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ORIGINAL CONTRIBUTION

‘Designer oils’ low in n-6:n-3 fatty acid ratio beneficially modifies cardiovascular risks in mice

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Abstract Cardiovascular benefits of dietary n-3 fatty acids have been shown. However, benefits of n-3 fatty acids as part of a high fat, low n-6:n-3 fatty acid ratio diet has not been fully characterized. Aim of this study is to investigate cardiovascular and metabolic benefits of ‘designer oils’ containing a low ratio of n-6:n-3 fatty acids in C57BL/6 mice. Three groups of C57BL/6 mice were fed an atherogenic diet supplemented with either a fish oil- or flaxseed oil-based ‘designer oil’ with an approximate n-6:n-3 fatty acid ratio of 2:1 (treated groups, $n = 6$ each) or with a safflower oil-based formulation with a high ratio (25:1) of n-6:n-3 fatty acids (control group, $n = 6$) for 6 weeks. Food intake, body weight, and blood lipid levels were monitored regularly. Fatty acid profile of the heart tissues was assessed. Histological assessment of liver samples was conducted. At the end of the study body weight and food intake was significantly higher in the flax group compared to control. The levels of 20:5n-3 and 22:6n-3 was significantly increased in the heart phospholipids in both flax and fish groups compared to control; tissue 20:4n-6 was significantly reduced in the fish group compared to control. Significant liver pathology was observed in the control group only. Lowering dietary ratio of n-6:n-3 fatty acids may significantly reduce cardiovascular and metabolic risks in mice regardless of the source of n-3 fatty acids.

Keywords α-Linolenic acid · Cardiovascular · Designer oil · Docosahexaenoic acid · Eicosapentaenoic acid

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Introduction

Cardiovascular disease is the leading cause of death and disability in the western world, with diet playing an important role. Epidemiological, animal, and clinical studies have reported cardiovascular benefits of dietary α-linolenic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). The benefits of n-3 fatty acids (FA) are multiple and include improvements in blood lipid profile [15], blood pressure [6], and inflammatory system [11]. ALA can at least partially be converted to EPA/DHA endogenously by the elongase and desaturase enzymes [7]; this limited conversion may be species- and tissue-specific [3, 9]. EPA (n-3) can compete with arachidonic acid (AA, 20:4n-6) for the cyclooxygenase (COX) and lipoxygenase (LOX) enzymes for the formation of eicosanoids [18]. Therefore, n-3 FA intake will result in reduced production of pro-inflammatory and pro-aggregatory series 2 eicosanoids, which are produced from AA, and more series 3 eicosanoids [18]. This is one main reason for recent recommendation of reducing the dietary n-6:n-3 FA ratio to 2-4:1 [18].

Thus, we have generated 'designer oils' that contain a desirable fatty acid profile. The purpose of this study was to investigate the effects of these 'designer oils' on cardiovascular and metabolic risk factors in a well-recognized animal model.

Methods

Animals and diets

A total of 18 C57BL/6 mice (approximately 5-week-old) were purchased from Central Animal Facility (Winnipeg, MB, Canada). After 1 week of adaptation, the animals were randomly divided into three experimental groups; the experiments were carried out over 6 weeks. We [21] have shown that a period of at least 4 weeks is adequate to observe the effects of dietary oil treatments on lipid metabolism.

Three different oil formulations were made using flaxseed oil, fish oil, safflower oil, and beef tallow as previously described [17]. PicoLab mouse chow was supplemented with 2% (w/w) cholesterol and 0.2% (w/w) cholic acid and used as "atherogenic diet." The "control group" received the atherogenic diet supplemented with 10% (w/w) safflower-based oil formulation high in n-6:n-3 FA ratio (25:1). The "flax group" or "fish group" received the same atherogenic diet supplemented with 10% (w/w) flaxseed- or fish oil-based oil formulations, respectively, low in n-6:n-3 FA ratio (approximately 2:1). The flaxseed oil based diet had 15.9% w/w ALA and the fish oil based diet had 7.0 and 4.6% w/w EPA and DHA, respectively. Body weight and food intake were measured regularly. One mouse in the flax group had to be sacrificed prematurely (at week 4) due to dehydration, apparent signs of jaundice, and weight loss, which was likely due to gallstones found at autopsy. This condition may be related to addition of cholic acid to the diet. At the end of the study, the animals were sacrificed using CO_2 gas and final blood samples were taken from the heart. Heart tissues were collected and stored at −80 °C until analysis; liver samples were weighed and fixed in formalin for histological examinations. This study was approved by the Animal Care Committee on the use of animals in Research at the University of Manitoba, Winnipeg, Canada.

