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Use of manufactured foods enriched with fish oils as a means of increasing long-chain *n*-3 polyunsaturated fatty acid intake

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The objectives of the present study were to determine the feasibility of using manufactured foods, enriched with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as a means of increasing the intake of these *n*-3 polyunsaturated fatty acids (PUFA), and to determine the effect of the consumption of these foods on postprandial lipaemia and other metabolic responses to a high-fat mixed test meal. Nine healthy, normotriacylglycerolaemic, free-living male volunteers (aged 35–60 years) completed the randomized, controlled, single-blind, crossover study. The study consisted of two periods (each of 22 d) of dietary intervention, separated by a 5-month washout period. During these two periods the subjects were provided with the manufactured foods enriched with EPA and DHA (*n*-3 enriched) or identical but unenriched foods (control). A mixed test meal containing 82 g fat was given to the fasted subjects on day 22 of each dietary intervention period. Two fasting, and thereafter hourly, blood samples were collected from the subjects for an 8 h period postprandially. Plasma triacylglycerol, total and HDL-cholesterol, non-esterified fatty acids (NEFA), glucose and immunoreactive insulin levels, post-heparin lipoprotein lipase (*EC* 3.1.1.34) activity and the plasma free fatty acid and phospholipid fatty acid compositions were measured. A mean daily intake of 1·4 g EPA + DHA (0·9 g EPA, 0·5 g DHA) was ingested during the *n*-3-enriched dietary period, which was significantly higher than the intake during the habitual and control periods ($P < 0·001$) assessed by a 3 d weighed food intake. A significantly higher level of EPA + DHA enrichment of the plasma fatty acids and phospholipids ($P < 0·001$) after the *n*-3-enriched compared with the control intervention periods was also found. The energy intake on both of the dietary intervention periods was found to be significantly higher than on the habitual diet ($P < 0·001$), with an increase in body weight of the subjects, which reached significance during the *n*-3 PUFA-enriched dietary intervention period ($P < 0·04$). The palatability of the enriched foods was not significantly different from that of the control foods. Significantly higher fasting plasma HDL-cholesterol and glucose concentrations were found after the *n*-3 PUFA-enriched compared with the control intervention period ($P < 0·02$ and $P < 0·05$ respectively). No significant differences were found for the postprandial lipid and hormone measurements, except for significantly lower levels of NEFA at 60 min after the *n*-3-enriched intervention period ($P < 0·04$). Enriched manufactured foods were a feasible vehicle for increasing *n*-3 PUFA intake. However the nature of the foods provided as the *n*-3 vehicle may have contributed to the increased body weight and higher energy intakes which were adverse consequences of the intervention. These factors, together with the short duration of the study may have been reponsible for the failure to observe significant plasma triacylglycerol reductions in response to daily intakes of 1·4 g EPA + DHA.

***n*-3 Polyunsaturated fatty acids: Eicosapentaenoic acid: Docosahexaenoic acid**

Epidemiological studies have related the high habitual intakes of fatty fish with a low incidence of ischaemic heart disease in the Eskimo population of Greenland (Bang & Dyerberg, 1972; Bjerregaard & Dyerberg, 1988) and the Japanese population (Hirai *et al.* 1980). Many studies have investigated the effects of supplementation with marine *n*-3 polyunsaturated fatty acids (PUFA; especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) on human plasma lipids and lipoproteins and have consistently found a lowering of plasma triacylglycerol (TAG) concentrations, although the effects of these agents on cholesterol and lipoprotein levels have been varied (Harris, 1989). In addition to the potential beneficial effects of *n*-3 PUFA on circulating lipids, these fatty acids have beneficial haemostatic effects which may contribute to a decreased risk from CHD. These include reduced platelet aggregation, reduced blood pressure, decreased viscosity and a lower incidence of cardiac arrhythmias (Weber & Leaf, 1991).

Despite the apparent consistency of findings in relation to physiological effects of long-chain *n*-3 PUFA, the detailed results from many of the studies are contradictory, and difficult to compare. The probable explanation for this is the large number of factors which determine the extent of the *n*-3 PUFA effect. These include: the population studied, the dose of *n*-3 PUFA ingested, the period of intervention, the source of *n*-3 PUFA, background diet and diet during the intervention period. However despite these problems, the weight of evidence suggests that *n*-3 PUFA (EPA and DHA) confer the protective effect associated with high fish intakes (Harris, 1989).

In many countries, the average level of fish consumption is very low. Moreover, the proportion of the population consuming fish is low. Some people do not like to eat fatty fish, others have reservations due to the possible toxic effects of pesticides and/or heavy metals in fatty fish. Middle-aged men and women have low intakes, and pregnant and lactating women often avoid fatty fish despite their increased requirements for long-chain *n*-3 PUFA (Conner *et al.* 1991). In the UK, the average daily fish consumption is 26 g, of which only 25 % is oily fish, the primary source of *n*-3 PUFA (Gregory *et al.* 1990). According to the present UK recommendations the intake of *n*-3 PUFA should amount to at least 0·2 g daily (Department of Health, 1994). The low intake and low proportion of the population who ingest oily fish makes the task of increasing the intake of *n*-3 PUFA difficult. However, advances in food technology have allowed the successful incorporation of fish oils (EPA and DHA) into a variety of foods, while still maintaining their palatability, acceptability and shelf-life. The aim of the present study was to investigate the feasibility of using normal manufactured foods, which had been enriched with EPA and DHA, to increase moderately the quantity of these long-chain *n*-3 PUFA ingested over a 22 d period. The absorption, effect on the composition of plasma phospholipids and any changes in fasting and postprandial lipids, lipoproteins and hormone levels were investigated in nine middle-aged, normotriacylglycerolaemic men with a low habitual fatty fish intake.

