ADRIANE MOREIRA MACHADO

EFFECT OF YACON FLOUR (*Smallanthus sonchifolius*) CONSUMPTION ON BODY WEIGHT, BODY COMPOSITION, INTESTINAL PERMEABILITY AND METABOLIC CHANGES IN EXCESS BODY WEIGHT ADULTS

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

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(Orientadora)

"Talvez não tenha conseguido fazer o melhor, mas lutei para que o melhor fosse feito. Não sou o que deveria ser, mas Graças a Deus, não sou o que era antes". (Marthin Luther King)

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LISTA DE ABREVIATURAS

%E	Percentage of energy
%L	Percentage of lactulose
%M	Percentage of manitol
µl	Microliters
µm	Micrometer
ALT	Alanine aminotransferase
AMPK	Adenosine-monophosphate-activated-protein-kinase
AOAC	Association of Official Analytical Chemists
AST	Aspartate amino transferase
BMI	Body mass index
CAT	Catalase
CHO	Carbohydrate
CI	Conicity index
CON	Control group
CRP	C-reactive protein
DXA	Dual-energy X-ray absorptiometry scan
EER	Estimated Energy Requirement
FOS	Fructooligosaccharides
FRAP	Ferric reducing antioxidant power
g	Grams
GAE	Gallic acid equivalents
GEE	Generalized estimating equation model
GSRS	Gastrointestinal symptom assessment scale
GST	Glutathione S-transferase
HDL-c	High density lipoprotein cholesterol
HOMA-IR	Homeostasis Model Assessment index
HPAEC-PAD	High-performance anion-exchange chromatography with pulsed amperometric detection
Kcal	Kilocalories
Kg	Kilograms

L/M ratio	Lactulose/mannitol ratio
L	Liters
LDL-c	Low density lipoprotein cholesterol
LIP	Lipides
MDA	Malondialdehyde
Min	Minutes
mL	Milliliters
mm	Millimeter
mM	Millimolar
NLR	Neutrohils/lymphocytes ratio
NO	Nitric oxide
PGC-1α	Peroxisome-proliferator-activated-receptor-1α-coactivator
PLR	Platelets/lymphocytes ratio
PTN	Protein
SCFA	Short chain fatty acids
TEAC	Trolox equivalent antioxidant capacity
TG	Triglycerides
VLDL	Very low density lipoprotein
YAC	Yacon flour group

RESUMO

MACHADO, Adriane Moreira, D.Sc., Universidade Federal de Viçosa, fevereiro de 2019. **Efeito do consumo da farinha de yacon (*Smallanthus sonchifolius*) no peso, na composição corporal, na permeabilidade intestinal e alterações metabólicas em adultos com excesso de peso.** Orientadora: Rita de Cássia Gonçalves Alfenas.

A raiz de yacon é uma fonte natural de frutooligossacarídeos (FOS) e compostos fenólicos. No entanto, apresenta vida de prateleira curta devido ao seu elevado conteúdo de água. Neste sentido, a farinha de yacon torna-se promissora, pois além de aumentar a vida de prateleira do yacon, apresenta maior concentração de compostos bioativos em uma menor porção de produto quando comparado à raiz. O efeito do consumo crônico de farinha de yacon sobre marcadores metabólicos ainda não foi avaliado em adultos com excesso de peso. Assim, avaliamos os efeitos do consumo crônico desta farinha aliada a uma dieta com restrição calórica no peso, na composição corporal, no consumo alimentar, na função intestinal, em variáveis bioquímicas, na permeabilidade intestinal, na concentração fecal de ácidos graxos de cadeia curta (AGCC) e em marcadores de estresse oxidativo e inflamatórios em indivíduos com excesso de peso. Para tanto, foi conduzido um estudo randomizado de braços paralelos que envolveu 26 adultos (11 homens e 15 mulheres, com idade média 31,3 ± 8,5 anos) com excesso de peso corporal (índice de massa corporal médio 30,4 ± 2,4 kg/m²). Os voluntários foram alocados em um dos dois grupos experimentais (controle (n=13) ou farinha de yacon (n=13)), receberam uma dieta com restrição calórica (-500 kcal/dia) e consumiram diariamente, durante 6 semanas, uma bebida contendo ou não 25g de farinha de yacon. Ao início e ao final do período experimental foram realizadas avaliações antropométricas, de composição corporal, da pressão arterial, de marcadores bioquímicos, da função intestinal, do consumo alimentar, da permeabilidade intestinal e de marcadores inflamatórios e de estresse oxidativo. A capacidade antioxidante da farinha foi avaliada também *in vitro*. Além disso, foi realizada a caracterização química da farinha bem como a determinação do teor de FOS e fenólicos totais. O consumo diário de 25g de farinha de yacon foi bem tolerado, não causou efeitos adversos gastrointestinais, aumentou o consumo de fibra alimentar, melhorou a consistência das fezes, reduziu a frequência de constipação e promoveu maior perda de peso corporal, do perímetro da cintura, do índice cintura-estatura, do diâmetro abdominal sagital e da gordura corporal. Somados a estes resultados, observou-se também uma redução nas concentrações de AGCC e proteína carbonilada,

além do aumento na capacidade antioxidante total do plasma. Diante dos resultados observados no presente estudo, pode-se inferir que a farinha de yacon é uma boa fonte de fibras (FOS) e parece ter um efetivo positivo no manejo do excesso de peso corporal.

ABSTRACT

MACHADO, Adriane Moreira, D.Sc., Universidade Federal de Viçosa, February, 2019. **Effect of yacon flour (*Smallanthus sonchifolius*) consumption on body weight, body composition, intestinal permeability and metabolic changes in excess body weight adults.** Adviser: Rita de Cássia Gonçalves Alfenas.

Yacon root is a natural source of fructooligosaccharides (FOS) and phenolic compounds. However, it has short shelf life due to its high water content. In this sense, the yacon flour becomes promising, because in addition to increasing the shelf life of the yacon, it presents a higher concentration of bioactive compounds in a smaller portion of product when compared to the root. The effect of yacon flour consumption on metabolic markers has not been studied in excess body weight adult humans. Thus, we evaluated the effect of the consumption of yacon flour associated with an energy restricted diet on body composition, food intake, intestinal function, biochemical variables concentration, intestinal permeability, fecal short chain fatty acids, oxidative stress and inflammatory markers concentrations in adults with excess body weight. A randomized, parallel arm study involving 26 adults (11 men and 15 women, mean age 31.3 ± 8.5 years) with excess body weight (mean body mass index 30.4 ± 2.4 kg / m²) was conducted. Subjects were allocated to one of the two experimental groups (control (n= 13) or yacon flour (n = 13)), received the prescription of energy-restricted diets (-500 kcal / day) and daily consumed a breakfast drink not containing or containing 25 g of yacon flour. At the beginning and at the end of the study, anthropometrics, body composition, blood pressure, biochemical analyses, intestinal function, food intake, intestinal permeability, inflammatory and oxidative stress markers concentrations were assessed. Antioxidant capacity was evaluated *in vitro*. Furthermore, the flour chemical characterization, FOS and total phenolics contents were evaluated. Daily consumption of 25g of yacon flour was well tolerated, did not cause adverse gastrointestinal effects, increased dietary fiber consumption, improved stool consistency, reduced constipation frequency and promoted greater body weight, waist circumference, waist to height index, sagittal abdominal diameter, and body fat reductions. In addition to these results, there was also a reduction in fecal SCFA and protein carbonyl concentrations besides increasing plasma total antioxidant capacity. Considering the results observed in the present study, it can be inferred that yacon flour is a good source of fiber (FOS) and it seems to have a positively manage excess body weight.

1. INTRODUCTION

Excess of body weight is directly associated with increased prevalence of diseases, such as type 2 diabetes, hypertension, cardiovascular diseases and cancer (ABBASI; BLASEY; REAVEN, 2013; ABDULLAH et al., 2010; DORRESTEIJN; VISSEREN; SPIERING, 2012; MUSSO; GAMBINO; CASSADER, 2011; WORLD HEALTH ORGANIZATION, 2018). Excessive fat and sugar consumption, besides low dietary fiber consumption, combined with physical inactivity have contributed to the increasing prevalence of excess body weight (HRUBY et al., 2016). Thus, the consumption of high fiber and of low calorie foods, which can help reduce food intake and thus reduce body weight is an interesting strategy to prevent / control excessive body weight.

Therefore, yacon (Smallanthus sonchifolius), a root from the Andes region, has been gaining prominence in the scientific environment. This root has a low caloric value, because it consists mainly of water (about 85% to 90%). In addition, it presents high fiber content (around 30-45g of fiber in 100g of dry matter) (CASTRO et al., 2013), mainly fructooligosaccharides (FOS), and phenolic compounds, mainly chlorogenic acid, an important antioxidant and anti-inflammatory compound (VILHENA; CâMARA; KAKIHARA, 2000).

Animal studies have associated yacon consumption as flour, extract or *in natura* (OLIVEIRA; BRAGA; FERNANDES, 2013; PARK et al., 2009) with the reduction of appetite, food consumption (HABIB et al., 2011; OLIVEIRA; BRAGA; FERNANDES, 2013), glycemia (OLIVEIRA; BRAGA; FERNANDES, 2013; PARK et al., 2009), triglycerides, very low density lipoproteins (VLDL), oxidative stress markers concentrations (HABIB et al., 2015; OLIVEIRA et al., 2016, 2017), as well as with insulin secretion improvement (HABIB et al., 2011, 2015).

Although scare, the results of human studies are often controversial. In subjects with reduced glucose tolerance (SCHEID et al., 2014) and type 2 diabetes (GORDILLO et al., 2012), lyophilized yacon supplementation (18g/day, 9 weeks) or yacon extract (3g/day, 90 days) significantly reduced fasting blood glucose. However, other authors (GENTA et al., 2009) did not observe change in fasting glycaemia in response to yacon extract supplementation (0,14 g FOS/kg of body weight/day, 120 days), although there was an improvement in insulin sensitivity, reduction in body weight and waist circumference, and an increase in the evacuation frequency in obese women. Acute consumption of yacon flour (21 g) seems to have no effect on glycemic response, appetite or food intake in healthy eutrophic subjects (ROCHA et al., 2018).

The results of recent studies identified the existence of an association between yacon consumption and intestinal barrier function improvement (GRANCIERI et al., 2017; VAZ-TOSTES et al., 2014). It has been suggested that overweight individuals have higher intestinal permeability (TEIXEIRA et al., 2012). antigens and macromolecules translocation, inflammation, oxidative stress and other complications (DELZENNE; CANI, 2011; SHOELSON; LEE; GOLDFINE, 2006).

Although promising for the obesity treatment, the results of the studies have shown that the in natura root is highly perishable (SANT'ANNA et al., 2015). Therefore, the use of yacon flour becomes an interesting strategy since it has a longer shelf life and it provides FOS higher FOS and phenolic compounds intake in a smaller portion compared with the *in natura* root, in addition to presenting greater practicality of use and incorporation in food preparation (PEREIRA et al., 2013; SCHER; RIOS; NOREÑA, 2009).

Studies with yacon flour in humans are scarce (ROCHA et al., 2018; VAZ-TOSTES et al., 2014) and in our search we did not identify any study that evaluated the effect of chronic consumption of yacon flour on body composition, intestinal permeability, fecal concentration of short chain fatty acids (SCFA), biochemical, oxidative stress and inflammatory markers concentrations in overweight adult subjects. Thus, the importance of conducting intervention studies to assess the effects and mechanisms of action of yacon flour in these markers is emphasized, aiming to improve the metabolic profile of these individuals. Therefore, our hypothesis is that yacon flour supplementation may cause beneficial systemic (reduced concentration of oxidative stress and inflammation markers, improvement in insulin sensitivity, glycemic control) and local effects (reduced intestinal permeability, body weight and body fat) and is therefore a useful strategy in the treatment of overweight.

This study is part of a larger project entitled "Effect of yacon flour consumption (*Smallanthus sonchifolius*) associated with an energy restricted diet on metabolic changes in overweight subjects". The effects of the acute consumption of yacon flour were evaluated by our group and the results are available in the thesis entitled "Characterization of yacon flour (*Smallanthus sonchifolius*) and its acute effect on glycemic response and food intake in excess body weight subjects".

2. OBJECTIVES

2.1 General objective

To evaluate the effects of the consumption of yacon flour on body weight, body composition, intestinal permeability and metabolic changes in overweight subjects: a randomized, double-blind, placebo-controlled clinical trial

2.2 Specific objectives

- To evaluate the yacon flour nutritional composition and its antioxidant capacity *in vitro*;
- To evaluate the effects of yacon flour consumption on anthropometry and body composition;
- To evaluate the effects of yacon flour consumption on biochemical variables, insulin resistance, oxidative stress and inflammatory profile;
- To evaluate the effects of yacon flour consumption on intestinal health.

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4. ARTIGOS

4.1 Original Research

Consumption of yacon flour improves body composition and intestinal function in overweight adults: a randomized, double-blind, placebo-controlled clinical trial

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ABSTRACT

Background & Aims: Yacon root is a natural source of fructooligosaccharides (FOS), and it has been studied for its potential effect as a functional food. However, FOS in the *in natura* root are rapidly hydrolyzed. Thus, the use of dehydrated products such as flour ensures stability of FOS. The effect of chronic consumption of yacon flour on body composition, food intake and of biochemical variables concentration has not yet been evaluated in humans. Thus, we evaluated the effects of yacon flour consumption on these variables associated with excess body weight. **Methods:** Twenty-six adults (31.3 ± 8.5 years) with excess weight (30.4 ± 2.4 kg / m², 40.16 ± 6.7% body fat) participated in this randomized, double-blind, six-week study. Subjects were randomly allocated to one of the experimental groups (control (n= 13) or yacon flour (n = 13)), received the prescription of energy-restricted diets (- 500 kcal / day) and daily consumed a breakfast drink not containing or containing 25 g of yacon flour (0.1 g of FOS / kg body weight). At the beginning and at the end of the study, biochemical analyses (glycemia, insulinemia, lipid profile and liver function markers), anthropometrics (weight, waist, hip and neck circumference and sagittal abdominal diameter), body composition (lean mass, total body fat, trunk fat, android fat and gynoid fat), blood pressure, intestinal function, and food intake were assessed. **Results:** Daily yacon flour consumption was well tolerated, did not cause adverse gastrointestinal effects, increased dietary fiber consumption, promoted greater body weight, waist circumference, waist to height index, sagittal abdominal diameter,

and body fat reduction, besides improving bowel function in comparison to the control group. **Conclusions:** Yacon flour served as a natural fiber supplement and proved to be an effective adjuvant to be used in nutritional strategies to control excess body weight.
Key words: yacon, fructooligosaccharides, adiposity, glycemia, gastrointestinal function

INTRODUCTION

Nearly two billion adults around the world are overweight. Of these, over 650 million are obese. Excess of body weight is directly associated with an increase in the prevalence of diseases, such as type 2 diabetes, hypertension, cardiovascular diseases and cancer (WORLD HEALTH ORGANIZATION, 2018). Excessive fat and sugar consumption and low dietary fiber consumption, combined with physical inactivity have contributed to the increasing prevalence of excess weight. On the other hand, the consumption of foods rich in fibers and of low caloric value that can help reduce food intake and thus can lower body weight is an interesting strategy to prevent / control this pathology.

In this sense, the yacon (*Smallanthus sonchifolius*) tuberous root, originating in the Andes region, has been gaining prominence in the scientific environment. That root has a low caloric value, since it contains more than 70% of water and it is rich in dietary fiber (approximately 45g in 100g dry matter), mainly fructooligosaccharides (FOS) (CAETANO et al., 2016). Due to the naturally high concentrations of FOS, yacon root has been extensively studied for its potential as a functional food (CAETANO et al., 2016). However, soon after yacon harvesting, FOS hydrolysis and increase in the amount of free sugars occur. On the other hand, dehydrated products of that root, such as flour, guarantees the existence of a natural product with great FOS stability (CAMPOS; AGUILAR-GALVEZ; PEDRESCHI, 2016). Additionally, the consumption of flour allows the ingestion of a greater amount of FOS in a smaller portion of food, when compared to the root i*n natura.*

Few studies have evaluated the functional effects of yacon flour in humans (ROCHA et al., 2018; VAZ-TOSTES et al., 2014). The effect of chronic flour consumption on body composition, food intake and biochemical variables concentrations has not yet been assessed in humans yet. However, yacon supplementation in the lyophilized form, in extract or syrup, exerted positive effects on body weight (GENTA et al., 2009), blood lipid profile (GENTA et al., 2009), glycemic response (GENTA et al., 2009; GORDILLO et al., 2012; SCHEID et al., 2014), and intestinal function (GENTA et al., 2009; GEYER et al., 2008; SCHEID et al., 2014). Additionally, fructans, present in large quantities in yacon, stimulate the secretion of endogenous intestinal peptides, favoring the reduction of food intake and the beneficial modulation of the intestinal microbiota (CAMPOS et al., 2012).

The benefits highlighted above suggest that yacon flour consumption may be effective to prevent and treat excess body weight and associated complications. Thus, the objective of the present study was to evaluate the effects of yacon flour consumption on variables associated with excess body weight, such as body composition, anthropometry, food intake, biochemical variables, intestinal function and blood pressure.

METHODOLOGY

Chemical composition of yacon flour

The chemical composition of yacon flour (Linea Verde Alimentos, Curitiba, Brazil) was determined using official methods of analysis of Association of Official Analytical Chemists (AOAC) (HORWITZ, 2002). Total carbohydrate content was calculated by difference, subtracting from 100 the sum of the values obtained for moisture, protein, fat and ashes. Dietary fiber content was obtained from AOAC enzymatic-gravimetric method, using Total Dietary Fiber Assay Kit (Sigma-Aldrich®). FOS content was determined according to the methodology described by Pedreschi et al. (PEDRESCHI et al., 2003) with some modifications. Total dietary fiber was determined adding the obtained dietary fiber plus FOS contents.

FOS determination was based on quantification of glucose, fructose, and sucrose present in the sample before and after enzymatic hydrolysis of FOS. Sugars were measured by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Metrohm, Herisau, Switzerland) through a column Metrosep Carb 1 (dimensions 150×4.0 mm) (Metrohm, Herisau, Switzerland). The mobile phase used was NaOH 0.2 mol L^{-1}, a flow rate of 0.5 mL min^{-1} and temperature 30 °C column. To determine the initial amounts of sugars, the yacon flour was diluted (0.3 g of flour in 30 mL of ultrapure water) and centrifuged at 7000g for 20 min. Before injection into the chromatograph, 300 μL of the supernatant were removed and diluted in 10 mL of ultrapure water. For enzymatic hydrolysis, 100 μL of the supernatant was removed and mixed with 50 μL of the inulinase solution (Megazyme, County Wicklow, Ireland) in 50mM acetate buffer, pH 4.5. The mixture was incubated in a 40 °C water bath C for 30 min. The sugars were identified and quantified by comparing the retention times of previously analyzed standards. The concentration of FOS was calculated according to Prosky & Hoebregs (PROSKY; HOEBREGS, 1999) and Pedreschi et al., (PEDRESCHI et al., 2003). The results were expressed as g fructans per 100 g of flour.

Subjects

Subjects were initially recruited through local advertisements, from August 2017 to May 2018. Subjects were adult men and women (31.3 ± 8.5 years old), with excess body weight (30.4 ± 2.4 kg / m², 40.16 ± 6.7% body fat), who regularly consumed breakfast, had mild low physical activity level and dietary restraint ≤ 14. Smokers, pregnant/lactating women, and people with: habitual alcohol consumption greater than 30 g/day; use of medications that affect glycemia or energy metabolism; use of medications, herbs or diets to reduce appetite and body weight; had a body weight gain or loss of at least 5 kg in the 3 months prior to the beginning of the study; recent change in the level of physical activity; aversion or intolerance to the food provided in the study; existence or history of endocrine, cardiovascular, arterial hypertension, liver and/or gastrointestinal diseases; report of eating disorders; use of laxatives or antibiotics in the three months prior to the start of the study; use of probiotics, prebiotics or symbiotics (> 2 times per week) in the month prior to the start of the study, and women with menstrual irregularity (three months prior to the beginning of the study) were not included in the study.

