

Freeze-dried jaboticaba peel powder improves insulin sensitivity in high-fat-fed mice

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Abstract

The peel of the native Brazilian fruit jaboticaba is rich in anthocyanins, which are known for their anti-obesity effects in animal models. The aim of the present study was to evaluate the effects of freeze-dried jaboticaba peel powder (FDJPP) on a number of metabolic parameters in a model of diet-induced obesity. Mice (*n* 8 per group) were initially fed on a high-fat diet (HFD, 35% w/w) for 4 weeks and then switched to a HFD supplemented with FDJPP (1, 2 or 4% w/w) for an additional 6 weeks. Energy intake, weight loss, glucose tolerance, insulin resistance and lipid profile were determined, and the results were evaluated using ANOVA and Tukey's tests. The FDJPP exerted no protective effect on HFD-induced weight gain, hyperleptinaemia and glucose intolerance. However, the supplementation was effective to reduce insulin resistance, as evidenced in the insulin tolerance test, and subsequently confirmed by improved signal transduction through the insulin receptor/insulin receptor substrate-1/Akt/forkhead box protein pathway and by the attenuation of HFD-induced inflammation in the liver, verified by lower expressions of IL-1 β and IL-6 and decreased phosphorylated I κ B- α protein levels in all jaboticaba-treated mice. These results suggest that FDJPP may exert a protective role against obesity-associated insulin resistance.

Key words: Jaboticaba: Obesity: Insulin resistance

Obesity has reached epidemic proportions in the world, with substantial adverse consequences for human health⁽¹⁾. A number of life-threatening diseases, such as type 2 diabetes mellitus, dyslipidaemia, CVD, hypertension and certain types of cancer, are associated with this condition⁽²⁾.

Nutritional factors are known to play an important role in the development of a number of diseases. The recent changes in human lifestyle, with the introduction of high-fat/simple carbohydrate-rich diets, are amongst the most important factors leading to the increased prevalence of obesity. Conversely, epidemiological data suggest that increased consumption of fruits and vegetables are positively associated with the prevention of metabolic disorders, including obesity^(3,4). The ability of some vegetable-derived nutrients to reduce the risk of chronic diseases has been associated, at least in part, with the occurrence of bioactive

non-nutrient compounds that are known to exert a wide range of biological activities^(5,6).

Anthocyanins, which belong to the flavonoid class, are an example of such bioactive compounds. They constitute the largest and probably the most important group of water-soluble natural pigments responsible for the vivid blue, purple and red colour of many vegetables and fruits⁽⁷⁾. Many reports have shown that anthocyanin exhibits an array of pharmacological properties, such as anti-inflammatory and anticarcinogenic activities, as well as preventive effects on CVD and degenerative disease. The majority of research has focused on the antioxidant properties of these dietary polyphenols⁽⁸⁾. Furthermore, it has been shown that phenolic compounds can exert modulatory action in cells by interacting with a wide range of molecular targets of intracellular signalling machinery to elicit their beneficial effects⁽⁹⁾.

Abbreviations: C, control group; FDJPP, freeze-dried jaboticaba peel powder; FoxO, forkhead transcription factor box; HF, high-fat group; HFD, high-fat diet; HFJ1%, high-fat diet plus 1% (w/w) freeze-dried jaboticaba peel powder group; HFJ2%, high-fat diet plus 2% (w/w) freeze-dried jaboticaba peel powder group; HFJ4%, high-fat diet plus 4% (wt/wt) freeze-dried jaboticaba peel powder; iGTT, intraperitoneal glucose tolerance test; I κ B- α , inhibitor of NF- κ B α ; IRS1, insulin receptor substrate 1; ITT, insulin tolerance test.

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Recent findings have suggested that the consumption of anthocyanins may reduce obesity and some of its associated disorders, at least in animal models^(10–12). However, additional *in vivo* studies have reported that whole powdered berries in high-fat diets, compared with purified anthocyanins, were not quite effective in preventing obesity. Actually, they showed the potential to increase obesity^(13,14).

Jaboticaba (*Myrciaria jaboticaba*) is an indigenous Brazilian fruit found in the largest part of the country, particularly in the Southeast. The mature fruit is round and has a thick, dark purple, astringent skin that covers a sweet, white, gelatinous flesh. The fruit is widely used in Brazil, where it is mostly consumed fresh and, to a smaller extent, as jams and liqueurs. A recent study published by our research group showed that cyanidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside are the major constituents found in the freeze-dried jaboticaba peel. The total content of anthocyanins analysed by HPLC was 2599.3 mg/100 g of freeze-dried powder and cyanidin 3-*O*-glucoside was the major anthocyanin (75.6% of total anthocyanins) (Table 1)⁽¹⁵⁾. These levels are comparable with those of other well-known fruit sources of anthocyanins. In spite of the high concentration of anthocyanins, few other studies have reported that several flavonoids, phenolic acids and terpenes are also present in jaboticaba^(16–18).

