High-Fructose Intake Impairs the Hepatic Hypolipidemic Effects of a High-Fat Fish-Oil Diet in C57BL/6 Mice

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Background: **Overnutrition of saturated fats and fructose is one of the major factors for the development of nonalcoholic fatty liver disease. Because omega-3 polyunsaturated fatty acids (n-3fa) have established lipid lowering properties, we tested the hypothesis that n-3fa prevents high-fat and fructose-induced fatty liver disease in mice.** ***Methods:*** **Male C57BL/6J mice were randomly assigned to one of the following diet groups for 14 weeks: normal diet (ND), high-fat lard-based diet (HFD), HFD with fructose (HFD + Fru), high-fat fish-oil diet (FOD), or FOD + Fru.** ***Results:*** **Despite for the development of obesity and insulin resistance, FOD had 65.3% lower ($P < 0.001$) hepatic triglyceride levels than HFD + Fru, which was blunted to a 38.5% difference ($P = 0.173$) in FOD + Fru. The lower hepatic triglyceride levels were associated with a lower expression of lipogenic genes LXRα and FASN, as well as the expression of genes associated with fatty acid uptake and triglyceride synthesis, CD36 and SCD1, respectively. Conversely, the blunted hypotriglyceride effect of FOD + Fru was associated with a higher expression of CD36 and SCD1.** ***Conclusions:*** **During overnutrition, a diet rich in n-3fa may prevent the severity of hepatic steatosis; however, when juxtaposed with a diet high in fructose, the deleterious effects of overnutrition blunted the hypolipidemic effects of n-3fa. (J Clin Exp Hepatol 2016;6:265–274)**

For the past decade, the growing prevalence of overweight and obesity is a major global health problem responsible for the rapid rise in the incidence of nonalcoholic fatty liver disease (NAFLD).[1] NAFLD is a multifactorial progressive liver disorder that is the result of hepatic insulin resistance combined with increased fatty acid uptake and *de novo* lipogenesis.[2] NAFLD is quickly becoming one of the most common liver diseases present in 20–30% of the Western population and increasing up to approximately 90% in morbidly obese individuals. The prevalence of NAFLD parallels the increase in obesity and type 2 diabetes mellitus (T2DM) with hepatic steatosis present in approximately 70% of patients with T2DM.[3,4]

Overnutrition, defined as the chronic consumption of a calories consisting of 40–60% fat and 15–20% fructose to mimic the prevailing diet in the western society,[5] is one of the major contributing factors for the increased incidence of obesity and related comorbidities. The American Heart Association's dietary guidelines recommend the consumption of a low saturated fat/high complex carbohydrate diet for weight loss and to reduce the risk for coronary heart disease.[6] In contrast to these recommendations, during the past 40 years, the consumption of fructose and rapidly absorbed carbohydrates has increased in most industrialized countries.[7] Moreover, low-fat diets that are high in refined carbohydrates have been tied to dyslipidemia, insulin resistance, and hypertension in comparison to high-fat diets that are low in refined carbohydrates or high in complex carbohydrates.[8,9]

The use of omega-3 polyunsaturated fatty acids (n-3fa), such as docosahexaenoic acid (DHA; C22:6) and eicosapentaenoic acid (EPA; C20:5), may provide health professionals a nonpharmacological therapeutic strategy for the treatment of NAFLD. EPA and DHA are commonly consumed through either diet (*e.g.*, fatty fish) or marine and synthetic-based supplements.[10] The American Heart Association recommends the consumption of an average of 1 g/day of EPA/DHA or two servings of fatty fish weekly.[11] In contrast, the World Health Organization recommends an intake of about 400–1000 mg/day.[12] A definitive therapeutic dosage of n-3fa for the treatment of NAFLD remains controversial, in part because an unclear understanding of the bioactive properties of n-3fa during

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Abbreviations: ACC1: acetyl-CoA carboxylase-1; CPT1a: carnitine palmitoyltransferase 1a; ChREBP: carbohydrate response element binding protein; FASN: fatty acid synthase; FFA: free fatty acid; LPL: lipoprotein lipase; LXRα: liver-X-receptor; MTTP: microsomal triglyceride transfer protein; n-3fa: omega-3 polyunsaturated fatty acids; NAFLD: nonalcoholic fatty liver disease; PPARα: peroxisome proliferator activated receptor α; PPARγ: peroxisome proliferator activated receptor γ; SCD1: stearoyl-CoA desaturase 1; SREBP1c: sterol response element binding protein; T2DM: type 2 diabetes mellitus; TRL: triglyceride-rich lipoproteins; VLDL: very low-density lipoprotein
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overnutrition and its interaction with other bioactive nutrients.