MATERIALS AND METHODS

Subjects

Nine healthy normotriacylglycerolaemic male volunteers were recruited from the staff at the University of Surrey, Guildford. The average age of the volunteers was 50 (SD 7·2) years (range 35–60 years) with BMI of 25·7 (SD 2·6) kg/m^2 (range 25–30 kg/m^2). The volunteers all satisfied the following inclusion criteria: they were between 35 and 60 years old, with a BMI between 22 and 30 kg/m^2, their habitual fat consumption was between 35 and 45 % of total energy and their habitual long-chain *n*-3 PUFA intake was $< 0{\cdot}5$ g/d.

They did not have any previous history of hyperlipidaemia, endocrine or liver disease or known alcoholism. Their alcohol intake was < 30 units per week (1 unit = 8 g alcohol) and they did not exercise aerobically for more than three 20 min sessions per week. None of the volunteers was following any form of therapeutic, or specialized, diet or taking dietary supplements of fatty acids. All subjects had a fasting blood sample collected before inclusion in the study for screening. The criteria for inclusion were: glucose < 6·5 mmol/l, total cholesterol < 7 mmol/l, TAG < 1·5 mmol/l and haemoglobin > 130 g/l.

Informed consent was obtained from each subject at the beginning of the study and ethical consent was obtained from the University of Surrey and the Royal Surrey County Hospital Research Ethics Committees.

Dietary intervention

The study design was a randomized, controlled, single-blind, crossover study which included two periods, each of 22 d duration, of dietary intervention using *n*-3 PUFA (EPA + DHA)-enriched and identical control foods; these were separated by a 5-month washout period. The food items used were bread, biscuits, cake, ice-cream, orange drink, milk shake, low-fat spread, pasta, mayonnaise, vinaigrette and milk-shake powder. Enrichment was achieved by incorporation of a fish-oil product (ROPUFA; Hoffman La Roche Ltd, Basel, Switzerland), either directly, or in a microencapsulated form, into the enriched food items during manufacture. The EPA and DHA content of all the food items used in this study were constant for the stated shelf-life (as confirmed by the manufacturers Hoffman La Roche). Control foods were prepared and packaged in an identical form to the enriched products to allow a blind protocol. The subjects were requested to incorporate the study foods into their habitual diets, exchanging them for their usual brands, in an attempt to maintain their habitual dietary intake. No foods, except fatty fish or those containing fatty fish, were excluded from their diets. The aim of the study was for the subjects to achieve a mean daily intake of 1·8 g EPA + DHA/d on the enriched diet. To this end an exchange system was used to allow the subjects choice in the foods that could be consumed to reach the objective. Each food portion was assigned an exchange value, where one exchange was equivalent to 0·2 g EPA + DHA (Pike *et al.* 1996). Subjects were asked to consume nine exchanges daily, equal to 1·8 g EPA + DHA/d. In addition to these foods, subjects were asked to consume a 113 g pot of smoked mackerel pâté (enriched diet) or tuna pâté (control diet) per week. These contained 1·6 g and 0·3 g EPA + DHA respectively. During the period of study the subjects were contacted frequently to help appropriate food choice, to answer any queries and to aid subject motivation, essential to the success of dietary intervention studies.

At the end of the 22 d intervention period the subjects were asked to score the acceptability and palatability of the individual food items using a linear analogue score ranging from 0 to 10, where 0 was 'not palatable' and 10 was 'very palatable'. They were also asked to score for compliance on a linear analogue scale where 0 was 'very compliant' and 10 was 'not at all compliant'. Before commencing the study and during the two periods of dietary intervention the subjects were asked to complete a 3 d weighed food intake (including 1 d of the weekend). These data were analysed using the FOODBASE nutrient analysis program (Version 1.2; Institute of Brain Chemistry and Human Nutrition, London).

Protocol of postprandial study days

On day 21 of the intervention period the subjects were asked not to undertake any strenuous exercise, and not to consume any alcohol or eat any food after 20·00 hours. On day 22 of the two intervention periods the subjects were asked to come into the Investigation Unit at the University of Surrey after a 12 h overnight fast. A cannula was inserted into the antecubital vein of the forearm, and two fasted blood samples were taken. The subjects were then given the test meal which consisted of 180 g white bread, 250 ml orange juice, 50 g Frosties (Kellogg's Company of Great Britain Ltd, Manchester, Greater Manchester), 150 ml whole milk, 65 ml double cream, 20 g sunflower margarine, 75 g cheddar cheese. The nutritional content of the test meal was 6·4 MJ energy, 43 g protein, 163 g carbohydrate and 82 g fat, which comprised 43 g saturated fatty acids, 8 g PUFA, 23 g monounsaturated fatty acids, 6 g *trans*-fatty acids and 2 g other fatty acids. The subjects consumed the meal within 20 min. Following the consumption of the meal the subjects were allowed free access to water, low-energy drinks and decaffeinated beverages without milk or sugar, but no food was consumed for 8 h.

