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Inflammatory state of periaortic adipose tissue in mice under obesogenic dietary regimens

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ABSTRACT

High-fat diet or high-sugar diet causes obesity and a chronic low-grade inflammation that leads to the development of diabetes and cardiovascular diseases. Inflammation of the surrounding fat of thoracic aorta namely periaortic adipose tissue (PAAT) has been associated with increased prevalence of vascular diseases in obesity. C57Bl/6 male mice (12 weeks of age) fed a whole grain-based commercial diet (WGD), refined carbohydrate diet (RCD), refined carbohydrate diet plus sweetened condensed milk *ad libitum* (RCD + CM) or high-fat diet (HFD) for eight weeks were studied. Serum fatty acid (FA) composition was evaluated by gas chromatography. The cellularity (as indicated by DNA and protein contents) and the inflammatory state (as indicated by the contents of TNF-α, IL-6, IL-1β, IL-10, VCAM-1, ICAM-1, leptin and adiponectin measured by ELISA) of the PAAT and thoracic aorta (TA) were evaluated. Both obesogenic regimens (RCD + CM and HFD) increased the content of total fatty acids (FA) in serum and the cellularity of the PAAT compared to WGD. RCD + CM increased serum monounsaturated fatty acid (MUFA) levels and HFD increased serum saturated fatty acid (SFA) levels compared to WGD. RCD (one of the diets used as control) and RCD + CM increased the levels of TNF-α, IL-1β, IL-10 and VCAM-1 in the PAAT compared to WGD. Mice fed with HFD showed decreased contents of TNF-α, VCAM-1 and IL-10 in the PAAT compared to animals fed RCD. The RCD raised the levels of SFA in serum, cellularity and inflammatory state in the PAAT compared to WGD. In conclusion, the effects of obesogenic dietary regimens on PAAT can be interpreted differently when the results are compared with WGD or RCD. We found marked changes in the PAAT and no significant modifications in TA indicating this adipose tissue as the major starting point of vascular diseases.

Abbreviations: PAAT, Periaortic adipose tissue; WGD, Whole grain-based commercial diet; RCD, Refined carbohydrate diet; RCD + CM, Refined carbohydrate diet with sweetened condensed milk *ad libitum*; HFD, High-fat diet; FA, Fatty acids; ICAM-1, Intercellular adhesion molecule-1; VCAM-1, Vascular adhesion molecule-1; SFA, Saturated fatty acids; PUFA, Polyunsaturated fatty acids; MUFA, Monounsaturated fatty acids; NFκB, Nuclear factor-κB; BAT, Brown adipose tissue; WAT, White adipose tissue; SEM, Standard error of the mean.

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1. Introduction

Excessive food energy intake and a sedentary lifestyle are the main cause of overweight and obesity [1]. Although energy balance is clearly important uncertainty remains however about the role of the main dietary components, fat and sugar, on the development of obesity and associated complications, namely type 2 diabetes, dyslipidemia and cardiovascular diseases [2]. Lomba et al. [3] demonstrated macronutrient proportion in the diet plays an important role in body weight gain and metabolic syndrome manifestations by affecting the expression of key genes of energy metabolism.

The chronic low-grade inflammation in obese patients has been associated to the development of diabetes and cardiovascular diseases [4,5]. Mice fed with a high-fat diet show increased body weight, raised abundance of inflammatory peritoneal macrophages and white adipose tissue inflammation [6,7]. Consumption of a high-refined carbohydrate-containing diet also induces inflammation in the liver and adipose tissue [8,9].

Periaortic adipose tissue (PAAT) is the surrounding fat of thoracic aorta and it has been associated with the occurrence of vascular diseases [10]. In humans and most animal models, the development of obesity leads to an increase of the PAAT mass [11]. Schlett et al. [12] demonstrated in humans a strong correlation between PAAT and visceral adipose tissue mass. Accumulation of fat around blood vessels may affect vascular function in a paracrine manner as PAAT cells secrete vascular relaxing factors and pro-inflammatory cytokines [13]. Sweazea et al. [14] demonstrated that different obesogenic dietary regimens, such as high-sucrose and high-fat diets, have varying effects on vascular reactivity and function in Sprague-Dawley rats. However, the involvement of the PAAT cells in the vascular inflammatory response to the main obesogenic components of the diet remains unknown.