Subjects were informed about the objectives of the study and signed a consent form before being included in the study. The protocol of the present study was approved by the Universidade Federal de Viçosa Ethics Committee (Process no.1,875,372 / CAAE no. 62047316.6.0000.5153) and was conducted in accordance with 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 Protocol

In this is double-blind, parallel, randomized, placebo-controlled clinical trial study with a duration of six weeks (± 5 days), the volunteers were randomly allocated to the control group or the yacon flour group. We adopted a tolerance of ± 5 days to the end the women's trial, to ensure that anthropometry/body composition were not assessed in the menstrual period.

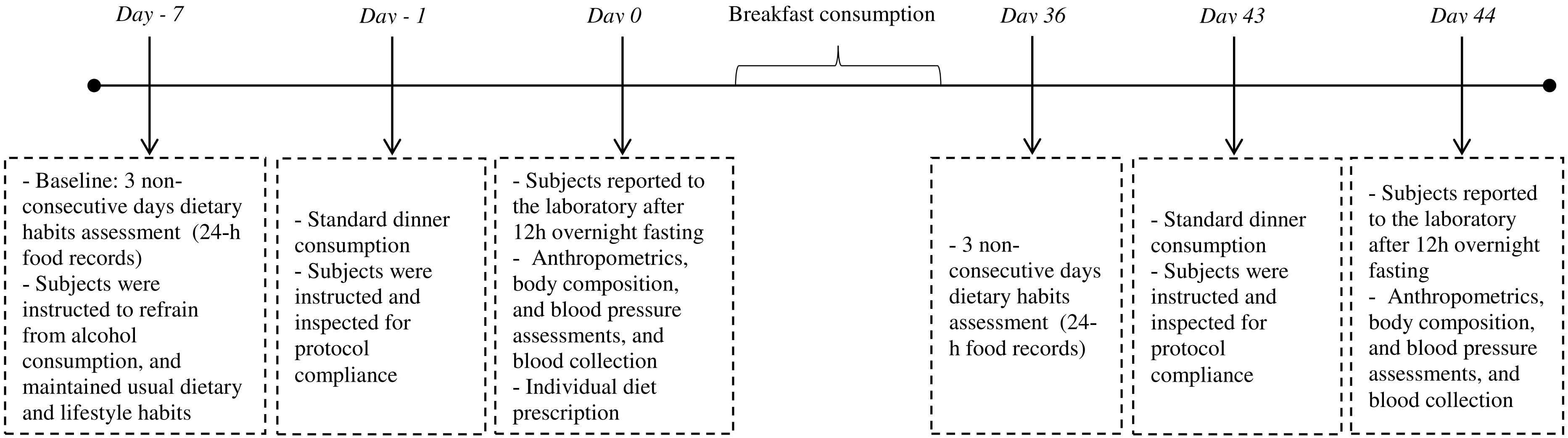
All participants were allocated (1:1) to receive either yacon flour or placebo (control). Block randomization technique (with block size equal to 4) was used to allocate the subjects into the groups. This technique was applied by a person who was not part of the research group.

During the intervention period, energy-restricted diets (- 500 kcal / day) were prescribed and subjects daily consumed 350 mL of a control drink without the addition of yacon flour (CON) or a test drink (YAC) containing yacon flour at breakfast. The other meals of the day were consumed in free living conditions.

Food intake, body composition (total body fat and specific regions (trunk, android and gynoid) and total lean mass), anthropometry (weight, waist, hip, thigh and neck circumferences and sagittal abdominal diameter), blood pressure, biochemical evaluation (glycemia, insulin, lipid profile and markers of liver function), stool consistency, gastrointestinal symptoms, and fecal pH were assessed before the beginning, and at the end of the study. Subjects were instructed to maintain their level of physical activity constant throughout the study, and were also instructed not to engage in strenuous physical activity within 24 hours prior to the biochemical assessments.

One week before beginning the study, subjects were instructed not to consume alcoholic beverages and not to alter their usual food intake. A standard dinner consisting of 200 ml of Tial ® nectar (grape flavor), 85 g of pasta and 10 g of Parmesan cheese (523 kcal, 57.4% CHO, 10.3% PTN, 32.3% LIP, 2.1g fibers) was supplied to be consumed the night before the assessments (Suppl. Figure1).

Suppl. Figure 1.



Breakfast

The amount of yacon flour (25g/day, providing 0.1g FOS/kg body weight/day) added to the YAC group drink was determined based on other studies and previous laboratory tests to avoid possible undesirable gastrointestinal effects due to excessive yacon flour ingestion (GENTA et al., 2009; SCHEID et al., 2014). Once purchased, the yacon flour was stored at 21°C and 64% humidity until the moment of use.

Corn starch was added to CON drink so that it presented similar energy and macronutrient contents as the YAC drink (Table 1). The drinks' nutritional composition was based on the nutritional information contained in the food labels and on the nutritional composition analysis of yacon flour (Table 2). Seven flavors of drinks (cappuccino, cocoa milk, coffee with milk, guava, mango, blackberry and passion fruit vitamins), presenting similar nutritional composition, were served to avoid monotony and increase adherence to the study protocol.

Table 1. Mean ± SD nutritional composition of the seven rotating breakfast drinks[1] tested

Nutritional composition	Control breakfast	Yacon flour breakfast
Energy (kcal)	418 ± 21.6	437 ± 23.9
Carbohydrate (g)	60.23 ± 5.18	63.14 ± 5.09
Protein (g)	14.15 ± 1.3	14.85 ± 1.3
Total fat (g)	13.38 ± 0.49	13.94 ± 0.41
Dietary fiber[2] (g)	2.63 ± 3.91[a]	13.72 ± 3.97[b]
FOS (g)	-	8.69

[1] Based on the information contained in the food labels of the ingredients added to prepare the drinks, and on the nutritional composition analysis of yacon flour determined by the researchers (Table 2). [2] Dietary fiber: soluble fiber (kit) + insoluble fiber (kit) + FOS. Means followed by distinct letters, in the same line, indicate inter-groups difference by Student's *t* test or Mann-Whitney U signed-rank test, $p < 0.05$. *FOS:* fructooligosaccharides.

Table 2 – Chemical characterization of the yacon flour used to prepare the breakfast drinks

Nutrients	Content (g / 100 g flour)
Carbohydrate[1] (g)	85.5
Protein (g)	2.8
Total fat (g)	0.9
Fiber[2] (g)	46.9
FOS (g)	34.7

[1] Carbohydrate: 100 - (protein + fat + ashes + moisture). [2] Fiber: soluble fiber (kit) + insoluble fiber (kit) + FOS. *FOS:* fructooligosaccharides.

The volunteers daily attended the Laboratory of Food Intake, Department of Nutrition and Health, Federal University of Viçosa – Brazil, from Monday to Friday to consume breakfast. On weekends, identical drinks were provided to be consumed at home. Subjects were requested to bring to the laboratory any quantities of food supplied but not ingested on weekends. In addition, adherence to the protocol on weekends was verified by asking the subjects about the consumption of the breakfast provided. A trained investigator, not involved on data collection and analyses, was responsible for assessing adherence to the protocol, preparing and serving the drinks. Drinks were served in colored cups to avoid visual identification of the type of drink tested. There was no description or dietary information on these cups.

Prescribed diets

Energy-restricted diets were prescribed (-500 kcal/day) (ASSOCIAÇÃO BRASILEIRA PARA ESTUDO DA OBESIDADE E DA SÍNDROME METABÓLICA, 2016) which were calculated considering the level of physical activity, the estimated energy requirement *(Estimated Energy Requirement* - EER) for each subject and the nutritional value of the daily drinks consumed. The diets prescribed to the experimental groups presented similar energy density, and macronutrients distribution (1,734 ± 494.2 kcal *vs.* 1,729 ± 471.1kcal, carbohydrate: 51.6 ± 3.6% E *vs.* 51.3 ± 2.4% E, protein: 21.2 ± 2.4% E *vs.* 21.0 ± 1.9% E, fat: 28.8 ± 2.4% E *vs.* 29.1 ± 2.0% E, for CON and YAC, respectively).

Dietary assessments

Food intake on the week before baseline, and during the experimenal period, was assessed using three non-consecutive days (2 week days and 1 weekend day) 24-h food records. Each dietary record was review with the subjects to ensure accuracy and completeness. Macronutrients and dietary fiber intakes were analysed by a two dietitians and the results of these analyses were checked by a third dietitian, using DietPro software (version 5.2i, Agromídia, MG, Brazil).

Anthropometric, body composition, and blood pressure measurements

Body weight was measured on a digital platform scale with a resolution of 50 g (Toledo®, Model 2096PP/2, SP, Brazil), while subjects were barefoot and wearing lightweight clothing. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Wiso, Chapecó, SC, Brazil). BMI was calculated by dividing body (kg) by height (m) squared. Waist circumference and sagittal abdominal diameter were measured in the midpoint between the last rib and iliac crest. Hip, thigh and neck circumferences were also measured. All variables were measured in triplicate and the average of the two nearest values of the three collected measurements was recorded. Waist/heigh, waist/hip, and conicity index (CI) were calculated following the formula: CI = [waist circumference (m)]/[0.109 √(body weight (kg)/height (m))]. Blood pressure was assessed using an automatic Omron HEM-7200 device (Omron Inc., Dalian, China) in both arms (MANCIA et al., 2007).

Dual-energy X-ray absorptiometry scan (DXA) (model Prodigy Advance, GE Healthcare Inc., Waukesha, WI) was performed to assess changes in body composition according to manufacturer's instructions. Values of lean mass, total body fat, and fat distribution (truncal, gynoid, and android regions) were obtained. Anthropometric and blood pressure measurements were assessed by a single investigator.

Categorization of bowel movements and evaluation of gastrointestinal symptoms

The Bristol scale was used to categorize bowel movements. This scale descriptively evaluates the shape and consistency of stools (MARTINEZ; AZEVEDO, 2012). Gastrointestinal symptoms presented during the study were evaluated using a gastrointestinal symptom assessment scale (GSRS) translated and validated for the Brazilian population (SOUZA et al., 2016).

Biochemical evaluation

Antecubital blood samples were collected after 12 hours of fasting. Samples were then centrifuged for serum and plasma separation (3500rpm, 4°C, 15min), and immediately frozen at -80°C until analysis. Glucose, triglycerides, total cholesterol, high density lipoprotein cholesterol (HDL-c), aspartate amino transferase (AST) and alanine aminotransferase (ALT) concentrations were quantified by an automated analyzer system (BS-200™ Chemistry Analyzer, Mindray) using commercially available colorimetric assay kits (K802, K117, K083, K071, K048 and K049, respectively; Bioclin®, MG, Brazil). Low density lipoprotein (LDL-c) concentration was calculated following the formula: LDL-c = total cholesterol - HDL-c - (triglycerides / 5). Insulin was determined by chemiluminescent immunoassay (*Access Ultrasensitive Insulin*). The Homeostasis Model Assessment index (HOMA-IR) was calculated and insulin resistance was identified according to the cutoff point established by Geloneze & Tambascia (GELONEZE; TAMBASCIA, 2006). The atherogenic index (TG / HDL-c ratio) (DOBIÁSOVÁ, 2006) was also calculated.

Statistical analysis

The present study had a statistical power of 95 % (MERA; THOMPSON; PRASAD, 1998) to detect a 7% reduction in the subjects' body fat, considering their baseline mean and standard deviation values. Body fat and body weight were measured as a primary outcome, whilst other anthropometrics and body composition variables, glycemic parameters, lipid profile, liver function markers, blood pressure, intestinal function, and food intake were measured as secondary outcomes.

Data was entered by two independent researchers to ensure reliability and accuracy. Statistical analyses were conducted using SPSS software (SPSS Inc., Chicago, IL, 2015, version 22.0.0.0). Delta (Δ) was calculated subtracting the baseline value from the final value obtained. Data normality and homoscedasticity were evaluated by the Shapiro-Wilk and Levene tests, respectively. The nutritional composition of the breakfast drinks tested and the description of the sample data at baseline were compare through Student's *t* test or Mann-Whitney U signed-rank test. The effect of the intervention was assessed by comparing the outcome variables intra and inter CON and YAC groups using generalized estimating equation model (GEE). For the variables with normal distribution, a connection identify function was used.

For the variables that did not follow normal distribution, gamma distribution with log link was used. The working correlation matrix used was unstructured and robust estimator covariance matrix. CON group was chosen as reference. Unstandardized Coefficients of the interaction term between time and the group, and the associated confidence interval (95% CI) were reported. We used a Bonferroni post-hoc to identify the differences on group, time and group*time interaction. These models were adjusted by baseline values. A $\alpha < 0.05$ was adopted as the level of statistical significance.

RESULTS

Subjects' characterization

Four hundred and four potential subjects were assessed for eligibility, but only thirty were selected. Of these, four subjects did not complete the study protocol (Figure 1). Thus, data from twenty-six subjects was included in the analyses.

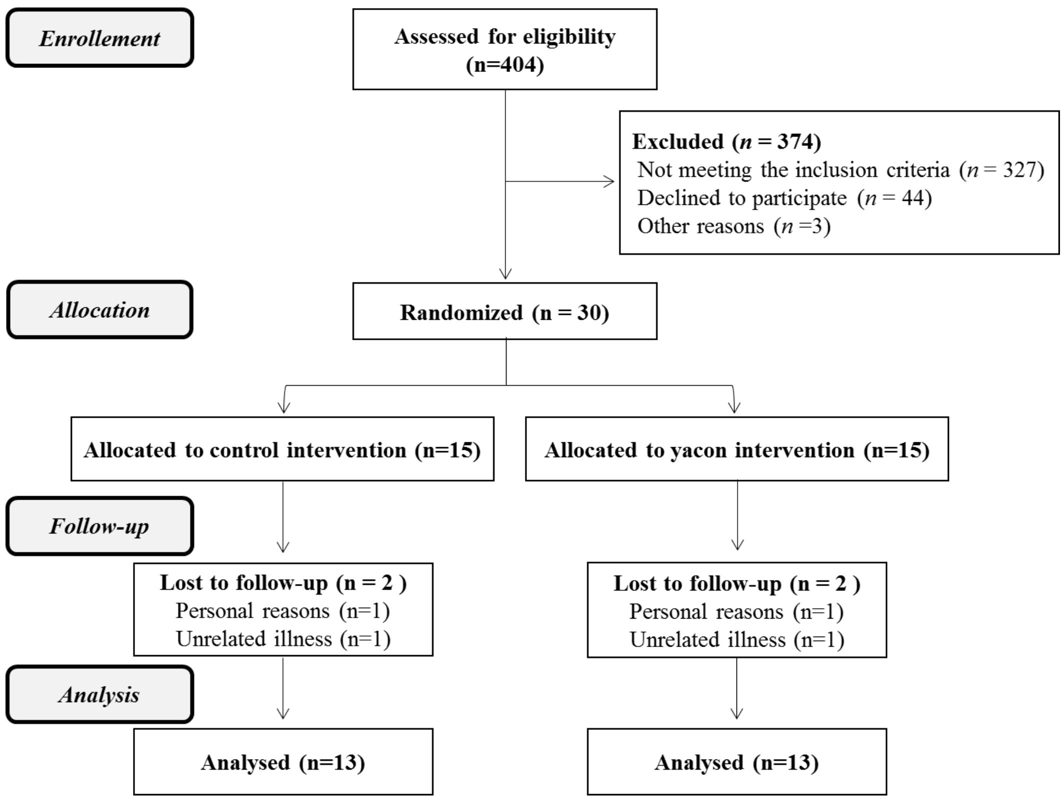


Figure 1: CONSORT diagram showing the flow of participants through the study.

Subjects' body composition, food intake and biochemical variables concentrations did not differ the groups at baseline. According to BMI, most (57.7 %) of the subjects were obese, and 42.3 % were overweight. However, according to body fat %, all subjects were considered obese. The subjects were 31.3 ± 8.5 years old, most (57.7 %) of them were women, and none of them had systolic blood pressure higher than 139 mmHg and diastolic blood pressure higher than 90 mmHg (Table 3). Thirty-eight percent had high cholesterol, 30.7 % high triglycerides and 19.2% high LDL, according to the Brazilian Society of Cardiology (FLAUDI et al., 2017). None of the subjects had blood glucose higher than 5.5 mmol/L. All subjects conducted low level of physical activity.

Table 3. Mean baseline characteristics of the subjects according to experimental groups

	Control group (n = 13)	**Yacon flour group (n = 13)**
Men / Women (n)	6/7	5/8
Age (years)	32.92 ± 9.68	29.77 ± 7.26
Body weight (kg)	84.93 ± 13.83	88.52 ± 15.21
Body mass index (kg/m^2)	30.08 ± 2.04	30.80 ± 2.85
Waist circumference (cm)	100.17 ± 8.18	102.62 ± 9.37
Sagittal abdominal diameter (cm)	19.12 ± 2.16	18.78 ± 2.26
Total body fat (kg)	32.56 ± 5.41	36.61 ± 8.15
Total lean mass (kg)[1]	48.28 ± 11.87	46.57 ± 10.00
Systolic blood pressure (mmHg)	112.66 ± 8,43	113.96 ± 11.03
Diastolic blood pressure (mmHg)	67.33 ± 7,17	69.30 ± 7.86
HOMA-IR	2.11 ± 0,68	2.11 ± 0.72

[1] Non-parametric data. Data are not significantly different by Student's *t* test or Mann-Whitney U signed-rank test, p > 0.05.

Two subjects from the YAC group and three from the CON group were considered insulin resistant. However, after removing these subjects from the analyses did not alter the results of the comparisons between groups. Therefore, these subjects were kept in their respective groups and thus, no subject was excluded from the analyses.

Food intake

In both groups, the average daily dietary fiber intake at baseline was below the recommended level (14g of fiber/1000kcal/day). At the end of six weeks, both groups reached the daily recommendation. However, on YAC group dietary fiber intake was 52% higher than CON group. Energy, carbohydrate, and fat intakes reduced in both groups (Table 4).

Anthropometry and body composition

Body weight, total body fat, waist-to-height ratio, waist-to-hip ratio, conicity index, sagittal abdominal diameter, besides waist, neck and thigh circumferences reduced in both groups after six weeks of intervention (Table 5). However, at the end of the experimental period, YAC group present lower body weight, gynoid fat, waist circumference, sagittal abdominal diameter and waist/height index than CON group (Figure 2). Besides, hip circumference and body fat in specific regions (trunk and android body fat) reduced, and total lean mass increased only in the YAC group.

Gastrointestinal effect

YAC subjects had higher frequency of reflux and heartburn episodes at baseline (Table 6). During the first days of intervention, approximately 15% of the YAC group subjects reported abdominal discomfort symptoms, such as flatulence and abdominal pain. These effects decreased after the second week and no significant difference was observed at the end of the experimental period. YAC group presented lower constipation frequency, and a higher frequency of softened stools at the end of the experimental period compare with CON group (Table 6). Besides, hard stools frequency reduced in the YAC group (Table 6).

Biochemical tests

The concentrations of most of the biochemical variables (glucose, insulin, lipid profile, and liver function markers) assessed in this study did not differ at baseline or after six weeks of intervention between groups (Suppl. Table 1). All YAC group subjects that had dyslipidemia at baseline reduced blood lipid profile concentration, and in 50% of them, the values reached normality. On the other hand, that reduction was verified in 69% of the CON group subjects with dyslipidemia, and of those, 40% reached normality.

Table 4. Dietary assessments at baseline and after the 6-week intervention according to experimental groups

	Control (n = 13)		Yacon flour (n = 13)		**β [2] (95% CI)**
	Baseline	**6 weeks**	**Baseline**	**6 weeks**	
Energy (kcal)	1,877 ± 158.2[a]	1,552 ± 109.5[b]	2,018 ± 124.5[a]	1,557 ± 94.6[b]	- 135.94 (-527.27; 255.37)
Carbohydrate (g)	239.1 ± 19.7[a]	191.6 ± 10.9[b]	270.1 ± 22.7[a]	188.8 ± 12.8[b]	-33.79 (-91.61; 24.02)
Protein (g)[1]	70.9 ± 8.9	77.2 ± 8.9	75.5 ± 4.9	77.9 ± 7.1	-0.05 (-0.33; 0.22)
Fat (g)[1]	69.5 ± 8.9[a]	53.0 ± 4.9[b]	70.6 ± 4.2[a]	54.5 ± 3.9[b]	0.01 (-0.25; 0.27)
Dietary Fiber (g)	17.2 ± 1.4[a,A]	25.5 ± 1.4[b,A]	24.9 ± 2.3[a,B]	38.8 ± 1.9[b,B]	5.59 (0.51; 11.69)

Values are means ± SEM. [1] Non-parametric data. [2] Unstandardized *beta* for comparison between-group at 6 weeks (reference category: control group). Means followed by different upper-case letters indicate between-group difference (baseline *vs.* baseline or 6 weeks *vs.* 6 weeks) by generalized estimating equation model (GEE), $p < 0.05$. Between-group comparisons at 6 weeks was adjusted for baseline. Means followed by different lowercase letters, in the same line, indicate intra-group difference by GEE, $p < 0.05$.