Plasma lipids

Blood samples were taken at baseline and week 4 from lightly anesthetized mice in the fed state as previously described [17]. Final blood collection was performed at the endpoint through cardiac puncture. Plasma was used for total cholesterol (TC) and triglycerides (TG) determination using standard enzymatic assays [16].

Tissue lipid analysis

Methods described by Folch et al. [5] were used to extract lipids from the heart tissues, plasma, and red blood cells (RBC). Thin-layer chromatography with G-silica gels was used to separate neutral lipids and phospholipids of hearts, respectively, as previously described [19]. Following methylation using BF_3-methanol (14%, w/w), FA analysis was conducted by gas chromatography (Shimadzu 17A, Japan) using Agilent DB225 capillary column (30 m × 0.25 mm internal diameter) [19]. Tissue concentration of TC and TG were analyzed using standard enzymatic assays according to manufacture's instructions (Diagnostic Chemicals Limited). Plasma and red blood cell (RBC) total fatty acid composition was analyzed as previously described [14].

Liver histology

Liver samples were collected at sacrifice and fixed in 10% buffered formalin. Tissues were sectioned at 5 μm thickness. Samples were stained with Hematoxylin & Eosin (H&E), Trichrome and periodic-acid schiff (PAS) procedures. Every section was examined for possible pathological changes using criteria set by the NASH Clinical Research Network Scoring System [12]; similarly the extent of glycogen contents of the liver tissues was evaluated using a scoring system previously described by Gomes et al. [8]. Overall, the light microscopic examinations of the liver tissue included histological features of steatosis (grade, location, type), fibrosis (stage), inflammation (lobular, portal), liver cell injury (ballooning, mega mitochondria) and other pathologies such as presence of Mallory's hyaline or glycogenated nuclei as previously described [20]. Finally, samples were inspected for evidence of cholestasis to ensure that the animals were not adversely affected by dietary cholic acid.

Statistical analysis

Data were analyzed using one-way ANOVA with Tukey test to determine differences among groups using SPSS for Windows version 11.5 (SPSS Inc, Chicago, IL). Repeated measures analysis was used to detect the effects of time on plasma lipids and body weight. Chi-square test was used to determine if distribution of histological scores of liver differed between groups. Data are expressed as mean ± standard deviation (SD). Differences among the groups were considered significant at $p < 0.05$.

Results

Body weight and food intake

Body weight increased steadily in all groups throughout the study course, which indicates tolerability of experimental diets. However, body weights of the flax group were significantly higher at weeks 5 and 6 as compared to controls. The flax group also had higher food intake as compared to either the control (37.5 ± 7.3 vs. 25.6 ± 3.7 g) or to the fish group (37.5 ± 7.3 vs. 29.3 ± 2.6 g). A slight decrease in the mean body weight in the control group at weeks 5 and 6 was skewed by the weight loss of two mice that developed signs of sickness. This may explain the reasons for higher mean body weight in the flax group at these time points.

Plasma lipids

Repeated measures analysis revealed comparable ($p = 0.081$) levels of plasma total cholesterol (TC) among the groups at baseline, week 4, and week 6 (Fig. 1, panel A). The levels of TC at week 6 were only reported in three animals in the control group due to technical difficulties. Plasma triglyceride (TG) levels were also comparable among groups throughout the study course (Fig. 1, panel B). However, all three groups of mice had significantly ($p < 0.01$) lower TG levels at week 4 as compared to baseline values. Compared to baseline data, both treatment groups maintained their significantly lower plasma TG levels at week 6.

Heart lipid and fatty acid composition

The effects of dietary treatments on heart FA composition were studied in total phospholipids (PL), free fatty acids (FFA), triglycerides (TG) and cholesteryl ester (CE) fractions. The fish group showed significantly lower n-6:n-3 FA ratio in the PL and FFA fractions as compared to those in the control; no significant differences in n-6:n-3 FA ratio were observed in TG and CE fractions among the groups. The order of the n-6:n-3 FA ratio in the individual heart PL fractions from highest to lowest were found in the control, flax and fish groups.

