: não se aplica.

11. Exclusão dos Indivíduos: Os indivíduos podem ser excluídos do projeto se não forem capazes de completar os requisitos de cada etapa.

12. Direitos dos Indivíduos para recusar-se a participar ou retirar-se do Estudo: Eu entendo que minha participação é voluntária e posso recusar-me a participar ou posso interromper minha participação em qualquer hora, sem penalização.

13. Direitos dos Indivíduos quanto à privacidade: Os resultados do estudo podem ser publicados, sem citação dos nomes envolvidos, havendo total proteção à participação dos indivíduos. Os resultados poderão estar disponíveis para a Agência Financiadora da Pesquisa, observando a privacidade dos nomes envolvidos.

14. Publicação da Informação: As informações coletadas referentes ao projeto estarão disponíveis para a Equipe envolvida no projeto e para a Agência Financiadora.

15. Informação Financeira:

A. Minha participação neste estudo não implica em contrato de trabalho.

B. Fui comunicado que qualquer enfermidade que surja durante o estudo, deverá ser tratada por conta própria, ou seja, o estudo que participo não assume nenhum compromisso no tratamento da mesma. Nestes casos, deverei comunicar à equipe do projeto todas as informações referentes à enfermidade e o seu tratamento.

C. Eu não receberei qualquer compensação financeira para participar do estudo.

16. Em caso de Emergência: Se existe alguma intercorrência decorrente da pesquisa, chamarei ao investigador principal no telefone: 31-899-2766 ou 31-8701-7718, em qualquer horário do dia ou da noite.

17. Assinaturas: O estudo foi discutido comigo e todas as questões foram respondidas. Eu entendo que perguntas adicionais relacionadas ao estudo devem ser dirigidas aos investigadores listados acima. Eu entendo que, se tenho dúvidas sobre direitos dos voluntários, posso contatar o Comitê de Ética da UFV. Eu concordo com os termos acima e acuso o recebimento de uma cópia deste consentimento.

_____	_____
Assinatura do Indivíduo	Data
_____	_____
Assinatura do Investigador	Data

Anexo 4: Instruções para avaliação da atividade física habitual e ficha de anotações do número de passos diários

Instruções para o uso do pedômetro:

- ➔ Usar o aparelho no cós da calça do lado direito em cima do osso do quadril;
- ➔ Retirar o aparelho ao andar de bicicleta, moto, trator e charrete;
- ➔ Certificar se o aparelho está zerado (botão amarelo) antes de usar;
- ➔ Iniciar o uso logo após acordar e retirá-lo antes de dormir;
- ➔ No final do dia (antes de dormir) abrir o aparelho, verificar o valor (número de passos) e anotar os dados de acordo com o dia que usou.

	Dia 1	Dia 2	Dia 3	Dia 4	Dia 5	Dia 6	Dia 7	Dia 8
Nº de Passos diário								

	Média do Dia 2 ao Dia 8
Nº de Passos diário	

Anexo 5: Instruções para avaliação da composição corporal pela absortometria de feixe duplo de raios-x e realização dos exames bioquímicos

Nome: _____

Data do exame: _____/_____/_____

Local: Divisão de Saúde (Hospital) da Universidade Federal de Viçosa

Recomendações para realização do exame:

- Jejum de 12 horas antes da realização do exame;
- Não realizar exercício físico nas 12 horas antes da realização do exame;
- Não ingerir álcool nas 48 horas (2 dias) antes da realização do exame;
- Usar roupas leves, sem metal.

Anexo 6: Técnica da medida da pressão arterial

1. Explicar o procedimento ao paciente, orientar que não fale e deixar que descanse por 5 a 10 minutos em ambiente calmo, com temperatura agradável. Promover relaxamento.

2. Certificar-se de que o paciente não está com a bexiga cheia; não praticou exercícios físicos há 60–90 minutos; não ingeriu bebidas alcoólicas, café, alimentos, ou fumou até 30 minutos antes; e não está com as pernas cruzadas.

3. Utilizar manguito de tamanho adequado ao braço do paciente, cerca de 2 a 3 cm acima da fossa antecubital, centralizando a bolsa de borracha sobre a artéria braquial. A largura da bolsa de borracha deve corresponder a 40% da circunferência do braço e o seu comprimento, envolver pelo menos 80%.

4. Sentar o indivíduo em uma cadeira com os pés firmemente plantados no chão e manter o braço do paciente em uma mesa para que o manguito esteja na altura do coração, livre de roupas, com a palma da mão voltada para cima e cotovelo ligeiramente fletido.

5. Colocar o braço do paciente na abertura do manguito, certificando-se que a borda inferior esteja aproximadamente 1,27 cm acima do cotovelo e que o marcador verde no manguito esteja acima da artéria braquial.

6. Puxar a extremidade do manguito para que ele todo envolva firmemente o braço e pressionar o material do gancho contra o lado do manguito.

7. Pressionar o botão ON/OFF.

8. Após aparecer o símbolo do coração no painel digital, pressione o botão Start. O paciente deve permanecer imóvel até que se complete a medição.

9. Quando a medida estiver concluída, o monitor exibe a pressão arterial e a taxa de batimentos cardíacos e desinfla o manguito automaticamente.

10. Registrar os valores das pressões sistólica e diastólica, complementando com a posição do paciente, o tamanho do manguito e o braço em que foi feita a medida. Não arredondar os valores de pressão arterial para dígitos terminados em zero ou cinco.

11. Esperar 1 a 2 minutos antes de realizar novas medidas. Pode ser necessário mais tempo de descanso entre as leituras, dependendo das características fisiológicas de cada indivíduo.

12. O paciente deve ser informado sobre os valores obtidos da pressão arterial e a possível necessidade de acompanhamento.

