PRISCILA VAZ DE MELO RIBEIRO

EFEITOS DA FARINHA DE YACON (*Smallanthus sonchifolius*) E DIETA RESTRITA EM CALORIAS NOS MARCADORES DE GLICAÇÃO E NA MICROBIOTA INTESTINAL EM ADULTOS COM EXCESSO DE PESO

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

Orientadora: Rita de Cássia G. Alfenas

Coorientadores: Leandro Licursi de Oliveira
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Aos meus pais, irmã, esposo e filha.

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RESUMO

RIBEIRO, Priscila Vaz de Melo, D.Sc., Universidade Federal de Viçosa, junho de 2021. **Efeitos da farinha de yacon (*Smallanthus sonchifolius*) e dieta restrita em calorias nos marcadores de glicação e na microbiota intestinal em adultos com excesso de peso**. Orientadora: Rita de Cássia Gonçalves Alfenas. Coorientadores: Leandro Licursi de Oliveira e Leidjaira Juvanhol Lopes.

Introdução: O excesso de peso se associa à inflamação, ao estresse oxidativo e à alteração da microbiota intestinal, resultando na manifestação de doenças crônicas. Os resultados de estudos recentes sugerem que os produtos finais de glicação avançada (AGEs) também podem contribuir para a obesogênese. A inclusão na dieta de alimentos capazes de melhorar as concentrações dos marcadores de doenças crônicas não transmissíveis constitui uma estratégia interessante para controle do excesso de peso. A batata yacon (*Smallanthus sonchifolius*) é considerada um alimento funcional, por ser fonte natural de frutooligossacarídeos (FOS). No entanto, os efeitos do seu consumo nas concentrações séricas de AGEs, de produtos de glicação precoce (EGPs), do receptor solúvel de AGEs (sRAGE) e na microbiota intestinal ainda não foram avaliados. **Objetivos:** Verificar como o consumo da farinha de yacon e dieta restrita em calorias afeta as concentrações séricas dos marcadores de glicação e a microbiota intestinal, além de verificar associações dessas variáveis com marcadores antropométricos, cardiometabólicos, de estresse oxidativo, composição corporal e inflamação em adultos com excesso de peso. **Métodos:** Vinte e seis adultos com excesso de peso foram incluídos neste ensaio clínico randomizado, paralelo, duplo-cego, controlado por placebo, de seis semanas. Os indivíduos foram alocados aleatoriamente no grupo controle (n=13) ou no grupo farinha de yacon (n=13) e consumiram diariamente uma bebida no desjejum, não contendo ou contendo 25 g de farinha de yacon (8,7 g de FOS). Dietas restritas em calorias foram prescritas para ambos os grupos. Marcadores cardiometabólicos, antropométricos, de composição corporal, glicação, estresse oxidativo, inflamação e a microbiota intestinal foram avaliados ao início e ao final do estudo. **Resultados: Artigo 1 -** As interações AGEs-RAGE podem ativar a via de sinalização fator nuclear kappa β e inibir a via PI3K-AKT em adipócitos, favorecendo a manifestação de doenças crônicas. Essa interação pode ser considerada uma nova explicação para a patogênese da

obesidade. **Artigo 2** - Os AGEs e os EGPs não aumentaram no grupo farinha de yacon. sRAGE diminuiu, independentemente do grupo. Alterações nos marcadores de glicação foram positivamente associadas a alterações na gordura corporal, insulina e HOMA-IR. **Artigo 3 -** As mudanças nos marcadores de glicação se associaram a mudanças no poder antioxidante de redução do ferro (FRAP), proteína carbonilada, pressão arterial sistólica, triglicerídeos e razão triglicerídeos/HDL-c no grupo yacon. Os EGPs no baseline se associaram negativamente a mudanças na gordura corporal total e ao malondialdeído após consumo da yacon. **Artigo 4** - Houve aumento dos gêneros *Bifidobacterium*, *Blautia, Subdoligranulum e Streptococcus* após o consumo da yacon e dieta restrita em calorias. No grupo yacon, também observamos uma correlação positiva entre as concentrações de ácidos graxos de cadeia curta e os gêneros *Coprococcus* e *Howardella*, além de uma correlação negativa entre os marcadores de glicação versus os gêneros *Ruminococcus* e *Prevotella*, respectivamente. **Conclusão:** O consumo de farinha de yacon e dieta restrita em calorias alterou seletivamente a microbiota intestinal e a avaliação dos marcadores de glicação pode ser uma estratégia útil para monitorar as respostas às intervenções alimentares em indivíduos com excesso de peso.

Palavras-chave: Produtos de glicação avançada alimentares. Biomarcadores cardiometabólicos. Estresse oxidativo. Excesso de peso. Frutooligossacarídeos. Microbiota intestinal. Produtos finais de glicação avançada. Receptor solúvel para produtos finais de glicação avançada. Yacon.

ABSTRACT

RIBEIRO, Priscila Vaz de Melo, D.Sc., Universidade Federal de Viçosa, June, 2021. **Effects of yacon flour (*Smallanthus sonchifolius*) and energy-restricted diet on glycation markers and intestinal microbiota in adults with excess body weight.** Adviser: Rita de Cássia Gonçalves Alfenas. Co-advisers: Leandro Licursi de Oliveira and Leidjaira Juvanhol Lopes.

Background: Excess body weight is associated with inflammation, oxidative stress and changes in the intestinal microbiota, resulting in the manifestation of chronic diseases. The results of recent studies suggest that advanced glycation end products (AGEs) may also contribute to obesogenesis. Diets containing foods capable of improving the concentrations of non-communicable chronic disease markers is an interesting strategy to control excess body weight. Yacon (*Smallanthus sonchifolius*) is considered a functional food, as it is a natural source of fructooligosaccharides (FOS). However, the effects of yacon consumption on serum concentrations of AGEs, early glycation products (EGPs), soluble receptor AGEs (sRAGE) and on the intestinal microbiota have not been evaluated. **Objectives**: Verify the effect of the consumption of yacon flour and energy-restricted diet on serum concentrations of glycation markers and intestinal microbiota, besides verifying associations between such variables with oxidative stress, inflammatory, body composition, anthropometric and cardiometabolic markers in adults with excess body weight. **Methods**: Twenty-six adults with excess body weight were included in this randomized, parallel, double-blind, placebo-controlled, six-week clinical trial. Subjects were randomly allocated into the control group (n=13) or the yacon flour group (n=13), and daily consumed a breakfast drink, not containing or containing 25 g of yacon flour (8.7 g of FOS). Energy-restricted diets were prescribed for both groups. Cardiometabolic, anthropometric, body composition, glycation, oxidative stress, inflammation and intestinal microbiota markers were evaluated at the baseline and the end of the study. **Results**: Article 1 - AGEs-RAGE interactions can activate the nuclear factor kappa β signaling pathway and inhibit the PI3K-AKT pathway in adipocytes, favoring the manifestation of chronic diseases. This interaction can be considered as a novel explanation for the pathogenesis of obesity. Article 2 - AGEs and EGPs did not increase in the yacon flour group. sRAGE decreased regardless of the group. Changes in glycation markers were positively

associated with changes in body fat, insulin and HOMA-IR. Article 3 - Changes in glycation markers were associated with changes in ferric reducing antioxidant power (FRAP), carbonylated protein, systolic blood pressure, triglycerides and triglycerides/HDL-c ratio in the yacon group. Baseline EGPs were negatively associated with changes in total body fat and malondialdehyde after yacon consumption. Article 4 - There was an increase in the genera *Bifidobacterium, Blautia, Subdoligranulum* and *Streptococcus* after yacon consumption and energy-restricted diet. In the yacon group, we also observed a positive correlation between the concentrations of short-chain fatty acids and the genera *Coprococcus* and *Howardella*, beside a negative correlation between the concentrations of glycation markers versus the genera *Ruminococcus* and *Prevotella*, respectively. **Conclusion**: Consumption of yacon flour and energy-restricted diet selectively altered the intestinal microbiota and the assessment of glycation markers may be a useful strategy for monitoring dietary interventions responses in subjects with excess body weight.

Keywords: dietary Advanced glycation end products. Cardiometabolic biomarkers. Oxidative stress. Overweight. Fructooligosaccharides. Gut microbiota. Advanced glycation end products. Soluble receiver for advanced glycation end products. Yacon.

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LISTA DE SIGLAS E ABREVIATURAS

AGCC - ácidos graxos de cadeia curta
AGEs - produtos finais de glicação avançada
ALT - alanina aminotransferase
AST - aspartato aminotransferase
ASVs - amplicon sequence variants
AU - unidade arbitrária
CDNB - 2,4-dinitroclorobenzeno conjugado com glutationa
CML - N"-carboxymethyllysine
DMT2 - diabetes *mellitus* tipo 2
DXA - dual-energy X-ray absorptiometry scan
EER - estimated energy requirement
EGPs - early glycation products
ERK - extracellular signal-regulated kinase
EROs - espécies reativas de oxigênio
FDR - false discovery rate
FRAP - ferric reducing antioxidant power
FOS - frutooligossacarídeos
GEE - modelo de equações de estimações generalizadas
GSH - glutationa
GST - glutationa S transferase
HDL-c - lipoproteína de alta densidade
HOMA-IR - homeostasis model assessment of insulin resistance
HPLC - cromatografia líquida de alta eficiência
iAUC - área sob a curva incremental
IgA - imunoglobulina A
IMC - índice de massa corporal
JNK - c-jun N-terminal kinase
LAP - lipid accumulation product
LDL-c - lipoproteína de baixa densidade
LPS - lipopolissacarídeo
MDA - malondialdeído
MG - metilglioxal

NBT - ensaio de nitroazul de tetrazólio

NF-kB - fator nuclear kappa B

ON - óxido nítrico

OTU - operational taxonomic units

PCR - proteína C reativa

RAGE - receptor para produtos finais de glicação avançada

SOD - superóxido dismutase

SPSS - statistical package for the social sciences

sRAGE - receptor solúvel para produtos finais de glicação avançada

TCA - ácido tricloroacético

TGO - transaminase glutâmico-oxalacética

TGP - transaminase glutâmico-pirúvica

TNF-α - fator de necrose tumoral alfa

TyG - índice triglicerídeos/glicose

VAI - índice de adiposidade visceral

SUMÁRIO

1. INTRODUÇÃO GERAL

O excesso de peso tem sido considerado um grande problema de saúde pública, em função do aumento da sua prevalência em todo o mundo (CHOOI; DING; MAGKOS, 2019). O percentual de gordura corporal aumentado em indivíduos com excesso de peso pode favorecer o desenvolvimento de estresse oxidativo e inflamação de baixo grau. Tais alterações precedem a manifestação de doenças crônicas como obesidade, diabetes *mellitus* tipo 2 e doenças cardiovasculares (BONDIA-PONS; RYAN; MARTINEZ, 2012; FURUKAWA et al., 2004; JUNG; CHOI, 2014).

Acredita-se que os produtos finais de glicação avançada (AGEs) e os seus receptores (RAGE e sRAGE) estejam envolvidos na patogênese do excesso de peso. Os AGEs endógenos e os originados dos alimentos (Reação de Maillard) são formados por reações não enzimáticas entre açúcares e proteínas, lipídeos ou ácidos nucléicos (VAN NGUYEN, 2006; URIBARRI et al., 2010). Inicialmente, ocorre condensação entre um grupo amino e um grupo carbonila, resultando na formação da base de Schiff seguida pelo rearranjo e formação dos produtos de Amadori ou produtos de glicação precoce (EGPs). Um desses subprodutos é o metilglioxal, um precursor altamente reativo de AGEs formado a partir do metabolismo da glicose e da frutose. Em estágio avançado, os produtos de Amadori sofrem reações de oxidação, formando AGEs (compostos irreversíveis) (VAN NGUYEN, 2006; MATAFOME et al., 2017). A formação de AGEs endógenos é exacerbada na hiperglicemia e no estresse oxidativo (GOH; COOPER, 2008). Há aumento da formação destes em alimentos submetidos a tratamentos térmicos, que utilizam altas temperaturas e baixa umidade, como fritar, assar e grelhar (AMES, 1998; DELGADO-ANDRADE et al., 2010, 2012). Acredita-se que o alto consumo de AGEs pode aumentar a quantidade total de AGEs séricos. Estima-se que 10% dos AGEs consumidos sejam prontamente absorvidos (BOTROS et al., 2017; DI PINO et al., 2016; GOLDBERG et al., 2004).

A importância clínica dos AGEs está relacionada à ativação de vias inflamatórias e do estresse oxidativo pelas interações desses AGEs com seu receptor específico ligado à membrana (RAGE) (BASTA, 2004; GAO et al., 2008). Após a ligação AGEs-RAGE, inicia-se uma cascata de sinalização inflamatória, culminando na ativação do fator nuclear kappa β (NF-kβ) e transcrição subsequente de citocinas inflamatórias, incluindo a transcrição do próprio RAGE (TANAKA et al., 2000). Esta

cascata cíclica contribui para a obesogênese (GAENS et al., 2014; SONG et al., 2014). Por outro lado, a forma solúvel do receptor RAGE (sRAGE), presente na circulação, parece atuar como um agente protetor, impedindo a interação entre AGEs-RAGE e os danos mediados pelos AGEs (FALCONE et al., 2005; LINDSEY et al., 2009).

Outro fator responsável pela gênese da obesidade é a disbiose (LEY et al., 2005; LEY, TURNBAUGH, KLEIN, 2006; TURNBAUGH et al., 2006, 2009), por favorecer a ocorrência de inflamação e estresse oxidativo (BLAUT; KLAUS, 2012). Está bem estabelecido que a microbiota intestinal tem um impacto fundamental nas funções metabólicas, imunológicas e endócrinas dos indivíduos (CLEMENTE et al., 2012; FLINT, SCOTT, LOUIS, 2012; JOYCE, GAHAN, 2014; MARCHESI et al., 2016). A inter relação microbiota saudável-hospedeiro é vista como mutualista, contribuindo para a saúde geral do hospedeiro (SOMMER; BÄCKHED, 2013). No entanto, as alterações na composição e função da microbiota podem resultar no desenvolvimento de vários estados de doença crônica. O excesso de peso, em particular, está associado à disbiose caracterizada por baixa diversidade bacteriana e mudanças específicas nas taxas bacterianas, que se correlacionam com marcadores metabólicos e inflamatórios (LE CHATELIER et al., 2013). Essas associações sugerem que os microrganismos do intestino contribuem para a inflamação sistêmica subclínica, que leva ao desenvolvimento de patologias como obesidade, resistência à insulina, diabetes tipo 2 e doença cardiovascular (ARSLAN, 2014; ESSER et al., 2014; KARLSSON et al., 2013; LOZUPONE et al., 2012; NIEUWDORP et al., 2014; QIN et al., 2012).

Acredita-se que a microbiota intestinal presente em indivíduos com excesso de peso pode favorecer o aumento da permeabilidade intestinal (BRUN et al., 2007; CANI, 2016), promovendo a translocação de compostos bacterianos, como o lipopolissacarídeo (LPS). O LPS é uma endotoxina derivada da membrana celular de bactérias gram-negativas, que pode contribuir para a inflamação sistêmica (CANI et al., 2007; MOREIRA et al., 2012; NEVES et al., 2013). Quando em concentrações aumentadas, o LPS também pode favorecer a ocorrência de resistência insulínica, hiperplasia adipocitária e diminuição da função das células β-pancreáticas. Essas alterações caracterizam um fenômeno denominado endotoxemia metabólica (CANI, 2016; KRAJMALNIK-BROWN et al., 2012).

Mudanças no estilo de vida, especialmente nos padrões alimentares, desempenham um papel central na prevenção e no controle do excesso de peso.

Assim, o consumo de alimentos capazes de reduzir a ingestão alimentar e melhorar marcadores metabólicos, constitui uma estratégia interessante. A yacon (*Smallanthus sonchifolius*) é uma planta herbácea da família Asteraceae, nativa das regiões andinas da América do Sul, que vem ganhando destaque no meio científico. Mais de 70% do peso fresco das raízes da yacon é água, enquanto a maior parte da matéria seca é de oligofrutanos ou frutooligossacarídeos (FOS). Devido a alta concentração de FOS, a yacon é amplamente estudada pelo seu potencial como alimento funcional (CAETANO et al., 2016). No entanto, logo após a colheita da yacon, a hidrólise do FOS se inicia, resultando na liberação de grandes quantidades de açúcares livres, como produtos de degradação da despolimerização de FOS (GRAEFE et al., 2004). Por outro lado, por meio da desidratação das raízes de yacon, obtém-se a farinha de yacon sem adição de conservantes ou produtos químicos, garantindo a produção de um produto natural com grande estabilidade do FOS (CAMPOS; AGUILAR-GALVEZ; PEDRESCHI, 2016).

Identificamos na literatura apenas quatro estudos em que foram avaliados os efeitos funcionais da farinha de yacon em humanos (MACHADO et al., 2019; MACHADO et al., 2020; ROCHA et al., 2018; VAZ-TOSTES et al., 2014). Os estudos mais recentes foram realizados pelo nosso grupo. Verificamos que o consumo diário de 25 g de farinha de yacon (0,1g FOS/kg) associado a dieta restrita em calorias durante 6 semanas foi eficaz no controle do excesso de peso corporal (MACHADO et al., 2019), aumentou a capacidade antioxidante do plasma, diminuiu o estresse oxidativo e os ácidos graxos de cadeia curta em adultos com excesso de peso (MACHADO et al., 2020). Por outro lado, o consumo agudo de 21g/dia de farinha de yacon (7,4g FOS) não afetou a resposta glicêmica, o apetite ou a ingestão de alimentos em indivíduos eutróficos e euglicêmicos (ROCHA et al., 2018). Já em crianças pré-escolares, sendo a maioria (62,5%) eutróficas, o consumo de 6, ou 7 ou 9g de farinha de yacon por dia (0,14g FOS/kg), durante 18 semanas, melhorou a resposta imune intestinal, sem afetar o estado nutricional de ferro e zinco (VAZ-TOSTES et al., 2014).

O consumo do xarope de yacon (0,14 g FOS/kg), durante 120 dias por mulheres obesas, reduziu o peso corporal, o perímetro da cintura, o índice de massa corporal (IMC), a concentração de lipoproteína de baixa densidade, a insulinemia sérica em jejum e o HOMA-IR. Além disso, aumentou a frequência de defecação e a sensação de saciedade (GENTA et al., 2009). O consumo de yacon em pó liofilizado (7,4g FOS)

por 9 semanas reduziu a glicemia, sem afetar negativamente o trânsito intestinal em idosos (SCHEID et al., 2014). Já o consumo de 20g de xarope de yacon (6,4g FOS), durante 2 semanas melhorou o trânsito intestinal em eutróficos (GEYER et al., 2008). Por fim, a ingestão de um produto a base de yacon (10g FOS) por 30 dias foi eficaz na melhoria dos sintomas de constipação em adultos e idosos (SANT'ANNA et al., 2015). No entanto, evidências sobre os efeitos da farinha de yacon nas concentrações séricas de AGEs, sRAGE, e na microbiota intestinal em indivíduos com excesso de peso ainda não foram publicadas. Portanto, o objetivo desse estudo foi avaliar os efeitos do consumo da farinha de yacon e dieta restrita em calorias nas concentrações séricas dos marcadores de glicação e na microbiota intestinal, além de verificar associações dessas variáveis com marcadores antropométricos, cardiometabólicos, de estresse oxidativo, composição corporal e inflamação em adultos com excesso de peso.

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2. OBJETIVOS

2.1. Objetivo geral

Avaliar os efeitos da farinha de yacon (*Smallanthus sonchifolius*) e dieta restrita em calorias nos marcadores de glicação e na microbiota intestinal em adultos com excesso de peso.

2.2. Objetivos específicos

- Analisar criticamente estudos nos quais foram avaliados os efeitos dos produtos finais de glicação avançada (AGEs) alimentares nas complicações associadas à obesidade e discutir os possíveis mecanismos que explicam os efeitos desses compostos sobre a progressão de doenças crônicas associadas ao excesso de peso.
- Avaliar os efeitos do consumo da farinha de yacon e dieta restrita em calorias nas seguintes variáveis:
    - Concentrações séricas de AGEs, produtos de glicação precoce (EGPs - hemoglobina glicada, albumina glicada, frutosil-liína, furosina e outras proteínas plasmáticas glicadas) e receptor solúvel dos AGEs (sRAGE);
    - Composição e diversidade das bactérias que compõem a microbiota intestinal;
- Avaliar a associação entre marcadores de glicação com mudanças nos marcadores cardiometabólicos, antropométricos, de composição corporal, do estresse oxidativo e de inflamação em adultos com excesso de peso submetidos à intervenção com farinha de yacon e dieta restrita em calorias.

3. ARTIGOS

Artigo	Título do artigo
Revisão	Effect of reducing dietary advanced glycation end products on obesity associated complications: a systematic review
Original 1	Effect of the consumption of yacon flour and energy-restricted diet on glycation markers and association between these markers with factors linked to obesity in adults with excess body weight: a randomized, double-blind, placebo-controlled clinical trial
Original 2	Glycation markers are associated with changes in body fat, and in cardiometabolic and oxidative stress markers in adults with excess body weight: a secondary analysis of data from a randomized clinical trial
Original 3	Consumption of yacon flour and energy-restricted diet increased the relative abundance of intestinal bacteria that may lead to positive metabolic effects in adults with excess body weight: a randomized, double-blind, parallel, placebo-controlled clinical trial

ARTIGO 1

Artigo publicado – Nutrition Reviews, Fator de impacto 6.500, Qualis Capes A1 – DOI: 10.1093/nutrit/nuz034

Effect of reducing dietary advanced glycation end products on obesity associated complications: a systematic review

ABSTRACT

Context: Consumption of dietary advanced glycation end products (AGEs) is related to oxidative stress, inflammation and other chronic conditions commonly associated with obesity.

Objective: To analyze the effects of dietary AGEs on complications associated with obesity.

Data sources: This systematic review was conducted and reported according to PRISMA. Terms 'advanced glycation end products', 'overweight' and 'obesity' were searched on the PubMed, Cochrane and Scopus databases. The last search was performed in October 2018.

Data extraction: There were included six studies that evaluated the effects of low-AGE and high-AGE diets consumed from one day to 12 weeks. A comparison of all the compiled data was conducted by the authors.

Data analysis: Circulating and urinary AGEs, besides soluble receptor for AGEs were considered as primary outcomes. Secondary results were cardiometabolic, inflammatory, glycemic, anthropometric and renal markers.

Conclusions: AGE-RAGE interactions can activate the NF-κB signaling pathway and inhibit the adipocytes PI3K-AKT pathway, which may explain their association with chronic diseases. This interaction can be considered as a novel explanation for obesity pathogenesis. AGEs can also be used as a biomarker for monitoring responses to dietary interventions in overweight and obese people.

Systematic Review Registration: PROSPERO registration no. CRD42018082745.

Keywords: Advanced glycation end product**,** RAGE, insulin resistance, NF-κB, ROS, pathway PI3K-AKT, dietary recommendations, dietary AGEs, obesity, renal injury and endothelial dysfunction.

INTRODUCTION

Obesity has reached epidemic proportions worldwide and has become a serious public health problem. The World Health Organization estimates that in 2016 more than 1.9 billion adults were overweight and that over 650 million of them were obese.[1] Obesity is a risk factor for the development of chronic diseases, such as cardiovascular diseases, diabetes and kidney disease.[2–4] Inflammation and oxidative stress are complications associated with obesity, which in turn are related to the genesis of chronic diseases.[5,6]

Changes in lifestyle, especially in dietary patterns, play a central role in obesity prevention and control. Recently, the consumption of foods rich in advanced glycation end products (AGEs) has been considered to play a fundamental role in chronic disease pathogenesis.[7,8] Since dietary AGEs increase obesity oxidative stress and inflammation, a reduction on AGEs intake seems to be beneficial, independently from the consumption of the usual energy restricted diets.

