ÂNGELA GIOVANA BATISTA

"ANTIOXIDANT STATUS AND LIPID PROFILE OF RATS FED HIGH-FAT DIET WITH FREEZE-DRIED JABOTICABA PEEL ADDED"

"STATUS ANTIOXIDANTE E PERFIL LIPÍDICO DE RATOS ALIMENTADOS COM DIETA HIPERLIPÍDICA ADICIONADA DE CASCA DE JABUTICABA LIOFILIZADA"

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Orientador: Prof. Dr. Mário Roberto Maróstica Júnior

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Dissertação de mestrado apresentada ao Programa de Pós-Graduação em Alimentos e Nutrição da Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas para obtenção do título de Mestra em Alimentos e Nutrição na Área de Concentração de Nutrição Experimental aplicada à Tecnologia de Alimentos.

Master dissertation presented to the Food and Nutrition Postgraduation Programme of the School of Food Engineering of the University of Campinas to obtain the M.D. grade in Food and Nutrition in the Concentration Area of Experimental Nutrition applied to Food Technology.

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Juliana Alves Macedo
Elizabethe Adriana Esteves
Luciana Gomes Malta
Nísia Andrade Villela Dessimoni Pinto
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BANCA EXAMINADORA

_____

Dr. Mário Roberto Maróstica Júnior

Orientador

_____

Dra. Juliana Alves Macedo

Membro

_____

Dra. Elizabethe Adriana Esteves

Membro

_____

Dra. Nísia Andrade Villela Dessimoni Pinto

Suplente

_____

Dra. Luciana Gomes Malta

Suplente

"Na verdade, há um espírito no homem,
e a inspiração do Todo-Poderoso o faz entendido."
Jó 32:8

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SUMÁRIO

RESUMO GERAL

O sobrepeso e a obesidade têm sido apontados como problemas de saúde pública e estão associados com o surgimento de doenças crônicas. O estresse oxidativo desencadeado pela ingestão de dietas hiperlipídicas estimula o desenvolvimento da síndrome metabólica, a qual é acompanhada de doenças cardiovasculares, dislipidemias, diabetes, resistência à insulina e outros. A casca de jabuticaba (*Myrciaria jaboticaba* (Vell.) Berg.) é rica em antocianinas, compostos fenólicos antioxidantes extensivamente referenciados pela literatura científica devido ao seu potencial de combater o estresse oxidativo *in vivo*. A proposta deste trabalho foi verificar a presença de biomarcadores do estresse oxidativo e perfil lipídico de ratos *Sprague-Dawley* alimentados com dieta hiperlipídica adicionada de casca de jabuticaba liofilizada (CJL). Trinta ratos foram distribuídos em 5 grupos de 6 animais: controle normal (N) – dieta normal AIN-93G; controle hiperlipídico (C) – dieta com 35% de lipídios (31% banha suína e 4% óleo de soja); jabuticaba 1 (J1), jabuticaba 2 (J2) e jabuticaba 4 (J4) – dieta hiperlipídica (C) adicionada de 1, 2 e 4% de CJL, respectivamente. Os animais dos grupos J1, J2 e J4 foram alimentados com dieta C nos primeiros 30 dias e depois receberam as dietas com CJL, completando 70 dias de experimento. Os animais foram mortos por decapitação, e exsanguinados para obtenção do soro, plasma e órgãos para liofilização. As análises da capacidade antioxidante total do plasma e órgãos, enzimas envolvidas no estresse oxidativo em órgãos, além de lipídeos séricos, hepáticos e fecais, foram analisados para verificação do efeito da adição de CJL às dietas. No segundo período do experimento (últimos 40 dias), a ingestão dietética dos animais J4 foi maior em relação ao grupo C. O grupo J2 mostrou os maiores níveis de lipídios hepáticos e fecais, e o grupo J4 a maior média nos níveis de colesterol hepático e fecal. Não houve diferenças significativas para triglicerídeos hepáticos, e os grupos J1 e J4 excretaram mais triglicerídeos via fezes em relação a C. Os parâmetros de avaliação antioxidante no plasma foram aumentados nos grupos alimentados com J2 e J4. Todas as dietas contendo CJL impediram a peroxidação lipídica hepática induzida pela dieta hiperlipídica, bem como aumentaram sua capacidade antioxidante. No cérebro foi percebida uma resposta dependente da dose de CJL: os valores de TBARS diminuíram com o aumento do teor de antocianinas na dieta. Os rins dos animais alimentados com J2 e J4 mostraram uma melhora do seu status antioxidante de acordo com análises de

ORAC e GSH. Os grupos J1 e J4 apresentaram os maiores valores de TEAC no baço. Assim, a inclusão na dieta CJL poderia reforçar as defesas antioxidantes sistêmicas, além de promover maior eficiência na excreção de triglicerídeos. Portanto, o produto da casca de jabuticaba pode ser uma alternativa natural para minimizar os danos do estresse oxidativo induzido pela obesidade.

Palavras chave: Jabuticaba. *Myrciaria jaboticaba* (Vell) Berg. Dieta hiperlipídica. Estresse oxidativo.

SUMMARY

Overweight and obesity have been identified as public health problems and are associated with chronic diseases. High-fat diet-induced oxidative stress stimulates the development of metabolic syndrome, which is accompanied by cardiovascular diseases, dyslipidemia, diabetes, insulin resistance and others. The peel of jaboticaba (*Myrciaria jaboticaba* (Vell.) Berg.) is rich in anthocyanins, phenolic antioxidants, which are referenced in the scientific literature because of its in vivo antioxidant potential. The aim of this study was to determine the presence of biomarkers of oxidative stress and to evaluate the lipid profile of rats fed high-fat diet with freeze-dried jaboticaba peel (FJP) added. Thirty rats were divided into 5 groups of 6 animals each: normal control (N) - normal diet AIN-93G; high-fat control (C) - a diet with 35% fat (31% lard and 4% soybean oil); jaboticaba 1 (J1), jaboticaba 2 (J2), and jaboticaba 4 (J4) - fat diet (C) with 1, 2 and 4% CJL added, respectively. The animals of J1, J2 and J4 groups received C diet during the first 30 days and after, they received the FJP diets, completing 70 days. The animals were killed by decapitation and exsanguinated to obtain the serum, plasma and organs for lyophilization. The total antioxidant capacity, oxidative stress-related enzyme activities of the tissues, and serum, liver and feces lipids were analyzed as indicators of FJP diets *in vivo* effects. In the second period of the experiment (last 40 days), food intake of J4 animals was higher than in C group. The J2 group showed higher levels of hepatic and fecal lipids and the animals from J4 group showed the highest hepatic and fecal cholesterol levels. There were no significant differences in hepatic triglycerides, but J1 and J4 groups excreted more triglycerides relative to C group. The antioxidant parameters evaluated in plasma were increased in the groups fed the J2 and J4 diets. All FJP diets prevented hepatic oxidative stress. Brain exhibited a dependent dose response as lipid peroxidation decreased with increasing of FJP content in the diet. The kidneys of J2 and J4-fed animals showed an improvement in their antioxidant status according to ORAC and GSH assays. The J1 and J4 groups showed the highest TEAC values in the spleen. Thus, the inclusion of FJP in the diet could enhance systemic antioxidant defenses, and promote greater efficiency in the excretion of triglycerides. This product could be a natural alternative to minimize the damage of obesity-induced oxidative stress.

Keywords: Jaboticaba. *Myrciaria jaboticaba* (Vell) Berg. High-fat diet. Oxidative stress.

INTRODUÇÃO GERAL

O sobrepeso e a obesidade têm sido apontados como problemas de saúde pública desde meados da década de 80 (WHO, 2000), e, paralelamente a este problema, a prevalência de doenças crônicas vem aumentando em todo o mundo. De acordo com dados da Organização Mundial da Saúde, o Brasil apresenta prevalência de 52,8% de excesso de peso em adultos (20 anos ou mais), sendo que 19,5% da população são obesos (WHO, 2011). Assim, acompanhando a tendência dos achados nas pesquisas (IBGE, 2010), em cerca de 10 anos, a população com excesso de peso representará 66% da população adulta do Brasil, assim como nos Estados Unidos, onde aproximadamente 30,2% dos homens norte-americanos e 33,2% das mulheres norte-americanas são obesos (WHO, 2011). Diante desta epidemia, as investigações sobre os determinantes deste quadro clínico e o interesse em terapias preventivas e de controle do peso e das morbidades advindas da obesidade vêm sendo intensificadas.

O consumo de frutas exóticas e seus derivados tem sido fortemente associado com o risco reduzido de desenvolver doenças crônicas como a obesidade (TSUDA et al., 2003), doenças cardiovasculares, diabetes tipo 2, resistência à insulina (BASU et al., 2010), doenças neurodegenerativas (PAPANDREAU et al., 2009), câncer (LEITE-LEGATTI et al., 2012) e outros. Há fortes evidências de que essas propriedades estão relacionadas com a presença de fitoquímicos, capazes de combater radicais livres e o estresse oxidativo, aumentado por processos fisiológicos e/ ou patológicos. Além disso, esses compostos presentes em sementes, cascas e polpas de frutas possuem efeitos anti-inflamatório, hipoglicemiante e hipolipidêmico (KIM et al., 2010; GUO et al., 2012).

As frutas vermelhas são destaque neste contexto por serem ricas em polifenóis e quantidades significativas de fibras. Estes compostos fenólicos purificados ou adicionados à dieta, bem como fibras dietéticas parecem promover efeitos benéficos para o organismo, tanto no tratamento como na prevenção de doenças crônicas (PRIOR, 2003).

A jabuticaba é uma fruta nativa, tropical do sudeste do Brasil. *Myrciaria jaboticaba* (Vell.) Berg. e *Myrciaria caulifolia* (DC) Berg são as variedades mais adequadas para o consumo *in natura*, bem como para a aplicação na indústria alimentar. O fruto possui diâmetro de cerca de 3-4 cm, contendo de um a quatro sementes no interior, sendo a sua casca roxa escura. Além disso, o fruto é altamente perecível, com período curto de comercialização após a colheita, devido ao elevado teor de água e açúcares e outros constituintes presentes na polpa. A casca é

fina e muito frágil; a polpa é doce com leve acidez, de ótimo sabor, e de cor variando do branco a translúcido (MATTOS, 1983; DONADIO, 2000).

A casca da jabuticaba, geralmente não é consumida e apresenta características que incitam o seu desenvolvimento tecnológico. Este material concentra a maioria dos constituintes do fruto de interesse na saúde, como: antocianinas (cianidina 3-glicosídeo e delfinidina 3-glicosídeo), quercetina, elagitaninos, ácido elágico, fibras solúveis, insolúveis, e polifenóis totais (Abe et al., 2012; Leite-Legatti et al., 2012). Nossos estudos têm demonstrado que um produto feito a partir da casca *M. jaboticaba*, a casca de jabuticaba liofilizada, quando incorporada na dieta de ratos saudáveis e obesos, beneficia o estado redox das células do plasma, melhora a inflamação em fígado e tecido adiposo, atenua a resistência à insulina, e corrobora para a diminuição de colesterol total, LDL e aumento de HDL (Leite et al., 2011; Dragano, 2011; Lenquiste et al., 2012).

Os produtos da jabuticabeira são ainda produzidos de maneira artesanal, em pequena escala. A indústria apresenta interesse nesta fruta, no entanto detêm-se à falta de matéria-prima, no curto período de comercialização pós-colheita e de informações sobre o processamento (BRUNINI et al, 2004). Desta forma, estudos que valorizem a utilização deste resíduo oriundo do processamento da jabuticaba, poderiam agregar valor à sua produção, além de incentivar o seu aproveitamento agroindustrial, e promover melhoras em parâmetros de saúde da população, com evidências de efeitos funcionais.

Diante do exposto, houve a hipótese de que possíveis alterações em biomarcadores do estresse oxidativo e do perfil lipídico de ratos *Sprague-Dawley* alimentados com dieta hiperlipídica pudessem ocorrer diante da adição de diferentes doses de casca de jabuticaba (*Myrciaria jaboticaba* (Vell) Berg.) liofilizada à dieta. Para constatação de tal hipótese, foram avaliados *in vivo*: o crescimento, parâmetros de ingestão dietética e o ganho de peso dos animais; os níveis séricos, hepáticos e fecais de colesterol total, triglicérides e lipídios totais; níveis de atividade de enzimas marcadoras do estresse oxidativo em diferentes tecidos; capacidade antioxidante e peroxidação lipídica tecidual.

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CHAPTER 1:

POLYPHENOL-RICH DIETS AND OXIDATIVE STRESS IN OBESITY: A REVIEW

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Ângela Giovana Batista, Mário Roberto Maróstica Júnior.

School of Food Engineering, University of Campinas (UNICAMP), P.O. Box 6121, 13083-862 Campinas-SP, Brazil.

ABSTRACT

Obesity is a healthy public problem linked to chronic diseases and oxidative stress. Investigations report that a chronic state of oxidative stress is also related to insulin resistance, dyslipidemias, diabetes and cardiovascular diseases. In this way, natural products-based extracts and diets have been used due to their antioxidant effects. Polyphenol-rich diets are capable to minimize oxidative stress and chronic diseases. Particularly, anthocyanin-rich diets could increase the antioxidant circulating compounds and consequently, improve the antioxidant defense of cells, attenuate inflammation, act in signaling pathways and modulate proteins expression. Berries, purple corn and purple sweet-potatoes are rich in cyanidin and delphinidin, however, they are not worldwide accessible. Brazilian berries, such as jaboticaba, are also good sources of anthocyanins, they are widely known in South America countries, with a recognized function on oxidative stress attenuation. Although, the minimum orally doses, which could exert functional effects, is not clear yet. This review aimed to link oxidative stress with obesity, co-morbidities and how polyphenol/anthocyanin-rich diets could minimize oxidative stress via exotic berries oral administration.

Keywords: High-fat diet. Oxidative stress. Berries. Brazilian exotic fruits.

1. INTRODUCTION

Overweight and obesity have been pointed as healthy public problems since the 80's, and in parallel, the prevalence of chronic diseases have been risen in the entire world (WHO, 2003).

In global terms, obesity is a risk factor that is associated with increased mortality and morbidity reaching 10% women and 14% men. In the regions of America this rate represents 23.5 and 29.7% for women and men, respectively (WHO, 2012). In the USA approximately 30.2% American men and 33.2% American women are obese (WHO, 2011). Faced with this epidemic, investigations on the determinants of this clinical problem and the interest in preventive therapies and control of obesity co-related morbidities have been intensified.

Genetics, environmental and psychosocial factors compose the triad determinant of obesity (KOPELMAN, 2000; RAVUSSIN, 1995). However, changes in lifestyle during the last decades suggests that environmental and psychosocial factors are the most influential in the development of obesity, since high-calorie foods and sedentary behavior are rising as common habits (GARDNER; RHODES, 2009). An unbalanced diet, based on fast foods, characterized by a high content of saturated and trans fats, is commonly reported by obese subjects (BRAY; POPKIN, 1998; BUETTNER; SCHOLMERICH; BOLLHEIMER, 2007; STENDER; DYERBERG, 2004).

Obesity also is established as a state of chronic oxidative stress, or an imbalance between free radicals and circulating antioxidants, which is responsible for many life threatening complications (KEANEY et al., 2003; VINCENT; TAYLOR, 2006). Dietary nutrients and specific antioxidant-rich foods may play an important role in the control and prevention of these complications in obesity (CAO; BOOTH et al., 1998; JAYAPRAKASAM et al., 2006; KAY; HOLUB, 2002; KIM et al., 2010).

The consumption of polyphenol-rich vegetables and specially berries, small globular fruits of colors ranging from red to purple, have been linked to some health benefits (CAO; RUSSELL et al., 1998; MAZZA et al., 2002; PRIOR et al., 2010). The berries are rich in flavonoid antioxidants, such as anthocyanins. These pigments are responsible for the fruit color with important functional effects (PRIOR et al., 2008). The increasing interest on anthocyanins is due to its property of attenuation of the oxidative stress and, consequently, decreasing biomarkers associated with chronic diseases such type 2 diabetes (GHOSH; KONISHI, 2007), insulin resistance (GUO et al., 2012; JAYAPRAKASAM et al., 2006), hypercholesterolemia

(KALT et al., 2008; KIM et al., 2010), neurodegenerative diseases (WILLIAMS; SPENCER, 2012), among others.

In this way, this review aimed to show the evidences that link obesity and related diseases with oxidative stress and the effect of polyphenol/anthocyanin-rich diets on oxidative stress/obesity-related biomarkers.

2. OXIDATIVE STRESS AND REACTIVE OXYGEN SPECIES

Free radicals are molecules highly reactive with unpaired electrons, which rapidly bind to nearby molecules (HALLIWELL; GUTTERIDGE, 2007). Reactive Oxygen Species (ROS) are molecules containing oxygen, which may be paired electrons or not, but are highly reactive in tissues. The reactive nitrogen species (RNS) could also be cited as free radicals with important biological role. Low concentrations of free radicals (mainly ROS and RNS) are important for maintaining the normal redox state of cells, tissue function and intracellular signaling process. In contrast, the excess of free radicals can damage lipids, proteins, DNA, impair cellular function, lead to cell death or the acceleration of ageing and age-related diseases (FINKEL; HOLBROOK, 2000; YU, 1994).