Blood samples were collected at 30 min and 60 min postprandially, and then hourly for 8 h. The blood samples were collected into heparinized tubes, except for a 1 ml sample which was collected into a fluoride oxalate tube for glucose analysis. The blood samples were spun at 7000 ***g***, the plasma was then frozen in portions at −20°, for future analysis. The samples for non-esterified fatty acid (NEFA) determination were divided into portions, and frozen within minutes to reduce degradation. At 8 h a 7500 IU bolus quantity of heparin was given to the subjects intravenously and 5 ml blood samples were taken at 5 and 15 min after the heparin injection, from the opposite arm. These samples were treated as described for the other samples collected.

Analytical procedures

Preparation of triacylglycerol-rich and triacylglycerol-poor lipoprotein fractions. Plasma samples were prepared as described earlier. Then 3·5 ml plasma was overlayered with an equal volume of saline (1·006 g NaCl/ml), and ultracentrifuged for $5{\cdot}0 \times 10^6 \boldsymbol{g}$ (Lindgren *et al.* 1972) at 24° in a 10 ml centrifuge tube to prepare the triacylglycerol-rich lipoprotein (TRL) fraction using essentially the method of Grundy & Mok (1976). The top 1·2 ml of the upper layer, the TRL fraction, was carefully removed, portioned and frozen at −20° for later analysis. The remaining sample (triacylglycerol-poor lipoprotein (TPL) fraction) was mixed, portioned and stored at −20° for future analysis.

Measurement of triacylglycerols, non-esterified fatty acids, glucose, total and HDL-cholesterol. TAG, NEFA, glucose, total and HDL-cholesterol were measured on the Cobas-Mira analyser (Roche Products Ltd, Welwyn Garden City, Herts.) using enzymic kits. TAG levels were measured in plasma, TRL and TPL fractions, using an enzymic colorimetric kit supplied by Roche Diagnostic Products Ltd (Welwyn Garden City, Herts). The plasma glucose, total and HDL-cholesterol levels were analysed using kits supplied by Roche Diagnostic Products Ltd and plasma NEFA using a kit supplied by WAKO Chemical GmbH (Germany) supplied by Alpha Laboratories Ltd (London). The mean intra-assay CV for the TAG, cholesterol, NEFA and glucose assays were 2·2, 2·4, 2·0 and 4·5 % respectively. The inter-assay CV for the TAG, cholesterol, NEFA and glucose assays were 7·3, 7·4, 4·0 and 5·4 % respectively.

Measurement of insulin. Immunoreactive insulin was measured using a double radioimmunoassay (Hampton, 1984). The inter-assay CV were 10 % at 40 IU/ml.

Measurement of lipoprotein lipase activity. Lipoprotein lipase (*EC* 3.1.1.34; LPL) activity was measured in 20 μl portions of plasma taken 5 and 15 min after the administration of an intravenous dose of heparin. LPL activity was measured with LPL activity being inhibited with 4 M-NaCl by a modified method described by Nilsson-Ehle & Schotz (1976) using the detection of liberated ^{3}H-labelled free fatty acids from ^{3}H-labelled triolein substrate emulsion (Amersham International, Bucks.), as the index of triolein hydrolysis. The intra- and inter-assay CV were 5·1 and 8·7 % respectively.

Statistical analysis

Mean, SD and SEM were calculated for each measurement. Differences in time response curves were analysed by repeated measures ANOVA followed by a Tukey–Kramer test to locate individual differences. Differences in the calculated areas under the time *v.* response curves were identified by ANOVA, followed by Duncan's multiple range test to locate individual differences and assess the level of statistical significance. Values of $P < 0.05$ were taken as the lowest level of significance.

RESULTS

Nutrient intake

The mean nutrient intake of the subjects on their habitual diet and during the intervention diet period (*n*-3 enriched) and the control diet period (control) are shown in Table 1. There were significant increases in the energy, carbohydrate and fat intakes on the *n*-3-enriched and the control intervention periods when compared with the habitual diet ($P < 0.001$). However the two intervention periods did not differ in their macronutrient content. The quantities of EPA and DHA, when considered separately and together, were significantly higher in the *n*-3-enriched, compared with both the control, and habitual periods ($P < 0.001$). The estimated average intake of EPA + DHA during the *n*-3-enriched dietary period was 1·4 g/d (0·9 g EPA/d, 0·5 g DHA/d), assessed by the 3 d weighed food intake.

Palatability and compliance

There were no significant differences in palatability scores between *n*-3-enriched (mean 6·9, SD 1·0) and control foods (mean 7·4, SD 0·9). The mean compliance scores reported by the volunteers were not significantly different when the *n*-3 enriched (mean 8·0, SD 1·8) and the control (mean 8·5, SD 1·4) period were compared. The values indicated good compliance and motivation of the subjects on both intervention periods. Similarly convenience scores of the dietary intervention periods did not reveal any significant differences between the two diets, with the mean values for the *n*-3-enriched and control periods being 5·1 (SD 3·0) and 5·7 (SD 3·1) respectively. There were also no significant differences found for all compliance, convenience or palatability scores recorded in the two arms of the crossover study which could have come about through seasonal differences.