Moreover, some studies have detected a positive association between visceral fat and elevated serum concentration of AGEs, suggesting a causal role of exogenous AGEs in metabolic syndrome, which in turn is independent from energy balance.[9] AGEs formation results from non-enzymatic reactions between reactive sugars and proteins (the Maillard reaction), and the formation depends directly on the temperature and the time used for food preparation. This reaction is activated during frying, roasting, grilling, and baking.[10–12] Therefore, the consumption of AGEs-rich foods should be restricted.[7]

The consumption of low-AGEs diets decreases circulating and urinary AGEs markers and improves anthropometric, glycemic, cardiometabolic, inflammatory and renal function markers in overweight and obese people. However, the mechanism of action of molecular AGEs in obesity associated complications remain unclear. The amount of AGEs considered safe for consumption also needs to be established. Therefore, it was carried out a systematic review according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) to analyze the effects of dietary AGEs on complications associated with obesity, as well as to discuss the molecular mechanisms related to the effects of these compounds in chronic diseases, and to establish a safe recommendation for AGEs ingestion.

METHODS

Protocol and Registration

This systematic review was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[19] (Annex I Checklist) and was registered in PROSPERO (registration number: CRD42018082745).

Literature Search

The participants, intervention comparators, outcomes, and study design (PICOS) criteria adopted in this study are shown in Table 1. Four authors (PVMR, JFT, MACC and JBM) independently searched for original articles that investigated the effects of dietary advanced glycation end products on obesity complications using the following electronic databases: MEDLINE (PubMed, www.pubmed.com), Cochrane (www.cochrane.org), and Scopus (www.scopus.com). Keywords were chosen from the Medical Subject Headings (MeSH) and Descriptors in Health Sciences (DeHS) using the following search strategy: ("glycosylation end products, advanced" OR "advanced glycation end products" OR "dietary advanced glycation end products" OR "circulating advanced glycation end products") AND (overweight OR obesity) NOT review*.

The search strategy was not restricted by date and language. The last search was done in October 9, 2018. A reverse hand-search was also performed to identify relevant articles cited in all selected studies.

Study Selection

The study selection was performed by five authors (PVMR, JFT, MACC, JBM and RCGA) in three phases: analysis of titles, abstracts and full texts. All clinical trials that assessed the effects of reducing dietary advanced glycation end products intake on obesity complications markers in overweight and obese were included.

Comments, reviews, letters, case reports, abstracts and unpublished articles were not included. Animal studies, *in vitro* studies, and epidemiological studies involving people with diseases other than being overweight or obese (e.g., metabolic syndrome, diabetes, and polycystic ovary syndrome) were excluded.

Data Extraction

After reading the selected studies, a comparison of all the compiled data was conducted by the authors (PVMR, JFT, MACC, JBM and RCGA) to guarantee its

integrity and reliability. Divergent decisions were settled by consensus. For each study included, the following information was extracted: title, author's name, year of publication, study purpose, subjects' characteristics, sample size, study design, intervention (low/high AGEs consumption), and study duration; in addition, the main results were extracted regarding circulating and urinary advanced glycation end products as well as cardiometabolic, inflammatory, glycemic, renal and anthropometric markers.

Assessment of Risk of Bias

Risk of bias was assessed using the Cochrane collaboration method.[20] The studies were judged on three levels of bias: high risk, low risk and unclear (when the information provided was not sufficient to make a clear judgment). The authors considered the following biases: random sequence generation and allocation concealment (selection bias), blinding of participants and staff (performance bias), and blinding of results evaluation (detection bias) and selective reporting (notification bias).[20]

Data Analyses

All studies reviewed in this article are summarized in a table according to their main characteristics and results concerning obesity-associated markers. The studies were organized chronologically by year of publication, starting with the first published study. Circulating and urinary advanced glycation end products carboxymethyl lysine (CML), carboxyethyl lysine (CEL), methylglyoxal (MG) and the soluble receptor for the advanced glycation end product (sRAGE) were considered as the primary outcomes. The secondary results were cardiometabolic (lipid profile and systemic arterial pressure), inflammatory (TNF-α, IL-6, MCP-1, PCR, NFκB), glycemic (insulin sensitivity, HOMA-IR, glycemia, fasting insulin), anthropometric (body mass index, waist circumference, waist-hip ratio) and renal (GFR, albumin, creatinine) markers. In addition, the interaction of dietary AGEs intake versus the study duration was analyzed.

RESULTS

Study Selection

After searching the PubMed, SCOPUS and Cochrane databases, 679 studies were identified. The 224 duplicates were identified among the databases and were removed, resulting in 455 articles. Then, 445 studies were excluded based on their titles since they were considered irrelevant to the topic of interest. After reading the summary of the remaining 10 studies, 6 met all criteria for the systematic review. The reasons for exclusion of the other studies are indicated in Figure 1.

Description of the Included Studies

A total of 172 healthy overweight and obese (mean BMI 31.7 ± 2.3 kg/m²) subjects participated in the six studies included in this review (Table 2).[13–18] All studies included both overweight and obese participants. [13–18] These studies had sample sizes varying from 11[13] to 73[15] participants. The mean age was 36 ± 7.7 years for healthy overweight and obese subjects. All studies were randomized and controlled, four were crossovers,[13,14,17,18] and two were parallel studies,[15] with a duration varying from 1 day[14] to 12 weeks.[16] The content of the AGEs in the test diets ranged from 10.7 mg/day[15] to 43 mg/day[17,18] and 3,302 kU/day[13] to 7,306 kU/day[16] in the chronic studies and 2.8 mg/meal in the acute study.[14] In the control diets, consumption of AGEs varied from 24.6 mg/day[15] to 59 mg/day[17,18] and 11.223 kU/day to 14.090 kU/day[16] and 5 mg in the acute study[14] (Table 2). In all studies, a normocaloric diet was prescribed to participants and a food menu (low-AGEs versus high-AGEs content) was provided to meet the participants' preferences and dietary habits. In addition, the test and control diets were similar in macronutrient content and total energy, differing only in relation to the AGEs content.

Main Results of the Studies

The main results obtained in this study were related to AGEs effects on urinary and circulation AGEs, inflammation and oxidative stress, insulin resistance, and chronic diseases markers. Analyzing the AGEs consumption between the groups, it was observed that low-AGEs diets have an average of 5,304 kU/d[13,16] or 32.23 mg/d[14,15,17,18] and that high-AGEs diets average 12,656 kU/da[13,16] or 43.53 mg/d.[14,15,17,18]

The consumption of low-AGEs diets reduced the urinary excretion of CML,[15] methylglyoxal-derived hydroimidazolone (MG-H1) [15,17] and N €–(carboxyethyl) lysine (CEL).[17] Similarly, there was a reduction in circulating AGEs (CML and MG)[16] within

the participants consuming a low AGEs diet. Regarding metabolic markers, low AGEs diets reduced plasma triglycerides and increased high density lipoprotein (HDL),[16] reduced blood glucose on an oral glucose tolerance test, fasting insulin and HOMA-IR,[15] while insulin sensitivity increased.[15,16] A restriction in AGEs intake also reduced body weight,[15,16] waist circumference,[15] waist-hip ratio,[15] body mass index (BMI)[15,16] and urinary albumin/creatinine ratios[13] but increased the estimated glomerular filtration rate (eGFR).[17]

On the other hand, the consumption of high AGEs diets reduced plasma CML, increased urinary CML[13] and postprandial plasma glucose peak,[14] increased urinary 8-isoprostans[13] and F2-isoprostanes,[14] monocytic chemotactic protein-1 (MCP-1), plasma cystatin C[13] and vascular cell adhesion molecule-1 (marker of endothelial activation),[14] in addition to reducing macrophage migration inhibitory factor (MIF).[13]

Risk Assessment of Bias

The major domains evaluated in the present study were the random generation allocation sequence and the incomplete results data; most of the studies presented a low risk of bias, and only two studies were unclear as to their random sequence generation of allocation.[13,15] All studies were randomized; the missing data were balanced between the intervention groups, and the ratios between the groups were similar. However, blinding of treatment allocations was not clearly presented in two studies.[13,15] Only studies by Harcourt et al.[13] and Macías-Cervantes et al.[16] presented a risk of unclear bias due to the blinding of the participants, staff and evaluation of the results. In addition, Mark et al.[15] did not clearly define the selective results report (Figure 2).[13–18]

DISCUSSION

Dietary Recommendations

Although there are studies in which the authors tested the effects of low or high-AGES diets were tested, the differences between the methods used to determine the AGEs content and the limited number of studies, the authors were unable to suggest a cutoff point to classify AGEs consumption as high or low. A low-AGEs diet was associated with positive effects on health (improves anthropometric, glycemic, cardiometabolic, inflammatory and renal function markers) and better outcomes compared with a high-AGEs diet. Despite the fact that the subjects of the studies

included in this review were prescribed isocaloric diets, a reduction in body weight was observed[14,15]. However, since the actual food intake was not evaluated, possible unintended modifications on the caloric or macronutrient intake can not be discarded. Moreover, the results of the studies suggest a link between a high-AGEs diet and adverse impacts on health, evidencing the need for establishing safe dietary intake recommendations.[21]

Harcourt et al.[13] and Macías-Cervantes et al.[16] calculated the AGEs consumption considering a database of almost 550 foods. In that report, the AGEs content was assessed as carboxymethyllysine, a chemical type of AGEs commonly used for that purpose, and expressed as AGEs kilounits/100 grams of food.[21–23] Conversely, the other four studies included in this review expressed the AGEs content in milligrams. This difference in units of AGE content does not allow a comparison of AGEs consumption between the studies. Thus, establishing a standard unit that allows determination of cutoffs for low- and high-AGEs diets is recommended. The results of the studies suggest that dietary AGEs restriction may be a therapeutic strategy to promote health. Therefore, it is fundamental to replace AGEs- rich foods with low-AGEs foods.

In addition to the concentration of reactants, the formation of AGEs in foods is influenced by the preparation technique used.[21,24] Meat and high-sugar, high-fat, and highly processed foods are prone to develop a high AGEs content.[21,25] High temperatures, low humidity and alkaline pH contribute to new AGEs formation, which includes grilling, searing, roasting, and frying methods.[21,24]

In contrast, dairy products, fruits and vegetables have lower AGEs contents. Higher humidity, lower temperatures and low-pH have a minor contribution to AGEs formation. Thus, steaming, stewing, boiling and poaching should be the preferred techniques used to prepare foods.[21,24] Before cooking, the use of lemon juice and vinegar may reduce AGEs formation.[21] Table 3 shows differences between food groups and cooking methods that contribute to a lower or higher AGEs content.[21–23]

Effect of Dietary AGEs on Inflammation and Oxidative Stress

Obesity is an inflammatory condition[26] and is associated with an imbalance between reactive oxygen species production and their detoxification through biological systems that remove or repair the damage caused by them.[27] Oxidative stress and low-grade inflammation precede the manifestation of chronic diseases such as

diabetes and cardiovascular diseases[28,29]. Since obese people are already at increased risk of developing chronic diseases it is imperative to explore and identify modifiable risk factors for chronic diseases pathogenesis. Food is the major environmental factor in direct contact with host defenses. It is currently suggested that substances producing AGEs from processed foods are a source of reactive oxygen species entering the body.[30–32]

AGEs are a class of pro-oxidant foods, and their content is increased by processing the food at high temperatures.[33,34] Pro-oxidant AGEs also act as "appetite enhancer" agents that simultaneously stimulate excessive food intake and inflammation and increase the risk of obesity and diabetes mellitus.[35]

Approximately 10% of dietary AGEs are absorbed by humans, with only one-third of them being excreted in urine and feces. Plasma concentration of AGEs appears to be directly influenced by diet and the ability of the body to eliminate them.[30] Recent evidence suggests that AGEs can also be formed intraluminally in the bowel through reactions between unabsorbed excess free fructose and partially digested proteins.[36]

AGEs can increase oxidative stress through the receptor for AGEs (RAGE). Activation of RAGE induces a signaling cascade event, including MAPK p38-JNK, JAK-STAT and CDC42-RAC, many of which are the result and cause of oxidative stress. Thus, they modulate global cellular responses to various stress conditions and increase cellular damage.[37] However, the RAGE-ligand interaction may activate several signaling cascades, which implies that different RAGE-ligands may induce different pathways (especially in different cell types). The consequences of such mechanisms can be critical in negative feedback pathways, responsible for the return of cellular behavior to equilibrium.[38]

Some authors observed that the restriction of dietary AGEs led to oxidative stress reduction and suppression of RAGE mRNA levels and protein concentrations in people and rats with diabetes.[39,40] Similarly, AGEs restriction reduced RAGE concentrations in healthy human peripheral blood mononuclear cells and in people with diabetes below their baseline, indicating that RAGE is regulated by AGEs in the external environment.[32,41]

Oxidative stress is an inflammatory mediator. Thus, the binding of AGEs to RAGE during intracellular signaling leads to the activation of the proinflammatory NF-κB transcription factor.[42] In turn, NF-κB activates the transcription of target genes, such as proinflammatory cytokines, adhesion molecules and RAGE.[42–44] Therefore, the

expression of RAGE is induced by NF-κB, and continuous NF-κB activation results in positive receptor regulation and guarantees the maintenance and amplification of the signal[44] (Figure 3).

AGEs and Insulin Resistance

The effects of AGEs and their receptors on adipose tissue are still unknown. *In vitro* and experimental evidence suggests that AGEs-RAGE interactions can attenuate insulin sensitivity in adipocytes.[45,46] In an *in vitro* study conducted in 3T3-L1 adipocytes, the presence of AGEs inhibited glucose uptake both in the presence and absence of insulin as well as increased the generation of intracellular reactive oxygen species (ROS) and the expression of monocyte-1 chemoattractive protein (MCP-1).[45]

Monden and colleagues[46] demonstrated that increased RAGE expression is associated with adipocyte hypertrophy, suppression of glucose transporter type 4 (GLUT-4), attenuation of insulin-stimulated glucose uptake, and reduction of IRS-1 phosphorylation. The authors confirmed their results in cells and rats by demonstrating that RAGE deficiency is associated with obesity resistance, increased expression of GLUT-4 and adiponectins, and decreased expression of MCP-1, resulting in increased insulin sensitivity in adipose tissue.[46]

The results of these studies suggest that AGEs-RAGE interactions in adipocytes inhibit glucose uptake through increased ROS, cytokines and other inflammatory molecules production as well as decreased phosphorylation of IRS-1, thereby inhibiting the PI3K-AKT pathway.[45–49]

Thus, based on the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway associated with the *in vitro* and experimental evidence,[45,46] the authors hypothesize that interactions between AGEs-RAGE may be involved in insulin resistance in adipose tissues through insulin signaling pathway downregulation (PI3K-AKT) (Figure 4).

Finally, due to the use of thermal processes by the food industry, the increased consumption of processed foods in the last 50 years has favored a greater consumption of dietary AGEs. Therefore, it is hypothesized that this dietary pattern change probably contributed to an increase in obesity, oxidative stress and inflammation, which can explain an increase in the manifestation of overweight-associated complications (diabetes mellitus, cardiometabolic disorders, and renal diseases) due to higher AGEs consumption.[35] Thus, this review provides new insights

into the role of dietary AGEs in the pathogenesis of obesity and associated complications.

AGEs and Chronic Diseases

Once host defenses are compromised and increased oxidative stress occurs, AGEs-RAGE may increase and perpetuate the inflammation condition, leading to obesity, diabetes mellitus and cardiovascular and kidney diseases.[42,50] The consumption of an AGE-s rich diet appears to lead to pathological consequences, such as weight gain, obesity and, consequently, metabolic syndrome.[51–53]

The kidney is the major organ for AGEs detoxification, both by filtration and active secretion and absorption, two processes that result in the net excretion of urine AGEs.[30,54] The kidneys are directly exposed to a greater circulating AGEs concentration than many other organs, a fact that may make them vulnerable to circulating lesions of reactive carbonyls and ROS.[55] A diet rich in AGEs can lead to renal damage by inducing proteinuria and/or high formation of pro-fibrotic transforming growth factor β1 (TGF-β1), accelerating atherosclerosis (through lipid peroxidation).[51,53] It has been shown that chronic ingestion of high-AGEs foods may predispose the kidney to chronic injury in the absence of diabetes mellitus, suppressing local anti-AGEs defenses, and inducing oxidative stress and inflammatory responses.[31,32]

Furthermore, AGEs-RAGE interactions are active in pathogenic pathways involved in the development and progression of atherosclerosis. The endothelium neutralizes the effects of different chemical or physical stimuli to maintain homeostasis. When this balance is disturbed, the endothelium becomes susceptible to invasion by leukocytes and lipids, representing the key steps in the formation of atherosclerotic plaque. On the other hand, the bioavailability and activity of the endothelium-derived vasodilator, nitric oxide (NO), has been shown to be reduced by AGEs.[56] The role of AGEs in endothelial dysfunction was verified in type 2 diabetics, in which serum AGEs were negatively associated with the degree of endothelium-independent vasodilatation.[57] Some mechanisms have been suggested to explain these associations. One of these is the induction of AGE-s related oxidative stress and NO inactivity.[58] In addition, eNOS can be used to reduce the activity of endothelial NOS (eNOS) through receptor-mediated phosphorylation of serine residues in eNOS and to increase the degradation of eNOS mRNA.[59] Additionally, AGEs may impair endothelial

balance by reducing the endothelial prostacyclin (PGI2) production and increasing endothelin-1 expression.[60]

Regarding the association between AGEs and diabetes, the consumption of an AGE-s rich diet for 6 months increased body weight, visceral fat and 8-isoprostane and decreased insulin and adiponectin sensitivity, leading to diabetes development in animals.[51] In another study, T cell activation occurred, increasing oxidative stress that led to pancreatic β-cell injury. Therefore, high AGEs consumption favors the occurrence of insulin resistance, visceral obesity, diabetes and metabolic syndrome.[61]

LIMITATIONS

Papers included in this review present strong points. Calorie and macronutrient consumption were controlled in both the test and control groups, decreasing the risk of biases. However, the papers also had several limitations: (1) the duration of the interventions varied among the studies, which may have been insufficient to obtain a real conclusion about the effects of AGEs on health outcomes; (2) the variables analyzed were different among the studies; (3) none of the studies presented the actual dietary AGEs content, only the mean value tested; and (4) the AGEs units used were different among the studies. All these factors make it difficult for us to compare the results obtained by the studies and to state recommendations about AGEs consumption.

Several AGEs are formed in food during preparation processes. However, it is difficult to quantify the total AGEs content since there is not a standard quantification method. Although we know the types of foods that may contain the highest AGEs content and the food processing methods that are capable of increasing the AGEs content, we cannot be sure of the type of foods that contribute the most to total dietary AGEs consumption.

CONCLUSIONS

The consumption of low-AGEs diets reduced the concentration of circulating and urinary excretion of AGEs markers, improved metabolic syndrome markers (reduced plasma triglycerides, blood glucose, fasting insulin and HOMA-IR, besides increasing HDL), reduced anthropometric variables (body weight, waist circumference, waist-hip ratio, BMI), urinary albumin/creatinine ratios, but increased the estimated glomerular filtration rate. On the other hand, high AGEs diets reduced plasma CML,

increased urinary CML and postprandial plasma glucose peak, increased the concentration of urinary oxidative stress (8-isoprostans and F2-isoprostanes), besides negatively affecting inflammation (increased monocytic chemotactic protein-1 (MCP-1)), renal function (plasma cystatin C (12) and cardiovascular diseases (vascular cell adhesion molecule-1 (marker of endothelial activation) markers.

The results of the chronic studies selected for this review indicate that AGEs dietary intake from 3302 to 7306 kU/day and from 10.7 to 43 mg/day for 2 to 12 weeks positively affected the concentration of markers related to overweight complications. However, the uncertain contradictions related to dosage of AGEs in food makes it difficult to establish how much AGEs would be safe to consume. Therefore, reducing the consumption of processed foods and changing food preparation methods are good strategies to promote health.

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Table 1. PICOS criteria for inclusion of studies

Parameter	Inclusion criteria
Participants	Overweight and obese adults
Intervention or exposure	Consumption of a low AGEs diet
Comparison	Consumption of a high AGEs diet
Outcome	Circulating and urinary AGEs; cardiometabolic, inflammatory, glycemic, renal and anthropometric markers
Study design	Clinical trials

Abbreviation: AGEs: Advanced glycation end products.

Table 2. Studies in which the effect of reducing dietary advanced glycation end products on overweight and obesity associated complications was assessed.

Reference	Sample	Intervention	Study Design	Duration	Main results
Harcourt et al. (2011)[13]	11 healthy overweight and obese men 30±9 years old BMI: 31.8±4.8 kg/m^2	Test: Low AGEs diet (3,302 kU / day) Control: High AGEs diet (14,090 kU /day)	Randomized, controlled and crossed	2 weeks (4 weeks washout)	Test group: ↓ urinary albumin/creatinine ratios Control group: ↑ plasma cystatin C, ↓ plasma CML, ↑ urinary CML, ↑ urinary 8-isoprostantes, ↑ plasma MCP-1, ↓plasma MIF
Poulsen et al. (2014)[14]	19 healthy overweight and obese individuals 34.8 ± 10.0 years old Sex: 16 women and 3 men BMI: 31.3 ± 5.1 kg/m^2	Test: Low AGEs diet (2.8 mg / meal) Control: High AGEs diet (5.0 mg / meal)	Randomized, single blind, controlled and crossover	1 day (2 weeks washout)	Test group: ↓ plasma ghrelin after intervention □ GLP-1 e PYY compared to the control group Control group: ↑ peak of postprandial plasma glucose; plasma VCAM-1; urinary F2-isoprostane after intervention compared to the test group

Mark et al. (2014)[15]	73 healthy overweight and obese women 39.6±1.4 years old BMI: 32.7±0.7 kg/m^2	Test: Low AGEs diet (10.7 mg/day) Control: High AGEs diet (24.6 mg/day)	Randomized, double-blind, controlled and parallel	4 weeks	Test group: ↓ weight, BMI, WP, waist-hip ratio, urinary excretion of CML and MG-H1, 2h glucose, fasting insulin, HOMA-IR and ↑ $S_{i0,120}$ after intervention in comparison to the control group
Macías-Cervantes et al. (2015)[16]	29 healthy overweight and obese men 43.9±6.2 years old BMI: 28.6±2.0 kg/m²	Test: Low AGEs diet (7,306 ± 2,811 kU/day) Control: habitual food intake (11,223 ± 4,147 kU/day)	Randomized, controlled and parallel	12 weeks	Both groups: ↓ weight, BMI e WP Test group: ↓ triglycerides and circulating AGEs (CML e MG) e ↑ HDL-c
Courten et al. (2016)[17]	20 healthy overweight and obese individuals 34.0±10.0 years old	Test: Low AGEs diet (43 (36–51) mg/day)	Randomized, double-blind, controlled and crossed	2 weeks (4 weeks washout)	Test group: Sensitivity to insulin, MG-H1 and urinary CEL eGFR Serum concentrations of CML, MG-H1, CEL, sRAGE, urinary CML, body weight and insulin secretion between diets

	Sex: 6 women and 14 men BMI: 31.3±3.8 kg/m²	Control: High AGEs diet (59 (49–68) mg/day)			
Baye et al. (2017)[18]	20 healthy overweight and obese individuals 34.0±10.0 years old Sex: 6 women and 14 men BMI: 31.3±3.8 kg/m²	Test: Low AGEs diet (43 (36–51) mg/day) Control: High AGEs diet (59 (49–68) mg/day)	Randomized, double-blind, controlled and crossed	2 weeks (4 weeks washout)	Test group: SBP, DBP, MAP e PP, TC, LDL, HDL, triglycerides, IL-6, MCP-1, TNF-α, PCR, NFκB compared to the control

Abbreviations: AGEs: Advanced glycation end products; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; PP: pulse pressure; TC: total cholesterol; LDL: low density lipoprotein; HDL: high density lipoprotein; IL-6: interleukin -6; MCP-1: monocytic chemotactic protein -1; TNF-α: tumor necrosis factor α; CRP: C-reactive protein; NFκB: nuclear factor kappa B; BMI: body mass index; WC: waist circumference; CEL: N€ -(carboxyethyl)lysin; MG-H1: methylglyoxal-derived hydroimadazolidine; CML: N€ -(carboxymethyl)lysine; sRAGE: soluble receptor for advanced glycation end product; $S_{i0,120}$: insulin sensitivity index; VCAM-1: vascular cell adhesion molecule-1; ICAM-1: Intracellular adhesion molecule-1; HOMA-IR: Homeostasis model assessment of insulin resistence; MIF: macrophage migration inhibitoy factor; eGFR: estimated glomerular filtration rate; GLP-1: glucagonlike-peptide-1; PYY: peptide YY; Unchanged.

Table 3. Factors that influence food AGEs content.