Table 5. Anthropometric, body composition and blood pressure at baseline and after the 6-week intervention according to experimental groups

	Control (n = 13)		Yacon flour (n = 13)		β [2] (95% CI)
	Baseline	6 weeks	Baseline	6 weeks	
Hip circumference (cm)	110.28 ± 1.11	109.53 ± 1.11	114.33 ± 7.03[a]	112.86 ± 1.76[b]	-0.72 (-1.66; 0.23)
Thigh circumference (cm)	62.13 ± 0.84[a]	61.08 ± 0.93[b]	63.82 ± 1.20[a]	62.45 ± 1.03[b]	-0.32 (0.38; -1.07)
Neck circumference (cm)	37.98 ± 0.97[a]	37.51 ± 0.95[b]	38.10 ± 0.98[a]	37.23 ± 0.89[b]	-0.24 (-0.88; 0.07)
Waist / hip index	0.90 ± 0.01[a]	0.88 ± 0.01[b]	0.89 ± 0.01[a]	0.86 ±0.01[b]	-0.01 (-0.02; 0.00)
Conicity index	1.29 ± 0.02[a]	1.26 ± 0.02[b]	1.30 ± 0.02[a]	1.26 ± 0.01[b]	-0.02 (-0.04; 0.01)
Total lean mass (kg)[1]	49.13 ± 3.10	48.77 ± 2.87	48.11 ± 3.07[a]	49.14 ± 3.12[b]	0.03 (-0.00; 0.06)
Total body fat (kg)	32.56 ± 1.44[a]	31.34 ± 1.45[b]	36.61 ± 2.17[a]	34.54 ± 2.17[b]	-0.85 (-2.09; 0.37)
Truncal fat mass (kg)[1]	15.31 ± 1.10	14.82 ± 1.01	17.54 ± 1.22[a]	15.49 ± 0.97[b]	-0.09 (-0.18; 0.01)
Android fat mass (kg)[1]	2.21 ± 0.21	2.09 ± 0.14	2.54 ± 0.17[a]	2.17 ± 0.12[b]	-0.10 (-0.22; 0.02)
Bone mineral density (g/cm²)	3.04 ± 0.12	3.03 ± 0.14	3.14 ± 0.16	3.13 ± 0.16	0.00 (-0.11; 0.11)
Systolic blood pressure (mmHg)	113.96 ± 2.93	111.92 ± 1.99	112.66 ± 2.33	110.41 ± 3.07	-0.21 (-6.95; 6.53)
Diastolic blood pressure (mmHg)[1]	69.30 ± 2.09	66.53 ± 1.44	67.33 ± 1.98	67.25 ± 3.11	0.04 (-0.08; 1.6)

Values are means ± SE. [1] Non-parametric data. [2] Unstandardized beta for comparison between-group at 6 weeks (reference category: control group). Means followed by different lowercase letters, in the same line, indicate intra-group difference by generalized estimating equation model (GEE), $p < 0.05$.

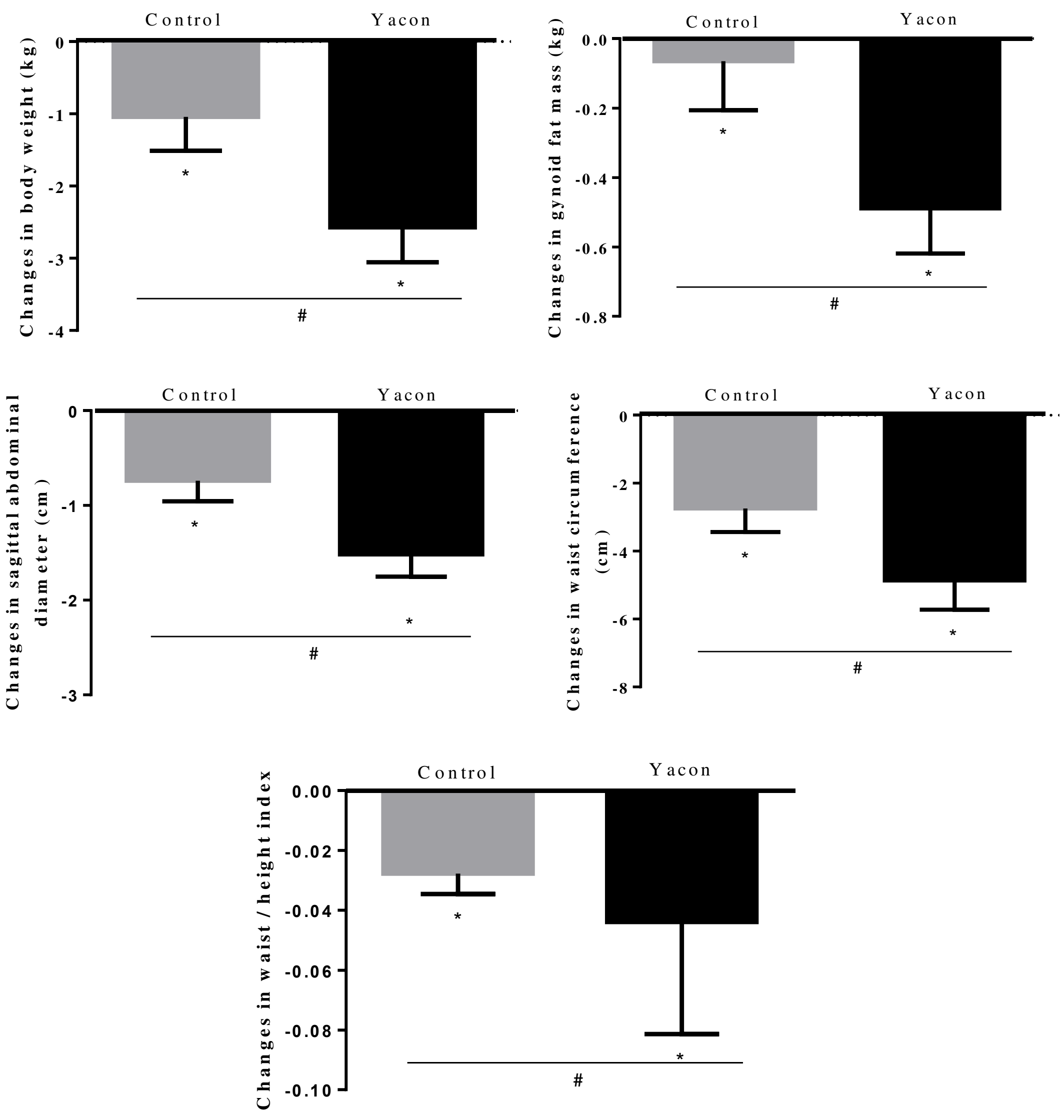


Figure 2. Mean ± SEM changes (Δ values = 6 weeks values - baseline values) in body weight, gynoid body fat, sagittal abdominal diameter, waist circumference and waist/height index in response to energy-restricted prescribed diet (- 500 kcal/d), considering a daily consumption of a drink at breakfast not containing (control, n=13) or containing yacon flour (25g, n=13). *Significant intra-group reduction (generalized estimating equation model (GEE), p <0.05). # Significant between-group differences at 6 weeks, adjusted for baseline (GEE, p <0.05).

Table 6. Gastrointestinal function markers at baseline and after the 6-week intervention according to experimental groups

	Control (n = 13)		Yacon flour (n = 13)		β [2] (95% CI)
	Baseline	**6 weeks**	**Baseline**	**6 weeks**	
Fecal pH	7.23 ± 0.11	7.48 ± 0.13	7.16 ± 0.14	7.32 ± 0.11	-0.08 (-0.47; 0.66)
Bristol scale[1]	3.38 ± 0.27	3.30 ± 0.25	3.92 ± 0.29	4.30 ± 0.25	0.12 (-0.06; 0.29)
Abdominal pain[1]	1.31 ± 0.22	1.46 ± 0.25	1.46 ± 0.23	2.03 ± 0.49	0.44 (-0.21; 1.09)
Heartburn[1]	1.07 ± 0.07[A]	1.23 ± 0.16	1.54 ± 0.17[B]	1.69 ± 0.27	-0.04 (-0.53; 0.45)
Reflux [1]	1.00 ± 0.00[A]	1.15 ± 0.14	1.30 ± 0.13[B]	1.15 ± 0.10	-0.27 (-0.56; 0.03)
Nausea[1]	1.00 ± 0.00	1.23 ± 0.16	1.15 ± 0.10	1.15 ± 0.14	-0.21 (-0.52; 0.11)
Flatulence	3.23 ± 0.36	3.00 ± 0.39	4.30 ± 0.44	3.84 ± 0.44	-0.23 (-1.62; 1.16)
Constipation[1]	1.38 ± 0.17	1.92 ± 0.36[A]	1.92 ± 0.44	1.46 ± 0.17[B]	-0.60 (-1.17; -0.02)
Loose stools[1]	1.69 ± 0.33	1.46 ± 0.30[A]	1.69 ± 0.22	2.53 ± 0.43[B]	0.55 (0.71; 1.03)
Hard stools[1]	1.84 ± 0.21	1.77 ± 0.29	2.61 ± 0.50[a]	1.61 ± 0.25[b]	-0.44 (-1.02; 0.14)
Feeling of incomplete evacuation[1]	2.07 ± 0.45	2.30 ± 0.49	2.23 ± 0.52	2.38 ± 0.46	-0.04 (-0.62; 0.55)

Values are means ± SEM. [1] Non-parametric data. [2] Unstandardized beta for comparison between-group at 6 weeks (reference category: control group). Means followed by different upper-case letters indicate between-group difference (baseline *vs.* baseline or 6 weeks *vs.* 6 weeks) by generalized estimating equation model (GEE), $p < 0.05$. Between-group comparisons at 6 weeks was adjusted for baseline. Means followed by different lowercase letters, in the same line, indicate intra-group difference by GEE, $p < 0.05$.

Suppl. Table 1.

Metabolic variables at baseline and after the 6-week intervention according to experimental groups

	Control (n = 13)		Yacon flour (n = 13)		β [2] (95% CI)
	Baseline	**6 weeks**	**Baseline**	**6 weeks**	
Glucose (mmol/L)	5.10 ± 0.07	5.05 ± 0.11	4.98 ± 0.10	5.02 ± 0.11	-1.84 (-2.15; 5.84)
Insulin (pmol/L)[1]	1.33 ± 0.11	1.19 ± 0.16	1.36 ± 0.10	1.36 ± 0.15	0.11 (-0.14; 0.38)
HOMA-IR[1]	2.11 ±0.18	1.90 ± 0.28	2.11 ± 0.19	2.17 ± 0.30	0.13 (-0.15; 0.42)
Total cholesterol (mmol/L)[1]	4.94 ± 0.25	4.93 ± 0.27	4.62 ± 0.16	4.50 ± 0.19	-0.02 (-0.10; 0.05)
HDL-c (mmol/L)[1]	1.30 ± 0.09	1.32 ± 0.08	1.26 ± 0.09	1.27 ± 0.09	-0.02 (-0.09; 0.05)
LDL-c (mmol/L)[1]	3.03 ± 0.24	3.02 ± 0.25	2.71 ± 0.18	2.66 ± 0.21	-0.02 (-0.12; 0.08)
Triglycerides (mmol/L)[1]	1.32 ± 0.20	1.27 ± 0.18	1.41 ± 0.18	1.26 ± 0.11	-0.07 (-0.30; 0.16)
Triglycerides/HDL-c[1]	1.18 ± 0.25	1.09 ± 0.22	1.18 ± 0.17	1.08 ± 0.13	-0.01 (-0.28; 0.25)
AST (iU/L)	22.77 ± 1.26	23.46 ± 1.26	21.46 ± 1.06	23.38 ± 1.00	1.23 (-1.62; 4.08)
ALT (iU/L)[1]	15.77 ± 2.08	15.76 ± 2.02	13.46 ± 1.39	15.53 ± 1.67	0.14 (-0.15; 0.43)

Values are means ± SEM. [1] Non-parametric data. [2] Unstandardized beta for comparison between-group at 6 weeks (reference category: control group). No significant difference occurred intra-group or between-groups (by generalized estimating equation model, $p>0.05$). To convert from mmol/L to mg/dL: multiply mmol/L by 38.67 for total, HDL, and LDL cholesterol; multiply mmol/L by 88.57 for triglycerides; multiply mmol/L by 18 for glucose. *HDL-c:* high-density lipoprotein cholesterol, *LDL-c:* low-density lipoprotein cholesterol, *AST:* aspartate amino transferase, *ALT:* alanine amino transferase.

DISCUSSION

Our results show that daily consumption of 25 g of yacon flour (0.1 g FOS / kg body weight / day) was well tolerated and can be used as a nutritional strategy to aid in the control of excess body weight. Although about 15% of the subjects reported of abdominal discomfort symptoms such as flatulence and abdominal pain during the first days of intervention, these effect reduced after the second week and no significant difference in such symptoms was observed between groups at the end of the experiment. Like other non-digestible carbohydrates, FOS is fermented by the intestinal bacteria, leading to the production of gases such as carbon dioxide and hydrogen, which can result in gastrointestinal tract adverse effects. The severity of these effects appears to be dose-dependent. Doses less than 0.29 g FOS / kg body weight / day have been shown to be well tolerated, resulting in few adverse gastrointestinal effects (GENTA et al., 2009; SANT'ANNA et al., 2015).

In the present study, yacon flour supplementation (YAC group) resulted in a greater increase in fiber consumption, besides leading to lower body weight, gynoid body fat, waist circumference, waist / height index and sagittal abdominal diameter than CON group. In addition, a reduction of body fat in specific regions (trunk and android) and an increase in total lean mass was observed only in the yacon flour supplemented group.

The greater weight loss and body composition changes in response to the consumption of yacon flour could be explained in part by the lower energy intake by the YAC group. Although not significant, energy intake reduction was 40% higher in the YAC compared to the CON group (-460.9 ± 463.8 kcal *vs.* -324.9 ± 567.3 kcal, respectively), although both groups received prescriptions of diets with the same energy restriction (-500 kcal/d). Dietary fiber supplementation, such as yacon flour, seems to attenuate food intake by various mechanisms (DAUD et al., 2014; PARNELL; REIMER, 2009). The most widely reported mechanism is related to FOS regulation of endogenous intestinal peptides production. FOS appears to increase peptide YY concentration, while suppressing ghrelin and leptin secretions (PARNELL; REIMER, 2009), thus reducing appetite and food intake.

FOS may also increase the activity of adenosine-monophosphate-activated-protein-kinase (AMPK) in muscle and liver tissue (DEN BESTEN et al., 2013; GAO et al., 2009). That enzyme increases energy and fatty acid metabolism by a mechanism associated with the peroxisome-proliferator-activated-receptor-1α-coactivator (PGC-1α) (GAO et al., 2009). Thus, that effect may have contributed to greater reductions of body weight, sagittal abdominal diameter and body fat in certain regions of the body in the YAC group.

The results of the present study suggest that yacon may be an adjuvant in intestinal constipation treatments. Subjects who consumed yacon flour had a lower stool consistency and constipation frequency compare with subjects in the control group. The occurrence of intestinal constipation is common in overweight individuals (POURHOSEINGHOLI et al., 2009), and it is positively related to increased colon cancer risk (ROBERTS et al., 2003). That condition may affect intestinal microbiota, decreasing the number of beneficial bacteria (eg bifidobacteria) and increasing the number of potentially pathogenic microorganisms and fungi. FOS fermentation produces short chain fatty acids (propionate, butyrate and acetate), resulting in a decrease in the colon pH, which in turn affects the microbiota composition. Under low pH conditions the growth of pH-sensitive pathogenic bacteria, such as Enterobacteria and Clostridia, is suppressed while there is an increase of *Bibidobacterium* and *Lactobacillus* (SANT'ANNA et al., 2015). Acidification also favors water retention in the intestinal lumen as a way to preserve the osmotic pressure, promoting an increase in stools viscosity and humidity, besides reducing intestinal constipation (OKU; NAKAMURA, 2017).

Similar results to the ones verified in the present study have been observed with yacon consumption. In a study involving lightly dyslipidemic obese premenopausal women, consumption of yacon syrup (0.14g FOS/kg body weight/day) for 120 days increased satiety, reduced body weight and waist circumference, in addition to increasing the evacuation frequency (GENTA et al., 2009). The addition of a yacon-based product (10g FOS/day, approximately 0.15g FOS/kg body weight) to orange juice for 30 days also increased evacuations, improved stools consistence and reduced constipation score in constipated adults/elderly individuals (SANT'ANNA et al., 2015). In another study, the consumption of yacon syrup (20g/day, containing 6.4g FOS) significantly reduced intestinal transit time in healthy subjects (GEYER et al., 2008).

Consumption of 25g of yacon flour did not affect most of the concentration of the biochemical variables evaluated in this study. However, all dyslipidemic subjects had an improvement in the lipid profile at the end of the study compared to baseline, and in half of them, the values reached normality. It should be noted however, that except of the lipid profile, our subjects had all the other biochemical variables assessed were within normal ranges. Thus, we believe that yacon flour may exert its beneficial effects only in metabolically decompensated individuals. Data from an animal study corroborate with our hypothesis. Diabetic and healthy animals (control) received 1.06 g yacon root extract / kg body weight / day (equivalent to 0.34 g of FOS / kg body weight / day) for 14 days. Although diabetic animals had glycemia 170 % higher at baseline compared to controls, yacon supplementation brought it to near to normal values. However, no change in glucose concentrations was observed in control mice (BIAZON et al., 2016).

In subjects with reduced glucose tolerance (SCHEID et al., 2014), insulin-resistant and dyslipidemic (GENTA et al., 2009) and in subjects with diabetes (GORDILLO et al., 2012), yacon supplementation in lyophilized form (0.1 g FOS / kg body weight / day for 9 weeks), in extract (3 g extract / day for 90 days, concentration of FOS was not available) or syrup (0.14 g FOS / kg body weight), significantly reduced the concentrations of variables associated with glucose metabolism (glycemia, insulin, and HOMA-IR) and lipid profile (LDL-c). In a previous study conducted by our group (ROCHA et al., 2018), glycemic response was not affected in response to yacon flour consumption (0.13 g FOS / kg body weight / day) in healthy euglycemic individuals.

The results of the present study suggest that yacon flour can be used as a dietary fiber supplement and that the consumption of 25 g / day by adult subjects (0.1g FOS/kg body weight/day) appears to be an interesting strategy to help control excess body weight. In addition to being well tolerated, that supplementation increased fiber consumption, improved bowel function. It also increased body weight, body fat, waist circumference, waist / height ratio and sagittal abdominal diameter reduction, besides increasing total lean mass.

Our study has several strengths. First, we evaluated a food source of FOS, rather than a synthetic source. In addition, we chose to test yacon flour because it presented greater FOS content stability than yacon root. Second, our study was double-blind and double-typed data. In addition, we used strict eligibility criteria, we used DXA to evaluate body composition, and breakfast was consumed in the laboratory to guarantee adherence to the protocol. Finally, our study differed from most studies already published for having included men and women in the sample. On the other hand, overweight individuals tend to underestimate the amount of food consumed. Thus, the use of the food records to assess food intake was a limitation. Despite of that, food records are widely used by other authors, since it is easy to apply and allow subjects to remain in free living conditions.

In summary, daily consumption of 25g of yacon flour (0.1 g FOS / kg body weight / day) for 6 weeks by overweight subjects was well tolerated and allowed dietary fiber intake to become adequate, resulted in lower constipation frequency and improved stools consistency, besides leading to lower body weight, body fat, waist circumference, waist-to-weight ratio and sagittal abdominal diameter than the CON group. In addition, it did not negatively affect the concentrations of the biochemical variables assessed. Thus, yacon flour seems to be a suitable nutritional strategy to be used in association with energy restriction diets to control excess body weight.