Among the major ROS, we could highlight: Superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), peroxyl radical ($RO_2^{\bullet}$), alkoxyl ($RO^{\bullet}$), singlet oxygen ($^1O_2$), ozone (O_3) and hydroxyl radical ($^{\bullet}OH$). The latter one could be considered the most reactive and dangerous to the cells (VINCENT; INNES; VINCENT, 2007; YU, 1994). These oxidants are produced as a result of normal metabolism in the intracellular mitochondria and peroxisomes, as well as a variety of cytosolic enzyme systems. Moreover, a number of external agents can trigger the production of ROS (YU, 1994).

Thus, oxidative stress is characterized by the insufficient capacity of biological systems to neutralize overproduction of free radicals, which can be induced by habits such as smoking (PARK; PARK; GWAK, 1998), alcoholism (DAS; VASUDEVAN, 2007), physical activity absence, high-calorie diet (BURNEIKO et al., 2006; MATSUZAWA-NAGATA et al., 2008), among others. The high-calorie diet is strongly associated with cases of overweight and obesity and, consequently to oxidative stress (GARDNER; RHODES, 2009). Possible mechanisms generating oxidative stress in these subjects were extensively reviewed by Vincent and Taylor (VINCENT; TAYLOR, 2006).

Obesity-related oxidative stress is involved with several chronic diseases, such as cardiovascular (KEANEY et al., 2003), type 2 diabetes; insulin resistance (GHOSH; KONISHI, 2007; GUO et al., 2012; MATSUZAWA-NAGATA et al., 2008), high blood pressure (ROBERTS et al., 2001; ROBERTS et al., 2000), and osteoarthritis (TANAKA et al., 1998). In addition, the increasing of these co-morbidities has become a major challenge for health care professionals to combat, who have sought bioactive compounds in the solution to such problems (LEITE et al., 2011; PRIOR et al., 2010; TSUDA et al., 2003).

3. BIOMARKERS OF OXIDATIVE STRESS AND METHODS FOR MEASURING ANTIOXIDANT ACTIVITY

Biomarkers are biological indicators of the presence or probability of occurrence of a specific disease in clinical or non-clinical subjects (BAKER, 2005). The measurement of biomarkers helps in the diagnosing of changes in physiological state of the body, facilitating treatments or preventive interventions.

In cases of oxidative stress, with high ROS production and deficiency of antioxidants, biomolecules are oxidized, generating specific metabolites or biomarkers that can be identified and measured. Products of lipid peroxidation induced by free radicals, tissue and circulating lipid levels, enzymes activities with antioxidant role, and total antioxidant capacity of tissues has been extensively investigated with the aim to create alternative therapies for prevention and chronic diseases controlling (BURNEIKO et al., 2006; CAO; BOOTH et al., 1998; HAN, K. H. et al., 2007; JENSEN et al., 2008; KIM et al., 2010; LEE, C. Y.; CHENG; SIM, 2007; LEITE et al., 2011; LENQUISTE et al., 2012; PRIOR et al., 2003; VINCENT; TAYLOR, 2006; WILLIAMS; SPENCER, 2012; YANG et al., 2008).

A sophisticated antioxidant defense system is related with the activities of enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (Gpx) and glutathione reductase (Grd). They are responsible for neutralization and control of the ROS levels to maintain physiological homeostasis. The homeostatic production of hydrogen peroxide, hydroxyl and superoxide radicals can be controlled by this enzyme system. Thus, quantifying the expression and the activity of the enzymes may also be considered parameters of in vivo oxidative state (Figure 1.A) (JACOB, 1995).

SOD converts $O2^{\bullet-}$ in H_2O_2. In turn, enzymes CAT and Gpx transform H_2O_2 in water, preventing the formation of hydroxyl radical, which has no enzymatic antioxidant defenses. By

turning H_2O_2 in water, Gpx oxidize reduced glutathione (GSH) in GSSG (oxidized glutathione). However, glutathione reductase (Grd) is the reverse reaction in the presence of nicotinamide adenine dinucleotide phosphate (NADPH), converting GSSG into GSH, which results in continuous cycle, when GSH is oxidized again. Therefore, the GSH levels are also indicators of system enzymatic antioxidant (JACOB, 1995; VINCENT et al., 2007).

The investigation of lipid peroxidation indicators is important to identify the expression of oxidation of polyunsaturated fatty acids present in the cell membrane (Figure 1.B). Thiobarbituric acid reactive species (TBARS) or malondialdehyde (MDA), and determination of hydroperoxides (Perox Index) in tissues are the more common ones in the in vivo studies. Isoprostanes, like 8-epiPGF_2 also are important biomarkers of lipid peroxidation derivates of arachidonic acid and could be analyzed in tissues and urine (DOTAN; LICHTENBERG; PINCHUK, 2004).

Circulating polyphenols and other dietary antioxidant compounds could be largely distributed in the tissues and act as exogenous antioxidants, neutralizing free radicals, reducing the oxidative stress and supporting the endogenous antioxidant system. Hence, total antioxidant capacity assays could indicate the redox status of cells (Figure 1.C). Recently, ORAC (Oxygen Radical Absorbance Capacity) (PRIOR et al., 2003), TEAC (Trolox Equivalent Antioxidant Capacity) (RE et al., 1999), and FRAP (Ferring Reducing Antioxidant Power) (BENZIE; STRAIN, 1996), are the most used in clinical and non-clinical studies.

The ORAC assay measures the capacity of peroxyl radical scavenging in a biological system, using values for the temperature, pH and radical, close to the physiological conditions (PRIOR et al., 2003). Thus, assays based in non-physiological radicals such as ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) have been unused to predict the in vivo antioxidant capacity. Regarding this concept, recent techniques have replaced these ones, such as Cap-e (cell-based antioxidant protection assay), ROS assay (JENSEN et al., 2008) and CAA. The CAA (cellular antioxidant activity) is a novel and also a promissory test. The assay measures the inhibition of peroxyl radical-induced oxidation of dichlorofluorescein by antioxidants in cell culture. Thus, this method simulates physiological conditions such as the ORAC one, beyond that it counts with the cell metabolism, being a further advantage. However, this test was made only with food extracts so far (WOLFE; LIU, 2007).

Indicators of insulin resistance are also given in Figure 1.D. Plasma insulin concentrations and insulin secretion rates have been evaluated to predict insulin resistance. The clearance of plasma

glucose as a function of time after insulin injection indicates insulin sensitivity, so called insulin tolerance test or ITT, which can be expressed by the constant rate for glucose disappearance (K_{ITT}). The iGTT or intraperitoneal glucose tolerance test is analyzed as ITT after glucose injection (DRAGANO et al., 2012; GUO et al., 2012). A mathematical model of the glucose/ insulin interactions has been used to indicate the level by which they combine to give hyperglycemia with low, normal or raised basal plasma insulin concentrations: Homeostatic Model Assessment (HOMA-IR) (MATTHEWS et al., 1985; PRIOR et al., 2010).

Lipidomics are also a good new tool to investigate lipid biomarkers that act as cellular messengers, and how these molecules are part of signaling and metabolic processes in cells and other important biological processes (WIEST; WATKINS, 2007). Gas chromatography and high-performance liquid chromatography techniques coupled with mass spectrometry detector have been used to investigate these lipid biomarkers, such as fatty acyls, glycerolipids, phospholipids, sterols and sphingolipids. In addition, the analysis of fatty acids profiles in tissues has become important to understand their roles in pathologies such as type 2 diabetes and nonalcoholic fatty liver disease. Fatty acid methylated esterified (FAMEs) and non-esterified fatty acids (NEFA) have been used as biomarkers (HAN, L. D. et al., 2011; WU et al., 2011) (Figure 1.E).

The lipoprotein lipid transporters in the plasma are indicators of dyslipidemias and cardiovascular diseases, which are increased in obese subjects (CASTELLANI, 2004). The commercial enzymatic kits are used to analyze the plasmatic and hepatic levels of triglycerides, cholesterol and also NEFAs (Figure 1.F) (CASTRO et al., 2012; LENQUISTE et al., 2012). These kits are economically advantageous, easier and faster to acquire. Moreover, they allow a rapid analysis of blood, since the lipoproteins assays must to be done in a short term after exsanguination.

High levels of LDL-cholesterol and increased oxidative stress are associated with increased levels of oxidized LDL (oxLDL). Free radicals could oxidize esterified cholesterol, which is up-taken by macrophage and starts the atherosclerostic cascade. The level of oxLDL could be analyzed by monoclonal antibody-based ELISA kit (BOON et al., 2012).

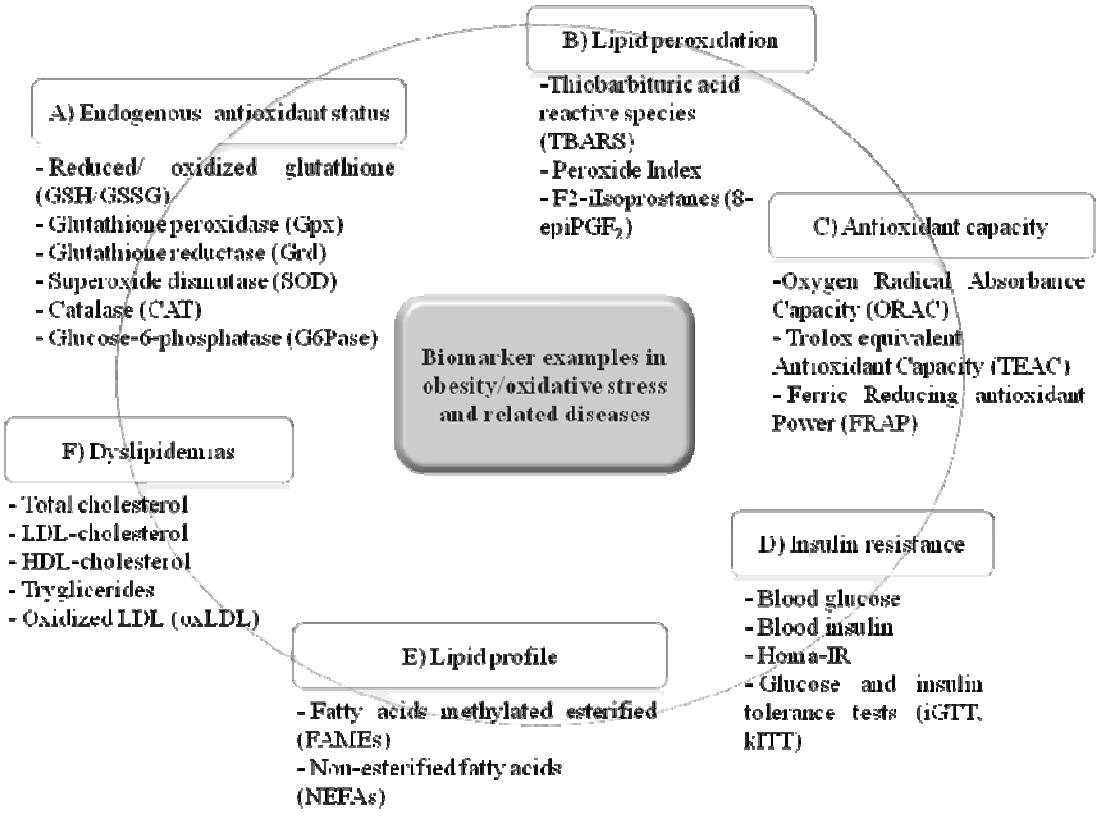

Figure 1 - Biomarkers of obesity-induced oxidative stress and insulin resistance.

4. HIGH-CALORIE DIET-INDUCED OXIDATIVE STRESS IN ANIMAL MODELS

Lard contains 37% saturated fat and 46% monounsaturated fat (CASTRO et al., 2012) and because of this, it has been used in diets (25 to 60% kcal) to induce metabolic disorders in experimental animal models (BUETTNER et al., 2007). Lard-based high-fat diets are responsible for the increasing oxidative stress and reduction of the activity of the enzymes involved in the antioxidant endogenous system in animal tissues (NOEMAN; HAMOODA; BAALASH, 2011). In fact, this is deeply linked to the developing of overweight, obesity, dyslipidemias (YANG et al., 2008), hepatic steatosis, inflammation, (MATSUZAWA-NAGATA et al., 2008), hyperglycemia, insulin resistance, (GUO et al., 2012) and osteoporosis (XIAO et al., 2010).

A study with C57BL/6J mice fed a high-fat diet (40% lipids) showed a high production of ROS, corroborating to high levels of inflammatory cytokines (e. g. TNF-α), and circulating or hepatic free fatty acids. In this case, the diet-induced oxidative stress was triggered by the

increase of ROS genes production in the liver, retroperitoneal adipose tissue even before of insulin resistance and obesity appearance (MATSUZAWA-NAGATA et al., 2008).

Yang et al. verified that mice fed a diet containing 20% lard showed 43% increasing in plasmatic TBARS, while this increasing in liver tissue stretches 56%. The plasmatic antioxidant capacity was also decreased in high-fat-fed animals, but no differences were showed in the SOD activity (YANG et al., 2008). Xiao et al. also showed that the genetic expression of antioxidant system enzymes such as SOD and Gpx were 1.5 times decreased in femur of high-fat-fed mice (21% lard and cholesterol) (XIAO et al., 2010). Indeed, the lard intake induce systemic oxidative stress also achieving liver, heart and kidneys, with a significant decrease in endogenous antioxidant activities (GSH, Gpx, CAT), and increased MDA levels (NOEMAN et al., 2011).

High-fat (39% kcal) and high-sugar (40% kcal) diets were also capable of inducing oxidative stress in female Sprague-Dawley (SD) (ROBERTS et al., 2000). The animals showed an increasing in renal oxide nitric synthase activity (30%), high plasmatic levels of H_2O_2 and reduced plasma antioxidant capacity (ROBERTS et al., 2005).

Given the facts that high-calorie diet could induce oxidative stress, there is the interest of researching alternatives to remedy the harm resulting from this and, thus, prevent the development of chronic degenerative not arising. It is known that nutritional therapy based on antioxidants bioactive compounds present in plants, may serve as natural alternative for the prevention and control of these diseases (ESTEVES et al., 2011; JENSEN et al., 2008; LEE, S. J.; CHOI; SEO, 2009; LEITE-LEGATTI et al., 2012).

5. DIETARY BIOACTIVE COMPOUNDS AND ITS ROLE ON ENHANCEMENT OF ANTIOXIDANT STATUS IN VIVO

Phenolic compounds are a large group of secondary metabolites of plants. They could scavenge free radicals due to their capacity of electrons donation. However, their antioxidant effect depends of their stability in different tissues, as well as the number and position of hydroxyls in the molecule (ROBARDS et al., 1999). These compounds, specially flavonoids, are also capable to reduce oxidative stress by the inhibition of lipid peroxidation in the cell membrane, improving endogenous antioxidant system, enzyme modulation and metals quelation (MLADENKA et al., 2010).

The phenolic compounds are the major contributor to dietary antioxidant capacity (PANTELIDIS et al., 2007). A study reported that the consumption polyphenols from fruits

represent daily 450 mg GAE among the North-americans (CHUN et al., 2005). However, little is known about the minimum daily requirement for these bioactive functional effects. Studies have shown that only the long-term consumption of vegetables contributes significantly (5-25%) to the total antioxidant capacity in vivo (CAO; RUSSELL et al., 1998).

A non-clinical study showed that diets with 0.2% polyphenols extract for 6 weeks decreased biomarkers of diabetes, hepatic steatosis, hepatic lipid peroxidation and heart oxygen production in obese rats (FEILLET-COUDRAY et al., 2009). In addition, it was reported that mulberry leaves (135 µg rutin day^{-1}) and rutin (2 mg day^{-1}) extracts increased the antioxidant status in plasma, liver, adrenal glandule, kidneys and spleen of immobilized rats, during 9 days (LEE, C. Y. et al., 2007).

A long-term clinical study demonstrated that a fruit and vegetable-rich diet (4.8 mM trolox equivalents by ORAC) administrated during 15 days, and a year later, was capable to increase the serum antioxidant capacity in men and women relative to those that received a diet with 3.3 mM TE (CAO; BOOTH et al., 1998). A short-term clinical study (2 week + 2 week wash-out + 2 week) showed that a diet with high antioxidant capacity ameliorated the inflammation and hepatic functions but there were no changes in the antioxidant status (VALTUENA et al., 2008). Therefore, the dose of polyphenols, their capacity to increase the antioxidant status and the period of treatment, seems to be the main factors to elucidate the functional effect of these compounds.

