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ORIGINAL ARTICLE

Consumption of an n-3 polyunsaturated fatty acid-enriched dip modulates plasma lipid profile in subjects with diabetes type II

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Objective: Recent developments in micro-emulsification technology have allowed the fortification of foods with long-chain n-3 polyunsaturated fatty acid (PUFA) without the undesirable fish odour/taste and with reasonable shelf life. The effects of supplementing the diets of people with diabetes type II with a hummus-based dip enriched with long-chain n-3PUFA on plasma fatty acid composition and lipid levels were examined.
Design: A pre- and post-intervention study.
Setting: This study was conducted at the University of Newcastle, Australia.
Subjects: Participants were recruited via advertisements on the University of Newcastle notice boards and in the local newspapers. Following initial response to study advertisements, information statements were mailed out to 29 potential participants. Thirteen participants were eligible and consented to participate in the trial. There were no dropouts as all the 13 participants completed 6-week intervention trial.
Methods: Free-living male and female subjects with diabetes type II ($n=13$) consumed the n-3PUFA-enriched dip for a period of 6 weeks. Fasting blood samples were collected pre- and post-intervention for analyses of fatty acids and plasma lipids.
Results: Following 6 weeks of consuming the enriched dip, all the long-chain n-3PUFA (20:5n-3, 22:5n-3 and 22:6n-3) were significantly ($P<0.05$) elevated in the plasma lipids. This represented an increase in 20:5n-3 content by 117%, an increase in 22:5n-3 content by 15% and an increase in 22:6n-3 content by 80% over the baseline values before dip consumption. A significant reduction ($P<0.05$) in the plasma triglyceride levels from 1.93 (1.08–2.09) mmol/l at baseline to 1.27 (0.93–2.22) mmol/l after 6 weeks was also apparent following the consumption of the n-3PUFA-enriched dip. Plasma cholesterol was unchanged; however, low-density lipoprotein (LDL)-cholesterol (2.46 ± 0.21 versus 2.72 ± 0.22 mmol/l, $P<0.034$) and high-density lipoprotein (HDL)-cholesterol (1.16 ± 0.09 versus 1.22 ± 0.09 mmol/l, $P<0.042$) were significantly increased following the dietary intervention.
Conclusions: These results demonstrate that n-3PUFA are readily bioavailable from the fortified dip matrix and alter the plasma lipid profile.
Sponsorship: This study was conducted without a dedicated fund source.
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Introduction

Epidemiological and experimental evidence suggests that the consumption of long-chain n-3 polyunsaturated fatty acids (PUFA) is associated with a reduced risk of cardiovascular disease, certain types of cancer, inflammatory disease (rheumatoid arthritis, asthma, lupus and ulcerative colitis), diabetes mellitus, multiple sclerosis and clinical depression

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(He *et al*., 2004; Ruxton, 2004; Ruxton *et al*., 2004). These effects are mediated by alterations in circulating lipid levels, eicosanoids, cytokines and physico-chemical properties of the cellular membranes (Balk *et al*., 2006; Mori and Woodman, 2006). Long-chain n-3 fatty acids are pleiotropic molecules with a broad variety of biological actions including hypotriglyceridaemic, anti-aggregatory, anti-inflammatory and anti-arrhythmic responses.

The US Food & Drug Administration (FDA) has granted a qualified health claim for dietary n-3PUFA supplements: 'Consumption of ω-3 fatty acids may reduce the risk of coronary heart disease. FDA evaluated the published data and determined that, although there is scientific evidence supporting the claim, the evidence is not conclusive' (US Food and Drug Administration, 2004). The UK has become the first country outside the US to grant an n-3 fish oil health claim that manufacturers throughout Europe have begun applying to their products. The claim, issued by the Joint Health Claims Initiative, made up of consumer protection groups, food law enforcers and members of the food industry, states: 'Eating 3 g weekly, or 0.45 g daily, long-chain n-3 PUFA, as part of a healthy lifestyle, helps maintain heart health' (Joint Health Claims Initiative, 2005). The importance of long-chain n-3PUFA as an essential nutrient for a healthy balanced diet has recently been recognized in Australia and New Zealand with their inclusion as a macronutrient recommendation in the draft document of nutrient reference values, previously known as recommended dietary intakes, which is due to be released shortly (National Health and Medical Research Council, 2006).