Food characteristics		**Cooking methods**	
High-AGEs	**Low-AGEs**	**High-AGEs**	**Low-AGEs**
Processed food	Grains	High-temperature	Low-temperature
Meat group	Vegetables	Grilling	Steaming
Fat group	Fruits	Searing	Stewing
Bakery products	Fat free dairy	Roasting	Boiling
Dairy	Soups	Frying	Poaching
		Low-humidity	High-humidity
		Alkaline pH	Low-pH

AGEs: Advanced glycation end products
Source: Uribarri et al. (2010)[21]; Barbosa et al. (2016)[22]; Tessier and Birlouez-Aragon (2012)[23]

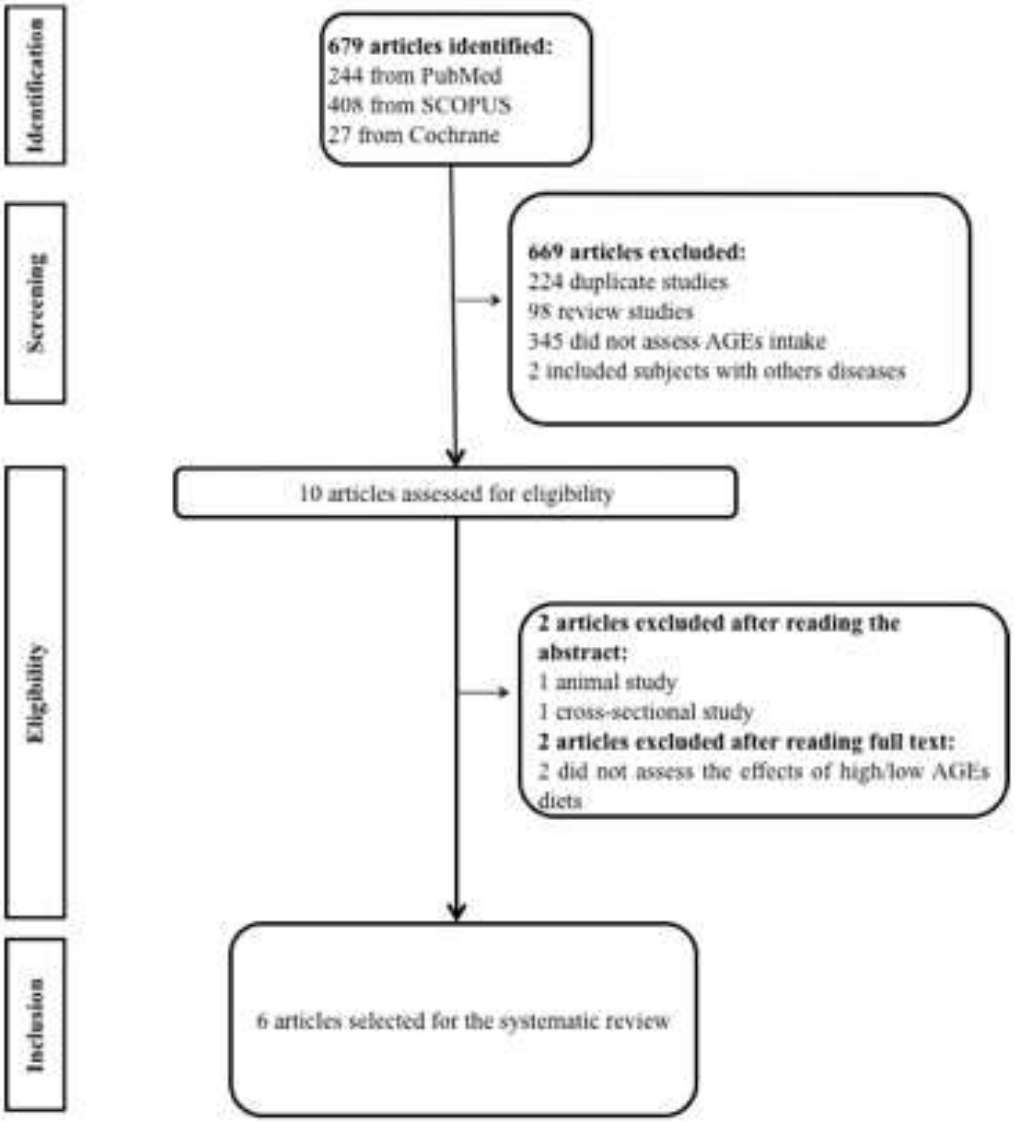

Figure 1. Flowchart of the studies selection process.

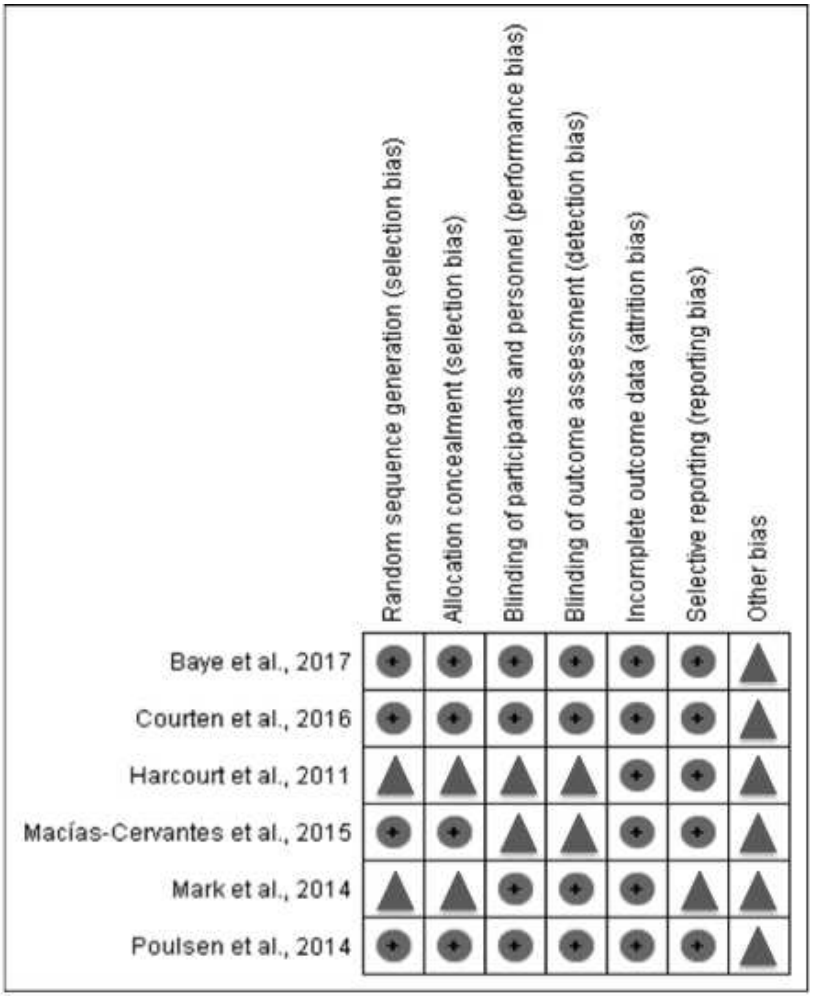

Figure 2. Risk of bias summary: review authors' judgments about each risk of bias item for each included study.
Legend: Circle: low risk; Triangle: unclear.

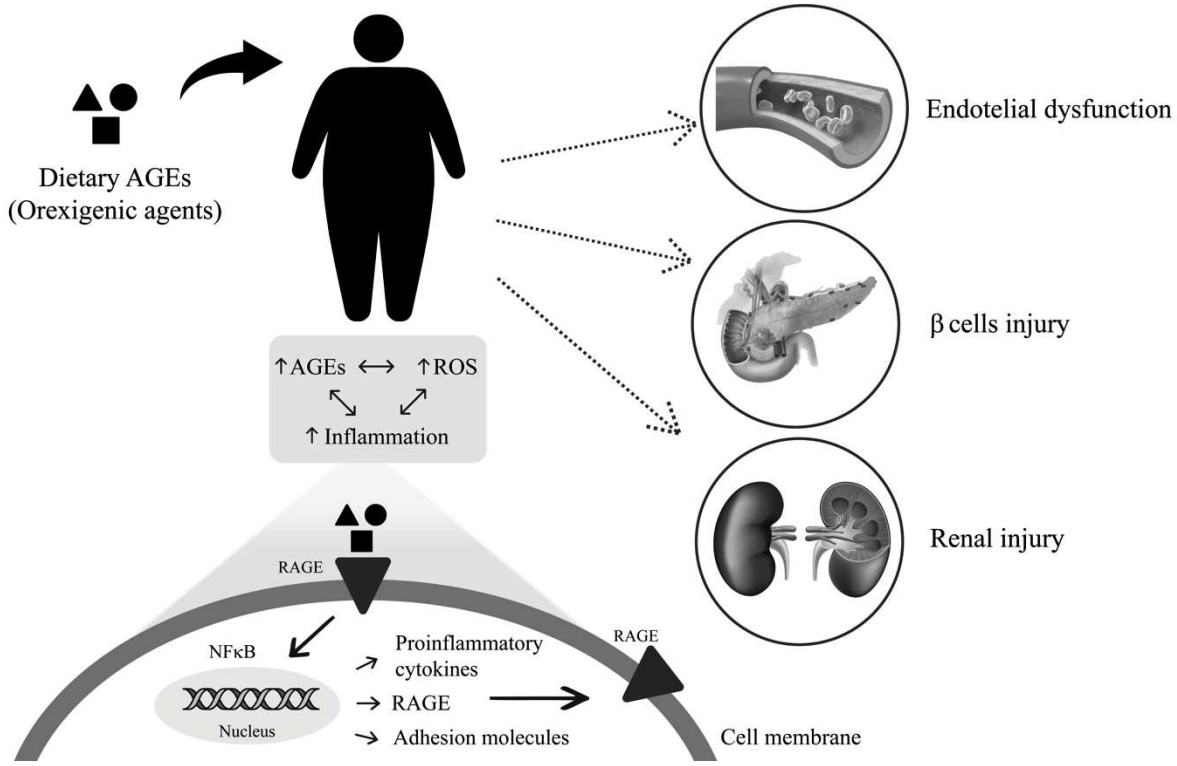

Figure 3. Mechanism related to the effect of dietary AGEs in overweight complications, through the increase of RAGE expression and activation of the NF-κB signaling pathway.
Legend: AGEs: advanced glycation end products; ROS: reactive oxygen species; RAGE: receptor for advanced glycation end product.

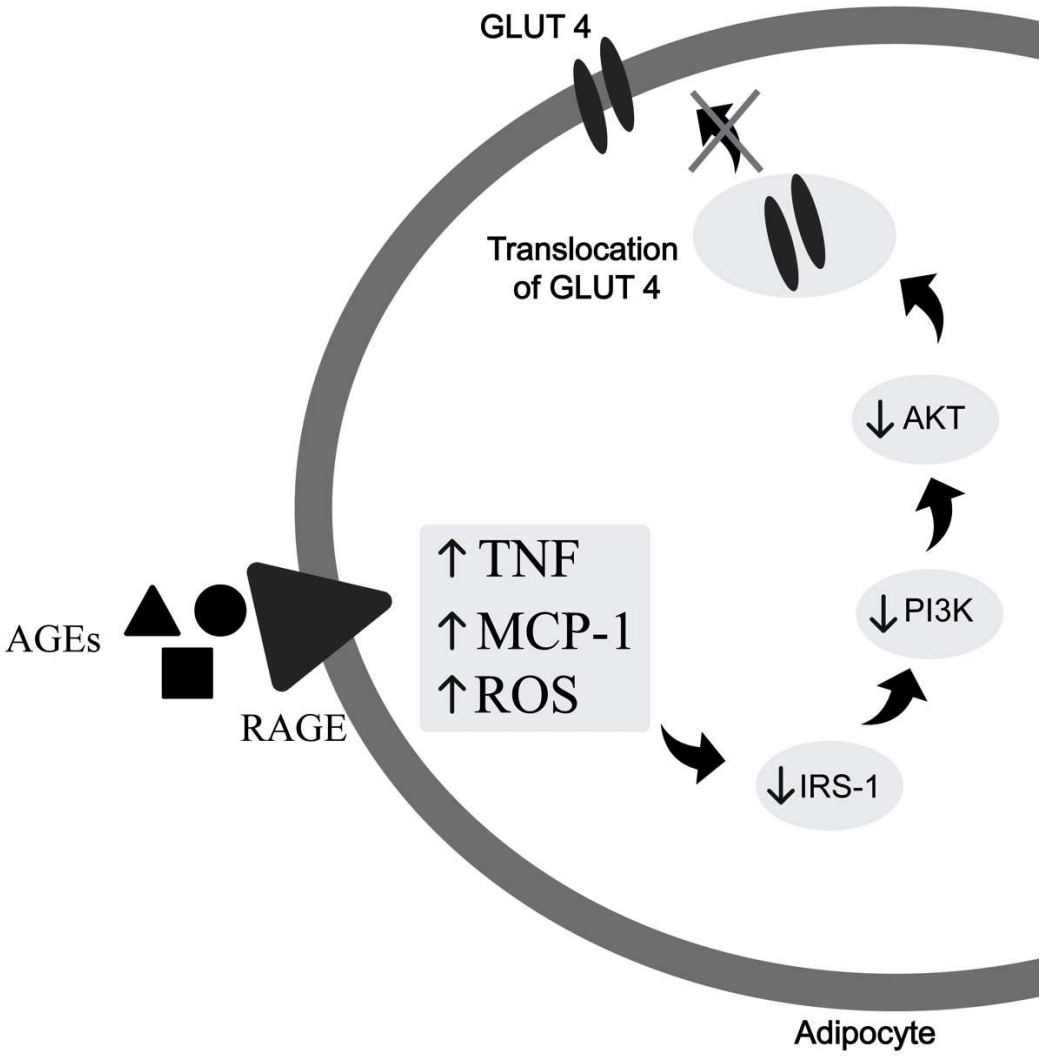

Figure 4. Proposed AGEs-RAGE interaction mechanism associated with increased oxidative stress and inflammation, inducing insulin resistance via PI3K-AKT signaling pathway in adipocyte.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Yes
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Pg. 1 - 2 Yes
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Pg. 3 Yes
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Pg. 3 Yes
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Pg. 4 Yes
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Pg. 4 - 5 Yes
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Pg. 4 Yes
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Pg. 4 Yes
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Pg. 5 Yes
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Pg. 5 Yes
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Pg. 5 Yes
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Pg. 5 - 6 Yes

Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Not applicable
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Not applicable
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Pg. 5 - 6 Yes
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Not applicable
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Pg. 6 and 26 Yes
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Pg. 6 - 9 Yes
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Pg. 9 Yes
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Pg. 7 -9 Yes
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Not applicable
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Pg. 9 Yes
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Not applicable
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Pg. 9 - 12 Yes

Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Pg. 15 - 16 Yes
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Pg. 16 - 17 Yes
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Pg. 17 Yes

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ARTIGO 2

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Effect of the consumption of yacon flour and energy-restricted diet on glycation markers and association between these markers with factors linked to obesity in adults with excess body weight: a randomized, double-blind, placebo-controlled clinical trial

Abstract

Objectives: Regardless of the positive effect of yacon on metabolic markers, this food contains fructose molecules, which can originate advanced glycation end products (AGEs). High AGEs serum concentrations can contribute to excess body weight. We evaluated the effect of yacon flour consumption and energy-restricted diet on glycation markers concentrations, and the association between these markers and factors linked to obesity in adults with excess body weight. Methods: Twenty-six adults with excess body weight were included in this randomized, parallel, double-blind, placebo-controlled, six-week clinical trial. Subjects were randomly allocated into the control group (n=13) or the yacon flour group (n=13), and daily consumed a breakfast drink, not containing or containing 25 g of yacon flour (8.7 g of FOS). Energy-restricted diets were prescribed for both groups. Biochemical markers, anthropometric variables, and body composition were evaluated at baseline and the end of the study. Results: AGEs and early glycation products did not increase in the yacon flour group. sRAGE decreased, regardless of the group. Besides, changes in AGEswere positively associated with changes in body fat ($\beta = 0.04$; $p = 0.038$) and, in sRAGE, with insulin ($\beta = 0.02$; $p = 0.035$) and HOMA-IR ($\beta = 0.01$; $p = 0.049$). Conclusions: The consumption of 25g of yacon flour associated with energy-restricted diet did not increase the concentrations of glycation markers. Changes in glycation markers were positively associated with changes consolidated anthropometric and biochemical markers related to overweight. Assessing glycation markers may be a useful strategy for monitoring dietary interventions responses in subjects with excess body weight.

Keywords: Advanced glycation end products; amadori products; fructooligosaccharides; overweight; sRAGE

Trial registration number and date of registration: RBR-6YH6BQ (01/23/2018)

1. INTRODUCTION

Abnormal or excessive accumulation of fat, in people with overweight, constitutes a risk factor for the development of several non-communicable chronic diseases [1,2]. The prevalence of excess body weight has increased and has become a major public health problem in many countries [3]. For that reason, there is a great interest of the cientific community to identify strategies capable of preventing or controlling excess body weight occurrence.

Advanced glycation end products (AGEs) seem to contribute to excess body weight and other chronic diseases manifestation [4–9]. AGEs can be produced endogenously through the Maillard non-enzymatic reaction between a reducing sugar and protein, lipid or nucleic acids [4,10]. Although AGEs concentrations may increase in the presence of hyperglycemia and oxidative stress [4,11,12], these compounds are also found in food [4].

The contribution of AGEs to excess body weight has mainly been attributed to their ability to bind to the AGEs receptor (RAGE), activating signaling pathways that activate inflammatory processes dependent on the nuclear factor kappa B (NF-kB), causing an increase in pro-inflammatory cytokines, adhesion molecules and the expression of RAGE itself [13,14], suggesting the occurrence of a positive inflammatory feedback. Furthermore, AGEs-RAGE interaction increases the formation of reactive oxygen species (ROS) and AGEs can also lead to loss of protein function after chemical modification [4]. On the other hand, the soluble AGEs receptor (sRAGE) has the same AGEs binding specificity as RAGE. Therefore, sRAGE can bind to AGEs, preventing the activation of the inflammation signaling cascade, and the occurence of oxidative stress [15,16].

Therefore, the adoption of strategies to manage excess body weight, such as lifestyle changes, consumption of energy-restricted diets [17], and the inclusion of foods capable of improving body composition and metabolic markers concentration may play a role in reducing AGEs concentrations. Thus, yacon (*Smallanthus sonchifolius*) is a herbaceous plant native to the Andean region of South America, and a rich source of fructooligosaccharides (FOS) and phenolic compounds, particularly chlorogenic acid [18,19]. Apparently, due to its low caloric value and high fiber content,

yacon is a promising food supplement that can be used to prevent and treat several chronic diseases, such as diabetes and obesity [20]. However, yacon contains FOS [18,21], which has short linear chains of fructose molecules [22] that can in turn originate methylglyoxal, a precursor of AGEs [4]. Therefore, the effect of increased yacon consumption on AGEs concentrations is not known.

In a study conducted by our research group, we observed that yacon flour consumption (25g-0.1g FOS/kg) associated with energy-restricted diet resulted in greater weight loss and improved body composition in individuals with excess body weight [23]. No human study has investigated the role of yacon consumption on glycation markers concentrations. Therefore, in the present study we evaluated how the consumption of yacon flour and energy-restricted diet affect the glycation markers concentrations and we also investigated the association between these markers and factors linked to obesity in adults with excess body weight.

2. SUBJECTS AND METHODS

2.1 Subjects

Eligible subjects were adults (20-59 years old) of both sexes who were excess body weight (body mass index (BMI) between 25 and 34.9 kg/m²) [24], regularly consumed breakfast, had a mild physical activity level, had food restriction/disinhibition ≤14 [25], were not diabetic and did not have a family history of diabetes or glucose intolerance.

Non-inclusion criteria were: smokers; consumption of more than two doses of alcohol /day (> 50g of ethanol/day); use of medications that affect blood glucose or energy metabolism; use of drugs, herbs or diets to reduce appetite and body weight; gain or loss of at least 5 kg three months prior to the beginning of the study; recent change in physical activity level; aversion or intolerance to the foods provided in the study; existence or history of endocrine, cardiovascular, arterial hypertension, liver and/or gastrointestinal diseases; eating disorders occurence; pregnant or lactating women; use of laxatives or antibiotics three months prior to the beginning of the study; use of probiotics, prebiotics or symbiotics, and women with menstrual irregularity three months before the beginning of the study.

The study protocol was approved by the Universidade Federal de Viçosa, Brazil Human Research Ethics Committee (nº 1.875.372). Subjects signed the informed consent form according to the recommendations of the Declaration of Helsinki [26]. The trial is registered in the Brazilian Registry of Clinical Trials (ReBEC) http://www.ensaiosclinicos.gov.br / (identifier: RBR-6YH6BQ).

2.2 Study design

This was a double-blind, randomized, parallel, six-week clinical trial. Participants were randomly allocated into the yacon flour group or the control group in the proportion of 1:1. Block randomization technique, used to allocate the subjects into the groups, was applied by a person who was not part of the research group. Energy-restricted diets were prescribed (- 500 kcal/day) [27], considering the nutritional composition of the drinks provided during the study. The prescribed diets had a similar content of macronutrients, according to the Acceptable Macronutrient Distribution Range [28]. Subjects did not receive any guidance on how to prepare the foods consumed during the study so that they would not be induced to change their AGEs consumption.

Subjects daily consumed in the laboratory 350ml of a drink breakfast containing 25 g of yacon flour (13.72 ± 3.97 g of total fibers and 8.7 g FOS) or not containing yacon flour (2.63 ± 3.91g of total fibers and 0g of FOS). On weekends, subjects received the ingredients of the drink to be consumed at home. The investigators assessed the protocol adherence. The full description of the nutritional composition of these drinks and the chemical characterization of yacon flour is described in Machado et al. (2019) [23]. All the other meals were consumed under free-living conditions. Participants were instructed to maintain the level of physical activity during the study.

Body composition, anthropometry, and biochemical variables; systolic and diastolic blood pressure; and glycation markers (AGEs, early glycation products - EGPs, and sRAGE) were assessed at baseline and after six weeks in each experimental group.

2.3 Breakfast drinks

Drinks with and without yacon flour had similar macronutrients and energy contents, differing only in total dietary fiber and FOS contents [23]. The ingredients used to prepare the drinks were the same, except for the addition of yacon flour (yacon

group) or not (control group). Yacon flour was replaced by corn starch in the control group drinks so that they would be nutritionally similar to those in the yacon group. These drinks also contained fruit pulp, cocoa powder or instant coffee, whole milk powder, sugar, oil, and water. The amount of yacon flour added to the drinks (25g) was based on other studies, considering the occurrence of possible undesirable gastrointestinal effects [29–31]. Daily intake of yacon flour was well tolerated, causing no adverse gastrointestinal effects.

2.4 Anthropometrics, body composition, and blood pressure measurements

Body weight was assessed using an electronic platform scale (Toledo®, Model 2096PP/2, SP, Brazil) with a capacity of 150 kg and an accuracy of 50 g. Height was measured using a stadiometer with a scale of 0–220 cm, precision 0.1 cm (Wiso, Chapecó, SC, Brazil). BMI was calculated by dividing body weight by height squared. Waist and hip circumferences were measured using a flexible inelastic tape. Waist circumference was measured at the smallest circumference, and hip circumference was measured at the highest prominence between the anterior iliac crest and the largest trochanter.

Body fat and fat-free mass were assessed using the Dual-energy X-ray absorptiometry scan (model Prodigy Advance, GE Healthcare Inc., Waukesha, WI). Blood pressure was assessed in both arms, using an automatic Omron HEM-7200 device (Omron Inc., Dalian, China), in duplicate [32].

2.5 Biochemical analyses

Venous blood samples were obtained after 12 h overnight fasting. Glucose, triglycerides, total cholesterol, and HDL-c concentrations were measured using a colorimetric assay (kit Bioclin, Quibasa Basic Chemical Ltda). LDL-c concentration was calculated using the following equation: LDL-c = total cholesterol - HDL-c - (triglycerides/5) [33]. Insulin was determined by chemiluminescent immunoassay (Access Ultrasensitive Insulin). Insulin resistance was calculated using the homeostasis model assessment index of insulin resistance (HOMA-IR) [34].

2.6 Glycation markers analyses

Serum concentrations of AGEs were assessed by fluorescence spectroscopy (λemission=460 nm, λexcitation=370 nm, SpectraMax M2e, SoftMax®Pro software)

using 50 µL of serum [35]. The AGEs content of the samples was corrected by the amount of protein [36].

