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Polyphenols-rich fruit in maternal diet modulates inflammatory markers and the gut microbiota and improves colonic expression of ZO-1 in offspring

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ABSTRACT

The increase in maternal *trans* fatty acid (TFAs) consumption during pregnancy and/or lactation leads to a pro-inflammatory state in the offspring. In contrast, polyphenol-rich fruit are promising modulators of inflammation. The aim of the present study was to verify whether supplementation of the maternal diet with jussara pulp changes the pro-inflammatory state in offspring exposed to TFAs during the intrauterine and lactation periods. Wistar rats were assigned to one of four groups and fed a control diet (C), the C diet with 0.5% jussara supplementation (CJ), a diet enriched with hydrogenated vegetable fats rich in TFAs (T) or the T diet supplemented with 0.5% jussara (TJ). The diets were maintained during pregnancy and lactation. Our data demonstrated that maternal intake of TFAs resulted in increased IL-6 protein expression in the retroperitoneal white adipose tissue (RET), MyD88 in the liver and a reduction in *Bifidobacterium* spp. in the colon of 21-day-old offspring. However, jussara supplementation (TJ group) restored the fecal content of *Bifidobacterium* spp., increased colonic ZO-1 mRNA expression and reduced NFκB pathway activation by decreasing MyD88, NFkB p65 phosphorylated (p-NFkB p65) subunit and TNFα receptor I (TNFR1) in the liver. These effects reduced IL-6 and TNF-α expression in the liver and IL-6 and TNFα mRNA expression in the RET. Additionally, jussara supplementation of the maternal diet increased the IL-10 profile in the RET and the IL-10/TNF-α ratio in the offspring's liver relative to the T group. The 0.5% jussara supplementation prevented the adverse effects of TFAs reducing low-grade inflammation *via* down-regulation of the NFkB signaling pathway. These effects are possibly associated with the better intestinal barrier integrity in the colon of 21-day-old offspring mediated by the gut microbiota. Thus, maternal diet supplementation could contribute to reduce chronic disease development in later life.

1. Introduction

Maternal nutrition during pregnancy and/or lactation causes permanent adaptations in the offspring that most likely occur due to epigenetic regulation and changes in metabolic programming (Barker, 1990). Fetal and/or neonatal exposure to lipids such as TFAs or saturated fatty acids (SFAs) can promote deleterious effects in the offspring's health; these effect can occur by increasing the pro-inflammatory status *via* activation of the TLR4-mediated NFκB signaling pathway involving the MyD88 adapter protein that is required for TLR-4 signaling (Mennitti et al., 2015; Pimentel et al., 2012). This process result in releases of NF-κB subunits p65 and p50 translocate into the nucleus and upregulate genes associated with pro-inflammatory cytokines (Lu, Yeh, & Ohashi, 2008; Takeda & Akira, 2004).

Additionally, maternal bacterial colonization has been shown to contribute to the development of intestinal microbiota in the offspring (Ma et al., 2014; Rautava, Luoto, Salminen, & Isolauri, 2012). Modulation of the gut microbiota was recently proposed to be an important factor associated with changes in intestinal permeability and the inflammatory status linked to chronic diseases (Caricilli & Saad, 2013; Kim, Gu, Lee, Joh, & Kim, 2012).

The low-grade inflammatory response and development of metabolic diseases has been associated with changes in the gut microbiota, especially with reduced numbers of colonic *Bifidobacterium* species (spp.) and higher plasma lipopolysaccharide (LPS) levels (Cani et al., 2007). The proposed mechanism involves the influence of these bacterial strains on tight junction and intestinal barrier functions (Cani et al., 2009; Caricilli et al., 2011).