This study was designed to examine the effects of high sucrose feeding and a high fat diet on inflammatory state of the PAAT and thoracic aorta of mice. The effects of the obesogenic regimens were compared with a whole grain-based commercial diet and a refined carbohydrate diet.

2. Materials and methods

2.1. Animal model

This study was approved by the local Ethics Committee and was conducted according to the Home Office Animals (Scientific Procedures) Act 1986. C57Bl/6 male mice (12-weeks-old) were fed a whole grain-based commercial diet (56% carbohydrates, 3.5% fat, 19% proteins; 3.5 kcal/g; *WGD*), a semisynthetic refined carbohydrate diet (76% carbohydrates, 9% fat, 15% proteins, in energy; 3.8 kcal/g; *RCD*) or a semisynthetic high-fat diet (26% carbohydrates, 59% fat, 15% proteins, in energy; 5.3 kcal/g; *HFD*) for 8 weeks. One group (*RCD + CM*) received a separated pot of sweetened condensed milk − CM (Italac, Brazil) (55% carbohydrates, 8.5% fat, 7.5% proteins) fortified with a mineral and vitamin mix (AIN93G) *ad libitum* during the same 8 weeks that another group received the semisynthetic refined carbohydrate diet [15]. All groups received food and water *ad libitum* during the study. Commercial chow was purchased from Nuvilab (Curitiba, Paraná, BR) and the two other diets were prepared in our laboratory (Table 1). Body weight and food intake were weekly recorded. At 20 weeks of age, following 3−5 h fasting, mice were killed by rising CO_2 tension in a small cage. Thoracic aorta and PAAT directly adjacent to the lesser curvature of the aortic arch were harvested, snap frozen, and stored at −80 °C until being used for the assays.

Table 1
Composition of the refined carbohydrate (RCD) and high-fat (HFD) diets.

Ingredients	RCD	HFD
	(g/kg of diet)	
Cornstarch	465.7	115.5
Dextrinized cornstarch	155	132
Sucrose	100	100
Casein	140	200
Choline bitartrate	2.5	2.5
L-Cystine	1.8	3
Vitamin mix AIN93 M	10	10
Mineral mix AIN93 M	35	35
Cellulose	50	50
Soybean oil	4	35
Lard	36	315

2.2. Gas chromatographic analysis of fatty acids

Fatty acid composition in serum (≈100 μL) was determined after lipid extraction and derivatization according to the AOAC Official Methods 996.06 and AOCS Ce 1 j-07 [16] with some modifications as described in previous study of our group [17].

2.3. Cellularity determination

The cellularity of the PAAT and thoracic aorta was estimated by measurement of total protein and DNA content in both tissues [18]. PAAT and thoracic aorta were homogenized in phosphate buffered saline (sodium chloride 137 mmol/L, potassium chloride 2.7 mmol/L, sodium phosphate dibasic 10 mmol/L, potassium phosphate monobasic 2 mmol/L, at pH 7.4; 300 μL and 150 μL, respectively), containing a protease inhibitor cocktail (Roche Diagnostics, Grenzach, Germany). Homogenates were centrifuged at 12,000 *g*, for 10 min, at 4 °C and the supernatant was collected for the measurements. The protein concentration was determined by the Bradford [19] method (BioRad, California, USA), using bovine serum albumin as standard. The DNA content was quantified by spectrophotometry in a Nanodrop (Thermo Scientific, Delaware, USA) with the purity determined by the 260/280 ratio.

2.4. Measurements of inflammation process associated proteins

The contents of TNF-α, IL-1β, IL-6, IL-10, VCAM-1, ICAM-1, adiponectin and leptin were determined in the homogenates of PAAT and thoracic aorta by ELISA according to the manufacturer's instructions (DuoSet kits, R&D System, Minneapolis, USA).

2.5. Statistical analysis

Results are expressed as mean ± standard deviation (SD). The groups were compared by one-way ANOVA followed by the Tukey post-test. Statistical analysis was performed using the GraphPad Prism v. 5.0 software (GraphPad Software, San Diego, CA, USA). $P < 0.05$ was considered to be significant.

3. Results and discussion

3.1. Obesogenic diets and serum free fatty acid composition

The obesogenic regimens (RCD + CM and HFD) herein reported has also been used in another study by our group [20]. We found both obesogenic diets significantly increase body weight gain, visceral fat tissue weight, plasma total cholesterol levels and glycemia and reduced glucose tolerance. In the present study, the obesogenic regimens markedly changed blood fatty acid levels and