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Basic nutritional investigation

Passiflora edulis peel intake improves insulin sensitivity, increasing incretins and hypothalamic satietogenic neuropeptide in rats on a high-fat diet

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ABSTRACT

Objective: This study aimed to investigate the effect of *Passiflora edulis* peel flour (PEPF) intake on hypothalamic neuropeptides messenger RNA expression, insulin sensitivity, and other metabolic parameters in Sprague-Dawley rats fed a high-fat (HF) diet.

Methods: Sprague-Dawley rats were divided in 3 groups: a control group, fed on a normal fat diet; a HF group, fed on a high-fat diet (35% fat [w/w]); and a high-fat *Passiflora* flour (HFPF) group, fed on a HF diet containing PEPF. The rats from the HFPF group as well as the HF group were kept on an HF diet for the first 4 wk to induce metabolic conditions related to obesity. Then the HFPF group was switched to a HF diet containing PEPF for additional 6 wk. Other groups were kept on normal-fat and HF diet without addition of PEPF during the whole period of experiment. The glucose tolerance and insulin sensitivity were evaluated through the glucose tolerance test (GTT) and the insulin tolerance test (ITT). Gut hormones and adipokines were measured through an immunoassay. The hypothalamic neuropeptides expression was assessed by real-time polymerase chain reaction.

Results: The PEPF intake increased the hypothalamic cocaine- and amphetamine-regulated transcript expression (CART) ($P < 0.05$), counteracted cumulative body weight gain ($P < 0.001$), decreased adiposity ($P < 0.05$) and leptin level ($P < 0.01$), whereas increased adiponectin ($P < 0.01$), glucose-dependent insulinotropic polypeptide ($P < 0.01$), and glucagon-like peptide-1 (GLP-1) ($P < 0.001$) improved the insulin sensitivity in diet-induced obesity rats by increasing the kITT (glucose disappearance rate) ($P < 0.01$), which was calculated during the ITT. Other gut hormones (peptide tyrosine tyrosine, pancreatic polypeptide, and amylin) and interleukins (IL) (IL-6, tumor necrosis factor-α, IL-1β, and monocyte chemoattractant protein-1) were not changed by the PEPF intake.

Conclusion: Our findings provide a further understanding of how the PEPF works as a dietary component to improve glucose homeostasis and demonstrate a molecular mechanism that may increase satiety by PEPF in diet-induced obesity.

Introduction

Obesity and related diseases, such as type 2 diabetes, have reached pandemic proportions and are among the leading causes of death worldwide [1]. Indeed, obesity and overweight are determinant factors for several metabolic diseases such as type 2 diabetes, impaired glucose tolerance, fatty liver disease, cardiovascular disease, some types of cancers, and different mental

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health conditions, which results is harmful for health and the economy, raising medical costs for treatment and causing productivity losses [2].

Dietary factors are important predictors for the risk of those diseases. The consumption of saturated/animal fat is associated with overweight that, in turn, deteriorates insulin sensitivity and glucose tolerance in humans and experimental animals [3,4]. On the other hand, increased intake of fiber-rich foods, fruits, and vegetables as well as limited amounts of total and saturated fats are important elements in the prevention of type 2 diabetes [5].

Indeed, food components, which may play a role in appetite, energy expenditure, or improvement of glucose homeostasis, have been the target of obesity-related research [6–8]. Different fibers, including pectin, have been shown capacity to prevent body weight gain, improve insulin sensitivity [7,9], and modulate gut hormones, mainly peptide tyrosine tyrosine (PYY) and glucagon-like peptide-1 (GLP-1) [6,10]. The gut hormones act on glucose homeostasis by affecting the insulin secretion and the control of food intake, functioning as mediators into the gut–-brain axis [11]. There is some evidence that GLP-1 action on satiety [12] involves the modulation of hypothalamic neuropeptides [6,13].

Passiflora edulis peel is a byproduct from the juice and pulp industry. Recently, several studies have reported the functional properties of *P. edulis* peel flour (PEPF), particularly its dietary fiber content and antioxidant capacity [14–16]. These features of PEPF have boosted research to evaluate its effects on health parameters, especially those related to antihyperglycemic action in animals [17,18] and humans [19]. Nevertheless, the physiological and molecular mechanisms by which PEPF improves the glucose homeostasis are still unclear. This study aimed to investigate the effect of PEPF on insulin sensitivity, adiposity, and metabolic parameters in rats fed on a high-fat (HF) (saturated/animal) diet. Additionally, we investigated whether these effects are associated with hypothalamic neuropeptides expression, which is related to food intake and energetic balance.

Methods and materials

PEPF

An available commercial dried and milled PEPF produced by M.W.A. Com. de Produtos Alimentícios Ltda (São José do Rio Preto, São Paulo, Brazil) was evaluated in this work.

Chemical characterization of PEPF

Proximate composition

The PEPF was evaluated regarding its nitrogen content (Kjeldahl method) [20], total lipids [21], ash (method 942.05) [20] and moisture (method 934.01) [20]. The dietary fiber (soluble and insoluble) was determined through the enzymatic-gravimetric method, according to the Association of Official Analytical Chemists [20]. The crude carbohydrate content was calculated using the percentile difference from all the other components.