The levels of DHA and EPA in the TG, FFA, and CE fractions were comparable between the flax and control groups. However, the hearts from the fish group had significantly higher EPA and DHA concentrations in the PL, TG, and FFA fraction as compared to those in the control group. Total amounts of DHA in the PL and TG fractions did not significantly differ between the flax and fish groups. PL fractions from the hearts in the control and flax groups had significantly higher levels of AA as compared to those in the fish group. Results of FA composition in heart tissues are summarized in Table 1.

Plasma and RBC fatty acid composition

At baseline, FA composition in both plasma and RBC was not significantly different among the groups, with the exception of total monounsaturated fatty acids in plasma; the fish group had significantly higher total monounsaturated fat compared to the flax group, whereas the control group did not differ from either fish or flax groups. FA composition of plasma and RBC at week 4 is comparative to fatty acid composition of heart at endpoint, as summarized in Tables 2 and 3. The fish group had significantly higher RBC EPA, DHA, total n-3 FA, and lower n-6:n-3 fatty acid ratio compared to either control or flax groups. Both the fish and flax groups had comparable reductions in RBC AA content compared to control. The flax group also

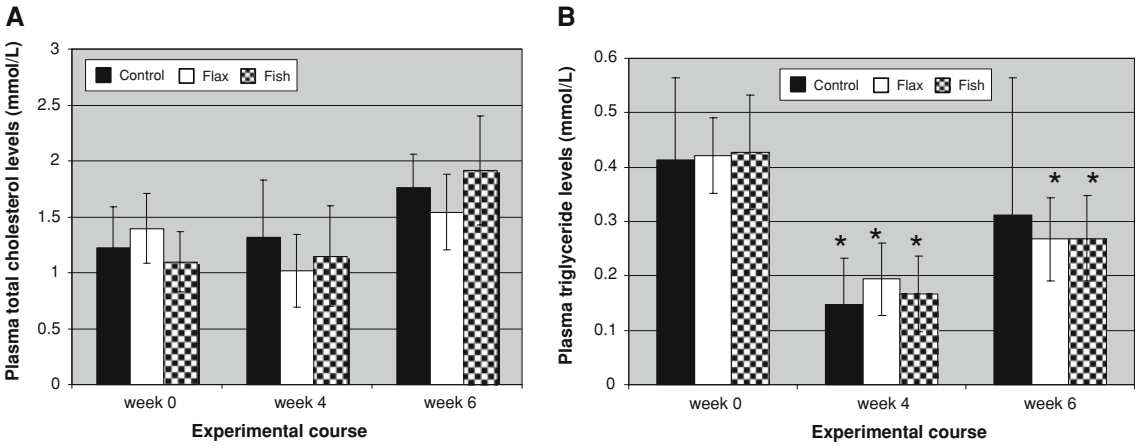

Fig. 1 Plasma total cholesterol (**a**) and plasma triglycerides (**b**) levels at baseline and during the experimental course. There were no significant differences among the groups at any time point. Repeated measures analysis revealed a significant effect (*$p < 0.05$) of time for triglyceride levels (**b**), but not for TC levels (**a**). $n = 5$–6 animals for each group, except $n = 3$ for the TC in the control group at week 6

Table 1 Fatty acid composition of heart lipid fractions of the three groups of experimental mice

