

Applied nutritional investigation

Oily fish reduces plasma triacylglycerols: a primary prevention study in overweight men and women

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Manuscript received May 5, 2006; accepted July 28, 2006.

Abstract

Objective: Previous studies have demonstrated benefits of high-dose long-chain ω -3 polyunsaturated fatty acid (LC ω -3 PUFA) supplements on metabolic risk. Effects of increased dietary ω -3 PUFA, via oily fish and/or plant-derived ω -3 PUFAs, are less clear and may be modulated by the ω -6: ω -3 PUFA of the habitual diet. This study examined the effect on cardiovascular disease risk markers of reducing dietary ω -6: ω -3 PUFA by changes in linoleic acid: α -linolenic acid (LA:LNA) and/or increasing LC ω -3 PUFA. It tested whether decreases in LA:LNA modulate effects of LC ω -3 PUFA.

Methods: One hundred forty-two subjects, recruited to a 24-wk randomized study, were assigned to a control group or one of four interventions. Intervention groups received two portions of oily fish (4.5 g eicosapentaenoic acid + docosahexanoic acid) or white fish (0.7 g eicosapentaenoic acid + docosahexanoic acid) per week, and replaced habitual household fats with ones high in sunflower (high LA:LNA) or rapeseed (low LA:LNA) oil.

Results: Modest dietary manipulations of ω -6 and ω -3 PUFAs resulted in significant group \times time interactions for serum triacylglycerols (TAGs; $P = 0.05$); at 24 wk the control and two oily fish groups showed lower TAG than did the white fish/sunflower group ($P = 0.05$). Reductions in TAG, associated with increased oily fish intakes, were maximized when combined with lower dietary LA:LNA. There were no significant changes in several other cardiovascular disease risk markers.

Conclusions: Two portions of oily fish per week led to significant reductions in TAG relative to consumption of two portions of white fish per week. Changes in TAG were maximized when combined with lower LA:LNA. © 2006 Elsevier Inc. All rights reserved.

Keywords:

ω -6 to ω -3 Polyunsaturated fatty acid ratio; Long-chain ω -3; Linoleic acid; α -Linolenic acid; Cardiovascular disease

Abbreviations:

AA, arachidonic acid; ACT, α_1 -antichymotrypsin; AGP, α_1 -Acid Glycoprotein; ANOVA, analysis of variance; APP, acute phase protein; AUC, area under the curve; BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; DHA, docosahexanoic acid; DXA, dual x-ray absorptiometry; EPA, eicosapentaenoic acid; Factor VIIa, factor VII activity; HDL-C, high density lipoprotein cholesterol; hs-CRP, C-reactive protein; IL-6, interleukin-6; LA, linoleic acid; LC ω -3 PUFA, long-chain ω -3 polyunsaturated fatty acids; LDL-C, low density lipoprotein cholesterol; LNA, α -linolenic acid; MRC HNR, Medical Research Council Human Nutrition Research; NSAID, non-steroidal anti-inflammatory drugs; OGTT, oral glucose tolerance test; PAI-1, plasminogen inhibitor activator-1; SD, standard deviation; TAG, triacylglycerols; TC, total cholesterol; TNF- α , tumour necrosis factor alpha

Introduction

Secondary prevention trials have shown long-chain ω -3 polyunsaturated fatty acids (LC ω -3 PUFA) from fish or supplements reduce premature mortality and sudden death in individuals with a history of myocardial infarction [1–3]

This study was supported by funding from the United Kingdom Food Standards Agency (Project Number NO2026) and Medical Research Council.

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and coronary artery disease [4]. Primary prevention studies have clearly demonstrated benefits of high-dose LC ω -3 PUFA supplements on metabolic risk factors; in particular reductions in triacylglycerols (TAGs) are widely accepted and extensively reported [5–9]. However, there are few consistent data for other health outcomes. Variable effects have been shown for low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) [10–12], and although there is some evidence of a beneficial effect on insulin sensitivity in non-diabetics [13], most studies have shown no significant effect [10,12,14].

Data on the effect of dietary changes in fatty acid intake in primary prevention studies are scarce [15] and equivocal. Most studies have focused on advice to increase fish intake. Increases in fish consumption have been shown to reduce TAGs in some intervention studies [16–18] but not in others [19]. Likewise cross-sectional data reporting links between fish consumption and diabetes risk or markers of insulin sensitivity have been inconsistent. Higher intakes have been associated with lower risk for impaired glucose tolerance and diabetes [20–22]; and although some studies have shown a positive association with blood glucose concentrations [22–24], others have shown no association [25].

In recent years there has been growing interest in the potential for plant-derived ω -3 PUFAs to mimic the effects of LC ω -3 PUFA in mediating cardiovascular disease (CVD) risk. Beneficial effects of α -linolenic acid (LNA) on thrombosis [26] and arterial compliance [27] have been shown at intakes between 2% and 5% energy intake (E) but this is greatly in excess of estimated habitual intakes (approximately 0.6% E) [28]. High intakes (2.3% E) have also been shown to have negative effects on CVD risk: reducing HDL-C and raising the LDL-C:HDL-C ratio [29]. The effect of more moderate intakes of dietary LNA on CVD outcomes has yet to be elucidated. Any beneficial effects of LNA may be dependent on its endogenous conversion to LC ω -3 PUFA (eicosapentaenoic acid [EPA] and docosahexanoic acid [DHA]). This process is limited and is specifically inhibited by high intakes of ω -6 linoleic acid (LA), which competes for the same enzymes of elongation and desaturation [30]. Thus the relative proportions of LA and LNA in the diet may influence the observed health effects. The Lyon Heart Health Study, a secondary prevention trial, showed a 70% reduction in cardiac death and non-fatal myocardial infarction in a group following a LNA-enriched Mediterranean diet with an overall dietary LA:LNA of 4:1 [2]. However, this was part of a complex dietary intervention and the precise contribution of the ω -6: ω -3 PUFA ratio is unclear.