Total and soluble pectin

The total pectin was extracted using Versene solution and 1.0 N NaOH solution until pH 11.5 for hydrolysis, followed by pH adjustment (5–5.5) and reaction with pectinase (Pectinase from *Aspergillus niger*, 1 U/mg; Sigma-Aldrich; St. Louis, USA). The sugar-free PEPF obtained using the 95% ethanol extraction was applied for soluble pectin determination after agitation and solubilization in water. After the carbazole reaction method [22], the total and soluble pectin were measured at 530 nm using a microplate reader (Synergy HT, Biotek, Winooski, VT, USA); with Gen5 2.0 data analysis software spectrophotometer. The galacturonic acid (Sigma-Aldrich; St. Louis, MO, USA) was employed as standard.

Phytochemical analysis

The phytic acid was determined in PEPF through the colorimetric method according to Latta and Eskin [23]. Tannin (met 952.03) and hydrogen cyanide (met 915.03) were analyzed in agreement with the Association of Official Analytical Chemists [20].

Animal trial and procedures

Animals and treatments

Male Sprague–Dawley rats (3 wk old) from the Multidisciplinary Center for Biological Investigation on Laboratory Animal Science–University of Campinas were used in this investigation, which was approved by the Institutional Animal Care and Use Committee (Permission no. 3070-1). The rats were treated in accordance with the institutional ethical guideline, allocated in individual cages, maintained in a room with relative humidity (60–70%) and controlled temperature (23°C) using a 12-h light–12-h dark cycle. The rats were fed on a American Institute of Nutrition (AIN)-93 G diet for 1 wk as an acclimatation period and then randomized into three groups: (control) rats were fed on a normolipidic diet based on AIN-93 G [24] containing 12% of protein [25]; HF rats were fed on a HF diet containing 4% (w/w) soybean oil and 31% (w/w) lard [25,26]; HFPF (HF *Passiflora* flour) rats were fed on a HF diet modified (50% of the cellulose was replaced by PEPF). The rats from the HFPF group as well as the HF group were kept on a HF diet for the first 4 wk to induce metabolic conditions related to obesity, according to the protocol previously described [25,27,28]. Then the HFPF group was switched to a HF diet containing PEPF for additional 6 wk. Diets' composition is displayed in Table 1. Access to food and drink was ad libitum. Food intake was monitored three times a week, and the rats were weighted once a week. After 10 experimental weeks, the fasting (12 h) rats were sacrificed by decapitation. Blood was collected, and tissues were dissected immediately and washed with saline. Epididymal and retroperitoneal adipose tissue were weighed to predict the adiposity. Blood and tissues were stored at −80°C for further analysis.

Intraperitoneal glucose tolerance test and insulin tolerance test

The peripheral sensitivity to insulin was measured through the intraperitoneal glucose tolerance test (iGTT) and the insulin tolerance test (ITT) methods after 9 and 10 wk on treatment, respectively. The glucose was determined in blood samples collected in the caudal vein of the animals, using Optium Xceed (Abbott Diabetes Care). The blood glucose was measured at baseline (food-deprived for 12 h), and then a glucose solution 25% (1.1 mmol/kg body weight) was injected into the peritoneal cavity. The samples were collected at 30, 60, 90, and 120 min after the injection of glucose solution to determine the rate of blood glucose concentrations, and then the area under curve (AUC) was calculated. For the ITT, the samples were collected after 5, 10, 15, 20, 25, and 30 min after the intraperitoneal injection of human insulin (0.75 U/kg body weight; Novolin R, Novo Nordisk; Bagsvaerd, Denmark). The insulin sensitivity was estimated by glucose disappearance rate (*k*ITT) from blood after the intravenous insulin injection during the ITT using the following formula: $0.693/t_{1/2}$. The glucose $t_{1/2}$ was calculated from the slope of the least-square analysis of the blood glucose concentrations during the linear decay phase [25].

RNA extraction and quantitative real-time polymerase chain reaction

The total RNA was extracted using the Trizol reagent (Invitrogen, Carlsbad, CA, USA). RNA concentration, purity, and integrity were confirmed spectrophotometrically by the use of a Nanodrop (ND-1000; Nanodrop Technologies, Wilmington, DE, USA). The first-strand cDNA was synthesized using SuperScript III

Table 1
Composition of experimental diets (g/Kg of diet)*

Ingredient	Control	HF	HFPF
Cornstarch	435.12	258.3	258.3
Casein (85.5%)	140.35	140.35	140.35
Dextrinized cornstarch	144.51	85.79	85.79
Sucrose	109.57	65.05	65.05
Soybean oil	70.00	40.00	40.00
Lard	-	310.00	310.00
Cellulose	50.00	50.00	25.00
PEPF	-	-	25.00
Mineral mix (AIN-93 G-MX)	35.00	35.00	35.00
Vitamin mix (AIN-93 G-VX)	10.00	10.00	10.00
L-Cystine	3.00	3.00	3.00
Choline bitartrate	2.50	2.50	2.50
Tert-butylhydroquinone	0.014	0.014	0.014
Energy density (kJ/g diet)	15.4	21.9	21.9

Control, control group fed on AIN 93 G diet; HF, high-fat group fed on AIN 93 G diet modified with 35% fat (w/w); HFPF, high-fat *Passiflora* flour group fed on AIN 93 G modified with 35% fat (w/w) added 2.5% (w/w) *Passiflora* edulis peel flour

* Diets were prepared according to American Institute of Nutrition for AIN 93-G [24] with modified protein content to 12%, according Dragano et al. (2013) [25].