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Replacement of soybean oil by fish oil increases cytosolic lipases activities in liver and adipose tissue from rats fed a high-carbohydrate diets☆

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Abstract

Several studies have demonstrated that fish oil consumption improves metabolic syndrome and comorbidities, as insulin resistance, nonalcoholic fatty liver disease, dyslipidaemia and hypertension induced by high-fat diet ingestion. Previously, we demonstrated that administration of a fructose-rich diet to rats induces liver lipid accumulation, accompanied by a decrease in liver cytosolic lipases activities. In this study, the effect of replacement of soybean oil by fish oil in a high-fructose diet (FRUC, 60% fructose) for 8 weeks on lipid metabolism in liver and epididymal adipose tissue from rats was investigated. The interaction between fish oil and FRUC diet increased glucose tolerance and decreased serum levels of triacylglycerol (TAG), VLDL-TAG secretion and lipid droplet volume of hepatocytes. In addition, the fish oil supplementation increased the liver cytosolic lipases activities, independently of the type of carbohydrate ingested. Our results firmly establish the physiological regulation of liver cytosolic lipases to maintain lipid homeostasis in hepatocytes. In epididymal adipose tissue, the replacement of soybean oil by fish oil in FRUC diet did not change the tissue weight and lipoprotein lipase activity; however, there was increased basal and insulin-stimulated *de novo* lipogenesis and glucose uptake. Increased cytosolic lipases activities were observed, despite the decreased basal and isoproterenol-stimulated glycerol release to the incubation medium. These findings suggest that fish oil increases the glycerokinase activity and glycerol phosphorylation from endogenous TAG hydrolysis. Our findings are the first to show that the fish oil ingestion increases cytosolic lipases activities in liver and adipose tissue from rats treated with high-carbohydrate diets.

Keywords: Fish oil; High-fructose diet; Nonalcoholic fatty liver; Adipose tissue; Cytosolic lipases activities

1. Introduction

A large number of studies have reported health benefits of the most important components of fish oil (FO), eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, both omega-3 fatty acids [1]. Besides its beneficial effects on inflammatory diseases [2–4], protection against cardiovascular diseases [5], anticancer effects [6] and contribution to brain development and function [7], FO intake has been studied as an adjuvant therapy for obesity and its comorbidities.

Ingestion of a high-fat diet containing lard induces obesity, metabolic syndrome and liver damage, along with hypertriglyceridemia, hepatic insulin resistance and steatosis [4,8]. On the other hand, the consumption of a high-fat diet containing FO does not induce these alterations. FO-fed mice have a significant decrease in body weight gain, epididymal adipose tissue weight, fasting blood glucose, HOMA-IR and serum cholesterol compared with saturated fat-fed mice [9]. The changes observed in the animals fed a high-fat diet containing lard are accompanied by an increase in hepatic lipogenesis and a decrease in beta-oxidation; meanwhile, in a high-fat diet containing FO, the opposite results are found, *i.e.* reduced lipogenesis and elevated beta-oxidation [8]. In adipose tissue, a high-fat diet containing lard promotes insulin resistance and infiltration of macrophages [4]. On the contrary, the group consuming a high-fat diet containing FO does not present increased body weight, adiposity or glucose intolerance, while in this group, insulin signaling, macrophage infiltration and inflammation are reduced in adipose tissue in comparison with the high-fat diet containing lard group [4]. Other studies performed in LDL-receptor-deficient mice show that ingestion of a high-fat diet containing FO decreases plasma lipid levels and increases lipid storage in white adipose tissue (WAT), as evidenced by increased total fat and perigonadal WAT weight and adipocyte hypertrophy, when compared with mice fed a high-fat diet containing olive oil [10]. An improvement in insulin sensitivity and reduced macrophage infiltration are also observed

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[10]. Furthermore, the increased adipose tissue lipid storage in FO-fed mice is associated with reduced lipid accumulation in the liver and a decreased atherosclerotic lesion area [10]. Dietary supplementation with long-chain polyunsaturated fatty acids (LC-PUFAs) of the n−3 series ameliorates the hepatic steatosis and liver injury in adult patients with nonalcoholic fatty liver [11]. The metabolic effects of DHA and EPA are largely mediated by peroxisome proliferator-activated receptor (PPAR) transcription factors, with PPARα and PPARδ (PPARβ) being responsible for the lipid-catabolizing effects of these n−3 LC-PUFAs [1,12]. PUFAs, including DHA, can also function as endogenous ligands of retinoid X receptor-α (RXR-α) while affecting lipid metabolism [12,13]. Besides that, phosphorylation and the consequent activation of AMP-activated protein kinase (AMPK) may be another pathway integrating the benefits of FO [12]. Liver AMPK phosphorylation inhibits lipogenesis and gluconeogenesis, while stimulating β-oxidation. In adipocytes, AMPK activation results in the inhibition of fatty acid synthesis and lipolysis, stimulation of glucose uptake and down-regulation of PPARγ [12].

