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ORIGINAL ARTICLE

Effects of dietary saturated and n-6 polyunsaturated fatty acids on the incorporation of long-chain n-3 polyunsaturated fatty acids into blood lipids

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BACKGROUND/OBJECTIVES: Omega-3 polyunsaturated fatty acids (n-3PUFA) are better absorbed when they are combined with high-fat meals. However, the role of different dietary fats in modulating the incorporation of n-3PUFA in blood lipids in humans has not been previously explored. Omega-6 polyunsaturated fatty acids (n-6PUFA) are known to compete with n-3PUFA in the metabolic pathways and for the incorporation into phospholipids, whereas saturated fats (SFA) may enhance n-3PUFA incorporation into tissues.

SUBJECTS/METHODS: In a randomized parallel-design trial, we aimed to investigate the long-term effects of n-3PUFA supplementation in subjects consuming a diet enriched with either SFA or n-6PUFA on fatty acid incorporation into plasma and erythrocytes and on blood lipid profiles (total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides).

RESULTS: Dietary supplementation with n-3PUFA co-administered with SFA for 6 weeks resulted in a significant rise in total cholesterol (0.46 ± 0.60 mmol/L; $P = 0.020$) and LDL-C (0.48 ± 0.48 mmol/L; $P = 0.011$) in comparison with combination with n-6PUFA. The diet enriched with SFA also induced a greater increase in eicosapentaenoic acid (2.07 ± 0.79 vs 1.15 ± 0.53; $P = 0.004$), a smaller decrease in docosapentaenoic acid (-0.12 ± 0.23 vs -0.30 ± 0.20; $P = 0.034$) and a similar increase in docosahexaenoic acid (3.85 ± 1.14 vs 3.10 ± 1.07; $P = 0.128$) percentage in plasma compared with the diet enriched with n-6PUFA. A similar effect was seen in erythrocytes. N-3PUFA supplementation resulted in similar changes in HDL-C and triglyceride levels.

CONCLUSIONS: The results suggest that dietary substitution of SFA with n-6PUFA, despite maintaining low levels of circulating cholesterol, hinders n-3PUFA incorporation into plasma and tissue lipids.

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INTRODUCTION

The consumption of saturated fatty acids (SFA) is often related to the development of chronic diseases, especially coronary heart disease (CHD) and other cardiovascular diseases (CVD),[1,2] despite conflicting results in recent meta-analyses.[3–5] Siri-Tarino *et al.*[5] and de Sousa *et al.*[3] suggested little association between SFA and CVD risk, whereas Mozaffarian *et al.*[4] suggested an improvement in CHD events by replacing SFA with omega-6 polyunsaturated fatty acids (n-6PUFA). SFA have also been associated with increased triglycerides, total cholesterol and low-density lipoprotein cholesterol (LDL-C),[6] although data from both epidemiological and interventional studies are often contradictory.[7,8] Notably, recent studies suggest the differentiation between dairy vs other food sources of saturated fats for their effects on blood lipids and CVD risk.[9–11] Data from animal studies have indicated that SFA increase triglycerides only when the diet is deficient in omega-3 polyunsaturated fatty acids (n-3PUFA).[12,13] However, the effects of n-3PUFA supplementation with background dietary SFA vs n-6PUFA have not been well explored in human trials.

Data on n-6PUFA are also heterogeneous. Some studies have demonstrated a negative association between n-6PUFA and blood lipid levels and cardiovascular disease,[14–16] whereas another study associated n-6PUFA with an increased risk for CHD, CVD and all-cause mortality.[17] Furthermore, n-6PUFA was shown to compete with n-3PUFA for the same enzymes in their metabolism, prevailing over n-3PUFA during elongation and desaturation.[18,19] Their imbalance was also shown to affect eicosanoid formation,[20] and cell membrane formation, fluidity and permeability.[21]

The anti-inflammatory and lipid-lowering effects of n-3PUFA are well known, as well as their association with decreased risk of many chronic diseases.[22,23] Although the competition between n-3 and n-6PUFA has been broadly discussed, the effects and importance of other dietary fatty acids on n-3PUFA incorporation into plasma and tissues have not yet been studied. In addition, n-3PUFA supplementation in the context of a background diet rich in either SFA or n-6PUFA has not been compared in humans, although animal studies have demonstrated that the combination of SFA and n-3PUFA was more effective in incorporating n-3PUFA into plasma than the combination of n-6PUFA and n-3PUFA.[13]

Therefore, in this study, we aimed to compare n-3PUFA supplementation in subjects consuming an SFA-enriched diet vs an n-6PUFA-enriched diet. Blood lipid levels (total cholesterol, high-density lipoprotein cholesterol (HDL-C), LDL-C and triglycerides) and fatty acid incorporation into plasma and erythrocytes

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were measured. Plasma and erythrocyte fatty acids were analysed to examine short- and long-term changes in fatty acid composition, respectively.