Plasma and phospholipid fatty acid enrichment

The mean EPA + DHA enrichments of the plasma and phospholipid fatty acids were not significantly different at the start of the two intervention periods. There was a significant increase in the EPA + DHA incorporation into both the plasma phospholipids and free fatty

Table 1. *The daily macronutrient, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) intakes of subjects consuming habitual,* n*-3-enriched and control diets*

(Mean values and standard deviations for nine subjects)

	Habitual diet		*n*-3-Enriched diet		Control diet	
Nutrients	Mean	SD	Mean	SD	Mean	SD
Energy (MJ)	9·2	1·1	12·0***	1·3	12·0***	1·9
Protein (g)	81	18	89	9	84	15
Carbohydrate (g)	290	52	397***	53	409***	87
Fat (g)	77	25	106***	29	102***	26
EPA (g)	0·04	0·03	0·86***†††	0·23	0·17	0·08
DHA (g)	0·08	0·05	0·50***†††	0·15	0·20	0·18
EPA + DHA (g)	0·12	0·07	1·36***†††	0·34	0·37	0·22

Mean values were significantly different from those for the habitual diet: *** $P < 0·001$.
Mean values were significantly different from those for the control diet: ††† $P < 0·001$.

acids during the enriched intervention period from day 1 to day 9 ($P < 0·001$), and a further smaller rise from day 9 to day 21, which did not reach significance. There was no significant difference in the incorporation of EPA + DHA into the plasma phospholipids or fatty acids during the control dietary period. The EPA + DHA enrichment of both the plasma fatty acids and phospholipids was significantly higher during (day 9) and immediately after (day 21) the *n*-3-enriched intervention period, compared with the control period ($P < 0·001$) as shown in Fig. 1.

Anthropometric data

Subjects' mean body weight increased during both the *n*-3-enriched and the control intervention periods by 1·2 (SD 1·4) kg (76·8 (SD 6·8) kg at the beginning, 78·0 (SD 7·3) kg at the end of the *n*-3-enriched study period) and 0·6 (SD 1·0) kg (77·6 (SD 7·7) kg at the beginning, 78·2 (SD 7·5) kg at the end of the control study period); this was found to be significant during the *n*-3-enriched period ($P < 0·04$). It was also found that, regardless of the diet type, there was a significant increase ($P < 0·007$) in the weight during arm 1 (*n* 5 on *n*-3-enriched and *n* 4 on control diet) compared with arm 2 (*n* 4 on *n*-3-enriched and *n* 5 on control diet), but there was no correlation between weight gain and energy intake. The starting weights of each subject on two diets were not significantly different.

Fasting biochemical data

The fasting plasma concentrations of TAG, total and HDL-cholesterol, glucose, insulin and the post-heparin LPL activity are shown in Table 2. The fasting concentrations of TAG, insulin and the post-heparin LPL activities were not different after the two intervention periods. The mean fasting glucose level after the *n*-3-enriched intervention period was higher than the habitual and after the control diet ($P < 0·05$). The total cholesterol concentrations were found to be significantly lower after both dietary intervention periods compared with the habitual diet ($P < 0·05$). The HDL-cholesterol level was also found to be significantly higher after the *n*-3-enriched compared with the control intervention period ($P < 0·02$).