A twin clinical study showed that continuous consumption of Mediterranean diet (rich in polyphenols) is strongly involved with lowering oxidative stress (DAI et al., 2008). This has been confirmed by the results of higher GSH/ GSSG in individuals with higher scores in the Mediterranean diet food frequency questionnaire. This study showed that the positive association of diet with reduced oxidative stress was independent of conventional risk factors, family influence, or genetic factors. In addition, the low incidence of cardiovascular disease in the Mediterranean region might be associated to the contribution of dietary antioxidant compounds to the in vivo antioxidant defense. Particularly, anthocyanin-rich diets have also been used to reduce the obesity-related oxidative stress, as shown in the next section.

5.1. ANTHOCYANINS: METABOLISM AND ANTIOXIDANT EFFECTS

Anthocyanins belong to the polyphenols group, responsible for blue, purple, and red color of many plant tissues and occur mainly as glycosides forms (LIEBERMAN, 2007). The

chemistry and distribution of anthocyanins have been studied and reviewed (VANZO et al., 2011). Resembling the majority of polyphenols, anthocyanins and its aglycone form (anthocyanidins), have important antioxidant powers. They could donate electrons or transfer hydrogen atoms from hydroxyl unities to free radicals. Cyanidin, delphinidin, peonidin, petunidin, malvidin and pelargonidin are common anthocyanidins found in vegetables, being cyanidin the more frequent. The antioxidant capacity will depend of the position and number of hydroxyls in the molecule (PRIOR, 2003).

The metabolism of anthocyanins is not yet totally clear. There is a hypothesis that cyanidin 3-glucoside (C3G) is hydrolyzed by β-glucosidase being chemically changed or not in the intestines, producing cyanidin (Cy), protocatechuic acid (PC) as main metabolites. Cy is unstable in the plasma, and may be degraded to PC, even if Cy is absorbed into the circulatory system (TSUDA; HORIO; OSAWA, 2000).

In the liver and kidneys, tissues where anthocyanins are largely distributed, other enzymes act. In a bioavailability study, C3G and its metabolite (peonidin 3-glucoside, P3G) were quickly (15 s) found in rat plasma, liver and kidneys after an intravenous dose of C3G (VANZO et al., 2011). The enzyme probably involved in the transformation of C3G to P3G (methylation) in these tissues is catechol *O*-methyl transferase (COMT) related in flavonoids metabolism (TSUDA; HORIO; OSAWA, 1999). The main vias of excretion was bile or urine (15 min) (VANZO et al., 2011). Conjugation of anthocyanins and other flavonoids with glucuronides (e.g. cyanidin 3-glucuronide – C3-glcd) are also a possible metabolism way (PRIOR, 2003). In fact, the major metabolic fate of anthocyanins in vivo seems to be the glucuronidation. As example, delphinidin may have been preferentially glucuronidated (MILBURY et al., 2006). The probably enzymes involved are the phase II enzyme, UDP-glucuronosyltransferases (UGTs) (RECHNER et al., 2002).

Previous studies have shown that anthocyanin-rich diets provide significant anthocyanins bioavailability. As aforementioned, anthocyanins could be found as native, or even in their aglycone form (anthocyanidins) in the tissues because of the clivage of the glycosides forms in small intestine (PRIOR, 2003; TALAVERA et al., 2005; VANZO et al., 2011). Metabolites and anthocyanidins could also transit the blood-brain barrier and be found in brain tissue until 18 h after the last feeding. Methylated and glucuronidated anthocyanin conjugates might be synthesized in brain, or in the liver, kidneys and, then transported to brain tissue (MILBURY et al., 2006). However, this hypothesis was not yet proved. Figure 2 shows a C3G metabolism scheme.

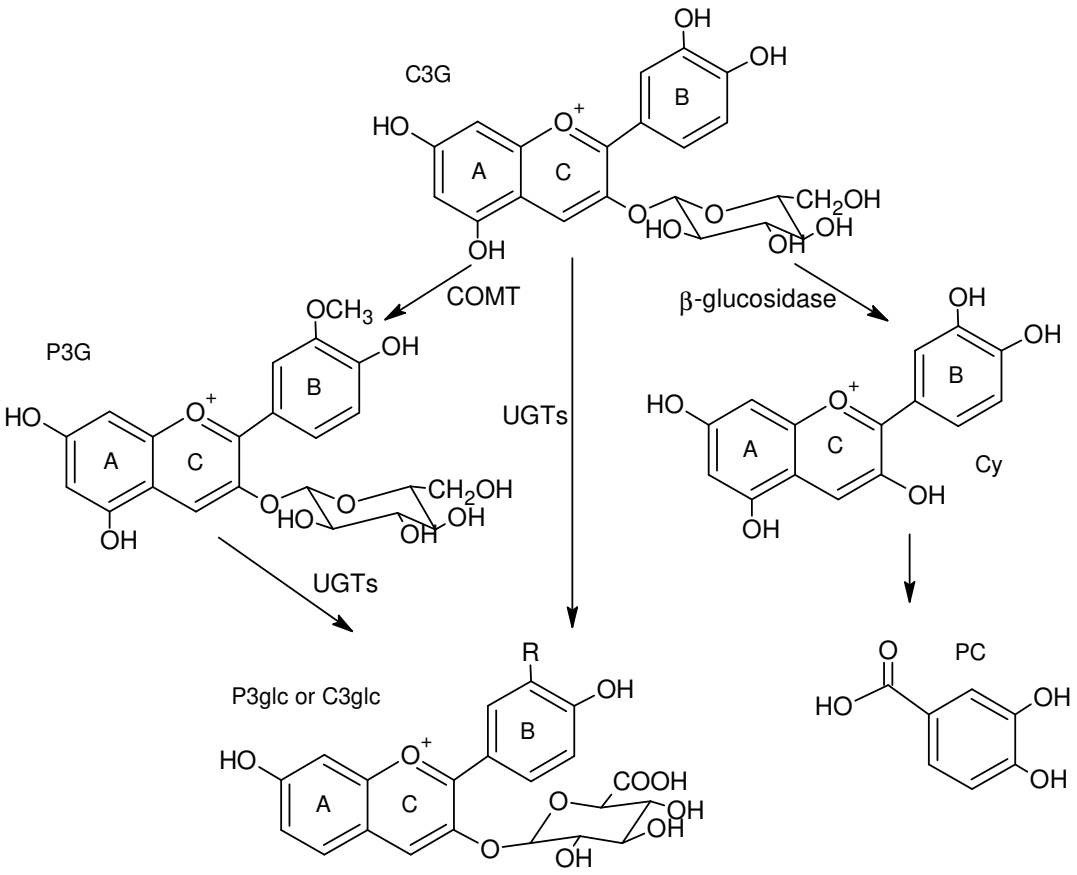

Figure 2 - Metabolism of cyanidin 3-glucoside (C3G). R= OCH_3 – peonidin 3-glucuronide (P3glc); R= OH - cyanidin 3-glucuronide (C3glc). When consumed orally, the C3G could be hydrolyzed by β-glucosidase in the small intestine, or absorbed as native form. When hydrolyzed, cyanidin (Cy) achieves blood stream, where it is strongly unstable. Cy molecules are also dissociated in procathecuic acid (PC) and other compounds. In liver, kidneys and possibly others tissues, C3G could be methylated in peonidin 3-glucoside (P3G) by catechol *O*-methyl transferase (COMT). C3G and P3G might be metabolized in C3-glc and P3-glc by UDP-glucuronosyltransferases (UGTs) (MILBURY et al., 2006; PRIOR, 2003; RECHNER et al., 2002; TSUDA et al., 1999).

In this way, given the animals tissues concentrations after anthocyanin-rich diets feeding, possibly, anthocyanins could interact with other molecules in signal transduction pathways (GUO et al., 2012), gene expression (TSUDA et al., 2000), scavenge free radicals, chelate metals (MLADENKA et al., 2010), ameliorate neuroinflammation, increase blood flow in brain, angiogenesis (WILLIAMS; SPENCER, 2012), and promote health effects. Although, the

oxidative stress-lowering effect is strongly correlated with the presence of anthocyanins and their metabolites in the tissues (MAZZA et al., 2002), this result will depend of their stability in different tissues, as well as the number and position of hydroxyls in the molecule as was described previously. A detailed in vivo functional effect of anthocyanins against obesity-induced oxidative stress in non-clinical studies is given on Table 1.

Clinical studies have also shown benefits of oral administration of anthocyanins against obesity-induced oxidative stress. First, a single-blinded crossover study with men (*n*= 8), receiving a diet containing 48.3% kcal fat, showed that the intake of freeze-dried wild blueberry powder (11.6 g kg^{-1} anthocyanins) dispersed in water (100g 500 mL^{-1}) promoted an increasing in serum antioxidant capacity: 8.5% $ORAC_{PCA,}$ and 4.5% ABTS assay at 1 h postprandial; and 15% at 4 h postprandial (KAY; HOLUB, 2002). Second, other single-blinded, crossover study with men (*n*= 5) fed a high-fat diet (49% kcal fat) and 0.2 g mL^{-1} blueberry powder supplemented water (25 different anthocyanins), promoted the bioavailability of 19 anthocyanins in the serum and an increased serum antioxidant capacity by $ORAC_{acetone}$ (MAZZA et al., 2002). Finally, a pilot and randomized, double-blinded, placebo-controlled, crossover study with women/ men (*n*=12), verified that the intake of 120 mL juice blend (0.47 µg mL^{-1} anthocyanins) was capable to increase the serum antioxidant capacity (2 h postprandial) and to reduce the liver TBARS levels (JENSEN et al., 2008).

In this context, the addition of fruits sources of these compounds in the diet could minimize and/ or control the obesity-induced oxidative stress. However, little is known about the possible mechanisms of action of these pigments as protective effect, nor what the minimum dose and frequency to be ingested to achieve satisfactory effects in combating chronic diseases. Another problem is the lack of non-clinical studies about toxicological effects; the concentrations used are often several orders of magnitude higher than the likely plasma concentration; and the mean presumable dietary intake in clinical studies are also unachievable.

Table 1 – Studies regarding anthocyanin oral administration and general benefits observed since 2000 (continues).

Experimental models	N	Treatment	Time	General results	Ref./ year
Wistar rats. Hepatic ischemia/reperfusion-induced oxidative stress.	4	Supplemented diet with 0.2% purified cyanidin 3-glucoside (>95%).	2 weeks	↓ liver and serum TBARS after 1h induction.	(TSUDA et al., 2000)
C57BL/6J mice. High-fat diet-induced oxidative stress (30% lard).	24	Supplemented high-fat diet with 2g kg^{-1} purple corn.	12 weeks	↓ liver lipids and triglycerides ↓ adipose tissue weight ↓ serum glucose, insulin, leptin ↓ leptin and TNF-α mRNA in adipose tissue ↓ mRNA level of lipogenic enzymes	(TSUDA et al., 2003)
C57BL/6J mice. High-fat diet-induced oxidative stress (60% kcal fat).	32	Supplemented high-fat diet with 0.1% *Cornus mas.* isolated anthocyanins	8 weeks	↓ 24% weight gain. ↓ insulin resistance. ↓ liver triglycerides.	(JAYAPRAKASAM et al., 2006)
Male F344/DuCrj rats. AIN93G diet.	20	Supplemented AIN-93G diet with 25% purple sweet-potato	4 weeks	↓ serum TBARS ↑ hepatic SOD mRNA	(HAN, K. H. et al., 2007)
Sprague-Dawley rats. High-fat diet-induced oxidative stress (16% lard).	32	Supplemented high-fat diet with 10% black soybean; and supplemented high-fat diet with 0.037% anthocyanins extract.	30 d	↓ weight gain. ↓ serum triglycerides, total cholesterol. ↑ serum HDL cholesterol.	(KWON et al., 2007)

Table 1 – Studies regarding anthocyanin oral administration and general benefits observed since 2000 (continues).

Experimental models	N	Treatment	Time	General results	Ref./ year
Pigs (Yorkshire x Landrace). 20% soya, oats and barley, 0.08% cholesterol and 9% fructose were added to diets.	24	Supplemented diet with 1.5% freeze-dried blueberry powder.	12 weeks	↓ 8% total cholesterol.	(KALT et al., 2008)
Sprague-Dawley rats. High-fat diet-induced oxidative stress (30% lard).	40	Supplemented diet with 5% grape peel.	4 weeks	↓ liver TBARS. ↑ serum antioxidant capacity (ABTS assay). ↑ hepatic G6Pase	(LEE, S. J. et al., 2009)
CBA/Hr mice. Commercial diet.	30	5 g per day of commercial food pellets containing 4 ml of 10% and 50% dilution of original cherry juice (CJ- rich in anthocyanins).	14 d	↓ liver TBARS in rats fed 10% CJ ↑ liver Gpx and blood SOD in rats fed both diet. Both treatment had anti-inflammatory properties by the inhibition of COX-2 activity.	(SARIC et al., 2009)
Kunming mice. D-galactose-induced aging	24	Sweet-potato extract, rich in anthocyanins (100 mg kg^{-1} d^{-1}).	12 weeks	↑ brain SOD (Cu/Zn) and CAT ↓ liver MDA ↑ neurocognition	(SHAN et al., 2009)
C57BL/6J mice. High-fat diet-induced oxidative stress (45% kcal fat).	36	Blueberry juice in place of drinking water	79 d	↓ serum leptin	(PRIOR et al., 2010)

Table 1 – Studies regarding anthocyanin oral administration and general benefits observed since 2000.

Experimental models	N	Treatment	Time	General results	Ref./ year
Golden Syrian hamsters. High-fat diet-induced oxidative stress (45% kcal fat).	40	Supplemented high-fat diet with 8% blueberry peel, or 2% dried blueberry peel extract (95% ethanol), or 6% blueberry peel byproduct.	3 weeks	↓ plasma VLDL and total cholesterol.	(KIM et al., 2010)
Wistar rats. AIN93M diet.	32	Supplemented AIN93M diet with 2 and 4% *M. jaboticaba*, rich in anthocyanins.	28 d	↑ plasma ORAC and ABTS	(LEITE et al., 2011)
Sprague-Dawley rats. High-fat diet-induced oxidative stress (31% lard)	32	Supplemented high-fat diet with 2 and 4% *M. jaboticaba*, rich in anthocyanins.	6 weeks	↑ HDL cholesterol ↓ HOMA-IR	(LENQUISTE et al., 2012)
Swiss mice. High-fat diet-induced oxidative stress (31% lard).		Supplemented high-fat diet with 2 and 4% *M. jaboticaba*, rich in anthocyanins.	6 weeks	↓ insulin resistance (kITT and improvement in insulin signal transduction). ↓ liver and adipose tissue IL-1β.	(DRAGANO et al., 2012)
C57BL/6J mice. High-fat diet-induced oxidative stress (58% kcal fat).	24	Supplemented high-fat diet with 0.2% cyanidin 3-glucoside (purity ≥98%)	5 weeks	↓ blood glucose and serum insulin. ↓ insulin resistance (iGTT, kITT and improvement in insulin signal transduction). ↓ 60% adipocytes death and macrophage infiltration. ↓ releasing of adipocytokines. ↓ serum and liver TNFα. ↓ hepatic steatosis.	(GUO et al., 2012)

5.2. BRAZILIAN BERRIES AND OTHER SOURCES OF ANTHOCYANINS

Blueberries, blackberries, strawberries, purple corn, purple potatoes, black soybeans, raspberries, red currants, gooseberries and cornelian cherries are all good sources of anthocyanins with reported functional effects and well-known around the world (KAHKONEN; HOPIA; HEINONEN, 2001; PANTELIDIS et al., 2007; PRIOR et al., 2008).

Brazil possesses a notable biodiversity and exotic fruits with extraordinary bioactive compounds. Along with several Brazilian anthocyanin-rich fruits, is açai, a wild berry from the palm species of *Euterpe oleracea* of the Amazon Forest region. Açai is already worldwide known and its consumption is related with antioxidant status improvement, hypocholesterolemic effect and anti-inflammatory activity (KANG et al., 2012; MERTENS-TALCOTT et al., 2008).

Bacaba (*Oenocarpus bacaba*) is also a palm tree from the Brazilian Amazon that provides purple-colored berries, rich in antioxidant compounds, such as cyanidin 3-glucoside. Few studies about bacaba are provided, but there is evidence that its phenolic extract played an antiproliferative role in cancer cells (FINCO et al., 2012).

Other highlighted palm tree is *Euterpe edulis* or jussara, whose berry fruits provide 1.3 g $100g^{-1}$ cyanidin 3-glucoside and 1.5 g $100g^{-1}$ cyanidin 3-rutinode. *Syzygium cumini,* is a berry that also shows compounds of interest, as delphinidin 3,5-diglucoside (256 mg 100 g^{-1}) and petunidin 3,5-diglucoside (245 mg 100 g^{-1}) (BRITO et al., 2007). The *S. cumini* are related to inhibition of hepatic lipid peroxidation and increase in liver GSH level and GST, SOD and CAT activities. *S. cumini* also possesses potent antioxidant and antidiabetic activity, possibly due to the antioxidant phytochemicals present in the fruit (ARUN et al., 2011).