In addition, we have previously found that the consumption of 1, 2 and 4% freeze-dried jaboticaba peel was able to reduce the hyperinsulinaemia and increased serum HDL-cholesterol in Sprague–Dawley rats fed a high-fat diet (HFD), but did not promote a reduction of the weight gain in the animals. However, the mechanisms involved remain to be elucidated⁽¹⁹⁾.

The present study was designed to investigate the effect and mechanisms possibly involved in using freeze-dried jaboticaba peel powder (FDJPP) on glucose tolerance and insulin resistance in high-fat-fed mice.

Experimental methods

Freeze-dried jaboticaba peel powder

Jaboticaba (*M. jaboticaba* Vell berg) was obtained directly from the producer (Aguai, São Paulo State, Brazil) during the main harvest season in September 2008. The fruit was manually washed with fresh water and the peel was separated and frozen at -20°C . The frozen peel was lyophilised and grounded into a fine powder by an electrical mill. The FDJPP was kept in airtight containers and stored at -80°C . The analysis of macronutrients, total protein, moisture and ash was performed according to methods described by the Association of Official Analytical Chemists⁽²⁰⁾. The total lipids were determined according to the Bligh & Dyer⁽²¹⁾ method and the determination of soluble and insoluble fibres was performed according to Asp *et al.*⁽²²⁾. The concentration of carbohydrate was calculated as the difference between 100 and the percentage total of the content of protein, fat, moisture and ash. The identification and quantification of anthocyanins in FDJPP were determined using MS and HPLC, as described previously by our group⁽¹⁵⁾.

Experimental animals and diets

Male Swiss inbred strain mice (21 d of age), originally imported from the Jackson Laboratory and currently bred at the University of Campinas Breeding Center (University of Campinas, SP,

Table 1. Composition of experimental diets*

Ingredients (g/kg diet)	C	HF	HFJ1 %	HFJ2 %	HFJ4 %
Casein†	151.5	151.5	151.5	151.5	151.5
Maize starch	428.0	251.3	253.3	255.3	259.3
Maltodextrin	142.2	83.4	83.4	83.4	83.4
Sucrose	107.8	63.3	63.3	63.3	63.3
Cellulose	50	50	48	46	42
Soyabean oil	70	40	40	40	40
Lard	0	310	310	310	310
Mineral mixture	35	35	35	35	35
Vitamin mixture	10	10	10	10	10
L-Cys	3.0	3.0	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5	2.5	2.5
<i>Tert</i> -butylhydroquinone	0.014	0.014	0.014	0.014	0.014
Jaboticaba peel powder	0	0	10	20	40
Total	1000	1000	1000	1000	1000
Anthocyanins (mg/kg diet)					
Cyanidin-3- <i>O</i> -glucoside	0	0	196.4	392.8	785.6
Delphinidin-3- <i>O</i> -glucoside	0	0	63.5	127	254
Total	0	0	259.9	519.8	1039.6
Energy density (kJ/g diet)	16.3	23.0	23.0	23.0	23.0

C, control group; HF, high-fat group; HFJ1 %, high-fat diet plus 1% (w/w) freeze-dried jaboticaba peel powder group; HFJ2 %, high-fat diet plus 2% (wt/wt) freeze-dried jaboticaba peel powder group; HFJ4 %, high-fat diet plus 4% (w/w) freeze-dried jaboticaba peel powder group.

*Diets were prepared according to the formulation of the American Institute of Nutrition for AIN-93G⁽²³⁾ with modified protein content to 12% according to Goena *et al.*⁽⁴⁵⁾.

†Amount was calculated based on protein content equal to 79.2%.

Brazil), were used in the study. The investigation was approved by the Ethics Committee for Animals (Permission: 2020-1/2010; University of Campinas, SP, Brazil) and followed the University guidelines for the use of animals in experimental studies. The animals were maintained at $21 \pm 3^\circ\text{C}$, on an artificial 12 h light–12 h dark cycle and housed in individual cages. They were then randomly divided into five groups, with eight mice per treatment (following an initial acclimatisation period of 7 d). All groups were balanced for initial body weight. The treatments were control group (C): mice were fed on a normal diet, based on the AIN-93G⁽²³⁾; control high-fat group (HF): mice were fed on a HFD, containing 4% (w/w) soyabean oil and 31% (w/w) lard; HFD plus 1% (w/w) FDJPP (HFJ1%); HFD plus 2% (w/w) FDJPP (HFJ2%) and HFD plus 4% (w/w) FDJPP (HFJ4%). Mice (HFJ1%, HFJ2% and HFJ4%) were initially fed on a HFD for 4 weeks and then switched to a HFD containing FDJPP for an additional 6 weeks. The diets were replaced at intervals of 3 d to prevent fat and anthocyanin oxidation. Because the FDJPP has significant amounts of fibre (20% insoluble fibre and 5% soluble fibre)⁽¹⁹⁾, the content of dietary fibre present in supplemented diets was adjusted to eliminate the influence of this important component over the present findings. The concentration of freeze-dried powder in the diets and anthocyanins content were determined according to preliminary studies conducted by our research group^(15,19). The composition of the diets, energy densities and anthocyanin content are presented in Table 1. Body weight and food intake were measured weekly.