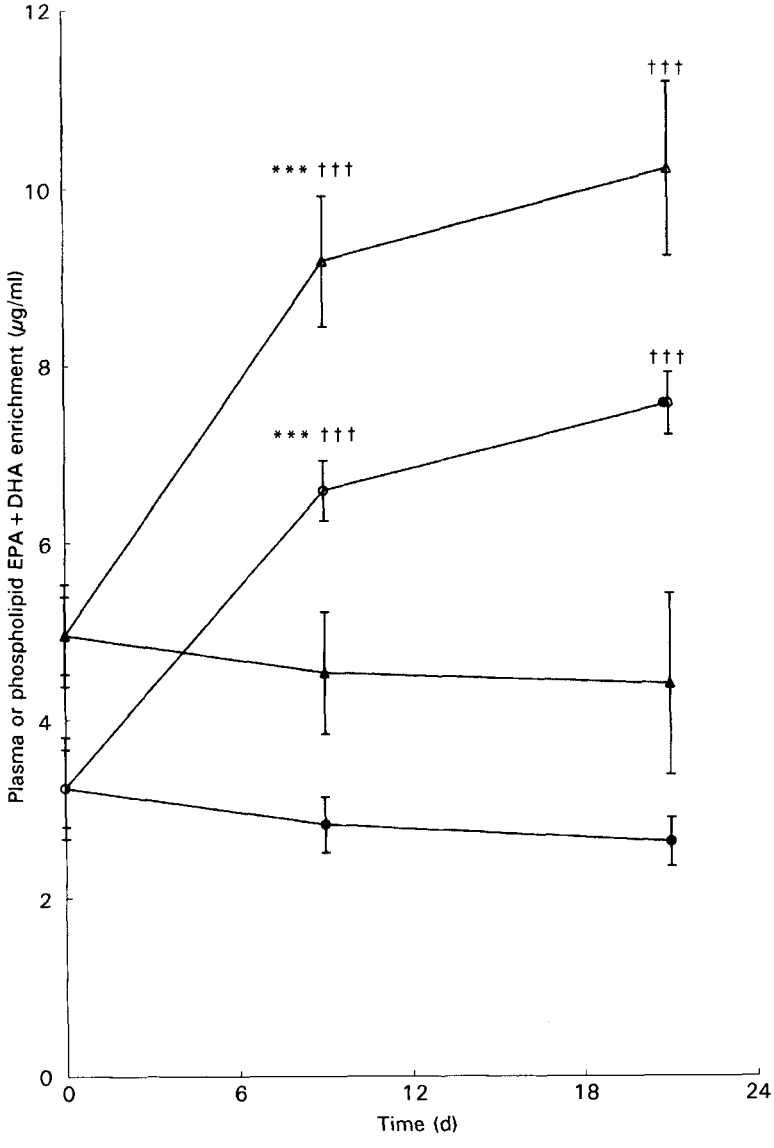

Fig. 1. Concentrations of eicosapentaenoic acid (EPA) and docosahexaneoic acid (DHA) in plasma (○, ●) and phospholipids (△, ▲) of subjects consuming an *n*-3 polyunsaturated fatty acid-enriched diet (○, △) or a control diet (●, ▲) for 22 d. Values are means for nine subjects with their standard errors represented by vertical bars. Mean values were significantly different from baseline: *** $P < 0{\cdot}001$. Mean values were significantly different from those for the control period: ††† $P < 0{\cdot}001$.

Postprandial biochemical data

There was no significant difference between the mean postprandial profiles for the TAG measured in any of the fractions (plasma, TRL and TPL fractions). Fig. 2(a) shows the postprandial profiles for the mean TRL TAG concentrations. The mean fasting TRL TAG levels were lower after the *n*-3-enriched compared with the control intervention period,

however this was not found to be significant. The postprandial profiles were very similar rising to a peak at 220 (SD 90) min and 202 (SD 96) min after the *n*-3-enriched and the control intervention periods respectively. The mean TRL TAG levels did not return to baseline levels 8 h after the meal for either intervention period. The patterns of postprandial TAG response were similar in all three fractions. However there was a tendency for the postprandial profiles after the *n*-3-enriched intervention period to be lower, compared with the control period, although this did not reach significance at any point.

Fig. 2(b) shows the postprandial NEFA responses. They followed a normal pattern, falling sharply after the consumption of the test meal and gradually rising to above postprandial levels by 8 h at the end of the study. At 60 min a significant difference was found between the NEFA responses, with the curve for the *n*-3-enriched intervention period falling to a lower level compared with the control intervention period ($P < 0{\cdot}04$).

The postprandial total cholesterol, glucose and insulin levels were not significantly different at any time point (results not shown). Higher postprandial cholesterol levels were generally observed after the control period and higher postprandial glucose levels after the *n*-3-enriched intervention period. These were not significant, and may reflect the different fasting values observed for these two variables after the two dietary intervention periods.

DISCUSSION

This study is one of the first to use normal foods enriched with long-chain *n*-3 PUFA as a means of increasing the EPA and DHA content of the diet, and offers an alternative to the use of fish-oil capsules or fish. The aim was to increase moderately the subjects' daily intakes of EPA and DHA (to about 1·8 g/d). This daily intake was chosen as it has been shown to reduce fasting and postprandial TAG levels significantly in studies using fish-oil supplements (Schmidt *et al.* 1990; Harris *et al.* 1991). It was also a level of enrichment predicted to be achievable using currently available enriched manufactured foods. Our

Table 2. *Mean fasting plasma concentrations of triacylglycerol (TAG), total and HDL-cholesterol, glucose, insulin and post-heparin lipoprotein lipase (EC 3.1.1.34; LPL) of male subjects after consuming their habitual diet, a control diet or an* n*-3-enriched diet*‡

(Mean values and standard deviations for nine subjects)

	Habitual diet		*n*-3-Enriched diet		Control diet	
	Mean	SD	Mean	SD	Mean	SD
TAG (mmol/l)	1·54	0·54	1·49	0·37	1·53	0·63
Total cholesterol (mmol/l)	5·99	0·62	5·52*	0·83	5·27*	0·54
HDL-cholesterol (mmol/l)	NC		1·04†	0·33	0·87	0·29
Glucose (mmol/l)	5·0	0·4	5·5*†	0·5	5·1	0·2
Insulin (pmol/l)	NC		89	58	87	81
Post-heparin LPL 5 min (mU)§	NC		133	34	117	24
Post-heparin LPL 15 min (mU)	NC		154	26	161	40

NC, no collection of sample.

* Mean values were significantly different from those for the habitual diet, $P < 0{\cdot}05$.

† Mean values were significantly different from those for the control diet, $P < 0{\cdot}05$

‡ For details of diets and procedures, see pp. 224–227.

§ 1 mU = 1 nmol oleate released/min per ml plasma at 37°.