Fatty acid	Phospholipids			Triglycerides			Free fatty acids			Cholesteryl esters		
	Control	Flax	Fish	Control	Flax	Fish	Control	Flax	Fish	Control	Flax	Fish
16:0	15.5 ± 3.5	14.0 ± 1.4	16.4 ± 3.4	15.3 ± 5.0	18.5 ± 1.6	19.7 ± 2.7	14.7 ± 3.6 a	15.8 ± 0.6 ab	18.8 ± 1.2 b	10.5 ± 7.4	4.1 ± 1.8	5.2 ± 3.2
16:1 (5 + 7)	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	3.6 ± 2.0	2.2 ± 0.8	2.4 ± 0.7	1.6 ± 0.7	1.3 ± 0.2	1.3 ± 0.3	2.5 ± 1.2 a	1.0 ± 0.3 b	1.1 ± 0.7 b
18:0	27.0 ± 5.8	25.6 ± 3.3	28.1 ± 5.9	6.6 ± 2.7	10.3 ± 6.6	9.7 ± 4.0	12.0 ± 3.6	10.0 ± 0.6	11.0 ± 0.9	5.1 ± 5.1	2.1 ± 0.9	3.1 ± 3.0
18:1 (7 + 9)	6.6 ± 1.2	7.0 ± 0.7	6.6 ± 1.6	29.8 ± 4.7	30.1 ± 6.5	25.8 ± 4.5	17.5 ± 2.6	19.2 ± 0.6	15.5 ± 2.6	14.5 ± 8.3 a	5.5 ± 3.2 b	4.2 ± 2.2 b
18:2 (6)	15.5 ± 4.8 a	14.3 ± 1.7 a	9.1 ± 1.8 b	35.8 ± 5.3 a	26.9 ± 4.6 b	25.8 ± 3.6 b	37.6 ± 3.6 a	35.2 ± 1.0 a	25.5 ± 1.9 b	11.9 ± 7.4 a	5.5 ± 3.0 ab	3.8 ± 1.4 b
20:0	0.8 ± 0.4	0.6 ± 0.1	0.6 ± 0.1	0.8 ± 0.8	1.4 ± 1.3	0.7 ± 0.7	0.4 ± 0.2 a	0.2 ± 0.0 ab	0.2 ± 0.0 b	0.8 ± 1.0	0.2 ± 0.1	0.2 ± 0.2
20:1	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	1.2 ± 0.6	1.2 ± 0.3	1.3 ± 0.3	0.7 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	ND	ND	ND
20:2 (6)	1.0 ± 1.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.4 ± 0.3	0.4 ± 0.1	0.9 ± 0.5	0.5 ± 0.1	0.5 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	ND
20:3 (6)	0.8 ± 0.2 a	0.9 ± 0.0 a	0.4 ± 0.0 b	0.5 ± 0.3	0.7 ± 0.5	0.3 ± 0.2	1.0 ± 0.1 a	1.0 ± 0.2 a	0.6 ± 0.1 b	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.2
20:4 (6)	5.1 ± 1.5 a	4.2 ± 0.7 a	2.4 ± 0.2 b	0.5 ± 0.3	0.3 ± 0.2	0.5 ± 0.4	4.6 ± 0.7 a	4.3 ± 0.4 ab	3.5 ± 0.5 b	0.5 ± 0.3	0.5 ± 0.3	0.4 ± 0.3
20:5 (3)	0.0 ± 0.0 a	0.2 ± 0.0 b	0.6 ± 0.1 c	0.0 ± 0.0 a	0.0 ± 0.0 a	0.6 ± 0.3 b	0.1 ± 0.0 a	0.4 ± 0.1 a	2.2 ± 0.4 b	ND	ND	ND
22:4 (6)	0.3 ± 0.1 a	0.1 ± 0.0 b	0.1 ± 0.0 b	0.2 ± 0.1	0.6 ± 0.7	0.2 ± 0.2	0.4 ± 0.1 a	0.2 ± 0.1 b	0.2 ± 0.1 b	ND	ND	ND
22:5 (3)	0.6 ± 0.1	0.9 ± 0.1	0.6 ± 0.5	0.2 ± 0.1 a	0.2 ± 0.2 a	1.1 ± 0.6 b	0.5 ± 0.1 a	1.2 ± 0.3 a	2.5 ± 0.7 b	0.0 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
22:6 (3)	16.3 ± 7.3 a	22.8 ± 6.6 ab	29.4 ± 7.5 b	0.6 ± 0.3 a	0.9 ± 0.8 ab	4.3 ± 3.5 b	4.1 ± 0.3 a	6.6 ± 0.6 a	13.8 ± 3.1 b	0.4 ± 0.5	0.8 ± 0.6	1.2 ± 1.2
n-6:n-3 ratio	1.7 ± 0.8 a	0.9 ± 0.4 ab	0.5 ± 0.2 b	49.1 ± 19.1	46.7 ± 47.3	6.2 ± 3.3	9.3 ± 1.0 a	5.0 ± 0.4 b	1.8 ± 0.6 c	20.1 ± 16.8	7.9 ± 3.2	4.5 ± 3.5

Values are mean ± SD ($n = 5$–6 animals for each group). Values with different letters in a row within a lipid fraction are significantly different, $p < 0.05$