There are several potential mechanisms to explain the links between ω -3 PUFA and CVD risk. In addition to their hypotriglyceridemic effect, LC ω -3 PUFAs are potent anti-arrhythmic agents [31,32] and have been shown to improve vascular function [33–37]. The effect on thrombosis risk is less clear [38–43]. There is growing evidence of a relation between inflammation and metabolic risk [44–46]. LC ω -3

PUFA intervention studies have shown reductions in a number of inflammatory mediators including eicosanoids [47], cytokines [48–52], and acute-phase proteins [53]. There is less evidence relating to other fatty acids, but one study has shown that at high doses LNA results in significantly lower levels of high-sensitivity C-reactive protein (hsCRP), interleukin 6 (IL-6), and serum amyloid A compared with LA-rich diets [54].

Together these studies suggest that CVD risk may be decreased by dietary strategies to reduce LA, increase LNA, and/or increase LC ω -3 PUFA intakes. This food-based intervention study examined the effect of reducing dietary ω -6: ω -3 PUFAs (by changes in LA:LNA or increases in LC ω -3 PUFA) on a wide range of CVD risk factors. It tested whether high consumption of LA attenuates the potential health benefits of LNA and investigated whether decreases in LA:LNA could enhance the metabolic benefits of oily fish, rich in LC ω -3 PUFA.

Subjects and study design

Population

Overweight or obese men and women were recruited from the community to participate in a 24-wk intervention study at Medical Research Council Human Nutrition Research (MRC HNR). The study was approved by the Cambridge local research ethics committee and all subjects gave written informed consent. All subjects were screened by telephone questionnaire and attended a brief medical examination to ensure they met the entry criteria, which included a body mass index (BMI) between 25 and 40 kg/m²; age between 35 and 65 y; not taking regular oil supplements; not currently prescribed non-steroidal anti-inflammatory drugs, aspirin, steroids, immunosuppressants, or lipid-lowering drugs; no known diagnosis of diabetes, hypertension, hyperlipidemia, asthma, or chronic inflammatory diseases; and, in female subjects, no known or planned pregnancies. The screening questionnaire also included a brief dietary assessment of the types and quantities of oils, spreads, and fish consumed in their habitual diet.

Baseline measurements were taken in 142 subjects and 134 completed the 24-wk intervention (Fig. 1). Reasons for withdrawing from the study included changes in health/medication (one subject), geographic location (one subject), work or other commitments (five subjects), and inability to tolerate a cannula (one subject). Another subject was excluded from the analysis due to the presence of undiagnosed type 2 diabetes at the start of the study (baseline fasting glucose level 12.3 mmol/L).

Study design

This was a 24-wk, randomized, controlled, dietary intervention study. Minimization was used to assign subjects to

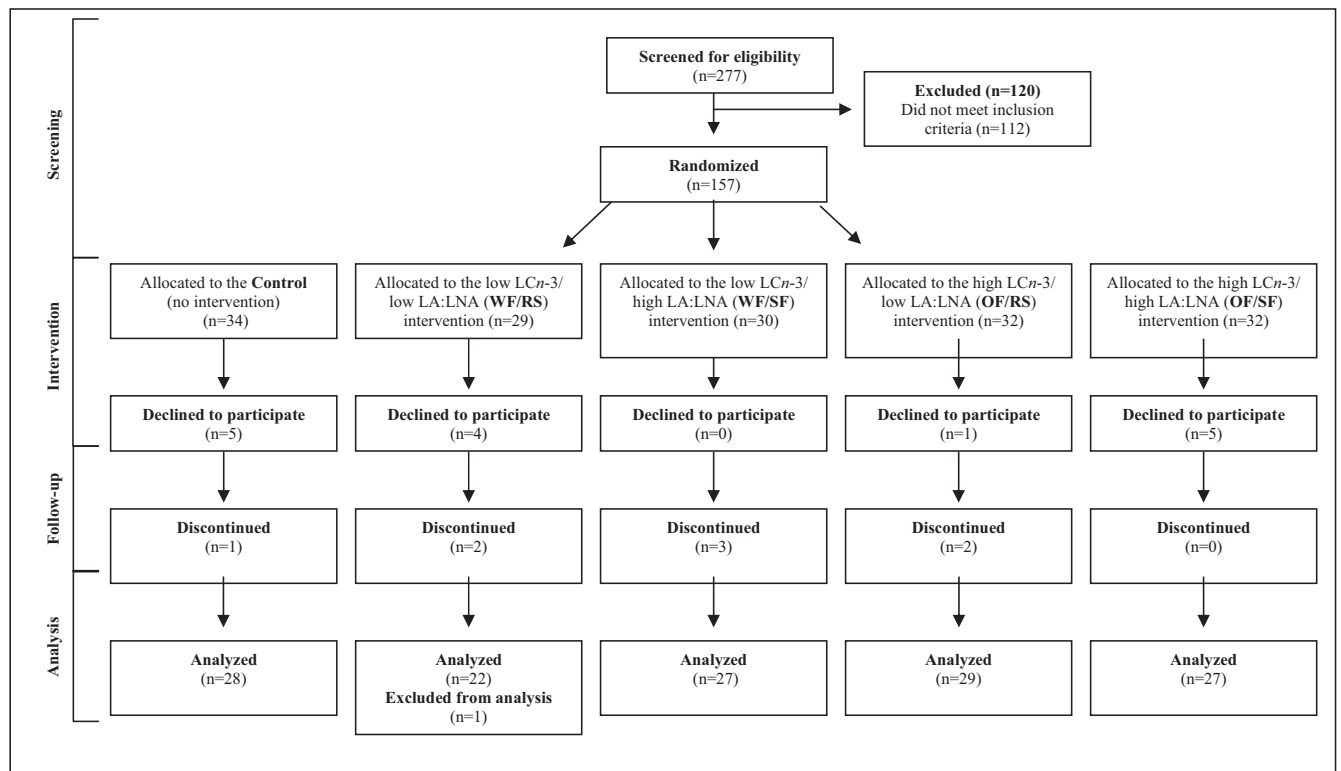


Fig. 1. Flow diagram of subject involvement in the study. LA, linoleic acid; LCn-3, long-chain ω -3; LNA, α -linolenic acid; OF, oily fish; RS, rapeseed; SF, sunflower; WF, white fish.