MATERIALS AND METHODS

Participants

The study population consisted of 33 healthy adults (26 women and 7 men) aged between 18 and 65 years. Participants were excluded if they were using lipid-lowering drugs (for example, statins); had consumed fish-oil supplements regularly within the past month; had regular consumption of two or more fish meals a week over the past month; had any history of congestive heart failure, stroke, myocardial infarction, coronary artery bypass graft or atherosclerotic CVD; had history of diabetes, gastrointestinal or liver disease; were smokers; or were pregnant or breastfeeding.

Study design

The study was a randomized controlled, parallel, dietary intervention trial. After an overnight fast, participants visited the Nutraceuticals Research Clinic facility at the University of Newcastle on two occasions: at baseline and after 6 weeks of dietary intervention with long-chain n-3PUFA (LCn-3PUFA) supplementation, either in combination with saturated fatty acids (SFA+LCn-3PUFA diet) or n-6PUFA (n-6PUFA+LCn-3PUFA diet). Participants on the SFA+LCn-3PUFA diet group consumed daily 24 g of butter, 40 g of white chocolate (providing 30 g of total fat and 20.9 g of saturated fat) and 4 × 1 g fish oil capsules (100 mg of eicosapentaenoic acid and 500 mg of docosahexaenoic acid each (EPAX 1050TG, Norway)); they were also advised on how to use more foods containing saturated fatty acids for cooking. Participants on the n-6PUFA+LCn-3PUFA diet consumed daily 20 g of margarine, 42 g of sunflower seeds (providing 30 g of total fat and 20 g of n-6PUFA) and 4 × 1g fish oil capsules; they were also advised on how to use more foods and oils containing n-6PUFA for cooking. All participants were advised not to change their physical activity status or any other aspect of their habitual diet. Subjects were allocated to one of the two diets using computer-generated random tables (www.randomization.com, seed 15 097 for men and seed 13 079 for women). A 3-day food record was used to measure nutrient intakes before intervention and changes during intervention. Food records were processed using FoodWorks 7 Professional (Xyris software). Participants also completed a physical activity questionnaire and a medical questionnaire, which included current medications and supplements, illnesses and alcohol consumption. Blood samples were collected at baseline and after 6 weeks of dietary intervention and assessed for blood lipid profile (total cholesterol, LDL-C, HDL-C and triglycerides) and fatty acids in plasma and erythrocytes. Participants refrained from strenuous exercise and alcohol consumption for 24 h and fasted for at least 10 h before each blood collection. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the University of Newcastle Human Research Ethics Committee (protocol H-2012-0117), and the study was registered with the Australia New Zealand Trial registry (registration identification number ACTRN12613000962730, http://www.anzctr.org.au/). All subjects gave written informed consent before participation.

Compliance was monitored by count back of fish oil capsules and other food products provided, interviewing volunteers about their food consumption at the end of the trial, evaluating their dietary records and analysis of plasma fatty acid composition. No participant showed any signs of intolerance to the supplements of the study diets.

Plasma lipid profile

Blood samples were collected in lithium heparin vacutainers. Plasma was immediately separated from erythrocytes by centrifugation (3000 *g* × 10 min at 4 °C) and analysed for lipid profile (total cholesterol, LDL-C and HDL-C and triglycerides) by the accredited Hunter New England Area Pathology Service.

Plasma and erythrocyte fatty acid composition

Blood samples were collected in EDTA vacutainers, and plasma was immediately separated from the erythrocytes by centrifugation (1000 *g* × 15 min at 4 °C) and stored at − 80 °C until analysis. Incorporation of fatty acids into plasma and erythrocytes was determined using gas chromatography after trans-esterification. Fatty acids were methylated according to the method by Lepage and Roy,[24] and C19:0 was used as an internal standard, and then the methyl ester products were separated, identified and quantified using a 30 m × 0.25 mm (DB-225) fused carbon–silica column, coated with cyanopropylphenyl (J & W Scientific, Folsom, CA, USA) and Hewlett Packard 6890 A series gas chromatographer with Chemstations Version A.04.02 for gas chromatographic analysis.[25] Fatty acid peaks were identified by comparison with a standard mixture of fatty acid methyl esters of known composition and concentration.

Fatty acid composition of the LCn-3PUFA supplement

Fish oil was weighed into culture tubes and diluted with toluene to a concentration of 10 mg/ml. A 100-µl aliquot of this solution was methylated and analysed for fatty acid composition, as described above for plasma and erythrocytes.

Statistical analysis

Sample-size calculation was based on an anticipated 30% (0.45 mmol/l) decrease in plasma triglycerides (primary outcome) over 6 weeks, with level of significance 0.05 and 80% power. Using a s.d. of 0.387 mmol/l in plasma triglycerides,[26] a minimum of 13 subjects would be required in each group.

