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Eicosapentaenoic acid (EPA) *vs*. Docosahexaenoic acid (DHA): Effects in epididymal white adipose tissue of mice fed a high-fructose diet☆

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ABSTRACT

Background: Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been demonstrated to be beneficial for many diseases, including those associated with the metabolic syndrome (e.g. insulin resistance and hypertension). Nevertheless, not only their actions are not entirely understood, but also their only effects were not yet elucidated. Therefore, we aimed to compare the effects of EPA and DHA, alone or in combination, on the epididymal white adipose tissue (WAT) metabolism in mice fed a high-fructose diet.

Methods: 3-mo-old C57Bl/6 mice were fed a control diet (C) or a high-fructose diet (HFru). After three weeks on the diets, the HFru group was subdivided into four new groups for another five weeks: HFru, HFru + EPA, HFru + DHA, and HFru-EPA + DHA (n = 10/group). Besides evaluating biometric and metabolic parameters of the animals, we measured the adipocyte area and performed molecular analyses (inflammation and lipolysis) in the epididymal WAT.

Results: The HFru group showed adipocyte hypertrophy, inflammation, and uncontrolled lipolysis. The treated animals showed a reversion of adipocyte hypertrophy, inhibition of inflammation with activation of anti-inflammatory mediators, and regularization of lipolysis. Overall, the beneficial effects were more marked with DHA than EPA.

Conclusion: Although the whole-body metabolic effects were similar between EPA and DHA, DHA appeared to be the central actor in WAT metabolism, modulating pro and anti-inflammatory pathways and alleviating adipocytes abnormalities. Therefore, when considering fructose-induced adverse effects in WAT, the most prominent actions were observed with DHA.

1. Introduction

The high consumption of fructose is now a global problem. Although present in small quantities in natural food, the large amounts of fructose seen in industrial products like corn syrup and sweetened beverages are now part of the individual's eating habits [1]. This high intake of fructose has been associated with insulin resistance, hypertension, and metabolic syndrome, also encompassing detrimental alterations in adipose tissue [2]. Although these effects may still be controversial [3], fructose appears to promote cardiovascular diseases, type 2 diabetes, and metabolic syndrome by increasing its uptake and metabolism in the liver [3,4].

White adipose tissue (WAT) is responsible not only for stocking energy but also for the production and secretion of essential metabolism

Abbreviations: AKT, protein kinase B; AMPK, AMP-activated protein kinase; aP2, adipocyte protein 2; ATGL, adipose triglyceride lipase; beta3-AR, *beta* 3-adrenergic receptor; BM, body mass; CD36, cluster of differentiation 36; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ERK, extracellular signal-regulated kinase; HOMA-IR, homeostasis model assessment of insulin resistance index; HSL, hormone-sensitive lipase; IL, interleukin; JNK, c-Jun N-terminal kinase; MCP, monocyte chemotactic protein; NFκB, nuclear factor *kappa* B; OGTT, oral glucose tolerance test; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acids; Q_A, numerical density per area; BP, blood pressure; TC, total cholesterol; TG, triglycerides; TNF, tumor necrosis factor; Vv, volume density; WAT, white adipose tissue

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mediators. The ingested fructose is driven mainly to the liver and the kidneys, although some considerable amount reaches WAT. This tissue can efficiently uptake fructose [5], and in WAT, fructose can alter its physiological function, inducing adipocyte hypertrophy, local insulin resistance, local inflammation [6], and dysregulated lipolysis [5].

The inflammation in WAT involves the secretion of cytokines (like tumor necrosis factor, TNF alpha, interleukin, IL6, and monocyte chemotactic protein, MCP1). Various inflammatory pathways regulate the cytokine secretion, including the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and nuclear factor *kappa* B (NFκB). Also, it involves the reduction of anti-inflammatory mediators like IL-10 and adiponectin that are controlled, among others, by peroxisome proliferator-activated receptor (PPAR) gamma. Moreover, local insulin resistance is characterized by several alterations that include a reduction of protein kinase B (AKT) phosphorylation, and, on the contrary, AMP-activated protein kinase (AMPK) phosphorylation improves glucose metabolism. Lastly, the dysregulated lipolysis is mediated by alterations in perilipin, adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL), which act in the lipid droplet promoting triglycerides (TG) hydrolysis into fatty acids [7–10].

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are n-3 polyunsaturated fatty acids (PUFA) that have beneficial effects on cardiovascular and inflammatory diseases, and also during metabolic syndrome and its comorbidities [11]. However, most studies have used fish oil as a source of these fatty acids. Therefore, distinct effects of EPA or DHA and their specific mechanisms remain to be fully understood [12]. Studies have already reported that EPA and DHA have some differential effects. Thus, DHA was more effective than EPA in reducing the hepatic alterations related to nonalcoholic steatohepatitis in $Ldlr^{-/-}$ mice [13]. In macrophages, DHA was more potent than EPA in diminishing inflammation [14], and in accordance, DHA attenuated systemic inflammation in a greater extent than EPA [15]. Therefore, EPA and DHA appear to have different roles that were not yet fully investigated, especially in WAT. Based on that, we aimed to evaluate the effects of EPA and DHA, alone or in combination, on the WAT metabolism in mice fed a high-fructose diet.