Western diets fall well-short of the recommended two to three oily fish servings per week (Gregory *et al*., 1990; British Nutrition Foundation, 1992; Kris-Etherton *et al*., 2000, 2002; Sugano and Hirahara, 2000; Meyer *et al*., 2003). Food formulators have thus developed other ways of increasing fish oil intake, and a wide range of products, including eggs, breads, crackers, milks, cheeses and juices, is expected to carry a long-chain n-3PUFA health claim in the near future.

A hummus-based dip product containing long-chain n-3PUFA, using a micro-emulsification technique to mask the undesirable fish odour and taste, has been developed. The aim of the study was to investigate the bioavailability of n-3PUFA from the dip matrix and the associated effects on blood lipids and some other cardiovascular risk factors. The study involved people with diabetes type II because they are known to have elevated levels of plasma triglyceride, and dietary strategies to reduce triglyceride levels may be beneficial for the prevention of cardiovascular complications in these individuals (Lombardo and Chicco, 2006).

Materials and methods

The studies described herein were approved by the Human Research Ethics Committee of the University of Newcastle. Thirteen male and female participants with established diabetes type II were recruited via advertisement. Diabetic patients taking fish oil supplements or lipid-lowering medications were excluded. Participants were asked to refrain from eating seafood for at least 2 weeks before and during the 6-week intervention period. Following initial response to study advertisements, information statements were mailed out to 29 potential participants. Thirteen participants were eligible and consented to participate in the trial. There were no dropouts as all the 13 participants completed 6-week intervention trial.

All participants consumed 100 g/day of long-chain n-3PUFA-enriched dip for a period of 6 weeks. This dip provided 1.3–1.4 g of long-chain n-3PUFA/100 g. The dip contained healthy ingredients such as chickpeas as a base with added ingredients such as olives, sun-dried tomatoes, jalapeno and capsicum. The total fat content of the dip was 6.4–8.4 g/100 g, dependent on the added ingredients, with no more than 20% (1.3–1.7 g/100 g) of this as saturated fatty acids. Participants were given the choice of choosing a jalapeno- and/or an olive-flavoured dip.

Participants were instructed not to make any change in the type of foods that they normally consumed or in their level of physical activity during the study. Venous blood samples were collected at the start of the dietary intervention and following 6 weeks of dip consumption. Blood samples were centrifuged at 3000 *g* for 15 min to separate plasma from red blood cells. Plasma samples were analysed for plasma cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, glucose, glycosylated haemoglobin (Hb_{A1c}) and C-reactive protein (CRP) using standard analytical techniques.

Plasma fatty acid profiles were determined using an established method by Lepage and Roy (1986). Frozen plasma samples were thawed at room temperature. To 100 μl of plasma, 2 ml of a methanol/toluene mixture (4:1 v/v), containing C21:0 (0.02 mg/ml) as an internal standard and butylated hydroxytoluene (0.12 g/l), was added and vortexed vigorously. Fatty acids were methylated by adding 200 μl of acetyl chloride dropwise whereas vortexing, followed by heating to 100°C for 1 h. After cooling, the reaction was stopped by adding 5 ml of 6% K_2CO_3 followed by vigorous mixing by vortex. The sample was centrifuged at 3000 *g* at 4°C for 10 min to facilitate separation of the layers. The upper toluene layer containing the fatty acid methyl esters was transferred to a 2 ml glass vial and crimp sealed with a Teflon-lined cap for analysis by gas chromatography (GC).