ND not detected

Table 2 Red blood cell fatty acid composition (% w/w) at baseline and week 4

	Baseline			Week 4		
	Control ($n = 6$)	Flax ($n = 6$)	Fish ($n = 6$)	Control ($n = 6$)	Flax ($n = 5$)	Fish ($n = 6$)
16:0	24.2 ± 1.9	24.8 ± 1.6	24.5 ± 2.3	24.7 ± 0.6	26.1 ± 2.3	24.7 ± 2.3
18:2 (6)	15.2 ± 2.4	14.5 ± 2.5	14.2 ± 1.8	17.5 ± 1.5 a	18.1 ± 1.8 a	13.1 ± 1.2 b
18:3n-3	0.4 ± 0.3	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.3	1.0 ± 0.6	0.3 ± 0.3
20:4n-6	14.6 ± 1.1	14.7 ± 0.7	14.3 ± 1.7	16.0 ± 0.7 a	7.4 ± 5.5 b	6.0 ± 5.0 b
20:5n-3	0.8 ± 0.6	0.7 ± 0.5	1.3 ± 0.4	0.6 ± 0.3 a	1.2 ± 1.1 a	7.4 ± 0.9 b
22:6n-3	8.1 ± 1.6	8.4 ± 0.7	8.0 ± 1.3	6.9 ± 0.3 a	7.7 ± 0.6 a	10.5 ± 0.8 b
Total saturated fatty acids	38.9 ± 2.5	39.8 ± 2.5	39.2 ± 3.4	38.3 ± 1.6	41.4 ± 3.6	39.9 ± 3.0
Total monounsaturated fatty acids	15.5 ± 0.7	15.5 ± 0.4	16.3 ± 0.8	14.9 ± 0.2 a	17.7 ± 1.6 b	15.8 ± 1.2 a
Total n-6 fatty acids	32.2 ± 0.9	31.4 ± 1.8	31.0 ± 1.7	36.7 ± 1.4 a	28.9 ± 3.9 b	23.0 ± 3.3 c
Total n-3 fatty acids	13.4 ± 1.9	13.3 ± 1.1	13.5 ± 2.9	10.1 ± 1.1 a	12.1 ± 1.7 b	21.4 ± 0.9 c
n-6:n-3 fatty acid ratio	2.4 ± 0.3	2.4 ± 0.2	2.4 ± 0.5	3.7 ± 0.4 a	2.4 ± 0.3 b	1.1 ± 0.1 c

Results are presented as mean ± standard deviation. Values with different letters in a row, within the same time, are considered significantly ($p < 0.05$) different

Table 3 Plasma fatty acid (% w/w) composition at baseline and week 4

	Baseline[a]			Week 4		
	Control ($n = 3$)	Flax ($n = 3$)	Fish ($n = 3$)	Control ($n = 6$)	Flax ($n = 6$)	Fish ($n = 6$)
16:0	24.4 ± 0.8	24.2 ± 0.7	24.3 ± 1.2	15.2 ± 2.3 a	16.0 ± 2.6 ab	13.8 ± 0.7 b
18:2 (6)	21.0 ± 5.0	24.2 ± 1.1	22.3 ± 0.5	30.1 ± 5.1	33.5 ± 4.1	29.8 ± 2.4
18:3n3	0.9 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.9 ± 0.8	2.0 ± 2.2	1.1 ± 1.5
20:4n6	10.3 ± 0.8	10.1 ± 0.8	10.4 ± 0.6	1.7 ± 1.6	3.8 ± 5.7	3.1 ± 6.8
20:5n3	1.1 ± 0.3	1.5 ± 0.1	1.2 ± 0.4	1.1 ± 0.7 a	2.3 ± 2.7 a	9.0 ± 3.8 b
22:6n3	6.2 ± 2.2	7.1 ± 0.3	7.0 ± 0.5	4.4 ± 1.7 a	5.1 ± 2.0[a]	7.8 ± 1.6 b
Total saturated fatty acids	43.8 ± 5.7	41.2 ± 1.0	41.2 ± 1.6	27.2 ± 5.9	24.6 ± 3.9	25.4 ± 1.0
Total monounsaturated fatty acids	13.4 ± 0.3 ab	12.6 ± 0.7 a	14.6 ± 1.0 b	19.1 ± 2.8 ab	19.6 ± 2.4 b	13.2 ± 5.5 a
Total n-6 fatty acids	33.2 ± 4.4	35.7 ± 2.0	34.3 ± 1.1	43.6 ± 6.3	43.7 ± 3.8	40.6 ± 4.4
Total n-3 fatty acids	9.6 ± 1.5	10.6 ± 0.4	10.0 ± 0.6	9.4 ± 3.1 a	11.7 ± 4.6 a	20.2 ± 4.1 b
n-6:n-3 fatty acid ratio	3.5 ± 0.2	3.4 ± 0.3	3.5 ± 0.3	5.2 ± 2.2 a	4.2 ± 1.2 ab	2.1 ± 0.7 b