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Tight junctions are structures formed by a complex of proteins located at the apical side of epithelial cell membranes. These proteins are associated with the peripheral membrane proteins known as zonules occludentes (ZO) that are involved in the regulation of paracellular permeability (Ménard, Cerf-Bensussan, & Heyman, 2010; Paris, Tonutti, Vannini, & Bazzoni, 2008). Specifically, ZO-1 plays an important role in tight junctions and has specific structural functions in cell barrier properties (Hamada, Shitara, Sekine, & Horie, 2010). Thus, alterations in their expression may contribute to disturbances of the tight junction barrier, thereby leading to enhanced intestinal permeability (Hamada, Kakigawa, Sekine, Shitara, & Horie, 2013) and the translocation of potential inflammatory bacterial products (Caricilli & Saad, 2013; Le Dréan et al., 2014). However, the molecular mechanism underlying this process remains to be elucidated in programing models.

In contrast, maternal intake of polyphenol-rich foods can protect the offspring against the development of chronic diseases later in life through mechanism that is related to inflammation or oxidative stress (de Bem et al., 2014; Mukai, Sun, & Sato, 2013; Vanhees et al., 2013).

The jussara (*Euterpe edulis* Mart.) is a species native to the Atlantic Forest/Brazil. The fruit has been cited as a new "super fruit" due to its nutritional composition and phytochemical constituents, including phenolic compounds and especially the predominant anthocyanins cyanidin 3-glucoside and cyanidin 3-rutinoside (Felzenszwalb, da Costa Marques, Mazzei, & Aiub, 2013; Silva, Rodrigues, Mercadante, & de Rosso, 2014) which are known for their antioxidant and anti-inflammatory properties (Esposito, Chen, Grace, Komarnytsky, & Lila, 2014; Lee et al., 2014). However, there have been few investigations into fruit antioxidant and anti-inflammatory effects *in vivo*.

In the present study, we hypothesized that the maternal intake of polyphenol-rich fruit would prevent TFA-induced adverse effects in their offspring. Hence, the aim was to investigate the role of supplementation of the maternal diet with jussara pulp on the pro-inflammatory state in 21-day-old offspring exposed to TFAs during the intrauterine and lactation periods.

2. Experimental methods

2.1. Experimental animals and diet

The Experimental Research Committee of the Federal University of Sao Paulo (protocol no 859814) approved all experimental procedures.

Wistar rats were kept under controlled conditions of light (12 h light/dark cycle) and temperature (22 ± 2 °C) with *ad libitum* access to water and food.

Twelve-week-old female Wistar rats of first-order parity were left overnight to mate. Copulation was verified the following morning by the presence of sperm in vaginal smears. On the first day of gestation, the rats were isolated in individual cages and randomly assigned to one of four groups receiving a control diet (C diet, C group), a control diet supplemented with 0.5% freeze-dried jussara powder (CJ diet, CJ group), a diet enriched with hydrogenated vegetable fat (T diet, T group) or a T diet supplemented with 0.5% freeze-dried jussara powder (TJ diet, TJ group).

The diets were prepared according to the recommendations of the American Institute of Nutrition (AIN-93G) (Reeves, 1997) and had similar caloric and lipid contents. The source of lipids for the C and CJ diets was soybean oil; the principal source for the T and TJ diets was partially hydrogenated vegetable fat rich in TFAs. The CJ and TJ diets were prepared by adding 5 g/kg of freeze-dried jussara powder to each diet (Table 1).

To determine the dose, we used previous data that showed no genotoxic and mutagenic effects of acai in mice (fruit with characteristics similar to jussara) (Ribeiro et al., 2010). The calculation was applied for the conversion of the rat dose to the adult human dose according to FAO (2005).

Table 1
Composition of the control diet (C), control diet supplemented with 0.5% freeze-dried jussara powder (CJ), diet enriched with hydrogenated vegetable fat, TFAs (T) and diet enriched with TFAs supplemented with 0.5% freeze-dried jussara powder (TJ) according to AIN-93.