GC analysis was conducted using a $30 \times 0.25\,mm^2$ (DB-225) fused carbon-silica column, coated with cyanopropylphenyl (J & W Scientific, Folsom, CA, USA). The temperatures of both the injector port and the detector port were set at 250°C (MacDonald-Wicks and Garg, 2004). The oven temperature was 170°C for 2 min, increased by 10°C/min to 190°C, held for 1 min, then increased by 3°C/min up to 220°C and maintained to give a total run time of 30 min. A split ratio of 10:1 and an injection volume of 3 μl were used. The chromatograph was equipped with a flame

ionisation detector and an auto-sampler. Sample fatty acid methyl ester peaks were identified by comparing their retention times with those of a standard mixture of fatty acid methyl esters and quantified using a Hewlett Packard 6890 Series gas chromatograph with ChemStation software Version A.04.02.

All participants weighed and recorded all foods and beverages consumed for a 3-day period at the commencement of and at the end of the 6-week intervention. Nutrient intakes were calculated using the Foodworks Professional Edition (version 3.02) software programme (Xyris Software Australia Pty Ltd, Brisbane, Australia).

Before statistical analysis, the data were assessed for linearity and homoscedasticity using the Scatterplot option and for normality using the Explore function of the Statistical Package for Social Sciences (SPSS), version 13.0 for Windows. The data were analysed using a paired *t*-test to compare means for each measurement at baseline with post-dip consumption (SPSS version 13.0 for Windows). Data that were not normally distributed (including triglyceride and CRP concentrations and percentage Hb_{A1c}) were analysed by the Wilcoxon signed-ranks test, also using SPSS version 13.0 for Windows.

Results

Thirteen people with diabetes type II, 10 male subjects and three female subjects, were enrolled in the study. The average body weight was 62.2 kg and the average height was 171 cm. The diabetic state of the participants was confirmed by elevated fasting glucose (Table 1). There was no change in body weight or the body mass index values following consumption of the dip for 6 weeks. The nutrient intakes remained unchanged during the 6-week intervention period (Table 2). Energy intake was 9.66 ± 1.14 MJ/day before intervention and 8.96 ± 0.57 MJ/day post-intervention; protein intake was 99.3 ± 6.8 and 94.9 ± 6.1 g/day; carbohydrate intake was 291 ± 56 and 248 ± 29 g/day; total fat intake was 71.8 ± 6.0 and 73.2 ± 6.1 g/day, respectively. No differences were noted in the saturated (27.9 ± 3.0 versus 28.0 ± 1.8 g/day, respectively), monounsaturated (25.9 ± 2.4 versus 27.5 ± 3.5 g/day, respectively) and polyunsaturated (10.9 ± 0.9 versus 11.3 ± 1.6 g/day, respectively) fatty acid content before and following consumption of the long-chain n-3PUFA-enriched dip (Table 2).

The dip provided 1.5–1.8 g/day of saturated (14:0, 6:0, 18:0, 22:0), 1.9–3.7 g/day of monounsaturated (16:1, 18:1 and 20:1), 1.6–1.7 g/day of *n*-6 (18:2, 18:3, 20:2 and 20:4) and 1.3–1.4 g/day of *n*-3 (18:3, 20:5, 22:5 and 22:6) fatty acids (Table 3) depending on the variety (jalapeno or olive flavour) of dip consumed. The dip was well-tolerated and the participants reported no adverse health effects consequent upon consuming 100 g of the dip daily for a period of 6 weeks.