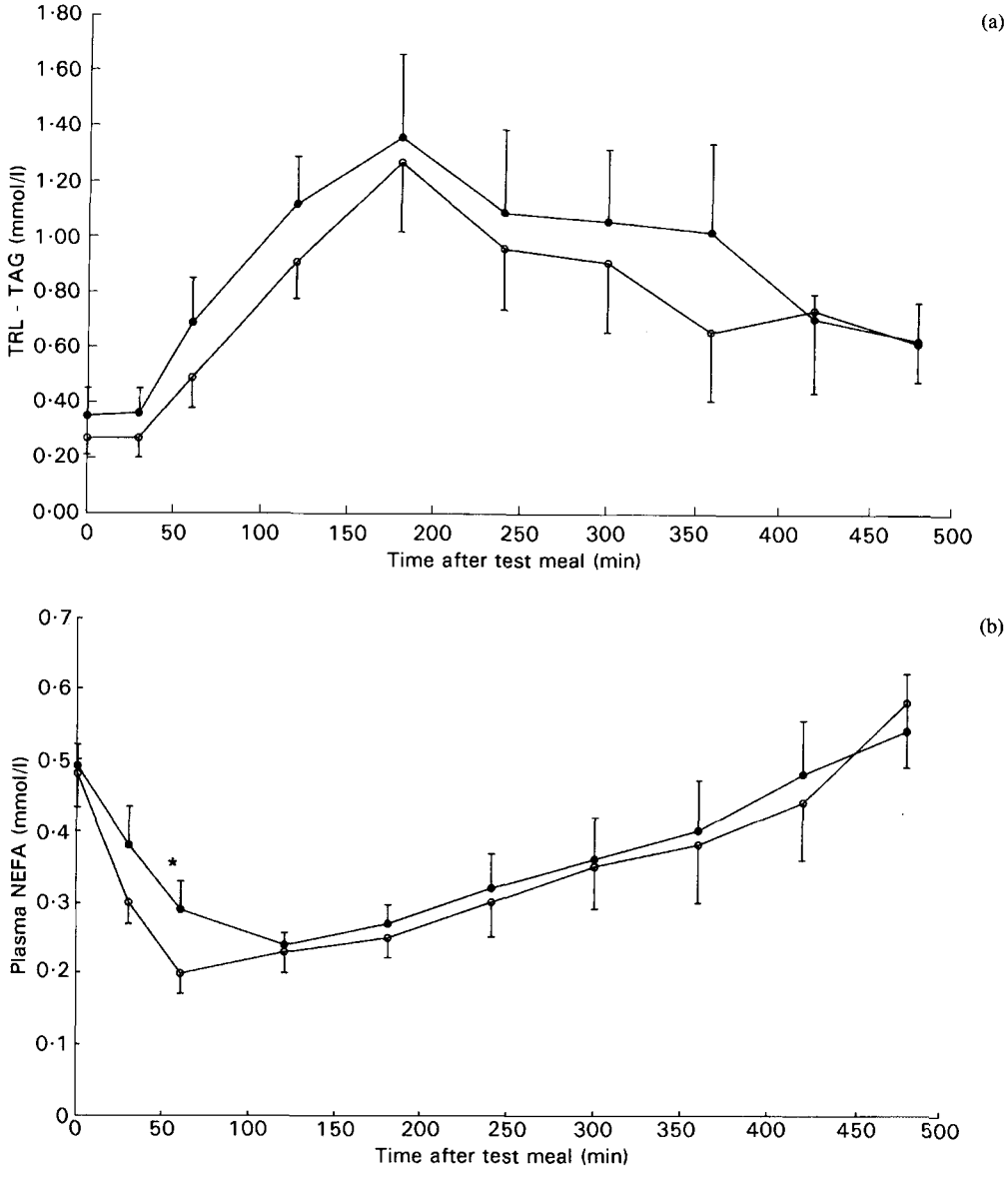

Fig. 2. Postprandial concentrations of (a) triacylglycerols (TAG) in the TAG-rich lipoprotein (TRL) fraction (mmol/l) and (b) plasma non-esterified fatty acids (NEFA; mmol/l) after a high-fat mixed test meal (82 g fat) consumed at time 0. Subjects had been consuming an *n*-3 polyunsaturated fatty acid-enriched diet (○) or a control diet (●) for 22 d before the test. Values are means for nine subjects, with their standard errors represented by vertical bars. Mean value was significantly different from that for the control period: * $P < 0{\cdot}04$.

results showed that it was feasible to increase the daily intake of EPA and DHA above the habitual level of 0·1 g/d using the foods available, consistent with other studies using spreads and fish-oil-enriched fish products (Roche, 1995; Gustafsson *et al.* 1996). However the mean daily intake of the subjects did not reach the target intake of 1·8 g/d. The mean palatability scores for the individual foods were not found to be significantly different

when the enriched foods were compared with the control foods and these results indicated that a range of *n*-3-enriched food products are acceptable vehicles through which EPA and DHA supplementation can be achieved without the use of capsules or fish.