The detection of early glycation products (EGPs) (glycated hemoglobin, glycated albumin, fructosyl-lyine, furosine and others glycated plasma proteins) was made to verify the reduction of tetrazolium nitroazul (NBT) [37]. In the NBT test, 20 µL of sample was mixed with 200 µL of NBT solution (100 mM sodium carbonate buffer (pH 10.8) containing 0.25 mM NBT). The glycation reaction was incubated at 37ºC for 30 minutes, and the reading was performed on a spectrophotometer (absorbance 525nm) (SpectraMax M2e) [35]. That method identifies EGPs concentrations as low as 0.04mM, with 2.45 and 0.74% coefficient of variation for repeatability and reproducibility [38].

sRAGE concentrations were determined using a commercial ELISA kit (Human RAGE Sigma-Aldrich®) specific for human sRAGE (intra-assay and inter-assay reproducibility CV of <10% and <12%, respectively). Duplicate samples and absorbed sRAGE standards were read at 450 nm (SpectraMax M2e, SoftMax®Pro software).

2.7 Statistical analysis

The present study had 81% statistical power, considering the AGEs concentrations after six weeks of intervention, with a 95% confidence interval, type I error $\alpha = 0.05$ and a type II error $\beta = 0.2$ [39–41]. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS), version 23.0 (SPSS, Inc., Chicago, IL, USA). Shapiro-Wilk test was used to assess data distribution normality. Student's t-test or Mann-Whitney U was used to identify differences in the deltas of the variables presented by the subjects in the yacon flour group and in the control group.

The effect of the intervention was assessed by comparing the glycation markers between groups before and after 6 weeks of intervention, using the generalized estimation equation (GEE) model. For variables with normal distribution, a identity link function was used. Gamma distribution with a log link was used for variables that did not present normal distribution. Unstructured covariance matrix was used as work correlation matrix. Bonferroni post-hoc was used to identify the differences in group, time, and group*time interaction.

Pearson or Spearman correlation was performed according to the variables distribution to assess the correlation between delta glycation markers versus delta anthropometric, body composition and biochemicals markers within experimental

groups. In addition, the correlations between delta sRAGE and delta biochemical markers presented in both groups.

Multiple linear regression was also performed considering delta glycation markers as independent variable and delta anthropometric/biochemical markers as dependent variables. A model was estimated for each independent variable (glycation markers) and adjustments were made for the values of the independent and dependent variable at baseline.

A significance level of 5% was addoped in all analysis.

3. RESULTS

Twenty-six subjects (11 men and 15 women, BMI of 30.44 ± 2.46 kg / m², total body fat of 40.16 ± 6.71% and 31.35 ± 8.54 years old) were included in this study. The CONSORT participants selection flow diagram was published earlier [23]. After six weeks of intervention, changes in body weight and BMI in the yacon group were greater than in the control group (Table 1). After six weeks of intervention, the daily consumption of dietary fiber corresponded to 25.5 ± 1.4g in the control group and 38.8 ± 1.9g in the yacon group ($p<0.05$).

AGEs and EGPs concentrations were not affected by the intervention in both experimental groups. On the other hand, sRAGE concentrations reduced after the intervention (baseline *versus* six weeks) in both experimental groups (Table 2).

Changes in AGEs concentrations were positively correlated with changes in BMI, waist circumference, gynoid body fat, total body fat and serum triglycerides in the yacon flour group. In addition, in the control group, changes in waist circumference and fat-free mass positively correlated with changes in AGEs concentrations (Table 3). When we evaluated the association between deltas anthropometric, body composition and biochemical markers (dependent variable) and delta AGEs concentrations (independent variable) by regression analysis in the yacon group, we observed that delta total body fat was positively associated with delta AGEs after adjusting for variables at baseline ($\beta = 0.04$; $p = 0.038$) (data not shown).

A positive correlation was verified between changes the concentrations of sRAGE and changes in insulin, HOMA-IR, and triglycerides considering the data from both groups (Figure 1). When assessing the association between delta biochemical markers (dependent variable) and delta sRAGE concentrations (independent variable)

by regression analysis, we observed that delta fasting insulin and HOMA-IR was positively associated with delta sRAGE after adjusting for baseline variables (β = 0.02; p = 0.035 and β = 0.01; p = 0.049, respectively) also considering the data from both groups (data not shown).

4. DISCUSSION

To our knowledge, this is the first study to investigate the effect of yacon flour consumption (*Smallanthus sonchifolius*) associated with an energy-restricted diet on the glycation markers serum concentrations and factors linked to obesity in adults with excess body weight. Daily consumption of 25g of yacon flour (8.7 g FOS) for six weeks did not increase AGEs and EGPs (early glycation products) concentrations. About 70-80% of yacon's dry weight is FOS [18,42], which are fructans made up of short linear chains of fructose molecules [22]. Methylglyoxal, a precursor of AGEs, is partly originated from fructose metabolism [4]. Therefore, we did not know if the ingestion of yacon flour could increase AGEs production.

In a study conducted out by our group, we observed that yacon flour consumption resulted in greater reduction in gynoid fat and anthropometric measurements (body weight, waist circumference, sagittal abdominal diameter and waist/height index) compared with the control group [23]. We believe that although yacon flour (FOS) may have caused an increase in AGEs concentrations, the observed reduction in anthropometrics may have caused a reduction in such concentrations. We also believe that weight loss due to the consumption of FOS may have resulted in greater satiety, reducing food intake and dietary AGEs, thus reducing the pool of energy substrate for endogenous AGEs formation. Besides, it is possible that the reduction in visceral adipose tissue observed in our previous study [23] may have led to a lower expression of RAGE, consequently resulting in less AGEs-RAGE bindings [43]. Thus, such opposite effects may have been responsible for the fact that AGEs and EGPs concentrations did not increase.

The consumption of an energy-restricted diet reduced AGEs concentrations in two three month randomized clinical trials [44,45] and in a prospective cohort in individuals with overweight [46]. The divergence in these results and the one verified in the present study may be due to the differences in the duration of the studies, type of intervention applied and method used to assess AGEs concentrations. The percentage of weight loss observed in these studies was 4.3% [44] and 6.7% [46], and

it corresponded to 2.9% in our previous study [23]. Although our study lasted for 1,5 month (six weeks), the duration of these studies corresponded to two [46] and three [44,45] months. Therefore, it is not known whether the consumption of yacon flour associated with an energy-restricted diet for more than six weeks would have affected the response we obtained in our study. If our study had been conducted for a longer period of time, we probably would have seen a higher weight loss, which in turn would stimulate a significant reduction in AGEs concentrations. We must also point out that the technique used to quantify AGEs concentrations differed between studies. While we and Gugliucci et al. (2009) [46] used a technique to quantify total fluorescent AGEs, Rodríguez et al. (2015) [44] assessed only εN-carboxymethyllysine (CML) concentrations by enzyme-linked immunosorbent assay (ELISA) and Deo et al. (2017) [45] evaluated CML a type of AGEs, using HPLC.

Moreover, in this study, we obtained positive correlations between delta AGEs concentrations *versus* delta anthropometric (waist circumference, BMI), body fat (gynoid body fat, total body fat) and biochemical (trygliceridemia) markers in the yacon flour group. Subjects of that group also presented greater weight loss and body composition markers reductions [23]. These results are interesting since AGEs are seen as new biomarkers, and it is relevant that they correlate with such well established obesity-related markers. In another study, the authors observed that reductions in AGEs concentrations were also positively correlated with triglycerides concentrations, waist circumference, and BMI in overweight subjects submitted to energy-restricted diet [35], as evidenced in the present study. Our correlation data and those verified by Gugliucci et al. (2009) [46] suggest that visceral and subcutaneous adipose tissue (represented by waist circumference, BMI, gynoid and total body fat) loss are related to reductions in AGEs concentrations, suggesting that these tissues may be important in regulating AGEs production, playing a role in the AGEs-RAGE axis mechanism.

In adipose tissue cells from subjects with obesity, there was an accumulation of carboxymethylisin, a type of AGEs, and higher RAGE expression compared with the adipose tissue from eutrophic subjects. RAGE overexpression was higher in visceral adipose tissue than in subcutaneous adipose tissue. Thus, the higher AGEs concentration and the activation of inflammatory pathways are mechanisms involved in adipokine dysregulation, contributing to insulin resistance in obesity [8]. In fact, visceral adipose tissue is an inflammatory fat deposit associated with increased risk of

metabolic disease, AGEs accumulation, and RAGE overexpression, which are involved in metabolic dysfunction.

We also observed a reduction in sRAGE in both experimental groups and a positive correlation between delta sRAGE and delta triglycerides, delta insulinemia and delta HOMA-IR. Norata et al. (2008) showed an inverse association between sRAGE concentrations *versus* BMI, waist/hip ratio, and fasting blood glucose in excess body weight healthy subjects [47]. The divergence in this results are probably due to the characterists presented by the interventions. While our subjects consumed yacon flour and energy-restricted diet, in the study conducted by Norata et al. (2008) no intervention was applied. sRAGE has been considered a new useful biomarker for chronic diseases diagnosis and prognosis [48]. Apparently, sRAGE acts as a RAGE endogenous inhibitor, since it binds to circulating AGEs, and inhibits tissue damages [49]. However, sRAGE protective effect and the mechanisms that regulate its concentrations are still under debate. In an epidemiological study conducted for four years, a positive correlation was observed between AGEs and sRAGE concentrations in 184 non-diabetic and normal weight subjects [50]. In a cross-sectional study involving 198 subjects with type 2 diabetes mellitus and high risk for vascular complications, a positive association was observed between sRAGE and peripheral neuropathy (a microvascular complication of diabetes) [51]. That indicates that sRAGE does not always have a protective role, since in the studies mentioned above there was an association between sRAGE and AGEs, which is know to exacerbate inflammation and oxidative stress [52], as well as sRAGE associated with diabetes complications [47]. Some author state that sRAGE is associated with inflammation [53–55]. The results of *in vitro* studies, suggest a new role for sRAGE on inflammation mediated by monocytes and neuthrophils survival and differenciation [56]. Therefore, future studies should be conducted to assess the actual role of sRAGE.

In subjects with chronic diseases, increased serum AGEs and RAGE expression is evidenced, due to increased oxidative stress and hyperglycemia. According to some authors, sRAGE concentrations may be a biomarker that can be used to reflect the deleterious effects of AGEs [51]. Increased tissue RAGE expression can result in increased sRAGE concentration to neutralize increased AGEs concentrations, thus preventing tissue damage [48,51]. However, the mechanism involved in sRAGE modulation is still not clear and should be further investigated. In the present study, sRAGE concentrations decreased despite AGEs concentrations

remained constant. That result suggests that the mechanism by which sRAGE modulates AGEs concentrations also needs to be futher explored.

Some authors observed body weight loss after energy-restricted diets and bariatric surgery, but different behaviors in terms of sRAGE concentrations [57–61]. Six months after the consumption of energy-restricted diets [57,58] and bariatric surgery [59] did not affect sRAGE concentrations in subjects with overweight. However, other authors observed a reduction in sRAGE concentrations after 12 months of bariatric surgery [60]. In another study, there was an increase in sRAGE concentrations after 24 months of bariatric surgery [61]. The results of these studies confirm that future studies should be conducted to clarify the effect of weight loss on sRAGE concentrations. However, we hypothesize that the discrepancies in the results of these studies may be due to diferences in the duration and the type of interventions applied (bariatric surgeries can lead to greater body weight loss than energy-restricted diets). Apparently, AGEs, RAGE, and sRAGE are three elements of a model in balance with positive and negative feedbacks that can be modified by different diseases [48]. However, the mechanisms that regulate their concentrations are still under debate, and little is known about the potential modulating role of diets.

Our study has several strengths. It was a randomized and controlled design study. We selected subjects without comorbidities, who were overweight or obese (not morbid obese) and who were not on pharmacological treatment. However, it is possible that the duration of our study was not long enough to result in signficant changes in glycation markers as we discussed earlier in this paper. We also quantified only serum glycation markers. Performing tissue analysis would have enabled us to evaluate RAGE expression, which would allow us to have a broader view of the effects resulting from weight loss in subjects with excess body weight. Besides, the assessment of serum AGEs concentrations do not reflect total AGEs concentration in the body. There is a fraction of AGEs that are linked to their receptors present in the tissue membranes, which may play a fundamental role in several chronic diseases pathogenesis [35]. However, since tissue analysis is more invasive, in our study we did not evaluate the fraction of AGEs linked to such receptors.

In conclusion, the consumption of 25g of yacon flour associated with energy-restricted diet for six weeks did not affect AGEs and EGPs concentrations. Changes in glycation markers concentrations positively associated with changes in consolidated anthropometric and biochemical markers related to excess body weight. These results

suggest that assessing the concentrations of glycation markers may be an useful strategy to monitore dietary interventions responses in subjects with excess body weight. Future long term studies (duration of at least two months) are needed to investigate the mechanisms that regulate the concentrations of these glycation markers and the effect of different types of foods in the concentrations of these markers.

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Tables

Table 1. Characteristics presented by the subjects at baseline, after the intervention (6 weeks) and changes from baseline (Δ) according to the experimental group

	Control group (n=13)			Yacon group (n=13)			
Variable, Units	**Baseline**	**6 weeks**	**Delta**	**Baseline**	**6 weeks**	**Delta**	**P_{inter}**
Weight, kg	84.93 ± 13.83	83.89 ± 13.54	-1.04 ± 1.69	88.52 ± 15.21	85.95 ± 14.67	-2.57 ± 1.74	0.032*
BMI, kg/m²	30.08 ± 2.04	29.72 ± 1.92	-0.37 ± 0.58	30.80 ± 2.85	29.91 ± 2.82	-0.89 ± 0.55	0.028*
Fat mass, kg	32.56 ± 5.41	31,51 ± 5,74	-1.22 ± 1.78	36.61 ± 8.15	33.39 ± 7,36	-2.08 ± 1.34	0.202
Percent fat, %	38.76 ± 5.96	38.07 ± 5.14	-0.96 ± 2.15	44.97 (29.31 – 49.78)	43.68 (26.55 – 49.20)	-1.29 ± 1.30	0.659
Waist circumference, cm	100.18 ± 8.19	88.66 ± 9.02	-2.66 ± 1.39	102.62 ± 9.38	88.13 ± 8.45	-3.69 ± 1.32	0.065
Fasting glucose, mg/dL	91.92 ± 5.27	90.92 ± 7.25	-1.00 ± 4.88	89.69 ± 6.91	90.54 ± 7.30	0.85 ± 5.89	0.393
Fasting Insulin, µUI/mL	9.29 ± 2.91	7.20 (4.50 – 19.40)	-0.96 ± 3.33	9.45 ± 2.78	9.10 (5.90 – 21.10)	0.05 ± 2.85	0.412
HOMA-IR, AU	2.11 ± 0.68	1.64 (0.99 – 4.79)	-0.21 ± 0.84	2.11 ± 0.72	1.95 (1.12 – 5.47)	0.06 ± 0.73	0.378
Triglycerides, mg/dL	87 (44 – 263)	94 (48 – 253)	-4.69 ± 37.02	106 (53 – 261)	112.23 ± 37.48	-13.15 ± 40.89	0.581
Cholesterol, mg/dL	189 (141 – 277)	192 (141 – 301)	-1.00 (-34.00 – 24.00)	178.85 ± 24.02	174.38 ± 27.53	-4.46 ± 22.80	0.670
HDL-cholesterol, mg/dL	50.38 ± 14.50	51.38 ± 12.82	1.00 ± 3.83	48 (33 – 86)	49 (35 – 85)	0.15 ± 5.96	0.585

LDL-cholesterol, mg/dL	106 (84 – 212)	104 (80 – 221)	-0.31 ± 15.22	104.92 ± 26.91	102.92 ± 30.40	-2.00 ± 15.36	0.780
Systolic blood pressure, mmHg	112.66 ± 8.43	111.92 ± 7.50	-2.04 ± 6.49	113.96 ± 11.03	110.42 ± 11.11	-2.25 ± 10.76	0.953
Diastolic blood pressure, mmHg	67.33 ± 7.17	66.54 ± 5.41	-2.77 ± 6.91	69.30 ± 7.86	62 (56 – 95)	-3.00 (-11.00 – 38.00)	0.979

Data are presented as means± standard deviation or median (interquartile range). Delta = value after 6 weeks - baseline value. BMI: body mass index, HOMA-IR: Homeostasis Model Assessment of Insulin Resistance, LDL-cholesterol: low-density lipoprotein, HDL-cholesterol: high-density lipoprotein. P*inter*: between groups of Δ values by Student's t test or Mann-Whitney U test, *p<0.05.

Table 2. Mean ± SD glycation markers concentrations at baseline and after 6 weeks of intervention, according to the experimental group

Variable, Units	Control (n=13)		Yacon flour (n=13)	
	Baseline	**6 weeks**	**Baseline**	**6 weeks**
AGEs, AU/mg	2.18 ± 0.31	2.20 ± 0.32	2.16 ± 0.36	2.05 ± 0.39
EGPs, AU	0.05 ± 0.02	0.05 ± 0.01	0.06 ± 0.03	0.05 ± 0.02
sRAGE, pg/ml	322.52 ± 122.78[a]	282.38 ± 106.95[b]	288.53 ± 104.16[a]	257.19 ± 81.36[b]

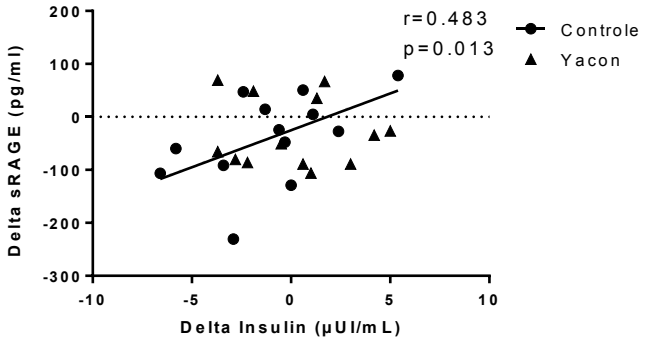
Values followed by different lowercase letters, in the same line, indicate intra-group difference by generalized estimating equation model (GEE), $p < 0.05$. AGEs: advanced glycation end products, EGPs: early glycation products, sRAGE: soluble receptors of advanced glycation end products.

Table 3. Correlations between delta AGEs concentrations *versus* delta anthropometric, body composition and biochemical markers, according to the experimental group

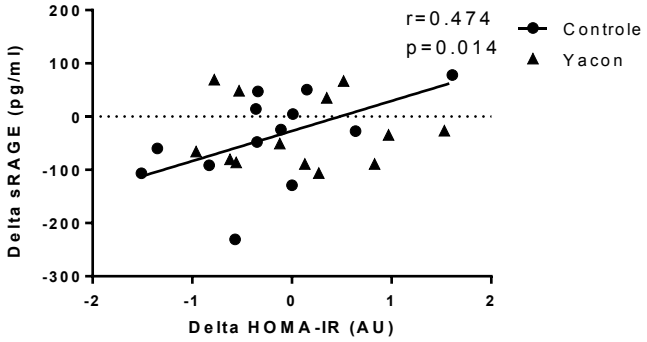
Variable, Units	Control (n=13)		Yacon flour (n=13)	
	r	p-value	r	p-value
ΔBMI, kg/m^2	0.496	0.085	0.557	**0.048**
ΔWaist circumference, cm	0.699	**0.008**	0.573	**0.041**
ΔGynoid body fat, kg	-0,103	0.777	0.691	**0.019**
ΔTotal body fat, kg	-0,271	0.420	0.680	**0.015**
ΔLean trunk mass, kg	0.645	**0.032**	0.168	0.602
ΔTriglycerides, mg/dL	0.554	0.050	0.560	**0.046**

Pearson or Spearman correlation according to variable distribution. Abbreviations: AGEs, advanced glycation end products; BMI, body mass index. Δ: delta (final – initial). Data in bold are p<0.05.

A



B



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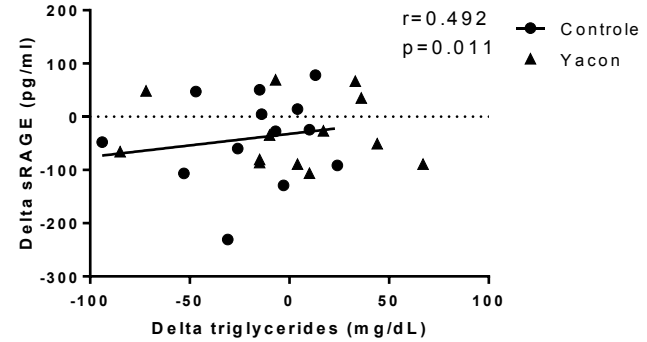

Figure 1. Pearson or Spearman correlations between delta sRAGE concentrations and delta biochemical markers concentrations (n=26). sRAGE: soluble receptors of advanced glycation end products. HOMA-IR: homeostasis model assessment of insulin resistance.

Artigo 3

Artigo submetido à revista Nutrition, Fator de impacto 3.639, Qualis Capes A1

Glycation markers are associated with changes in body fat, and in cardiometabolic and oxidative stress markers in adults with excess body weight: a secondary analysis of data from a randomized clinical trial

Abstract

Objective: To evaluate the association between glycation markers with anthropometric, body composition, cardiometabolic, inflammatory and oxidative stress markers in excess body weight adults undergoing an intervention with yacon flour and energy-restricted diet. **Methods:** We conducted a secondary analysis of data from a randomized, parallel, double-blind, placebo-controlled, six-week clinical trial. Twenty-six excess body weightadults daily consumed at breakfast a drink not containing (control group) (n = 13) or containing 25g of yacon flour (n = 13), and received the prescription of energy-restricted diets. At the beginning and end of the study, anthropometric, body composition, inflammatory, cardiometabolic, glycation (advanced glycation end products - AGEs, soluble receptor AGEs - sRAGE, AGEs/sRAGE ratio, and early glycation products - EGPs) and oxidative stress markers were assessed. Data were analyzed by linear regression. **Results**: Changes in glycation markers were associated with changes in ferric reducing antioxidant power (AGEs: β = -3.94; p = 0.036), carbonylated protein (EGPs: β = 42.99; p = 0.039), systolic blood pressure (EGPs: β = 476.60; p = 0.019), triglycerides (sRAGE: β = 0.34; p = 0.013) and triglycerides/HDL-c ratio (sRAGE: β = 0.01; p = 0.026 and AGEs/sRAGE ratio: β = -3.28; p = 0.047) in the yacon group. Baseline EGPs were negatively associated with changes in total body fat (β = -25.37; p = 0.029) and malondialdehyde (β = -56.43; p = 0.003) after yacon consumption. **Conclusion:** Glycation markers were associated with changes in body fat, and in cardiometabolic and oxidative stress markers in excess body weight adults in response to yacon flour consumption. Therefore, these glycation markers can be used as new biomarkers capable of identifying the possible beneficial effects of dietary interventions in excess body weightsubjects.

Trial Registration: ensaiosclinicos.gov.br Identifier: RBR-6YH6BQ. Registered 23 January, 2018.
Keywords: Advanced glycation end products, blood pressure, cardiometabolic markers, excess body weight, oxidative stress.

Introduction

Obesity, a multifactorial chronic disease defined as increased body fat accumulation, has an increased prevalence worldwide [1]. Adipose tissue is considered an endocrine organ that secretes adipokines, also known as cytokines, involved in the subclinical inflammatory response. These adipokines induce the production of reactive oxygen species (ROS), generating oxidative stress [2]. Thus, subclinical inflammation and oxidative stress are complications associated with excess body weight and are involved in the genesis of chronic diseases such as type 2 diabetes *mellitus* (T2DM), cardiovascular diseases, among others [3–5].