	Diet (g/100 g)			
Ingredient	C	CF	T	TF
Casein[a]	20.0	20.0	20.0	20.0
L-cystine [b]	0.3	0.3	0.3	0.3
Cornstarch [b]	62.0	62.0	62.0	62.0
Soybean oil [c]	8.0	8.0	1.0	1.0
Hydrogenated vegetable fat [d]	–	–	7.0	7.0
Butylhydroquinone [b]	0.0014	0.0014	0.0014	0.0014
Mineral mixture [e]	3.5	3.5	3.5	3.5
Vitamin mixture [f]	1.0	1.0	1.0	1.0
Cellulose[b]	5.0	5.0	5.0	5.0
Choline bitartrate[b]	0.25	0.25	0.25	0.25
Freeze-dried Juçara powder [g]	–	0.5	–	0.5
Energy (kcal/g)	4.00	4.02	4.00	4.02

[a] Casein was obtained from Labsynth, São Paulo, Brazil.
[b] L-cystine, cornstarch, butylhydroquinone, cellulose and choline bitartrate were obtained from Viafarma, São Paulo, Brazil.
[c] Oil was supplied from soybean (Lisa/Ind. Brazil).
[d] Hydrogenated vegetable fat was supplied from Unilever, São Paulo, Brazil.
[e] Mineral mix 9 mg/kg diet: calcium, 5000; phosphorus, 1561; potassium, 3600; sodium, 1019; chloride, 1571; sulfur, 300; magnesium, 507; iron, 35; copper, 6.0; manganese, 10.0; zinc, 30.0; chromium, 1.0; iodine, 0.2; selenium, 0.15; fluoride, 1.00; boron, 0.50; molybdenum, 0.15; silicon, 5.0; nickel, 0.5; lithium, 0.1; vanadium, 0.1 (AIN-93G, mineral mix, Rhoster, Brazil).
[f] Vitamin mix (mg/kg diet): thiamin HCL, 6.0; riboflavin, 6.0; pyridoxine HCL 7.0; niacin, 30.0; calcium pantothenate, 16.0; folic acid, 2.0; biotin, 0.2; vitamin B12, 25.0; vitamin A palmitate, 4000 IU; vitamin E acetate, 75; vitamin D3, 1000 IU; vitamin KI, 0.75 (AIN-93G, vitamin mix, Rhoster, Brazil).
[g] Freeze-dried Jussara powder: Jussara pulp (*Euterpe edulis* Mart.) was obtained from agroecological Project Juçara/IPEMA — Institute of Permaculture and Ecovillage of the Atlantic (Ubatuba, SP, Brazil) and by freeze-drying to powder using a lyophilizer.

Jussara pulp (*E. edulis* Mart.) was obtained from the agroecological Project Juçara/IPEMA — Institute of Permaculture and Ecovillage of the Atlantic (Ubatuba, SP, Brazil) and then freeze-dried to powder using a lyophilizer. The diets were stored at −20 °C. The centesimal composition of the diets is presented in Table 1. The fatty acid profile of the C and T diets was previously described by Pisani et al. (2008).

The dams' diets were maintained during pregnancy and lactation. After birth, litter sizes were adjusted to eight pups that remained with the mother. The offspring were decapitated when they reached 21-days-old. The liver, colon and RET were removed and the fecal content was isolated and stored at −80 °C.

2.2. Phytochemical analysis

Anthocyanins and other phenolic compounds from the jussara pulp were analyzed in a Shimadzu (Kyoto, Japan) high performance liquid chromatography (HPLC) device with quaternary pump (model LC-20 AD) and a photodiode array detector (PDA) (Shimadzu, model SPD-M20A). The extraction of all bioactive compounds was performed in triplicate. The anthocyanins were extracted from 20.0 g of jussara pulp with 100 mL of 0.5% HCl in methanol overnight in the dark (5 °C). The slurry was filtered, and the solids were washed with an additional 100 mL of 0.5% HCl in methanol at room temperature. The acidic methanol extracts were combined and concentrated in a rotary evaporator (Marconi, Piracicaba, Brazil). The experimental conditions for the separation, identification and quantification of jussara anthocyanins by HPLC–PDA were previously described by Silva et al. (2014). The anthocyanins were quantified using five-point analytical curve of cyanidin 3-glucoside (0.5–10.0 mg/mL). The phenolic compounds were extracted from 20.0 g of jussara pulp with 100 mL of methanol:water (8:2) by agitation provided by a homogenizer (Tecnal, Piracicaba, Brazil). The slurry was filtered and the solids were washed with an additional 50 mL of methanol water (8:2) and concentrated in a rotary evaporator