Myrciaria jaboticaba (Vell.) Berg. or jaboticaba sabará is other native plant from South America that produces globose fruits with a deep purple peel and white sweet pulp, known as Brazilian berry. Its popularity in Brazil is comparable with to grapes in Europe or the USA. Although not extensively consumed, the peel of the fruits concentrates the major amount of polyphenols such as ellagic acid, ellagitannins, quercetin, anthocyanins and have an expressive antioxidant activity (Table 2) (ABE; LAJOLO; GENOVESE, 2012; LEITE-LEGATTI et al., 2012; LIMA et al., 2011). The major anthocyanins found in jaboticaba peel is cyanidin 3-glucoside and delphinidin 3-

glucoside (approximately 2 g $100g^{-1}$ of total anthocyanins) (LEITE-LEGATTI et al., 2012).

Previous reports have been shown that 2% jaboticaba peel in the diet could play important in vitro and in vivo functional effects: High trolox equivalents content, antiproliferative effects against cancer cells, antimutagenic activity (LEITE-LEGATTI et al., 2012), an increased plasma antioxidant capacity (LEITE et al., 2011), serum HDL-cholesterol enhancement, less insulin resistance (LENQUISTE et al., 2012), anti-inflammatory effects in liver and adipose tissue (DRAGANO et al., 2012), and other effects not yet published.

Table 2 – Bioactive compounds of freeze-dried jaboticaba peel.

Chemical and bioactive compounds	Amount	Ref.*
Cyanidin 3-glucoside	1963.57 mg $100g^{-1}$	1
Delphinidin 3-glucoside	634.75 mg $100g^{-1}$	1
Total ellagic acid	22.5 to 43.95 g kg^{-1}	2
Quercetin	0.0056 g kg^{-1}	2
Total phenolic compounds**	556.3 g GAE kg^{-1b}	1
Total tannins	48 to 211 g kg^{-1}	2
ORAC anti-radical activity	25514.24 µmol TE g^{-1d}	1
Insoluble Fibers	20.00%	1
Soluble Fibers	5.00%	1
Vitamin C	298.23 mg $100g^{-1}$	3
Limonene and terpenes	-	4

*References: [1](LEITE-LEGATTI et al., 2012), [2] (ABE et al., 2012), [3](LIMA et al., 2011), [4](PLAGEMANN, 2012). **Folin Ciocalteau method.

6. CONCLUSIONS

Oxidative stress in obesity is a link for other chronic diseases. Polyphenols and anthocyanin-rich extracts or diet are strongly linked with reduced obesity-induced oxidative stress. Although, the minimum and maximum doses to be effective on the

improvement of antioxidant defense of cells, lose weight, and amelioration of insulin resistance are not a consent. Toxicological and clinical trials are needed to ensure the role of anthocyanins in physiological and pathology processes without adverse effects. The knowledge about plant species that provides anthocyanins in different regions is one way to better facilitate and guarantee the access to the population to these medicinal foods. The jaboticaba and açai are wild berries more common in Brazil than the others, and they show important in vitro and in vivo antioxidants properties that deserve attention of the health scientific researchers.

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CHAPTER 2:

BERRY PEEL (*MYRCIARIA JABOTICABA* (VELL.) BERG.) INTAKE IMPROVED TRIGLYCERIDES EXCRETION, BUT DID NOT AMELIORATED LIPID HEPATIC PROFILE IN HIGH-FAT-FED RATS

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Authors: Ângela Giovana Batista[1], Sabrina Alves Lenquiste[1], Carolin Moldenhauer[2], Juliana Teixeira Godoy[1], Soely Maria Pissini Machado Reis[1], Mário Roberto Maróstica Júnior[2].

[1]School of Food Engineering, University of Campinas (UNICAMP), P.O. Box 6121, 13083-862 Campinas-SP, Brazil.

[2] Christian-Albrechts-University - Faculty of Agricultural and Nutritional Science, 24098 Kiel, Germany.

ABSTRACT

Jaboticaba peel is known by its high polyphenols, anthocyanins and dietary fibers contents. The aim of this study was to evaluate the influence of high-fat diets added 1, 2 and 4% freeze-dried jaboticaba peel (FJP) on serum, liver and feces lipids profile of obese rats. Thirty *Sprague-Dawley* male rats were divided into 5 groups. Obesity was induced on four groups using a high-fat diet (35% lipids). Three of these groups received the same high-fat diet, each one added to 1, 2 and 4% FJP (J1, J2 and J4, respectively) in the last 40 experimental days. One group was used as high-fat fed control (C). Blood and liver were collected after 40 days of treatment and feces were collected in the last experimental week. The total cholesterol and triglycerides were evaluated in serum and liver and fecal total lipids were evaluated as well. In the second period of the experiment, J4 food intake was higher than the C group. There was no significant difference among experimental groups for total serum cholesterol and triglycerides levels. The J2 group showed the major hepatic and fecal lipids levels. The J4 showed the higher mean of hepatic and fecal cholesterol. The hepatic triglycerides showed no significant differences between groups, and the J1 and J4 demonstrated the higher values regarding triglycerides excretion via feces. The jaboticaba peel doses used did not show beneficial effects against hepatic steatosis or other lipid parameter evaluated, except for fecal triglycerides.

Keywords: *Myrciaria jaboticaba* (Vell.) Berg.; cholesterol; triglycerides, lipid profile, obesity.

1. INTRODUCTION

Myrciaria jaboticaba (Vell.) Berg. is a native plant from South America and produces globose fruits with a deep purple peel and white sweet pulp, known as Brazilian berry. Although not extensively consumed, the fruit peel concentrates the major amount of polyphenols such as ellagic acid, quercetin, anthocyanins and have an expressive antioxidant activity (ABE; LAJOLO; GENOVESE, 2012; LEITE-LEGATTI et al., 2012; LIMA, A. D. B. et al., 2011).

The polyphenol/ anthocyanin-rich diets have been related to the reduction of cardiovascular diseases (CVD) because their interference in the regulation of lipid metabolism (ESTEVES et al., 2011; KIM et al., 2010; LENQUISTE et al., 2012). There are reports that these diets could reduce total cholesterol, triglycerides blood levels (KWON et al., 2007) and increase the HDL-cholesterol levels in obese animals (LENQUISTE et al., 2012). The mechanism underlying these findings might be linked with the ability of these diets in increasing billiary cholesterol excretion and fecal excretion of lipids as well; reducing plasma lipids levels (KIM et al., 2010). Regarding the influence of anthocyanins in expression of hepatic enzymes, it is reported that cyanidin 3-glucoside, a powerful anthocyanin, suppressed those involved in the fatty acids and triglycerides synthesis. In this way, β-oxidation could be increased in liver and fatty acid synthesis is reduced. These mechanisms might also be associated with weight and fat loss in obese rats as well (JAYAPRAKASAM et al., 2006; PRIOR et al., 2008; TSUDA et al., 2003).

The jaboticaba peel is also a rich source of dietary fibers (LEITE-LEGATTI et al., 2012; LIMA, A. J. B. C., A. D.; ALVES, A. P. C.; ABREU, C. M. P.; DANTAS-BARROS, A. M. , 2008), nutrients with hypocholesterolemic role. Polyphenols and fiber-rich diets are capable to reduce total cholesterol and LDL-cholesterol (ESTEVES et al., 2011; KALT et al., 2008), suggesting that both substances might have synergistic role on blood lipids regulation. Moreover, diets supplemented with 1 and 2% of freeze-dried jaboticaba peel (FJP) improved antioxidant capacity in rats plasma, which indicates the potential of the compounds of FJP in attenuate oxidative stress (LEITE et al., 2011) and related damages (AVIRAM; FUHRMAN, 2002).

The previous ‘in vivo’ investigations that studied the administration of 1, 2 and 4% FJP in diets of obese animals reported that the ingredient was not effective in the reduction of energy intake, weight gain and body fat (MARQUES et al., 2012).

However, the obese animals that received diets added with 2 and 4% FJP showed increased HDL-cholesterol and reduced insulin levels (LENQUISTE et al., 2012). Possibly, changes in hepatic lipids contents and their excretion may was involved in this process. In this way, the aim of this study was evaluate the influence of high-fat diets added 1, 2 and 4% freeze-dried jaboticaba peel (FJP) on the lipids profile of serum, liver, feces and fecal pH of obese rats.

2. MATERIAL AND METHODS

2.1. ANIMAL, DIETS AND SAMPLES

Thirty weaned male *Sprague-Dawley* (SD) rats, weighting 58 ± 18.77 g were obtained from the Multidisciplinary Center for Biological Research at Unicamp (CEMIB). This experiment was approved by the Ethics Committee on Animal Experiments (CEEA / UNICAMP), protocol #2226-1, and followed all the ethical requirements of the Brazilian College of Animal Experimentation (COBEA). The animals were randomly assigned into five groups (n= 6) and remained at individual cages with food and water under the system of free access, temperature (22 ± 1 °C) and humidity (60 - 70%) controlled and light/ dark cycle of 12 h, throughout the experimental period.

Two control diets were given during the experiment: a normolipidic control diet (N), prepared in accordance with the American Institute of Nutrition (REEVES; NIELSEN; FAHEY, 1993), AIN-93G, with protein concentration modified to 12%; and a high-fat control diet (C): AIN 93G-modified with 12% protein and 35% fat: 4% vegetable oil (soybean) and 31% lard. Beyond the control diets, three experimental diets were given: high-fat diet added with freeze-dried jaboticaba peel (FJP) in three different concentrations (1, 2 and 4% w w^{-1}). Adjustments were done in order to get diets with same contents of calories and fibers (Table 1). Normal group (N) received normolipidic diet and control group (C), a high-fat diet throughout the experiment; groups J1, J2 and J4 received the high-fat diet during the first 4 weeks and diets added with FJP until the end of 10 weeks. Diet consumption was monitored every 2 days and weight gain once a week.

Table 1 - The composition of experimental diets (g kg^{-1}).

	N	C	J1	J2	J4
Energy (kcal kg^{-1})*	4252	5834	5809	5772	5784
Casein (78% protein)	153.85	153.85	153.85	153.85	153.85
Corn starch	426.63	249.82	249.82	249.82	249.82
Maltodextrin	141.68	82.91	82.91	82.91	82.91
Sucrose	107.33	62.92	62.92	62.92	62.92
Soybean oil	70.00	40.00	40.00	40.00	40.00
Lard	-	310.00	310.00	310.00	310.00
Cellulose	50.00	50.00	47.50	45.00	40.00
Mineral mix**	35.00	35.00	35.00	35.00	35.00
Vitamin mix**	10.00	10.00	10.00	10.00	10.00
L-Cystine	3.00	3.00	3.00	3.00	3.00
Choline bitartrate	2.50	2.50	2.50	2.50	2.50
Freeze-dried jaboticaba peel	-	-	10.00	20.00	40.00

* Energy values determined using Isoperibol Calorimeter 1261 instrument equipped with 1108 oxygen bomb (Parr Instrument Co, Moline, IL).**Reeves et al. (REEVES et al., 1993). N= normal diet (AIN-93G) group; C= high-fat control diet group; J1= high-fat diet + 1% freeze-dried jaboticaba peel (FJP); J2= high-fat diet + 2% FJP; and J4= high-fat diet + 4% FJP.

FJP is a powder obtained from freeze-drying process of *M. jaboticaba* peels, as described in a previous work (LEITE-LEGATTI et al., 2012) kindly provided by 'Bioactive Compounds, Nutrition and Health' researcher group. The FJP powder contains compounds of interest (soluble fibers 5%, insoluble fibers 20%, total polyphenols 556.30 g GAE kg^{-1}, delphinidin-3-*O*-glucoside 634.75 mg 100 g^{-1}, cyanidin-3-*O*-glucoside 1963.57 mg 100 g^{-1}) (LEITE-LEGATTI et al., 2012).

Blood was obtained from fasted rats (12 h) by decapitation. Blood samples were collected in appropriated tubes and centrifuged at 4000 rpm for 20 min. Serum was collected and stored at –80 °C until analyses. Liver was collected, washed, frozen and

dried in a freeze-dryer (LP1010, Liobras, São Carlos, São Paulo, Brazil). Liver was manually triturated and kept at -80 °C until analyses. The feces of the rats were collected in the last 7 d of experiment and dried in an oven, with forced air circulation at 65 °C for 24 h; and then manually triturated and kept at - 20 °C until analyses.

2.2. BIOCHEMICAL ANALYSES

Total lipids. The lipid content of liver analyzed using the Bligh and Dyer method (BLIGH, 1959) and the total lipid of feces were extracted using Soxhlet apparatus and petroleum ether (AOAC, 1995).

Cholesterol and triglycerides analyses. The lipid content of liver and feces were extracted using the method described by Folch (FOLCH, 1957). Chloroform: methanol (2:1) were added to the samples, mixed and sonicated. Thus, an aliquot of methanol was added and then centrifuged at 21,036 g for 10 min. The Folch solution was added to the supernatant, which was washed 3 times, dried in an oven at 37 °C and resuspended with isopropanol for the analyses. Total cholesterol and triglycerides contents on serum and extracts were determined using enzyme assay kits according to manufacturer's instructions (Laborlab, São Paulo, Brazil).

Fecal pH. The dried feces were diluted with deionized water (25 mg mL^{-1}), homogenized and the pH was measured in the solution under agitation using a pHmeter (Tecnal model TEC-5, Piracicaba, SP, Brazil) (ESTEVES et al., 2011).

2.3. STATISTICAL ANALYSES

The statistical analyses of body weight, weight gain and food intake data were done using two-way ANOVA and Bonferroni test, *a posteriori* ($P< 0.05$). The statistical analyses of lipids profile, fecal weight and pH were made as follows: parametric data were based on one-way ANOVA followed by a Tukey multiple comparisons test. Non-parametric data was submitted to a Kruskal-Wallis and Dunn multiple comparisons test. The limit of significance was set at $P< 0.05$. Parametric results were expressed as means ± standard error (SEM) and non-parametrics were reported as means. Statistical analyses were carried out using Statistica 7.0 (StatSoft Inc., Tulsa, USA) and GraphPad Prism 5.0 (GraphPad Software, Inc. La Jolla, CA, USA) softwares.

3. RESULTS

Serum and liver were collected after 40 days of diet treatment and feces in the last week of experiment. The lipid profile of samples was analyzed. As detailed below, the 1 and 4% jaboticaba peel addition in the diet improved triglycerides excretions, although no changes were observed in liver or serum.

Body weight, food intake and fecal parameters. There was no statistical difference among the experimental groups ($P > 0.05$) concerning total weight gain (117.6 to 192.8 g) and total food intake (650.47 to 745.16 g). However, in the second period of the experiment (last 6 weeks), food intake of the J4-fed animals (441.02 g) was higher than the C group (378.84 g). In detail, the animals treated with high-fat diet showed higher body weight values since the 5th experimental week ($P < 0.001$, Figure 1.A), weight gain was similar in the second period of the experiment ($P > 0.05$, Figure 1.B) and the food intake was increased in J4-fed animals relative to C group on the last week ($P < 0.05$, Figure 1.C).

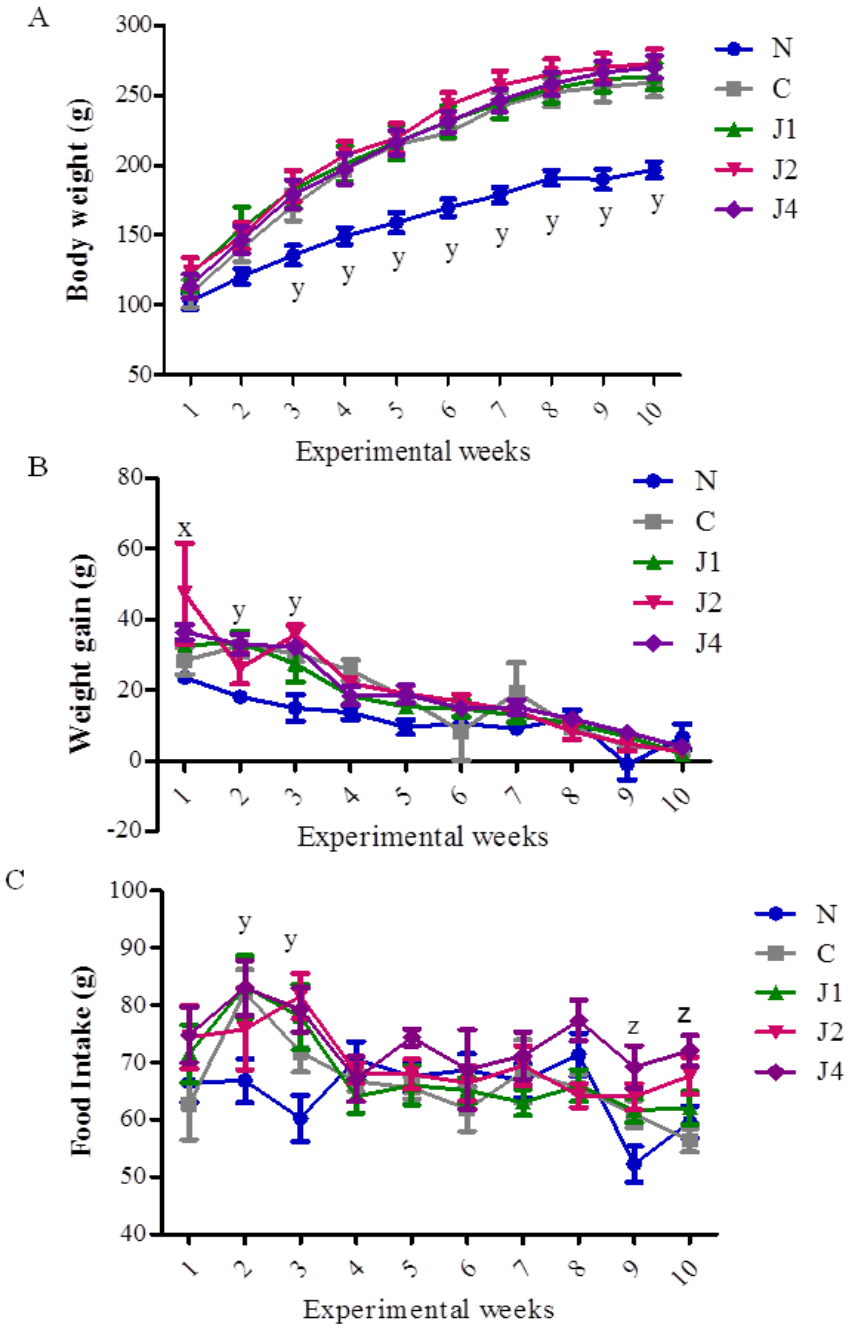

Figure 1 - Body weight and food intake. Body weight (A), weight gain (B) and food intake (C) in the 10 experimental weeks. N= normal diet (AIN-93G) group; C= high-fat control diet group; J1= animals fed high-fat diet added 1% freeze-dried jaboticaba peel (FJP); J2= animals fed high-fat diet added 2% FJP; and J4= animals fed high-fat diet added 4% FJP. Data were analyzed by two-way ANOVA and Bonferroni tests; y= N group were different among the experimental groups, x=J2 and Z=J4 ($P< 0.05$). All data were expressed as the mean ± SEM ($n= 6$).