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4.2 Original Research

Effects of yacon flour associated with an energy restricted diet on intestinal permeability, oxidative stress and inflammation markers concentrations in adults with excess body weight: a randomized, double blind, placebo controlled clinical trial

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ABSTRACT

Background and Aims Yacon (Smallanthus sonchifolius) flour is rich in bioactive compounds (phenolic compounds and fructooligosaccharides (FOS)), and may therefore reduce the risk of diseases associated with excess body weight. However, its effect on fecal short chain fatty acids (SCFA), intestinal permeability, oxidative stress and inflammation markers has not been studied in excess body weight adult humans. We evaluated the effect of the consumption of yacon flour associated with an energy restricted diet on these variables in adults with excess body weight. **Methods** Twenty-six excess body weight (30.4 ± 2.4 kg/m²) adults (11 men, 15 women; age 31.3 ± 8.5 y) were randomized to one of two groups (yacon flour or control; n = 13) on a double blind, placebo controlled clinical trial. Subjects received a breakfast drink containing or not 25 g of yacon flour associated with an energy restricted diet, for six weeks. The flour chemical characterization, FOS and total phenolics contents were evaluated. Antioxidant capacity was evaluated *in vitro* and *in vivo*. Intestinal permeability, oxidative stress and inflammatory markers were evaluated at baseline and at the end of the study. Intestinal permeability was quantified from urine samples. Oxidative stress and inflammatory markers concentrations were quantified from blood in fasting condition. **Results** Yacon flour was well tolerated. It presented an *in vitro* and *in vivo* antioxidant capacity, increased plasma total antioxidant capacity and reduced protein carbonyl concentrations. A reduction in SCFAs was also observed. **Conclusion** These results suggest that yacon flour is a potential food supplement with antioxidant capacity, which seems to be effective on body weight control.

Keywords: yacon; white blood cells; obesity; intestinal permeability; oxidative stress; inflammation

INTRODUCTION

Yacon root and its products seem to reduce body weight and body fat mass, improve oxidative stress and inflammation, thus reducing the risks of cardiovascular disease, type 2 diabetes and other chronic diseases (BIAZON et al., 2016; HABIB et al., 2015; MACHADO et al., 2018; OLIVEIRA et al., 2016, 2017, SATOH et al., 2013a, 2013b). Recent studies identified the existence of an association between yacon consumption and intestinal barrier function improvement (GRANCIERI et al., 2017; VAZ-TOSTES et al., 2014). These effects seem to occur due to its high fructooligosaccharides (FOS) and phenolic compounds content, mainly chlorogenic acid, present in yacon (CAMPOS et al., 2012; CASTRO et al., 2012).

FOS are prebiotics fermented by intestinal bacteria, leading to the production of lactic acid, short chain fatty acids (SCFAs), mainly acetic, propionic and butyric acid, and gases. Studies have suggested that FOS and its fermentation products may affect the composition of the microbiota (Lactobacillus and Bifidobacterium spp), promoting a healthier microvillus environment (BONET et al., 2010; CAMPOS et al., 2012; MIYAGUCHI et al., 2015). Prebiotic consumption may also increase the expression of tight junctions (CANI et al., 2009), which can reduce the passage of antigens, toxins and microorganisms though the enterocytes (SUZUKI, 2013). The increase in the tight junction proteins was correlated with reduced intestinal permeability, and reduced proinflammatory cytokines and oxidative stress markers expression (CANI et al., 2009). The phenolic compounds in turn contain hydroxyl groups capable of donating electrons and thus reduce free radicals (SUZUKI et al., 2006, 2008).

Few studies have evaluated the functional effects of yacon flour in humans (MACHADO et al., 2018; ROCHA et al., 2018; VAZ-TOSTES et al., 2014). To the best of our knowledge, this is the first clinical trial to investigate the effect of yacon flour consumption on intestinal short chain fatty acids (SCFAs) concentrations, intestinal permeability, oxidative stress and inflammatory markers concentrations in excess body weight adults. The aim of this study was to evaluate the effect of the consumption of yacon flour associated with an energy restricted diet on such variables in adults with excess body weight. We hypothesized that the addition of yacon flour to a diet would decrease gut permeability, oxidative stress and inflammation, favoring the control of obesity and its complications.

METHODOLOGY

Yacon flour composition

Yacon flour was purchased from Linea Verde Alimentos (Curitiba, Brazil) and was stored at 21°C and 64% humidity until the moment of use. Yacon flour chemical composition and FOS content was determined as previously described (MACHADO et al., 2018).

To determine the total phenolic content, a yacon flour extract was prepared, as described by Larrauri et al. (LARRAURI; RUPÉREZ; SAURACALIXTO, 1997), with some modifications. To obtain the extract, 12g of yacon flour were homogenized with 120ml of 50% methanol. That mixture stayed for 1 hour at room temperature. Subsequently, the sample was centrifuged (7,000xg, 15 min), the supernatant collected and kept under refrigeration (4°C). The residue was submitted to a second extraction with 120ml of 70% acetone at room temperature for 1 hour. After that time, the sample was centrifuged once more (7,000xg. 15 min) and the supernatant was transferred to a volumetric flask containing the first supernatant and the volume (200ml) was completed with distilled water. Before the analysis, the extract was filtered on filter paper, Whatman #1, in vacuum pressure, and concentrated in a rotary evaporator (MA 120, Marconi, São Paulo, Brazil) at 40°C, the concentrate was resuspended in distilled water to obtain a final volume of 50 ml.

Phenolic compounds content determination was carried out according to the methodology described by Singleton and Rossi (SINGLETON; ROSSI, 1965) with some adaptations. In an assay tube an aliquot (0.6ml) of the extract obtained (diluted 1/25) and 3 ml of the Folin-Ciocauteau reagent (diluted 1/10) were added and the mixture was stirred. After three minutes 2.4 ml of saturated sodium carbonate (7.5% m/v) were added. The mixture was allowed to rest in the dark for 1 hour and a UV-Visible (BEL Engineering UV-M51) spectrophotometer was read at 760nm. Gallic acid was used as standard and the results were expressed in milligrams of gallic acid equivalents (GAE) per 100 grams of yacon flour.

Antioxidant potential *in vitro*

DPPH • (2,2-Diphenyl-1-picrylhydrazyl) assay was performed according to the methodology proposed by Brand-Williams et al. (BRAND-WILLIAMS; CUVELIER; BERSET, 1995). A total of 0.5ml of the extract was mixed with 3.5ml of the DPPH• solution (60µmolL $^{-1}$). The samples were kept for one hour at room temperature in a dark environment. The absorbance was then read at 517nm in a UV-VISIBLE spectrophotometer (BEL Engineering UV-M51). The results were expressed as Trolox equivalent antioxidant capacity (TEAC) (μmol TEAC g^{-1} of flour). A total of 0.5ml of the extract was mixed with 3.5ml of the DPPH• solution (60μmolL $^{-1}$). The samples were kept for one hour at room temperature in a dark environment. The absorbance was then read at 517nm in a UV-VISIBLE spectrophotometer (BEL Engineering UV-M51). The results were expressed in antioxidant capacity of TEAC (μmol TEAC g^{-1} of flour).

For $ABTS^{•+}$ (2,2'-azinobis-3-ethyl-benzothiazoline-6-sultonated) radical formation a methodology described by Re et al. (RE et al., 1999) was used with some modifications. $ABTS^{•+}$ radical was formed from the addition of ABTS aqueous solution (7mmol L $^{-1}$) to a potassium persulfate solution (2.45mmol L $^{-1}$) in a ratio of 1:1. The mixture was heated in a water bath (40°C, 30 min), and the solution absorbance was corrected to 0.70 (± 0.05) at 734nm with the addition of 80% ethanol. An aliquot of 0.5ml of the extract, in different concentrations, was transferred to the test tubes with 3.5ml of the $ABTS^{•+}$ radical. The reaction occurred in the dark (6 min) and the absorbance was read at 734 nm in UV-Visible Spectrophotometer (UV-M51, BEL Engineering, Monza, Italy). The results were expressed as TEAC (μ mol TEAC. g $^{-1}$ of flour). All the analyses were performed in duplicate.

In vivo effects

Subjects

Four hundred and four (404) subjects were initially recruited through local advertisements flyers, radio, newspaper and electronic media, from August 2017 to May 2018. Eligible subjects were adult men and women (20-45 years), with excess body weight (BMI 25-35 kg/m²), who consumed regular breakfast, had low physical activity level and dietary restraint ≤ 14 (STUNKARD; MESSICK, 1985). Exclusion criteria were previously described (MACHADO et al., 2018).

From the 404 recruited subjects, 30 filled out the criteria for inclusion and were allocated in one of the study groups. Of these, four subjects did not complete the study protocol. Besides, intestinal permeability analysis from two subjects were lost (**Figure 1**). No exclusions happened due to protocol non-compliance.

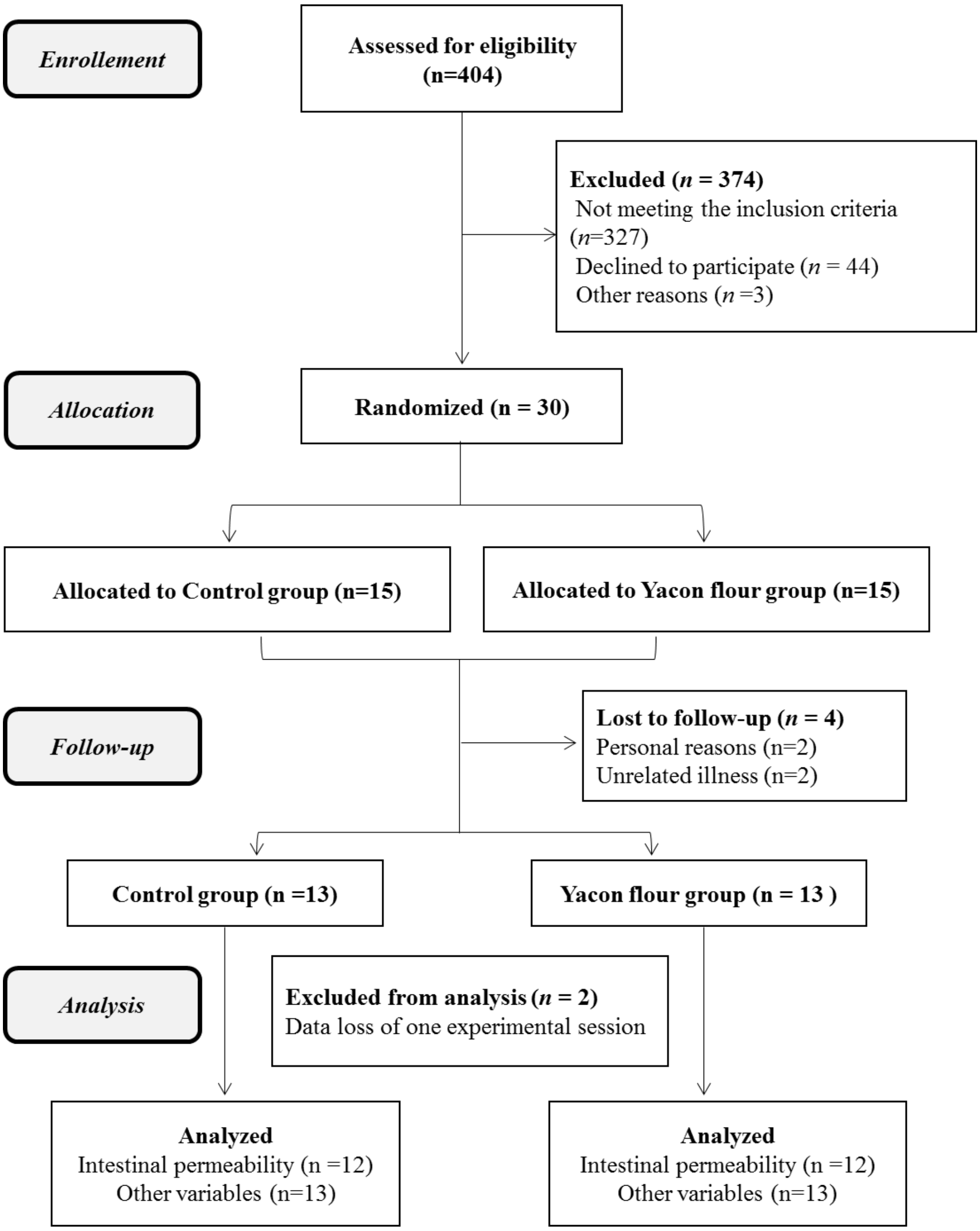

Figure 1. CONSORT study flow diagram

The study was conducted according to the Declaration of Helsinki and is registered in the Brazilian Clinical Trials Registry, http://www.ensaiosclinicos.gov.br/rg/RBR-6yh6bq/, number RBR-6YH6BQ. All procedures involving human subjects were approved by local ethical committee (Universidade Federal de Viçosa, number 62047316.6.0000.5153). Written informed consent was obtained from all subjects.

Study design

Details of the study design were described previously (MACHADO et al., 2018). In short, subjects completed a randomized, placebo controlled, double-blind clinical trial of 6 weeks (± 5 days). Potential subjects were screened for eligibility and randomly allocated to control group (CON, n = 13) or yacon flour group (YAC, n = 13), using the block randomization technique (with block size equal to 4). This technique was applied by an independent research group not involved in the study.

During the study, subjects daily attended the Laboratory of Food Intake, Department of Nutrition and Health, Federal University of Viçosa – Brazil to consume a breakfast drink, according to the group in which they were allocated, as part of the energy-restricted diet (-500kcal/day) individually prescribed to each subject. Diets were prescribed considering the nutritional composition of the estimated energy requirement, the level of physical activity and the breakfast shakes daily consumed in the laboratory during the study. CON and YAC prescribed diets had similar calories and macronutrients contents (MACHADO et al., 2018). Adherence to the prescribed diet was monitored by means of a 3-day (2 weekdays and 1 weekend) food records applied in the third and in the last week of the study. Subjects were instructed to maintain a constant level of physical activity throughout the study. If subjects presented infection and/or inflammation symptoms, or, if women had menstrual changes, the subject's permanence in the study was reevaluated. However, no volunteer was excluded because of these changes. The results of food intake, body composition, anthropometric, intestinal function and biochemical variables were previously published (MACHADO et al., 2018).

Breakfast

During the intervention period, subjects daily consumed at breakfast 350 mL of a control drink without yacon flour or a test drink containing yacon flour. The other meals were consumed in free living conditions.

Yacon drink had the maximum tolerable amount of yacon flour (25g/day) to make it palatable and to avoid possible undesirable gastrointestinal effects (GENTA et al., 2009; SCHEID et al., 2014). Yacon and control drinks contained similar energy and macronutrient contents (**Table 1**). To ensure similarity to YAC drink, corn stark was added to CON drink. Seven different flavor drinks were developed for consumption at breakfast: cappuccino, cocoa milk, coffee with milk, guava, mango, blackberry and passion fruit.

Table 1. Nutritional composition[1] of the seven rotating breakfast drinks served

	Energy (kcal)		CHO (g)		PTN (g)		LIP (g)		Fiber (g)[2]	
	CON	YAC	CON	YAC	CON	YAC	CON	YAC	CON	YAC
Guava vitamin	441.4	462.6	65.67	68.43	15.08	15.78	13.16	13.98	11.36	22.55
Blackberry vitamin	440.7	461.9	63.92	66.68	16.66	17.36	13.16	13.98	0.51	11.70
Passion vitamin	430.9	452.18	63.92	66.68	14.21	14.91	13.16	13.98	1.91	13.1
Mango vitamin	426.7	447.9	63.92	66.68	13.16	13.86	13.16	13.98	2.43	13.62
Cappuccino	394.7	412.9	55.44	58.80	13.41	14.11	13.26	13.48	0.75	11.7
Cocoa milk	394.74	412.9	55.44	58.80	13.41	14.11	13.26	13.48	0.75	11.7
Coffe with milk	397.43	411.6	53.55	55.90	13.16	13.86	14.51	14.73	0.75	11.7
Mean	418±21.6	437±23.9	60.2±5.2	63.1±5.1	14.1±1.3	14.8±1.3	13.4±0.5	13.9±0.4	2.6±3.9	13.7±3.9

[1] Based on the information contained in the food labels and on yacon flour nutritional composition analysis. [2] Dietary fiber: total fiber (soluble + insoluble) + fructooligosaccharides.

During the experimental period, subjects attended the laboratory daily on week days to consume their breakfasts according to the allocated group. Identical breakfasts were provided to be consumed at home on weekends. The subjects were instructed to consume the full portion and if for instance there were any leftovers, they were instructed to return these leftovers to the lab. A trained investigator, unrelated to data collection and analysis, was responsible for assessing adherence to the protocol, preparing and serving the drinks. Drinks were served in colored cups to avoid visual interference of the type of drink tested.

Faecal short chain fatty acids (SCFAs) concentrations

Faecal samples (500 mg) were homogenized in 1ml of Milli-Q water and centrifuged (12,000 xg, 10 min) and and the cell-free supernatants were treated as described by Siegfried et al. (SIEGFRIED; RÜCKEMANN; STUMPF, 1984).

The concentration of short chain fatty acids was determined by high performance liquid chromatography (HPLC). The samples were analyzed using a chromatograph Dionex Ultimate 3000 Dual coupled to a Shodex RI-101 refractive index (RI) detector maintained at 40°C, and Phenomenex ion exchange column Rezex ROA, 300x7.8mm maintained at 40°C. The mobile phase used was composed of 5mM sulfuric acid (H2SO4) with a flow rate of 0.7 ml/min. The organic acids used for calibration of the standard curve were: acetic, propionic, butyric and crotonic acids.

Intestinal Permeability

The subjects were instructed not to consume dietary sources of the lactulose and mannitol (TEIXEIRA et al., 2014) two days before the assessment. After 12 hours of fasting, the subjects attended the laboratory and were instructed to eliminate any residual urin. An iso-osmolar solution, containing 6.67 g of lactulose and 2.0 g of mannitol diluted in water to complete 150 mL of solution, was ingested by the subjects. The osmolarity of the solution is similar to that of plasma, in order to avoid any damage to the studied epithelium. Breakfast, containing or not containing yacon flour, was offered 2 hours after the solution was ingested. 800ml of water were given at regular intervals to the subjects. During six hours, all voiding product was collected (**Figure 2**). Next, 15ml of urine was transferred to a single vial containing 12 mg of thimerosal to prevent bacterial growth. The samples were stored in a freezer at -80°C until analysis.