Results are presented as mean ± standard deviation. Values with different letters in a row, within the same time, are considered significantly ($p < 0.05$) different

[a] Baseline sample sizes are $n = 3$ with each sample a pool of two samples due to limited plasma sample

had a significantly lower n-6:n-3 fatty acid ratio in RBC compared to control. In plasma, the fish group had significantly higher EPA, DHA, and total n-3 fatty acids compared to either control or flax group. However, the n-6:n-3 FA ratio in plasma did not significantly differ between the fish and flax groups.

Histology of liver

At sacrifice, the proportion of liver weight to body weight was significantly higher in the control group (10.7 ± 2.3%) compared to either flax (7.6 ± 0.4%) or fish (7.3 ± 0.9%) groups; the two treatment groups showed comparable values.

On histological examinations, the control group presented substantial fatty changes in the liver. This high degree of steatosis was present mainly in micro-vesicular pattern occupying approximately 66% of the liver parenchyma and primarily in panacinar locations. For such observations in the control group, we arbitrarily gave a total score of 7 to include the extent of steatosis (Score of 3), panacinar location (Score of 3) and micro-vesicular pattern (Score of 1). On the other hand, this scoring system revealed a score of 2 for either flax or fish group. The differences between the control groups and treated groups were not only related to the extent of lipid accumulation, but also due to both location and patterns

of lipid deposits, including peri-sinusoidal location (Score of 0) and the lack of micro-vesicular pattern (Score of 0). These histological findings are in agreement with our previous biochemical observations in which total liver lipid levels were significantly reduced in the liver of mice treated with these 'designer oils' as compared to controls [17].

Further investigation with PAS staining revealed depleted glycogen stores in the control group (Score 1 indicating weak reactivity and diffuse granulation), while glycogen stores were almost intact in the fish group (Score 3 indicating strong reactivity and multifocal granulation) and slightly reduced in the flax group (Score 2). Furthermore, only the control groups showed evidence of apparent glycogenated nuclei in the hepatocytes. Delicate spider-like patterns of fibrosis and bile pigment incontinence were also present in the control group. No evidence of fibrosis was observed in either of the treated groups. Evidence for cell injury characterized by ballooning and mega mitochondria features was only seen in the control group. All groups of animals showed mild inflammation in their liver tissues. None of the animals in any groups showed signs of either nonobstructive or obstructive cholestasis. There were no characteristic changes of cholestasis in the portal tracts-circle- (bile ductular proliferation, inspissated bile in bile ducts, portal tract edema, neutrophilic inflammation) or evidence of cholate stasis in periportal hepatocytes. Table 4 summarizes the scores of liver pathology and Fig. 2 illustrates histological features of the liver tissues in three experimental groups.

Discussion

This study has characterized the effects of low dietary ratios of n-6:n-3 FA, from different sources, on plasma lipids, heart FA profiles, and liver pathology. 'Designer oils' with low n-6:n-3 FA ratio as part of an atherogenic diet displayed beneficial effects on cardiovascular risk and tissue composition in mice. Diets supplemented with the 'designer oils' were apparently well tolerated by the animals. The flax group displayed greater weekly food intake as compared to the control and fish groups. We observed similar pattern of food intake in our previous study [17]. Consistent data on the effects of n-3 FA on appetite control and weight gain are lacking [2, 17, 22]. We speculate that flaxseed oil may influence hormones related to appetite, such as ghrelin or leptin. Another possibility is lost energy via an increase in uncoupling protein activity. We plan to investigate this phenomenon further in larger groups of animals; larger groups of animals would have given us a better power of differentiating statistical differences between the treated and control groups, especially in the face of disease development in a couple of animals. We also acknowledge the significant differences in food intake between groups as a potential confounding factor. However, we maintain that these differences do not fully account for the observed changes in lipid metabolism and liver histology.

