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Effects of ALA, EPA and DHA in high-carbohydrate, high-fat diet-induced metabolic syndrome in rats

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Abstract

We compared the cardiovascular, hepatic and metabolic responses to individual dietary n-3 fatty acids (α-linolenic acid, ALA; eicosapentaenoic acid, EPA; and docosahexaenoic acid, DHA) in a high-carbohydrate, high-fat diet-induced model of metabolic syndrome in rats. Additionally, we measured fatty acid composition of plasma, adipose tissue, liver, heart and skeletal muscle in these rats. The same dosages of ALA and EPA/DHA produced different physiological responses to decrease the risk factors for metabolic syndrome. ALA did not reduce total body fat but induced lipid redistribution away from the abdominal area and favorably improved glucose tolerance, insulin sensitivity, dyslipidemia, hypertension and left ventricular dimensions, contractility, volumes and stiffness. EPA and DHA increased sympathetic activation, reduced the abdominal adiposity and total body fat and attenuated insulin sensitivity, dyslipidemia, hypertension and left ventricular stiffness but not glucose tolerance. However, ALA, EPA and DHA all reduced inflammation in both the heart and the liver, cardiac fibrosis and hepatic steatosis. These effects were associated with complete suppression of stearoyl-CoA desaturase 1 activity. Since the physiological responses to EPA and DHA were similar, it is likely that the effects are mediated by DHA with EPA serving as a precursor. Also, ALA supplementation increased DHA concentrations but induced different physiological responses to EPA and DHA. This result strongly suggests that ALA has independent effects in metabolic syndrome, not relying on its metabolism to DHA.

Keywords: Metabolic syndrome; Omega-3; Fish oil; Chia oil; Cardiovascular

1. Introduction

The role of selected dietary fatty acids in the prevention or progression of chronic diseases has generated long-standing interest [1–5]. It is now generally accepted that the type of dietary fat plays a far more significant role in health and disease than the absolute amount [6–8]. Depending on the nutritional lifestyle, dietary fat includes varying quantities of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), mainly as n-3 and n-6 PUFA [6]. The Western diet, characterized by excessive amounts of SFA, n-6 PUFA and *trans* fatty acids with decreased intake of n–3 PUFA, has been implicated in chronic diseases such as obesity, diabetes, cardiovascular and renal diseases as well as inflammatory diseases such as arthritis [6–9]. We have reported that the chronic changes following a high-carbohydrate, high-fat diet in rats mimic the metabolic syndrome in humans [10] and, further, that interventions with natural products such as purple carrots [11], olive leaf extract [12], chia seeds [13] and rutin [14] can reverse these changes.

The three major dietary n-3 PUFA, α-linolenic acid (ALA; C18:3n-3), eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) may produce distinctly different responses on the risk factors for metabolic syndrome [15]. Although n-3 PUFA are a well-studied class of bioactive molecules, the effectiveness of ALA, the primary fatty acid of the n-3 pathway, in metabolic syndrome is less clear than its elongated metabolites, EPA and DHA. Further, it is still unclear if EPA and DHA have independent physiological effects as most studies have only studied EPA and DHA mixtures such as fish oil capsules or oily fish diet or supplements [15] rather than the individual fatty acids. Also, the paucity of comparative studies involving all three individual n-3 PUFA provides a weak basis for assuming different responses in the pathophysiology of chronic diseases [15].

Abbreviations: ALA, α-linolenic acid; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; DHA, docosahexaenoic acid; DXA, dual energy X-ray absorptiometry; EPA, eicosapentaenoic acid; ITT, insulin tolerance test; IVSd, interventricular septal thickness in diastole; IVSs, interventricular septal thickness in systole; LDH, lactate dehydrogenase; LV, left ventricle; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; LVPWd, left ventricular posterior wall thickness in diastole; LVPWs, left ventricular posterior wall thickness in systole; MUFA, monounsaturated fatty acid; NEFA, non-esterified fatty acid; OGTT, oral glucose tolerance test; PUFA, polyunsaturated fatty acid; SCD, stearoyl-CoA desaturase; SFA, saturated fatty acid.

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In high-carbohydrate, high-fat-diet fed rats, we have shown that chia seed, one of the richest dietary sources of ALA, improved insulin sensitivity and glucose tolerance, reduced visceral adiposity, decreased hepatic steatosis and reduced cardiac and hepatic inflammation and fibrosis without changes in plasma lipids or blood pressure [13]. Chia seed supplementation induced lipid redistribution with lipid trafficking away from the visceral fat and liver with increased accumulation in the heart. These effects were associated with the depletion of MUFA, the products of stearoyl-CoA desaturase-1 (SCD-1) activity, in the heart, liver and adipose tissue together with an increase in SFA concentrations. The C18:1*trans*-7 was preferentially stored in the adipose tissue; the relatively inert C18:1n-9 was stored in sensitive organs such as the liver and the heart, and C18:2n-6, the parent fatty acid of the n-6 pathway, was preferentially metabolized. Based on these results, we suggested that these effects were mediated by ALA rather than by metabolism to EPA or DHA [13].

In this study, we compared the effects of ALA-rich chia oil and fish oils enriched with either EPA or DHA on cardiovascular, hepatic and metabolic parameters in a diet-induced rat model of the human metabolic syndrome. Additionally, we investigated the changes in fatty acid composition of plasma, adipose tissue, liver, heart and skeletal muscle after dietary supplementation with these n-3 PUFA. In addition to serving as a control to the high-carbohydrate, high-fat diet (24% fat, mostly *trans* and SFA without n-3 PUFA), a corn starch-rich diet (0.8% fat, with comparable proportions of SFA, MUFA and PUFA without *trans* and little n-3 PUFA) also served as a model of a low fat diet to ascertain the responses to the individual n-3 PUFA without interactions with other dietary fatty acids (Table 1).