Intraperitoneal glucose tolerance test and insulin tolerance test

An intraperitoneal glucose tolerance test (iGTT) and insulin tolerance test (ITT) were performed on food-deprived (6 h) non-anaesthetised mice after 9 and 10 weeks of treatment, respectively. Blood glucose levels were measured with an Optium™ mini (Abbott Diabetes Care) handheld glucometer using appropriate test strips. For the iGTT, a solution of 20% glucose (2.0 g/kg body weight) was administered into the peritoneal cavity. Blood samples were collected from the tail vein at 30, 60, 90 and 120 min for the determination of glucose concentrations. The AUC of glucose was calculated. For the ITT, glucose blood levels were sampled at 5, 10, 15, 20, 25 and 30 min following intraperitoneal injection of human insulin (0.75 U 4.5 nmol/kg; Novolin R, Novo Nordisk). The constant rate for glucose disappearance during the ITT (K_{ITT}) was calculated using the formula $0.693/t_{1/2}$. The glucose $t_{1/2}$ was calculated from the slope of the least-square analysis of the plasma glucose concentrations during the linear decay phase⁽²⁴⁾.

Serum, liver and adipose tissue samples

After 10 weeks of diet consumption, the fasting (12 h) mice were anaesthetised (sodium thiopental 50 mg/kg body weight) and blood samples were taken from the inferior vena cava. Serum was obtained by centrifugation (1500 g for 15 min at 4°C) and immediately frozen at -80°C until use. The liver and epididymal

adipose tissue were removed and processed according to the subsequent analysis.

Serum sample analysis

The serum TAG, total cholesterol and HDL-cholesterol were assayed enzymatically using commercial kits (Laborlab). Serum leptin was measured by ELISA using a commercial kit (Mouse leptin ELISA kit; Millipore).

Protein immunoblotting analysis

For evaluation of cytokine expression, protein activity and insulin signal transduction in the liver and adipose tissue, the abdominal cavities of anaesthetised mice were opened, the portal vein exposed and 100 μl (10^{-6} mol/l) of insulin or saline solution were injected. Fragments ($3.0 \times 3.0 \times 3.0$ mm) of liver (at 30 s) and epididymal adipose tissue (at 2 min) were excised after the infusion of insulin and immediately homogenised in extraction buffer (1% Triton X-100, 100 mM-Tris (pH 7.4), containing 100 mM-sodium pyrophosphate, 100 mM-sodium fluoride, 10 mM-EDTA, 10 mM-sodium vanadate, 2 mM-phenylmethanesulphonylfluoride and 0.1 mg/ml aprotinin) at 4°C with a Polytron® System PT 10-35 (Brinkmann Instruments) operated at maximum speed for 15 s. The extracts were centrifuged at 9000 g at 4°C for 40 min to remove insoluble material, and the protein concentration of the supernatant was determined by the Biuret method⁽²⁵⁾. The immunoblotting was performed on tissue extracts as previously described by Thirone *et al.*⁽²⁶⁾, with minor modifications. The proteins were separated by SDS-PAGE, transferred to nitrocellulose membranes and blotted with antibodies against phospho-insulin receptor β (IR) (#3070; Cell Signaling), phospho-insulin receptor substrate (IRS)-1 (#3026; Cell Signaling) and phosphorylated-forkhead transcription factor box (FoxO) (#9464; Cell Signaling), phospho-Akt (sc-7985-R; Santa Cruz Biotechnology), phospho-inhibitor of NF- κB α (I κB - α) (sc-7977-R; Santa Cruz Biotechnology) and IL-1 β (sc-1252; Santa Cruz Biotechnology). Specific bands were labelled by chemiluminescence and visualisation was performed by exposure of the membranes to RX films. Band intensity was quantified by digital densitometry (ScionCorp) and normalised with β -actin.

RNA extraction and quantitative real-time PCR

Total RNA was extracted using a commercially available acid-phenol reagent, Trizol (Invitrogen). RNA integrity was confirmed by non-denaturing agarose gel electrophoresis. First-strand complementary DNA was synthesised using SuperScript III RT and random hexamer primers, as described in the manufacturer's protocol (Invitrogen). Quantitative PCR was run to determine the expression of IL-1 β (Rn0058,0432_m1; Applied Biosystems) and IL-6 (Mm012,10733_m1; Applied Biosystems) in the liver of treated mice using the primer supplied by Applied Biosystems. The reference gene was glyceraldehyde-3-phosphate dehydrogenase (#4352339E; Applied Biosystems). Real-time PCR analysis of gene expression was carried out in an ABI Prism 7500 sequence detection

system (Applied Biosystems). Each PCR contained 25 ng of reverse-transcribed RNA and was run according to the manufacturer's recommendations using the TaqMan PCR master mix. Real-time data were analysed using the Sequence Detector System 1.7 (Applied Biosystems). Results were expressed as relative transcript amounts⁽²⁷⁾.