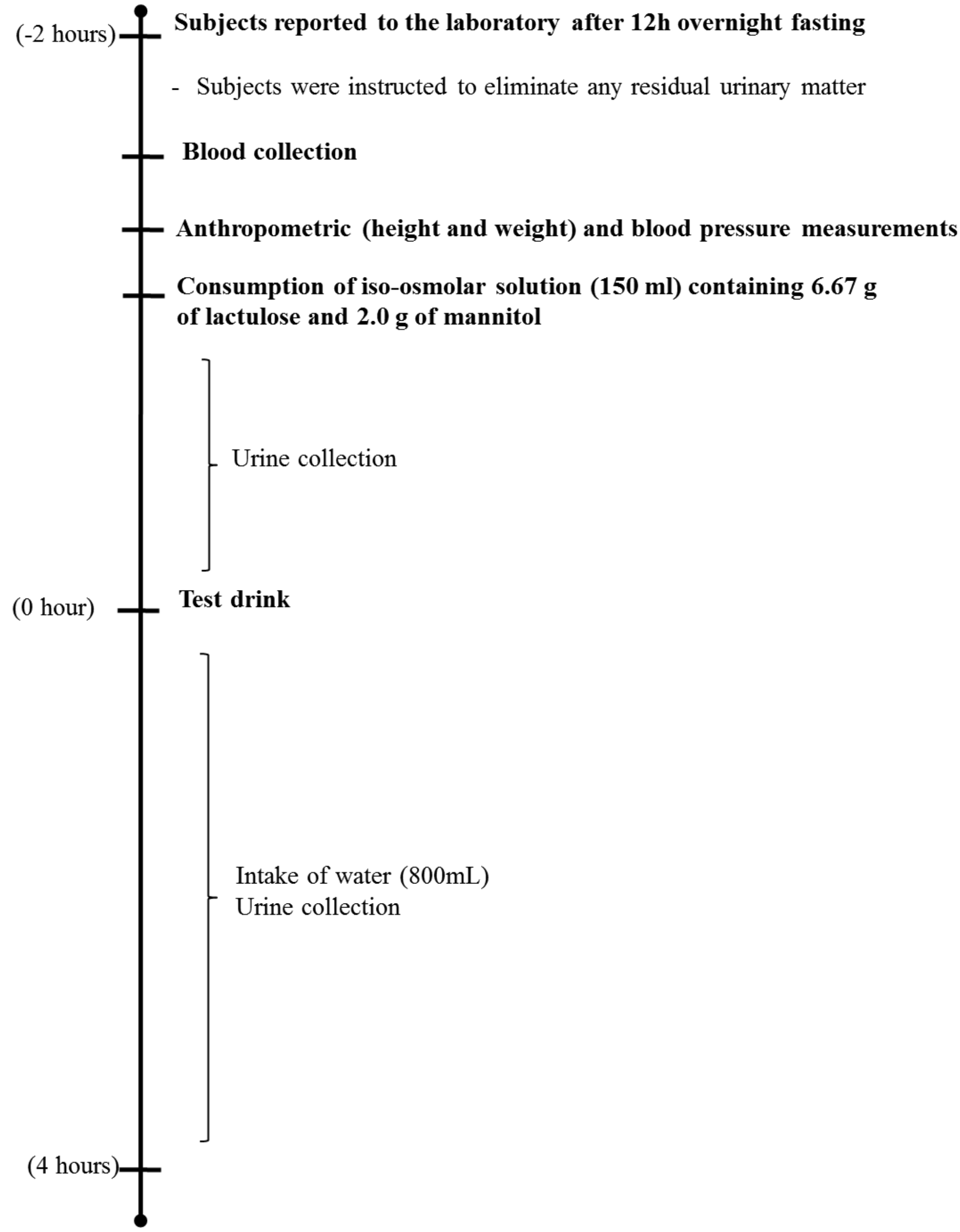

Figure 2. Data collection protocol

Two milliliters of stored urine samples were taken into a water bath (56°C, 10 min), centrifuged (10,000 rpm, 7 min) and filtered through a microporous membrane (0.22 μmx13mm, Millipore, USA). Urinary lactulose and mannitol excretions were analyzed by HPLC, Dionex Ultimate 3000 Dual coupled to a Shodex RI-101 refractive index detector maintained at 40°C, and Phenomenex Rezex ROA ion exchange column, 300x7.8mm maintained at 40°C. The volume of filtered urine injected was 20μl. The mobile phase used was 5mM of sulfuric acid with a flow rate of 0.7ml/min. Standardization curves with lactulose and mannitol standards were used to determine sugar concentration in the urine samples. The net amount of sugar excreted was calculated by multiplying the determined concentration of each sugar in the urine by the total volume of urine collected over the course of 6 hours. Then the sugar dose administered was used to calculate the percentage of lactulose (%L) and mannitol (%M) excreted in the urine. These results were used to calculate the proportion of lactulose/mannitol (L/M) (TEIXEIRA et al., 2012; VILELA et al., 2008).

Metabolic biomarkers

The subjects were instructed not to consume alcoholic drinks and not to change their usual food intake one week before the evaluations and were also instructed not to engage in strenuous physical activity within the previous 24 hours. In the night before the evaluations a standard dinner was supplied to be consumed (200 ml of Tial® nectar (grape flavor), 85 g of pasta and 10 g of Parmesan cheese - 523 kcal, 57.4% CHO, 10.3% PTN, 32.3% LIP, 2.1g fibers).

Antecubital blood samples were collected after 12 hours of fasting. Immediately after collection, blood samples were sent to the laboratory to complete blood count (leukocytes, lymphocytes, neutrophils and platelets). The remaining of the blood was centrifuged for serum and plasma separation (3500rpm, 4°C, 15min), and immediately frozen at -80°C until analyses.

CRP was assessed by the quantitative method based on immunoturbidimetric, using commercially available kit for ultrasensitive CRP (Bioclin® MG, Brazil). The neutrophils/lymphocytes (NLR) and the platelets/lymphocytes (PLR) ratios were calculated.

Oxidative stress

The oxidative stress markers concentrations were evaluated in plasma. Catalase activity (CAT) was measured according to Hadwan and Abed (HADWAN; ABED, 2016) with modifications. The catalase activity was expressed in U per milligram of protein. The activity of glutathione S-transferase (GST) was assessed and was carried out according to the formation of 2,4-dinitrochlorobenzene with glutathione conjugate (HABIG; PABST; JAKOBY, 1974). GST activity was expressed in μmole/min/g. Lipid peroxidation was assessed by malondialdehyde (MDA) concentrations following the methodology prescribed by Buege and Aust (BUEGE; AUST, 1978) with modifications. MDA concentrations were expressed as μmol/ml of plasma. Protein oxidation was evaluated by the quantification of protein carbonyl using the method described by Mekrungruangwong et al. (MEKRUNGRUANGWONG et al., 2012) with modifications. Protein carbonyl was expressed in nmol/ml. Nitric oxide (NO) production was determined indirectly by the total nitrite dosage of the samples, using Griess's reagent (GRISHAM; JOHNSON; LANCASTER, 1996). Nitric oxide concentrations were expressed in µmol/mg of protein. Antioxidant capacity of the plasma was evaluated by the ferric reducing antioxidant power (FRAP) assay (BENZIE; STRAIN, 1996). FRAP was expressed in µM trolox.

Statistical analysis

The present study presented a statistical power of 99.9 % (α=0.05) (Dean et al., 2006) to detect a reduction of 15% in the FRAP concentration, considering our subjects *baseline* data.

Statistical analyses were conducted using SPSS software (SPSS Inc., Chicago, IL, 2015, version 22.0). The thresholds for lower and upper outliers were defined as follows: lower thresholds = lower quartile - (1.5 × interquartile range) and upper threshold = upper quartile + (1.5 × interquartile range). Data normality and homoscedasticity were evaluated by the Shapiro-Wilk and Levene tests, respectively. The description of the sample data at baseline was compare through Student's t test or Mann-Whitney U signed-rank test. The effect of the intervention was assessed by comparing the outcome variables intra and inter CON and YAC groups using generalized estimating equation model (GEE) adjusted by sex. We used Bonferroni post-hoc to identify the differences on group, time and group*time interaction when required. For the variables with normal distribution, a connection identify function was used. For the variables that did not follow normal distribution, gamma distribution with log link was used. The working correlation matrix used was unstructured and robust estimator covariance matrix. These models were adjusted by baseline values. A $\alpha < 0.05$ was adopted as the level of statistical significance.

RESULTS

Yacon flour chemical characterization

The portion of yacon flour tested in the present study (25 g) contained 0.69 g of protein, 0.21 g of fat, 1.23 g of ashes and 21.36 g of total carbohydrate. It contained 11.72 g of dietary fiber, of which 8.67 g were FOS. Total phenolic compounds content was 180 mg of GAE.

***In vitro* antioxidant capacity**

Yacon flour antioxidant capacity was 54.2 μmol TEAC. g $^{-1}$ of flour by the DPPH method and 22.18 μmol TEAC. g $^{-1}$ of flour by the ABTS method.

***In vivo* outcomes**

Included participants were mostly female (57.7%), with 31.3 ± 8.5 years old and had a BMI (±SD) of 30.44 ± 2.44 kg/m^2. No adverse effects were reported by subjects from either experimental group. Two subjects from YAC group and one from CON group had concentrations of CRP above 10 mg/L, suggesting the presence of acute inflammation or infection at baseline. However, the removal of these subjects from the analyses did not alter the results of the comparisons. Therefore, the subjects were kept in their respective groups.

The concentrations of acetic, propionic and butyric acid significantly reduced in the YAC group. At the end of the six weeks of intervention, subjects in the YAC group presented lower acetic acid concentration in the feces than the ones in the CON group (**Figure 3**).

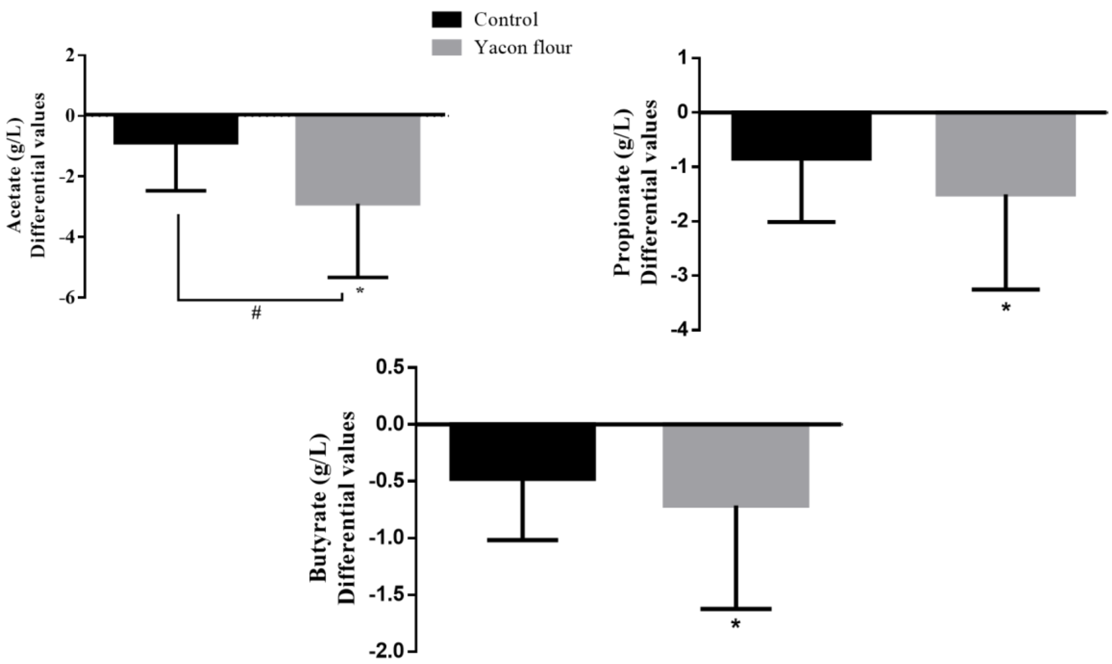

Figure 3. Mean ± SEM changes (Δ values = 6 weeks values - baseline values) in short chain fatty acids (acetate, propionate, butyrate) in response to consumption of a drink containing (control, n=13) or containing yacon flour (25g, n=13) allied to energy-restricted diet (- 500 kcal/d). *Significant intra-group reduction (generalized estimating equation model (GEE), p <0.05). # Significant between-group differences at 6 weeks, adjusted for baseline (GEE, p <0.05).

No differences were detected in the urinary lactulose or mannitol excretion, the lactulose/mannitol ratio, CRP, NLR and PLR concentrations, platelet count and white blood cell counts between baseline groups or after six weeks of intervention (**Tables 2 and 3**). YAC group subjects had a 67% reduction in NLR and in half of them the values reached were below the established cut-off point (<1.84). On the other hand, only 33% of the subjects who consumed the control drink had a reduction in NLR.

The antioxidant enzymes CAT and GST activities, the oxidation product malondialdehyde and nitric oxide concentrations were not affected during the study. There was an increase in the plasma antioxidant capacity (FRAP method) and reduction in the concentrations of protein carbonyl in the YAC group (**Table 4**).

Table 2. Intestinal permeability assessments at baseline and after intervention (6 weeks) according to experimental groups*

	Control group (n = 12)		**Yacon flour group (n = 12)**		P_{inter}
	Baseline	**6 weeks**	**Baseline**	**6 weeks**	
	Adjusted mean ± SEM	**Adjusted mean ± SEM**	**Adjusted mean ± SEM**	**Adjusted mean ± SEM**	
% Lactulose excretion	0.92 ± 0.43	2.92 ± 0.29	1.34 ± 0.44	1.79 ± 0.35	0.429
% Mannitol excretion	4.61 ± 1.56	3.95 ± 0.85	5.54 ± 1.92	3.13 ± 0.90	0.484
L/M ratio	0.32 ± 0.08	0.64 ± 0.06	0.40 ± 0.16	0.66 ± 0.13	0.698

*Data are given as adjusted mean and SEM per treatment group. No significant difference occurred intra-group or between-groups (by generalized estimating equation model, p>0.05). *P inter:* between-group comparisons at 6 weeks was adjusted for baseline.

Table 3. Inflammatory markers assessments at baseline and after intervention (6 weeks) according to experimental groups*

	Control (n=13)		**Yacon flour (n = 13)**		P_{inter}
	Baseline	**6 weeks**	**Baseline**	**6 weeks**	
	Adjusted mean ± SEM	**Adjusted mean ± SEM**	**Adjusted mean ± SEM**	**Adjusted mean ± SEM**	
Leukocytes (mm^3)	6338.58 ± 474.68	6266.96 ± 538.22	6952.60 ± 334.45	6788.12 ± 423.70	0.804
Neutrophils (mm^3)	3657.07 ± 363.34	3773.76 ± 466.87	3951.24 ± 351.18	3879.20 ± 317.55	0.560
Lymphocytes (mm^3)	2355.27 ± 208.02	2094.36 ± 157.24	2448.14 ± 248.51	2424.11 ± 213.34	0.206
Platelet ($10^3/mm^3$)	206.06 ± 131.85	198.58 ± 134.17	208.90 ± 170.23	216.19 ± 164.83	0.160
Neutrophil/lymphocyte rate	1.61 ± 0.21	1.81 ± 0.19	1.76 ± 0.24	1.63 ± 0.20	0.100
Platelet/Lymphocyte rate	91.20 ± 7.84	97.86 ± 8.22	91.02 ± 11.45	92.23 ± 11.56	0.443
C reactive protein	2.52 ± 0.84	2.58 ± 0.86	4.89 ± 1.07	5.23 ± 1.17	0.623

*Data are given as adjusted mean and SEM per treatment group. No significant difference occurred intra-group or between-groups (by generalized estimating equation model, p>0.05). *P inter:* between-group comparisons at 6 weeks was adjusted for baseline.

Table 4. Oxidative stress markers assessments at baseline and after intervention (6 weeks) according to experimental groups*

	Control (n=13)		Yacon flour (n = 13)		P_{inter}
	Baseline	6 weeks	Baseline	6 weeks	
	Adjusted mean ± SEM	Adjusted mean ± SEM	Adjusted mean ± SEM	Adjusted mean ± SEM	
Catalase (U/mg)	1359.09 ± 8.99	1532.11 ±134.11	1444.3 ± 121.5	1698.64 ± 129.67	0.661
GST (µmol/min/g)	1.50 ± 0.21	1.54 ± 0.18	1.38 ± 0.17	1.44 ± 0.20	0.642
FRAP (µmol/L)	708.75 ± 54.60	727.10 ± 63.21	755.20 ± 50.69[a]	834.38 ± 47.37[b]	0.235
MDA (µmol/mL)	7.42 ± 1.34	0.76 ± 0.98	7.15 ± 1.14	8.36 ± 1.00	0.247
Nitric oxide (µmol/mg)	2.11 ± 0.80	1.50 ± 0.43	1.27 ± 0.28	1.65 ± 0.34	0.078
Protein carbonyl (nmol/mL)	3.05 ± 0.45	2.44 ± 0.34	3.03 ± 0.36[a]	1.94 ± 0.33[b]	0.315

FRAP, ferric reducing antioxidant power; GST, glutathione S-transferase; MDA, malondialdehyde

*Data are given as adjusted mean and SEM per treatment group. Means followed by different lowercase letters, in the same line, indicate intra-group difference by generalized estimating equation model, $p < 0.05$. *P inter:* between-group comparisons at 6 weeks was adjusted for baseline.

DISCUSSION

To the best of our knowledge, this study provides the first clinical evidence that six weeks of yacon flour consumption reduces oxidative stress status and fecal SCFAs in adults with excess body weight.

Yacon is a source of phenolic compounds (720 mg GAE/ 100g of flour), mainly chlorogenic acid, that could neutralize free radicals and improve oxidative stress (BIAZON et al., 2016; CASTRO et al., 2012; HABIB et al., 2015; OLIVEIRA et al., 2016, 2017). These compounds contain hydroxyl groups, which can donate electrons and thus reduce free radicals production, avoiding biomolecules oxidation (SUZUKI et al., 2006, 2008). Several methods have been developed to determine food antioxidant potential. However, to measure the antioxidant activity of food extracts, at least two test systems have been recommended (RUFINO et al., 2010). For this reason, the antioxidant activity of the yacon flour was measured using the ABTS and DPPH methods. The total antioxidant activity of the yacon flour was 54.2 μmol Trolox/ g of flour by the DPPH method and 22.18 μmol Trolox/g of flour by the ABTS method. It is well known that the antioxidant capacity *in vitro* of food do not necessarily represent its antioxidant potential *in vivo* (BIAZON et al., 2016). Therefore, we used several biochemical assays to determine the *in vivo* antioxidant properties of yacon flour.

In our study, after six weeks of yacon flour consumption, the plasma FRAP of the YAC group was significantly increased while the protein carbonyl level was significantly decreased than the baseline value, in contrast to the CON group, which no differences were observed. Similar results were observed in animal models. Yacon extract supplementation (1.06mg/kg body weight/day, providing 0.34g of FOS/kg body weight/day) for 14 days reduced the concentrations of protein carbonyl in liver of diabetic rats (BIAZON et al., 2016). In another study with diabetic animals, a reduction in the concentration of oxidative stress markers was also observed after 90 days of yacon flour supplementation (0.34g FOS/kg body weight/day) (HABIB et al., 2015). In hypercholesterolemic animals, similar results were observed. Oliveira et al. (OLIVEIRA et al., 2016, 2017) provided yacon root or leaf (20 and 40mg/kg body weight/day, respectively) extracts for 14 days and observed reductions in the concentrations of oxidation products (malondialdehyde and protein carbonyl) in plasma and increase in antioxidant defense (catalase, superoxide dismutase and glutathione peroxidase) in erythrocytes.

An increase in plasma antioxidant capacity provides greater protection against free radicals. There is also a reduction in protein carbonyl concentration, which helps reduce key events in the development of cardiovascular disease (AGUDELO-OCHOA et al., 2016). The fact that the plasma FRAP increased after the consumption of yacon flour and no change has been observed in antioxidant enzymes suggests that the substances presents in yacon flour may increase antioxidant capacity without requiring change in the antioxidant defense system.

We observed lower butyrate, acetate and propionate after six weeks of yacon flour consumption and lower acetate compare to CON group. Although the reduction in SCFAs concentrations appears to be a negative results, it has been shown that 95% of the SCFAs produced by bacterial fermentation are absorbed by colonocytes during intestinal transit (GOMES; COSTA; ALFENAS, 2015). Besides, many metabolites are intermediates (eg, acetate) and therefore are associated with the metabolic activity of bacterial producers and/or bacterial users (GOMES; COSTA; ALFENAS, 2015). Another issue to be considered is the fact that fecal SCFAs concentrations seem to be associated with the nutritional status of the subjects (obese or euthrophic). The authors of previous studies have reported higher fecal SCFAs concentrations in excess body weight subjects compared with eutrophic subjects (SCHWIERTZ et al., 2010; TEIXEIRA et al., 2013). In addition, positive correlations between SCFAs and metabolic syndrome risk factors have been reported (TEIXEIRA et al., 2013). Since we have previously demonstrated that yacon flour consumption reduced central obesity (MACHADO et al., 2018), we believe that this reduction in SCFAs concentration may be associated with changes in body composition observed or changes in the composition of the gut microbiota (decrease in bacterial producers or an increase in bacterial users of specific SCFA).

Yacon flour consumption did not affect intestinal permeability. To our knowledge, the effect of yacon consumption on human intestinal permeability hasn't been assessed by any other author. In animals with colon cancer, yacon flour consumption (7.5% FOS, during 8 weeks) reduced urinary mannitol and lactulose excretion (GRANCIERI et al., 2017). It should be considered, however, that in the present study yacon flour was consumed by adults with excess body weight and that did not have a significant intestinal integrity impairment, such as in colon cancer. In addition, differences in FOS concentration and in the intervention duration may have contributed to the differences in the outcomes.

Daily consumption of yacon flour associated with an energy restricted diet also did not affect the concentration of most of the inflammatory variables evaluated in this study. However, 67% of subjects who consumed yacon flour showed a reduction in NLR at the end of the study compared to baseline, and in half of them the values were below the proposed cut-off point (1.84) (BUYUKKAYA et al., 2014). Buyukkaya et al. (BUYUKKAYA et al., 2014) observed that subjects with LNR> 1.84 had higher glucose concentration and plasma C-reactive protein and greater number of criteria for metabolic syndrome. Thus, a reduction of NLR as observed in our study may indicate a beneficial effect of yacon in reducing the inflammatory status and improving general health status.