From the 33 subjects initially randomized to the diets (18 on the SFA +LCn-3PUFA diet and 15 on the n-6PUFA+LCn-3PUFA diet), 7 failed to adhere to the intervention (compliance $< 80\%$) and 1 had erythrocytes data missing, therefore, those subjects were excluded from the data analysis. Twenty-five healthy subjects were then included in the statistical analysis, 13 subjects on the SFA+LCn-3PUFA diet (2 men and 11 women) and 12 on the n-6PUFA+LCn-3PUFA diet (3 men and 9 women). All data are presented as mean and s.d. Kruskal–Wallis test was used for baseline comparisons. Data at baseline and 6 weeks were compared within groups using paired *t*-test and Wilcoxon signed-rank test, for parametric and non-parametric data, respectively. Data on change were compared between groups using unpaired *t*-test or Kruskal–Wallis test, for parametric and non-parametric data, respectively. For all tests, a *P*-value < 0.05 was considered statistically significant. Stata IC 11.2 (StataCorp LP) was used to perform the statistical analysis.

RESULTS

Twenty-five healthy subjects (13 on the SFA+LCn-3PUFA diet and 12 on the n-6PUFA+LCn-3PUFA diet) were included in the statistical analysis. Nutrient consumption of subjects before and during intervention is described in Table 1. Participants consuming the SFA+LCn-3PUFA diet increased significantly their saturated fat consumption ($P = 0.009$), with no significant change in n-6PUFA and monounsaturated fatty acid (MUFA) consumption ($P = 0.917$ and $P = 0.584$, respectively). On the contrary, participants on the n-6PUFA+LCn-3PUFA reduced their consumption of saturated fats ($P = 0.002$), while increasing n-6PUFA ($P = 0.002$), and did not change MUFA consumption. None of the groups increased total fat or protein consumption, and both groups increased LCn-3PUFA and carbohydrate consumption significantly to the same extent (Table 1).

The fatty acid composition of the n-3PUFA supplements provided to the study subjects is described in Table 2; the capsules contained $> 70\%$ total LCn-3PUFA (14.3% eicosapentaenoic acid (20:5n-3) and 58.8% docosahexaenoic acid (22:6n-3)).

Subjects' plasma and erythrocyte fatty acids before and after intervention are described in Tables 3 and 4, respectively. Percentages of fatty acids in plasma (Table 3) and erythrocytes (Table 4) were not different at baseline between the diet groups, except for participants in the n-6PUFA+LCn-3PUFA diet group presenting with significantly lower docosahexaenoic acid (22:6n-3) in plasma than participants in the SFA+LCn-3PUFA group before intervention (Table 3). After the n-6PUFA+LCn-3PUFA diet, there was a significant increase in linoleic acid (18:2n6) in plasma but not in erythrocytes, whereas there was no significant change in 18:2n6 after the SFA+LCn-3PUFA diet. Arachidonic acid (20:4n6) in erythrocytes decreased to the same extent after both diets, although in plasma it decreased only after the n-6PUFA+LCn-3PUFA diet.

Table 1. Daily nutrient consumption of subjects, before intervention and during intervention

	Diet	*Before intervention*	*After intervention*	*ΔChange*	P[a]
Energy (kJ)	SFA+LCn-3PUFA	8532 ± 419	8358 ± 1501	− 160 ± 1227	0.174
	n-6PUFA+LCn-3PUFA	8846 ± 2452	8821 ± 1331	404 ± 2080	
Protein (% energy)	SFA+LCn-3PUFA	17.3 ± 3.4	16.7 ± 2.8	− 0.6 ± 3.6	0.744
	n-6PUFA+LCn-3PUFA	19.9 ± 7.3	19.3 ± 4.7	− 0.6 ± 4.1	
Carbohydrates (% energy)	SFA+LCn-3PUFA	36.0 ± 3.9	40.7 ± 4.7	4.7 ± 4.7**	0.703
	n-6PUFA+LCn-3PUFA	37.4 ± 6.4	41.4 ± 5.1	4.0 ± 5.5*	
Total fat (% energy)	SFA+LCn-3PUFA	20.0 ± 7.4	17.5 ± 5.1	− 2.5 ± 5.2	0.744
	n-6PUFA+LCn-3PUFA	16.0 ± 5.9	14.2 ± 3.6	− 1.8 ± 5.1	
Saturated fat (g/100 g fat)	SFA+LCn-3PUFA	43.2 ± 7.0	48.3 ± 4.5	5.1 ± 5.9*	< 0.001
	n-6PUFA+LCn-3PUFA	38.0 ± 5.8	27.3 ± 3.6	− 10.6 ± 5.1**	
Monounsaturated fat (g/100 g fat)	SFA+LCn-3PUFA	32.6 ± 5.7	33.4 ± 3.6	0.8 ± 5.4	0.192
	n-6PUFA+LCn-3PUFA	34.3 ± 4.9	31.7 ± 3.1	− 2.6 ± 5.9	
Polyunsaturated fat (g/100 g fat)	SFA+LCn-3PUFA	12.4 ± 3.7	12.2 ± 2.5	− 0.2 ± 2.9	< 0.001
	n-6PUFA+LCn-3PUFA	15.1 ± 3.7	34.8 ± 4.4	19.7 ± 4.4**	
Linoleic acid (g/100 g fat)	SFA+LCn-3PUFA	10.2 ± 3.7	7.8 ± 2.6	− 2.4 ± 2.4**	< 0.001
	n-6PUFA+LCn-3PUFA	11.8 ± 2.4	30.5 ± 3.8	18.7 ± 3.8**	
Long-chain n-3PUFA (g/100 g fat)	SFA+LCn-3PUFA	0.6 ± 0.7	2.9 ± 1.0	2.3 ± 0.9**	0.355
	n-6PUFA+LCn-3PUFA	0.4 ± 0.6	2.4 ± 0.5	2.0 ± 0.7**	
Cholesterol (mg)	SFA+LCn-3PUFA	278 ± 100	278 ± 68	35 ± 50	0.201
	n-6PUFA+LCn-3PUFA	353 ± 213	318 ± 141	− 31 ± 165	