Data analysis and statistics

Analyses were performed using Statsoft Statistica 7 software. Initially, all data were subjected to the Shapiro–Wilk's and Levene's test to verify the data distribution and variance homogeneity, respectively. When necessary, the data were log-transformed to fulfil parametric requirements. Parametric data were evaluated using one-way ANOVA and non-parametric data using Kruskal–Wallis followed by *post hoc* testing for significance ($P < 0.05$, by Tukey–Kramer or Dunnett's test, respectively). Results were expressed as means with their standard errors for parametric data and medians and ranges for non-parametric data.

Results

Body weight, food and energy intake

As expected, after 10 weeks, body mass gain was significantly higher in mice fed on the HFD compared with the low-fat control diet (C). This difference was evidenced after the second experimental week (Fig. 1). The addition of FDJPP to the HFD did not protect the animals against HFD-induced body mass gain, while the cumulative body mass gain in mice fed on HFJ2% was significantly greater, 31.62 (SEM 0.9) and 23.25 (SEM 1.40) g, respectively. In all the remaining groups receiving FDJPP, body mass increased when compared with the HFD, but this was not significant (Fig. 1).

The overall food intake (g/mouse per d) did not differ among control and high-fat groups throughout the experiment (data not shown). It is important to notice that control mice consumed the same amount of food (weight) as mice on the HFD. However, energy intake was higher on the HF diets, approxi-

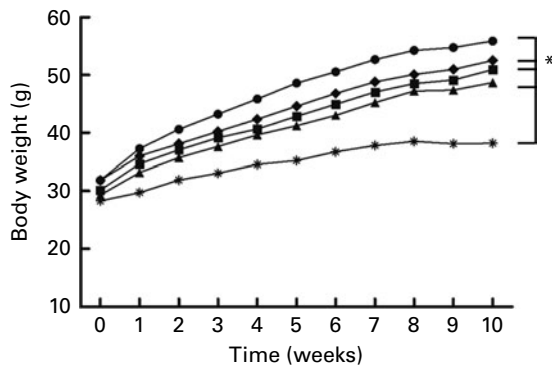


Fig. 1. Body weight trajectories of experimental groups for 10 weeks. Control (C, —); high-fat (HF, —▲—); high-fat diet plus 1% (w/w) freeze-dried jaboticaba peel powder (HFJ1%, —■—); high-fat diet plus 2% (w/w) freeze-dried jaboticaba peel powder (HFJ2%, —●—) and high-fat diet plus 4% (w/w) freeze-dried jaboticaba peel powder (HFJ4%, —◆—) groups. Values are means with their standard errors, n 8. *Values were significantly different from the C group ($P < 0.001$).

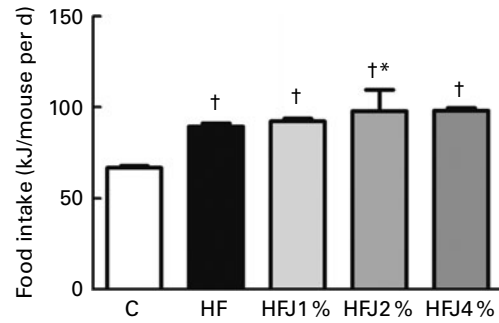


Fig. 2. Energy intake (kJ/mouse per d) for 10 weeks. Control (C); high-fat (HF); high-fat diet plus 1% (w/w) freeze-dried jaboticaba peel powder (HFJ1%); high-fat diet plus 2% (w/w) freeze-dried jaboticaba peel powder (HFJ2%) and high-fat diet plus 4% (w/w) freeze-dried jaboticaba peel powder (HFJ4%) groups. Values are medians and ranges, n 8. *Median values were significantly different from the HF group ($P < 0.05$). †Median value was significantly different from the C group ($P < 0.001$).

mately 96.3 kJ/d. Mice from the control group consumed 68.3 kJ/d. In mice fed on the HFD plus 2 and 4% FDJPP, the energy intake was significantly greater than the HF group (Fig. 2). Thus, a higher energy intake was associated with a greater cumulative weight gain throughout the experiment in these treatments.

Serum lipids and leptin concentrations

Fasting serum TAG, total cholesterol and HDL-cholesterol were not altered by level of fat or FDJPP in the diet (data not shown). Serum leptin levels increased with body weight (control group *v.* HFD groups); nevertheless, this trend did not reach statistical significance, probably due to the small number of animals used in this analysis (n 4) (Fig. 3).

Glucose intolerance and insulin resistance

The AUC values for plasma glucose levels during the iGTT were significantly increased in all HFD groups compared with the control group (Fig. 4(a)). These results demonstrated that glucose intolerance in control HFD-fed mice was not ameliorated by FDJPP treatments.

The contribution of insulin sensitivity to glucose intolerance was investigated by determining the clearance of plasma glucose as a function of time after insulin injection (ITT). This measure of whole-body insulin sensitivity can be expressed by constant rate for glucose disappearance (K_{ITT}); larger values indicate greater tissue insulin sensitivity. Notably, mice fed on the HFD plus FDJPP showed a significant increase in K_{ITT} values compared with the mice fed the HFD, and the K_{ITT} did not differ from the control group (Fig. 4(b)). These data suggest that FDJPP may prevent HFD-induced insulin resistance in mice.