The J2 and J4 animals showed high fecal excretion in dry weight on last week (Figure 2.A). The fecal pH of C, J1 and J2 group were lower compared to N group (P< 0.001). The J2-fed animals showed the lowest levels of fecal pH (Figure 2.B).

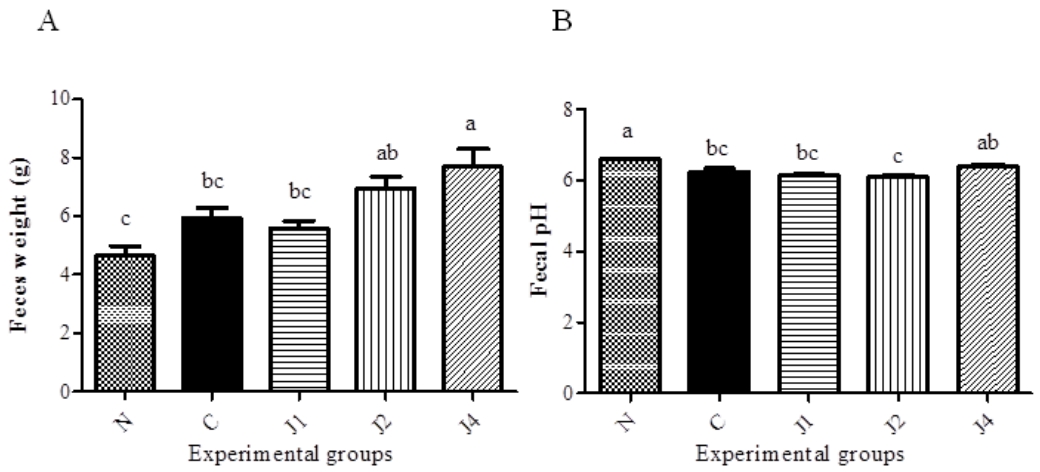

Figure 2 - Fecal parameters. (A) Feces weight in dried basis, and (B) fecal pH of the last experimental week. N= normal diet (AIN-93G) group; C= high-fat control diet group; J1= animals fed high-fat diet added 1% freeze-dried jaboticaba peel (FJP); J2= animals fed high-fat diet added 2% FJP; and J4= animals fed high-fat diet added 4% FJP. Data was analyzed by one-way ANOVA and Tukey test; different letters (a, b, c) represents statistical difference among the experimental groups (P< 0.05). All data were expressed as the mean ± SEM (n= 6).

Serum analyses. There was no significant differences (P> 0.05) among experimental groups for total serum cholesterol (69.24 to 80.38 mg dL^{-1}) and serum triglycerides levels (39.67 to 49.49 mg dL^{-1}).

Total lipid analyses. The high-fat diet-fed animals showed higher hepatic lipid contents, especially the J2 group, which showed the highest means relative to N. As expected, the lipid excretion was numerically increased in all high-fat groups and J2 showed the higher values, statistically different from the N group and similar to the C group (Figure 3).

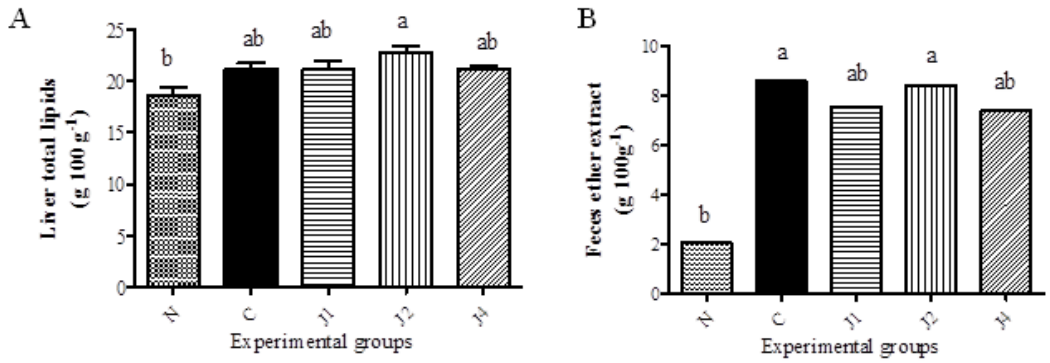

Figure 3 - Hepatic and fecal lipids contents of the experimental animals. A) Liver total lipids; B) feces ether extract. N= normal diet (AIN-93G) group; C= high-fat control diet group; J1= animals fed high-fat diet added 1% FJP; J2= animals fed high-fat diet added 2% FJP; and J4= animals fed high-fat diet added 4% FJP. Different letters in columns represents statistical difference among the experimental groups. The parametric data (ANOVA and Tukey tests) were expressed as the mean ± SEM (n= 6); P< 0.05.

Liver and feces cholesterol analyses. Differently from N, J4 showed the higher mean for hepatic and fecal cholesterol similar to group C, although, hepatic cholesterol levels of the J1 and J4 groups tended to lower values (Figure 4.A and 4.B). Except for J2, the high fat groups also excreted more cholesterol than N (P< 0.05).

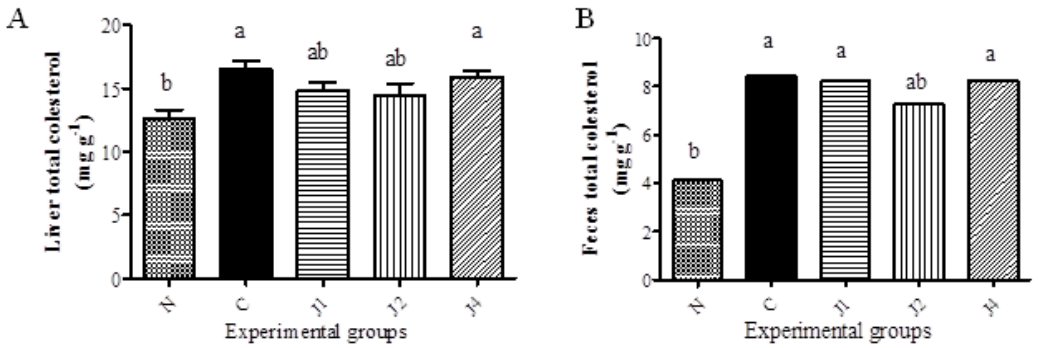

Figure 4 - Hepatic and fecal cholesterol contents of the experimental animals. A) Liver total cholesterol; B) feces total cholesterol. N= normal diet (AIN-93G) group; C= high-fat control diet group; J1= animals fed high-fat diet added 1% FJP; J2= animals fed high-fat diet added 2% FJP; and J4= animals fed high-fat diet added 4% FJP. Different letters in columns represents statistical difference among the experimental groups. The parametric data (ANOVA and Tukey tests) were expressed as the mean ± SEM; the non-parametric data (Kruskal-Wallis and Dunn tests) were expressed as the mean (n= 6); $P< 0.05$.

Triglycerides analyses. The hepatic triglycerides showed no significant differences ($P>$ 0.05) among groups, but the absolute values showed that the liver of high-fat-fed animals concentrated more triglycerides than N (Figure 5.A). The J1 and J4 demonstrated the higher values regarding triglycerides excretion via feces ($P<$ 0.05), and the J4 values were similar to the N group (Figure 5.B).

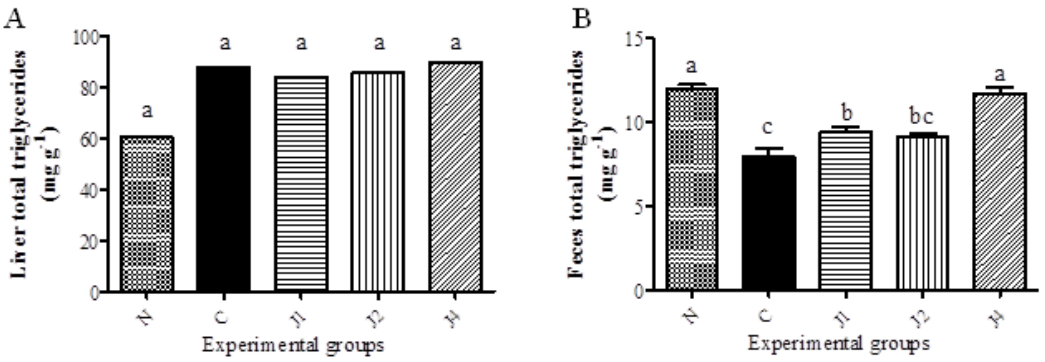

Figure 5 - Hepatic and fecal triglycerides contents of the experimental animals. A) Liver total triglycerides; B) feces total triglycerides. N= normal diet (AIN-93G) group; C= high-fat control diet group; J1= animals fed high-fat diet added 1% FJP; J2= animals fed high-fat diet added 2% FJP; and J4= animals fed high-fat diet added 4% FJP. Different letters in columns represents statistical difference among the experimental groups. The parametric data (ANOVA and Tukey tests) were expressed as the mean ± SEM; the non-parametric data (Kruskal-Wallis and Dunn tests) were expressed as the mean (n= 6); P< 0.05.

4. DISCUSSION

This study indicates that *M. jaboticaba* peel intake could have enhanced fecal triglycerides excretion in obese rats, but the hepatic and serum lipid profiles were not reduced. The FJP powder contains bioactive compounds that could be responsible for these findings, as dietary fibers and polyphenols (LEITE-LEGATTI et al., 2012). Although, the diets used in our experiment possessed the same fiber content, the differences observed might be linked to the proportion of soluble fibers associated with the FJP addition, or with the amount of polyphenols and/ or substances not yet known. Moreover, the lard added to the high-fat diets is characterized by high amounts of saturated fatty acids, which could harm the lipid metabolism in rats, increasing skeletal muscle fat accumulation, cholesterol esters and triglycerides concentration in the liver, blood, and decreasing the concentration of HDL-cholesterol in blood. In addition, the saturated fats are more efficiently stored in the adipose tissue, which leads to conclude that lard in this work was the main responsible for the obesity induction (CASTRO et al., 2012; ZIVKOVIC; GERMAN; SANYAL, 2007).

Regarding serum lipids, some studies with high-fat-fed rodents treated with anthocyanins sources have pointed out controversial results (KWON et al., 2007; PRIOR et al., 2010; TSUDA et al., 2003). Kwon et al. showed that SD rats fed 10% black soybean or 0.037% anthocyanins extract showed reduced serum total cholesterol and triglycerides levels (KWON et al., 2007). However, studies with obese mice treated with anthocyanin-rich drink or diet corroborated our data (PRIOR et al., 2010; TSUDA et al., 2003). Some investigations have reported that polyphenols have reduced total cholesterol in animals fed atherogenic diets (KALT et al., 2008; KIM et al., 2010), or even in healthy animal studies (ESTEVES et al., 2011; SOUZA et al., 2010). In a previous study, J2 and J4 diets were capable to increase HDL-cholesterol (LENQUISTE et al., 2012). Thus, even total-cholesterol levels in FJP-fed animals were not reduced; an increase in HDL-cholesterol, as shown in a previous study, could be related to a reduced risk of CVD (AVIRAM; FUHRMAN, 2002).

The hepatic steatosis and fecal steatorrhea were expected in high-fat-fed animals and the FJP ingestion was not capable to reduce the total lipid values in liver or to increase its excretion. Studies have shown that different concentrations of anthocyanins could minimize the lipid content in liver but no changes were shown in feces (8% blueberry peel and 2 mg kg^{-1}) (KIM et al., 2010; TSUDA et al., 2003). Although the hepatic lipids content of J2-fed animals were higher in comparison with N, previous studies showed that the lipid peroxidation index was the opposite (see chapter 3). In this way, the addition of 2% FJP in the high-fat diet might have protected the liver against oxidative stress-induced obesity (LEE; CHOI; SEO, 2009).

Scientific reports have been shown that anthocyanins oral administration did not interfere significantly in hepatic cholesterol levels in animals fed high-fat diets (JAYAPRAKASAM et al., 2006; KIM et al., 2010). In this work, the J4 and C groups demonstrated the highest values of hepatic cholesterol. However, this data could imply that FJP might have a protector effect, since J4 showed high food intake values in comparison with C. Concomitantly, J4 ingested more fat and showed no proportional damages.

Commonly, anthocyanin-rich diets are capable to reduce hepatic triglycerides levels in high-fat-fed animals (JAYAPRAKASAM et al., 2006; TSUDA et al., 2003), although some work showed controversial results (KIM et al., 2010). We found no significant differences relative to hepatic triglycerides among the experimental groups,

but the absolute values were higher in high-fat-fed animals. On the other hand, FJP diets improved the triglycerides excretion of rats fed J1 and J4 diets. The triglycerides levels of J4 obese animals were comparable to the healthy animals receiving N.

The dry weight of the fecal output was increased in J2 and J4-fed animals, probably because of their highest food intake in the last week. Though, there is an evidence that the suitable proportion of dietary fibers (soluble and insoluble) is associated with functional effects, such as cholesterol-lowering and blood glucose control. Soluble fibers, mainly, are capable to complex dietary constituents, which may drag them in larger amounts, corroborating to high fecal weight (BATISTA et al., 2011). Therefore, it might be inferred that the inclusion of FJP in the diets was capable to influence nutrient up-take, such as lipids.

Curiously, the J2-fed animals showed the lowest fecal pH values. As observed in the lipids excretion profile data (Figure 3), the increased fatty acid content of feces might be responsible for this result. Another hypothesis is that soluble fiber and polyphenols content of FJP may favor the production of short chain fatty acids (SCFA) by gut microbiota. A greater increase in SCFA production and potentially a greater delivery of SCFA, specifically butyrate, to the distal colon may result in a protective effect. Moreover, the production of propionate (another SCFA) enhances the inhibition of cholesterol synthesis by liver (WONG et al., 2006). Thus, the combination of fecal lipid content and the ideal concentrations of polyphenols and dietary fibers in the diet might be responsible for the lowest fecal pH showed by the J2 animals in relation to the other groups.

4. CONCLUSION

The addition of 1, 2 and 4% FJP in the high-fat diets was not capable to decrease hepatic and serum cholesterol and triglycerides levels of obese animals. The triglycerides excretion via feces was improved in animals fed 1 and 4% FJP. Concomitantly, the fecal pH values were decreased in animals fed the J2 diet, possibly due the increased fecal lipids content and a great balance between polyphenol and dietary fibers contents. Thus, more studies considering different fat concentrations in diets and longer treatment with FJP could better define its role on changes in lipid profile of obese rats.

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CHAPTER 3:

INTAKE OF JABOTICABA BERRY PEEL ATTENUATES OXIDATIVE STRESS IN TISSUES OF RATS WITH HIGH-FAT INDUCED OBESITY

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Ângela Giovana Batista[a], Sabrina Alves Lenquiste[a], Cinthia Baú Betim Cazarin[a], Juliana Kelly Silva[a], Anderson Luiz Ferreira[b], Mário Roberto Maróstica Júnior[a].

[a]School of Food Engineering, University of Campinas (UNICAMP), P.O. Box 6121, 13083-862 Campinas-SP, Brazil.

[b] Biology Institute. University of Campinas (UNICAMP). Campinas-SP, Brazil.