Similar to previous observations [10, 17], both flax and fish groups showed a significant reduction in plasma TG at week 4, as compared to baseline data. It was interesting to note that the control diet—high in n-6:n-3 FA ratio—also reduced plasma TG levels compared to baseline. Previous studies have compared the effects of low n-6:n-3 FA ratios from single sources on plasma lipids [23]. However, the present study showed that both sources of n-3 FA as a part of an equally low ratio of n-6:n-3 FA had comparable effects on plasma TG or TC, consistent with previous findings [13, 15, 17]. In the present study, we were unable to determine plasma HDL cholesterol concentrations due to limited quantities of samples; however, in our previous study we reported a significant increase in HDL cholesterol levels in mice fed with a fish-oil-based formulation [22]. The reasons for comparable effects of all of three diets on TC and TG in the present study are currently unknown, but we speculate that it may be due to the simultaneous introduction of the high fat diet from the high carbohydrate basal chow diet in all groups.

Table 4 Severity of pathological changes in the liver tissues from experimental groups

Histological features	Score[a]		
	Control ($n = 6$)	Flax ($n = 5$)	Fish ($n = 5$)
Steatosis	7 a	2 b	2 b
Fibrosis	1 a	0 b	0 b
Inflammation	1	1	1
Cell injury (ballooning and mega mitochondria)	1 a	0 b	0 b
Glycogen content	1 a	2 b	3 c

Different letters between groups within a row indicate significant differences ($p < 0.001$) between groups, according to chi-square test

[a] Scores determined by an independent observer based on published guidelines as described in the text; numbers reported indicate the mean of 5–6 observations which were identical for all tissues within a group, resulting in no standard variation