a control group or one of four parallel intervention groups and to ensure that the treatment arms were balanced for age, BMI, gender, and habitual consumption of oily fish based on three categories (0, ≤ 1 , or > 1 portion/wk). LC ω -3 PUFA intakes were manipulated by providing intervention groups with two portions of white fish (approximately 0.7 g of EPA + DHA/wk) or oily fish (4.5 g of EPA + DHA/wk). Each fish group was split into two further arms in which usual household fat spreads and cooking oils were replaced by those based on sunflower or rapeseed oil to manipulate LA:LNA (sunflower spread 27:1; sunflower oil 63:0.1; rapeseed spread 3:1; rapeseed oil 2:1). Thus the five groups consisted of a seasonal control (no intervention) group, a low LC ω -3 PUFA and low LA:LNA (white fish/rapeseed) group, a low LC ω -3 PUFA and high LA:LNA (white fish/sunflower) group, a high LC ω -3 PUFA and low LA:LNA (oily fish/rapeseed) group, and a high LC ω -3 PUFA and high LA:LNA (oily fish/sunflower) group.

Subjects collected the study foods from the investigators at MRC HNR at 4-wk intervals. The fish was obtained from local supermarkets and volunteers selected items from a range, including frozen fish (white fish, cod or prawns; oily fish, salmon and mackerel) fishcakes (white fish, cod; oily fish, salmon), and tinned fish (white fish, tuna; oily fish, salmon). The spreads and oils were generously provided by Matthews Foods Plc. (Ossett, West Yorkshire, UK).

Due to the nature of food-based interventions, it was not possible for the principal investigators or subjects to be

blinded to the randomization. However, the specific hypothesis under study was not made explicit to subjects to reduce the possibility of concomitant changes in habitual diet. Moreover, laboratory analysts, dietary coders, and the clinical scientist and statistician involved in the study were blinded to the randomization, thus effectively removing most investigator bias.

Subjects were required to attend three half-day investigations, at 0, 12, and 24 wk, for anthropometric, body composition, and fasting blood sample measurements. Within 7 d of these visits, subjects returned to the unit for a second fasting blood sample. All subjects were encouraged to maintain their usual physical activity habits throughout the study period. Before each of the investigation days, subjects were asked to complete a prospective 7-d diet diary with quantities of foods described in household measurements or portion sizes estimated from photographic examples.

Methods

Anthropometry and body composition analysis

Height was measured to the nearest 0.1 cm using a stadiometer (Karrimetre, Raven Equipment Ltd., Dunmow, Essex, UK). Weight was measured to the nearest 0.1 kg using Seca Alpha 770 digital scales (Cross and Co. Medical

Products Ltd., Lancaster, UK). Measurements were made with subjects wearing light clothing and without shoes. Waist circumference was measured in the standing position to the nearest 0.5 cm and standardized as the minimum measurement between the lower rib margin and the top of the pelvic brim. Body composition was measured by dual-energy X-ray absorptiometry. A whole-body dual-energy X-ray absorptiometric scan was performed using a Hologic QDR-100W scanner (Hologic Inc., Waltham, MA, USA) and analyzed to estimate bone mineral, fat, and fat-free soft tissue mass.

Blood collection

Subjects were instructed to fast from 2000 h the evening preceding their investigation day. Water was allowed throughout the fast. Fasting blood samples were collected between 0800 and 1000 h, from the antecubital fossa vein in the forearm, using venipuncture if no further samples were to be collected, or an in-dwelling cannula where more samples were due to be taken throughout the same morning. Samples were put onto ice immediately when required. Serum samples were allowed to coagulate before putting onto ice. A citrate sample (collected for analysis of fibrinogen) was kept at room temperature throughout the processing procedure. All samples were centrifuged within 1 h of collection at 3000 rpm for 20 min at 4°C (iced samples) or 20°C (room-temperature samples) and frozen immediately at –80°C. Laboratory analysis was conducted in the Nutritional Biochemistry Laboratory, MRC HNR (Cambridge, UK) unless otherwise stated.

CVD risk factors

Blood pressure (BP) was measured while the subject was seated and after a 10-min rest, using an automated Dinamap Pro 200 machine (Critikon Ltd., Basingstoke, Hampshire, UK) and recorded as the average of two measurements, made at least 5 min apart.

All lipid analyses were performed at the Department of Clinical Biochemistry at Addenbrooke's Hospital (Cambridge, UK). To improve precision, two measurements of fasting lipids (<7 d between repeats) were taken for each subject and the average was used in subsequent data analysis. All analysis was carried out with the Dimension clinical chemistry system (Dade Behring, Newark, NJ, USA). Between-run coefficients of variation for TAG are 3.2% at 1.0 mmol/L and 1.1% at 2.2 mmol/L. Interassay coefficients of variation for total cholesterol (TC) are 1.3% at 3.2 mmol/L and 1.2% at 7.5 mmol/L and for HDL-C are 3.3% at 0.6 mmol/L and 2.1% at 1.5 mmol/L. The Friedewald formula was used to calculate LDL-C from TC, TAG, and HDL-C fractions [55].

Analyses of hemostatic factors were carried out at the MRC Epidemiology and Medical Care Unit, Queen Mary and Westfield College (London, UK). Samples were col-

lected in sodium citrate tubes. Factor VII activity (factor VIIa) was measured using a one-stage clotting assay [56]. Fibrinogen was measured using a thrombin-clotting assay, modified from Clauss [57]. Plasminogen activator inhibitor-1 (PAI-1) activity was measured using the Coatest PAI-1 assay kit (Chromogenix Coatest PAI; Quadratch Diagnostics, Epsom, Surrey, UK).

Insulin sensitivity

All measurements of plasma insulin and glucose were carried out by the Department of Clinical Biochemistry at Addenbrooke's Hospital. To improve precision, two measurements of fasting insulin and glucose (<7 d between repeats) were taken for each subject and the average was used in subsequent data analysis. On one occasion, an oral glucose tolerance test (OGTT) was performed; after collection of a fasting blood sample, subjects were given a test load consisting of 75 g of glucose in 250–300 mL of cold water consumed within 5 min [58]. Blood samples for glucose and insulin analyses were collected every 30 min for 120 min from the time of beginning the drink.