ABSTRACT

The antioxidant status of rats fed high-fat diets containing different doses of freeze-dried jaboticaba peel (FJP) was evaluated. Obesity was induced in four groups using a high-fat diet (35% fat). Three of these groups received the same diet with 1, 2 and 4% FJP added (J1, J2 and J4, respectively). The plasma trolox equivalents (TEAC) were increased in the J2 and J4-fed animals. The antioxidant enzyme parameters also indicated a protective effect against oxidative stress in the plasma. All the FJP diets prevented lipid peroxidation in the liver as induced by a high-fat diet, and increased its antioxidant defenses. The brain exhibited a dependent dose response: lipid peroxidation decreased with an increasing FJP content in the diet. The antioxidant status of the kidneys of J2 and J4-fed animals increased. Thus, FJP contains bioactive compounds and could be a natural alternative to minimize the oxidative stress-induced damage arising from obesity.

Key words: *Myrciaria jaboticaba* (Vell.) Berg; obesity; antioxidant defenses; lipid peroxidation

1. INTRODUCTION

Overweight and obesity have become major public health problems, and the prevalence of related chronic diseases is increasing throughout the world (WHO, 2012). Obesity may involve a state of chronic oxidative stress, which could be an important factor underlying the development of related co-morbidities (VINCENT; INNES; VINCENT, 2007).

Oxidative stress happens when the generation of reactive oxygen species (ROS) increases and the antioxidant defense of the cell is insufficient to repair the oxidative damage. An excess of ROS, over-produced in obesity, can harm proteins, lipids, nucleic acids and cause cell damage and even death. In addition, obesity impairs the enzyme antioxidant system, with depletion of the superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPx) activities, and also impairs in non-enzymatic oxidative system, consisting of the reduced thiol (GSH), vitamins and minerals (NOEMAN; HAMOODA; BAALASH, 2011; VINCENT et al., 2007)

Dietary nutrients and specific foods, rich in polyphenols (e.g. anthocyanins, flavonols), could play an important role in the prevention and control of complications arising from oxidative stress, increasing the circulation of antioxidant compounds (LEITE et al., 2011; LENQUISTE et al., 2012). They are capable of neutralizing reactive species, when metabolized in the tissues, due to their favorable number of hydroxyls and their position (MAZZA et al., 2002; PRIOR, 2003; VANZO et al., 2011). Moreover, there is evidence that anthocyanin-rich diets could decrease weight gain and the diseases resulting from a gain in body fat (TSUDA et al., 2003).

Animals or humans treated with anthocyanin sources showed an increase plasma antioxidant capacity and improved tissue lipid peroxidation (LEE, S. J.; CHOI; SEO, 2009; LEITE et al., 2011; MAZZA et al., 2002). Moreover, anthocyanins could influence cell signaling intermediaries (GUO et al., 2012) and increase the antioxidant enzyme activities (GPx, SOD and CAT) in tissues such as the brain and liver of animals fed anthocyanin-rich diets (LEE, S. J. et al., 2009; SHAN et al., 2009).

Myrciaria jaboticaba (Vell) Berg., popularly known as jaboticaba, is a Brazilian berry with a deep purple peel. Freeze-dried jaboticaba peel (FJP) shows considerable polyphenol and anthocyanin contents with important *in vivo* antioxidant properties (LEITE et al., 2011). Cyanidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside are the

major anthocyanins present in FJP and could enhance the antioxidant power of diets (LEITE-LEGATTI et al., 2012). Moreover, recent studies have reported that jaboticaba fruit contains high contents of ellagic acid, ellagitannins, significant amounts of quercetin (ABE; LAJOLO; GENOVESE, 2012) and some volatile compounds (PLAGEMANN, 2012) that could contribute to its antioxidant power.

Some studies attempted to elucidate the action mechanisms, and the protective and dose-response effects of the aforementioned bioactive compounds, but the results were inconclusive. It is possible that the metabolism of phenolic compounds, may influence their bioactivities and properties *in vivo* (PRIOR, 2003; TALAVERA et al., 2005). The jaboticaba fruit has been little explored from a scientific point of view, especially regarding to its chemical constituents and *in vivo* functional effects, and the present study is the first to investigate the effects of jaboticaba peel on oxidative stress indicators. The authors hypothesized that the intake of different FJP concentrations could protect the tissues against oxidative stress in obese rats.

2. MATERIAL AND METHODS

2.1. CHEMICALS AND BIOCHEMICALS

Reduced l-glutathione, Glutathione reductase, disodium salt of oxidized glutathione, β-nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate (NADPH), hypoxanthine, xanthine oxidase, nitrotetrazolium blue chloride (NTB), 5′5′-dithio-bis-2-nitrobenzoic acid (DTNB), bovine serum albumin (BSA), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS); (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (TROLOX); 2,2′-azobis(2-methylpropionamidine) dihydrochloride (APPH), 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ), and 2-thiobarbituric acid (TBA) were all obtained from Sigma–Aldrich (São Paulo – Brazil). Fluorescein sodium salt and metaphosphoric acid were purchased from Vetec Química Fina (São Paulo – Brazil). The malondialdehyde standard (MDA #10009202) was purchased from Chayman Chemical Company (Michigan, USA).

2.2. RAT STUDY

2.2.1. ANIMAL AND DIETS

Thirty weaned male *Sprague-Dawley* rats were used in this study following all the ethical recommendations, and the protocol (#2226-1) was approved by the UNICAMP Ethics Committee, Brazil. The rats were housed under conditions of constant temperature (22 °C ± 2), relative humidity (60 – 70 %) and a standard dark cycle (19 – 07 h),and randomized into five groups of 6 animals each: a control group (N or normal), fed a semi-purified diet for 10 weeks (REEVES; NIELSEN; FAHEY, 1993); a high-fat control group (C), fed a high-fat diet for 10 weeks; and the FJP groups (J1, J2 and J4), which were fed the high-fat diet for the first 4 weeks and then the high-fat diet added 1, 2 and 4% FJP, respectively, for the following 6 weeks. The ingredients used in the normal diet (N) were: casein (15.4%); corn starch (42.66%); maltodextrin (14.17%); sucrose (10.73%); cellulose (5.0%); soy oil (7.0%); mineral mix (3.5%), vitamin mix (1.0%); L-cystine (0.3%); and choline substrate (0.25%) (REEVES et al., 1993). The ingredients used in the control diet (C) were corn starch (24.98%); maltodextrin (8.29%), sucrose (6.29%); soybean oil (4.0%); and lard (31%). The groups denominated J1, J2 and J4 were fed the C diet added 1, 2, and 4% FJP, respectively. The amount of cellulose was adjusted to 4.75, 4.5, and 4.0% for the same groups. The rats were given free access to water and food. The weight gain and diet intake were determined weekly. FJP is powder obtained from freeze-drying process of *M. jaboticaba* peels, described previously (LEITE-LEGATTI et al., 2012), kindly provided by 'Bioactive Compounds, Nutrition and Health' researcher group. The FJP powder contains compounds of interest (fibers 25.00%, total polyphenols 556.30 g GAE kg^{-1}, delphinidin-3-*O*-glucoside 634.75 mg 100 g^{-1}, cyanidin-3-*O*-glucoside 1963.57 mg 100 g^{-1}, ORAC 25514.24 μmol TE g^{-1}) (LEITE-LEGATTI et al., 2012).

2.2.2. SAMPLING

Blood was obtained from the fasted rats (12 h) by decapitation. The blood samples were collected in EDTA tubes and centrifuged at 2000 g for 20 min. The plasma was collected, bubbled through nitrogen gas and stored at –80 °C until analyzed. The spleen, liver, kidneys, pancreas and the whole brain were removed, washed,

weighed, frozen in liquid nitrogen, and kept at - 80 °C. Tissue homogenates (pancreas, kidneys and liver) were prepared in a ratio of about 100 mg wet tissue per 1 mL of 50 mmol phosphate buffer (pH 7.4) or 5% trichloroacetic acid (TCA) solution using a homogenizer (MA102/Mini, Marconi, Piracicaba - SP, Brazil). The homogenates were used in the antioxidant enzyme and GSH assays. The liver and kidneys were also freeze-dried, as also the spleen and whole brain. The organs were manually ground and kept at -80 °C until analyzed for lipid peroxidation and antioxidant capacity.

2.3. BIOCHEMICAL ANALYSES

All the absorbance and fluorescence readings for the biochemical analyses were determined in a Synergy HT, Biotek microplate reader (Winooski, USA); with Gen5™ 2.0 data analysis software.

2.3.1. LIPID PEROXIDATION IN THE TISSUES

TBARS (Thiobarbituric Acid Reactive Substances) assay. The malondialdehyde (MDA) levels in the plasma, liver, kidneys, spleen and brain were determined using the method described by Ohkawa, Ohishi and Yagi (OHKAWA; OHISHI; YAGI, 1979), with adaptations. The freeze-dried tissues were sonicated in acetate buffer (pH 3.5, 10 mg mL^{-1}) on ice or 100 µL in the case of plasma. The samples were mixed with 8.1% sodium dodecyl sulfate, TBA powder, 20% acetic acid and 5% sodium hydroxide. After heating at 95 °C for 60 min, the samples were cooled in an ice bath for 10 min and centrifuged at 10,000 g, 10 min, 4^{o} C. The resulting MDA-TBA adducts were quantified at 532 nm, using a 96-well microplate. A standard curve (0.625 – 50 µmol L^{-1} MDA) was prepared using the MDA standard.

2.3.2. ENZYMATIC AND NON-ENZYMATIC ENDOGENOUS ANTIOXIDANT SYSTEMS IN THE PANCREAS, KIDNEYS AND LIVER

Superoxide dismutase activity. The phosphate buffer homogenates were used to determine SOD activity in the tissues (WINTERBOURN et al., 1975). One hundred microliters of appropriately diluted samples were added to a 96-well microplate. One hundred and fifty microliters of a previously prepared solution (0.1 mmol L^{-1}

hypoxanthine, 0.07 U xanthine oxidase, and 0.6 mmol L^{-1} NTB in phosphate buffer in 1: 1: 1 proportions) were added just before the readings. The reading was taken at 560 nm and the reaction monitored for 10 min. The SOD activity was calculated from the area under the curve (AUC) and expressed as U mg^{-1} protein.

Glutathione peroxidase activity. GPx activity in the tissues was quantified in phosphate buffer homogenates by the method described in Flohe and Gunzler (FLOHE; GUNZLER, 1984). The decrease in absorbance was monitored at 365 nm after induction by 0.25 mmol L^{-1} H_2O_2 in the presence of 10 mmol L^{-1} reduced glutathione, 4 mmol L^{-1} NADPH and 1 U of GR enzymatic activity. The results were expressed as nmol NADPH consumed min^{-1} mg^{-1} protein.

Glutathione Reductase activity. GR activity was measured in phosphate buffer homogenates according to Carlberg and Mannervik (CARLBERG; MANNERVIK, 1985), following the decrease in absorbance at 340 nm induced by oxidized glutathione in the presence of NADPH in phosphate buffer. The results were expressed as nmol NADPH consumed min^{-1} mg^{-1} protein.

Reduced thiol (GSH) contents. The GSH levels in the tissues were determined in TCA homogenates by Ellman's reaction using DTNB (ELLMAN, 1959). The intensity of the yellow color was read at 412 nm and GSH (2.5 – 500 nmol mL^{-1}) was used as the external standard. The reduced thiol concentrations were expressed as nmol GSH μg^{-1} protein.

The protein concentrations of all the tissue homogenates were determined using the Bradford method (BRADFORD, 1976).

2.3.3. FREE RADICAL SCAVENGING CAPACITY IN THE PLASMA, LIVER, BRAIN, KIDNEYS AND SPLEEN

Sample preparation. The plasma samples obtained above were treated with ethanol: ultrapure water (2: 1) and 0.75 mol L^{-1} metaphosphoric acid (LEITE et al., 2011). The freeze-dried tissues were mixed with the same solvents. The samples were centrifuged at 21,036 g for 5 min at 4 °C, the supernatant removed and appropriately diluted in phosphate buffer (pH 7.4), when necessary. These extracts were analyzed by the ORAC, TEAC and FRAP in vitro assays.

ORAC (Hydrophilic Oxygen Radical Absorbance Capacity) assay. Twenty microliters of sample or standard solution, 120 µL of fluorescein diluted in phosphate buffer (pH 7.4), and 60 µL of AAPH were added to black microplates in the dark. Trolox was used as the standard and the microplate reader with fluorescent filters as previously described, was used under the following conditions: excitation wavelength, 485 nm; emission wavelength, 520 nm (PRIOR et al., 2003). The ORAC values were expressed as µmol trolox equivalent (µmol TE) per gram of lyophilized tissue or liter of plasma using the standard curves (2.5 - 50.0 µmol TE L^{-1}) for each assay. The linearity between the net AUC and the concentration was checked for all the samples and the fluorescence readings were used for the appropriate calculations (LEITE-LEGATTI et al., 2012).

TEAC (Trolox Equivalent Antioxidant Capacity) assay. The tissues TEAC levels were determined based on the method of Rufino et al. (RUFINO et al., 2010) with modifications. The ABTS solution was prepared by mixing 5 mL of 7.0 mmol L^{-1} ABTS and 88 µL of 145 mmol L^{-1} potassium persulfate solution, which was allowed to react for 12- 16 h, at room temperature in the dark. Ethanol (99.5%) was added to the solution until absorbance of 0.700 ± 0.05 was obtained at 734 nm. Trolox was used as the reference antioxidant. ABTS solution was added to the sample or standard (10 – 800 µmol TE L^{-1}) solutions and allowed to react for 6 min before reading at 734 nm at room temperature.

FRAP (Ferric Reducing Antioxidant Power) assay. The ferric reducing ability of the tissues was determined using the FRAP method (RUFINO et al., 2010), with adaptations. The FRAP reagent was prepared with 300 mmol L^{-1} acetate buffer (pH 3.6), 10 mmol L^{-1} TPTZ in a 40 mmol L^{-1} HCl solution and 20 mmol L^{-1} $FeCl_3$. The sample or standard solutions, ultrapure water and FRAP reagent were mixed in microtubes, and incubated in a water bath for 30 min at 37 ºC. After cooling to room temperature, the absorbance of the samples and standard were read at 595 nm. A trolox standard curve was prepared using the same concentrations used in the TEAC assay.

2.4. DIET ANALYSES

The total anthocyanin contents were assayed in the FJP diets using the pH-differential method, according to Leite-Legatti et al. (LEITE-LEGATTI et al., 2012). The results were expressed as mg cyanidin 3-glucoside 100 g^{-1} diet.

The antioxidant capacity of all diets was evaluated by the ORAC assay as described above. The antioxidants were extracted from the diets with ethanol: ultrapure water (2: 1), the proteins precipitated with 0.75 mol L^{-1} metaphosphoric acid and the results expressed as non-protein trolox equivalents (NP TE µmol L^{-1}).

2.5. STATISTICAL ANALYSES

The parametric results were expressed as the means ± standard error (SEM) and the non-parametric data by the means. The statistical analyses of the parametric data were based on a one-way ANOVA followed by a Tukey multiple comparisons test. The non-parametric data were submitted to the Kruskal-Wallis and Dunn multiple comparisons test. The limit of significance was set at $P< 0.05$. The statistical analyses were carried out using the GraphPad Prism 5.0 (GraphPad Software, Inc. La Jolla, CA, USA) software.

3. RESULTS

The plasma and tissues were collected after 40 days of diet treatment with three different concentrations of freeze-dried jaboticaba peel and enzymatic and non-enzymatic parameters were analyzed. The addition of 2 and 4% FJP improved plasma, liver, brain, kidneys and spleen antioxidant status as detailed below.

Weight and intake parameters. The animals fed on the high-fat diet showed a 52% increase in weight gain (absolute values) as compared to the standard group. The FJP-diets intake did not attenuate the weight gain of the rats during the last 40 days of treatment ($P > 0.05$). The rats fed the J4 diet increased their total weight gain in relation to the N group (Table 1).

There was no statistical difference ($P > 0.05$) in total food intake among the groups, but in the second period of the experiment, rats from group J4 showed a higher food intake than the C group. This could demonstrate that the J4 diet probably was more palatable than the C diet. The J4-fed animals showed the highest antioxidant capacity and anthocyanins daily intake (pH differential method). The daily NP TE consumed by the J4 animals was higher than for all the other groups (Table 1). In addition, the regression analysis showed that the ORAC antioxidant capacity of the FJP diets depended on the total anthocyanin contents ($r^2 = 0.9953$, $P < 0.001$; $y = -2.0978 + 1.0382x$).

The tissue weights were not altered by the dietary FJP treatment, when compared to the C group. The weights of the brains and spleens of the obese rats were lower than those of the healthy rats. The kidneys of the FJP-fed animals were smaller than those of the lean and high-fat control animals (Table 2).