Abbreviations: n-6PUFA: omega-6 polyunsaturated fatty acids; SFA: saturated fatty acids. Data are presented as mean ± s.d. ($n = 13$ for the SFA+LCn-3PUFA diet and $n = 12$ for the n-6PUFA+LCn-3PUFA diet). Significant difference between values before and after intervention, $^*P < 0.05$ and $^{**}P < 0.01$. [a]*P*-values for comparison between diets.

Table 2. Fatty acid composition of the LCn-3PUFA supplement (% from total fatty acids detected)

Fatty acid	
Palmitic (16:0)	2.27 ± 0.01
Palmitoleic (16:1n-7)	0.47 ± 0.01
Stearic (18:0)	4.93 ± 0.02
Oleic (18:1n-9)	7.67 ± 0.02
Cis-Vaccenic (18:1n-7)	1.57 ± 0.02
Linoleic (18:2n-6)	1.20 ± 0.01
γ-Linolenic (18:3n-6)	0.11 ± 0.00
α-Linolenic (18:3n-3)	0.05 ± 0.00
Arachidic (20:0)	0.34 ± 0.01
Dihomo-γ-linolenic (20:3n-6)	0.58 ± 0.01
Arachidonic (20:4n-6)	3.34 ± 0.02
Eicosapentaenoic acid (20:5n-3)	14.33 ± 0.10
Docosahexaenoic acid (22:6n-3)	58.77 ± 0.10

Data are presented as mean ± s.d.

There was a significant increase in eicosapentaenoic acid (20:5n3) and docosahexaenoic acid in plasma and erythrocytes after consumption of the SFA+LCn-3PUFA and the n-6PUFA+LCn-3PUFA diet. However, the increase in 20:5n-3 was about two times higher after the SFA+LCn-3PUFA diet than after the n-6PUFA +LCn-3PUFA diet, whereas the increase in 22:6n-3 was similar after both diets. There was also a significant decrease in erythrocyte docosapentaenoic acid (22:5n-3) after both diets and in plasma 22:5n-3 after the n-6PUFA+LCn-3PUFA diet; plasma 22:5n-3 decreased over two times more after the n-6PUFA+LCn-3PUFA diet.

Subject characteristics, blood lipid profiles and systemic inflammation (high-sensitivity C-reactive protein – hsCRP) are described in Table 5. At baseline, there was no difference between groups in any of these characteristics (Table 5). After intervention, there was a significant increase in body mass index after consumption of both diets: 1.1% after the SFA+LCn-3PUFA diet ($P = 0.012$) and 1.8% after the n-6PUFA+LCn-3PUFA diet ($P = 0.019$), which was not significantly different between diet groups. However, the increase in body mass index did not result in a significant increase in percentage body fat. Triglycerides decreased significantly after both the SFA+LCn-3PUFA and n-6PUFA+LCn-3PUFA diets ($P = 0.013$ and $P = 0.002$, respectively); no significant difference in change in triglycerides was observed between the two diets. Total and LDL-C increased significantly after the SFA+LCn-3PUFA diet ($P = 0.021$ and $P = 0.011$, respectively), although they did not change significantly after the n-6PUFA+LCn-3PUFA diet. Hence, there was a significant difference in the change after each of the diets. HDL-C increased after both diets (4.8% after SFA+LCn-3PUFA and 8.6% after n-6PUFA+LCn-3PUFA); however, the increase was significant only after consumption of the n-6PUFA+LCn-3PUFA diet ($P = 0.036$), and there was no significant difference in HDL-C change between the two diet groups after dietary intervention. There was also no change in hsCRP after the intervention in either group.