Advanced glycation end products (AGEs) are heterogeneous compounds formed from the non-enzymatic reaction between reducing sugars and proteins, lipids or nucleic acids [6,7]. AGEs formation is a process that involves several steps in a series of reactions. In the first step, there is the glycation of a protein that leads to the formation of Schiff base (intermediate products such as methylglyoxal). This unstable product undergoes rearrangements to form the Amadori products or early glycation products (EGPs). Some of the known EGPs are glycated hemoglobin, fructosamine, fructosyl-lyine, and furosine. These products undergo other reactions to irreversibly form AGEs [8]. Serum concentrations of AGEs increase in situations of hyperglycemia and oxidative stress [9]. Thus, once they bind to the membrane receptor for AGEs (RAGE), AGEs are related to the genesis of obesity and its complications, as they exacerbate subclinical inflammation. AGEs-RAGE interaction leads to the nuclear factor κB (NF-κB) activation, which in turn leads to the transcription of inflammatory

cytokines, adhesion molecules, and RAGE [10]. Therefore, the increase in AGEs concentrations increases RAGE expression, increasing ROS and inflammatory cytokines, resulting in a vicious cycle that aggravates oxidative stress and inflammation, promoting the progression of obesity [11]. On the other hand, the soluble receptor for AGEs (sRAGE) can also bind to AGEs, preventing AGEs-RAGE binding and avoiding the inflammatory signaling cascade [12]. However, sRAGE concentration in response to chronic diseases and nutritional interventions, as well as the mechanisms involved in these processes, still need further clarification [13].

Dietary strategies to control body weight and the concentrations of oxidative stress, inflammation, and cardiometabolic markers are of interest. Yacon is a tuberous root considered a functional food due to its high content of fructooligosaccharides (FOS) [14] and phenolic compounds, mainly chlorogenic acid [15]. Other authors have identified five derivatives of caffeic acid [16], vitamin C and potassium in yacon's roots [17]. Besides, tryptophan, which has antioxidant properties, is the most abundant amino acid in yacon [18]. In a study conducted by our research group, consumption of 25g of yacon flour for six weeks increased plasma antioxidant capacity and decreased oxidative stress (carbonylated protein) in excess body weight adults [19]. In another study, subjects with T2DM showed a reduction in tumor necrosis factor-alpha (TNF-α) after consuming 100g/day of yacon for five months [20].

We observed in a recently published article that consumption of 25g yacon flour and energy-restricted diet did not increase AGEs and EGPs concentrations. Changes in AGEs were positively associated with changes in body fat and, in sRAGE, with insulin and HOMA-IR [21]. However, the association between delta glycation markers (AGEs, EGPs, sRAGE, AGEs/sRAGE) versus delta cardiometabolic, inflammatory and oxidative stress markers, as well as the association of glycation marker concentrations

baseline versus delta anthropometric, body composition marker in excess body weight subjects in response to yacon consumption has not been investigated yet. It is relevant to assess these associations, because glycation markers are new biomarkers and it is necessary to verify whether it is associated with classical cardiometabolic, oxidative stress and inflammation markers related to the development of chronic diseases. Thus, the objective of this study was to evaluate the association between such variables in excess body weightadults undergoing intervention with yacon flour and energy-restricted diet.

Material and methods

Subjects

Eligible subjects were adults (20-59 years old) of both sexes who were excess body weight (body mass index (BMI) between 25 and 34.9 kg/m²), regularly consumed breakfast, had a mild physical activity level, had food restriction/disinhibition ≤14, were not diabetic and did not have a family history of diabetes or glucose intolerance.

Non-inclusion criteria were: smokers; consumption of more than two doses of alcohol /day (> 50g of ethanol/day); use of medications that affect blood glucose or energy metabolism; use of drugs, herbs or diets to reduce appetite and body weight; gain or loss of at least 5 kg three months prior to the beginning of the study; a recent change in physical activity level; aversion or intolerance to the foods provided in the study; existence or history of endocrine, cardiovascular, liver and/or gastrointestinal diseases; high blood pressure; eating disorders occurence; pregnant or lactating women; use of laxatives, antibiotics, probiotics, prebiotics or symbiotics three months prior to the beginning of the study; and women with menstrual irregularity three months before the beginning of the study.

The study protocol was approved by the Universidade Federal de Viçosa, Brazil Human Research Ethics Committee (number 1.875.372). Subjects signed the informed consent form according to the recommendations of the Declaration of Helsinki. The trial is registered in the Brazilian Registry of Clinical Trials (ReBEC) http://www.ensaiosclinicos.gov.br / (identifier: RBR-6YH6BQ).

Experimental design

This is a study based on secondary data obtained from a double-blind, randomized, parallel, six-week clinical trial study. Details of that study the have been described previously [22]. All participants were allocated (1:1) to receive either yacon flour or control. Block randomization technique was used to allocate the subjects into the groups, and was applied by a person who was not part of the research group. Twenty-six excess body weight adults attended the laboratory daily (from Monday to Friday), and received a shake containing 25 g of yacon flour (8.7 g FOS) or not containing yacon flour (0 g of FOS) for breakfast, and energy-restricted diet (restriction of 500 kcal/day). On weekends, the subjects received the ingredients to prepare and consume the drink at home. The investigators assessed the protocol adherence. The full description of the nutritional composition of these drinks and the chemical characterization of yacon flour is described in another article [22]. The other meals were consumed under free-living conditions. Subjects were instructed to maintain the level of physical activity during the study.

Body composition, anthropometry, biochemical variables (glycation, cardiometabolic, oxidative stress, and inflammatory markers), and systolic and diastolic blood pressure were assessed at baseline and after six weeks in each experimental group.

Anthropometrics, body composition and glycation markers

The methodology used in the evaluation of anthropometric, body composition [19,23] and glycation markers [21] are published. AGEs/sRAGE ratio was calculated by dividing AGEs (AU) by sRAGE (pg/ml).

Oxidative stress and inflammation markers

Catalase, glutathione S transferase, superoxide dismutase, malondialdehyde, carbonylated protein, nitric oxide, and ferric reducing antioxidant power (FRAP) concentrations were assessed. Samples of antecubital blood were collected after 12 hours of fasting. Blood was centrifuged to separate serum and plasma (3500rpm, 4 ° C, 15min) and immediately frozen at -80 ° C until analyses. All markers were evaluated in the plasma.

Catalase activity was determined using the method described by Hadwan, Abed (2016) [24] with modifications. Glutathione S transferase (GST) activity was evaluated using the method of Habig, Pabst (1974) [25]. The method used to determine superoxide dismutase enzyme (SOD) activity was based on the reduction of superoxide (O_2-) in hydrogen peroxide (H_2O_2) and O_2, using pyrogallol [26]. Malondialdehyde (MDA) was determined following the methodology prescribed by Buege, Aust (1978) [27] with adaptations. Carbonylated protein was determined using the adapted method described by Mekrungruangwong et al. (2012) [28]. Plasma concentration of total protein was measured using the Bradford method (1976) [29]. The production of nitric oxide (ON) was indirectly determined by measuring the total nitrite content of the samples, using the Griess reagent [30]. FRAP test was performed according to the methodology proposed by Benzie, Strain (1996) [31].

For the analysis of inflammation markers, blood samples were sent to the laboratory for analysis of the complete blood count (leukocytes, lymphocytes, neutrophils and platelets). C-reactive protein (CRP) was evaluated by the quantitative method based on immunoturbidimetry, using a commercially available kit for ultra-sensitive CRP (Bioclin® MG, Brazil). Neutrophil/lymphocyte, platelet/lymphocyte, neutrophil/leukocyte and lymphocyte/leukocyte ratios were calculated.

Cardiometabolic risk markers

Glucose, triglycerides, total cholesterol, and HDL-c concentrations were measured using a colorimetric assay (kit Bioclin, Quibasa Basic Chemical Ltda). LDL-c concentration was calculated using the equation: LDL-c = total cholesterol - HDL-c - (triglycerides/5) [32]. Insulin was determined by chemiluminescent immunoassay (Access Ultrasensitive Insulin). Insulin resistance was calculated using the following indices: assessment index of the insulin resistance homeostasis model (HOMA-IR), triglyceride/glucose index (TyG) and triglycerides/HDL-c. HOMA-IR index was calculated using the following equation: fasting insulin (μU/L) x fasting glucose (nmol / L)/22.5 [33]. TyG index was calculated according to the following equation: (ln [fasting triglycerides (mg/dL) x fasting blood glucose (mg/dL)])/2 [34]. TG/HDL-c ratio was calculated dividing triglycerides (mg/dL) by HDL-c (mg/dL) [35], and LDL-c/HDL-c ratio was calculated dividing LDL-c (mg/dL) by HDL-c (mg/dL).

Visceral adiposity index (VAI) was calculated using the following equations: Men = [WC (cm) / (39.69 + 1.88 × BMI (kg / m2))] × (triglycerides (mmol / l) /1.03) × (1.31 / HDL-c (mmol / l)); Women = [WC (cm) / (36.58 + 1.89 × BMI (kg / m2))] × (triglycerides (mmol / l) /0.81) × (1.52 / HDL-c (mmol / l)) [36]. Lipid accumulation product (LAP)

index was calculated using the equation: Men = (WC (cm)) - 65) × (triglycerides (mM)); Women = (WC (cm) - 58) × (triglycerides (mM)) [37].

Blood pressure was assessed in both arms, using the Omron HEM-7200 automatic device (Omron Inc., Dalian, China), in duplicate, and the mean of both assessments was used.

Statistical analysis

All data were analyzed using the Statistical Package for Social Sciences (SPSS) software, version 23.0 (SPSS, Inc., Chicago, IL, USA). Numerical variables were expressed as mean ± standard deviation or median and interquartile range. Shapiro-Wilk test was performed to assess the normality of the variables. Paired Student's t test or Wilcoxon was performed to identify possible differences between the values obtained at baseline and after the sixth week of intervention.

Multiple linear regression was performed considering delta glycation markers as independent variable and delta oxidative stress, cardiometabolic and inflammatory markers as dependent variable (delta = baseline value - value after six weeks). A model was estimated for each independent variable (glycation markers) and adjustments were made for the values of the independent and dependent variable at baseline. Multiple linear regression was also conducted to test the association between EGPs at baseline (independent variable) versus delta anthropometric, body composition, cardiometabolic, inflammatory and oxidative stress markers (dependent variable).

All analyzes were stratified according to the experimental group, adopting a significance level of 5%.

Results

The study included 26 excess body weightsubjects (57.7% women; BMI = 30.44 ± 2.46 kg / m²), and that were 31.35 ± 8.54 years old. After six weeks of intervention with or without yacon flour and energy-restricted diet, BMI, waist circumference, and body fat decreased significantly. Besides, FRAP increased and carbonylated protein concentrations decreased in the yacon group (Figure 1).

When we evaluated the association between delta glycation markers and delta oxidative stress, inflammatory and cardiometabolic markers in the yacon group, we observed that changes in MDA, neutrophil/lymphocyte ratio, and neutrophil/leukocyte ratio were positively associated with changes in the concentrations of AGEs. However, after adjusting for the baseline variables, there was a loss of statistical significance. On the other hand, delta FRAP was negatively associated with delta AGEs, even after adjustments (β = -3.94; p = 0.036). Delta sRAGE was positively associated with delta triglycerides (β = 0.34; p = 0.013) and with triglycerides/HDL-c ratio (β = 0.01; p = 0.026) after adjusting for the baseline variables. In contrast, the neutrophil/leukocyte delta was positively associated with delta sRAGE only in the crude model (Table 1).

As for delta EGPs marker, there was a positive association after adjustments with delta carbonylated protein (β = 42.99; p = 0.039) and delta systolic blood pressure (β = 476.60; p = 0.019). In turn, delta AGE/sRAGE ratio was negatively associated with delta triglyceride/HDL-c ratio in the adjusted model (β = -3.28; p = 0.047) (Figure 2).

By linear regression analysis, delta total body fat (β = -25.37; p = 0.029) and delta MDA (β = -56.43; p = 0.003) were negatively associated with EGPs at baseline in the adjusted models (Table 2).

There were no statistically significant associations between glycation markers and delta anthropometric, body composition, oxidative stress, inflammatory, and

cardiometabolic markers for the control group in the regression models (data not shown).

Discussion

To the best of our knowledge, this is the first study to assess associations between delta glycation markers (AGEs, EGPs, sRAGE, and AGEs/sRAGE) versus changes in oxidative stress, inflammatory and cardiometabolic markers, as well as the association of glycation marker concentrations baseline versus delta anthropometric, body composition marker in excess body weight adults consuming yacon flour and energy-restricted diet. In the yacon group, changes in sRAGE positively associated with delta triglycerides and with delta triglycerides/HDL-c ratio, besides greater changes in EGPs were positively associated with carbonylated protein and delta systolic blood pressure. On the other hand, negative associations were verified between changes in AGEs and changes in FRAP, besides changes in AGEs/sRAGE ratio versus changes in triglycerides/HDL-c ratio in that same group. These results point out the association between glycation markers versus consolidated cardiometabolic risk markers, suggesting that these glycation markers should be assessed to evaluate the responses to dietary interventions in clinical studies.

In clinical practice, triglycerides and systolic blood pressure are among the classic cardiometabolic risk markers [38]. Carbonylated protein, an oxidative stress marker, is also related to the development of cardiovascular diseases [39]. Thus, the positive association between changes in cardiometabolic markers and glycation markers identified in the present study may be directly related to the genesis of cardiovascular diseases and obesity in our subjects.

We believe that the high content of FOS and phenolic compounds in the yacon flour favored a reduction in anthropometric markers, carbonylated protein, and an increase in FRAP in our previous study [22]. Thus, the associations we verified indicate that the improvement of these markers (reduction in carbonylated protein, increase in FRAP) may be followed a reduction on glycation markers, which would be a positive effect since AGEs are inflammation mediators.

AGEs are formed in the final stages of the Maillard reaction in food and biological systems. Initially, there is condensation between an amino group and a carbonyl group, resulting in the formation of the Schiff base followed by the rearrangement and formation of Amadori products or EGPs. In the advanced stage, Amadori's products undergo oxidation reactions, forming AGEs (irreversible compounds) [6]. AGEs favor the manifestation of several types of chronic diseases by exacerbating oxidative stress/inflammation. The binding of AGEs to RAGE results in oxidative stress induction, increased expression of inflammatory cytokines and adhesion molecules, by activating the nuclear factor κB (NF-κB) [40]. Therefore, the AGEs-RAGE interaction triggers processes linked to vascular and inflammatory complications that characterize the disorders in which oxidative stress and inflammation are established [41].

In a study involving overweight subjects submitted to energy-restricted diet (1200 kcal, 47% protein, 7% fat, and 40% carbohydrate) for two months, the reduction in AGEs concentration (fluorescent AGEs assessed by spectrofluorometer) positively correlated with changes in BMI, waist circumference and triglycerides [42]. In another study, overweight subjects with and without T2DM submitted to a low fat and energy-restricted diet (~ 1400 to 1700 kcal/day) for three months, there was no correlation between changes in AGEs (N "-carboxymethyllysine (CML) assesed by ELISA) versus

cardiometabolic markers [43]. There was also no correlation between AGEs concentrations (CML, Nepsilon-(carboxyethyl)Lysine (CEL), and pentosidine assessed by high performance liquid chromatography - mass spectrometry) and cardiometabolic markers in overweight subjects, after the consumption of AGEs restricted diet (43mg/day) for two weeks [44]. The divergences in these results may be due to the different methodologies used to assess the AGEs concentrations (spectrofluorometer for plasma fluorescent AGEs versus ELISA Kit for CML versus high performance liquid chromatography-mass spectrometry - HPLC for CML, CEL, and pentosidine). Spectrofluorometer identifies only AGEs with aromatic rings, failing to identify CML and CEL. Through CML methodology (ELISA) only a single type of AGEs (CML) is evaluated. Through HPLC, three types of AGEs (CML, CEL and pentosidine) are assessed [8].

In a study involving subjects with metabolic syndrome there was a positive correlation between AGEs (methylglyoxal - MG and CML) versus SOD and lipid peroxidation products, as well as a negative correlation with antioxidant markers [45]. We also observed a positive association between EGPs versus cardiometabolic markers (systolic blood pressure) and oxidative stress (carbonylated protein). Changes in concentrations of AGEs were negatively associated with changes in concentrations of FRAP in our study, indicating a higher antioxidant capacity of plasma to provide protection against free radicals.

We also observed a positive association between changes in sRAGE and cardiometabolic markers, as well as a negative association between changes in AGEs/sRAGE ratio and TG/HDL ratio. Apparently, sRAGE is a variation of RAGE, and it seems to prevent AGEs from binding to RAGE and activating the inflammatory cascade [46]. However, we and other authors [47–50] did not verify the beneficial effect

of sRAGE. Low serum concentrations of sRAGE were observed in subjects with chronic diseases [51–53]. However, sRAGE concentrations may be elevated in diabetes [54–56] and in chronic kidney disease [57]. Human studies have indicated positive associations of sRAGE versus albuminuria [47], AGEs [48,49] and peripheral neuropathy [50]. The authors of an in vivo and in vitro study noted that sRAGE can also trigger inflammation by binding to Mac1, activating NF-κB, thus contributing to the development of vascular complications. The authors of that study believe that depending on the inflammatory medium (presence / absence of AGEs), the interactions of sRAGE with ligands may lead to an anti-inflammatory or pro-inflammatory effect [58]. Future studies should be conducted to evaluate the properties of sRAGE and the mechanisms by which this molecule regulates inflammatory responses. Apparently, although AGEs and sRAGE are elevated in chronic diseases, AGEs elevation is greater than sRAGE elevation. Thus, more AGEs will bind to RAGE and activate the inflammatory signaling cascade. Therefore, sRAGE cannot be used as a single risk biomarker to assess chronic diseases. Instead, it must be evaluated in conjunction with other biomarkers [13,59].

The authors of a recent study [13] proposed the use of AGEs/sRAGE ratio as a marker for various types of chronic diseases. It is hypothesized that an increase in that ratio, not the isolated concentrations of AGEs or of sRAGE, is a biomarker for chronic diseases. In the present study, we observed that changes in AGEs/sRAGE ratio were negatively associated with lower changes in the triglycerides/HDL-c ratio in the yacon group. That result confirms the association between glycation markers and cardiometabolic markers. The reason why that association was verfied only in the yacon group deserves further investigation. As far as we know, no other study has

evaluated the association between the AGEs/sRAGE ratio and cardiometabolic markers.

We also observed a negative association between EGPs at baseline versus delta total body fat and malondialdehyde concentrations after yacon consumptiom. These results indicate that individuals with higher concentrations of EGPs at the beginning of the study had lower reductions in total body fat and malondialdehyde in response to yacon flour consumption. Once AGEs bind to RAGE there is an increases in the formation of reactive oxygen species (ROS), such as malondialdehyde, through the activation of NADPH oxidase, protein kinase-C, p21, extracellular signal-regulated kinase (ERK), p38 and c-jun N-terminal kinase (JNK), leading to the translocation of NF-κB to the nucleus and resulting in the transcription of inflammatory markers [11]. These results suggest that the increase in oxidative stress can be favored by the EGPs metabolic pathway, and that AGEs and ROS are related to obesity and are risk factors for cardiovascular diseases [2,60]. As far as we know, no other study has explored the relationship between EGPs and oxidative stress and cardiometabolic markers in humans.

Our study has strengths, as it was a randomized and placebo-controlled clinical trial in which we evaluated different types of glycation markers (AGEs, sRAGE, EGPs and AGEs/sRAGE ratio), as well as several cardiometabolic and oxidative stress markers. However, we did not have the opportunity to evaluate classic inflammatory markers, such as tumor necrosis factor α (TNF-α), interleukin-6, monocyte chemoattractant protein-1, NF-κB concentrations, which would help us to have a wider view of the inflammatory aspects related to these glycation markers.

Conclusions

In conclusion, changes in glycation markers were associated with changes in cardiometabolic and oxidative stress markers in excess body weight adults. Higher EGPs concentrations at baseline were associated with lower reductions in total body fat and malondialdehyde concentration after the intervention with yacon. Therefore, the associations between consolidated obesity and cardiometabolic risk markers observed in this study suggest that glycation markers can be used as new biomarkers capable of identifying possible changes in response to dietary interventions in excess body weightsubjects. Future studies should evaluate associations between sRAGE, AGEs, EGPs and AGEs/sRAGE ratio versus oxidative stress, inflammatory and cardiometabolic markers in subjects presenting other pathologies.

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Figures

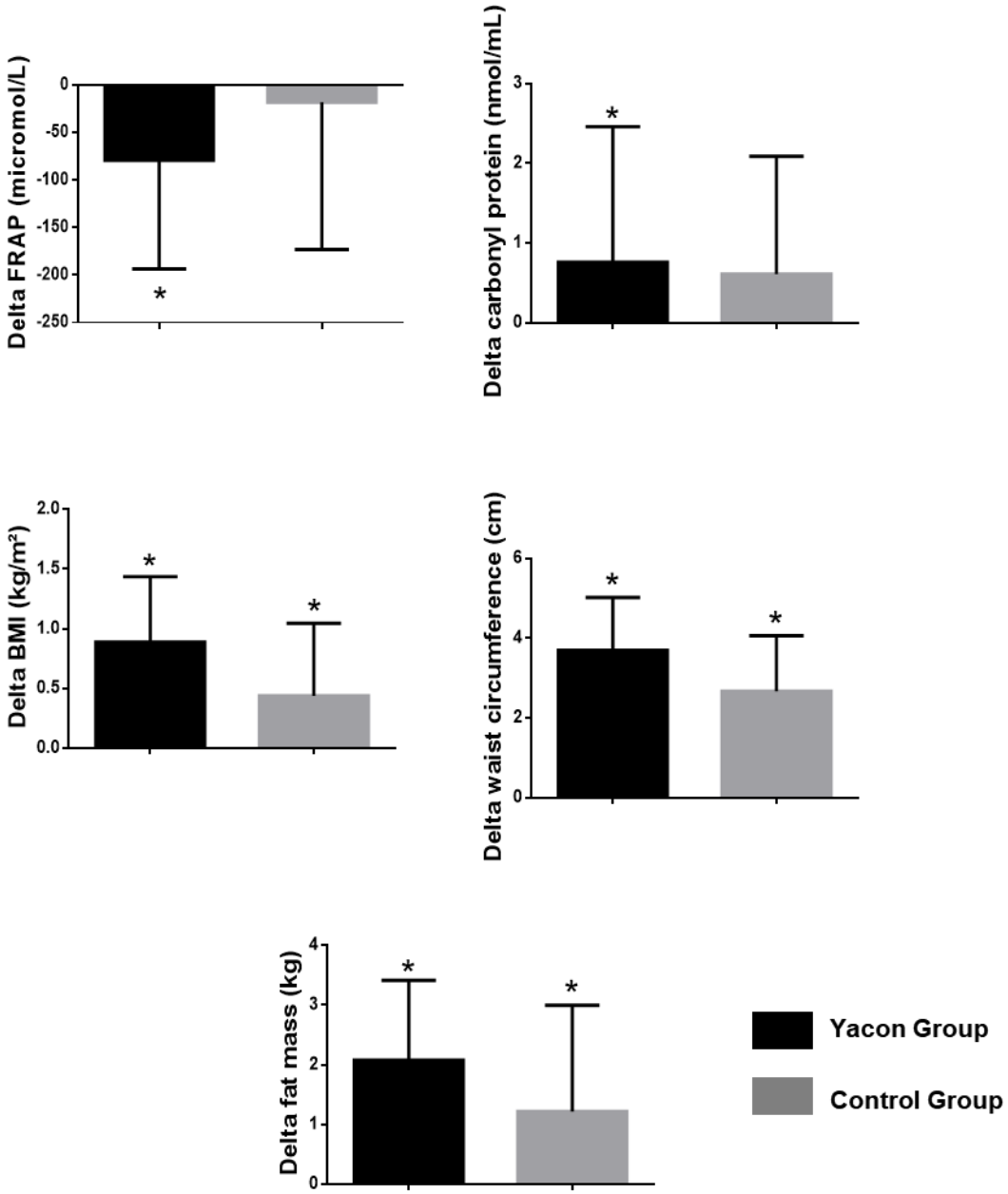

Figure 1. Delta oxidative stress, anthropometric and body composition markers concentrations in response to the consumption of yacon flour (Yacon group; n=13) or no yacon flour (Control group; n=13) and energy-retricted diet for six weeks. Data are presented as mean and standard deviation. Delta = baseline value - value after six weeks of intervention. * Baseline vs. six weeks by Paired Student's t test or Wilcoxon test, $p < 0.05$. FRAP: ferric reducing antioxidant power, BMI: body mass index.

