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Basic nutritional investigation

Early and prolonged intake of partially hydrogenated fat alters the expression of genes in rat adipose tissue

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Abstract

Objective: Our previous study indicated that partially hydrogenated fat (PHF) diets, rich in *trans*-isomers, alter plasma lipids and increase the lipogenesis rate on adipose tissue in rats at a young age. In the present study we investigated the effects of dietary PHF on the expression of genes associated with glucose and lipid metabolism in rat adipose tissue.

Methods: Female Wistar rats were fed normolipidic diets containing PHF (rich in *trans*-fatty acids and poor in polyunsaturated fatty acids [PUFAs]), soy oil (rich in ω-6 PUFAs), and fish oil (rich in ω-3 PUFAs) during gestation and lactation; young male pups were fed the same diets from weaning until 120 d of life. The mRNA expression of peroxisome proliferator-activated receptor-γ, tumor necrosis factor-α, resistin, adiponectin, and leptin were analyzed in retroperitoneal adipose tissue (RET) using real time polymerase chain reaction.

Results: The PHF group showed the highest triacylglycerol, glucose, and insulin levels and the lowest plasma adiponectin level. The RET of PHF incorporated *trans*-fatty acids, whereas fish oil and soy oil groups had increased ω-3 and ω-6 PUFAs, respectively. In the RET the PHF group had the highest resistin and tumor necrosis factor-α levels and the lowest adiponectin and peroxisome proliferator-activated receptor-γ gene expressions, whereas the fish oil group had the highest peroxisome proliferator-activated receptor-γ and the lowest leptin gene expression.

Conclusion: Prolonged intake of PHF has a negative effect on the expression of genes in RET when compared with diets with ω-6 and ω-3 PUFAs. These changes may be an effect of the smaller proportions of PUFAs in this fat, instead of being only caused by *trans*-fatty acids. Published by Elsevier Inc.

Keywords: Trans-fatty acids; Polyunsaturated fatty acids; Adipokines; Peroxisome proliferator-activated receptor-γ; Adipose tissue; Rats; Glucose; Lipid homeostasis

Introduction

In recent years, adipose tissue has been regarded as a multifunctional organ, which produces and secretes countless peptides and bioactive proteins, known as adipokines or adipocytokines [1]. Alterations in the mass of adipose tissue, as occurs in obesity, affect the production of most substances secreted by the adipocytes [2]. Although these alterations are frequently associated with metabolic dysfunctions and increased risk of cardiovascular disease [3],

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the role of adipose tissue in the development of these alterations, considering its endocrine function, is still under investigation. The concentrations of several adipokines increase with obesity and have been related to hypertension (angiotensinogen) [4], damage to fibrinolysis (plasminogen activator inhibitor-1) [5], and resistance to insulin (acylation-stimulating protein, tumor necrosis factor-α [TNF-α], interleukin-6, and resistin) [1,6]. Furthermore, insulin resistance is related to leptin resistance and decreased plasma adiponectin levels [7]. Nevertheless, leptin and adiponectin have additional physiologic functions, namely, although leptin controls food intake and energy expenditure, adiponectin has a strong antiatherogenic action [8].

The influence of high-fat consumption and the fat content of a diet on the development of non-transmissible chronic disease has been the focus of intense scientific research [9]. The amount and quality of lipid intake highly influence cellular functions by modulating the processes of differentiation, growth, and metabolism. Fatty acids may influence the expression of adipokines such as leptin, resistin, or adiponectin directly by interaction with transcription factors or indirectly by unknown mechanisms possibly linked to fatty acid oxidation synthesis, or storage [10]. Peroxisome proliferator-activated receptors (PPARs) are among the most abundant classes of nuclear receptor transcription factors and play a critical role in the regulation of fatty acid metabolism and adipose tissue function. PPAR-γ is mainly expressed in adipocytes, activated by fatty acids, and participates in the adipogenesis process and energy homeostasis, modulating adipose tissue hormone and cytokine expression or secretion of adipokines [11].

Cardiovascular and metabolic diseases are associated with obesity and with alterations in the production of adipokines, such as leptin, resistin, adiponectin, TNF-α, plasminogen activator inhibitor-1, and haptoglobin [12]. Because fatty acids are the main components of adipose tissue, it is of essential interest to clarify the biological effects of different types of fatty acids on the expression of relevant adipokines. Dietary *trans*-fatty acids (TFAs) increase the production of proinflammatory cytokines such as interleukin-6 and TNF-α [13,14]. In addition, a high intake of TFAs has been shown to promote insulin resistance [15] and to alter blood lipid profile and adiposity [16,17]. In contrast, a high intake of ω-3 polyunsaturated fatty acids (PUFAs) has active anti-inflammatory effects [18,19], and it has been proposed that the ω-6/ω-3 fatty acid ratio increase, currently found in the occidental diet, contributes to many of the metabolic alterations present in non-transmissible chronic disease [20].

To our knowledge, there are currently no studies showing the effects of partially hydrogenated fat (PHF) ingestion in a normolipidic diet during gestation and lactation and during offspring growth on the expression of PPAR-γ and specific genes associated with glucose and lipid metabolism. Moreover, most studies related to lipid metabolism have been designed for the postweaning period and/or high-fat diets [21–23]. However, maternal intake of PHF can affect the metabolism of mammalian offspring [24,25].

The main objective of the present study was to investigate whether early and prolonged exposure to PHF could modify blood lipid profiles and mRNA expression of PPAR-γ and some adipokines linked to insulin resistance and development of non-transmissible chronic disease. In addition, because the PHF diet is also poor in PUFAS, we compared the effects of these diets with the effects of diets rich in ω-6 and ω-3 PUFAs, soy oil (SO; control group, rich in ω-6 PUFAs) and fish oil (FO; rich in ω-3 long-chain [LC] PUFAs). Regarding the FO diet, there are multiple well-documented health benefits of this diet on glucose and lipid levels, with concomitant improvement in insulin signaling [9,18,19,20].

Materials and methods

Animals, diets, and general procedures

Adult virgin female Wistar rats weighing 180 to 220 g were obtained from the animal breeding unit of the Institute of Nutrition, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil. Animals were kept in controlled light and dark (12 h each) and temperature (24 ± 1°C) conditions. After mating, pregnant rats were housed in individual cages and divided into three groups that were fed isoenergetic (4.1 kcal/g of dry diet) and normolipidic diets: the SO group received a diet containing 7% SO (rich in ω-6 PUFAs), the PHF group received a diet containing 6% partially hydrogenated vegetable oil (rich in TFAs) plus 1% SO, and the FO group received 6% FO (rich in ω-3 LC-PUFAs) plus 1% corn oil. The composition of each diet complied with the recommendations of the American Institute of Nutrition [26]. SO and corn oil were added to adjust each diet to the minimum requirement for essential fatty acids. Diets were prepared as pellets and stored at 4°C until use. After weaning (day 21 of life), the mothers were excluded from the study and six male pups per each dam were fed the same diets as their mothers (Table 1) up to day 120 of life. The fatty acid compositions of the diets are summarized in Table 2. The pups had free access to food and water during the entire period of the study. Food consumption was measured daily and body mass weekly. The pups were sacrificed by guillotine on day 120 of life after an overnight fast. Blood was collected in tubes containing 100 μL of Na_2-ethylenediaminetetra-acetic acid (1 mmol/L) and the plasma was separated by centrifugation. Plasma aliquots were frozen at -70°C. Samples of retroperitoneal white adipose tissue (RET) were quickly extracted and stored at -70°C for further determination of gene expression. All experimental protocols and procedures were approved by the university's experimental research committee.