It should be emphasized that the subjects in this study did not present metabolic complications, as in studies in which there was a reduction on inflammatory markers after yacon supplementation (OLIVEIRA et al., 2016, 2017; SATOH et al., 2013a). Thus, it is possible that yacon flour exerts significant effects on inflammatory markers only in metabolically decompensated individuals. Results from healthy animal studies receiving standard diet supplemented with yacon corroborate our hypothesis. Supplementation with yacon flour (0.34 and 6.8g of FOS/kg body weight/day) did not affect leukocyte, lymphocyte, platelet or neutrophil counts in healthy animals (BONET et al., 2010; GENTA et al., 2005).

To our knowledge, this is the first study evaluating the effects of yacon flour consumption associated with an energy restricted diet on intestinal permeability, oxidative stress, and inflammation markers in adults with excess body weight. Yacon flour was chosen as our test food because its FOS and phenolic compounds contents are more stable than the ones in yacon root. Our data were obtained in a double-blind manner and were double-typed. In addition, we used strict eligibility criteria to select our subjects and the consumption of the test food (yacon flour) was done in the laboratory to guarantee adherence to the protocol. Our intervention did not produce adverse effects. Subjects presented a good adhesion and acceptability to our protocol. However, the limitations of our study were that we did not assess the intestinal microbiota and the fact that SCFAs concentrations were assessed in the feces. However, it is difficult to precisely access SCFAs production because the value obtained in the feces is a balance between its production and absorption. In addition, evaluating fecal SCFAs concentration has been one of the most widely method used by other researchers.

CONCLUSION

The consumption of 25g of yacon flour associated with an energy restricted diet for 6 weeks by adults with excess body weight reduced fecal SCFAs concentrations, and protein carbonyl concentrations, besides increasing total antioxidant capacity of the plasma. Thus, the consumption of yacon flour may be an interesting strategy to control oxidative stress in overweight subjects. Whether the beneficial effects of this short-term yacon flour supplementation will maintain in the longer term is not clear. Assessing the efficacy of yacon flour in the long-term period is therefore warranted. Furthermore, we believe that in individuals with metabolic complications, the consumption of yacon flour may lead to additional effects, as demonstrated in animal models studies. New studies should be conducted to verify that.

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5. CONCLUSION

- Yacon flour is a good source of fiber and phenolic compounds;
- The consumption of 25g / day of yacon flour by overweightsubjects for six weeks was well tolerated, did not cause adverse gastrointestinal effects, increased dietary fiber intake, improved stool consistency and reduced constipation;
- In addition, it contributed to improve body composition, increasing fat-free mass, besides favoring the reduction of body weight, body fat in specific regions (gyno, android and trunk), waist circumference and hip, waist / height index and sagittal abdominal diameter;
- Both groups with (YAC) and without yacon meal (CON) reduced energy intake compared with baseline, although energy intake and macronutrients did not differ between such groups. However, the reduction in the mean energy intake of YAC was 40% higher than the reduction observed for CON, suggesting a possible role of this flour on apetite suppression;
- Yacon flour has an antioxidant potential, verified *in vitro* and *in vivo*. Its consumption increased the total antioxidant capacity of the plasma and reduced the concentrations of carbonylated protein evaluated in the first day and after six weeks of intervention;
- A reduction in fecal SCFA concentrations was observed in response to the consumption of 25g / day of yacon flour for six weeks by overweight subjects. Additional analyzes of the intestinal microbiota may help in the interpretation of these results;
- Given the results obtained, we believe that more studies are needed to elucidate the unanswered questions. However, our results indicate that the consumption of yacon flour associated with an energy-restricted diet has positive effects on health.

6. APÊNDICES

6.1 Apêndice 1 – Questionário de triagem pelo telefone

Nome: ______________________________ Idade:________(20 a 50 anos) Data: ____/____/_____

Peso (kg): ______ Estatura (m): _______ IMC (Kg/m²) (25 a 35kg/m²): ________ Fuma: (   ) Sim (   ) Não

Gestante /Lactante: (   ) Sim (   ) Não   Consome bebida alcoólica? (   ) Sim (   ) Não   Frequência:__________

Pratica alguma atividade física? (   ) Sim (   ) Não   Qual? ______________________Frequência:__________

Intolerância/alergia a algum alimento? (   ) Sim (   )Não. Quais? ______________________________

Possui diagnóstico de alguma doença? (   ) Sim (   ) Não. Quais? ______________________________

(diabetes, doenças cardiovasculares, hipertensão arterial, distúrbios da tireoide, doenças hepáticas e gastrointestinais, colesterol alto)

Faz uso de algum medicamento? (   ) Sim (   ) Não Quais?______________________________

Tem disponibilidade de vir ao laboratório consumir café da manhã durante 6 semanas? (   ) Sim (   ) Não

Faz uso de: aveia, chia, linhaça, granola, kefir, Yakult, activia ...? (   ) Sim (   ) Não

Se sim, quantas vezes por semana?______________________________

Fez uso de antibiótico ou laxante nos últimos 3 meses? (   ) Sim (   ) Não

Agendada para próxima etapa? (   ) Sim (   ) Não - Telefone para contato: ______________________

Responsável pela entrevista: ______________________________

6.2 Apêndice 2 - Questionário de triagem

Nome:____ Código de ID: ___________
Telefone: ________________ e-mail:_______________________________________

PARTE A – DADOS PESSOAIS		CODIFICAÇÃO
A.1 Sexo do entrevistado (observar e marcar)	(1) masculino (2) feminino	
A.2 Data de nascimento	______/_____/_____	____/____/____
A.3 Qual é a situação conjugal atual? (**ler as alternativas**)	(1) Casado (2) Solteiro (3) Divorciado/separado (4) Viúvo (9) IGN	
A.4 Em Viçosa você mora (**ler as alternativas**)	(1) Com família (2) Com amigos (3) Sozinho	
A.5 Quantas pessoas moram na casa? (**incluindo o entrevistado**)	___________ 99 (IGN)	
A.6 Qual a renda familiar? (**incluir salário das pessoas que moram na casa e recebimento de bolsa**)	R$__________	
A.7 Qual a sua escolaridade?	(1) Ensino fundamental completo (2) Ensino fundamental incompleto (3) Ensino Médio completo (4) Ensino Médio incompleto (5) Ensino Superior completo (6) Ensino Superior incompleto (7) Pós-graduação completa (8) Pós-graduação incompleta (9) Nenhuma	
PARTE B – DADOS GERAIS		
B.1 Você está usando alguma medicação? (**se a resposta for sim, pergunte quais e a dosagem**)	(1) Sim (2) Não Quais/dose: _____________________________ __________________________________ _____	
B.2 Você faz uso de algum medicamento ou chá para emagrecer?	(1) Sim (2) Não Quais/dose: _____________________________ __________________________________ _____	
B.3 Nos últimos três meses você fez uso de laxante ou antibiótico?	(1) Sim (2) Não Quais/dose: _____________________________ __________________________________ _____	
B.4 Você fuma?	(1) Sim (2) Não	
B.5 Você costuma tomar bebida alcóolica? (**espere a resposta e marque o que for relatado, não leia as alternativas, se a**	(1) Sim (2) Não	

resposta for não, marque NA na pergunta B.6 e pule para a pergunta B.7)		
B.6 Quantas DOSES DE BEBIDAS ALCÓOLICAS você toma em uma semana normal? (1 dose = ½ garrafa /1 lata de cerveja, 1 taça de vinho (150 mL) ou 1 dose de uísque/conhaque/cachaça/vodca)	(1) nenhuma (2) de 1 a 7 doses (3) de 8 a 14 doses (4) 15 doses ou mais (5) NA	
B.7 Você está grávida ou amamentando? (**Perguntar só para mulheres**)	(1) Sim (2) Não (3) NA	
B.8 Sua menstruação veio regularmente, sem alteração de fluxo e data nos últimos três meses? (**Perguntar só para mulheres**)	(1) Sim (2) Não (3) NA Data da última menstruação: ____/____/_____	
B.9 Você alterou seu nível de atividade física no último mês? (**Se a resposta for não, pule para parte C**)	(1) Sim (2) Não (3) Não sabe	
B.10 Qual foi a alteração?	(1) iniciou (2) interrompeu (3) frequência (4) intensidade (5) NA	
PARTE C – HISTÓRIA CLÍNICA		
Você ou algum de seus familiares já tiveram ou tem alguma das seguintes doenças?		
C.1 Hipertensão arterial (**se nem o entrevistado ou alguém da família apresentar a doença, marcar não. Se alguém apresentar a doença, ver as opções**)	(1) Não (2) O próprio entrevistado (3) Pai (4) Mãe (5) Avós	
C.2 Diabetes (**Anotar o tipo**)	(1) Não (2) O próprio entrevistado (3) Pai (4) Mãe (5) Avós	
C.3 Hipoglicemia	(1) Não (2) O próprio entrevistado (3) Pai (4) Mãe (5) Avós	
C.4 Cardiopatia (angina, arritmia, infarto, sopro, insuficiência cardíaca)	(1) Não (2) O próprio entrevistado (3) Pai (4) Mãe (5) Avós	
C.5 Neuropatia (neuropatia periférica: fraqueza, dormência, dor nas mãos e pés)	(1) Não (2) O próprio entrevistado (3) Pai (4) Mãe (5) Avós	
C.6 Hepatopatia (problema no fígado)	(1) Não (2) O próprio entrevistado (3) Pai (4) Mãe (5) Avós	
C.7 Nefropatia (problema renal)	(1) Não (2) O próprio entrevistado (3) Pai (4) Mãe (5) Avós	
C.8 Dislipidemia (colesterol, triglicérides, LDL, HDL alterados) Obs: especificar	(1) Não (2) O próprio entrevistado (3) Pai (4) Mãe (5) Avós	
C.9 Obesidade	(1) Não (2) O próprio entrevistado (3) Pai (4) Mãe (5) Avós	
C.10 Câncer (qual tipo)	(1) Não (2) O próprio entrevistado (3) Pai (4) Mãe (5) Avós	
C.11 Hipo ou Hipertireoidismo (problema na tireoide)	(1) Não (2) O próprio entrevistado (3) Pai (4) Mãe (5) Avós	
C.12 Outros problemas hormonais (Síndrome de Cushing – excesso de cortisol...)	(1) Não (2) O próprio entrevistado (3) Pai (4) Mãe (5) Avós	
C.13 Síndrome do ovário policístico (**Só para mulheres**)	(1) Não (2) O próprio entrevistado (3) Pai (4) Mãe (5) Avós	
C.14 Doenças intestinais (Doença celíaca, diverticulite, Doença de Chron, Síndrome de intestino irritável, outras)	(1) Não (2) O próprio entrevistado (3) Pai (4) Mãe (5) Avós	
C.15 Constipação	(1) Não (2) O próprio entrevistado (3) Pai (4) Mãe (5) Avós	

C.16 Transtorno alimentar (anorexia, bulimia, compulsão alimentar)	(1) Não (2) O próprio entrevistado (3) Pai (4) Mãe (5) Avós	
C.17 Depressão	(1) Não (2) O próprio entrevistado (3) Pai (4) Mãe (5) Avós	
C18 Você ou algum de seus familiares tem alguma outra doença, que não foi listada anteriormente?	(1) Sim (2) Não Quem?___________________________	
C.19 Quando foi o último exame que você realizou?	____/____/____	____/____/____
C.20 Seus exames de sangue apresentaram alguma alteração? **<u>(se a resposta for sim, pergunte quais)</u>**	(1) Sim (2) Não Quais: ___________________________ _________________________________	
PARTE D – INFORMAÇÕES DIETÉTICAS E NUTRICIONAIS		
D.1 Você está adotando alguma dieta específica para aumento/ redução do peso?	(1) Sim (2) Não	
D.2 Você já seguiu alguma dieta específica para aumento/redução de peso? **<u>(se a resposta for sim, pergunte a quanto tempo atrás e por quanto tempo seguiu)</u>**	(1) Sim (2) Não A quanto tempo atrás? ______________ Por quanto tempo? ________________	
D.3 Você planeja adotar alguma dieta específica para aumento/redução de peso?	(1) Sim (2) Não	
D.4 Você está participando de alguma outra pesquisa? (**<u>se a resposta for sim, pergunte quais)</u>**	(1) Sim (2) Não Quais: ___________________________	
D.5 Você participou de alguma outra pesquisa? (**<u>se a resposta for sim, pergunte quais e a quanto tempo atrás)</u>**	(1) Sim (2) Não Quais: ___________________________ A quanto tempo atrás? ______________	
D.6 Você mudou seu hábito alimentar no último mês?	(1) Sim (2) Não (3) Não sabe	
D.7 Você tem alguma aversão alimentar? (**<u>se a resposta for sim, pergunte quais)</u>**	(1) Sim (2) Não Quais: ___________________________	
D.8 Você tem alguma intolerância alimentar**?** (**<u>se a resposta for sim, pergunte quais)</u>**	(1) Sim (2) Não Quais: ___________________________	
D.9 Você tem alergia a algum alimento ou composto dietético? (**<u>se a resposta for sim, pergunte quais)</u>**	(1) Sim (2) Não Quais: _________________________________	
D.10 Você é vegetariano?	(1) Sim (2) Não	
D.11 Você faz uso de suplementos ou vitaminas? (**<u>se a resposta for sim, pergunte quais)</u>**	(1) Sim (2) Não Quais: _________________________________	
D.12 Você faz uso de probióticos (Actvia, Leite fermentado, *Lactobaccilus,* Actimel, Kefir)? (**<u>se a resposta for sim, pergunte quais, se a resposta for não marque NA na pergunta D.13 e pule para a pergunta D.14)</u>**	(1) Sim (2) Não Quais: ___________________________	
D.13 Com que frequência?	(1) 1 a 3 dias (2) de 4 a 6 dias (3) todos os dias (4) quase nunca (5) nunca (6) NA	
D.14 Você faz uso de prebióticos (Aveia, Yacon, Granola, Chia, Amaranto, Linhaça, Biomassa de Banana Verde)? (**<u>se a resposta for sim, pergunte quais, se a resposta for não marque NA na pergunta D.15 e pule para a pergunta D.16)</u>**	(1) Sim (2) Não Quais: ___________________________	

D.15 Com que frequência?	(1) 1 a 3 dias (2) de 4 a 6 dias (3) todos os dias (4) quase nunca (5) nunca (6) NA	
D.16 Você faz uso de simbióticos? **(se a resposta for sim, pergunte quais, se a resposta for não marque NA na pergunta D.17 e pule para a pergunta D.18)**	(1) Sim (2) Não Quais: ____________________________	
D.17 Com que frequência?	(1) 1 a 3 dias (2) de 4 a 6 dias (3) todos os dias (4) quase nunca (5) nunca (6) NA	
D.18 O que você utiliza para adoçar as bebidas ou preparações?	(1) açúcar refinado (2) açúcar cristal (3) açúcar mascavo (4) adoçante artificial (5) açúcar de coco (6) mel (7) nenhum (8) mais de um tipo Quais? ( ) ( ) ( )	
D.19 Que tipo de gordura você costuma usar no preparo das refeições?	(1) óleo de soja/milho (2) azeite (3) outro óleo (4) bacon/banha (4) manteiga (5) margarina (6) mais de um tipo Quais? ( ) ( ) ( )	
Indique as horas do dia em que você NORMALMENTE consume refeições e lanches. Coloque a letra R para refeições e L para lanches sob cada hora do dia. AM (manhã e início da tarde) 1 2 3 4 5 6 7 8 9 10 11 12 — — — — — — — — — — — — PM (tarde e noite) 1 2 3 4 5 6 7 8 9 10 11 12 — — — — — — — — — — — —		
D.20 Você gosta de vitamina de maracujá?	(1) Sim (2) Não (3) NA	
D.21 Você gosta de vitamina de manga?	(1) Sim (2) Não (3) NA	
D.22 Você gosta de vitamina de goiaba?	(1) Sim (2) Não (3) NA	
D.23 Você gosta de vitamina de amora?	(1) Sim (2) Não (3) NA	
D.24 Você gosta de cappuccino?	(1) Sim (2) Não (3) NA	
D.25 Você gosta de leite com chocolate?	(1) Sim (2) Não (3) NA	
PARTE E – AVALIAÇÃO ANTROPOMÉTRICA E VARIÁVEIS CLÍNICAS		
E.1 Você ganhou peso nos últimos 3 meses? (**se a reposta for sim, perguntar quantos kg)**	(1) Sim (2) Não (3) Não sabe Quantos kg? ________________________	
E.2 Você perdeu peso nos últimos 3 meses? (**se a reposta for sim, perguntar quantos kg)**	(1) Sim (2) Não (3) Não sabe Quantos kg? ________________________	
E.3 Peso habitual	______ kg	
E.4 Peso atual (mensuração)	______ kg	
E.5 Altura atual (mensuração)	______ cm	
E.6 IMC	______ kg/m²	
E.7 % gordura corporal (mensuração BIA)	______ %	
E.8 Pressão arterial sistólica	________ mmHg	
E.9 Pressão arterial diastólica	________ mmHg	
E.10 Three Factor	_________	

6.3 Apêndice 3 – Termo De Consentimento Livre e Esclarecido

O Sr.(a) está sendo convidado(a) como voluntário(a) a participar de uma pesquisa que irá avaliar o efeito de uma intervenção alimentar sobre alterações metabólicas em indivíduos com excesso de peso. Nesta pesquisa pretendemos avaliar os efeitos do consumo de uma bebida associado à uma dieta hipocalórica no controle do peso em indivíduos com excesso de peso. O motivo pelo qual pretendemos estudar esse tema é a possível relação entre o consumo dos ingredientes da bebida e benefícios à saúde tais como redução do peso corporal, redução da glicemia, melhora do perfil lipídico, entre outros.

Para participar do estudo, o Sr.(a) deverá comparecer ao Laboratório de Estudos em Ingestão Alimentar (LEIA), localizado no Departamento de Nutrição e Saúde da Universidade Federal de Viçosa, em um horário pré-definido de acordo com sua disponibilidade para realização de uma triagem. Nessa triagem, adotaremos os seguintes procedimentos: medidas de peso, altura, percentual de gordura corporal (bioimpedância elétrica) e pressão arterial; avaliação da glicemia capilar (por punção digital); aplicação de questionários de triagem contendo perguntas relativas aos dados pessoais, ao estilo de vida e ao histórico pessoal e familiar de doenças; questionário de avaliação do nível de restrição/desinibição alimentar e questionário de avaliação do nível de atividade física habitual. A triagem terá duração de aproximadamente 1h30.

O estudo terá duração de seis semanas e o Sr.(a) deverá comparecer diariamente ao laboratório, em horário pré-determinado, para fazer a ingestão do café da manhã. No início (dia 0), na metade do estudo (3 semanas) e ao final do estudo (seis semanas) serão realizadas uma avaliação da ingestão alimentar (registro alimentar de 24h); avaliação antropométrica e de composição corporal. No início e ao final do estudo será realizada a coleta de sangue (20mL), urina e fezes. O Sr. (a) receberá uma refeição padronizada (macarrão, queijo e suco), os quais iremos fornecer, que deverá ser consumida na noite anterior a coleta de sangue e urina. O Sr. (a) deverá comparecer ao laboratório após 10 horas de jejum onde um profissional capacitado coletará o sangue. A coleta de urina será realizada pelo Sr. (a). Nesse dia o Sr.(a) deverá permanecer seis horas no laboratório. Após esse período o Sr. (a) receberá uma refeição (sanduíche natural de frango e suco) e será liberado para exercer suas atividades normais. Além da coleta de sangue e urina, será solicitado ao Sr. (a) que colete amostras de fezes (em sua residência) em coletores universais estéreis próprios, os quais iremos fornecer.

Em relação aos riscos/desconfortos do estudo, serão realizadas algumas ações para reduzi-los. A coleta de sangue será realizada individualmente por profissional capacitado a fim de reduzir qualquer desconforto possível. Para a coleta de sangue serão utilizados apenas materiais descartáveis. Durante a extração de sangue o Sr (a) poderá sentir o local da punção (picada) na dobra do cotovelo dolorido e poderão aparecer pequenos hematomas (roxo), como em qualquer outra coleta de sangue que possa ter feito no passado. A aplicação dos questionários e as avaliações antropométricas e de composição corporal serão realizadas em ambiente adequado e de forma individualizada utilizando-se as técnicas padronizadas e preconizadas na literatura científica. Com relação aos questionários caso o Sr. (a) sinta algum constrangimento, devido a determinada pergunta, poderá se negar a responder uma, duas ou até mesmo todas as perguntas se as mesmas lhe causarem qualquer incomodo. A avaliação da glicemia sanguínea por punção digital será realizada com materiais descartáveis e as punções feitas em partes menos sensíveis dos dedos, para evitar qualquer desconforto. Caso ocorra algum mal estar durante a coleta de sangue, como por exemplo, desmaios ou queda de pressão a equipe o conduzirá a unidade de atendimento médico mais próxima. De acordo com os relatos na literatura, não há riscos para o indivíduo devido ao consumo da bebida teste. No entanto, caso ocorra algum desconforto a equipe de pesquisa se responsabilizará pelos cuidados com os voluntários.

