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Chronic fish oil supplementation partially reverses renal alterations in mice fed with a high-fat diet ☆

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ABSTRACT

The objective of this study was to investigate the alterations in renal morphology and function in an animal model of obesity, and to determine the possible effect of fish oil (FO) supplementation on these alterations. Six-week-old male mice were fed during eight weeks with regular rodent chow (R) or a high-fat diet (HFD), 53.09% fat. After this, they were supplemented orally for 30 days with FO. The weight gain of HFD group was almost three times higher than R. Albuminuria induced by HFD was significantly reduced by FO. The reduction in fractional sodium excretion (FE_{Na+}) observed in HFD group was reversed by FO. The HFD was able to increase in almost 100% the concentration of TNF-α in renal tissue, an effect reversed by FO. Intrarenal expression of vimentin was significantly higher in tubulointerstitial cells of HFD group, an effect reversed by FO. In summary, FO partially reverses renal alterations induced by an HFD.

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1. Introduction

The prevalence of obesity and overweight has rapidly increased to pandemic proportions, especially in children (Börgeson & Sharma, 2013; Kopple & Feroze, 2011). Obesity and overweight are associated with insulin resistance, diabetes, dyslipidaemia, atherosclerosis, and hypertension, which are factors of metabolic syndrome (Declèves, Mathew, Cunard, & Sharma, 2011). Obesity increases the risk of cardiovascular disease, and acts directly or indirectly (via diabetes and hypertension) in the development of kidney disease (Börgeson & Sharma, 2013; Ejerblad et al., 2006).

Previous studies in humans and animal models of obesity have demonstrated that fat intake can be an important factor responsible for the increase in adiposity (Hariri & Thibault, 2010; Woods, Seeley, Rushing, D'Alessio, & Tso, 2003). Studies in rats and mice have indicated a positive relationship between the level of fat in the diet and the increase in body weight or fat gain. In addition, the ingestion of a high-fat diet (HFD) for a determined period can induce obesity and related metabolic syndromes in animal models (Deji, Kume, & Araki, 2011; Ghibaudi, Cook, Farley, Van Heek, & Hwa, 2002; Hariri & Thibault, 2010; Srinivasan & Ramarao, 2007). Microalbuminuria, focal and segmental glomerulosclerosis, and altered sodium handling are some of the complications related to obesity that could lead to chronic kidney disease (CKD) (Declèves et al., 2011; Hall, 2003; Praga & Morales, 2006; Tesauro et al., 2012).

These renal alterations are associated with systemic and local inflammatory states, such as the increase in secretion of pro-inflammatory cytokines including tumour necrosis factor (TNF)-α, interleukin (IL)-6, and transforming growth factor (TGF)-β; reduction in anti-inflammatory adipokine (adiponectin) secretion; and activation of the renin–angiotensin–aldosterone system and the sympathetic nervous system, all of which are induced by obesity (Hall, 2003; Jia, Carrero, Lindholm, & Stenvinkel, 2012; Tesauro et al., 2012).

Omega-3 polyunsaturated fatty acids (n-3 PUFA), mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) obtained from marine fatty fish and fish oil, and alpha-linolenic acid extracted from plant sources, have been shown to exhibit anti-inflammatory and anti-proliferative properties. n-3 PUFA inhibit inflammation by reducing the production of pro-inflammatory cytokines such as the monocyte chemoattractant protein (MCP)-1 and TNF-α, which could result in the prevention or inhibition of inflammatory signalling pathway (NF-κB and ERK) activation, which characterizes chronic metabolic diseases, such as obesity, diabetes, and kidney disease (Diaz Encarnacion et al., 2011; Lorente-Cebrián et al., 2013; Wu et al., 2012). In addition, n-3 PUFA supplementation was observed to attenuate tubulointerstitial injury in a CKD animal model, by decreasing NAD(P)H oxidase, TGF-β, connective tissue growth factor, α-smooth muscle actin (α-SMA), fibronectin, and MCP-1 activation, and attenuating ERK 1/2 and NF-κB activation (An, Kim, Cho, & Vaziri, 2009). Thereby, n-3 PUFA assisted in the reduction of oxidative stress, inflammation, fibrosis, and epithelial-to-mesenchymal transition, processes that would culminate in end-stage renal failure (An et al., 2009; Hong & Lu, 2013).

As obesity is associated with renal injury, the anti-inflammatory and anti-proliferative properties of n-3 PUFA could be useful in its prevention. So, the aim of this study was to investigate the alterations in renal morphology and function in an animal model of obesity (induced by an HFD), and to determine the possible effect of fish oil supplementation on these alterations.

2. Materials and methods

2.1. Animals, diets and supplementation

All experimental procedures were carried out in accordance with the ethical principles established by the Experimental Brazilian Council (COBEA) and approved by the local Animal Ethics Committee (protocol n°476B). Six-week-old male Swiss mice were maintained under controlled temperature (22 ± 3 °C) and humidity, in a 12 h/12 h light/dark cycle. The following diets were used: commercial regular chow (Nuvilab CR-1 from Nuvital Nutrients, Curitiba, Brazil, 67.3% carbohydrate, 23.5% protein and 9.2% fat) and a manipulated high-fat diet (26% carbohydrate, 15% protein and 53.09% fat) (Araújo et al., 2007). The exact composition of the high-fat diet was: corn starch 115.5 g/kg; casein 200 g/kg; dextrinated starch 132 g/kg; sucrose 100 g/kg; soybean oil 35 g/kg; lard 315 g/kg; cellulose 50 g/kg; mineral mix 35 g/kg; vitamin mix 10 g/kg; L-cystine 3 g/kg; choline 5 g/kg. The caloric content of the diets was 3 kcal/g for regular and 5.3 kcal/g for high-fat diet. Initially, animals were randomly divided into two groups: regular chow (R) and high-fat diet (HFD), and received their respective diets for eight weeks. After this period, animals were divided into four groups for fish oil (FO) supplementation: R (regular chow); RFO (regular chow plus FO); HFD (high-fat diet); HFO (high-fat diet plus FO) for additional period of four weeks. The FO used was a mixed marine triacylglycerol preparation containing 180 g eicosapentaenoic acid (EPA) and 120 g docosahexaenoic acid (DHA) per kg. The oil supplement was administered at a dose of 1 g/kg body weight per day and was given orally as a single bolus using a precision microliter pipette. Body weight was determined every 2 days for adjustment of the fish oil supplementation. Food was administered daily and the remaining food was weighed to calculate the average food intake. Fish oil supplementation increased the overall caloric intake by only 1.5–2.3% of control or obese animals, respectively. In a previous study of our group, working with the same dose, we observed that food consumption was not modified by FO supplementation (Fernandez, Piechnik, Fabris, Malnic, & Fernandes, 2004).

2.2. Fatty acid composition of renal tissue

The quantification of fatty acid composition in renal tissue was performed by high-performance liquid chromatography (HPLC) in three steps: extraction, saponification and derivatization of fatty acids, as previously described (Bonatto et al., 2015). First, total lipids were extracted from renal tissue using chloroform-methanol (2:1 v/v) according to Folch, Lees and Stanley (1957). After centrifugation and removal of lipid phase, 1 mL of Folch solution was added (methanol 48,98%; water 47,96%; chloroform 3,06%; $MgCl_2$ 0,017%; $CaCl_2$ 0,02%; NaCl 0,37%) three times, isolated and lipid phase evaporated until dry under gentle