2. Materials and methods

2.1. Animals and diet

All procedures followed the standard guidelines for animal experimentation (NIH Publication number 85-23, revised 1996) and were approved by the local Ethics Committee for Animal Experimentation (Protocol Number CEUA/041/2015). The mice were maintained under controlled conditions (20 ± 2 °C, 12 h/12 h dark/light cycle) in ventilated cages (NexGen system, Allentown Inc., PA, USA), and had free access to food and water, which were monitored daily. Fifty C57Bl/6 male mice with three months of age were randomly assigned to two groups: a control group (C) and a high-fructose group (HFru). After three weeks of the respective diets, animals from the HFru group were further subdivided into four groups, forming a total of five groups, as described below. We used n = 10/group, with n = 5 used for adipocyte area and the other n = 5 for molecular analysis.

a) Control group (C): 76% of the total energy content of carbohydrates;
b) High-fructose group (HFru): 47,43 g/100 g diet of fructose, 76% of the total energy content of carbohydrates;
c) High-fructose + eicosapentaenoic acid group (HFru-EPA): 2% of the total energy content of EPA;
d) High-fructose + docosahexaenoic acid group (HFru-DHA): 2% of the total energy content of DHA;
e) High-fructose + eicosapentaenoic and docosahexaenoic acid group (HFru-EPA + DHA): 2% of the total energy content of EPA + DHA.

These diets were administrated for another five weeks, totalizing eight weeks of the experiment (three weeks on diets plus five weeks of EPA and DHA treatment). The diets were elaborated with purified nutrients by PragSolucoes (Jau, SP, Brazil) based on the American Institute of Nutrition's recommendations (AIN 93 M) [16] (Table 1). All diets contained soybean oil, as recommended by the AIN93 for the adequate supply of essential fatty acids [16]. EPA (Carb - FE22647; purity ≥ 96%) and DHA (Carb - FD01734; purity ≥ 98%), in the form of free fatty acids, were purchased from Carbosynth (Berkshire, UK). The diets were stored in the freezer and changed every day to avoid lipid degradation. Moreover, the antioxidants were raised from 0.008 g/kg in the C diet to 0.060 g/kg in the supplemented diets.

Table 1
Composition and energy content of the diets (based on the AIN 93 M recommendations).

Ingredients (g/kg)	C	HFru	HFru-EPA	HFru-DHA	HFru-EPA + DHA
Casein (≥ 85% of protein)	140.0	140.0	140.0	140.0	140.0
Cornstarch	620.7	146.4	146.4	146.4	146.4
Sucrose	100.0	100.0	100.0	100.0	100.0
Fructose	–	474.3	474.3	474.3	474.3
Soybean oil	40.0	40.0	31.53	31.53	31.53
EPA	–	–	8.47	–	4.235
DHA	–	–	–	8.47	4.235
Fiber	50.0	50.0	50.0	50.0	50.0
Vitamin mix	10.0	10.0	10.0	10.0	10.0
Mineral mix	35.0	35.0	35.0	35.0	35.0
L-Cystin	1.8	1.8	1.8	1.8	1.8
Choline	2.5	2.5	2.5	2.5	2.5
Total mass (g)	1000.0	1000.0	1000.0	1000.0	1000.0
Proteins (% Energy)	14	14	14	14	14
Carbohydrates (% Energy)	76	76	76	76	76
Fructose (% Energy)	–	50	50	50	50
Lipids (% Energy)	10	10	10	10	10
EPA (% Energy)	–	–	2	–	1
DHA (% Energy)	–	–	–	2	1
Energy content (kcal/kg)	3811	3811	3811	3811	3811

Abbreviations: control (C), high-fructose (HFru), high-fructose plus eicosapentaenoic acid (HFru-EPA), high-fructose plus docosahexaenoic acid (HFru-DHA), and high-fructose plus eicosapentaenoic and docosahexaenoic acids (HFru-EPA + DHA) diets.

2.2. Body mass and blood pressure

Body mass (BM) was measured weekly. For systolic blood pressure (BP) evaluation, the animals were trained for two weeks in constraint conditions before the measurement of BP to minimize their stress. Every other week, BP was measured by tail-cuff plethysmography (Letica LE 5100, Harvard/Panlab, Barcelona, Spain) in conscious mice.

2.3. Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was achieved at the end of the experiment using a 25% glucose solution (1.0 g/kg) administered by orogastric gavage after a 6-h fasting period. Blood was obtained from the tail vein. Glycemia was measured (glucometer Accu-Check, Roche, SP, Brazil) before glucose administration (0 min, which was considered the basal glycemia) and 15, 30, 60, and 120 min after glucose administration. The results are presented as the 'area under the curve' (GraphPad Prism version 7.03, La Jolla, CA, USA).

2.4. Blood analyses

At sacrifice, animals were deprived of food for six hours, and then they were deeply anesthetized (150 mg/kg sodium pentobarbital intraperitoneal). Blood samples were obtained, the serum was separated