Plasma cholesterol tended to be higher but not significantly so (4.43 ± 0.27 versus 4.65 ± 0.26 mmol/l, $P = 0.061$)

Table 2 Average values for nutrient intakes from 3-day weighed food records at the beginning and end of the 6-week experimental period[a]

Nutrient	*Baseline*[b] (n = 1)	*Post-intervention*[c] (n = 1)	P-*value*[d]
Energy (MJ/day)	9.66 ± 1.14	8.96 ± 0.57	0.4
Protein (g/day)	99.26 ± 6.8	94.88 ± 6.1	0.5
Carbohydrate (g/day)	291 ± 56	248 ± 29	0.2
Sugar (g)	97.2 ± 7.3	96.9 ± 7.9	1.0
Starch (g)	169 ± 34	136 ± 19	0.1
Fibre (g)	30.9 ± 3.6	26.1 ± 2.2	0.1
Fat (g)	71.81 ± 6.0	73.19 ± 6.8	0.8
Saturated fat (g)	27.87 ± 3.0	28.00 ± 1.8	1.0
Monounsaturated fat (g)	25.93 ± 2.4	27.48 ± 3.5	0.6
Polyunsaturated fat (g)	10.91 ± 0.9	11.29 ± 1.6	0.8
Cholesterol (mg)	253 ± 36	237 ± 20	0.7

[a]Values are presented as mean ± s.e.m.
[b]Values are the average of the 3-day (2 weekdays and 1 weekend day) weighed food records in the first week of the experimental period.
[c]Values are the average of the 3-day (2 weekdays and 1 weekend day) weighed food records in the last week of the experimental period.
[d]The *P*-value is considered to be significant at $P < 0.05$.

Table 1 Demographic details, lipid profiles and glycaemic control before and following 6-week consumption of the long-chain-n-3PUFA-enriched dip

Measurement	*Baseline*	*Post-intervention*	*Mean change*	P-*value*
Body weight (kg)	82.44 ± 3.14	82.27 ± 2.97	-0.16 ± 0.42	0.702
Body mass index	28.45 ± 1.03	28.42 ± 3.09	-0.03 ± 0.14	0.802
Total cholesterol (mmol/l)	4.43 ± 0.27	4.65 ± 0.26	0.22 ± 0.10	0.061
LDL-cholesterol (mmol/l)	2.46 ± 0.21	2.72 ± 0.22	0.26 ± 0.11	0.034
HDL-cholesterol (mmol/l)	1.16 ± 0.09	1.22 ± 0.09	0.06 ± 0.03	0.042
Total/HDL-cholesterol	4.05 ± 0.36	4.08 ± 0.41	0.03 ± 0.10	0.774
Triglyceride (mmol/l)	1.93 (1.08–2.09)	1.27 (0.93–2.22)	-0.25 ± 0.16	0.038
CRP (mg/l)	5.9 ± 2.6	4.1 ± 1.1	-1.8 ± 1.61	0.203
Fasting glucose (mmol/l)	8.04 ± 0.58	8.14 ± 0.63	0.10 ± 0.56	0.860
Hb_{A1c} (%)	6.40 (6.10–7.85)	6.70 (6.20–7.65)	-0.04 ± 0.11	0.752

Abbreviations: CRP, C-reactive protein; $Hb_{A1c,}$ glycosylated haemoglobin; HDL-cholesterol, high-density lipoprotein cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol.
Values are mean ± standard error of the mean (s.e.m.), except values for triglyceride concentration and Hb_{A1c} which are medians with 25th and 75th percentiles.

Table 3 Fatty acid composition (mg/100 g) of the *n*-3PUFA-enriched dip. Values are the average of at least triplicate samples taken at random

Fatty acid	*Olive dip*	*Jalapeno dip*
C14:0	256	241
C16:0	1144	934
C16:1*n*-7	284	258
C18:0	303	259
C18:1*n*-9	3119	1421
C18:1*n*-7	199	144
C18:2*n*-6	1676	1606
C18:3*n*-6	10	10
C18:3*n*-3	173	84
C20:0	39	28
C20:1*n*-9	87	74
C20:2*n*-6	10	11
C20:4*n*-6	32	32
C20:5*n*-3	546	530
C22:5*n*-3	76	74
C22:6*n*-3	596	585
Total saturated fatty acids	1759	1476
Total MUFA	3708	1920
Total *n*-6PUFA	1723	1654
Total *n*-3PUFA	**1391**	**1273**
Total lipid content	8640	6380