Insulin signalling pathway

In order to evaluate possible mechanisms responsible for the enhancement of insulin sensitivity of FDJPP, we examined insulin signalling through the IR/IRS1/Akt/FoxO pathway in the liver and adipose tissue of mice. Impairment in all insulin signalling cascade steps was detected in the HF control group. As shown in Fig. 5, significant improvement in insulin signal

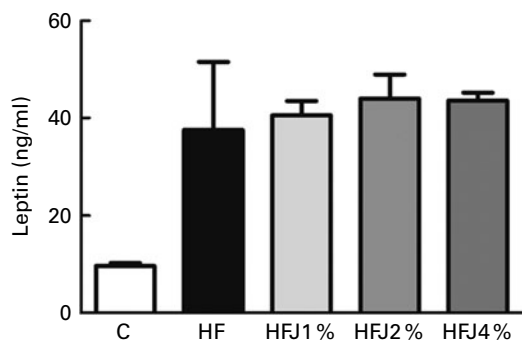


Fig. 3. Serum leptin levels of control (C); high-fat (HF); high-fat diet plus 1% (w/w) freeze-dried jaboticaba peel powder (HFJ1%); high-fat diet plus 2% (w/w) freeze-dried jaboticaba peel powder (HFJ2%) and high-fat diet plus 4% (w/w) freeze-dried jaboticaba peel powder (HFJ4%) groups. Values are medians and ranges, *n* 4. Statistical differences were not found among the experimental groups.

transduction was observed through the increased insulin-mediated tyrosine phosphorylation of IR and IRS-1, serine phosphorylation of Akt in the liver and adipose tissue and threonine phosphorylation of FoxO in the liver of mice treated with FDJPP when compared with the HF control mice.

Inflammation

Because previous studies have shown that chronic activation of intracellular proinflammatory pathways within the insulin target cells might contribute to the obesity-related insulin resistance, we evaluated whether FDJPP could modulate HFD-induced inflammation. Remarkably, the mRNA levels of the cytokines IL-6 and IL-1 β from the liver of the HFJ groups were significantly reduced, except for the mRNA levels of IL-1 β found in the HFJ2% group (Fig. 6(a) and (b)). This effect was accompanied by a significant decrease in the phosphorylated I κ B- α protein levels in the liver of all mice treated with FDJPP compared with the HF mice (Fig. 7). These improvements were comparable with those of the lean control mice.

Discussion

Recently, much attention has been focused on natural bioactive phytochemicals present in food that may provide

desirable health benefits. The potential of bioactive components to treat or prevent obesity is under intense exploration, and this represents a strategy for developing safe anti-obesity approaches^(8,27). Emerging reports have provided evidence of the importance of anthocyanins as dietary antioxidants for the prevention of oxidative damage. Lately, several studies have gradually focused on their beneficial effects in preventing obesity and diabetes^(10–13,28).

The present study demonstrates that supplementation of a HFD with jaboticaba peel does not produce significant protection against body weight gain and, in fact, tends to increase cumulative weight gain, accompanied by no difference in food and energy intake. We also observed no changes in serum lipid content and leptin levels in mice fed on the FDJPP-enriched diets. Similarly, recent studies have also reported that whole freeze-dried powders of concord grapes, blueberry and black raspberry were ineffective in preventing obesity and, in some cases, they could increase body weight gain and adiposity relative to a HFD^(13,14,29,30). However, purified anthocyanins from various sources, including purple maize, Cornelian cherry, black soybean coats, blueberries and strawberries, have shown positive responses in HFD-induced obesity, such as decrease in body weight gain and improvement in lipid profile, hyperleptinaemia and hyperglycaemia to levels similar to control mice^(10–14). Reasons for the different response between whole foods and purified extracts of anthocyanins are still not fully elucidated. The possible therapeutic effects of anthocyanins are certainly dependent on sufficient bioavailability and not on the exact amount of anthocyanins consumed⁽³¹⁾. Other components present in the whole food matrix may possibly reduce the rate of absorption and bioactivity of anthocyanins, counteracting in some way to prevent any protective effect against obesity.

The FDJPP supplementation did not attenuate HFD-induced hyperglycaemia either, which was reflected in a larger incremental iGTT_{AUC} of the plasma glucose. The pathogenesis of glucose intolerance is complex and is mainly a function of the interplay between insulin sensitivity and endocrine pancreatic function⁽³²⁾. In the present study, we demonstrated that mice fed on HFJ diets for 6 weeks were protected from insulin resistance based on a significant increase in K_{ITT} when compared with the HF group. The K_{ITT} value reflects the rate