For insulin measurements, plasma samples were collected using lithium heparin pretreated monovettes (Sarstedt, UK). Samples were assayed on a 1235 AutoDELFLIA automatic immunoassay system using reagents supplied by DAKO Ltd. (Ely, Cambridgeshire, UK). Cross reactivity with intact proinsulin is <0.5% at 2736 pmol/L, 32–33 split proinsulin 1% at 2800 pmol/L, and C-peptide <0.1% at 5280 pmol/L. Typical between-run coefficients of variation are 3.1% at 29.0 pmol/L, 2.1% at 79.4 pmol/L, 1.9% at 277 pmol/L, and 2.0% at 705 pmol/L, respectively. The limit of detection has been shown to be 1.3 pmol/L. A prototype of the assay has previously been described [59].

Plasma samples, collected in fluoride-treated monovettes (Starstedt, UK), were analyzed for glucose with the hexokinase-glucose-6-phosphate dehydrogenase method, using the Dimension clinical chemistry system. Typical between-run coefficients of variation are 1.4% at 3.7 mmol/L and 1.1% at 26 mmol/L, respectively. Indices describing insulin sensitivity were based on fasting and post-glucose-load measurements of glucose and insulin from the OGTT. Fasting and subsequent time-point measurements were used to calculate area under the curve (AUC) values, using the trapezoidal rule, for insulin and glucose.

Inflammatory status

Serum cytokines (tumor necrosis factor- α [TNF- α] and IL-6) were measured using an enzyme-linked immunosorbent assay principle (Quantikine kit, R&D Systems, Abingdon, UK) using high-sensitivity kits. The interassay coefficient of variations for TNF- α were 19.3%, 14.9%, and 15.6% at mean concentrations of 1.5, 9.0, and 19.4 pg/mL, respectively, and those for IL-6 were 40.3%, 10.3%, 14.9%,

and 11.1% at mean concentrations of 0.76, 3.2, 6.5, and 22.2 pg/ml respectively.

The acute-phase proteins hsCRP, sialic acid, orosomucoid (α_1 -acid glycoprotein), and α_1 -antichymotrypsin (ACT) were measured. High-sensitivity CRP analysis was performed by the Department of Clinical Biochemistry at Addenbrooke's Hospital using a high-sensitivity assay. High-sensitivity CRP was measured in serum using a turbidimetric immunoassay (minimum detectable level 0.5 mg/L) using the Dimension clinical chemistry system. The interassay coefficients of variation were 1.7% at 12 mg/L and 2.7% at 50 mg/L. Serum sialic acid was analyzed using a colorimetric assay (Roche, Welwyn Garden City, UK). The interassay coefficients of variation were 1.1% and 0.9% at mean concentrations of 45.4 and 98.6 mg/dL. α_1 -Acid glycoprotein was measured in serum using an immunoturbidimetric assay based on immunologic agglutination (Roche). The interassay coefficients of variations were 4.9% at 81.7 mg/dL and 5.0% at 136.7 mg/dL. ACT was measured using an immunochemical method (Dako, Denmark) on a Hitachi 912 Clinical Analyzer (Roche Diagnostics Ltd., Lewes, East Sussex, UK). The interassay coefficients of variation were 5.5% at 0.257 g/L and 4.6% at 0.609 g/L. Leptin was measured with an enzyme-linked immunosorbent assay method (R&D Systems Quantikine Human Leptin Kit); the minimum detectable level of leptin was <7.8 pg/mL. The between-run coefficients of variation were 9.8% at the high control, 7.2% at medium control, and 12.4% at low control.

Dietary intakes and fatty acid status

Nutrient intake was calculated from the diet diaries by using an in-house database (DIDO) of the composition of foods consumed in the United Kingdom diet, based on *McCance and Widdowson's the Composition of Foods* [60] and *Fatty Acid Supplement* [61]. This database was specifically extended to estimate intakes of fatty acids by the incorporation of standardized recipes and manufacturers' ingredient information [62].

Total plasma fatty acids were measured by using a modified Folch extraction [63] and capillary gas chromatography. Individual fatty acids were expressed as a percentage of total plasma fatty acids. The coefficients of variation were 2.2% for LA, 6.5% for LNA, 8.6% for EPA, and 6.7% for DHA.

Statistics

Data was analyzed using SPSS (SPSS Inc., Chicago, IL, USA) and SAS (SAS Institute, Cary, NC, USA) and is expressed as mean \pm SD for normally distributed data or as geometric mean \pm SD (calculated before transformation) for \log_e -transformed data.

Differences between genders and groups in baseline variables were tested with SPSS independent sample *t* test and

one-way analysis of variance with Bonferroni correction for multiple comparisons. Independent *t* tests were also used to examine differences in reported fish/oil intakes between the white and oily fish/sunflower and rapeseed oil groups, respectively.

To model the effect of intervention on fatty status and health outcomes, we used random effects analysis (also known as subject-specific model). For each outcome variable, the treatment group (group) and investigation time point (time) effects of the intervention were examined. Group \times Time interactions and gender were added as covariates in the model. Univariate analysis was used to compare differences between groups at each time point (0, 12, and 24 wk) to explore the origin of group \times time interactions. All analysis was performed with PROC MIXED in SAS 8.2.

With 30 subjects per group and an estimated 20% dropout rate, the study was powered to detect a 0.8 SD change in outcome variables at 80% power.

Results

Baseline characteristics

The intervention groups were well matched for age, BMI, and waist circumference (Table 1). Men had a significantly higher mean BMI ($P < 0.05$) and larger waist circumference ($P < 0.01$) than did women. The female/male ratio was similar across all treatment groups.

Fatty acid intakes

Complete dietary records at 0, 12, and 24 wk were collected for 123 subjects. Reported mean baseline energy intakes for males, 9.6 ± 2.3 MJ, and females, 7.5 ± 1.3 MJ, were compared with estimates of predicted energy needs based on estimated basal metabolic rates of 8.4 MJ (males) and 6.4 MJ (females) and a physical activity level of 1.55. There was a bias toward under-reporting of energy intakes (approximately 25%), which was of a similar magnitude in female and male subjects and comparable to that calculated for the UK National Diet and Nutrition Survey [64].