DISCUSSION

This study was designed to evaluate the effect of diets containing either SFA or n-6PUFA on blood lipid profile and incorporation of LCn-3PUFA into plasma and erythrocyte membranes. The diet enriched with SFA induced a greater increase in 20:5n-3, while inducing a similar increase in 22:6n-3 compared with the n-6PUFA-enriched diet. This fact indicates that dietary substitution of SFA with n-6PUFA-rich vegetable oils may hinder 20:5n-3 incorporation into plasma and erythrocytes, without modulating 22:6n-3 uptake and incorporation into tissues.

Plasma and erythrocyte 20:5n-3 were increased to a greater extent after the SFA-enriched diet than after the n-6PUFA diet, despite the same level of dietary n-3PUFA supplementation in the two groups. These findings support competition between n-6PUFA and n-3PUFA, which would have impaired 20:5n-3 incorporation into plasma and erythrocytes when participants consumed an n-6PUFA-enriched diet and favoured 20:5n-3 incorporation in the group consuming the SFA-enriched diet. In addition, it is likely that the SFA-enriched diet allows greater conversion of dietary 18:3n-3 to 20:5n-3 compared with the n-6PUFA-enriched diet, because of less competition for delta-6 and/or delta-5 desaturase enzymes.[12,27] In contrast, plasma and erythrocyte 22:6n-3 percentage increased to the same extent irrespective of the background dietary fat. Subjects consuming the SFA-enriched diet presented significantly higher baseline plasma

Table 3. Plasma fatty acids (% from total fatty acids detected) before and after intervention

Fatty acids	*Diet*	*Before intervention*	*After intervention*	*ΔChange*	P[a]
16:0	SFA+LCn-3PUFA	22.55 ± 2.32	21.37 ± 1.39	−1.18 ± 1.25**	0.957
	n-6PUFA+LCn-3PUFA	21.33 ± 1.72	20.55 ± 1.70	−0.79 ± 1.67	
16:1n-7	SFA+LCn-3PUFA	2.60 ± 1.08	1.82 ± 0.85	−0.79 ± 0.46**	0.957
	n-6PUFA+LCn-3PUFA	2.16 ± 0.78	1.35 ± 0.43	−0.81 ± 0.68**	
18:0	SFA+LCn-3PUFA	8.15 ± 1.66	8.24 ± 0.63	0.09 ± 1.55	0.724
	n-6PUFA+LCn-3PUFA	7.70 ± 0.52	8.02 ± 0.68	0.32 ± 0.60	
18:1n-9	SFA+LCn-3PUFA	20.56 ± 1.34	18.56 ± 1.41	−1.99 ± 1.24**	0.017
	n-6PUFA+LCn-3PUFA	21.35 ± 2.52	17.43 ± 2.13	−3.93 ± 2.36***	
18:1n-7	SFA+LCn-3PUFA	1.91 ± 0.27	1.60 ± 0.19	−0.31 ± 0.29**	0.644
	n-6PUFA+LCn-3PUFA	1.90 ± 0.30	1.65 ± 0.32	−0.25 ± 0.23**	
18:2n-6	SFA+LCn-3PUFA	28.43 ± 4.17	27.71 ± 3.71	−0.72 ± 2.39	0.008
	n-6PUFA+LCn-3PUFA	29.96 ± 4.04	32.91 ± 4.01	2.95 ± 4.74*	
18:3n-6	SFA+LCn-3PUFA	0.68 ± 0.33	0.35 ± 0.09	−0.32 ± 0.33**	0.210
	n-6PUFA+LCn-3PUFA	0.64 ± 0.17	0.47 ± 0.13	−0.17 ± 0.12**	
18:3n-3	SFA+LCn-3PUFA	0.80 ± 0.20	0.67 ± 0.06	−0.13 ± 0.22*	0.892
	n-6PUFA+LCn-3PUFA	0.84 ± 0.26	0.71 ± 0.16	−0.14 ± 0.29	
20:0	SFA+LCn-3PUFA	0.40 ± 0.06	0.46 ± 0.08	0.06 ± 0.07*	0.219
	n-6PUFA+LCn-3PUFA	0.37 ± 0.06	0.43 ± 0.11	0.06 ± 0.10*	
20:1n-9	SFA+LCn-3PUFA	0.22 ± 0.04	0.16 ± 0.03	−0.05 ± 0.05**	0.272
	n-6PUFA+LCn-3PUFA	0.22 ± 0.05	0.19 ± 0.08	−0.02 ± 0.05*	
20:2n-6	SFA+LCn-3PUFA	0.27 ± 0.07	0.21 ± 0.05	−0.06 ± 0.05**	0.312
	n-6PUFA+LCn-3PUFA	0.29 ± 0.06	0.24 ± 0.13	−0.04 ± 0.15*	
20:3n-6	SFA+LCn-3PUFA	0.31 ± 0.07	0.24 ± 0.05	−0.07 ± 0.07*	0.301
	n-6PUFA+LCn-3PUFA	0.30 ± 0.03	0.27 ± 0.09	−0.03 ± 0.07*	
20:4n-6	SFA+LCn-3PUFA	8.62 ± 1.87	8.30 ± 1.55	−0.32 ± 1.16	0.097
	n-6PUFA+LCn-3PUFA	9.03 ± 0.81	7.94 ± 1.02	−1.09 ± 1.00*	
20:5n-3	SFA+LCn-3PUFA	1.26 ± 0.53	3.33 ± 0.84	2.07 ± 0.79**	0.004
	n-6PUFA+LCn-3PUFA	1.04 ± 0.27	2.19 ± 0.58	1.15 ± 0.53**	
22:5n-3	SFA+LCn-3PUFA	0.79 ± 0.22	0.68 ± 0.12	−0.12 ± 0.23	0.034
	n-6PUFA+LCn-3PUFA	0.95 ± 0.16	0.65 ± 0.12	−0.30 ± 0.20**	
22:6n-3	SFA+LCn-3PUFA	2.45 ± 0.71	6.30 ± 1.01	3.85 ± 1.14**	0.128
	n-6PUFA+LCn-3PUFA	1.92 ± 0.36[b]	5.01 ± 0.98	3.10 ± 1.07**	