Abbreviations: MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

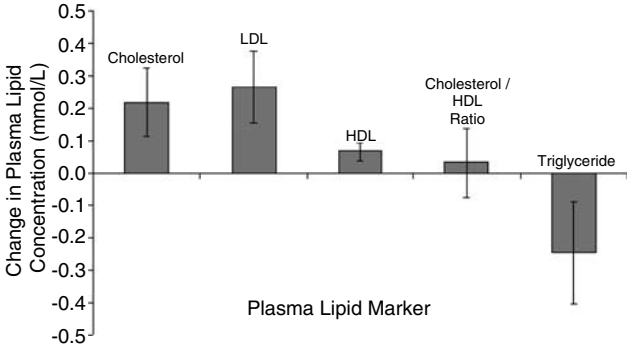

Figure 1 Change in plasma lipid concentration (total, LDL- and HDL-cholesterol and triglyceride) (mmol/l) and the ratio of total cholesterol to HDL-cholesterol from baseline to 6 weeks post-consumption of the long-chain n-3PUFA-enriched dip. Values are mean ± s.e.m.

following consumption of the long-chain-n-3PUFA-enriched dip. Both LDL-cholesterol (2.46 ± 0.21 versus 2.72 ± 0.22 mmol/l, $P < 0.034$) and HDL-cholesterol (1.16 ± 0.09 versus 1.22 ± 0.09 mmol/l, $P < 0.042$) were significantly increased following the dietary intervention (Table 1, Figure 1). The ratio of plasma cholesterol to HDL-cholesterol remained unchanged following 6-week consumption of the enriched dip. Plasma triglyceride values were significantly reduced (−0.25 mmol/l, $P = 0.038$) by dip consumption (Table 1, Figure 1). Fasting glucose level, percentage Hb_{A1c} and CRP concentration were not affected by the dietary intervention.

Table 4 Fatty acid composition of plasma (mean ± s.e.m.) lipids before and following 6-week consumption of the long-chain *n*-3PUFA-enriched dip

Fatty acid	*Plasma fatty acid composition (wt/wt %)*		
	Baseline	*Post-intervention*	P-*value*
C14:0	0.93 ± 0.09	0.86 ± 0.07	0.385
C16:0	20.94 ± 0.39	20.83 ± 0.56	0.841
C16:1*n*-7	2.21 ± 0.16	2.12 ± 0.20	0.443
C18:0	8.52 ± 0.43	8.33 ± 0.46	0.349
C18:1*n*-9	24.10 ± 0.72	23.25 ± 0.80	0.292
C18:1*n*-7	1.75 ± 0.08	1.64 ± 0.06	0.122
C18:2*n*-6	22.49 ± 1.18	21.70 ± 1.08	0.205
C18:3*n*-6	0.55 ± 0.05	0.48 ± 0.05	0.060
C18:3*n*-3	0.86 ± 0.08	0.64 ± 0.05	0.007
C20:0	0.37 ± 0.08	0.30 ± 0.02	0.353
C20:1*n*-9	0.67 ± 0.43	0.22 ± 0.02	0.326
C20:2*n*-6	0.22 ± 0.03	0.19 ± 0.02	0.136
C20:3*n*-6	1.70 ± 0.10	1.49 ± 0.09	0.011
C20:4*n*-6	6.51 ± 0.43	6.03 ± 0.27	0.117
C20:5*n*-3	1.38 ± 0.18	2.99 ± 0.47	0.000
C22:5*n*-3	0.77 ± 0.05	0.89 ± 0.08	0.037
C22:6*n*-3	2.42 ± 0.24	4.34 ± 0.54	0.000

The fatty acid composition of the plasma lipids reflected the long-chain n-3PUFA enrichment in the dip matrix. All the long-chain n-3PUFA (18:3, 20:5, 22:5 and 22:6) were increased following 6-week consumption of the enriched dip (Table 4). Saturated, monounsaturated and n-6 polyunsaturated fatty acids were not affected by the ingestion of the long-chain-n-3PUFA-enriched dip.

