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Gastrointestinal hormone modulation after a double-blind interventional study with unavailable carbohydrates

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ABSTRACT

The intake of unavailable carbohydrates—functional ingredients—has presented an inverse relationship with the risk for non-communicable diseases. Inulin and unripe banana flour (UBF) (source of resistant starch—55%) are among these ingredients. The aim of this work was to evaluate the impact of regular and discontinued intake of inulin or UBF on the plasma levels of gastrointestinal hormones and energy intake in healthy volunteers. A medium-term clinical assay was conducted with healthy volunteers, both males and females (n = 33), who were oriented to consume soup with added inulin (INU group), UBF (UBF group) or maltodextrin (Control group) three times a week for six weeks. Prototypes of two different types of frozen soups were provided by a food industry. The plasma concentration of satiety-related gastrointestinal hormones was evaluated before and at the end of the intervention. Blood collection was performed 180 min after the consumption of breakfast ad libitum. The energy intake was evaluated at the subsequent meal (180 min). UBF consumption (8 g) caused significant changes in the plasmatic levels of the gastrointestinal hormones when compared to the period before the intervention: there was a lower increase in ghrelin (T0, T60, T120 and T180 min) and a decrease in insulin (T0 and T180 min), hormones related to hunger, when at high levels, as well as an increase in peptide YY (PYY) at all timepoints. When comparing the Control and UBF groups at the end of the intervention, the latter presented a reduction in ghrelin (T0, 120 and 180 min) and insulin (T0 and 180 min) and an increase in PYY (T30 and 180 min). The consumption of inulin (8 g), compared to the period before and at the end of the intervention, resulted in a lower increase in ghrelin (T0, T120 and T180 min) and a decrease in insulin (T180 min). PYY also increased at all timepoints, which indicates higher satiety. When the Control and INU groups were compared at the end of the intervention, the INU group presented reductions in ghrelin (T0, 120 and 180 min) and insulin (T180 min) and an increase in PYY (T180 min). At the subsequent meal, there was a reduction in energy intake of approximately 15% (129 kJ) for the UBF and 12% (130 kJ) for the INU groups. Both inulin and UBF present positive effects on gastrointestinal hormones and energy intake and may be used for producing products that stimulate healthy eating habits.

1. Introduction

Several trials suggest that dietary fiber (DF) may be related to the hormones involved in the hunger/satiety mechanism; other studies associated this component with the decrease of some intestinal disorders (Klosterbuer, Roughead, & Slavin, 2011). DF components, such as resistant starch (RS) and inulin-type fructans, are substrates for colonic fermentation, of which the main products are short chain fatty acids (SCFA) (acetic, propionic and butyric acids) and the gases hydrogen (H_2), carbon dioxide, and in some individuals, methane (Topping & Clifton, 2001). The SCFA produced have a broad action spectrum, from local beneficial effects on the intestinal epithelium (Conlon et al., 2012; Goñi & López-Oliva, 2006; Hamer et al., 2008), to more complex systemic effects that need to be clarified, such as the impact on glucose homeostasis and the regulation of lipid metabolism (Kelly, 2009; Roberfroid et al., 2010). The consumption of different types of DF can also change the profile of satiety-related gastrointestinal hormones and energy intake (Roberfroid et al., 2010; Sánchez et al., 2012), so it is important to increase the supply of food and ingredient sources of DF and to encourage their consumption.

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Resistant starch (RS) has been studied for its potential beneficial effect on health. RS metabolism takes 5–7 h, which can reduce postprandial plasma glucose and insulin and increase satiety (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010). Unripe banana flour (UBF) is rich in RS (Faisant et al., 1995; Tribess et al., 2009), and its application in short-term clinical trials in healthy volunteers showed positive response relating to hunger/satiety and improvement in bowel function (Dan, 2011; Menezes et al., 2010). These positive attributes of UBF indicate the possibility of using this ingredient in the preparation of foods aimed at reducing the risk of certain non-communicable diseases (NCD).

Along with other unavailable carbohydrates, inulin-type fructans may also be responsible for several health benefits (Roberfroid et al., 2010). Researchers have observed improvement in mineral absorption and the immune system, reducing the risk of colon cancer (Morris & Morris, 2012), modulation of intestinal microbiota and a decrease in constipation, in addition to the effects of the production of SCFA (Nair, Kharb, & Thompkinson, 2010). However, the relationship of these carbohydrates with the increase in satiety and weight control is still being investigated, and it may be associated with lower energy density, the rate of gastric emptying and gastrointestinal hormone modulation (Smith & Tucker, 2011). In a short-term preliminary trial performed by Menezes and Giuntini (2010), effects were observed shortly after the consumption of ready-to-eat frozen meals with inulin added on the hormones related to hunger/satiety, in addition to a decrease in energy intake in subsequent meals and an improvement of bowel function. However, there are no trials assessing the ingestion according to food consumption patterns, i.e., a regular but not necessarily daily intake.

The aim of this work was to evaluate the impact of regular and discontinued intake of inulin or UBF on plasma levels of gastrointestinal hormones and energy intake in healthy volunteers.