Previous study from our group has shown that administration of a fructose-rich diet (FRUC diet, 60%) increases serum triacylglycerol (TAG) levels and the lipid content of hepatocytes, but decreases serum insulin levels and the activity of liver cytosolic lipases [14]. In adipose tissue, FRUC diet consumption increases lipogenesis, evaluated *in vitro* using ^3H_2O, and decreases the lipoprotein lipase (LPL) activity, without changing the glycerol release into the incubation medium and the cytosolic lipases activities [14]. In the present work, our main objective was to evaluate the effect of replacement of soybean oil by FO in FRUC diet on hepatic steatosis, the rate of VLDL-TAG secretion and cytosolic lipases activities, and adipose tissue glucose uptake, lipogenesis, lipolysis, cytosolic lipases and LPL activities in rats.

2. Materials and methods

2.1. Animals and treatment

Wistar male rats, aged 8–10 weeks old, were obtained from the Breeding Center of the Federal University of Minas Gerais and kept in cages in an environmentally controlled room with a 14/10 h light/dark cycle, at 25 ± 2 °C and with free access to tap water and food. The rats were divided into four experimental groups: those fed an AIN-93M diet containing soybean oil (AIN-SO) or fish oil (AIN-FO), both composed of 14.8% protein, 71.2% carbohydrate and 4% lipid (Table 1); and those fed a high-fructose (FRUC) diet containing soybean oil (FRUC-SO) or fish oil (FRUC-FO), both composed of 14.8% protein, 76.2% carbohydrate and 4% lipid (Table 1). The rats were fed for 8 weeks with the experimental diets and their body weight and food intake were carefully monitored at regular intervals. The experiments were carried out on fed or, when specified, fasted (12 h) rats between 08.30 and 10.30. For tissue removal, rats were killed by decapitation. The animals were treated according to the ethical guidelines of our institution and the experimental protocol was approved by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais (protocol n. 242/2010).

Table 1
Diet composition (g/100 g).

	AIN-SO	AIN-FO	FRUC-SO	FRUC-FO
Corn starch	45.8	45.8	16.3	16.3
Fructose	-	-	60	60
Casein	14.8	14.8	14.8	14.8
Dextrin starch	15.5	15.5	15.5	15.5
Sucrose	10	10	10	10
Soybean oil	4	-	4	-
Fish oil	-	4	-	4
Fiber	5	5	-	-
Mineral mix	3.5	3.5	3.5	3.5
Vitamin mix	1	1	1	1
L-cysteine	0.18	0.18	0.18	0.18
Choline bitartrate	0.25	0.25	0.25	0.25
Carbohydrate (%)	71.2	71.2	76.2	76.2
Protein (%)	14.8	14.8	14.8	14.8
Lipids (%)	4	4	4	4
Energy (kJ/g)	15.9	15.9	16.7	16.7

2.1.1. Hepatic histological analysis

Liver samples were fixed in 4.5% glutaraldehyde dissolved in 0.1 M phosphate buffer, pH 7.3, for 24 h. The tissue was dehydrated, embedded in paraffin and cut into sections (3 μm). Liver sections were then stained with eosin and hematoxylin for histological and morphometric analyses. Images of liver sections were captured using a digital camera coupled to an optical microscope. The mean nuclear diameter of the hepatocytes was obtained from the measurement of 30 nuclei per field, per animal, randomly selected. For this purpose, a micrometer ruler coupled to 10x ocular was used, in a final magnification of 1000x. In same magnification, the volumetric proportions of nucleus and cytoplasm of hepatocytes and the proportion of lipids in these cells were estimated using a reticulum with 441 intersections. For each animal approximately 1000 points were analyzed on hepatocytes obtaining the ratio between nucleus and cytoplasm. From the cell volume and the proportion of lipids present in the hepatocytes, the volume occupied by the lipid droplets was calculated.