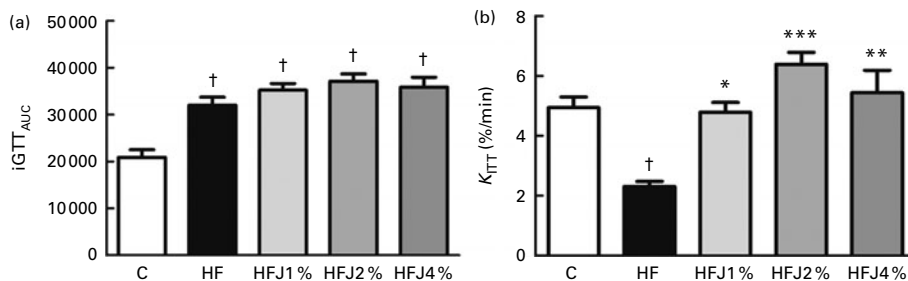


Fig. 4. (a) Glucose AUC during intraperitoneal glucose tolerance test (iGTT_{AUC}) and (b) K_{ITT} during insulin tolerance test were determined in control (C); high-fat (HF); high-fat diet plus 1% (w/w) freeze-dried jaboticaba peel powder (HFJ1%); high-fat diet plus 2% (w/w) freeze-dried jaboticaba peel powder (HFJ2%) and high-fat diet plus 4% (w/w) freeze-dried jaboticaba peel powder (HFJ4%) groups after 9 and 10 weeks of the experiment, respectively. Values are means with their standard errors of *n* 6 mice. Mean values were significantly different from the HF group: **P*<0.05; ***P*<0.01; and ****P*<0.001. †Mean values were significantly different from the C group (*P*<0.001).