Quanto aos benefícios, ao final do estudo o Sr.(a) receberá uma avaliação do seu estado nutricional e de saúde, bem como orientações para melhorar sua alimentação habitual. Cada voluntário receberá um planejamento alimentar individualizado e será conscientizado quanto à importância da ingestão de uma alimentação adequada. Além disso, os participantes do estudo terão acesso aos seus dados das avaliações antropométrica, composição corporal e bioquímica. O resultado destas avaliações será discutido com cada participante.

Para participar deste estudo o Sr.(a) não terá nenhum custo, nem receberá qualquer vantagem financeira. Apesar disso, caso sejam identificados e comprovados danos provenientes desta pesquisa, o Sr.(a) tem assegurado o direito à indenização. O Sr.(a) tem garantida plena liberdade de recusar-se a participar ou retirar seu consentimento, em qualquer fase da pesquisa, sem necessidade de comunicado prévio. A sua participação é voluntária e a recusa em participar não acarretará qualquer penalidade ou modificação na forma em que o Sr.(a) é atendido(a) pelo pesquisador. Os resultados da pesquisa estarão à sua disposição quando finalizada. O Sr.(a) não será identificado(a) em nenhuma publicação que possa resultar. Seu nome ou o material que indique sua participação não serão liberados sem a sua permissão.

Este termo de consentimento encontra-se impresso em duas vias originais, sendo que uma será arquivada pelo pesquisador responsável, no “Laboratório de Estudos em Ingestão Alimentar – LEIA - DNS/UFV” e a outra será fornecida ao Sr.(a). As amostras e questionários coletados no presente estudo serão armazenados para análises relativas a este projeto e ainda, poderão ser utilizados em outras pesquisas da mesma área de estudo. Os resultados desse projeto e de outros futuros serão apresentados, comunicados e/ou publicados no meio científico, mas sempre preservando sua confidencialidade e privacidade. Os dados e instrumentos utilizados na pesquisa ficarão arquivados com o pesquisador responsável por um período de 5 (cinco) anos após o término da pesquisa, e depois desse tempo serão destruídos. Os pesquisadores tratarão a sua identidade com padrões profissionais de sigilo e confidencialidade, atendendo à legislação brasileira, em especial, à Resolução 466/2012 do Conselho Nacional de Saúde, e utilizarão as informações somente para fins acadêmicos e científicos.

Eu,____
______________contato ____,
fui informado(a) dos objetivos da pesquisa de maneira clara e detalhada, e esclareci

minhas dúvidas. Sei que a qualquer momento poderei solicitar novas informações e modificar minha decisão de participar se assim o desejar. Declaro que concordo em participar. Recebi uma via original deste termo de consentimento livre e esclarecido e me foi dada a oportunidade de ler e esclarecer minhas dúvidas.

Viçosa, ______ de ______________ de 20___.

_______________________________	_______________________________________
Participante	Pesquisador

Nomes e números dos telefones dos investigadores:
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Em caso de discordância ou irregularidades sob o aspecto ético desta pesquisa, você poderá consultar:
CEP/UFV – Comitê de Ética em Pesquisa com Seres Humanos
Universidade Federal de Viçosa - Edifício Arthur Bernardes, piso inferior
Av. PH Rolfs, s/n – Campus Universitário - Cep: 36570-900 Viçosa/MG
Telefone: (31)3899-2492 - Email: cep@ufv.br - www.cep.ufv.br

7. ANEXOS

7.1 Anexo 1 - Artigo original publicado na Clinical nutrition ESPEN

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Randomized Controlled Trial

Consumption of yacon flour improves body composition and intestinal function in overweight adults: A randomized, double-blind, placebo-controlled clinical trial

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A R T I C L E I N F O

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S U M M A R Y

Background & aims: Yacon root is a natural source of fructooligosaccharides (FOS), and it has been studied for its potential effect as a functional food. However, FOS in the *in natura* root are rapidly hydrolyzed. Thus, the use of dehydrated products such as flour ensures stability of FOS. The effect of chronic consumption of yacon flour on body composition, food intake and of biochemical variables concentration has not yet been evaluated in humans. Thus, we evaluated the effects of yacon flour consumption on these variables associated with excess body weight.

Methods: Twenty-six adults (31.3 ± 8.5 years) with excess weight (30.4 ± 2.4 kg/m^2, 40.16 ± 6.7% body fat) participated in this randomized, double-blind, six-week study. Subjects were randomly allocated to one of the experimental groups (control (n = 13) or yacon flour (n = 13)), received the prescription of energy-restricted diets (- 500 kcal/day) and daily consumed a breakfast drink not containing or containing 25 g of yacon flour (0.1 g of FOS/kg body weight). At the beginning and at the end of the study, biochemical analyses (glycemia, insulinemia, lipid profile and liver function markers), anthropometrics (weight, waist, hip and neck circumference and sagittal abdominal diameter), body composition (lean mass, total body fat, trunk fat, android fat and gynoid fat), blood pressure, intestinal function, and food intake were assessed.

Results: Daily yacon flour consumption was well tolerated, did not cause adverse gastrointestinal effects, increased dietary fiber consumption, promoted greater body weight, waist circumference, waist to height index, sagittal abdominal diameter, and body fat reduction, besides improving bowel function in comparison to the control group.

Conclusions: Yacon flour served as a natural fiber supplement and proved to be an effective adjuvant to be used in nutritional strategies to control excess body weight.

7.2 Anexo 2- Questionário Internacional De Atividade Física.

Nome:_____________________________Data: ___/___ /___ Idade: ____ Sexo: F ( ) M ( ).
Quantas horas você trabalha por dia: ____ Quantos anos completos você estudou: _____

Nós estamos interessados em saber que tipos de atividade física as pessoas fazem como parte do seu dia a dia. Este projeto faz parte de um grande estudo que está sendo feito em diferentes países ao redor do mundo. Suas respostas nos ajudarão a entender que tão ativos nós somos em relação à pessoas de outros países. As perguntas estão relacionadas ao tempo que você gasta fazendo atividade física em uma semana **ULTIMA SEMANA**. As perguntas incluem as atividades que você faz no trabalho, para ir de um lugar a outro, por lazer, por esporte, por exercício ou como parte das suas atividades em casa ou no jardim. Suas respostas são MUITO importantes. Por favor, responda cada questão mesmo que considere que não seja ativo. Obrigado pela sua participação!

Para responder as questões lembre que:
- Atividades físicas **VIGOROSAS** são aquelas que precisam de um grande esforço físico e que fazem respirar **MUITO** mais forte que o normal
- Atividades físicas **MODERADAS** são aquelas que precisam de algum esforço físico e que fazem respirar **UM POUCO** mais forte que o normal

SEÇÃO 1- ATIVIDADE FÍSICA NO TRABALHO

Esta seção inclui as atividades que você faz no seu serviço, que incluem trabalho remunerado ou voluntário, as atividades na escola ou faculdade e outro tipo de trabalho não remunerado fora da sua casa. **NÃO** incluir trabalho não remunerado que você faz na sua casa como tarefas domésticas, cuidar do jardim e da casa ou tomar conta da sua família. Estas serão incluídas na seção 3.

1a. Atualmente você trabalha ou faz trabalho voluntário fora de sua casa?
( ) Sim ( ) Não – Caso você responda não **Vá para seção 2: Transporte**
As próximas questões são em relação a toda a atividade física que você fez na **ultima semana** como parte do seu trabalho remunerado ou não remunerado. **NÃO** inclua o transporte para o trabalho. Pense unicamente nas atividades que você faz por **pelo menos 10 minutos contínuos**:

1b. Em quantos dias de uma semana normal você **anda,** durante **pelo menos 10 minutos contínuos**, **como parte do seu trabalho**? Por favor, **NÃO** inclua o andar como forma de transporte para ir ou voltar do trabalho.
_______dias por **SEMANA**
( ) nenhum - **Vá para a seção 2 - Transporte**.

1c. Quanto tempo no total você usualmente gasta **POR DIA** caminhando **como parte do seu trabalho** ? ____ horas ______ minutos

1d. Em quantos dias de uma semana normal você faz atividades **moderadas,** por **pelo menos 10 minutos contínuos,** como carregar pesos leves **como parte do seu trabalho**?
_______dias por **SEMANA**

( ) nenhum - **Vá para a questão 1f**

1e. Quanto tempo no total você usualmente gasta **POR DIA** fazendo atividades moderadas **como parte do seu trabalho**? _____ horas ______ minutos

1f. Em quantos dias de uma semana normal você gasta fazendo atividades **vigorosas,** por **pelo menos 10 minutos contínuos,** como trabalho de construção pesada, carregar grandespesos, trabalhar com enxada, escavar ou subir escadas **como parte do seu trabalho:**
_______dias por **SEMANA** ( ) nenhum - **Vá para a questão 2a.**

1g. Quanto tempo no total você usualmente gasta **POR DIA** fazendo atividades físicas vigorosas **como parte do seu trabalho**? _____ horas ______ minutos

SEÇÃO 2 – ATIVIDADE FÍSICA COMO MEIO DE TRANSPORTE

Estas questões se referem à forma típica como você se desloca de um lugar para outro, incluindo seu trabalho, escola, cinema, lojas e outros.
2a. O quanto você andou na ultima semana de carro, ônibus, metrô ou trem?
________dias por **SEMANA**
( ) nenhum - **Vá para questão 2c**

2b. Quanto tempo no total você usualmente gasta **POR DIA andando de carro, ônibus, metrôou trem**? _____horas _____minutos

Agora pense **somente** em relação a caminhar ou pedalar para ir de um lugar a outro na ultima semana.

2c. Em quantos dias da ultima semana você andou de bicicleta por **pelo menos 10 minutos contínuos** para ir de um lugar para outro? (**NÃO** inclua o pedalar por lazer ou exercício)
_____ dias por **SEMANA**
( ) Nenhum - **Vá para a questão 2e**.

2d. Nos dias que você pedala quanto tempo no total você pedala **POR DIA** para ir de um lugar para outro? _______ horas _____ minutos

2e. Em quantos dias da ultima semana você caminhou por **pelo menos 10 minutos contínuos** para ir de um lugar para outro? (**NÃO** inclua as caminhadas por lazer ou exercício)
_____ dias por **SEMANA**
( ) Nenhum - **Vá para a Seção 3**.

2f. Quando você caminha para ir de um lugar para outro quanto tempo **POR DIA** você gasta? (**NÃO** inclua as caminhadas por lazer ou exercício) _______ horas _____ minutos

SEÇÃO 3 – ATIVIDADE FÍSICA EM CASA: TRABALHO, TAREFAS DOMÉSTICAS E CUIDAR DA FAMÍLIA

Esta parte inclui as atividades físicas que você fez na ultima semana na sua casa e ao redor da sua casa, por exemplo, trabalho em casa, cuidar do jardim, cuidar do quintal, trabalho de manutenção da casa ou para cuidar da sua família. Novamente pense ***somente*** naquelas atividades físicas que você faz **por pelo menos 10 minutos contínuos**.

3a. Em quantos dias da ultima semana você fez atividades **moderadas** por pelo menos 10 minutos como carregar pesos leves, limpar vidros, varrer, rastelar **no jardim ou quintal.**

________dias por **SEMANA**

( ) Nenhum - **Vá para questão 3c**.

3b. Nos dias que você faz este tipo de atividades quanto tempo no total você gasta **POR DIA** fazendo essas atividades moderadas **no jardim ou no quintal**? _______ horas _____ minutos

3c. Em quantos dias da ultima semana você fez atividades **moderadas** por pelo menos 10 minutos como carregar pesos leves, limpar vidros, varrer ou limpar o chão **dentro da sua casa**.

_____ dias por **SEMANA**

( ) Nenhum - **Vá para questão 3e.**

3d. Nos dias que você faz este tipo de atividades moderadas **dentro da sua casa** quanto tempo no total você gasta **POR DIA**? _______ horas _____ minutos

3e. Em quantos dias da ultima semana você fez atividades físicas **vigorosas no jardim ou quintal** por pelo menos 10 minutos como carpir, lavar o quintal, esfregar o chão:

_____ dias por **SEMANA**

( ) Nenhum - **Vá para a seção 4.**

3f. Nos dias que você faz este tipo de atividades vigorosas **no quintal ou jardim** quanto tempo no total você gasta **POR DIA**? _______ horas _____ minutos

SEÇÃO 4 - ATIVIDADES FÍSICAS DE RECREAÇÃO, ESPORTE, EXERCÍCIO E DE LAZER.

Esta seção se refere às atividades físicas que você fez na ultima semana unicamente por recreação, esporte, exercício ou lazer. Novamente pense somente nas atividades físicas que faz **por pelo menos 10 minutos contínuos**. Por favor, **NÃO** inclua atividades que você já tenha citado.

4a. **Sem contar qualquer caminhada que você tenha citado anteriormente**, em quantos dias da ultima semana você caminhou **por pelo menos 10 minutos contínuos no seu tempo livre**?

_____ dias por **SEMANA**

( ) Nenhum - **Vá para questão 4c**

4b. Nos dias em que você caminha **no seu tempo livre**, quanto tempo no total você gasta **POR DIA**? _______ horas _____ minutos

4c. Em quantos dias da ultima semana você fez atividades **moderadas no seu tempo livre** por pelo menos 10 minutos, como pedalar ou nadar a velocidade regular, jogar bola, vôlei, basquete, tênis :

_____ dias por **SEMANA**

( ) Nenhum - **Vá para questão 4e.**

4d. Nos dias em que você faz estas atividades moderadas **no seu tempo livre** quanto tempo no total você gasta **POR DIA**? _______ horas _____ minutos

4e. Em quantos dias da ultima semana você fez atividades **vigorosas no seu tempo livre** por pelo menos 10 minutos, como correr, fazer aeróbicos, nadar rápido, pedalar rápido ou fazer Jogging:
_____ dias por **SEMANA**
( ) Nenhum - **Vá para seção 5.**

4f. Nos dias em que você faz estas atividades vigorosas **no seu tempo livre** quanto tempo no total você gasta **POR DIA**? _______ horas _____ minutos

SEÇÃO 5 – TEMPO GASTO SENTADO

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

5a. Quanto tempo no total você gasta sentado durante um **dia de semana**?
______horas ____minutos

5b. Quanto tempo no total você gasta sentado durante em um **dia de final de semana**?
______horas ____minutos

7.3 Anexo 3 – Questionário para avaliação do nível de restrição e desinibição alimentar

1- Quando eu sinto o cheiro de um bife fritando, ou vejo um pedaço suculento de carne, eu encontro muita dificuldade para não comê-lo, se eu tiver acabado de fazer uma refeição.	V	F
2- Eu geralmente como muito em ocasiões sociais, gosto de festas e picnics.	V	F
3- Eu geralmente estou faminto por isso como mais de três vezes por dia.	V	F
4- Quando eu como minha cota de calorias, eu normalmente me sinto bem em não comer mais nada	V	F
5- Fazer dieta é muito difícil para mim porque sinto muita fome.	V	F
6- Eu intencionalmente como pequenas refeições para ajudar no controle do meu peso	V	F
7- Às vezes, alguns alimentos têm sabor tão bom que consigo comer mesmo quando não estou com fome	V	F
8- Visto que estou sempre com fome, às vezes desejo que enquanto estou comendo, um especialista me diga se comi o suficiente ou se poderia comer mais alguma coisa.	V	F
9- Quando estou ansioso (a), costumo comer mais do que normalmente como.	V	F
10- A vida é muito curta para perdê-la fazendo dieta.	V	F
11- Quando meu peso aumenta ou diminui, faço dieta	V	F
12- Sempre que sinto muita fome tenho que comer alguma coisa.	V	F
13- Quando estou com alguém que come muito, eu também como muito.	V	F
14- Eu tenho uma boa noção de quantas calorias têm os alimentos mais comuns.	V	F
15- Às vezes, quando eu começo a comer, não consigo parar.	V	F
16- Não é difícil para eu deixar resto no prato.	V	F
17- Em determinados horários do dia, eu fico com fome porque tenho o hábito de comer nesses horários	V	F
18- Quando estou fazendo dieta, se eu como algo que não é permitido, eu intencionalmente como menos por um período de tempo para compensar.	V	F
19- Quando estou com alguém que está comendo, as vezes sinto fome suficiente para comer também	V	F
20- Quando me sinto deprimido, eu sempre como muito	V	F
21- Eu divirto comendo muito e fico deprimido contando calorias ou vigiando meu peso.	V	F
22- Quando eu vejo uma guloseima, eu freqüentemente fico com fome e tenho que comer imediatamente.	V	F
23- Eu freqüentemente paro de comer antes de estar completamente cheio, como forma consciente de limitar a quantidade de comida ingerida.	V	F
24- Eu sinto tanta fome que meu estômago, freqüentemente, parece um buraco sem fundo.	V	F
25- Meu peso mudou pouco durante os últimos 10 anos.	V	F
26- Eu estou sempre faminta, por isso é difícil para eu parar de comer antes de acabar a comida no meu prato.	V	F
27- Quando eu me sinto sozinha, eu me consolo comendo.	V	F
28- Eu conscientemente vomito uma refeição com objetivo de não ganhar peso.	V	F
29-Eu, algumas vezes, tenho muita fome pela tarde ou à noite.	V	F
30- Eu como qualquer coisa que quero, quando eu quero.	V	F
31- Sem pensar em comida, eu agüento ficar muito tempo sem comer.	V	F
32- Eu conto calorias como meio consciente de controlar meu peso.	V	F
33- Eu não como alguns alimentos porque eles podem me engordar.	V	F
34- Eu estou sempre com fome o suficiente para comer por muito tempo.	V	F
35- Eu presto muita atenção às mudanças no meu corpo.	V	F

36- Enquanto estou fazendo dieta, se eu como um alimento que não é permitido, eu, muitas vezes, como outros alimentos com elevado teor calórico. V F

PARTE 2

Por favor responda as seguintes questões marcando um "x" na resposta apropriada para você.

37- Com que freqüência você faz dieta com intenção de controlar seu peso?
1 - raramente 2 - algumas vezes 3 - freqüentemente 4 - sempre

38- Poderia a flutuação de peso de 2.kg afetar a maneira como você vive sua vida?
1 - não totalmente 2 - pouco 3 - moderadamente 4 - muito

39- Qual a freqüência que você sente fome?
1 - somente na hora das refeições 2 - algumas vezes entre as refeições 3 - freqüentemente entre as refeições 4 - quase sempre

40- Sua sensação de culpa por comer muito ajuda você a controlar sua ingestão de alimentos?
1 - nunca 2 - raramente 3 - freqüentemente 4 - sempre

41- Quão difícil seria para você parar de comer a meio caminho de terminar o jantar e ficar sem comer nas próximas quatro horas?
1 – fácil 2 - pouco difícil 3 - moderadamente difícil 4 - muito difícil

42- Você tem consciência sobre o que você está comendo?
1 - não totalmente 2 - pouco 3 - moderadamente 4 - extremamente

43- Qual a freqüência que você tem resistido a alimentos tentadores?
1 - quase nunca 2 - raramente 3 - freqüentemente 4 - quase sempre

44- Qual a probabilidade de você comprar alimentos de baixa caloria?
1 - improvável 2 - pouco provável 3 - moderadamente provável 4 - muito provável

45- Você come moderadamente diante de outros e sozinho come grande quantidade de alimentos?
1 - nunca 2 - raramente 3 - freqüentemente 4 - sempre

46- Qual a probabilidade de você, conscientemente, comer lentamente com objetivo de reduzir o quanto você come?
1 - improvável 2 - pouco provável 3 - moderadamente provável 4 - muito provável

47- Com qual freqüência você dispensa uma sobremesa porque você já está satisfeita?
1 - quase nunca 2 - raramente 3 - no mínimo uma vez por semana 4 - quase todo dia

48- Qual a probabilidade de você comer conscientemente menos do que você quer?
1 - improvável 2 - pouco provável 3 - moderadamente provável 4 - muito provável

49- Você costuma comer mesmo sem estar com fome?
1 - nunca 2 - raramente 3 - Algumas vezes 4 - ao menos uma vez por semana

50- Na escala de 0 a 5, onde 0 quer dizer sem restrição alimentar (comer tudo que você quer, sempre que você quer) e 5 significa restrição total (limita constantemente a ingestão de alimentos e nunca cede) qual o número você poderia dar para você mesmo?