Abbreviations: n-6PUFA: omega-6 polyunsaturated fatty acids; SFA: saturated fatty acids. Data are presented as mean ± s.d. ($n = 13$ for the SFA+LCn-3PUFA diet and $n = 12$ for the n-6PUFA+LCn-3PUFA diet). Significant difference between values before and after intervention, *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. [a]*P*-values for comparison between diets. [b]Significant difference for values before intervention between dietary groups $P < 0.05$.

22:6n-3 than subjects in the n-6PUFA+LCn-3PUFA diet, which may have rendered relatively little room for a further increase in the group consuming the SFA+LCn-3PUFA diet. In addition, it has been previously established that in humans conversion of dietary 18:3n-3 to 22:6n-3 is very minimal; therefore, the competition offered by n-6PUFA at the desaturase enzyme level may only be applicable for 20:5n-3 but not for 22:6n-3 synthesis.

Subjects consuming either diet presented comparable reduction in plasma triglycerides over the study period. A similar effect was also observed by Mori *et al.*,[28] when comparing diets containing 30% fat (Polyunsaturated: Saturated fat > 1.0) or 40% fat (Polyunsaturated: Saturated fat < 0.3) supplemented with fish or fish oil capsules, indicating that the background dietary fat does not affect the triglyceride-lowering effect of n-3PUFA, although in the study by Mori *et al.* [28] the difference in fat content between diets may have confounded the results. Indeed, consumption of n-3PUFA supplements has been shown by other authors[22,29] to decrease plasma triglycerides, although the usual fat consumption of the study subjects is not reported. N-3PUFA reduce triglycerides by inhibiting the synthesis and secretion of very low-density lipoprotein cholesterol (VLDL-C), decreasing lipogenesis and increasing lipid oxidation.[30] Furthermore, the n-3PUFA effect on triglyceride levels seems to be dose[31,32] and time dependent. In a 12-week intervention, men consuming 2.6, 3.6, 5.2 and 6.6 g of n-3PUFA and following a 40% fat diet (20% SFA, 16% Monounsaturated fat and 4% PUFA) had a 14%, 20%, 32% and 43% decrease in serum triglycerides, respectively.[28] Another 6-week intervention in mildly hypercholesterolaemic men supplemented with 20:5n-3 (3.8 g) or 22:6n-3 (3.7 g) showed 18% and 20% decrease in triglycerides, respectively.[33] In addition, Milte *et al.*[32] observed a 23% reduction in triglycerides for every 1 g/day increase in 22:6n-3 intake in healthy adults over 12 weeks.

After 6 weeks of consuming the SFA-enriched diet, subjects presented with an increase in LDL-C, which was not seen after consuming the n-6PUFA-enriched diet. Saturated fats,[29,34] as well as LCn-3PUFA,[35] have been shown to increase plasma LDL-C because of their capacity to downregulate low-density lipoprotein receptor (LDL-r)[30,36] and consequently reduce the uptake of LDL-C by the liver. N-3PUFA can also induce the conversion of VLDL to smaller VLDL particles, which are converted to LDL particles; small VLDL particles are also more efficiently taken up by LDL-r than LDL particles[28,37] and they compete with LDL particles for hepatic clearance, decreasing LDL uptake by the liver.[38,39] N-6PUFA, on the contrary, have been shown to increase LDL-r transcription and synthesis,[34,38] increasing the uptake of LDL-C by the liver and reducing plasma LDL-C. In this study, combining LCn-3PUFA with an n-6PUFA-enriched diet did not change plasma LDL-C levels, despite the cholesterol-lowering effects of n-6PUFA and the potential LCn-3PUFA capacity to increase LDL-C. N-6PUFA consumption appears to have, therefore, suppressed the ability of LCn-3PUFA to increase LDL-C levels, whereas SFA appear to have enhanced the n-3PUFA-driven increase in LDL-C. However, it is uncertain from the results presented whether the increase in LDL-C is because of an increase in LDL particle number or because of an increase in particle size, as LCn-3PUFA have been shown to increase LDL particle size.[33,40] LDL particle size may be important, as larger buoyant LDL particles have been shown to be less atherogenic compared with the smaller dense LDL particles in one study.[41] However, others have failed to demonstrate such an effect.[42] Determination of LDL particle size following