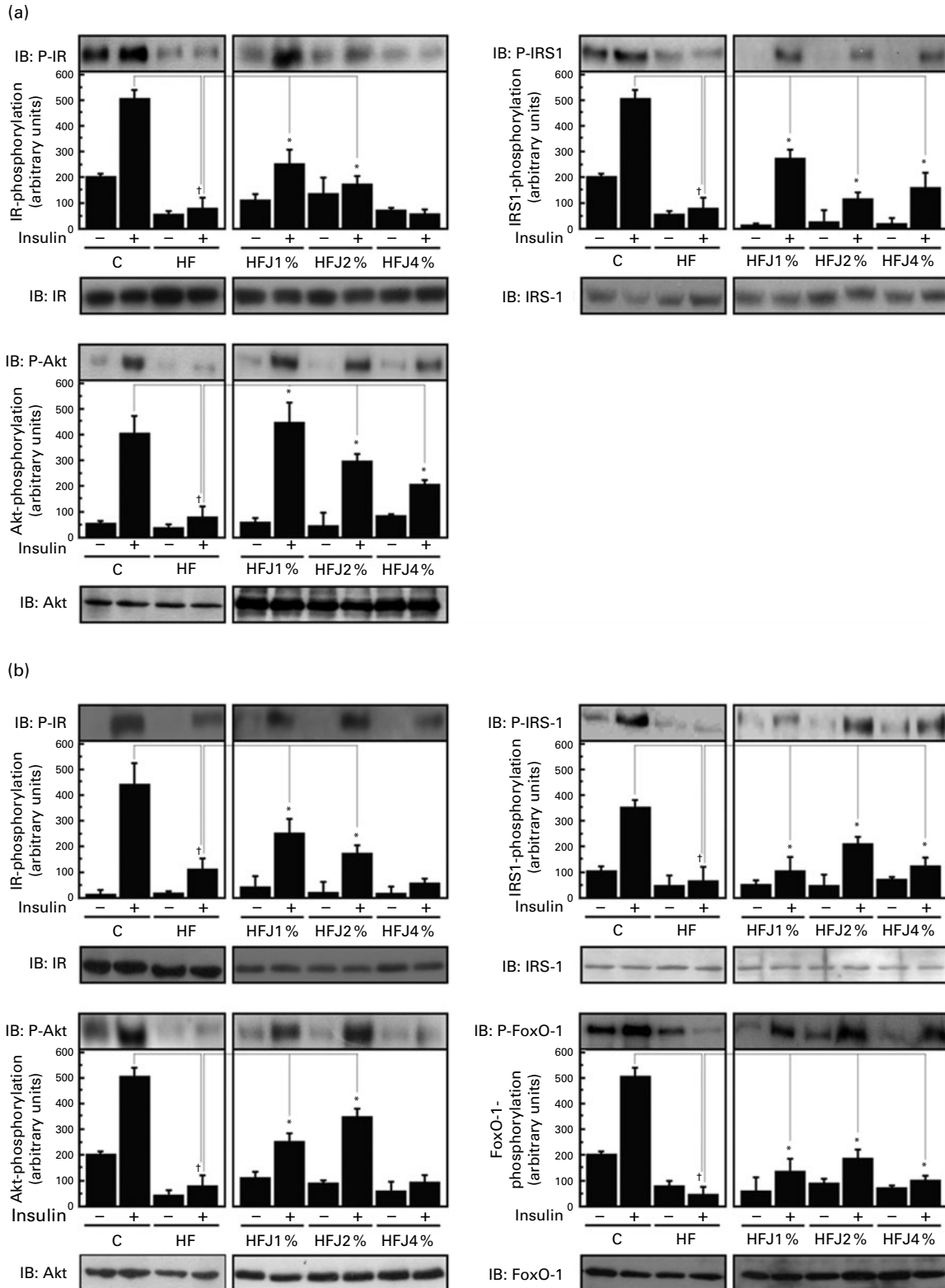


Fig. 5. Representative immunoblots (IB) of insulin signalling proteins in (a) adipose tissue and (b) liver of control (C); high-fat (HF); high-fat diet plus 1% (w/w) freeze-dried jaborcaba peel powder (HFJ1%); high-fat diet plus 2% (w/w) freeze-dried jaborcaba peel powder (HFJ2%) and high-fat diet plus 4% (w/w) freeze-dried jaborcaba peel powder (HFJ4%) groups before (-) or after (+) insulin stimulation. *Values were significantly different from the HF group ($P < 0.05$). †Values were significantly different from the C group ($P < 0.001$). IR, insulin receptor; IRS1, insulin receptor substrate 1.

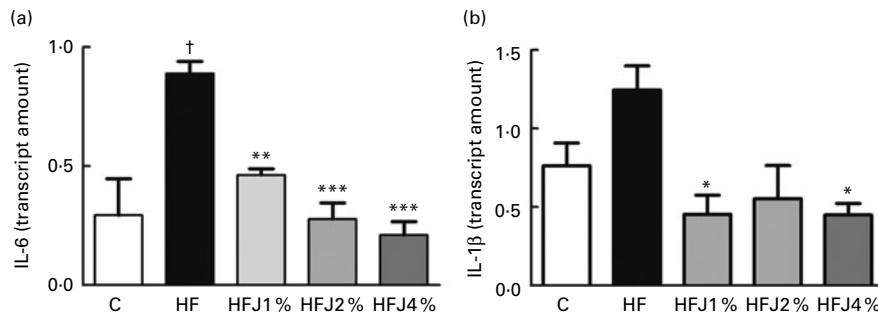


Fig. 6. Transcript amount of (a) IL-6 and (b) IL-1 β in the liver of control (C); high-fat (HF); high-fat diet plus 1% (w/w) freeze-dried jaboticaba peel powder (HFJ1%); high-fat diet plus 2% (w/w) freeze-dried jaboticaba peel powder (HFJ2%) and high-fat diet plus 4% (w/w) freeze-dried jaboticaba peel powder (HFJ4%) groups was determined by real-time PCR. Values are means with their standard errors, n 5. Mean values were significantly different from the HF group: * $P < 0.05$; ** $P < 0.01$; and *** $P < 0.001$. † Mean value was significantly different from the C group ($P < 0.001$).

of glucose removal by increased glucose uptake in insulin-sensitive tissues, particularly the skeletal muscle, and suppression of hepatic glucose production in response to exogenous insulin.

To address the molecular action and possible mechanisms by which FDJPP treatments ameliorate insulin sensitivity, we evaluated key molecular steps of the insulin signalling machinery in the liver and the adipose tissue of the treated mice. The effects of insulin on cell metabolism are mediated by a heterotetrameric receptor expressed in the liver, the adipose tissue and the skeletal muscle, among others. Binding of insulin to its receptor initiates a cascade of events that includes receptor autophosphorylation on tyrosine residues and tyrosine phosphorylation of IRS proteins that subsequently activate the subsequent signalling pathways: tyrosine phosphorylation of IRS1, phosphorylation and activation of Akt and translocation of GLUT4 to facilitate the transport of glucose into cells. Akt also mediates the inhibitory effects of insulin on hepatic glucose output by inducing phosphorylation of the transcriptional factor FoxO, resulting in inhibition of key gluconeogenic enzymes⁽³³⁾. The present results show that the impaired signal transduction through IR/IRS1/Akt/FoxO pathway is completely restored in liver and adipose tissue from the FDJPP-treated mice compared with the HFD mice.

It is now commonly accepted that obesity is characterised by a broad inflammatory response. Many inflammatory mediators, overproduced by expanded adipose tissue, may have an impact on insulin action, contributing significantly to the establishment of insulin resistance. TNF- α , IL-6, IL-1 β and other cytokines, activate various serine kinases, such as Jun N-terminal kinase and I κ B. These can phosphorylate IRS proteins and possibly other insulin signalling molecules interfering with the normal insulin action and creating a state of cellular insulin resistance^(34,35).

Some reports showed that anthocyanins have anti-inflammatory activity potential, and the down-regulation of some inflammatory adipocytokine expression can contribute to amelioration of the diabetic state^(30,36). Accordingly, the insulin-sensitising effect of FDJPP in the present study is associated with attenuated inflammation, observed by lower expressions of IL-1 β and IL-6 accompanied by decreased

phosphorylated I κ B- α protein levels in liver of all treated mice in a dose-independent manner. Similarly, DeFuria *et al.*⁽³⁰⁾ demonstrated that blueberry supplementation can protect against whole-body insulin resistance by inhibiting the early inflammatory events in adipose tissue, induced by HFD in mice.