2. Materials and methods

2.1. Test ingredients

UBF was prepared with the pulp of unripe banana, *Musa acuminata* (group AAA), sub-group *Cavendish* (called "Nanicão" in Brazil), maturity stage I, not subjected to ripening chamber, from Vale do Ribeira (São Paulo, Brazil) and marketed in CEAGESP/SP. The production of UBF on a semi-industrial scale was performed according to the process proposed by Tribess et al. (2009). The source of fructans (inulin) used was the product Beneo GR from Beneo-Orafti™—Tienen, Belgium. Maltodextrin (code MOR-REX 1920, Ingredion) was used as a placebo.

Ready-to-eat frozen soups were used as the vehicles of the test ingredients. BRF S.A. (São Paulo, Brazil) produced the prototypes of the ready-to-eat frozen soups in a pilot plant (semi-industrial scale). Two types of ready-to-eat frozen soups were produced (control and added inulin), in two flavor options, with an approximate energy input of 1100 kJ (260 kcal). For the UBF group, the control soup with UBF added at the time of consumption was used.

The ready-to-eat soups were subjected to specific chemical and microbiological analyses by the BRF S.A. Central Lab, and they met all microbiological limits established by RDC 12/01 from ANVISA (Brasil, 2001) for the category of Ready-to-eat Frozen Meals. The soup samples were lyophilized (Freeze Dryer, model Super Modulyo 220 TC60 Tray Cell, Thermo Fisher Scientific, Waltham, MA, USA), grinded to particles <0.250 mm and stored at −20 °C prior to chemical analyses.

The soups, intended for consumption, were stored at −20 °C until they were gradually delivered to volunteers, who were instructed to keep them in a domestic freezer throughout the intervention and heat them in the microwave.

2.2. Chemical composition

The determination of the moisture of the samples was performed in a vacuum oven at 70 °C (AOAC 920.151); the protein content was determined through the total nitrogen, using the micro-Kjeldahl technique (AOAC 960.52), with a conversion factor of 6.25. The lipids were determined by Soxhlet (AOAC 920.39), and the ash was determined by calcination in a muffle at 550 °C to a constant weight (AOAC 923.03) (Horwitz & Latimer, 2006).

RS was quantified based on the method AOAC 2002.02 (McCleary, McNally, & Rossiter, 2002), and to quantify the total starch (TS), the method from Cordenunsi and Lajolo (1995) was used. The content of free glucose was determined by the enzymatic method (glucose oxidase/peroxidase/ABTS) (Bergmeyer & Bernet, 1974). The following reference materials were used: corn and potato RS (Megazyme International Ireland Ltd., Wicklow, Ireland; Megazyme K-RSTCL), in house standard cooked carioca beans were used for the determination of RS and potato starch (Sigma S-2004) was used for the determination of TS. The concentration of available starch (AS) was obtained by the difference between total and resistant starch (AS = TS − RS). In the lyophilized, grinded (60 mesh) and degreased samples, the soluble, insoluble and total fiber contents were determined by the enzymatic-gravimetric method AOAC 991.43 (Lee, Prosky, & Devries, 1992), with modifications proposed by McCleary and Rossiter (2004) to avoid overlapping of the RS values (pre-treatment of samples with DMSO in a 100 °C water bath for 30 min to solubilize the RS present). The total content of DF or unavailable carbohydrates was calculated by summing the content of fiber without RS and the RS content.

2.3. Gastrointestinal hormones

The hormones ghrelin, insulin and peptide YY (PYY) were analyzed (duplicate) through specific LINCOplex® kits (Linco Research Inc., St Charles, MO, USA) according to Luminex™ xMAP technology (Luminex Corporation, Austin, TX, USA).

Blood samples were collected in Vacutainer® tubes containing EDTA. Next, blood aliquots (1 mL) were transferred to Eppendorf tubes containing Pefabloc® (Sigma Aldrich, Switzerland) (1 mg/mL of blood) and DPPIV inhibitor (Millipore) (10 μL/mL of blood) and were centrifuged (800 g) at 4 °C, for 15 min. Plasma was stored in an ultra-freezer until the analysis. The values were expressed in pg/mL of plasma or serum.

2.4. Clinical assay

The trial was a double-blind, parallel, placebo-controlled clinical trial, with a 6 week duration. Before and at the end of the intervention, gastrointestinal hormones and the energy intake were assessed.

Volunteer screening—33 healthy participants were selected, from both genders, aged between 18 and 39 years old, and who usually consume frozen meals.

Selection criteria—the subjects were in good health (reported the absence of hyperthyroidism, renal and gastrointestinal diseases, and had neither a previous diagnosis of diabetes *mellitus* nor a family history of diabetes), and all subjects were free of the use of medication affecting the digestion and absorption of food (antibiotics) during the study period.

Non-inclusion criteria—overweight/obese subjects (BMI ≥ 25 kg/m^2) and underweight (BMI ≤ 18,6 kg/m^2) subjects, according to the World Health Organization criteria (WHO, 1997), reported disease, pregnancy, breastfeeding or treatment of any kind, with possible eating disorders.

Experimental design—the 33 volunteers were divided into three groups: Control Group—received control soup and individual servings of placebo (maltodextrin—2 g); UBF Group—received control soup and individual servings of UBF (8 g); Inulin Group—received control soup