Discussion

Functional foods enriched with long-chain n-3PUFA that are currently available to consumers include breads, dairy products, eggs and meats. One of the limitations of these foods is that they need to be consumed in large quantities to meet a dietary recommendation of 200 mg/day for healthy adults or even in larger amounts to meet a dietary recommendation of 1000 mg/day of long-chain n-3PUFA for people at high risk of cardiovascular disease (Garg *et al*., 2006a, b). We have developed a low-fat (total fat 6.4–8.6%), low-saturated fat (1.5–1.8 g/100 g), low sodium (<200 mg/100 g) humus-based dip containing relatively high amounts of long-chain-n-3PUFA using a micro-emulsification technique to mask the undesirable fish odour and taste. One tablespoon (20 g) of this dip is sufficient to meet daily requirements (200 mg/day) for healthy adults, and three to four tablespoons (75 g) can provide 1 g/day of long-chain n-3PUFA for people with cardiovascular risk factors. One hundred grams of the dip provides long-chain PUFA equivalent to the amount obtained from 4 × 1 g of common brands of fish oil capsules. The aims of this study were to test

the bioavailability of long-chain n-3PUFA from the dip matrix and to test the effectiveness of the dip enriched with long-chain n-3PUFA on plasma lipid levels. People with diabetes type II are known to have elevated levels of plasma triglyceride and dietary strategies to reduce triglyceride levels may be beneficial for the prevention of cardiovascular complications in these individuals (Lombardo and Chicco, 2006). The results presented demonstrate that the long-chain n-3PUFA present in the enriched dip was incorporated into the plasma lipids. The parent fatty acid of the n-3PUFA family (18:3n-3) was significantly lower in the plasma lipids following the dip intervention. This is likely to be a reflection of the foods, other than the dip, consumed during the intervention period as the fortified dip provided adequate amounts of 18:3n-3 (2.0% or 173 mg/100 g in the olive and 1.3% or 84 mg/100 g in the jalapeno-flavoured dip). Previous studies using fish oil capsules (containing long-chain n-3PUFA; 20:5n-3, 22%n-3 and 22:6n-3) as a supplement has not demonstrated such a change in plasma 18:3n-3 levels (Garg *et al*., 2006a, b). Consumption of the dip was also accompanied by changes in the plasma lipid profile, typical of the effects of long-chain *n*-3PUFA when supplemented in capsule form.

As a major aim of the study was to demonstrate the uptake of long-chain n-3PUFA when consumed in a dip matrix, the fatty acid composition of the plasma total lipids was determined, because it represents incorporation into all lipid fractions including phospholipids, non-esterified fatty acids, triglycerides and cholesteryl esters. A 6-week intervention period was considered to be adequate to detect alterations in the plasma lipids and fatty acid composition and to provide evidence that up to 100 g/day of the enriched dip could be comfortably consumed by participants over that period of time. Participants reported no difficulties in consuming the amount of dip and reported favourably on the product. Two flavours of the dip were made available to the participants, which may have assisted in improved compliance. Compliance in the study, as determined by return of the empty containers and indeed evident from the fatty acid composition of plasma lipids, was 100%. The dip was well tolerated by the study participants with no detectable fishy odour/eructation and no gastrointestinal disturbances reported as in the case of fish oil capsules (Kolanowski *et al*., 1999).

Six-week consumption of the n-3PUFA-enriched dip had no adverse effects on glycaemia control, as evident by no significant ($P>0.05$) change in fasting glucose or Hb_{A1c} values. Low-grade sub-clinical inflammation, as determined by plasma levels of CRP, also remained unchanged following consumption of the dip. As CRP is also a common biomarker for obesity, diabetes and cardiovascular disease (Dandona *et al*., 2004), it appears that the dip can be safely consumed by people with these conditions as there were no adverse effects of the dip on CRP levels.