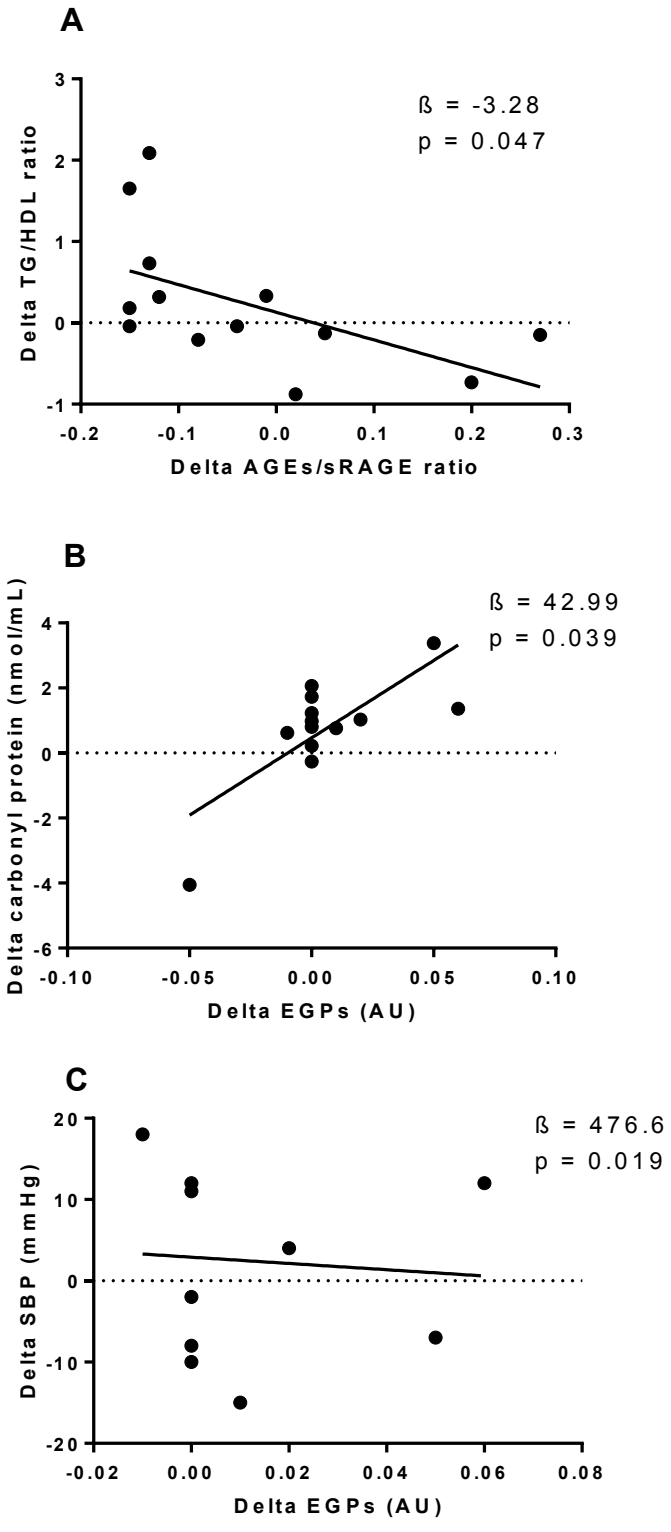

Figure 2. Association between delta AGEs/sRAGE and delta TG/HDL ratio (A), and delta EGPs versus delta carbonyl protein (B) and delta SBP (C) in subjects with excess body weight consuming yacon flour and energy-retricted diet for six weeks (n = 13). Beta value is the result of linear regression adjusted by the baseline values. AGEs:

advanced glycation end products, sRAGE: soluble receptors of advanced glycation end products and SBP: systolic blood pressure.

TABLES

Table 1: Association between delta advanced glycation end products and soluble receptors of advanced glycation end products (independent variable) versus delta oxidative stress, inflammation, and cardiometabolic markers (dependent variable) in subjects consuming yacon flour and energy-retricted diet for six weeks (n = 13)

	ΔAGEs		**ΔsRAGE**	
Dependent variables	**β**	***p*-value**	**β**	***p*-value**
ΔFRAP (μmol/L)				
Crude	-2.75	0.030*	-0.39	0.476
Adjusted	-3.94	0.036*	-0.43	0.583
ΔMalondialdehyde (μmol/mL)				
Crude	0.12	0.022*	0.01	0.751
Adjusted	0.04	0.387	0.00	0.820
ΔNeutrophil/lymphocyte ratio				
Crude	0.01	0.047*	0.00	0.082
Adjusted	-0.00	0.804	0.00	0.835
ΔNeutrophil/leukocyte ratio				
Crude	0.00	0.034*	0.00	0.021*

Adjusted	0.00	0.782	0.00	0.692
ΔTG/HDL ratio				
Crude	0.00	0.794	0.01	0.057
Adjusted	0.01	0.371	0.01	0.026*
ΔTriglycerides				
Crude	0.31	0.533	0.34	0.063
Adjusted	0.68	0.101	0.34	0.013*

Multiple linear regression. Adjusted: adjusted by the variables at baseline. Δ = baseline value - value after 6 weeks of intervention. AGEs: advanced glycation end products, sRAGE: soluble receptors of advanced glycation end products, FRAP: ferric reducing antioxidant power. *$p < 0.05$.

Table 2: Association between baseline early glycation products (EGPs) (independent variable) and delta cardiometabolic risk and oxidative stress markers (dependent variables) in subjects consuming yacon flour and energy-retricted diet (n = 13)

Dependent variables	**EGPs baseline**	
	β	***p*-value**
ΔTyG		
Crude	-2.96	0.356
Adjusted	-1.26	0.641
ΔTriglycerides		
Crude	-404.19	0.259
Adjusted	-132.49	0.583
ΔLAP		
Crude	-261.42	0.218
Adjusted	-86.81	0.541
ΔFat mass		
Crude	-23.09	0.035*
Adjusted	-25.37	0.029*
ΔMDA		
Crude	-93.37	0.015*
Adjusted	-56.43	0.003*

Multiple linear regression. Adjusted: adjusted by the variables at baseline. Δ = baseline value - value after 6 weeks of intervention. EGPs: early glycation products, TyG: triglycerides/glucose index, LAP: lipid accumulation product, MDA: malondialdehyde. *p < 0.05.

Artigo 4

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Consumption of yacon flour and energy-restricted diet increased the relative abundance of intestinal bacteria that may lead to positive metabolic effects in adults with excess body weight: a randomized, double-blind, parallel, placebo-controlled clinical trial

Abstract

Purpose: The dysbiosis can favor the occurence of obesity and associated chronic diseases. Some strategies have been proposed to modulate the intestinal microbiota. However, the effects of yacon on the intestinal microbiota in subjects with excess body weight have not been investigated yet. Thus, the objective of this study was to evaluate the effects of yacon flour consumption and energy-restricted diet in the intestinal microbiota in adults with excess body weight. **Methods**: Twenty-one adults with excess body weight were included in this randomized, parallel, double-blind, placebo-controlled, six-week clinical trial. Subjects daily consumed at breakfast a drink containing 25g of yacon flour (n = 11) or not containing yacon (control group) (n = 10) and received the prescription of energy-restricted diets. Fecal samples were collected on the first and on last day of the study. 16S rDNA sequencing was assessed to evaluate the effect of yacon fermentation on intestinal microbiota bacterial composition. **Results:** There was an increase in the genera *Bifidobacterium*, *Blautia*, *Subdoligranulum*, and *Streptococcus* after the consumption of yacon and energy-restricted diet. In the yacon group, we also observed a positive correlation between the concentrations of short-chain fatty acids (SCFA) versus the genera *Coprococcus* and *Howardella*, beside a negative correlation between the concentrations of advanced glycation end products and early glycation products versus the genera *Ruminococcus* and *Prevotella*, respectively. **Conclusion**: Therefore, in the yacon group *Bifidobacterium*, *Blautia*, *Subdoligranulum*, *Streptococcus* increased. Glycation markers and SCFA also correlated with bacterial genera (*Ruminococcus*, *Prevotella,*

Coprococcus and *Howardella*) related to chronic diseases, such as obesity. **Register number:** RBR-6YH6BQ. Registered 23 January, 2018.

Keywords: fructooligosaccharides, intestinal microbiota, obesity, prebiotic, *Smallanthus sonchifolius***,** 16S rDNA sequencing.

Introduction

Excess body weight, defined as the accumulation of body fat, is a global epidemic. According to the World Health Organization, the prevalence of obesity has almost tripled since 1975 and currently, more than 1.9 billion adults are overweight, with more than 650 million clinically obese. Excess body weight can be caused by excessive caloric intake, sedentary lifestyle, genetic predisposition, among other factors [1]. Recently, the intestinal microbiota has been included in the etiology of obesity [2, 3].

The human intestine is densely populated by diverse microbial communities that are essential to health. Under normal physiological conditions, the intestinal microbiota is in equilibrium. The interruption of that equilibrium alters the microbial composition and diversity, a phenomenon called dysbiosis, which has been associated with the pathogenesis of many diseases, such as obesity and its complications, such as insulin resistance and even colorectal cancer [3–7]. Dysbiosis may favor an increase in intestinal permeability, favoring the translocation of compounds such as lipopolysaccharide into the bloodstream, contributing to increased systemic concentrations of endotoxins and inflammatory markers [3, 8, 9]. These changes seen in subjects with excess body weight characterize a phenomenon called metabolic endotoxemia [9–11]. In a clinical trial, increased paracellular permeability was observed in women with obesity compared with lean women, and intestinal permeability markers were positively correlated with metabolic syndrome risk factors [12].

Scientists have been interested in identifying effective strategies to modulate the intestinal microbiota to prevent diseases [13, 14]. One of these strategies is the consumption of prebiotics and dietary fibers, as they can be metabolized by the intestinal microbiota [15]. Yacon (*Smallanthus sonchifolius*) is a tuberous root native to the Andean regions of South America, considered a functional food due to its high

content of fructooligosaccharides (FOS) [16, 17], a dietary fiber with prebiotic properties [15, 18]. Prebiotics are substrates used by microorganisms, which can modify the composition and activity of the intestinal microbiota, leading to health benefits [19]. FOS is not broken down by human digestive enzymes, and it is therefore fermented in the colon resulting in short-chain fatty acids (SCFA) production. As a result, bifidobacteria are preferentially stimulated to grow, increasing the number of health-promoting bacteria and reducing the number of harmful bacteria [18, 20, 21].

In a thirty-day clinical trial involving adults and elderlies with normal body weight, there was an increase in *Bifidobacterium* and a reduction in *Clostridium* and *Enterobacteria*, after the consumption of orange juice added with a yacon based product (10g FOS/inulin per day) [22]. Other authors observed the beneficial effect of prebiotics on the intestinal microbiota of individuals with overweight [23, 24]. In a parallel, double-blind, placebo-controlled study involving women with obesity, the consumption of 16g of powdered supplement (50% oligofructose and 50% inulin) for three months increased *Bifidobacterium* and *Faecalibacterium* and reduced *Bacteroides intestinalis*, *Bacteroides vulgatus*, and *Propionibacterium*, showing that the supplement positively changed the intestinal microbiota, which may have a positive impact on metabolites involved in obesity [23]. In another study, with the same design as the previous study, the consumption of an inulin-type fructan bar (6 g of oligofructose + 2 g of chicory root inulin) for twelve weeks led to an increase in *Bifidobacterium* in adults with excess body weight [24].

However, evidence on the effects of yacon consumption on the intestinal microbiota in subjects with excess body weight has not been published yet. Thus, the aim of our study was to investigate the effects of yacon flour consumption associated with an energy-restricted diet on the intestinal microbiota in adults with excess body weight.

Materials and Methods

Participants

Four hundred and four subjects were recruited from the local community and were assessed for eligibility criteria. The inclusion criteria were: adults (20 - 59 years old) of both sexes with excess body weight (body mass index (BMI) between 25 and 34.9 kg/m²) [25], regular breakfast ingestion, light level of physical activity, food

restriction/disinhibition ≤ 14 [26], non-diabetic and without a family history of diabetes or glucose intolerance.

The exclusion criteria were: smokers; consumption of more than 2 doses of alcohol /day (> 50g of ethanol/day); use of medications that affect blood glucose or energy metabolism; use of medications, herbs, or diets to reduce appetite and body weight; gain or loss of at least 5 kg three months prior to the beginning of the study; a recent change in the level of physical activity; aversion or intolerance to the foods provided in the study; existence or history of endocrine, cardiovascular, arterial hypertension, liver and/or gastrointestinal diseases; report of eating disorders; pregnant or lactating women; use of laxatives or antibiotics three months prior to the beginning of the study; use of probiotics, prebiotics or symbiotics, women with menstrual irregularity.

Thirty subjects were considered eligible and were randomized to the control (n = 15) or yacon group (n = 15). After six weeks of intervention, twenty-six subjects completed the study protocol (n = 13 yacon group and n = 13 control group). However, when performing bioinformatics analyses, three subjects from the control group and two from the yacon group were removed, due to the low number of sequences (low throughput) obtained from the sequencer, and outliers. Therefore, ten subjects were allocated in the control group and eleven in the yacon group (Figure 1).

The study was conducted according to guidelines established in the Declaration of Helsinki [27] and all procedures involving participants were approved by the Universidade Federal de Viçosa, Brazil Human Research Ethics Committee (protocol number: 1.875.372). The trial is registered in the Brazilian Registry of Clinical Trials (ReBEC) http://www.ensaiosclinicos.gov.br/ (identifier: RBR-6YH6BQ).

Study design

For a broader overview of the study design and procedures, see Machado et al. [28]. In summary, this was a randomized, double-blind, parallel, placebo-controlled, six week clinical trial.

Subjects attended the research unit for an initial screening visit, when questionnaires were applied, and fasting capillary blood glucose, anthropometry (body weight and height), body composition (% of body fat by tetrapolar bioimpedance), and blood pressure were evaluated. Eligible subjects were then allocated by the block

randomization technique to one of the two interventions (yacon flour group or control group). Energy-restricted diets were prescribed for both groups. Subjects were advised to not consume alcohol, and to not change their usual food intake, not to consume prebiotics, probiotics and symbiotics, and not to change the level of physical activity during the study. Body composition, anthropometry, biochemical markers, food intake, and intestinal microbiota were assessed at baseline and after six weeks in each experimental group.

Interventions

Subjects daily consumed at breakfast 350ml of beverages containing 25 g of yacon flour (13.72 ± 3.97 g of total fibers and 8.7 g FOS) or 0 g of yacon flour (2.63 ± 3.91 g of total fibers and 0 g of FOS). Beverages were similar in terms of macronutrients and energy, differing in total fiber and FOS content [28]. The ingredients used to prepare the drinks were the same, except for adding (yacon group) or not adding (control group - added corn starch) yacon flour. Corn starch was added to the control group drinks so that they would be nutritionally similar to those in the yacon group. The full description of the nutritional composition of these beverages and the chemical characterization of the yacon flour can be seen in another article [28]. Beverages were offered to the subjects in identical glasses and had a similar appearance. Energy-restricted diets (restriction of 500 kcal/day) [25] were prescribed to the subjects of both experimental groups. The other meals were consumed in free-living conditions.

Anthropometric and metabolic markers

The methodology used in the evaluation of anthropometric, biochemical, and SCFA markers has already been published [28, 29]. AGEs serum concentrations analysis was performed by fluorescence spectroscopy (λemission=460 nm, λexcitation=370 nm, SpectraMax M2e, SoftMax®Pro software) using 50 µL of serum [30]. The detection of early glycation products (EGPs) (glycated hemoglobin, glycated albumin, fructosyl-lyine, furosine and others glycated plasma proteins) was assessed through the reduction of tetrazolium nitroazul (NBT) [31]. sRAGE concentrations were determined using a commercial ELISA kit (Human RAGE Sigma-Aldrich®) specific for human sRAGE (intra-assay and inter-assay reproducibility CV of <10% and <12%,

respectively). Duplicate samples and absorbed sRAGE standards were read at 450 nm (SpectraMax M2e, SoftMax®Pro software).

Fecal samples collection and DNA extraction

Subjects were instructed to collect fecal samples as close as possible to the moments of the analyses and to keep the material under refrigeration (4ºC) until its delivery to the laboratory. Fecal samples were kept at -80ºC until the time of preparation for the analyses of intestinal microbiota, fecal pH and SCFA.

The extraction of total DNA from fecal samples was performed using a standardized protocol adapted [32]. In microtubes, 50 µL of samples were added with 500 µL of PBS buffer. After vortex homogenization, 700 µL of lysis buffer was added and homogenized. The microtubes were inverted to homogenize and left in the dry bath (65ºC) for 5 minutes. Next, the microtubes were homogenized by vortexing, with 700 µL of precipitation solution added. The tubes were poured and incubated at 4ºC for 10 minutes. Then, the tubes were centrifuged for 10 minutes (11000 to 15000 rpm), and 700 µL of supernatant was transferred to another Eppendorf. An aliquot of 700 µL of isopropanol was added, homogenized, and incubated at 4ºC for 10 min. Then, the tubes were centrifuged for 10 minutes and the supernatant was discarded. It was necessary to let the content of the tubes dry for 5 minutes at room temperature, resuspend it in 25 µL of distilled water, and leave in a dry bath (65ºC) for 5 minutes. Finally, after centrifugation for 5 minutes, 20 µL of the supernatant was removed and added to another Eppendorf. The quality and quantity of the extracted DNA were obtained using µDropTM Plate (Thermo Fisher Scientific, Finland) and stored at - 20 ° C.

16S rDNA bacterial gene sequencing

Sequencing of the V4 hypervariable region of the 16S rDNA gene from members of the Bacteria domains was performed by the company Argonne National Laboratory® (Ilinois, United States). The libraries were prepared using Reagent Kit v3 (Illumina) and sequencing was done on the MiSeq 2 x 151 bp platform (Illumina, San Diego, California, USA).

The strings were demultiplexed using the Qiime 2 program (version 2020.8) [34]. Then, adapters, barcodes, and primers were removed using the “cutadapt trim-

paired" command. Subsequently, the low-quality sequences and chimeras were removed, and the remainder were grouped into ASVs (Amplicon Sequence Variants). Rare ASVs, which have occurred only once (singletons) were removed.

Sequences were aligned using the MAFFT algorithm, and the alignment was used for phylogenetic reconstruction with the fastree algorithm. The entire procedure was performed on Qiime 2 v.2020.8. The sequences were recorded taxonomically using the SILVA 138 database [33]. The raw sequences were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under the access number PRJNA681193.

Statistical analysis

The present study had 94% statistical power, considering the *Bifidobacterium* genus after six weeks of intervention, with a 95% confidence interval, type I error $\alpha = 0.05$ [34–36]. Statistical Package for Social Sciences (SPSS), version 23.0 (SPSS, Inc., Chicago, IL, USA) was used to evaluate the effect of metabolic variables between groups (control versus yacon). The normality of the distribution of all variables was assessed according to the Shapiro-Wilk test, and a significance of 5%.

Differences between baseline and final values were assessed using the paired Wilcoxon test. On the other hand, for comparisons between groups (Yacon and Control), the Kruskall-Wallis test was used. Statistical analyzes were performed using the software R 3.6.3 [37].

P-values of the paired Wilcoxon test were corrected to control the false discovery rate (FDR) [38], when the test was applied to assess the significant differences of individual groups in terms of genus in the microbiota of both groups after six weeks. For the p-corrected value, significance was set at <0.2 [39, 40].

Spearman's correlation was performed to analyze the correlations between bacterial genera and anthropometric/biological markers. The analyses were performed using the software R 3.6.3. The level of significance adopted was $p < 0.05$.

Results

Participants

Subjects of the two experimental groups did not differ in terms of the characteristics presented at baseline (Table 1).

Intestinal Microbiota

1,230,539 raw reads were obtained. After removing the low-quality sequences (254,522) and chimeras (11,789), the remaining 964,048 sequences were grouped into 1,705 ASVs. Rarefaction curves (Supplementary Figure 1 - S1A) and the Goods Coverage index (Figure S1B) showed that sampling and sequencing were sufficient to access the diversity of the microbiota present in the samples.

Principal component analysis (Figure 2) showed that the microbiota present before and after the intervention in both experimental groups did not differ (PERMANOVA's p-value = 0.975).

Bacterial communities did not vary in terms of equitability and the diversity index, estimated by Shannon, for both experimental groups. However, microbial richness, estimated by the Chao1 index, decreased in the control group, while in the yacon group it remained unchanged (Figure 3). Supplementary Table 1 (S1) shows all the wealth and diversity indexes evaluated.

Taxonomic analyses of the bacterial community in the group that consumed yacon flour revealed the existence of 864 ASVs that were classified into 14 phyla, 24 classes, 51 orders, 89 families and 227 genera. We observed 841 ASVs, 13 phyla, 19 classes, 38 orders, 72 families and 204 genus in the control group. ASVs that showed a relative abundance greater than 0.5% after the consumption of each intervention are shown in Supplementary Table 2 (S2). However, no significant changes were observed between the experimental groups regarding the verified ASVs.

At the Genera level, we observed an increase in *Bifidobacterium* ($p = 0.029$, FDR = 0.182), *Blautia* ($p = 0.000$, FDR = 0.019), *Subdoligranulum* ($p = 0.002$, FDR = 0.024) and *Streptococcus* ($p = 0.009$, FDR = 0.078) after consuming yacon associated with energy-restricted diet (Figure 4). In the control group, there were no changes after six weeks of intervention (Figure 5).

When considering the two predominant phyla, Firmicutes and Bacteroidetes, we did not observe significant changes before and after the consumption of yacon flour or between the experimental groups. We also did not observe significant changes in the Firmicutes/Bacteroidetes ratio within and between experimental groups (Figure 6).

Correlations between intestinal bacteria and anthropometric and metabolic markers

Spearman correlation analyses were performed to assess the potential link between significant changes in the composition of the intestinal microbiota induced by the prebiotic and the host metabolism (Figure 7). In the yacon group, we observed a positive correlation between the concentrations of short-chain fatty acids (SCFA) and the genera *Coprococcus* and *Howardella*, beside a negative correlation between the concentrations of advanced glycation end products (AGEs) and early glycation products versus the genera *Ruminococcus* and *Prevotella*, respectively.

Discussion

As far as we know, this is the first randomized, parallel, double-blind clinical trial to investigate the effects of yacon flour consumption associated with an energy-restricted diet on the intestinal microbiota in adults with excess body weight. Consumption of 25g of yacon flour (8.7g FOS) in an energy-restricted diet for six weeks increased relative abundance of the genera *Bifidobacterium*, *Blautia, Subdoligranulum*, and *Streptococcus*.

Nutritional interventions with prebiotics can modulate the intestinal microbiota having a positive impact on health [41]. Increased relative abundance of the genera *Bifidobacterium* was observed by us and by the authors of other human clinical trials in response to prebiotics [22, 23, 42–46]. It has been observed that bifidobacteria tend to decrease in obesity and diabetes [47, 48], and increase after the ingestion of prebiotics [9, 49]. Bifidobacteria can reduce intestinal permeability, reducing serum LPS concentrations (endotoxemia) in mice. Besides, the genus *Bifidobacterium* was positively correlated with glucose tolerance improvement, glucose-induced insulin secretion, and normal inflammatory profile (secretion of pro-inflammatory cytokines from the adipose tissue). For that reason, it seems that the intestinal microbiota can control chronic diseases, such as obesity and diabetes, by reducing the occurrence of endotoxemia and inflammation. Consumption of FOS is an interesting strategy to increase bifidobacteria, preventing the harmful effects of chronic diseases [9, 49]. In humans, oligofructose and inulin supplementation improved chronic inflammation clinical characteristics in subjects with colitis and Chron's disease [50, 51].

Fermentable carbohydrates can also control obesity and chronic diseases by modulating the intestinal endocrine function. Nutrients with prebiotic properties, such as FOS, can change the intestinal microbiota, promoting an increase in the number of intestinal cells that produce GLP-1 and GLP-2, modulating the activation of the intestine and adipose tissue endocannabinoid system.Thus, these effects favor reductions in intestinal permeability, metabolic endotoxemia, and systemic inflammation. Besides, increased GLP-1 production favors food intake, body fat, blood glucose, and insulin resistance reductions [41].

Supplementation of inulin-type fructans with prebiotic properties in mice fed a high-fat diet modulated the intestinal microbiota, increasing the abundance of bifidobacteria. Prebiotics that promoted intestinal fermentation neutralized GPR43 overexpression induced in adipose tissue by a high-fat diet, which in turn was correlated with a beneficial effect on adiposity and with the potential decrease in processes activated by PPARγ. These two genes are implicated in the adipocytes fat accumulation. Thus, the authors concluded that by modifying the intestinal microbiota, prebiotics can neutralize high-fat diet induced obesity, endotoxemia and related metabolic changes. Thus, the results of these studies suggest that the increase in bifidobacteria may have been followed by adipocytes fat accumulation and metabolic endotoxemia occurrence control [52].