The mean daily intake of EPA and DHA, calculated from the weighed food intake, was 1·4 g/d during the *n*-3-enriched dietary period which did not reach the target intake of 1·8 g/d. Apparent failure to achieve the target intake was thought to be due to a number of factors. First the limitations of the 3 d weighed food intake method for accurate fatty acid intake assessment. For an individual to undertake a weighed food record for accurate determination of fatty acid content of their diet, it would require a period of about 27 d (the time estimated for accurate determination of cholesterol), which was considered too restrictive for the subjects involved with this study, as a level of precision necessary for a metabolic study was not appropriate in the free-living situation and may have altered subjects' food intakes (Bingham, 1987). In addition, the days during which the fish pâté was consumed were not often chosen by subjects as their dietary recording days. This source of EPA + DHA represented a significant quantity of the weekly *n*-3 PUFA intake, and lack of a record of this food intake may explain the lower than expected recorded *n*-3 PUFA intake. Lastly, although the subjects were given full, clear instructions on the procedure of dietary records it was observed that the subject who consumed the lowest quantity of EPA + DHA (0·6 g/d), according to the dietary records, had the third highest enrichment of EPA + DHA in the plasma fatty acids and fifth highest enrichment of EPA + DHA in the plasma phospholipids in the group. This sheds further doubt on the credibility of self-recorded dietary data as a means of assessing nutrient intake, particularly of these low levels of dietary fatty acids.

The plasma fatty acid enrichment data indicated that the enriched foods were being consumed. It was observed that the plasma EPA + DHA fatty acid levels were at their highest on day 22 of the study, and may have continued to increase if the duration of the intervention periods had been longer. This suggests that the tissue was not saturated by the test fatty acids. Harris *et al.* (1991) reported a peak level of fish-oil incorporation into platelet phospholipids after supplementation for 1 month, with various doses of fish oil, with no increase for the subsequent 5 months of supplementation. It was observed that the *n*-3 PUFA plasma phospholipid enrichment was not significantly different at the beginning of the two intervention periods, confirming that the 5-month washout period was sufficient time for the incorporated EPA + DHA to be replaced with fatty acids from the subjects' habitual diet in the plasma and phospholipids, between study periods. However no data were available for the fatty acid enrichment of the membranes, which would be essential to assess the true period of time required for an adequate washout period.

All of the subjects gained weight and found difficulty consuming the full number of exchanges required to achieve a daily intake of 1·8 g EPA + DHA/d. The main reason for this was that several of the study foods were not routinely eaten by the subjects; for example, milk shake, vinaigrette, ice-cream, cakes and biscuits. When these foods were added to the diet, failure to compensate by reduction in other foods led to an increased energy intake, accounting for the weight gain observed. The most significant weight gain was observed after phase 1, during which five of the subjects were on the *n*-3-enriched diet. This difference in weight gain during the two study phases could be explained by the subjects gaining expertise in exchanging the study foods for their usual foods during phase 1, which they used more successfully during phase 2, preventing such a marked dietary energy increase. The data obtained in the present study suggest that if enriched foods are to be used to achieve levels of intake of greater than 1 g/d, then higher levels of enrichment in individual foods, and a wider range of enriched food products, are needed. It would be

advisable to enrich foods with a higher fat content such as spreads and oils which are used habitually in most people's usual diet.

The moderate intake of EPA + DHA in the present study did not have the expected hypotriacylglycerolaemic effect. Previous studies using low doses of *n*-3 PUFA (0·74 g EPA, 0·44 g DHA and 0·34 g EPA, 0·15 g DHA) in the form of supplements have reported a significant TAG-lowering effect (Schmidt *et al.* 1990; Harris *et al.* 1991), although there seems to be a critical quantity, as studies using very low doses of *n*-3 PUFA (0·36 g EPA, 0·24 g DHA and 0·65 g *n*-3 PUFA) for an 8-week period showed no effect (Davidson & Gold, 1988; Lervang *et al.* 1993).

The quantity of EPA and DHA consumed is known to be important in determining the physiological effects of these fatty acids. Li & Steiner (1991) demonstrated this when they gave 3, 6 or 9 g EPA/d for 21 d, which led to dose-related gains of 4·5, 5·6 and 7·6 g EPA/100 g total platelet phospholipid fatty acids respectively, similar to findings of other groups (Blonk *et al.* 1990; Harris *et al.* 1991). The mean daily intake of 1·4 g/d found in the present study resulted in a significant increase in EPA + DHA incorporation into both the plasma fatty acids and phospholipid fatty acids (changes of 4·3 μg/ml and 5·2 μg/ml respectively). This compares very well with the data reported by von Schacky *et al.* (1985) which showed that a daily intake of approximately 2 g EPA + DHA in the form of cod-liver-oil supplements led to an increase of EPA + DHA incorporation into the plasma phospholipids in the order of 5 μg/ml. These findings suggest that the *n*-3 PUFA incorporation from the enriched foods was comparable with that achieved with supplements and should, therefore, have provided the predicted reduction in fasting and postprandial TAG levels.

A previous study which investigated the effect of 0·72 g EPA/d and 0·36 g DHA/d incorporated into either a high- or low-fat enriched spread for a 12-week period, also reported no effect on fasting plasma TAG levels (Roche, 1995). In that study the platelet phospholipid *n*-3 PUFA incorporation during the 12-week period illustrated that those subjects who were consuming the high-fat fish-oil-enriched spread, had a more rapid, and a higher level of EPA + DHA incorporation into platelet phospholipids, compared with those subjects ingesting the same quantity of fish oils in the form of a low-fat spread (Roche, 1995). These findings are interesting in relation to the enriched foods used in the present study, since it may be that the fish oil is more readily absorbed and incorporated into the platelet phospholipids if it is ingested with a high-fat food. The manufactured, enriched foods which were used in our study, except for the spread (600 g fat/kg), were low-fat foods, with some (bread, pasta and orange juice) being almost fat-free. Whether the lack of a biochemical effect of our moderately low *n*- 3 PUFA intake of 1·4 g/d could be due to the form in which the fish oil was ingested cannot be determined from this study. Further studies on bioavailability of, and metabolic responses to, diets in which *n*-3 PUFA are produced in different types of foods, are needed.