Energy intake and percentage energy from protein, carbohydrate and fat remained constant during the study. Fibre and alcohol intake as well as saturated fat level in the diet remained unchanged during the 6-week intervention period. These results demonstrate that the effects on plasma lipids and fatty acid composition following dip consumption were specifically a consequence of the dip ingredients. Moreover, although the levels of physical activity were not determined, all the participants were clearly instructed not to alter their physical activities during the trial. Finally, none of the participants reported any change in medication over the 6-week intervention period. The total fat content of the participants' diet remained unchanged despite an additional 6.0–8.5 g of fat originating from 100 g of the long-chain n-3PUFA dip. Apparently, this oil intake was compensated for by adjustments in the fats consumed from other foods, indicating a replacement of possibly otherwise bad fats with healthy oil from the dip, keeping the total lipid intake constant as evident by increased, although non-significant, intake of monounsaturated and PUFA during the intervention period (Table 2).

Consumption of 1.3–1.4 g/day of long-chain n-3PUFA in the dip matrix for a period of 6 weeks resulted in a 35% reduction in the median plasma triglyceride values. A critical appraisal of 10 available randomized controlled trials addressing the efficacy of long-chain n-3PUFA as secondary agents for the prevention of hypertriglyceridaemia revealed that the average decrease in plasma triglyceride was 29% (Lewis *et al*., 2004). Mechanisms for the reduction of plasma triglyceride involve a significant decrease in very LDL synthesis in the liver (Harris *et al*., 1997) and an increased triglyceride clearance as indicated by an increase in fractional catabolic rate (Simopoulos, 1999). The results presented here suggest that the enriched dip may serve as a safe and effective alternative means of treating hypertriglyceridaemia, *in lieu* of lipid-lowering medications. The results presented are also consistent with a meta-analysis of 26 different trials in diabetic studies that showed that long-chain n-3PUFA supplementation is associated with a significant decrease in plasma triglyceride in a dose-dependent manner (Friedberg *et al*., 1998).

A small but statistically significant increase in LDL-cholesterol was noted following dietary supplementation with the long-chain n-3PUFA-enriched dip. This is consistent with published reports (Lu *et al*., 1999; Damsgaard *et al*., 2006). A recent meta-analysis using a multivariate approach revealed that long-chain n-3PUFA supplementation increased total, LDL- and HDL-cholesterol (Castro *et al*., 2005). In our study, total cholesterol was increased but the difference did not reach significance ($P>0.05$), whereas HDL-cholesterol was significantly elevated following consumption of the dip for 6 weeks. The ratio of total cholesterol to HDL-cholesterol was not affected by the dietary intervention. The clinical significance of the increase in LDL- and HDL-cholesterol following n-3PUFA supplementation in some studies including this study, in the absence of no change in the ratio of total cholesterol to HDL-cholesterol, remains unclear.

In summary, short-term (6 weeks) consumption of a long-chain n-3PUFA-enriched dip product allows incorporation of long-chain n-3PUFA into plasma lipids and exhibits the known lipid-modifying effects. Participants were asked to refrain from eating seafood for at least 2 weeks before and during the 6-week intervention period to ensure that the results obtained truly reflect the effects of the long-chain n-3PUFA dip supplement, not an artefact of the diet. However, this may have made the effects look stronger than it truly is, by creating an artificially low base-line plasma level of 20:5n-3 and 22:6n-3. Nevertheless, the developed functional food appears to be an ideal way to conveniently meet the dietary recommendation of 200 mg/day of long-chain n-3PUFA (from just 20 g of the enriched dip) or even a recommendation of 450 mg/day (from 35 g of the enriched dip) as stipulated in the UK Health Claims guidelines. A larger longer-term placebo-controlled, randomized clinical trial is warranted to confirm the lipid-lowering potential of the enriched dip.

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