There were no significant gender or group differences in baseline intakes of LA, LNA, EPA, or DHA. Respective mean contributions to % E were 4.8 ± 1.57 for LA, 0.50 ± 0.16 for LNA, 0.03 ± 0.07 for EPA, and 0.06 ± 0.13 for DHA. Average LA:LNA was 9:1 and total LC ω -3 PUFA (EPA + DHA) intake was 0.19 ± 0.40 g/d. At baseline, there were no group differences in total reported daily intakes of fat spreads (16.35 ± 11.28 g/d), cooking oils (1.83 ± 1.97 g/d), white fish (22.81 ± 24.33 g/d), or oily fish (16.94 ± 21.75 g/d).

Reported intakes of the study foods, from dietary records, were used to assess relative compliance to the intervention across the four treatment groups (Table 2).

Table 1
Baseline characteristics (n = 141)*

Characteristic	All subjects		Intervention groups				
	Female (n = 92)	Male (n = 49)	Control (n = 29)	WF/RS (n = 24)	WF/SF (n = 30)	OF/RS (n = 31)	OF/SF (n = 27)
Age (y)	50 ± 9	52 ± 8	51 ± 9	51 ± 9	51 ± 9	50 ± 9	50 ± 8
BMI (kg/m ²)	30.3 ± 3.9	32.0 ± 4.0 [†]	31.2 ± 4.5	30.5 ± 3.5	30.0 ± 4.1	30.9 ± 3.7	31.9 ± 4.2
Waist (cm)	90.0 ± 9.6	108.2 ± 10.3 [‡]	95.8 ± 15.3	94.9 ± 13.2	95.5 ± 13.1	97.9 ± 12.4	97.0 ± 11.7
Female:male ratio	—	—	19:10	15:9	20:10	20:11	18:9

BMI, body mass index; OF, oily fish; RS, rapeseed; SF, sunflower; WF, white fish

* Data are expressed as mean ± SD for all variables and were analyzed using independent sample *t* test (to test gender differences) and one-way analysis of variance (to test group differences).

[†] Significantly different between females and males ($P < 0.05$).

[‡] Significantly different between females and males ($P < 0.01$).

Reported intakes of spread were similar in those assigned to the rapeseed and sunflower arms at 12 and 24 wk. Reported intakes of white and oily fish interventions were similar at 12 wk; however, at 24 wk, intake of white fish in the white fish groups was significantly higher than that of oily fish in the oily fish groups ($P < 0.01$).

Fatty acid status

At baseline there were no significant differences across intervention groups in percentage of plasma ω -6 LA and arachidonic acid (AA) or in ω -3 LNA, EPA, and DHA. Percentage mean values across all subjects were 27.92 ± 4.42 for LA; 3.14 ± 0.77 for AA; 0.69 ± 0.19 for LNA; 1.0 ± 0.82 for EPA, and 2.38 ± 0.76 for DHA (data for percentage of LNA and EPA were log_e transformed to normalize their distribution). Similarly, no significant differences across groups were shown for baseline LA:LNA or total ω -6: ω -3; mean values were 42:1 and 8:1, respectively.

Significant group × time interactions were shown for plasma LA ($P < 0.05$), LNA ($P < 0.001$), AA ($P < 0.01$), EPA ($P < 0.001$), and DHA ($P < 0.001$) and were reflected in significant differences across groups in LA:LNA and LC

ω -3 PUFA (log_e transformed to normalize distribution) at 12 and 24 wk. In comparison with the rapeseed groups, both sunflower groups showed significantly higher LA:LNA ($P < 0.05$) at 24 wk; the white fish/sunflower group also demonstrated a higher ratio than the control group ($P < 0.05$; Fig. 2). At the same time point, both oily fish groups showed significantly higher plasma concentrations of LC ω -3 than the white fish groups and control group ($P < 0.001$; Fig. 3). Changes in fatty acid intakes calculated from diet dairies correlated well with changes in plasma fatty acid status described above [62].

Anthropometry and body composition

Across all subjects, there was no significant change in weight over time, but there was a significant group × time interaction ($P < 0.01$). Although the white fish/sunflower group showed significant decreases in weight between 0 and 24 wk (−0.92 kg, $P < 0.01$), there was an increase in weight with the oily fish/sunflower intervention (+1.35 kg, $P < 0.05$). Significant group differences existed at 12 and 24 wk (Table 3). Weight was therefore added as an addi-

Table 2
Reported intakes of study foods at 12 and 24 weeks*

Study food	Intakes (g/d)			
	Rapeseed WF/RS + OF/RS	Sunflower WF/SF + OF/SF	White fish WF/RS + WF/SF	Oily fish OF/RS + OF/SF
12 wk	n = 52	n = 52	n = 50	n = 54
Study spread	15.27 ± 10.65	14.34 ± 8.66	—	—
Study oils	2.67 ± 2.31	1.87 ± 2.42	—	—
Study fish	—	—	52.38 ± 29.86	49.03 ± 21.74
24 wk	n = 50	n = 49	n = 47	n = 52
Study spread	13.71 ± 9.29	14.37 ± 10.52	—	—
Study oil	2.70 ± 3.27	1.64 ± 1.92	—	—
Study fish	—	—	59.11 ± 26.74	42.96 ± 23.69 [†]

OF, oily fish; RS, rapeseed oil; SF, sunflower oil; WF, white fish

* Data expressed as mean ± SD for all variables and analysed using independent sample *t* test.

[†] Significantly different between OF and WF groups ($P < 0.01$).