Table 1 - Growth and food intake parameters.[A]

Parameters	Period	N	C	J1	J2	J4
Weight gain (g)	Total	117.6	179.2	174.4	174.5	192.8[b]
Food intake (g)	Total	650.47 ± 9.71	662.31 ± 17.38	680.52 ± 20.69	687.40 ± 27.44	745.16 ± 36.29
	2nd	386.28 ± 7.03	378.84 ± 9.16	383.80 ± 10.43	399.71 ± 15.18	441.02 ± 22.09[d]
ACN intake[B] (mg day^{-1})	2nd	-	-	3.44 ± 0.09	3.9.8 ± 0.15	8.83 ± 0.44[h,i]
ACN intake (mg 100 g^{-1} day^{-1})		-	-	1.33 ± 0.04	1.53 ± 0.02[f]	3.30 ± 0.6[h,j]
Total TE[C] intake (µmol day^{-1})	2nd	49.64	21.99	54.05	102.32[e]	219.41[a,e]
Total TE intake (µmol 100 g^{-1} day^{-1})		25.99	8.64	20.95	38.32[d]	81.95[e,g]
NP[D] TE intake (µmol day^{-1})	2nd	35.37 ± 0.64	6.60 ± 0.16[b]	14.36 ± 0.39[b,c]	21.24 ± 0.81[d,e,f]	68.59 ± 3.44[b,e,h,j]
NP TE intake (µmol 100 g^{-1} day^{-1})		17.88 ± 0.64	2.556 ± 0.15[b]	5.469 ± 0.39[b,e]	8.08 ± 0.77[b,e,h]	24.89 ± 2.76[b,e,h,j]

[A]N= normal diet (AIN-93G) group; C= high-fat control diet group; J1= high-fat diet + 1% freeze-dried jaboticaba peel (FJP); J2= high-fat diet + 2% FJP; and J4= high-fat diet + 4% FJP. 2nd=last 40 days of experiment. [B]ACN= total anthocyanins; [C]TE= trolox equivalents [D]NP TE= Non-protein trolox equivalents. The parametric data (ANOVA and Tukey tests) were expressed as the mean ± SEM; the non-parametric data (Kruskal-Wallis and Dunn tests) were expressed as the mean (n= 6). [a]P< 0.01 and [b]P< 0.001 are compared to the values of the N group. [c]P< 0.05; [d]P< 0.01 and [e]P< 0.001 are compared to the values of the C group. [f]P< 0.05, [g]P< 0.01 and [h]P< 0.001 are compared to the values of J1 group; and. [i]P< 0.01 and [j]P< 0.001 are compared to the values of the J2 group.

Table 2 - Tissue weights in the experimental group (% body weight).[A]

Tissues	N	C	J1	J2	J4
Liver	3.88 ± 0.03	5.01 ± 0.04[b]	4.44 ± 0.07	4.62 ± 0.04	5.07 ± 0.05[b]
Kidneys	1.02	0.70	0.68[b]	0.68[a]	0.69[a]
Brain	0.96 ± 0.02	0.76 ± 0.02[c]	0.75 ±0.02[c]	0.76 ± 0.02[c]	0.72 ± 0.02[c]
Pancreas	0.34	0.26	0.28	0.26[b]	0.26
Spleen	0.22 ± 0.01	0.18 ± 0.01[c]	0.20 ± 0.01[a]	0.19 ± 0.01[c]	0.19 ± 0.01[b]

[A]N= normal diet (AIN-93G) group; C= high-fat control diet group; J1= high-fat diet + 1% freeze-dried jaboticaba peel (FJP); J2= high-fat diet + 2% FJP; and J4= high-fat diet + 4% FJP. The parametric data (ANOVA and Tukey tests) were expressed as the mean ± SEM; the non-parametric data (Kruskal-Wallis and Dunn tests) were expressed as the mean (n= 6). [a]P< 0.05; [b]P< 0.01 and [c]P< 0.001 compared to the values of the N group.

Plasma antioxidant status. The TEAC assay showed that the plasma antioxidant capacity was higher in the J2 and J4 groups as compared to the C group. A significant (P< 0.001) increase in the plasma antioxidant capacity of the J1 group in comparison to group N was confirmed by the ORAC method. The FRAP assay showed no statistical differences between the groups (Figure 1.A). Although, the TBARS levels in high-fat-fed groups were higher than those fed the normal diet. The J2-fed animals showed a tendency to reduced TBARS levels (Figure 1.B).

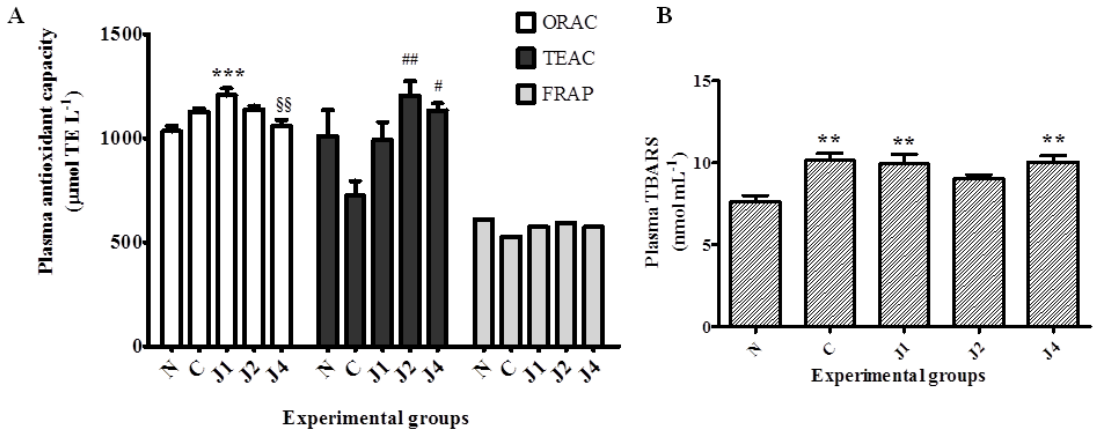

Figure 1. Plasma antioxidant status and lipid peroxidation. A) Total antioxidant capacity as evaluated by the ORAC, TEAC and FRAP methods. B) Lipid peroxidation by the TBARS assay. N= normal diet (AIN-93G) group; C= high-fat control diet group; J1= high-fat diet + 1% freeze-dried jaboticaba peel (FJP); J2= high-fat diet + 2% FJP; and J4= high-fat diet + 4% FJP. The parametric data (ANOVA and Tukey tests) were expressed as the mean ± SEM; the non-parametric data (Kruskal-Wallis and Dunn tests) were expressed as the mean (n= 6). * Indicates statistical differences from N; $^{\#}$indicates statistical differences from C; and §from J1 (1 code= $P< 0.05$; 2 codes= $P< 0.01$; and 3= $P< 0.001$).

Liver antioxidant status. The obese conditions of the high-fat control group were probably responsible for the high TBARS values and low antioxidant capacity levels in the livers of these animals. Nevertheless, the addition of FJP to the diets improved de antioxidant status according to the ORAC method (Figure 2.A) and ameliorated the lipid peroxidation levels in the rat livers. The TBARS levels were reduced 23.61% in the animals from group J2 and about 31% in the J1 and J4-fed animals (Figure 2.B). A linear trend line showed significant correlation coefficient ($P< 0.01$) between the liver TBARS and ORAC levels (r^2= 0.2492; r= -0.4992; P= 0.0052; y= 41.111 – 2.0886x).

The diets added FJP increased the liver GPx and SOD activities (Figures 2.C and D), which corroborates the antioxidant status of the tissue. The addition of 2% FJP to the high-fat diets seemed to be the most efficient dose to increase GPx, since the J2 animals showed a 3.98 times increase in GPx as compared to the control group. The GSH contents did not differ ($P> 0.05$) among the experimental groups (values ranging from 41.32 ± 3.88 to 61.50 ± 10.22 nmol μg^{-1} protein). The animals fed the high-fat

diets showed the lowest GR activities (14.63 ± 17.19 nmol min^{-1} μg^{-1} protein) as compared to the N group (23.06 nmol min^{-1} μg^{-1} protein), but not in the case of the J4 animals (17.98 nmol min^{-1} μg^{-1} protein).

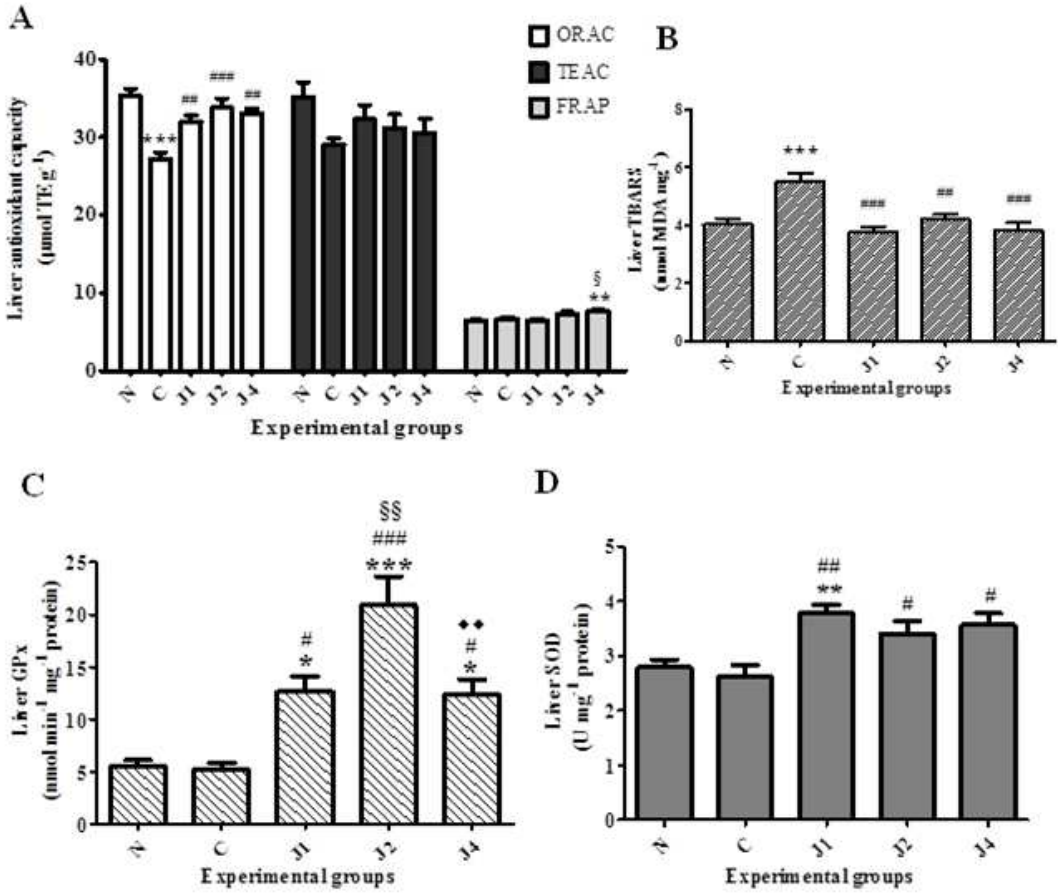

Figure 2. Liver antioxidant status and lipid peroxidation. A) Total antioxidant capacity evaluated by ORAC, TEAC and FRAP methods. B) Lipid peroxidation by the TBARS assay. C) Glutathione peroxidase activity. D) Superoxide dismutase activity. N= normal diet (AIN-93G) group; C= high-fat control diet group; J1= high-fat diet + 1% FJP; J2= high-fat diet + 2% FJP; and J4= high-fat diet + 4% FJP. The parametric data (ANOVA and Tukey tests) were expressed as the mean ± SEM (*n*= 6). *Indicates statistical differences from N; #indicates statistical differences from C; §from J1; and ♦from J2 groups (1 code= $P< 0.05$; 2 codes= $P< 0.01$; and 3= $P< 0.001$).

Brain antioxidant status. There was no difference in brain antioxidant status among the groups according to ORAC assay. However, it was noticeable that the high-fat diet promoted a decrease in brain antioxidant values according to the TEAC assay. Interestingly, the addition of 1% FJP to the diet (group J1) reversed the effect caused by the high fat diet, as shown in Figure 3.A. The brain FRAP values were higher in groups J2 and J4 as compared to the C group (Figure 3.A) and the brain lipid peroxidation was ameliorated by the FJP diets. Indeed, among the high-fat-fed groups, the J2 and J4 groups showed the lowest means for TBARS, exhibiting a dependent dose response pattern (Figure 3.B). In addition, the total antioxidant activity as measured by FRAP was significantly correlated ($P< 0.001$) with the lipid peroxidation values according to TBARS assay ($r^2 = 0.4514$; r = -0.6719; $P = 0.00005$; y = 8.4991 – 0.3681x).

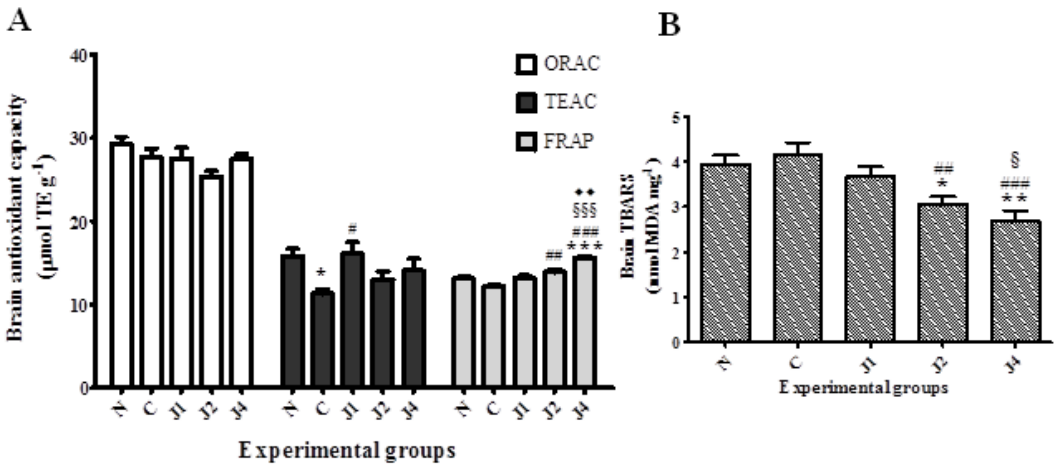

Figure 3 – Brain antioxidant capacity and lipid peroxidation. A) Total antioxidant capacity evaluated by ORAC, TEAC and FRAP methods. B) Lipid peroxidation by TBARS assay. Results expressed on a dry weight basis. N= normal diet (AIN-93G) group; C= high-fat control diet group; J1= high-fat diet + 1% FJP; J2= high-fat diet + 2% FJP; and J4= high-fat diet + 4% FJP. The parametric data (ANOVA and Tukey tests) were expressed as mean ± SEM (n= 6). *Indicates statistical differences from N; #indicates statistical differences from C; §from J1; and •from J2 groups (1 code= $P<$ 0.05; 2 codes= $P<$ 0.01; and 3= $P<$ 0.001).

Kidney antioxidant status. As observed in Figure 4.A, the ORAC antioxidant capacity of kidneys increased in the rats fed the FJP diets in relation to the C group. The TEAC assay also showed an improvement in the antioxidant capacity of the kidneys in the J2

and J4 animals, although the FRAP assay showed no significant differences ($P > 0.05$). According to the TBARS assay, there was an increase of 24.45% in lipid peroxidation in the kidneys of the C animals (2.90 nmol MDA mg^{-1}) in comparison to the N-fed ones (2.33 nmol MDA mg^{-1}). The values for TBARS in the kidneys were similar among the groups and values ranging from 2.58 to 2.78 nmol MDA mg^{-1}.

The GSH values increased in the high-fat-fed animals. Furthermore, groups J2 and J4 showed 39.29 and 84.57% increases in GSH, respectively, as increasing compared to group C (Figure 4.B). The FJP-fed animals showed similar SOD activities as compared to group C, but the animals fed 4% FJP (1.47 U mg^{-1} protein) showed a decrease in the activity of this enzyme ($P < 0.05$), when compared to group N (2.20 U mg^{-1} protein).

Spleen antioxidant status. The ORAC results showed that the spleens of the animals in J4 group had lower trolox equivalents as results compared to J2, despite the reverse response in TEAC. According to the TEAC results, the J4 group showed an increase in antioxidant activity relative to groups N, C and J2 (Figure 4.C), but the FRAP and TBARS results did not confirm these findings.