Therefore, our study confirms the bifidogenic effect of yacon, which is rich in FOS. The chemical structure of the FOS has 2 to 10 fructose molecules connected by a β- (1,2) glycosidic bond and 1 glucose molecule linked by an α- (1,2) bond [53]. The human small intestine lacks enzymes to hydrolyze FOS bonds. However, FOS can be fermented by most strains of *Bifidobacterium* and some *Lactobacillus* [54, 55]. Consumption of FOS can increase SCFA production, leading to an increase in bifidobacteria [56, 57]. Yacon stimulates the growth of bifidogenic bacteria, which stimulates the balance of the immune system, preventing the growth of pathogenic bacteria in the intestinal microbiota [15, 58]. Apparently, the inhibition process used by these bacteria is mainly due to the production of acetic and lactic acids [59, 60]. In our study, the consumption of yacon flour and energy-restricted diet increased the genera *Blautia, Subdoligranulum*, and *Streptococcus*, which belong to the phylum Firmicutes. In another stuy, the consumption of prebiotics for three months Firmicutes abundance increased in women with obesity [23]. The author of most studies on the relationship

between the obesity and/or diabetes host phenotype versus the intestinal microbiota report changes in the abundance of phyla, genus or species of bacteria [61–63]. The first studies that described changes in the intestinal microbiota in individuals with obesity observed an increase in the phylum Firmicutes and a reduction in the phylum Bacteroidetes in obese versus lean individuals [63–65]. However, the Firmicutes/Bacteroidetes ratio in subjects with excess body weight appears to be controversial. In another study, similar abundance of Bacteroidetes were observed in lean and obese subjects [66], or even an increase in the proportion of that phylum in subjects with overweight [48, 67]. While Firmicutes were positively associated with fiber intake and negatively associated with body fat [68], bacteroidetes were positively associated with body fat and negatively associated with fiber intake [68]. Differences in the methodology used for bacterial analysis (16S rDNA versus Real-Time Quantitative PCR versus fluorescent in situ hybridization) and the fact that the study was conducted with animal versus human may explain the discrepancies in the results obtained different authors. 16S rDNA sequencing technology is the most robust method, which has a good depth (it can capture most of the diversity), resolution (it can identify up to genus level), and sensitivity (it can detect even the most rare species) [69].

In our study, we identified in the yacon group an increase in the genera *Blautia* and *Subdoligranulum*, known to include butyrate-producing bacteria [70, 71]. Intestinal microbiota can ferment non-digestible carbohydrates, such as FOS, converting them to SCFA. The most important SCFA are acetate, propionate, and butyrate. SCFA can provide calories when they are oxidized, favoring body weight increase and fat gain, contributing to adipogenesis in animal models [64, 72]. However, apparently the additional calories supplied to the host by the fermentation of prebiotics are not sufficient to induce body weight gain [70]. Fermentable carbohydrates, such as prebiotics, can neutralize most metabolic changes linked to obesity (glucose and lipid metabolism improvement; body weight, body fat and LPS reductions; and modulation of intestinal peptides that regulate food intake) [73–76]), although they are fermented to SCFA [41].

In human clinical trials, the consumption of low-fat diet increased the abundance of the genus *Blautia* [71], which was negatively associated with changes in serum total cholesterol and LDL-c [71, 77]. Less relative abundance of that genus was observed

in subjects with T2DM than in healthy subjects [78, 79]. The results of these few studies identified in the literature suggest that the genus *Blautia* seems to have a positive impact on markers related to excess body weight and associated diseases.

Metagenomic data from subjects with overweight and clinical variables from two cohorts indicated that the abundance of *Subdoligranulum* was positively correlated with microbial richness, HDL-cholesterol, and adiponectin concentrations. In that study, abundance of *Subdoligranulum* was negatively correlated with mass fat, adipocyte diameter, insulin resistance, leptin, besides insulin, CRP and IL6 concentrations in humans [80]. In obese and diabetic rats, the intake of prebiotics (oligofructose) increased the abundance of *Subdoligranulum* by almost four times [73]. Besides, the *Subdoligranulum* genus negatively correlated with glycated hemoglobin (HbA1c) and positively correlated with HDL cholesterol [81]. Thus, based on these observations and associations identified between *Subdoligranulum* and markers related to excess body weight, it seems that this genus may favor obesity control and metabolic health improvement.

In the present study, we also observed the increase in the genus *Streptococcus* in the yacon group. *Streptococcus* is located in the intestinal mucosa and have the ability to lower the pH locally, thus contributing to its protection against pathogenic microorganisms [82]. In adolescents with overweight, it was observed that the high consumption of fructose, from industrialized foods (high fructose corn syrup - HFCS), was negatively associated with beneficial bactéria that belonged to the genus *Streptococcus*, including the species *Streptococcus thermophilus*. [83]. *Streptococcus thermophilus* can ferment lactose, sucrose, and fructose [84]. However, the effect of fructose intake on the genus *Streptococcus* has not been explored yet. In subjects submitted to bariatric surgery (Roux-en-Y gastric bypass), an increase in *Streptococcus spp* was observed [85]. However, other authors observed a positive association between *Streptococcus bovis* and metabolic disorders in children with overweight [86]. In another study in adults with overweight, the genus *Streptococcus* was positively correlated with BMI [87]. In subjects with T2DM and coronary heart disease [88] and in subjects with excess body weight [89] an increase in the genus *Streptococcus* was observed which negatively correlated with HDL-c [88]. Furthermore, that genus was positively associated with serum uric acid, glucose, and HOMA index [90]. These results suggest that future studies should be conducted to

assess the behavior of the genus Streptococcus in the control of excess body weight and of associated diseases.

We observed negative associations between the genera *Ruminococcus* and *Prevotella* versus advanced glycation end products (AGEs) and early glycation products (EGPs) concentrations, respectively, in response to the consumption of yacon flour and energy-restricted diet. Thus, the greater the abundance of *Ruminococcus* and *Prevotella*, the lower the AGEs and EGPs concentrations will be. AGEs and EGPs are known to be related to the genesis of obesity and its associated complications by exacerbating subclinical inflammation [91]. Bacteria of the genus *Ruminococcus* produce butyrate, the main source of energy for intestinal cells, which exert anti-inflammatory function [92]. High fiber consumption increased the abundance of Prevotella in mice, improving glucose metabolism. It was observed that in these animals, the gene G6pc located in the proximal colon was affected [93]. That gene encodes glucose-6-phosphatase, a key enzyme in intestinal gluconeogenesis that induces beneficial effects on glucose and energy homeostasis [94]. In the present study, we also verified that SCFA (acetate, butyrate, and propionate) were positively associated with the genera *Coproccus* and *Howardella*, both belonging to the phylum Firmicutes. *Coproccus* is known to be a producer of butyrate [92]. It was observed in an *in vitro* study that butyrate directly induced lipolysis in 3T3L1 adipocytes [95]. SCFA, including butyrate, can induce the production of intestinal-derived serotonin [96], which plays a role in increasing lipolytic enzyme activity [97]. In diet-induced obese animal models, butyrate attenuated weight gain and improved insulin resistance [98–100].

Finally, we would like to emphysize the applicability of yacon flour in clinical practice. Yacon flour is a low-cost food. Since it is dry, it can be stored for relatively long period, making it a practical and very convenient food. The results obtained in this study indicate that the consumption of a small quantity (25g/day) for a relatively short period (6 weeks) increased some bacterial genera from the intestinal microbiota, which are related to the control of excess weight, without causing any undesirable gastrointestinal effects.

There are many strengths in this study, including its robust design: a randomized, double-blind, placebo-controlled, parallel clinical trial in humans. Besides, this is the first study to investigate the effects of yacon flour consumption associated with an energy-restricted diet on the intestinal microbiota in adults with excess body

weight, using Next Generation sequencing technology (16S rDNA sequencing). That methodology allowed the characterization of the entire microbial community, instead of evaluating few bacterial taxa, such as it use to happen when predecessor methodologies were used [69]. However, in this study, we focused only on the composition of the intestinal microbiota and did not evaluate its functionality. Some authors have shown that not only the microbiota composition but also its functionality plays a role in the metabolic state [101, 102]. Future studies are needed to evaluate the influence of yacon flour consumption associated with an energy-restricted diet on intestinal microbiota functionality in subjects with excess body weight, besides the effect of that type of intervention on the composition and functionality in different populations with other diseases chronic.

In conclusion, in this study, the consumption of 25g of yacon flour associated with an energy-restricted diet by subjects with excess body weight increased the genera *Bifidobacterium*, *Blautia*, *Subdoligranulum*, and *Streptococcus*. Glycation markers concentrations (AGEs and EGPs) were negatively associated with the relative abundance of the genera *Ruminococcus* and *Prevotella*, respectively, in the group that consumed yacon. Besides, SCFA were positively associated with the genera *Coprococcus* and *Howardella* in the yacon group. Therefore, yacon flour associated with an energy-restricted diet selectively changed the intestinal microbiota composition in adults with excess body weight, which may be an interesting strategy to be adopted to control obesity and related diseases. Future studies should be conducted to elucidate which phyla/specific bacterial genera correlate with the increase in body fat, which may preceed the discovery of new “targets” in the treatment of obesity.

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Tables

Table 1. Characteristics presented by the subjects at baseline according to the experimental group

Variable (units)	Control Group (n=10)	Yacon Group (n=11)
Age (years)	33.30 ± 10.65	28.36 ± 6.83
Weight (kg)	88.82 ± 13.47	89.56 ± 16.43
BMI (kg/m²)	30.55 ± 1.79	30.85 ± 3.07
Waist circumference (cm)	93.25 ± 9.42	92.06 ± 9.86
Fat mass (kg)	33.90 ± 5.00	36.32 ± 8.90
Percent fat (%)	38.69 ± 6.28	40.74 ± 7.73
Fasting Insulin (µUI/mL)	9.41 ± 3.18	9.57 ± 3.00
Fasting glucose (mg/dL)	92.00 ± 5.77	89.36 ± 7.10
HOMA –IR (AU)	2.15 ± 0.74	2.14 ± 0.78
Cholesterol (mg/dL)	192.60 ± 40.92	178.45 ± 25.03
HDL-cholesterol (mg/dL)	50.90 ± 16.41	45.18 ± 8.10
LDL-cholesterol (mg/dL)	116.10 ± 39.41	109.82 ± 25.42
Triglycerides (mg/dL)	127.50 ± 74.32	117.45 ± 48.41
Systolic blood pressure (mmHg)	115.25 ± 12.38	112.20 ± 9.24
Diastolic blood pressure (mmHg)	69.90 ± 8.88	67.30 ± 7.76

Results are given as mean ± standard deviation. Data are not significantly different by Student's t test, $p > 0.05$. BMI: body mass index, HOMA-IR: Homeostasis Model Assessment of Insulin Resistance, LDL-cholesterol: low-density lipoprotein, HDL-cholesterol: high-density lipoprotein.

Table S1: Indexes of richness and diversity according to the experimental group at baseline and after 6 weeks of intervention

Indexes	Control Group (n=10)			Yacon Group (n=11)		
	Baseline	**6 weeks**	**p-value**	**Baseline**	**6 weeks**	**p-value**
Chao1	146.27 (118.43 - 62.34)	108.12 (91.68 - 130.00)	0.36	111.41 (77.17 - 139.02)	100.00 (78.90 - 118.72)	0.04
ACE	149.43 (119.32 - 169.03)	109.06 (92.11 - 128.37)	0.32	112.39 (79.27 - 141.07)	95.36 (78.80 - 123.09)	0.04
Shannon	3.46 (2.93 - 3.82)	3.35 (3.06 - 3.65)	0.41	3.04 (2.55 - 3.42)	3.42 (3.14 - 3.56)	0.63
Simpson	0.94 (0.90 - 0.95)	0.93 (0.89 - 0.95)	0.46	3.08 (0.85 - 0.93)	0.93 (0.90 - 0.94)	0.32
Inv Simpson	16.35 (10.18 - 21.84)	13.61 (9.55 - 19.17)	0.17	9.41 (6.68 - 14.69)	14.10 (10.08 - 18.091)	0.28
Fisher	28.34 (20.10 - 34.21)	22.14 (16.17 - 25.54)	0.36	21.38 (13.95 - 26.85)	18.74 (14.54 - 23.61)	0.15

Data are presented as median (interquartile range). p value by Wilcox's test., *p<0.05. Chao1 = Richness estimator of Chao; ACE = Abundance-based Coverage Estimator; Inv Simpson = Inverse of classical Simpson diversity estimator.

Table S2: Most abundant ASVs (abundance> 0.5%, after six weeks), at baseline and after six weeks of intervention, according to the experimental group

Yacon Group (n=11)		
Taxonomy	**Baseline**	**Six weeks**
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Subdoligranulum;NA	11.78±14.45	13.13±16.01
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Faecalibacterium;NA	15.6±10.99	9.29±8.4
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Subdoligranulum;NA	5.04±3.52	5.64±4.51
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Faecalibacterium;NA	6.42±5.97	5.18±5.94
D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Verrucomicrobiales;D_4__Akkermansiaceae;D_5__Akkermansia;NA	1.9±5.07	3.46±8.81
D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Verrucomicrobiales;D_4__Akkermansiaceae;D_5__Akkermansia;D_6__uncultured bacterium	3.24±5.97	3.42±6.66
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Agathobacter;NA	8.73±9.49	3.31±4.02
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Faecalibacterium;NA	2.09±2.28	2.84±3.35
D_0__Bacteria;D_1__Firmicutes;D_2__Negativicutes;D_3__Selenomonadales;D_4__Veillonellaceae;D_5__Dialister;NA	0.3±0.52	1.52±4.15
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__[Eubacterium] coprostanoligenes group;D_6__human gut metagenome	1.76±3.59	1.35±2.23
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Christensenellaceae;D_5__Christensenellaceae R-7 group;D_6__gut metagenome	0.37±0.68	1.29±2.56
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-002;NA	1.49±1.99	1.29±1.3
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-002;D_6__uncultured organism	1.09±0.95	1.2±0.81

D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Faecalibacterium;D_6__metagenome	0.07±0.14	1.03±2.3
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Roseburia;NA	1.06±1.15	0.97±1.3
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Faecalibacterium;NA	0.23±0.62	0.89±2.94
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Bacteroidaceae;D_5__Bacteroides;NA	1.11±1.46	0.85±1.77
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-013;D_6__uncultured organism	0.12±0.18	0.83±2.4
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcus 1;D_6__metagenome	0.03±0.07	0.74±2.25
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Prevotellaceae;D_5__Alloprevotella;D_6__uncultured organism	0.19±0.53	0.68±2.06
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;NA;NA	0.31±0.53	0.67±1.45
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__[Eubacterium] ruminantium group;D_6__uncultured bacterium	0.08±0.15	0.67±1.53
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Prevotellaceae;D_5__Alloprevotella;NA	0.75±2.5	0.65±2.17
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__[Eubacterium] coprostanoligenes group;D_6__uncultured organism	0.68±0.99	0.65±0.97
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcus 2;D_6__uncultured bacterium	0.89±1.5	0.64±1.7
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Bifidobacteriales;D_4__Bifidobacteriaceae;D_5__Bifidobacterium;NA	0.35±0.44	0.63±0.74
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-002;NA	0.54±1.56	0.62±2.07
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__CAG-56;D_6__uncultured bacterium	0.09±0.18	0.62±1.42
D_0__Bacteria;D_1__Firmicutes;D_2__Negativicutes;D_3__Selenomonadales;D_4__Veillonellaceae;D_5__Dialister;NA	0.41±1.35	0.58±1.93

D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-014;NA	0.26±0.51	0.56±1.06
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Escherichia-Shigella;NA	0.79±1.95	0.52±0.86
D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rhizobiales;D_4__Beijerinckiaceae;D_5__Microvirga;NA	0±0	0.52±1.71
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia;D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__Catenibacterium;NA	0.3±0.62	0.51±1.01
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Prevotellaceae;D_5__Prevotella 9;NA	0.28±0.7	0.51±0.96
Control Group (n=10)		
Taxonomy	**Baseline**	**Six weeks**
D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Verrucomicrobiales;D_4__Akkermansiaceae;D_5__Akkermansia;D_6__uncultured bacterium	6.53±15.76	11.52±21.54
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Agathobacter;NA	6.4±4.36	8.75±10.89
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Subdoligranulum;NA	7.37±10.72	5.58±7.64
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Subdoligranulum;NA	7.07±5.79	5.33±9.03
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Faecalibacterium;NA	8±6.52	4.81±4.65
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Faecalibacterium;NA	7.02±6.24	3.35±4.17
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__[Eubacterium] coprostanoligenes group;D_6__human gut metagenome	1.13±2.35	2.69±6.24
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-002;NA	3.08±2.89	2.01±1.75
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-002;D_6__uncultured organism	2.36±2.59	1.94±2.17

D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Verrucomicrobiales;D_4__Akkermansiaceae;D_5__Akkermansia;NA	0.7±1.72	1.76±5.03
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Butyrivibrio;NA	0.38±0.53	1.4±3.77
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Faecalibacterium;NA	2.5±4.08	1.4±3.07
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Prevotellaceae;D_5__Prevotella 9;NA	2.05±4.41	1.33±2.21
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Prevotellaceae;D_5__Prevotella 9;NA	1.05±1.74	1.33±2.97
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-002;NA	0.46±1.23	1.2±2.69
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Faecalibacterium;NA	1.11±1.06	1.1±2.2
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia;D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__Catenibacterium;NA	0.43±0.72	1.09±2.28
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Bacteroidaceae;D_5__Bacteroides;NA	0.65±0.8	0.94±1.1
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Bacteroidaceae;D_5__Bacteroides;D_6__Bacteroides uniformis	0.6±1	0.87±1.74
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Bacteroidaceae;D_5__Bacteroides;NA	0.31±0.81	0.78±2.32
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;NA;NA	0±0	0.68±2.16
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Christensenellaceae;D_5__Christensenellaceae R-7 group;NA	0.56±0.89	0.67±1.42
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-014;NA	0.06±0.18	0.67±2
D_0__Bacteria;D_1__Firmicutes;D_2__Negativicutes;D_3__Selenomonadales;D_4__Veillonellaceae;D_5__Dialister;D_6__uncultured bacterium	1.75±4.11	0.65±1.41
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Roseburia;NA	0.92±1.37	0.62±0.71

D_0__Bacteria;D_1__Tenericutes;D_2__Mollicutes;D_3__Mollicutes RF39;D_4__gut metagenome;D_5__gut metagenome;D_6__gut metagenome	0.03±0.09	0.61±1.84
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Anaerostipes;NA	0.17±0.29	0.61±1.23
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospiraceae UCG-007;D_6__uncultured bacterium	0.06±0.2	0.6±1.43
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__uncultured;D_6__gut metagenome	0.67±1.29	0.53±0.6
D_0__Bacteria;D_1__Tenericutes;D_2__Mollicutes;D_3__Mollicutes RF39;NA;NA;NA	0.63±1.97	0.51±1.62
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__[Eubacterium] eligens group;NA	0.26±0.52	0.51±0.89
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Christensenellaceae;D_5__Christensenellaceae R-7 group;D_6__gut metagenome	0.45±0.64	0.51±0.9
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Christensenellaceae;D_5__Christensenellaceae R-7 group;NA	0.26±0.5	0.5±1.17
D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Verrucomicrobiales;D_4__Akkermansiaceae;D_5__Akkermansia;D_6__uncultured bacterium	6.53±15.76	11.52±21.54

Figures

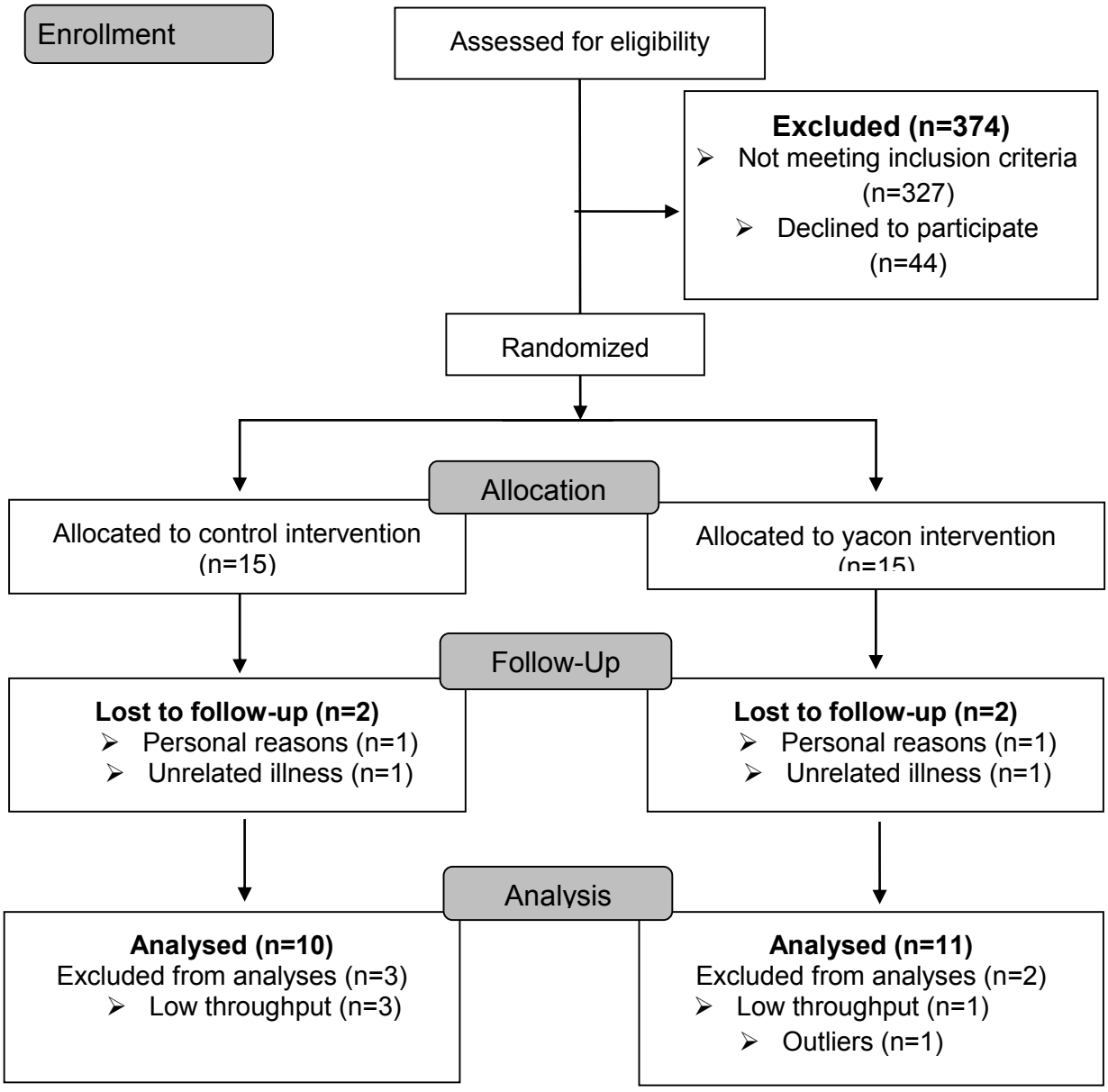

Figure 1. CONSORT diagram showing the flow of subjects through the study.