Table 4. Fatty acids (% from total fatty acids detected) in erythrocytes before and after intervention

Fatty acids	*Diet*	*Before intervention*	*After intervention*	*ΔChange*	P[a]
16:0	SFA+LCn-3PUFA	23.05 ± 1.11	22.67 ± 0.74	-0.39 ± 0.50*	0.644
	n-6PUFA+LCn-3PUFA	22.92 ± 0.66	22.41 ± 0.62	-0.51 ± 0.54**	
16:1n-7	SFA+LCn-3PUFA	0.39 ± 0.20	0.33 ± 0.13	-0.06 ± 0.08*	0.546
	n-6PUFA+LCn-3PUFA	0.33 ± 0.09	0.28 ± 0.06	-0.05 ± 0.05**	
18:0	SFA+LCn-3PUFA	18.74 ± 0.87	19.35 ± 0.73	0.61 ± 0.44**	0.073
	n-6PUFA+LCn-3PUFA	18.76 ± 0.84	19.13 ± 0.46	0.36 ± 0.87	
18:1n-9	SFA+LCn-3PUFA	13.65 ± 0.81	13.09 ± 0.65	-0.56 ± 0.36**	0.327
	n-6PUFA+LCn-3PUFA	13.58 ± 0.64	12.80 ± 0.81	-0.77 ± 0.43**	
18:1n-7	SFA+LCn-3PUFA	1.47 ± 0.14	1.40 ± 0.12	-0.07 ± 0.08*	0.531
	n-6PUFA+LCn-3PUFA	1.48 ± 0.17	1.44 ± 0.16	-0.04 ± 0.10	
18:2n-6	SFA+LCn-3PUFA	10.52 ± 1.14	9.03 ± 1.10	-1.48 ± 0.74**	< 0.001
	n-6PUFA+LCn-3PUFA	10.60 ± 0.87	10.94 ± 1.08	0.34 ± 0.83	
18:3n-6	SFA+LCn-3PUFA	0.11 ± 0.05	0.09 ± 0.02	-0.02 ± 0.04	0.411
	n-6PUFA+LCn-3PUFA	0.09 ± 0.03	0.09 ± 0.01	0.01 ± 0.03	
18:3n-3	SFA+LCn-3PUFA	0.25 ± 0.05	0.20 ± 0.03	-0.04 ± 0.03**	0.785
	n-6PUFA+LCn-3PUFA	0.23 ± 0.04	0.19 ± 0.03	-0.04 ± 0.05*	
20:0	SFA+LCn-3PUFA	0.56 ± 0.08	0.62 ± 0.06	0.05 ± 0.05**	0.015
	n-6PUFA+LCn-3PUFA	0.54 ± 0.08	0.54 ± 0.06	0.00 ± 0.05	
20:1n-9	SFA+LCn-3PUFA	0.30 ± 0.04	0.26 ± 0.02	-0.04 ± 0.02**	0.019
	n-6PUFA+LCn-3PUFA	0.33 ± 0.07	0.30 ± 0.06	-0.02 ± 0.01**	
20:2n-6	SFA+LCn-3PUFA	0.14 ± 0.04	0.12 ± 0.03	-0.03 ± 0.02**	0.161
	n-6PUFA+LCn-3PUFA	0.15 ± 0.04	0.11 ± 0.03	-0.04 ± 0.02**	
20:3n-6	SFA+LCn-3PUFA	0.34 ± 0.05	0.27 ± 0.03	-0.07 ± 0.03**	< 0.001
	n-6PUFA+LCn-3PUFA	0.34 ± 0.03	0.30 ± 0.03	-0.04 ± 0.02**	
20:4n-6	SFA+LCn-3PUFA	19.19 ± 1.32	17.42 ± 1.12	-1.77 ± 0.51**	0.849
	n-6PUFA+LCn-3PUFA	19.81 ± 0.98	17.99 ± 0.96	-1.81 ± 0.61**	
20:5n-3	SFA+LCn-3PUFA	1.20 ± 0.30	2.30 ± 0.48	1.10 ± 0.37**	< 0.001
	n-6PUFA+LCn-3PUFA	1.20 ± 0.36	1.71 ± 0.41	0.51 ± 0.27**	
22:5n-3	SFA+LCn-3PUFA	3.66 ± 0.58	3.33 ± 0.66	-0.33 ± 0.59*	0.744
	n-6PUFA+LCn-3PUFA	4.07 ± 0.76	3.46 ± 0.72	-0.61 ± 0.72*	
22:6n-3	SFA+LCn-3PUFA	6.43 ± 1.11	9.52 ± 1.31	3.09 ± 0.92**	0.301
	n-6PUFA+LCn-3PUFA	5.59 ± 1.18	8.31 ± 1.08	2.71 ± 1.16**	