The duration of supplementation in a study is also an important consideration. Saynor & Gillott (1992) demonstrated that dietary supplementation with 3 g *n*-3 PUFA/d for 4 years resulted in a time-related reduction in plasma TAG concentrations. These results were also supported by Schmidt *et al.* (1992) with a shorter study period of 9 months. The duration of the present study was relatively short with only a 22 d intervention period, however a significant reduction in plasma TAG has been reported after only 1 month by other groups (Schectman *et al.* 1989). The lack of a significant reduction in plasma TAG levels in the present study could be explained by the combination of the relatively low dose, short duration of the study and form of *n*-3 PUFA, and the significant weight gain during the *n*-3-enriched intervention period.

The significantly lower NEFA levels at 60 min postprandially after the *n*-3-enriched intervention period illustrated another potential beneficial effect of the low-dose fish-oil intake. Studies performed by Frayn *et al.* (1996) have illustrated that high circulating fasting and postprandial NEFA levels may be linked to a number of chronic disease conditions. The mechanism of their action is unclear. It is suggested that the lower levels of NEFA early in the postprandial profile reflect a greater insulin-induced suppression of lipolysis during the *n*-3 PUFA enrichment. In the present study the levels of insulin were unchanged after the enriched diet, however this observation may reflect an increase in insulin sensitivity, due to the postulated incorporation of EPA + DHA into membrane phospholipids, resulting in a reduced NEFA release. The mechanism of the reduction in NEFA requires further investigation.

Despite the lack of a significant effect of the *n*-3 PUFA intake on TAG concentrations, effects on fasting and postprandial lipid levels were observed. Higher fasting HDL-cholesterol values were found after the *n*-3-enriched intervention period compared with both the habitual diet and the control intervention period, and after both dietary intervention periods the total cholesterol levels were reduced. It has been reported that fish-oil intervention in placebo-controlled trials generally increases HDL-cholesterol levels by 5–10 % (Harris, 1989). The mechanism whereby *n*-3 PUFA increase HDL levels has not yet been elucidated. However it is believed to be related to the down-regulation of the enzymes: cholesteryl ester transfer protein and lecithin-cholesterol acyltransferase (*EC* 2.3.1.43; Abbey *et al.* 1990). In reviewing the effect of *n*-3 PUFA supplementation on LDL-cholesterol levels, Harris (1989) concluded that the effect was negligible in normolipidaemic subjects, unless intakes were high. The reduction in total cholesterol after both intervention periods in the present study was unexpected as the total energy intake and fat content of both diets were apparently higher when compared with their habitual intake, which would be expected to have an adverse effect on total cholesterol levels. Possible explanations for the findings are that there was under-reporting of habitual intakes or that subjects altered the types of foods consumed.

A significant increase in fasting glucose was observed after the *n*-3-enriched diet, although no significant difference was observed in the fasting insulin, postprandial glucose or insulin levels after either of the intervention periods. The effect of chronic *n*-3 PUFA intake on hypoglycaemic control is controversial. Some studies of glucose-intolerant and type II diabetic subjects have reported a detrimental effect on fasting and postprandial glucose control, with no significant effect on insulin levels, using relatively large doses of *n*-3 PUFA (Friday *et al.* 1989; Vessby & Boberg, 1990). Other less-controlled studies which have also shown this effect, have been criticized, with the negative effect on glucose metabolism being explained by the extra energy supplied by increased fat intake (Borkman *et al.* 1989). Whether our findings for glucose tolerant normotriacylglycerolaemic men reflect adverse effects of low-dose EPA + DHA on fasting glucose levels is unclear, but they appear to be inconsistent with apparent enhanced sensitivity of the anti-lipolytic response to insulin suggested by the NEFA findings.

This study has illustrated that it is feasible to use manufactured enriched foods as a means of increasing the EPA + DHA intake in a free-living population. The foods were found to be palatable and well tolerated, but a greater level of incorporation of EPA + DHA into the foods would be preferable for future studies. A significantly higher incorporation of EPA + DHA into the plasma fatty acids and phospholipids indicated that these long-chain *n*-3 PUFA were absorbed, although the efficiency of absorption could not be determined. The moderately low dose (1·4 g/d) of EPA + DHA for a 22 d period did not result in the expected significant hypotriacylglycerolaemic effect. This may have been due

to the relatively short intervention period and the significant weight gain achieved during the *n*-3-enriched intervention period. However a significant increase in fasting HDL-cholesterol concentration and a significant reduction in postprandial NEFA was observed after the *n*-3-enriched period, suggesting that even short periods of low doses of *n*-3 PUFA have potential beneficial effects in normotriacylglycerolaemic middle-aged men.

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