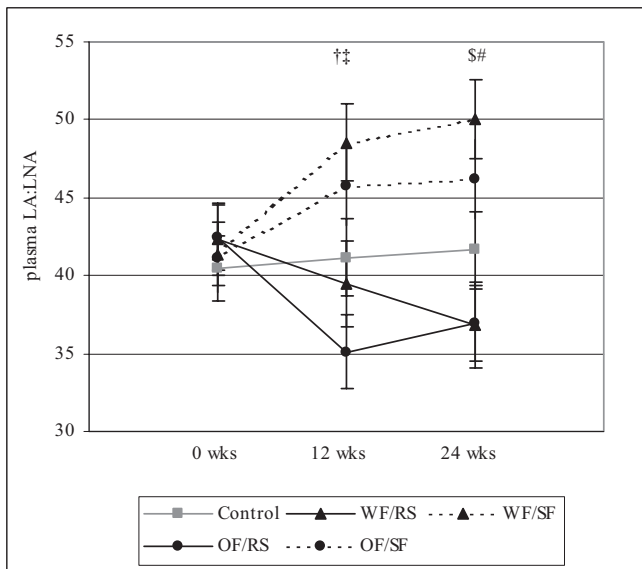


Fig. 2. Plasma LA:LNA during the intervention. Data are presented as mean \pm SE and were analyzed using random effects models of the change in outcome, adjusted for baseline values and gender. There was a significant group \times time interaction, ^{††}indicates both SF groups $>$ OF/RS ($P < 0.01$); [‡]indicates WF/SF $>$ WF/RS and control ($P < 0.05$); [§]indicates both SF groups $>$ both RS groups ($P < 0.05$); [#]indicates WF/SF $>$ control ($P < 0.05$). LA, linoleic acid; LNA, α -linolenic acid; OF, oily fish; RS, rapeseed; SF, sunflower; WF, white fish.

tional covariate into the random effects model used in the analysis of all health outcome measurements.

No significant between-group differences were found for percentage of body fat (dual energy X-ray absorptiometry) and waist circumference at any time point.

CVD risk factors

There were no differences in baseline TAG across treatment groups, although men had significantly higher serum levels than women (1.43 ± 0.74 versus 1.17 ± 0.59 mmol/L, $P < 0.01$, data for TAG were \log_e transformed to normalize their distribution). There was a significant effect of dietary treatment on changes in TAG during the dietary intervention (group \times time, $P = 0.05$); at 12 wk the oily fish/rapeseed showed lower TAG levels than the white fish/sunflower group ($P = 0.05$), and at 24 wk the control, oily fish/rapeseed, and oily fish/sunflower groups showed significantly lower ($P = 0.05$) TAG concentrations than the white fish/sunflower group (Table 4).

The separate effects of the LA:LNA ratio and LC ω -3 PUFA on TAG were examined. There was no significant time \times group interaction after collapsing data from both sunflower and rapeseed groups, but a significant interaction was shown when data for the two white fish and oily fish groups were combined ($P < 0.05$). Figure 4 shows contrasting increases and decreases in TAG with the white fish and oily fish interventions, respectively. At 24 wk the white fish and oily fish groups showed significantly different TAG

concentrations ($P < 0.05$), but neither was significantly different from the intermediate control group.

At baseline there were no significant group differences in systolic BP, diastolic BP, TC, HDL-C and LDL-C, or hemostatic factors factor VIIa, PAI-1, and fibrinogen. Men had significantly higher systolic and diastolic BP values than women (131.0 ± 15.4 versus 123.8 ± 18.0 mmHg and 79.4 ± 9.6 versus 71.3 ± 9.6 mmHg, $P < 0.05$ and $P < 0.001$, respectively); in addition, men were found to have higher PAI-1 concentrations than women (21.78 ± 9.92 versus 14.57 ± 8.02 AU/mL, $P < 0.001$). Women had higher HDL-C levels than men (1.47 ± 0.35 versus 1.21 ± 0.29 mmol/L, $P < 0.001$). Random effects analysis showed no significant group \times time interactions for systolic or diastolic BP, TC, LDL-C, HDL-C (Table 4), or hemostatic factors (data not shown).

Insulin sensitivity

At baseline there were no significant between-group differences in fasting glucose, fasting insulin, AUC glucose, or AUC insulin. Men had significantly higher mean values than women for each of these variables: glucose (5.55 ± 0.70 versus 5.30 ± 0.47 mmol/L, $P < 0.05$), insulin (64.36 ± 50.09 versus 44.03 ± 24.82 pmol/L, $P < 0.001$), AUC glucose (916.5 ± 203.1 versus 820.8 ± 177.0 mmol/L \cdot min⁻¹, $P < 0.01$), AUC insulin ($45\,000 \pm 40\,071$ versus $30\,343 \pm 17\,390$ pmol/L \cdot min⁻¹, $P < 0.001$; data for

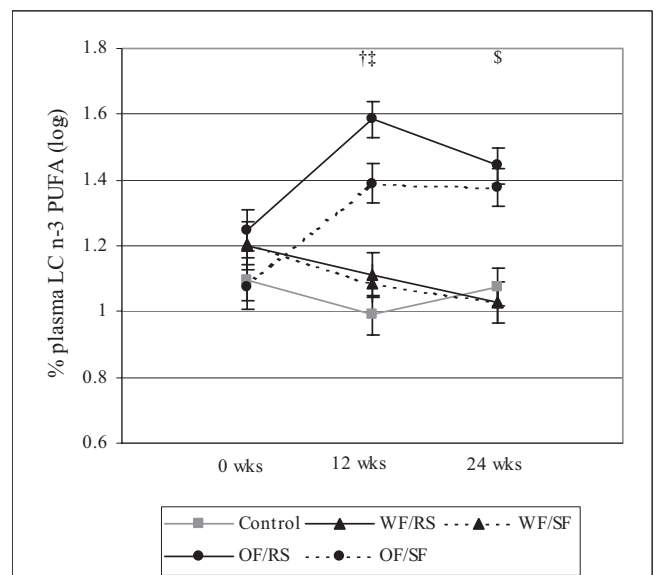


Fig. 3. Plasma LC ω -3 PUFA during the intervention. Data are presented as \log_e -transformed mean values (SE) and were analyzed using random effects models of the change in outcome, adjusted for baseline values and gender. There was a significant time \times group interaction, ^{††}indicates both OF groups $>$ both WF groups and control ($P < 0.01$); [‡]indicates OF/RS group $>$ OF/SF group ($P < 0.05$); [§]indicates both OF groups $>$ both WF groups and control ($P < 0.001$). LA, linoleic acid; LC n-3 PUFA, long-chain ω -3 polyunsaturated fatty acid; LNA, α -linolenic acid; OF, oily fish; RS, rapeseed; SF, sunflower; WF, white fish.