Anexo 7: Ficha de anotação dos dados de avaliação antropométrica, da pressão arterial, composição corporal e bioquímica

Nome: _____ Data Nascimento: ___/___/___

1) Avaliação Hemodinâmica:

Avaliação Clínica	Medida 1	Medida 2	Medida 3	Média (mmHg)
Pressão Arterial (mmHg)				

2) Avaliação Bioquímica:

Parâmetros Bioquímicos	Resultados
Colesterol Total (mg/dL)	
LDL (mg/dL)	
HDL (mg/dL)	
Ácidos graxos livres (mg/dL)	
Triglicerídeos (mg/dL)	
Glicemia (mg/dL)	
Insulina (µm/L)	
HOMA-IR	
LDL oxidada (U/L)	
F2 isoprostano	
8-OHdG	
Creatinina	

3) Avaliação Antropométrica e composição corporal

Variável Antropométrica	Medida 1	Medida 2	Medida 3	Média
Perímetro da Cintura (cm) Ponto Médio				

Variáveis Antropométricas	Medida	Variáveis de composição corporal	Medida
Peso (kg)		% Gordura Corporal Total	
Estatura (cm)		% Gordura Região Andróide	
Índice de Massa Corporal (Kg/m^2)		% Gordura Região Ginóide	

Anexo 8: Questionário de frequência do consumo alimentar

Alimentos e Grupos	Tamanho da porção			Frequência	
	Pequeno	Médio	Grande	Número de vezes	D: Dia S: Semana M: Mês
				0-10	D S M
Leite e derivados					
Leite integral					
Leite desnatado					
Iogurte convencional					
Iogurte light					
Queijo branco					
Queijo amarelo					
Requeijão convencional					
Requeijão light					
Pães e substitutos					
Pão francês					
Pão de forma convencional					
Pão de forma Light					
Pão integral					
Pão de queijo					
Biscoito salgado					
Biscoito polvilho					
Biscoito de maisena					
Biscoito recheado diet					
Biscoito recheado convencional					
Biscoito Waffer diet					
Biscoito Waffer convencional					
Bolo diet					
Bolo convencional					
Gorduras					
Margarina convencional					
Margarina Light					
Manteiga					
Maionese convencional					
Maionese light					
Azeite					
Cereais					
Arroz					
Arroz integral					
Arroz temperado					
Batata Frita					
Mandioca Frita					

Batata cozida					
Mandioca cozida					
Angu					
Milho Verde					
Macarrão					
Lasanha					
Macarrão instantâneo					
Coxinha					
Quibe					
Esfiha/ enroladinho					
Empada					
Pastel					
Pizza					
Farinha					
Farofa					
Frutas					
Laranja					
Banana					
Maçã					
Pêra					
Mamão					
Melancia/ melão					
Abacaxi					
Uva					
Outras frutas_____					
Leguminosas					
Feijão					
Feijão tropeiro					
Soja					
Verduras/ legumes					
Alface					
Agrião					
Repolho					
Espinafre					
Couve					
Couve flor, brócolis					
Cenoura crua					
Cenoura cozida					
Abóbora cozida					
Tomate					
Beterraba					
Chuchu					
Abobrinha					
Quiabo					

Carnes					
Carne bovina magra _____					
Carne bovina gorda _____					
Carne moída					
Carne suína magra _____					
Carne suína gorda _____					
Bacon, torresmo					
Frango sem pele _____					
Frango com pele _____					
Peixes_____					
Linguiça					
Salsicha					
Ovo cozido					
Ovo frito/ omelete					
Presunto, mortadela					
Hambúrguer					
Bebidas					
Refrigerante diet					
Suco artificial diet					
Refrigerante convencional					
Suco artificial convencional					
Doces, miscelâneas					
Chocolates_____					
Arroz doce _____					
Doce de leite _____					
Doces de fruta _____					
Sorvete_____					
Pipoca _____					
Achocolatado_____					
Chips®					
Outros					
Café com açúcar					
Café sem açúcar					
Café com adoçante					

Anexo 9: Ficha dos resultados dos exames físicos e bioquímicos entregue aos participantes após o cumprimento de todas etapas do estudo

Resultado: Pesquisa Saúde e Qualidade de Vida	
Nome:	**Idade:**
Exames Laboratoriais (jejum): *Data exame: ___/___* Glicose: ____ mg/dL Valor desejável (<99 mg/dL) Colesterol Total: ____ mg/dL Valor desejável (<200 mg/dL) Colesterol HDL: _____ mg/dL Valor desejável (>40 mg/dL) Colesterol LDL: _____ mg/dL Valor desejável (<125 mg/dL) Triglicerídeo: _____ mg/dL Valor desejável (<150 mg/dL) Resistência à insulina (HOMA): _____ Valor desejável (<2,71) *Considerações*:	
Antropometria e composição corporal: Peso: ____Kg Estatura: ____m IMC: ____ kg/m^2 Valor desejável (18,5 a 25 kg/m^2) Circ. da cintura: ____ cm Valor desejável (< 90 cm) *Considerações*:	Tecido Gorduroso = ____ % (< 20%) Tecido magro = ____ kg Dens. Mineral Óssea: ____ t-score
Parâmetro Hemodinâmico: Pressão Arterial: ___x___ mmHg Valor desejável (< 135 x 85 mmHg) *Considerações:*	
Nível de atividade física: Número de passos médio:_____ (Ideal: > 10.000 passos) *Considerações:*	
Considerações e indicações gerais:	

Qualquer dúvida entre em contato com: Paula: (31) 8699-7287 ou Alessandro: (31) 8701-7718

Muito obrigada pela participação! Investir na saúde é investir em sua vida!