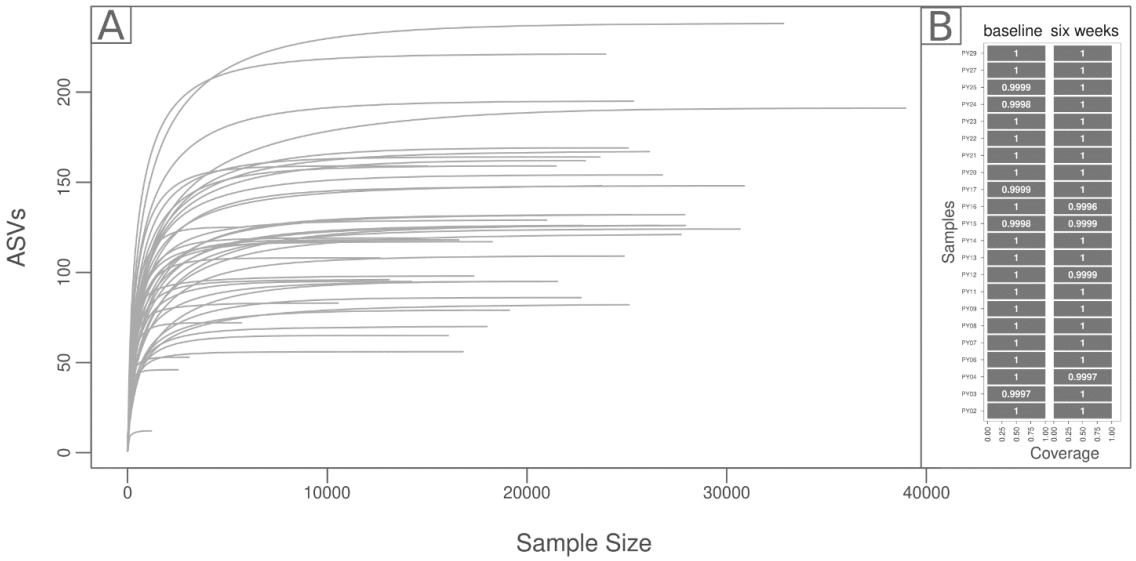

Figure S1. (A) Rarefaction curves of samples. (B) Goods Coverage index showing the sampling effort. The high values of Goods coverage (> 0.95) and the “plato” of rarefaction curves display that the sampling effort was enough to capture all the diversity in the samples.

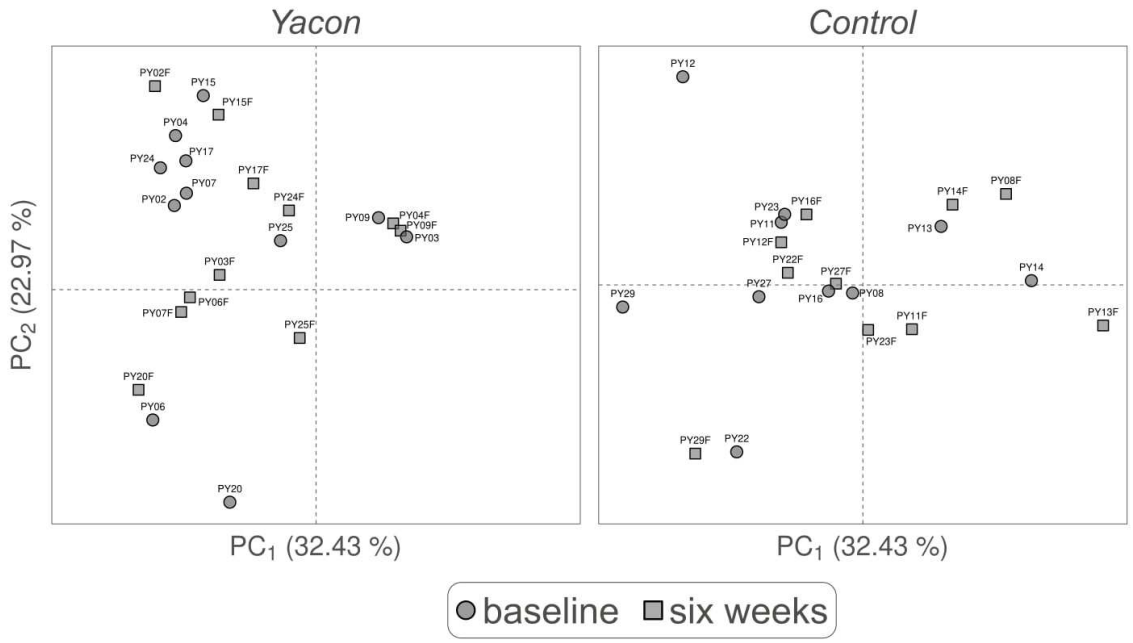

Figure 2. Principal Coordinate Analyses (PCoA) based on the Bray-Curtis distances among samples. Values in parenthesis represent the percent of explanation of each axis.

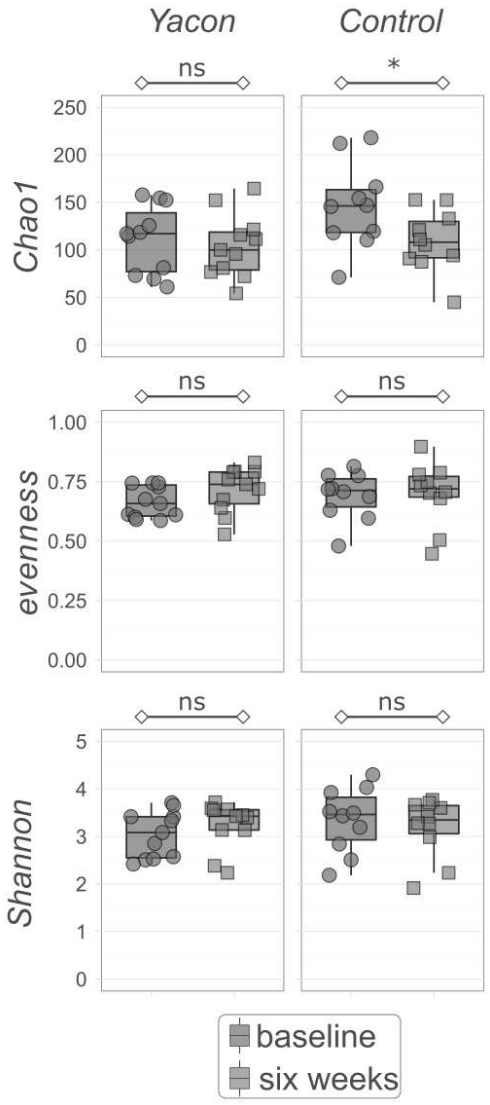

Figure 3. Estimator of richness (Chao1), evenness and Shannon's diversity from samples. * and ns stand, respectively, for significant and not significant at 0.05 confidence level by Wilcox' test.

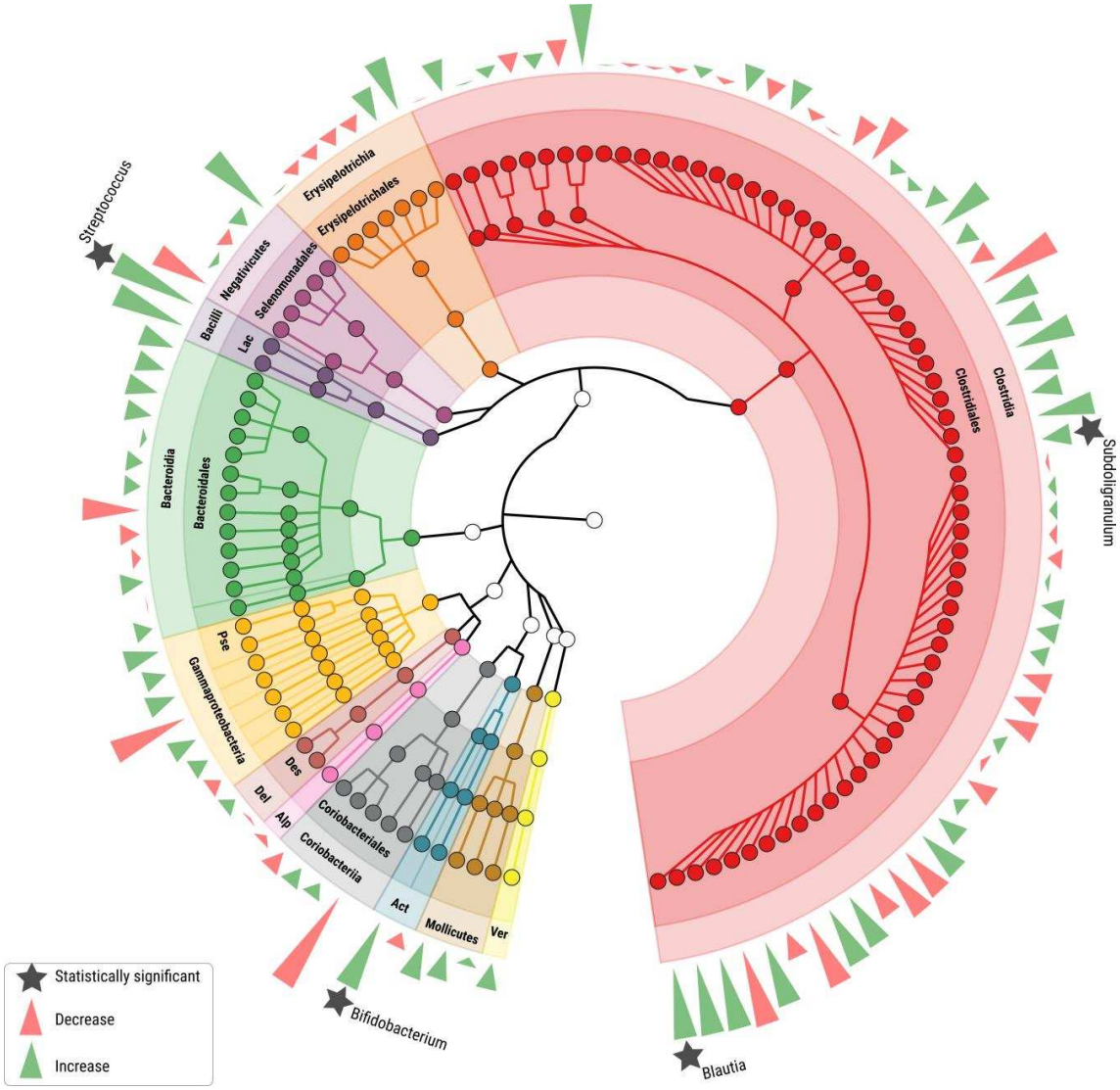

Figure 4. Taxonomic composition after six weeks of consumption of yacon flour associated with energy restricted diet in excess body weight subjects. Each node represents a taxon. From the innermost to the outmost, the nodes represent, respectivelly: kingdom, phylum, class, order, family and genus. The height of the external triangles represents the magnitude of change of taxon abudannce between baseline and six weeks after the study, and the color, red or green, indicate, respectively, decrease and increase in the genus abundance after the end of the study compared with baseline. Triangles with stars repersent significant changes at confidence level of 0.05.

Lac = Lactobacillales; Pse = Pseudomonadales; Des = Desulfovibrionales; Alp = Alphaproteobacteria; Del = Deltaproteobacteria; Act = Actinobacteria; Ver = Verrucomicrobiae

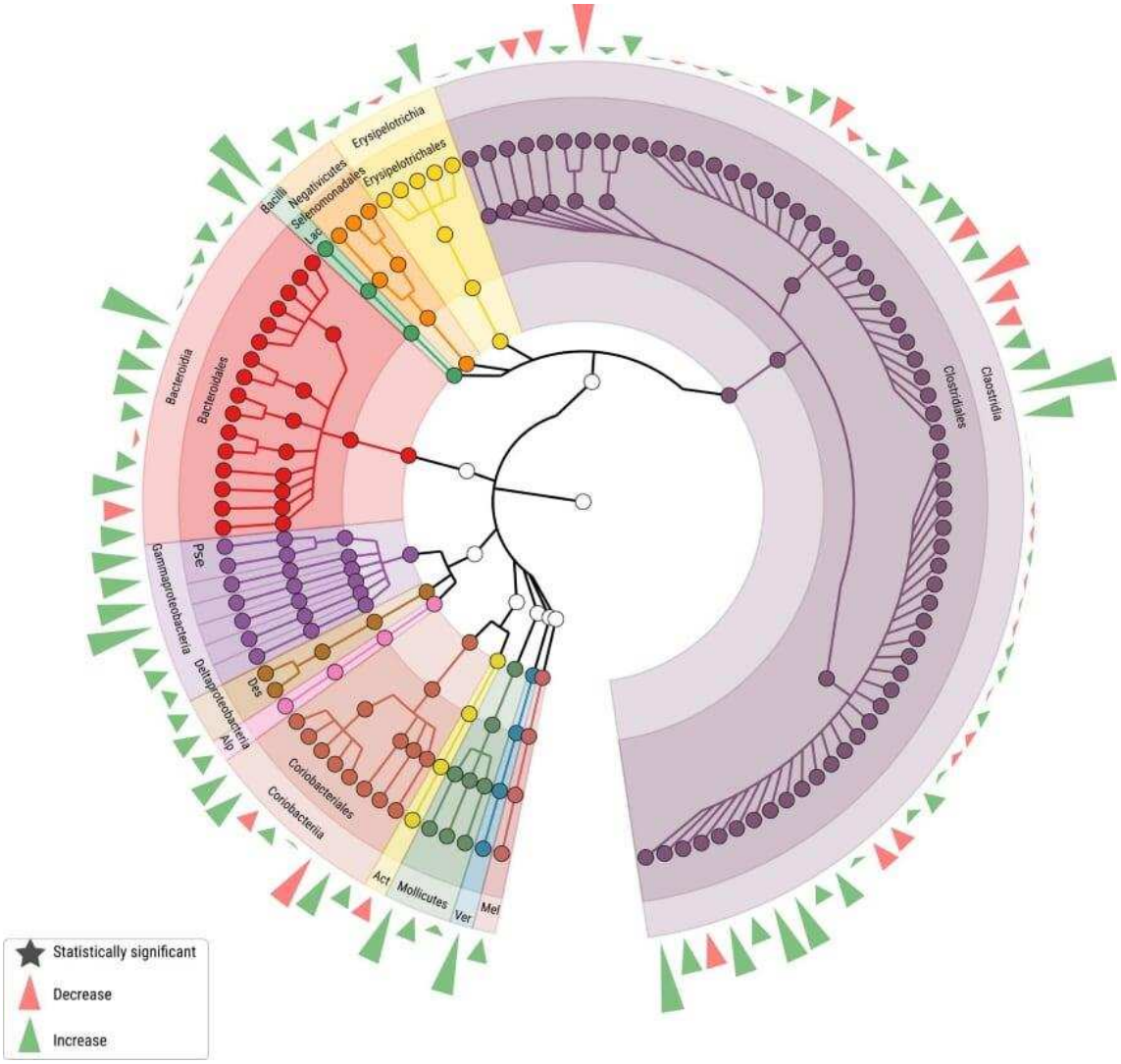

Figure 5. Taxonomic composition of consumption of energy restricted diet in excess body weight subjects. Each node represents a taxon. From the innermost to the outmost, the nodes represent, respectivelly: kingdom, phylum, class, order, family and genus. The height of the external triangles represent the magnitude of change between baseline and six weeks after the treatment, and the color, red and green, indicate, respectively, decrease and increase in the genus abundance after the end of the study compared with baseline. Triangles with stars repersent significant changes at confidence level of 0.05.

Lac = Lactobacillales; Pse = Pseudomonas; Des = Desulfovibrionales; Alp = Alphaproteobacteria; Act = Actinobacteria; Ver = Verrucomicrobiae; Mel = Melainabacteria

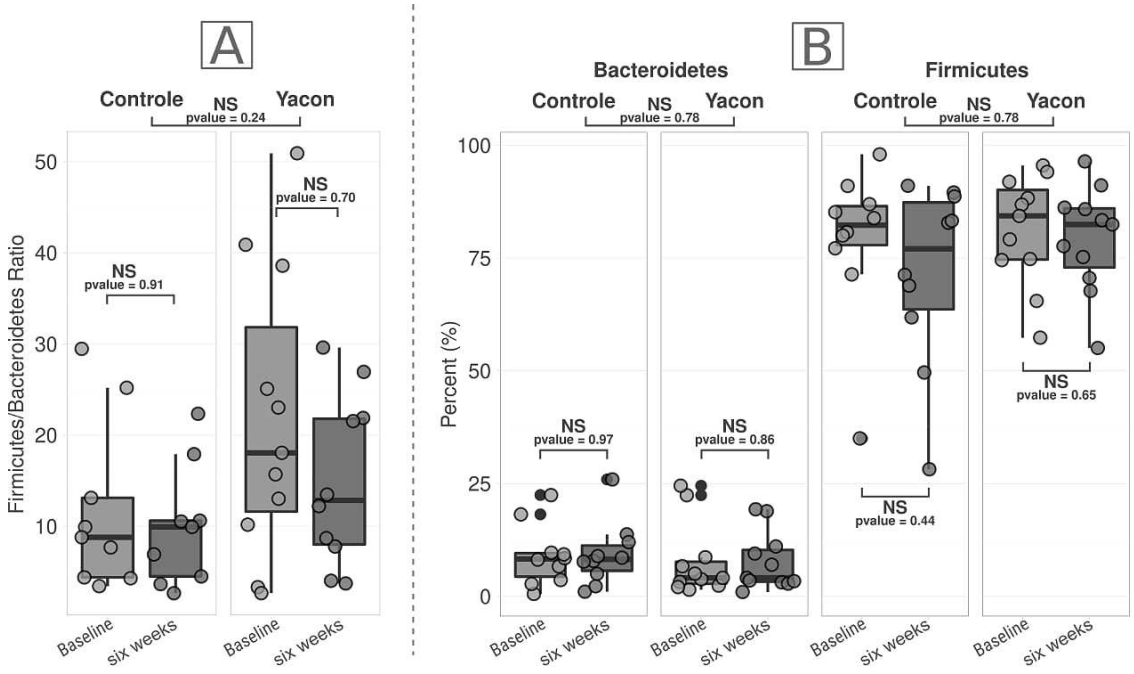

Figure 6. Percent of the Firmicutes and Bacteriodetes genera and Firmicutes/Bacteriodetes ratio before and after six weeks of consumption of yacon flour associated with an energy-restricted diet (yacon group n = 11) or only energy-restricted diet (control group n = 10) in excess body weight subjects.

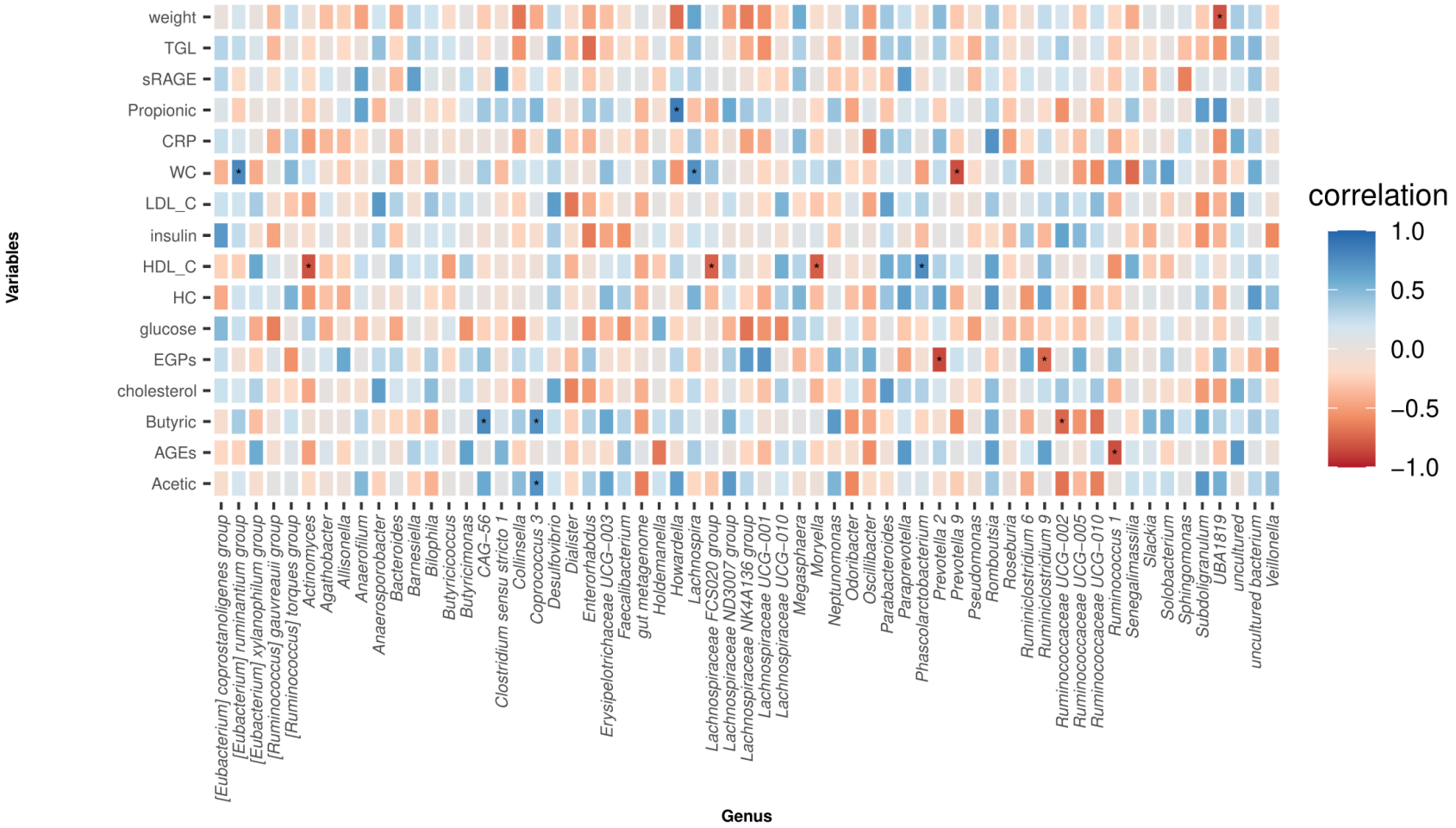

Figure 7. Heat map of the Spearman r correlations between the gut bacteria significantly modified by the consumption of yacon flour and energy restricted diet versus anthropometric/biological markers in excess body weight subjects. TGL: triglycerides; sRAGE: sRAGE: soluble receptors of advanced glycation end products; PCR: C reactive protein; WC: waist circumference; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; HC: hip circumference; EGP: early glycation products; AGE: advanced glycation end products.

4. CONCLUSÕES GERAIS

Os resultados dos estudos já publicados indicam que a ingestão de produtos finas de glicação avança (AGEs) de 3.302 kU/dia a 7.306 kU/dia e de 10,7 mg/dia a 43 mg/dia, por 2 a 12 semanas, afetou positivamente a concentração de marcadores relacionados às complicações do excesso de peso. No entanto, a utilização de diferentes unidades de AGEs (kU/100 g de alimento versus miligramas) não permitiu que comparássemos o consumo de AGEs entre os estudos, tonando difícil estabelecer um nível seguro de consumo de AGEs. Apesar disso, reduzir o consumo de alimentos processados e aqueles submetidos a altas temperaturas e baixa umidade (fritar, assar, grelhar), bem como aumentar o consumo de alimentos com conteúdos mais baixos em AGEs (frutas, vegetais, grãos, laticínios desnatados) e aqueles preparados em baixa temperatura e alta umidade (cozinhar no vapor e cozinhar por ebulição) são boas estratégias para promover saúde.

Verificamos no presente estudo que o consumo de 25g de farinha de yacon associado a uma dieta restrita em calorias por seis semanas em adultos com excesso de peso não afetou as concentrações de AGEs e produtos de glicação precoce (EGPs). Além disso, nós verificamos aumento dos gêneros *Bifidobacterium*, *Blautia, Subdoligranulum e Streptococcus* em resposta a esse tratamento.

Nós observamos, ainda, que as concentrações de marcadores de glicação se associaram positivamente a marcadores antropométricos e bioquímicos consolidados relacionados ao excesso de peso. Mudanças nos marcadores de glicação se associaram a mudanças em marcadores cardiometabólicos e de estresse oxidativo, e maiores concentrações dos EGPs no baseline se associaram a menores reduções na gordura corporal total e na concentração de malondialdeído em adultos com excesso de peso após a intervenção com yacon. As concentrações de marcadores de glicação (AGEs e EGPs) se associaram negativamente ao número de ASVs dos gêneros *Ruminococcus* e *Prevotella,* no grupo que consumiu yacon. Os AGCC se associaram positivamente aos gêneros *Coprococcus* e *Howardella.*

Os resultados dos nossos estudos de intervenção sugerem que a avaliação das concentrações de marcadores de glicação pode ser uma estratégia útil para monitorar as respostas às intervenções alimentares em indivíduos com excesso de peso. Estudos futuros são necessários para investigar os mecanismos que regulam as

concentrações desses marcadores de glicação e o efeito de diferentes tipos de alimentos nas concentrações desses marcadores.