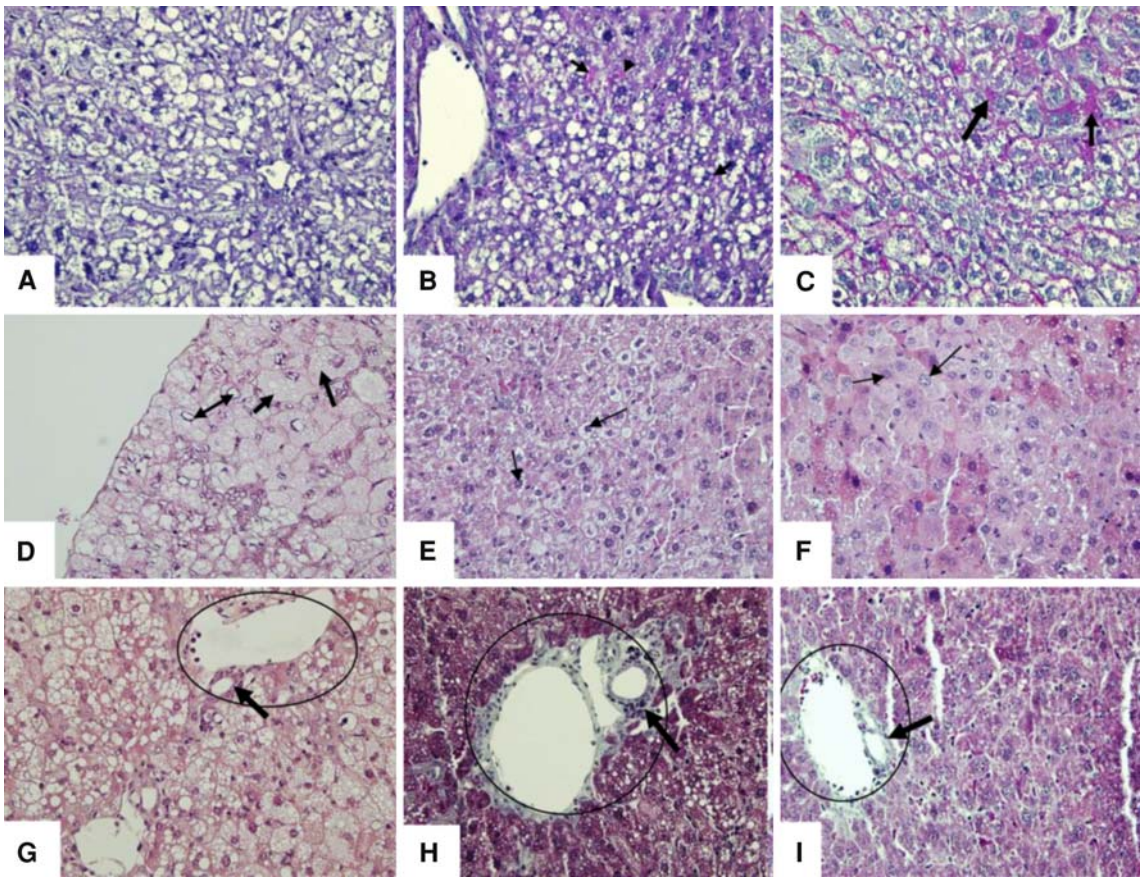

Fig. 2 Representative photomicrographs illustrating pathological changes in the liver tissues among the experimental groups. Glycogen contents of the liver tissues are illustrated by PAS stain; compared to controls (**a**), flax (**b**) and fish (**c**) groups show apparently normal glycogen contents. The pattern of micro-vesicular fatty changes (**d**, *arrow*) and empty looking apparently glycogenated nuclei (**d**, *double headed arrow*) are shown in the control group (**d**, *arrow*), while bland looking nuclei (**e**, **f**, *arrow*) are present in the flax and fish groups (**e**, **f**). **d**–**f** H&E stain. None of the animals in any groups showed signs of either. Nonobstructive or obstructive cholestasis (control, **g**; flax, **h**; fish, **i**). There were no characteristic changes of cholestasis in the portal tracts-circle- (bile ductular proliferation, inspissated bile in bile ducts, portal tract edema, neutrophilic inflammation) or evidence of cholate stasis in periportal hepatocytes. Bile ducts are indicated by *arrows* in **g**–**i**

Overall, both treatment diets substantially influenced heart lipid composition compared to control. Similar findings with regard to plasma and RBC fatty acid composition compared to heart were seen. Lower n-6:n-3 FA ratios and higher EPA/DHA concentrations in heart of the flax group (compared to control) may suggest a conversion of ALA to EPA/DHA [8, 10]. Our data suggest that dietary ALA cannot be incorporated into the tissue phospholipids at an identical rate to that of dietary EPA/DHA. Additionally, dietary ALA did not significantly reduce tissue AA in most lipid fractions to a level comparable to that achieved by fish oil (EPA/DHA). Nevertheless, a trend toward increased cardiac tissue EPA and DHA as a result of ALA feeding was observed. This incorporation of the long-chain n-3 FA in cardiac cell membrane in both treatment groups may help explain the anti-arrhythmic effects of flaxseed oils [1] and fish oils [4].

A reduction in liver to body weight ratio was observed in the flax and fish groups, which may likely be a reflection of the lower lipid contents observed previously in the two treatment groups [17]. Furthermore in the current study, histological evaluation revealed a significantly higher degree of steatosis in the control group compared to both fish and flax groups. Further histological analysis revealed hepatic glycogen depletion in the control group. It is possible that accumulation of lipids causes depletion of glycogen. It is also possible that these alterations in liver histology were associated with alterations in carbohydrate metabolism resulting in increased consumption and reduced synthesis of glycogen. It would be of interest to further investigate the association between these changes in hepatic tissues and glucose metabolism and insulin resistance. Additional studies are also needed to determine the role of inflammation in hepatic steatosis and insulin resistance.

Marginal increases in heart DHA levels were observed in the mice fed with flaxseed oil-based ‘designer oil’, likely

indicating ability of the heart tissue to convert ALA to DHA in mice. However, it must be noted that the flaxseed based designer oil did not increase heart tissue EPA and DHA to a comparable level of the fish group, further indicating that preformed EPA and DHA rather than ALA is more effective at increasing heart tissue EPA and DHA. This may suggest that longer time is needed to increase heart n-3 FA contents. Addition of the 'designer oils' to the diets of the mice did not result in any major alterations in the liver tissues. However, consumption of safflower oil-based oil formulation in the control group was associated with both liver steatosis and reduced glycogen content, which may indicate the development of insulin resistance in the control group, but not in the treated groups. Thus, further investigations are needed to evaluate whether these pathological changes influence metabolic function, specifically insulin sensitivity and plasma glucose.

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