Abbreviations: n-6PUFA: omega-6 polyunsaturated fatty acids; SFA: saturated fatty acids. Data are presented as mean ± s.d. ($n = 13$ for the SFA+LCn-3PUFA diet and $n = 12$ for the n-6PUFA+LCn-3PUFA diet). Significant difference between values before and after intervention, $^{*}P < 0.05$ and $^{**}P < 0.01$. [a]*P*-values for comparison between diets.

Table 5. Subject characteristics before and after intervention

	Diet	*Before intervention*	*After intervention*	*ΔChange*	P[a]
Body mass index	SFA+LCn-3PUFA	23.61 ± 4.14	23.88 ± 4.26	0.27 ± 0.28*	0.100
	n-6PUFA+LCn-3PUFA	23.15 ± 2.31	23.56 ± 2.36	0.41 ± 0.43*	
Percentage body fat	SFA+LCn-3PUFA	29.09 ± 10.93	29.61 ± 11.03	0.52 ± 1.30	0.913
	n-6PUFA+LCn-3PUFA	24.93 ± 7.49	25.28 ± 7.06	0.35 ± 1.13	
Triglycerides	SFA+LCn-3PUFA	1.01 ± 0.50	0.82 ± 0.24	-0.19 ± 0.32*	0.288
	n-6PUFA+LCn-3PUFA	0.97 ± 0.33	0.73 ± 0.19	-0.24 ± 0.20**	
Total cholesterol	SFA+LCn-3PUFA	5.53 ± 0.73	5.99 ± 0.84	0.46 ± 0.60*	0.019
	n-6PUFA+LCn-3PUFA	5.28 ± 0.58	5.13 ± 0.54	-0.16 ± 0.44	
LDL-C	SFA+LCn-3PUFA	3.45 ± 0.61	3.92 ± 0.79	0.48 ± 0.48*	0.005
	n-6PUFA+LCn-3PUFA	3.40 ± 0.53	3.22 ± 0.60	-0.17 ± 0.42	
HDL-C	SFA+LCn-3PUFA	1.62 ± 0.34	1.69 ± 0.31	0.08 ± 0.16	0.404
	n-6PUFA+LCn-3PUFA	1.45 ± 0.24	1.58 ± 0.35	0.13 ± 0.17*	
Ratio of total cholesterol/HDL-C	SFA+LCn-3PUFA	3.55 ± 0.74	3.64 ± 0.73	0.09 ± 0.34	0.011
	n-6PUFA+LCn-3PUFA	3.73 ± 0.69	3.41 ± 0.81	-0.33 ± 0.45*	
hsCRP	SFA+LCn-3PUFA	3.06 ± 4.26	3.21 ± 4.93	0.15 ± 1.93	0.352
	n-6PUFA+LCn-3PUFA	1.09 ± 1.49	0.99 ± 0.77	-0.10 ± 1.42	

Abbreviations: n-6PUFA: omega-6 polyunsaturated fatty acids; SFA: saturated fatty acids. Data are presented as mean ± s.d. (n = 13 for the SFA+LCn-3PUFA diet and n = 12 for the n-6PUFA+LCn-3PUFA diet). Triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and high-sensitivity C-reactive protein (hsCRP) are presented as mmol/L. Significant difference between values before and after intervention, $^{*}P < 0.05$ and $^{**}P < 0.01$. [a]*P*-values for comparison between diets.

co-administration of LCn-3PUFA with SFA or n-6PUFA merits further investigation and is of utmost importance in evaluating the full atherogenic potential of the type of background fat consumed. In addition, whether the background dietary fat alters the ability of LCn-3PUFA to modulate oxidized LDL-C levels is also worthy of further investigation.

Strengths of the study include compliance to the diet and stability in lifestyle (physical activity and general health)

throughout the trial period. Limitations include the lack of control diets, which are high in SFA and n-6PUFA but without LCn-3PUFA supplementation. However, diets high in SFA and n-6PUFA have been previously compared,[43,44] and their effects on lipid levels have been broadly discussed. Small sample size and short study duration may also have limited the findings. Nonetheless, the study design that we have used has allowed us to extend knowledge in this area.

Overall, the study findings support our working hypothesis that LCn-3PUFA status is an important determinant of the lipid modulating potential of dietary saturated and n-6PUFA.[45] Evaluation of LDL particle size and oxidizability is an important future direction in order to fully evaluate the atherogenic potential of major dietary fats.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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