0 - Come tudo que você quer, quando que você quer
1 - Freqüentemente come tudo que você quer, quando você quer
2 - Muitas vezes come tudo que você quer, Quando você quer
3 - Muitas vezes limita ingestão de alimentos, mas freqüentemente cede
4 - Freqüentemente limita ingestão de alimentos, mas raramente cede
5 - Constantemente limita ingestão de alimentos, nunca cede

51- Até que ponto esta declaração descreve seu comportamento alimentar? Eu começo fazer dieta pela manhã, mas devido algum número de coisas que acontecem durante o dia, pela tarde eu me rendo e como o que eu quero e prometo a mim mesma (o) começar, novamente, a dieta amanhã.

1 - não parece comigo	2 - parece um pouco comigo	3 - me descreve muito bem	4 - me descreve perfeitamente

7.4 Anexo 4 – Questionário Quantitativo De Frequência Alimentar

Leite e derivados	**Quantas vezes você come**	**Unidade**	**Quantidade em medida caseira**
Leite integral	N 1 2 3 4 5 6 7 8 9 10	D S M	
Leite desnatado	N 1 2 3 4 5 6 7 8 9 10	D S M	
Leite semi-desnatado	N 1 2 3 4 5 6 7 8 9 10	D S M	
Leite fermentado	N 1 2 3 4 5 6 7 8 9 10	D S M	
Iogurte convencional (sabores)	N 1 2 3 4 5 6 7 8 9 10	D S M	
Iogurte natural	N 1 2 3 4 5 6 7 8 9 10	D S M	
Iogurte light	N 1 2 3 4 5 6 7 8 9 10	D S M	
Queijo branco	N 1 2 3 4 5 6 7 8 9 10	D S M	
Queijo amarelo	N 1 2 3 4 5 6 7 8 9 10	D S M	
Requeijão convencional	N 1 2 3 4 5 6 7 8 9 10	D S M	
Requeijão light	N 1 2 3 4 5 6 7 8 9 10	D S M	
Creme de ricota	N 1 2 3 4 5 6 7 8 9 10	D S M	
Cream cheese	N 1 2 3 4 5 6 7 8 9 10	D S M	
Cottage	N 1 2 3 4 5 6 7 8 9 10	D S M	
Ricota	N 1 2 3 4 5 6 7 8 9 10	D S M	
	N 1 2 3 4 5 6 7 8 9 10	D S M	
Pães e substitutos			
Pão francês	N 1 2 3 4 5 6 7 8 9 10	D S M	
Pão de forma convencional	N 1 2 3 4 5 6 7 8 9 10	D S M	
Pão de forma Light	N 1 2 3 4 5 6 7 8 9 10	D S M	
Pão integral	N 1 2 3 4 5 6 7 8 9 10	D S M	
Pão de queijo	N 1 2 3 4 5 6 7 8 9 10	D S M	
Biscoito salgado	N 1 2 3 4 5 6 7 8 9 10	D S M	
Biscoito polvilho	N 1 2 3 4 5 6 7 8 9 10	D S M	
Biscoito doce	N 1 2 3 4 5 6 7 8 9 10	D S M	
Biscoito recheado diet	N 1 2 3 4 5 6 7 8 9 10	D S M	
Biscoito recheado convencional	N 1 2 3 4 5 6 7 8 9 10	D S M	
Biscoito Waffer diet	N 1 2 3 4 5 6 7 8 9 10	D S M	
Biscoito Waffer convencional	N 1 2 3 4 5 6 7 8 9 10	D S M	
Bolo diet	N 1 2 3 4 5 6 7 8 9 10	D S M	
Bolo convencional ou broa	N 1 2 3 4 5 6 7 8 9 10	D S M	
Rosquinha doce	N 1 2 3 4 5 6 7 8 9 10	D S M	
Cereal matinal	N 1 2 3 4 5 6 7 8 9 10	D S M	
Granola	N 1 2 3 4 5 6 7 8 9 10	D S M	

Aveia	N 1 2 3 4 5 6 7 8 9 10	D S M	
Tapioca	N 1 2 3 4 5 6 7 8 9 10	D S M	
	N 1 2 3 4 5 6 7 8 9 10	D S M	
Gorduras			
Margarina convencional	N 1 2 3 4 5 6 7 8 9 10	D S M	
Margarina Light	N 1 2 3 4 5 6 7 8 9 10	D S M	
Manteiga	N 1 2 3 4 5 6 7 8 9 10	D S M	
Maionese convencional	N 1 2 3 4 5 6 7 8 9 10	D S M	
Maionese light	N 1 2 3 4 5 6 7 8 9 10	D S M	
Azeite	N 1 2 3 4 5 6 7 8 9 10	D S M	
Óleo composto	N 1 2 3 4 5 6 7 8 9 10	D S M	
Óleo vegetal __________________	N 1 2 3 4 5 6 7 8 9 10	D S M	
Gordura de porco	N 1 2 3 4 5 6 7 8 9 10	D S M	
Óleo de coco	N 1 2 3 4 5 6 7 8 9 10	D S M	
	N 1 2 3 4 5 6 7 8 9 10	D S M	
Cereais			
Arroz	N 1 2 3 4 5 6 7 8 9 10	D S M	
Arroz integral	N 1 2 3 4 5 6 7 8 9 10	D S M	
Arroz temperado	N 1 2 3 4 5 6 7 8 9 10	D S M	
Batata Frita	N 1 2 3 4 5 6 7 8 9 10	D S M	
Mandioca Frita	N 1 2 3 4 5 6 7 8 9 10	D S M	
Batata cozida	N 1 2 3 4 5 6 7 8 9 10	D S M	
Mandioca cozida	N 1 2 3 4 5 6 7 8 9 10	D S M	
Angu	N 1 2 3 4 5 6 7 8 9 10	D S M	
Milho Verde (qual forma)	N 1 2 3 4 5 6 7 8 9 10	D S M	
Macarrão	N 1 2 3 4 5 6 7 8 9 10	D S M	
Lasanha	N 1 2 3 4 5 6 7 8 9 10	D S M	
Macarrão instantâneo	N 1 2 3 4 5 6 7 8 9 10	D S M	
Coxinha	N 1 2 3 4 5 6 7 8 9 10	D S M	
Quibe	N 1 2 3 4 5 6 7 8 9 10	D S M	
Esfiha/ enroladinho	N 1 2 3 4 5 6 7 8 9 10	D S M	
Empada	N 1 2 3 4 5 6 7 8 9 10	D S M	
Pastel	N 1 2 3 4 5 6 7 8 9 10	D S M	
Pizza	N 1 2 3 4 5 6 7 8 9 10	D S M	
Farinha(especificar)	N 1 2 3 4 5 6 7 8 9 10	D S M	
Farofa	N 1 2 3 4 5 6 7 8 9 10	D S M	
	N 1 2 3 4 5 6 7 8 9 10	D S M	
Frutas			
Laranja	N 1 2 3 4 5 6 7 8 9 10	D S M	

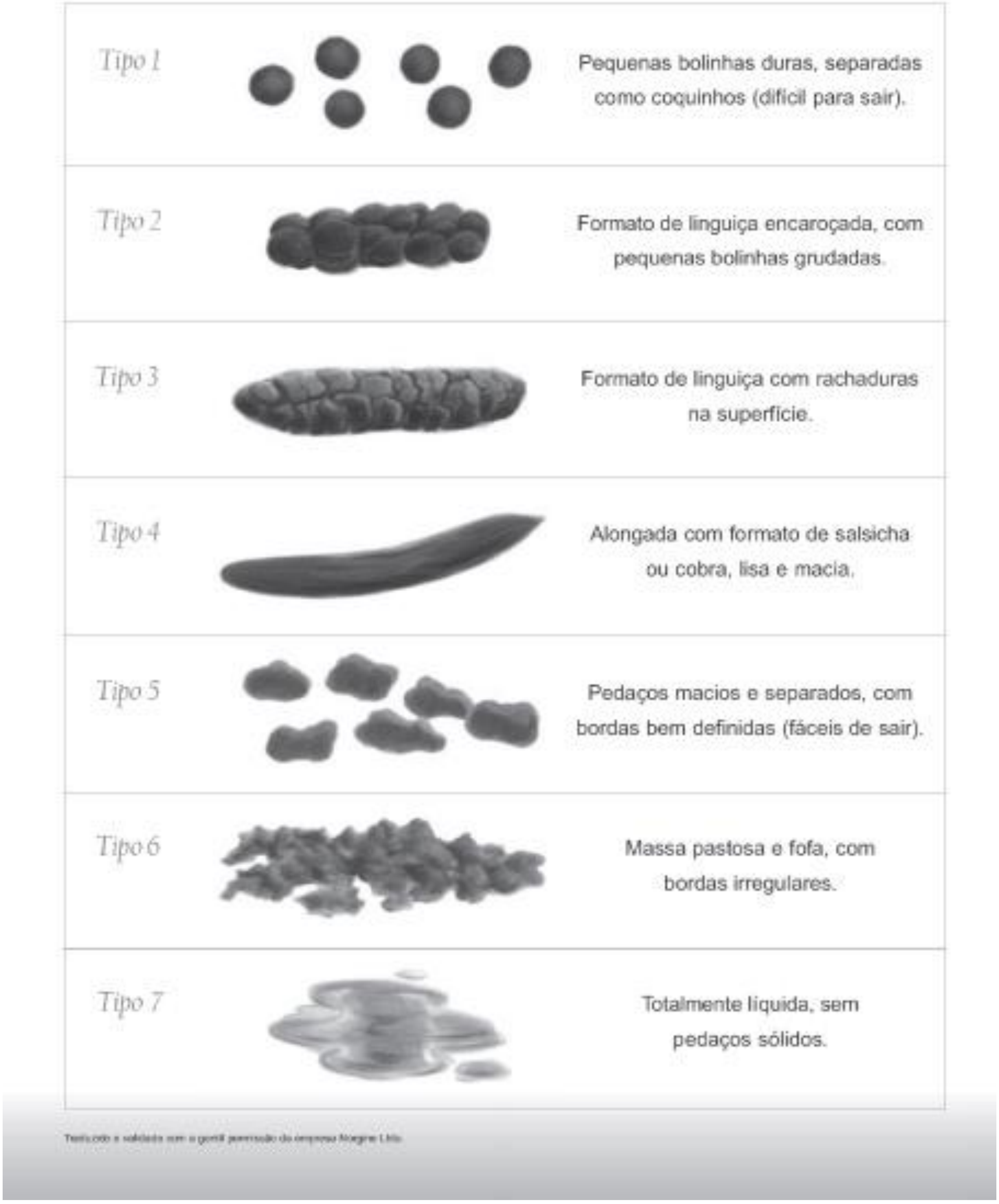
Banana	N 1 2 3 4 5 6 7 8 9 10	D S M	
Maçã	N 1 2 3 4 5 6 7 8 9 10	D S M	
Pêra	N 1 2 3 4 5 6 7 8 9 10	D S M	
Goiaba	N 1 2 3 4 5 6 7 8 9 10	D S M	
Manga	N 1 2 3 4 5 6 7 8 9 10	D S M	
Mamão	N 1 2 3 4 5 6 7 8 9 10	D S M	
Melancia/ melão	N 1 2 3 4 5 6 7 8 9 10	D S M	
Mexerica	N 1 2 3 4 5 6 7 8 9 10	D S M	
Abacaxi	N 1 2 3 4 5 6 7 8 9 10	D S M	
Uva	N 1 2 3 4 5 6 7 8 9 10	D S M	
Ameixa seca	N 1 2 3 4 5 6 7 8 9 10	D S M	
Uva passa	N 1 2 3 4 5 6 7 8 9 10	D S M	
Damasco	N 1 2 3 4 5 6 7 8 9 10	D S M	
Outras frutas________________	N 1 2 3 4 5 6 7 8 9 10	D S M	
Suco de laranja natural	N 1 2 3 4 5 6 7 8 9 10	D S M	
Suco de outras frutas__________	N 1 2 3 4 5 6 7 8 9 10	D S M	
	N 1 2 3 4 5 6 7 8 9 10	D S M	
Leguminosas			
Feijão(qual tipo?)	N 1 2 3 4 5 6 7 8 9 10	D S M	
Feijão tropeiro	N 1 2 3 4 5 6 7 8 9 10	D S M	
Soja	N 1 2 3 4 5 6 7 8 9 10	D S M	
Grão de bico	N 1 2 3 4 5 6 7 8 9 10	D S M	
Lentilha	N 1 2 3 4 5 6 7 8 9 10	D S M	
	N 1 2 3 4 5 6 7 8 9 10	D S M	
Verduras/legumes			
Alface	N 1 2 3 4 5 6 7 8 9 10	D S M	
Agrião	N 1 2 3 4 5 6 7 8 9 10	D S M	
Repolho	N 1 2 3 4 5 6 7 8 9 10	D S M	
Espinafre	N 1 2 3 4 5 6 7 8 9 10	D S M	
Couve(Qual a forma de preparo)	N 1 2 3 4 5 6 7 8 9 10	D S M	
Couve flor, brócolis	N 1 2 3 4 5 6 7 8 9 10	D S M	
Cenoura crua	N 1 2 3 4 5 6 7 8 9 10	D S M	
Cenoura cozida	N 1 2 3 4 5 6 7 8 9 10	D S M	
Abóbora cozida	N 1 2 3 4 5 6 7 8 9 10	D S M	
Tomate	N 1 2 3 4 5 6 7 8 9 10	D S M	
Beterraba	N 1 2 3 4 5 6 7 8 9 10	D S M	
Chuchu	N 1 2 3 4 5 6 7 8 9 10	D S M	
Abobrinha	N 1 2 3 4 5 6 7 8 9 10	D S M	
Quiabo	N 1 2 3 4 5 6 7 8 9 10	D S M	

Pepino	N 1 2 3 4 5 6 7 8 9 10	D S M	
Sopas______________________	N 1 2 3 4 5 6 7 8 9 10	D S M	
	N 1 2 3 4 5 6 7 8 9 10	D S M	
Carnes			
Carne bovina magra _________	N 1 2 3 4 5 6 7 8 9 10	D S M	
Carne bovina gorda __________	N 1 2 3 4 5 6 7 8 9 10	D S M	
Carne Moída	N 1 2 3 4 5 6 7 8 9 10	D S M	
Carne suína magra __________	N 1 2 3 4 5 6 7 8 9 10	D S M	
Carne suína gorda ___________	N 1 2 3 4 5 6 7 8 9 10	D S M	
Bacon, torresmo	N 1 2 3 4 5 6 7 8 9 10	D S M	
Frango sem pele ____________	N 1 2 3 4 5 6 7 8 9 10	D S M	
Frango com pele ____________	N 1 2 3 4 5 6 7 8 9 10	D S M	
Peixes____________________	N 1 2 3 4 5 6 7 8 9 10	D S M	
Lingüiça(qual o tipo)	N 1 2 3 4 5 6 7 8 9 10	D S M	
Salsicha	N 1 2 3 4 5 6 7 8 9 10	D S M	
Ovo cozido	N 1 2 3 4 5 6 7 8 9 10	D S M	
Ovo frito/ omelete	N 1 2 3 4 5 6 7 8 9 10	D S M	
Presunto, mortadela	N 1 2 3 4 5 6 7 8 9 10	D S M	
Hambúrguer	N 1 2 3 4 5 6 7 8 9 10	D S M	
	N 1 2 3 4 5 6 7 8 9 10	D S M	
Bebidas			
Refrigerante diet	N 1 2 3 4 5 6 7 8 9 10	D S M	
Suco artificial diet	N 1 2 3 4 5 6 7 8 9 10	D S M	
Refrigerante convencional	N 1 2 3 4 5 6 7 8 9 10	D S M	
Suco artificial convencional	N 1 2 3 4 5 6 7 8 9 10	D S M	
Café com _________________	N 1 2 3 4 5 6 7 8 9 10	D S M	
Chá com __________________	N 1 2 3 4 5 6 7 8 9 10	D S M	
	N 1 2 3 4 5 6 7 8 9 10	D S M	
Doces/guloseimas			
Chocolates_________________	N 1 2 3 4 5 6 7 8 9 10	D S M	
Arroz doce ________________	N 1 2 3 4 5 6 7 8 9 10	D S M	
Doce de leite _______________	N 1 2 3 4 5 6 7 8 9 10	D S M	
Doces de fruta ______________	N 1 2 3 4 5 6 7 8 9 10	D S M	
Sorvete____________________	N 1 2 3 4 5 6 7 8 9 10	D S M	
Pipoca ____________________	N 1 2 3 4 5 6 7 8 9 10	D S M	
Achocolatado_______________	N 1 2 3 4 5 6 7 8 9 10	D S M	
Chips ®	N 1 2 3 4 5 6 7 8 9 10	D S M	
	N 1 2 3 4 5 6 7 8 9 10	D S M	
Frutos secos e oleaginosas			

Amendoim e derivados	N 1 2 3 4 5 6 7 8 9 10	D S M	
Castanhas	N 1 2 3 4 5 6 7 8 9 10	D S M	
Nozes	N 1 2 3 4 5 6 7 8 9 10	D S M	
Pistache	N 1 2 3 4 5 6 7 8 9 10	D S M	
Amêndoas	N 1 2 3 4 5 6 7 8 9 10	D S M	
Linhaça	N 1 2 3 4 5 6 7 8 9 10	D S M	
	N 1 2 3 4 5 6 7 8 9 10	D S M	
Outros			
	N 1 2 3 4 5 6 7 8 9 10	D S M	
	N 1 2 3 4 5 6 7 8 9 10	D S M	
	N 1 2 3 4 5 6 7 8 9 10	D S M	

D (diária), S (semanal), M (mensal), N (nunca).

7.5 Anexo 5 – Escala de Bristol



7.6 Anexo 6 – Avaliação dos sintomas gastrointestinais

Data: ____/___/_____ Etapa: ________________________

Nome:___________________________________

Por favor, para cada questão **dê uma nota de 1 a 7**, conforme a escala abaixo:
Escala de resposta (em intensidade ou frequência):
1. Nenhum desconforto / nenhuma vez
2. Desconforto mínimo / raras vezes
3. Desconforto leve / pouquíssimas vezes
4. Desconforto moderado / poucas vezes
5. Desconforto moderadamente severo / algumas vezes
6. Desconforto forte / muitas vezes
7. Desconforto muito forte / muitíssimas vezes

1. Você teve dores abdominais durante a semana passada? (Dor se refere a todos os tipos de dores no estômago ou de intestino/barriga).
Resposta: _________________ Observação:_________________

2. Você sentiu azia durante a semana passada? (Por azia queremos dizer uma dor em queimação ou desconforto em seu peito).
Resposta: _________________ Observação:_________________

3. Você sentiu refluxo ácido durante a semana passada? (Por refluxo ácido queremos dizer: regurgitação ou fluxo de fluido azedo ou amargo na boca).
Resposta: _________________ Observação:_________________

4. Você sentiu náuseas durante a semana passada? (Por náuseas queremos dizer uma sensação de mal estar iminente – parece que vai vomitar).
Resposta: _________________ Observação:_________________

5. Você eliminou gases ou teve flatulência durante a semana passada? (Eliminar gases ou flatulência refere-se à liberação de ar ou gás a partir do intestino).
Resposta: _________________ Observação:_________________

6.Você teve constipação/prisão de ventre durante a semana passada? (Constipação refere-se a uma capacidade reduzida de defecar).
Resposta: _________________ Observação:_________________

7. Você teve/apresentou fezes moles durante a semana passada? (Se as fezes foram alternadamente duras e moles, essa questão refere-se apenas ao quanto você se sentiu incomodado pelas fezes moles).
Resposta: _________________ Observação:_________________

8. Você teve/apresentou fezes duras durante a semana passada? (Se as fezes foram alternadamente duras e moles, essa questão refere-se apenas ao quanto você se sentiu incomodado pelas fezes duras).
Resposta: _________________ Observação:_________________

9. Ao ir ao banheiro durante a semana passada, você teve a sensação de não esvaziar completamente o intestino? (A sensação de que depois de terminar uma defecação, ainda há mais fezes que precisam ser eliminadas).
Resposta: _________________ Observação:_________________