2.2. Oral glucose tolerance test

A glucose tolerance test was carried out after 12 h of food deprivation by oral administration of glucose (2 g/kg body weight). Tail blood was collected at -1, 15, 30, 60, 90 and 120 min, and blood glucose concentrations were measured with a glucometer (Accu-Chek® Advantage; Roche, USA).

2.3. VLDL-TAG secretion

In 12 h-fasted rats, blood samples were taken before and 2 h after intravenous administration of Triton WR 1339 (400 mg/kg, Sigma & Aldrich, USA), an LPL inhibitor. Serum TAG concentration was assayed by an enzymatic procedure with a commercial kit (Labtest®, Santa Luzia, Brazil).

2.4. In vitro lipogenesis

Portions of epididymal adipose tissue (200 mg) were incubated at 37 °C for 3 h in buffer containing (mM): 137 NaCl, 5 KCl, 4.2 $NaHCO_3$, 1.3 $CaCl_2$, 0.5 $MgCl_2$, 0.5 $MgSO_4$, 0.5 KH_2PO_4 and 20 HEPES (pH 7.4), plus 1% BSA, 5 mM glucose and 150 μCi/mL of ^3H_2O as a radioisotopic tracer. At the end of the incubation period, [^{3}H]-total lipids were extracted with chloroform–methanol 2:1 [15] and lipid counting was performed by adding a toluene–diphenyloxazole scintillation fluid (5 g/L). Rates of lipid synthesis were calculated as previously described [16].

2.5. Adipocyte isolation and glucose uptake

Adipocytes were isolated from epididymal fat pads by the method used by Rodbell [17]. Digestion was carried out at 37 °C with constant shaking for 45 min. Cells were filtered through nylon mesh and washed three times with buffer containing (mM): 137 NaCl, 5 KCl, 4.2 $NaHCO_3$, 1.3 $CaCl_2$, 0.5 $MgCl_2$, 0.5 $MgSO_4$, 0.5 KH_2PO4 and 20 mM HEPES (pH 7.4), plus 1% BSA. Aliquots of adipocytes in suspension were incubated for 45 min at 37 °C in the presence or absence of insulin (25 ng/mL). The uptake of 2-deoxy-[^{3}H]glucose (2DOG) was used to determine the rate of glucose transport, as previously described [18]. Briefly, glucose uptake was initiated by the addition of 2DOG (0.2 μCi/tube) for 3 min. Thereafter, cells were separated by centrifugation through silicone oil and cell-associated radioactivity was determined by scintillation counting. Non-specific association of 2DOG was determined by performing parallel incubations in the presence of 15 mmol/L phloretin, and this value was subtracted from glucose transport activity in each condition.

2.6. Lipolysis measurements

Portions of epididymal adipose tissue (200 mg) were incubated at 37 °C for 1 h in buffer containing (mM): 137 NaCl, 5 KCl, 4.2 $NaHCO_3$, 1.3 $CaCl_2$, 0.5 $MgCl_2$, 0.5 $MgSO_4$, 0.5 KH_2PO_4 and 20 HEPES (pH 7.4), plus 1% BSA and 5 mM glucose in the presence or absence of 0.1 μM isoproterenol (ISO), a non-specific beta-receptor agonist. The effects of 50 ng/mL insulin on ISO-stimulated lipolysis were also determined. Glycerol release was measured and used as lipolytic index, as previously described [19].

2.7. Enzyme activity measurement

2.7.1. Lipoprotein lipase activity

Samples of epididymal adipose tissue (150 mg) were homogenized in buffer containing 0.2 M Tris, pH 8.3, containing 5000 UI/mL heparin, 12 mM deoxycholate and 10% Triton-x100 [20]. The mixture of substrate contained 69 mg of triolein, 3.3 mg of lysolecithin in chloroform and 12.5 μCi of [9,10-^{3}H]triolein in toluene [21]. Solvents were evaporated under a gentle stream of N_2, glycerol was added and the mixture was sonicated 5 × 60 s with a 60 s interval between sonications. After 24 h, the reaction mixture was prepared with two parts of LPL buffer, containing 0.2 M Tris, pH 8.3, 6% BSA, 0.15 M NaCl, two parts of substrate mixture and one part of serum from 24 h-fasted rats as a source of apo-CII. The serum was pretreated by heating at 55 °C to inactivate non-specific plasma proteases. For each assay, the sample was incubated with reaction mixture for 60 min at 37 °C with shaking. Blank and inhibited (1 M NaCl) tubes were included. The reactions were stopped by addition of 3.25 mL methanol/chloroform/