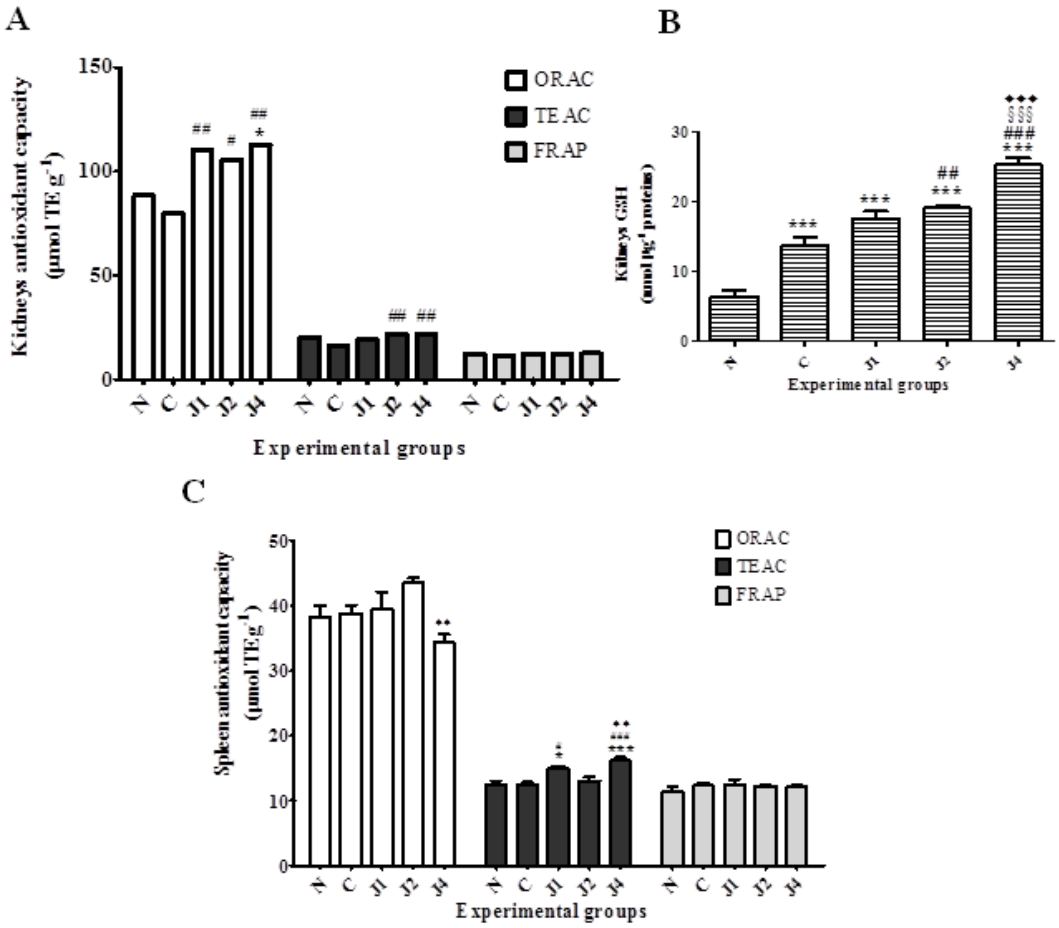

Figure 4. Tissues antioxidant capacity and lipid peroxidation. A) Kidneys total antioxidant capacity as evaluated by the ORAC, TEAC and FRAP methods. B) Kidneys lipid peroxidation by the TBARS assay. C) Spleen antioxidant capacity as evaluated by the ORAC, TEAC and FRAP methods. Results expressed on a dry weight basis. N= normal diet (AIN-93G) group; C= high-fat control diet group; J1= high-fat diet + 1% FJP; J2= high-fat diet + 2% FJP; and J4= high-fat diet + 4% FJP. The parametric data (ANOVA and Tukey tests) were expressed as the mean ± SEM; the non-parametric data (Kruskal-Wallis and Dunn tests) were expressed as the mean (n= 6). *Indicates statistical differences from N; $^{\#}$indicates statistical differences from C; §from J1; and $^{\bullet}$from J2 groups (1 code= P< 0.05; 2 codes= P< 0.01; and 3= P< 0.001).

Pancreas antioxidant status. The GSH content of the J4 animals (6.30 nmol μg^{-1}) increased in comparison to J1 (4.27 nmol μg^{-1} protein) and J2 (4.42 nmol μg^{-1} protein), but no differences were found in comparison to group C (5.11 nmol μg^{-1} protein). On

the other hand, the SOD units in group J4 (1.97 U mg^{-1} protein) were lower than in groups N (2.60 U mg^{-1} protein) and C (1.75 U mg^{-1} protein).

4. DISCUSSION

This study indicated that *M. jaboticaba* peel contained *in vivo* antioxidant properties, which could confirm protection against obesity-induced chronic diseases. Obesity may be a state of chronic oxidative stress with an overproduction of ROS (e. g. hydrogen peroxide, peroxyl, superoxide and hydroxyl radicals), a possible mechanism underlying the development of diabetes and cardiovascular diseases (VINCENT et al., 2007). This is the first study that determined the ORAC of different tissues from animals fed high-fat and high-fat anthocyanin-rich diets. The ORAC assay measures the capacity of peroxyl radical scavenging in a biological system, using values for the temperature, pH and radical close to the physiological conditions (PRIOR et al., 2003).

Indeed, polyphenol sources have shown protective effects against oxidative stress induced in different ways (LEE, C. Y.; CHENG; SIM, 2007; LEITE et al., 2011; MAZZA et al., 2002). Polyphenols may protect cells against oxidative stress by ROS scavenging and transient metals chelations (PRIOR, 2003). Recently, studies have reported that such compounds (cyanidin 3-glucoside) could interact with the receptors or enzymes involved in cell signaling transduction (GUO et al., 2012). In this way, they could ameliorate hepatic steatosis, inflammation in adipose tissue and enhance the insulin sensitivity of obese mice, consequently modifying the redox status of the cells. However, the minimum dose physiologically capable of promoting these functional effects without compromising normal cell functions is not yet clear.

An investigation reported that a concentration of 13.09 ng mL^{-1} of total anthocyanins was found in human serum 4 h after the consumption of freeze-dried blueberries (1.16 g $100g^{-1}$ total anthocyanins) (MAZZA et al., 2002). In addition, the authors proved a significant correlation between the serum anthocyanin content and postprandial antioxidant status. In the present study, a daily consumption of 1.53 and 3.30 mg cyanidin 3-glucoside 100 g^{-1} body weight (J2 and J4 diets, respectively) enhanced the plasma antioxidant status even under 12 h fasting conditions (Table 2 and Figure 1). Moreover, besides cyanidin and delphinidin, which are the anthocyanins detected in the freeze-dried jaboticaba peel (LEITE-LEGATTI et al., 2012), other compounds such as quercetin, ellagitannins, ellagic acid (ABE et al., 2012) and terpenes

(PLAGEMANN, 2012) could be the active compounds responsible for the antioxidant status enhancement in obese rats fed the FJP diets.

The higher plasma antioxidant status found in the J2 and J4 animals could explain previous studies that showed increased cardio protective parameters in obese rats fed a FJP diet (LENQUISTE et al., 2012). Thus, plasma antioxidant defense was enhanced by the intake of FJP and this was probably responsible for the amelioration of obesity related diseases. Moreover, 2% FJP was also capable of increasing the antioxidant potential in healthy rats (LEITE et al., 2011) showing that it could prevent diseases under normal conditions.

Jaboticaba peel is rich in polyphenols such as cyanidin, delphinidin 3-glucoside, ellagitannins, ellagic acid, quercetin (ABE et al., 2012) and other compounds that could increase the *in vivo* antioxidant status (LEITE et al., 2011). The majority of the polyphenols are metabolized by enzymes in the small intestine (e. g. catechol-*O*-methyl-transferase and UDP-glucuronosyltransferase) and by colonic microorganisms, hepatocytes and kidney cells. Polyphenol degradation provides methylated as well as glucoronide forms. Certain flavonoids, especially anthocyanins, could also be found in their native, or even in their aglycone forms in the referred-to tissues besides in the serum/ plasma, due to cleavage of the glycoside forms in the small intestine (PRIOR, 2003; TALAVERA et al., 2005; VANZO et al., 2011). In addition, some studies have reported that anthocyanins are capable of crossing the blood-brain barrier and appearing in the cortex and cerebellum brain (MILBURY et al., 2006; TALAVERA et al., 2005). Thus, the presence of polyphenols, anthocyanins and their metabolites in the tissues may be the principal reason for the increase in the antioxidant defense system (MAZZA et al., 2002), as shown in this study.

The liver weights of the C and J4-fed animals were equally higher than those of the N group, probably because of their higher diet consumption. Nevertheless, the visible hypertrophy of the J4 liver (Table 2) did not seem to impact the antioxidant status of the tissue, possibly because of the higher intake of anthocyanins and trolox equivalent that compensated the damage caused by the fat intake. The oxidative stress parameters of the livers of the FJP-fed animals showed that all doses of jaboticaba promoted oxidative protection of this tissue, especially 2% FJP in the diet, as observed in the ORAC, TBARS and GPx results. Based on the results, it can be seen that the liver antioxidant defenses in the rats fed high-fat diets with added 1, 2 and 4% FJP, were

similar to those of normal diet-fed animals. The scientific literature corroborates the findings concerning decreased TBARS levels in the livers of anthocyanin-fed animals (FEILLET-COUDRAY et al., 2009; LEE, S. J. et al., 2009). The ORAC data was shown to correlate with the TBARS data, improving the antioxidant defenses of the liver, and although not statistically significant, the TEAC results were improved for the diets with added FJP, in agreement with other reports in the literature (LEE, S. J. et al., 2009).

In contrast with some studies (FEILLET-COUDRAY et al., 2009; LEE, S. J. et al., 2009), hepatic GSH showed no differences among the groups evaluated, possibly because of the use of different methods or the absence of protein precipitation. The liver GPx activity was expressively higher in the FJP-fed animals, which seemed to contribute to an improvement in the endogenous antioxidant defenses. SOD and GPx act by removing the superoxide radical and hydrogen peroxide, respectively, and thus prevent the formation of the hydroxyl radical, a potent ROS responsible for much cell damage (VINCENT et al., 2007). Liver GR activity was reduced in obese rats, although it tended to increase in those that received 4% FJP. Thus, the liver could be the main tissue benefited by the intake of FJP.

Concomitantly, the brain/ body weight ratio was significantly reduced in the high-fat-fed animals (C, J1, J2 and J4 groups), which corroborates other findings in the literature (MOROZ et al., 2008). Obesity is linked with neuronal death, increased risk of developing Alzheimer disease, and diabetes could be a key mechanism underlying their development in obese animals (LENQUISTE et al., 2012; MOROZ et al., 2008). Thus, the FJP diets did not influence such data, but hypothetically might delay the initiation or progression of obesity-induced brain atrophy, if administered in a chronic way before further weight gain. More investigations are required to elucidate this hypothesis.

The present data suggested a better brain antioxidant defense for animals that received diets with added FJP, based on the results of the TEAC, FRAP and TBARS assays. The antioxidant status of the brain was not altered by obese conditions (except according to the TEAC results), but the higher doses of FJP ameliorated lipid brain peroxidation and ferric reduction. In addition, the decrease in the MDA levels was significantly correlated with the increase in TE according to the FRAP assay. This data might be attributed to the capacity of some flavonoids, such as anthocyanins, to cross the blood-brain barrier (MILBURY et al., 2006; TALAVERA et al., 2005) and interact

with intracellular neuronal and glial signaling pathways. Thus they ameliorate neural-inflammation, and protecting the neurons against apoptosis due to their antioxidant effects, leading to an increase in blood flow and angiogenesis (WILLIAMS; SPENCER, 2012).

Even under fasting conditions, anthocyanins are capable of affecting the nervous tissues, demonstrating slow metabolism (MILBURY et al., 2006), different from the effects on the blood, liver and kidneys (VANZO et al., 2011). An anthocyanin enriched diet could also increase endogenous antioxidant defense (SHAN et al., 2009), confirming the ability of the FJP diets to promoting healthy brain tissues.

In this work, the animals fed the FJP diets showed apparent atrophied kidneys (Table 2). Obesity and other stress conditions lead to kidney hypertrophy and polyphenols might contribute to these contradictory data (LEE, C. Y. et al., 2007). Kidneys are responsible for the catabolism of the polyphenols and the anthocyanins are widely distributed in this tissue according to some polyphenols bioavailability studies (TALAVERA et al., 2005; VANZO et al., 2011). In fact, the non-enzymatic antioxidant parameters increased in the J1, J2 and J4 groups, but lipid peroxidation was higher and did not alter with the use of the jaboticaba treatment. Kidney lipid peroxidation may be a consequence of increasing ROS production by renal tissue damaged by high intrarenal pressures (NOEMAN et al., 2011). The present data supported an increase in GSH in the kidneys of obese rats, which is possibly linked to the abnormalities found in the cell redox status. Rats treated with 4% jaboticaba peel showed an improvement in GSH, but not in SOD activity.

The administration of polyphenol-rich extracts could enhance the spleen antioxidant defenses (LEE, C. Y. et al., 2007). In our work, only the TEAC results showed an increase in antioxidant power of the spleen. The present findings also suggested a visible spleen atrophy arising from the obesity (Table 2), which could be allied with changes in its physiological functions, such as immunological cell recycling. No significant changes concerning the antioxidant status of the pancreas was observed. Regarding the SOD units in the pancreas, the lower levels found in the J4 group were associated with the increased weight gain, since obesity impaired the enzymatic oxidative system.

In summary, we concluded that the oxidative stress arising from obesity was minimized in many tissues by the intake of jaboticaba peel. However, the doses used

did not promote a reduction in weight gain as suggested by other investigations about anthocyanins and obesity (TSUDA et al., 2003). The diets with added 2 and 4 % jaboticaba peel showed better in vivo antioxidant properties, leading to plasma, liver, brain and kidney antioxidant status enhancement, probably due to the presence of cyanidin and delphinidin 3-glucoside which were identified in the FJP. The regular consumption of jaboticaba peel could increase the circulation of polyphenols in the body, which could possibly improve enzyme activities, free radical scavenging power and lipid peroxidation in obese animals, protecting their cells against oxidative stress damage.

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CONCLUSÃO GERAL

A partir dos resultados deste trabalho, infere-se que dietas ricas em polifenóis, especialmente em antocianinas, estão fortemente associadas à atenuação do estresse oxidativo induzido pela obesidade. Assim como em trabalhos anteriores, a casca de jabuticaba liofilizada, fonte de compostos bioativos como cianidina e delfinidina 3-glicosídeo, mostrou potencial de atuação na modulação de diversos processos celulares fisiológicos por métodos *in vitro* e *in vivo*.

O presente trabalho mostrou que a adição de 1, 2 e 4% de casca de jabuticaba liofilizada (CJL) nas dietas hiperlipídicas não alterou o colesterol sérico e hepático e os níveis de triglicerídeos dos animais experimentais. Entretanto, a excreção de triglicerídeos por meio das fezes foi maior nos animais alimentados com dietas contendo 1 e 4% de CJL. Adicionalmente, os valores de pH fecal foram reduzidos nos animais alimentados com dieta J2.

Ademais, mesmo que não tenham ocorrido mudanças de grande impacto no perfil lipídico e ganho de peso dos animais alimentados com CJL adicionado às dietas, o estresse oxidativo foi consideravelmente minimizado nos tecidos dos animais testes. As dietas com adição de 2 e 4% de CJL foram também capazes de elevar as defesas antioxidantes do plasma, cérebro, fígado e rins, provavelmente devido à biodisponibilidade de compostos bioativos presentes na casca através da administração da dieta, como discutido anteriormente.

Por fim, o consumo regular de casca de jabuticaba liofilizada (2 e 4% na dieta) poderia aumentar as concentrações dos polifenóis circulantes no corpo, o que favorece o aumento da atividade de enzimas antioxidantes, o sequestro de radicais livres e atenuação da peroxidação lipídica em animais obesos. Estes parâmetros indicam maior preservação celular contra os danos do estresse oxidativo advindo da obesidade. Estudos não-clínicos e clínicos adicionais são necessários para se verificar a aplicação de doses mínimas e máximas de concentração de CJL na dieta em que se detecte tais efeitos funcionais. Os mecanismos de ação celular e molecular propostos para estes também estão inseridos nesta perspectiva.

ANEXO

UNICAMP

CEUA/Unicamp

Comissão de Ética no Uso de Animais
CEUA/Unicamp

C E R T I F I C A D O

Certificamos que o Protocolo nº **2226-1**, sobre **"Avaliação do efeito do pó liofilizado da casca de jabuticaba sobre o estresse oxidativo, o tratamento da obesidade e parâmetros de saúde *in vivo*"**, sob a responsabilidade de **Prof. Dr. Mário Roberto Maróstica Junior / Sabrina Alves da Silva**, está de acordo com os Princípios Éticos na Experimentação Animal adotados pelo Colégio Brasileiro de Experimentação Animal (COBEA), tendo sido aprovado pela Comissão de Ética no Uso de Animais – CEUA/Unicamp em **30 de setembro de 2010**.

C E R T I F I C A T E

We certify that the protocol nº **2226-1**, entitled "_____", is in agreement with the Ethical Principles for Animal Research established by the Brazilian College for Animal Experimentation (COBEA). This project was approved by the institutional Committee for Ethics in Animal Research (State University of Campinas - Unicamp) on **September 30, 2010**.

Campinas, 30 de setembro de 2010.

Profa. Dra. Ana Maria A. Guaraldo
Presidente

Fátima Alonso
Secretária Executiva

CEUA/UNICAMP
Caixa Postal 6109
13083-970 Campinas, SP – Brasil

Telefone: (19) 3521-6359
E-mail: comisib@unicamp.br
http://www.ib.unicamp.br/ceea/