2. Materials and methods

2.1. Rats and diets

The experimental groups consisted of 96 male Wistar rats (9–10 weeks old) supplied by The University of Queensland Biological Resources unit and individually housed in a temperature-controlled, 12-h light/dark cycle environment with ad libitum access to water and the group-specific rat diet at the University of Southern Queensland Animal House. All experimentation was approved by the Animal Experimentation Ethics Committees of The University of Queensland and the University of Southern Queensland under the guidelines of the National Health and Medical Research Council of Australia. The rats were randomly divided into eight separate groups (n=12 each) and fed with corn starch (C; weighing 340±2 g), corn starch+ALA-rich chia oil (CA; 339±2 g), corn starch+EPA oil (CE; 338±1 g), corn starch+DHA oil (CD; 337±2 g), high-carbohydrate, high-fat (H; 339±3 g), high-carbohydrate, high-fat+ALA-rich chia oil (HA; 336±2 g), high-carbohydrate, high-fat +EPA oil (HE; 332±2 g) and high-carbohydrate, high-fat+DHA oil (HD; 337±1g). Measurements of body weight and food and water intakes were taken daily and feed efficiency (%) calculated as described in our previous study [13].

Table 1
Composition and fatty acid profiles of the diet and the oil supplements (chia oil, EPA oil, and DHA oil)

Composition	Basal diets		Oil supplements[a]		
	C	H	Chia oil	EPA oil	DHA oil
Ingredients					
Corn starch, g/kg	570.0	-	-	-	-
Powdered rat feed[b], g/kg	155.0	155.0	-	-	-
HMW salt mixture[c], g/kg	25.0	25.0	-	-	-
Fructose, g/kg	-	175.0	-	-	-
Beef tallow, g/kg	-	200.0	-	-	-
Condensed milk, g/kg	-	395.0	-	-	-
Water, ml/kg	250.0	50.0	-	-	-
Total energy density, kJ/g	11.2	17.9	37.0	37.8	37.8
Macronutrient composition, g/kg					
Total Carbohydrate	600.2	515.7	-	-	-
Total Fat[d]	8.1	239.0	-	-	-
Total Protein	31.8	58.1	-	-	-
Total Fiber	7.4	7.4	-	-	-
Total Vitamins	0.3	0.3	-	-	-
Total Minerals	0.1	0.4	-	-	-
Ash	0.6	0.0	-	-	-
Total Moisture	296.3	124.0	-	-	-
Fatty acid (g/100 g of total recovered fatty acid) (n=3/group)					
C14:0	10.0±0.9	3.7±0.1	0.0±0.0	0.0±0.0	0.0±0.0
C16:0	17.5±0.4	24.6±0.5	7.1±0.07	0.0±0.0	0.0±0.0
C16:1n-7	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
C18:0	0.6±0.0	24.2±0.2	3.8±0.02	0.0±0.0	0.0±0.0
C18:1n-9	34.5±2.3	0.9±0.5	7.6±0.01	0.0±0.0	0.0±0.0
C18:1*trans*-7	0.0±0.0	40.8±0.8	0.0±0.0	0.0±0.0	0.0±0.0
C18:2n-6	30.5±0.8	2.7±0.1	21.3±0.01	0.0±0.0	0.0±0.0
C18:3n-3	4.7±0.1	0.1±0.1	59.7±0.03	0.0±0.0	0.0±0.0
C20:0	0.0±0.0	0.2±0.1	0.3±0.0	0.8±0.01	0.0±0.00
C20:4n-6	0.0±0.0	0.0±0.0	0.0±0.0	3.9±0.01	0.8±0.01
C20:3n-3	0.0±0.0	0.0±0.0	0.0±0.0	1.2±0.01	0.3±0.01
C20:5n-3	0.0±0.0	0.0±0.0	0.0±0.0	86.6±0.05	5.6±0.03
C22:2n-6	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.0	6.2±0.00
C22:4n-6	0.0±0.0	0.0±0.0	0.0±0.0	0.5±0.0	2.9±0.02
C22:5n-3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
C22:6n-3	0.0±0.0	0.0±0.0	0.0±0.0	6.7±0.02	84.2±0.03
Total SFA	28.3±1.4	53.1±0.7	11.3±0.05	0.8±0.01	0.0±0.0
Total MUFA	35.7±2.3	44.1±0.6	7.6±0.01	0.0±0.0	0.0±0.0
Total PUFA	36.0±0.9	2.8±0.0	81.1±0.04	98.9±0.01	93.8±0.00

[a] Oil supplemented diets prepared by adding 30 ml of respective oil in the basal diets replacing equivalent amounts of water.
[b] Meat-free rat and mouse feed (Speciality Feeds; Glen Forrest, WA, Australia) contains (g/kg of feed): carbohydrate, 707.1; proteins, 194.0; fat, 48.0; fiber, 48.0; total vitamins, 2.1 and total minerals, 0.85.
[c] Hubble, Mendel and Wakeman salt mixture (Hubbell RB, Mendel LB, Wakeman AJ. New salt mixture for use in experimental diets. *J Nutr.* 1937;14:273–85.) (MP Biochemicals, Seven Hills, NSW, Australia).
[d] Primarily derived from powdered rat feed in the C diet.