In addition, systemic oxidative stress, a persistent imbalance between the production of highly reactive molecular species and antioxidant defenses, correlates with fat accumulation in human subjects and mice. The hypothesis that oxidative stress is a causative factor in the development of insulin resistance has been supported by several studies that showed that reversal of the imbalance between reactive oxygen species and antioxidants improves insulin resistance⁽³⁷⁾.

Reactive oxygen species can activate a number of cellular stress-sensitive pathways that cause cellular damage and are linked to insulin resistance and chronic inflammation. The

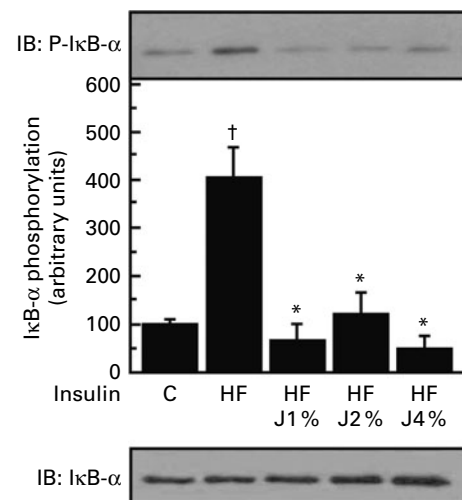


Fig. 7. Phosphorylation of I κ B in the liver of control (C); high-fat (HF); high-fat diet plus 1% (w/w) freeze-dried jaboticaba peel powder (HFJ1%); high-fat diet plus 2% (w/w) freeze-dried jaboticaba peel powder (HFJ2%) and high-fat diet plus 4% (w/w) freeze-dried jaboticaba peel powder (HFJ4%) groups was determined by immunoblot (representative blot). β -Actin was used as the loading protein (lower panel). Results of scanning densitometry are expressed as arbitrary units. Values are means with their standard errors, n 5. * Mean values were significantly different from the HF group ($P < 0.05$). † Mean value was significantly different from the C group ($P < 0.001$).

chronic and/or increased production of these reactive molecules or a reduced capacity for their elimination can lead to the activation of multiple stress-sensitive serine/threonine kinase signalling cascades such as $\text{I}\kappa\text{B}-\beta$, resulting in reduced insulin action⁽³⁸⁾.

Our previous study demonstrated that the addition of 1 and 2% of FDJPP to a control diet improved the antioxidant capacity of rat's plasma (1.7 times by the Trdox equivalent antioxidant capacity method and 1.3 times by the oxygen radical absorbance capacity assay). Taking all this information into account, FDJPP seems to exert a protective role against HFD-induced insulin resistance in mice in different ways. This might be due to the ability of FDJPP anthocyanins and/or other related bioactive antioxidants to exert their effects as free radical scavengers, preventing to some degree the increase in reactive oxygen species levels. They could also modulate cellular stress signalling processes during inflammation, acting as signalling agents by inhibiting the NF- κ B signal pathway, thus, preventing the development of insulin resistance in treated mice, in accordance with the results shown here.

It is worth highlighting that FDJPP is a complex byproduct and its phytochemical composition has not been fully elucidated. Thus, the beneficial health-promoting effects observed here and in previous studies may not be solely attributed to the high total anthocyanin content. Although in lower concentrations, studies have revealed that jabuticaba is also a dietary source of other phenolic compounds like quercetin and ellagic acid^(17,39). Results, both in cell culture and in animal models, have shown that quercetin has anti-obesity potential, improving glucose homeostasis, and it is able to modulate inflammation through a variety of mechanisms⁽⁴⁰⁾. Similarly, recent studies have found that pomegranate flowers and fruit extracts, which also contain abundant anthocyanins and hydrolysable tannins, have strong antioxidant, anti-inflammatory properties, appetite-suppressant activity and they have improved insulin sensitivity in diabetic animals^(41–43). Therefore, these bioactive compounds may also have contributed to the effects observed in the present study. Despite the fact that FDJPP is a remarkable source of dietary fibre, in the present work, the influence of this component on the results was unlikely to be due to the adjustments made in the supplemented diets.

Here, we demonstrated that impaired glucose tolerance observed in mice fed with the HFJ diets is not due to insulin resistance. It is well-known that dietary antioxidants, including anthocyanins, protect pancreatic β -cells from glucose-induced oxidative stress. One possibility is that the FDJPP treatment for 6 weeks is not efficient to reverse the β -cell dysfunction, as observed after exposure to high glucose levels, elevated NEFA levels or both during the first 4 weeks on the HFD. It is important to emphasise the fact that previous studies have shown that the β -cells are an additional target for the oxidative stress. These cells are particularly sensitive to reactive oxygen species because they are low in antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase^(38,44).

Conclusion

A HFD containing FDJPP, rich in anthocyanins, was ineffective in altering the effects of the HFD on body weight and glucose intolerance; however, the supplementation was efficient in correcting diet-induced insulin resistance, which may be associated, at least in part, to the reduced inflammation in the liver of the treated mice. These findings raise issues that require further investigation.

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