Table 3
Anthropometric data during the intervention*

Variable	Control (n = 28)	WF/RS (n = 22)	WF/SF (n = 27)	OF/RS (n = 29)	OF/SF (n = 27)	Time	Group	Group × time
Weight (kg)								
0 wk	86.1 ± 16.9	84.7 ± 16.1	85.6 ± 14.1	90.6 ± 12.7	90.3 ± 15.0	0.1984	0.0764	0.0062
12 wk	85.4 ± 16.8	83.1 ± 15.6 ^{§§}	84.9 ± 14.1 ^{†§}	90.2 ± 12.8	91.3 ± 14.4 [†]			
24 wk	85.4 ± 16.7	83.9 ± 15.2 [§]	84.7 ± 14.6 ^{†§§}	89.9 ± 13.5	91.7 ± 14.2 [†]			
Waist (cm)								
0 wk	95.8 ± 15.3	94.9 ± 13.2	95.5 ± 13.1	97.9 ± 12.4	97.0 ± 11.7	0.0006	0.5183	0.0972
12 wk	95.3 ± 14.6	94.1 ± 13.5	94.8 ± 13.1	98.3 ± 12.4	98.1 ± 10.9			
24 wk	96.6 ± 14.2	95.7 ± 13.5	95.9 ± 13.9	98.2 ± 12.8	98.8 ± 11.4			
DXA (%)								
0 wk	40.0 ± 7.4	37.4 ± 8.6	38.3 ± 8.7	37.7 ± 8.0	39.0 ± 10.6	0.0114	0.5705	0.0155
12 wk	39.4 ± 7.5	38.0 ± 8.2	37.9 ± 8.9	37.6 ± 8.1	39.4 ± 10.6			
24 wk	39.8 ± 7.3	38.4 ± 8.1	37.7 ± 9.5	37.8 ± 8.5	39.9 ± 10.2			

DXA, dual x-ray absorptiometry; OF, oily fish; RS, rapeseed; SF, sunflower; WF, white fish

* Data are presented as mean ± SD for all outcomes and were analyzed using random effects models of the change in outcome, adjusted for baseline values and gender. Time and group effects and time × group interactions were determined for comparisons of the five intervention groups.

Where significant time × group effects exist, there were [†]significant differences within the group versus baseline [§]significant differences versus the same time point in the OF/RS group ^{§§}significant differences versus the same time point in the OF/SF group.

insulin and AUC insulin were log_e transformed to normalize their distribution). There were no significant group × time interactions in markers of insulin sensitivity (data not shown).

Inflammatory status

At baseline there were no significant differences between intervention groups in plasma cytokines, acute-phase proteins, or leptin. Men were found to have higher mean TNF-α levels than women (1.73 ± 0.99 versus 1.50 ± 0.73 pg/ml, *P* < 0.05) and women had higher mean values for ACT (0.31 ± 0.05 g/L versus 0.29 ± 0.04 g/L, *P* < 0.01) and leptin (29.37 ± 14.50 versus 11.99 ± 9.32 ng/ml, *P* < 0.01; data for TNF-α were log_e transformed to normalize their distribution). There were no significant group × time interactions for any of the inflammatory markers measured (data not shown).

Discussion

This study has shown that it is possible to achieve significant changes in fatty acid status with simple food-based manipulations and that this is associated with significant effects on TAG. At 24 wk of the dietary intervention the white fish/sunflower group demonstrated significantly lower plasma LC ω-3 PUFA levels than both oily fish groups and higher TAG levels than both oily fish groups and the control group.

Long-chain ω-3 PUFA supplement studies typically show reductions in TAG of 25% in those with normal TAG levels [65]. However, only one primary prevention trial has previously shown a significant lowering of TAG levels with modest increases in LC ω-3 PUFA by consumption of two

portions of oily fish per week [17]. In that crossover study, healthy male subjects (*n* = 118) reported eating an average of 317 g/wk of oily fish (including mackerel, salmon, and trout) for a 3-mo period and little or no oily fish for another 3 mo. Significant decreases in TAG, of 6.7%, were shown on the oily fish diet. Reported intakes of oily fish were similar in the oily fish groups in the present study with similar effects on TAG, i.e., the percentage decrease in plasma TAG in the oily fish groups combined was 6.6%. In the present study the effect was greatest in those with lower dietary LA:LNA (oily fish/rapeseed, 10.4%, versus oily fish/sunflower, 2.8%). This is a marked trend, although not significant, and suggests there may be dose-response effects of combining oily fish with different background LA:LNA. On this basis the lowest (or a negative) effect on TAG might be expected with the white fish/sunflower group, with an intermediate effect of the white fish/rapeseed group, but this trend was not observed. It is possible that the reduction in LC ω-3 PUFA in the white fish groups, resulting from the substitution of white for oily fish, had a greater effect on changes in TAG, thus over-riding the effects of manipulations to the LA:LNA ratio.

The sample size was powered to detect a 0.8 SD (0.66 mmol/L) change in plasma TAG. This was a conservative calculation based on a between-subject SD and it was expected that the SD in this repeated measures design would be lower and thus smaller changes might be detected. Mean values for plasma TAG across all subjects was 1.25 mmol/L with an SD of 0.66, and thus the study was underpowered to detect the 0.12 mmol/L reduction in plasma TAG shown in the oily fish/rapeseed group. However, this reduction may be clinically significant; a 1 mmol/L increase in TAG has been shown to represent a 14% increase in CVD risk in men and a 37% increase in women [66].