The primary treatment strategy for patients with NAFLD is lifestyle modification with an emphasis on weight loss, increased physical activity, and dietary modifications. Diet appears to be central to development of obesity-induced hepatic steatosis; however, the causal relationship remains to be elucidated. Presently, there are no specific dietary guidelines for the treatment of NAFLD due to an incomplete understanding of the pathophysiology of this condition; however, the development of safe, nontoxic therapeutic options is needed to reverse the metabolic disturbances observed in NAFLD.[10] We hypothesized that a high-fat fish-oil diet will prevent hepatic steatosis induced by overnutrition of a diet high in fat and fructose. To test our hypotheses, we examined the contrasting dietary effects of fish oils and fructose on hepatic steatosis by examining the expression of hepatic genes that regulate lipid metabolism. Our specific aims for this project were to determine the therapeutic potential of fish oils for the treatment of NAFLD during continued overnutrition, as well as to develop a better understanding of the bioactive properties of fish oils and fructose when combined on genes central to development of NALFD during an overnutrition state.

MATERIALS AND METHODS

Experimental Animals and Protocols

All experimental procedures were approved by the Institutional Animal Care and Use Committee at Southern Illinois University Edwardsville. All animal experimentations were conducted in accordance with accepted standards of humane use and care of laboratory animals for biomedical research published by the National Institutes of Health (No. 85-23, Revised 1996). Male C57BL/6J mice were acquired from Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age and maintained at a 12-h light:12-h dark cycle. Animals (n = 2–3/cage) were housed in an individually ventilated cage system and fed *ad libitum* on a standard low-fat diet (D12450B) until 8 weeks of age. Research Diets, Inc. (New Brunswick, NJ) formulated all diets for this study.

At 8 weeks of age, mice were randomly placed in one of the three dietary treatment groups for 14 weeks. Diet formulations are shown in Table 1. The following are the three diets: a normal diet (D12450B, 10% of fat calories from lard and soybean oil; ND; n = 8), a high-fat diet (D12492, 60% of fat calories from lard and soybean oil; HFD; n = 10), and a high-fat Menhaden fish-oil diet (D09020505, 60% of fat calories at a ratio of 63.0:27.7:9.3 from Menhaden fish oil:

Table 1 Diet Ingredients and Relative Composition of the Low-fat and High-fat Diets.

Variable	ND		HFD		FOD	
	Grams	% kcal	Grams	% kcal	Grams	% kcal
Kcal/g		3.85		5.24		5.24
Protein		20.0		20.0		20.0
Casein, 30 Mesh	200	98.5	200	98.5	200	98.5
L-Cystine	3	1.5	3	1.5	3	1.5
Carbohydrate		70.0		20.0		20.0
Corn starch	315	45.0	0	0	0	0
Maltodextrin	35	5.0	125	64.5	125	64.5
Sucrose	350	50.0	68.8	35.5	68.8	35.5
Fat		10.0		60.0		60.0
Soybean oil	25	55.6	25	9.3	25	9.3
Lard	20	44.4	245	90.7	75	27.7
Menhaden oil	0	0	0	0	170	63.0
Saturated		22.7		32.1		30.7
Monounsaturated		29.9		35.9		29.4
Polyunsaturated		47.4		32.0		45.2
Mineral Mix S10026	10	0	10	0	10	0
Dicalcium phosphate	13	0	13	0	13	0
Calcium carbonate	5.5	0	5.5	0	5.5	0
Potassium citrate	16.5	0	16.5	0	16.5	0
Vitamin mix V10001	10	<1%	10	<1%	10	<1%
Choline bitartrate	2	0	2	0	2	0

Note. Macronutrient composition of diets as provided by the manufacturer. ND, normal diet; HFD, high-fat diet; FOD, high-fat Menhaden fish-oil diet.