Although the aim of the study was to maintain subjects'

Table 4
Clinical and biochemical data for cardiovascular disease risk during the intervention*

Variable	Control (n = 28)	WF/RS (n = 22)	WF/SF (n = 27)	OF/RS (n = 29)	OF/SF (n = 27)	Time	Group	Group × time
TAG [†] (mmol/L)								
0 wk	1.21 ± 0.71	1.17 ± 0.48	1.44 ± 0.83	1.21 ± 0.51	1.24 ± 0.66	0.5956	0.1353	0.0504
12 wk	1.27 ± 0.74	1.11 ± 0.48	1.33 ± 0.52	1.07 ± 0.33 [‡]	1.25 ± 0.82			
24 wk	1.15 ± 0.54 [‡]	1.25 ± 0.62	1.44 ± 0.53	1.09 ± 0.30 [‡]	1.20 ± 0.68 [‡]			
TC (mmol/L)								
0 wk	5.27 ± 0.90	5.13 ± 0.90	5.70 ± 1.12	5.67 ± 0.76	5.38 ± 0.85	0.0143	0.0693	0.1412
12 wk	5.29 ± 1.15	4.95 ± 0.88	5.27 ± 1.27	5.54 ± 1.06	5.47 ± 0.97			
24 wk	5.31 ± 1.04	5.05 ± 0.71	5.74 ± 0.99	5.88 ± 0.76	5.51 ± 0.82			
LDL-C (mmol/L)								
0 wk	3.34 ± 0.83	3.26 ± 0.84	3.58 ± 0.90	3.78 ± 0.79	3.38 ± 0.77	0.0774	0.0702	0.3238
12 wk	3.35 ± 1.05	3.17 ± 0.81	3.39 ± 1.06	3.76 ± 0.95	3.45 ± 0.83			
24 wk	3.40 ± 0.92	3.14 ± 0.67	3.67 ± 0.87	3.97 ± 0.80	3.50 ± 0.76			
HDL-C (mmol/L)								
0 wk	1.37 ± 0.42	1.35 ± 0.36	1.43 ± 0.38	1.34 ± 0.26	1.42 ± 0.36	<0.0001	0.5099	0.2702
12 wk	1.28 ± 0.42	1.28 ± 0.31	1.28 ± 0.30	1.32 ± 0.30	1.42 ± 0.44			
24 wk	1.40 ± 0.44	1.34 ± 0.31	1.42 ± 0.33	1.42 ± 0.29	1.45 ± 0.41			
Systolic BP (mmHg)								
0 wk	123.1 ± 23.4	126.0 ± 17.1	123.2 ± 13.7	129.5 ± 15.6	129.9 ± 15.9	<0.0001	0.9452	0.2416
12 wk	119.6 ± 19.8	122.0 ± 16.2	122.8 ± 15.6	124.6 ± 17.5	122.7 ± 15.0			
24 wk	120.9 ± 20.3	123.0 ± 16.9	118.4 ± 19.1	120.9 ± 14.7	120.7 ± 14.7			
Diastolic BP (mmHg)								
0 wk	72.6 ± 12.1	73.3 ± 10.6	73.0 ± 10.2	77.3 ± 9.6	74.2 ± 9.2	<0.0001	0.4148	0.2446
12 wk	68.3 ± 12.6	67.6 ± 9.1	70.0 ± 10.0	74.0 ± 10.2	71.4 ± 8.6			
24 wk	69.1 ± 10.1	70.4 ± 11.0	67.9 ± 11.3	70.7 ± 8.0	68.9 ± 8.8			

BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OF, oily fish; RS, rapeseed; SF, sunflower; TAG, triacylglycerol; TC, total cholesterol; WF, white fish

* Data are presented as mean ± SD or geometric mean ± SD for transformed data and were analyzed using random effects models of the change in outcome, adjusted for baseline values, gender, and weight. Time and group effects and time × group interactions were determined for comparisons of the five intervention groups.

[†] Log_e transformed for statistical analysis.

[‡] Where a significant time × group interaction exists, there were significant differences versus the same time point in the WF/SF group.

weight during the course of the study, changes were shown to differ across groups. Although the white fish/sunflower group showed weight loss, increases in weight were demonstrated with the oily fish/sunflower intervention. These findings did not reflect reported energy intakes during the intervention, which were shown to decrease in both groups and, moreover, smaller reductions were reported in the white fish/sunflower group (data not shown). It is unlikely that the substitution of oily fish or high ω -6 sunflower oil has implications for CVD risk that are related to increases in weight; both sunflower groups and oily fish groups showed weight changes in different directions. Changes in physical activity may offer an explanation for the changes in weight during the study, but no measurements of physical activity were taken and subjects were encouraged to maintain their habitual patterns during the course of the study.

In this study there were no significant differences across treatment groups in a number of other markers of CVD risk including TC, HDL-C, or LDL-C. LA has been shown to enhance the clearance and downregulate the production of LDL-C [67] and to have a protective role on the cholesterol effects of saturated fatty acids at intakes <5% E. In contrast, there is evidence that dietary substitution of LNA for LA may have a detrimental effect on blood lipid profiles,

with high LNA intakes lowering HDL-C at high doses (>2% E) [68]. However, in the present study, substitution of low for high LA:LNA fat spreads and oil in the rapeseed groups, resulting in intakes of LA (4.9% E) and LNA (0.8% E) at 24 wk, showed no effect on blood lipid profiles.

Most studies have shown no effect of EPA and DHA on TC levels [65]. The data on LDL-C are less consistent, with some reporting modest increases and others showing no effect. These inconsistencies may be dose related or due to differences in baseline health status, i.e., 4 g/d of DHA significantly increased LDL-C in mildly hyperlipidemic men [10] but had no similar effect in type 2 diabetics [69]. Disparate findings may also arise from the large interindividual variability shown in blood lipid profiles, making it difficult to demonstrate any subtle shifts with dietary intervention that may be clinically important. No measurements of lipoprotein subfractions or LDL particle size were made in this study. In other studies favorable shifts in HDL subclasses toward the larger HDL₂ and LDL particles have been found with LC ω -3 PUFA [10,69], providing a mechanism for potential antiatherogenic effects. The lack of effect on blood coagulation and fibrinolytic factors is consistent with most other studies examining the antithrombotic role of LNA [70] and LC ω -3 PUFA [42,43]. Likewise, no

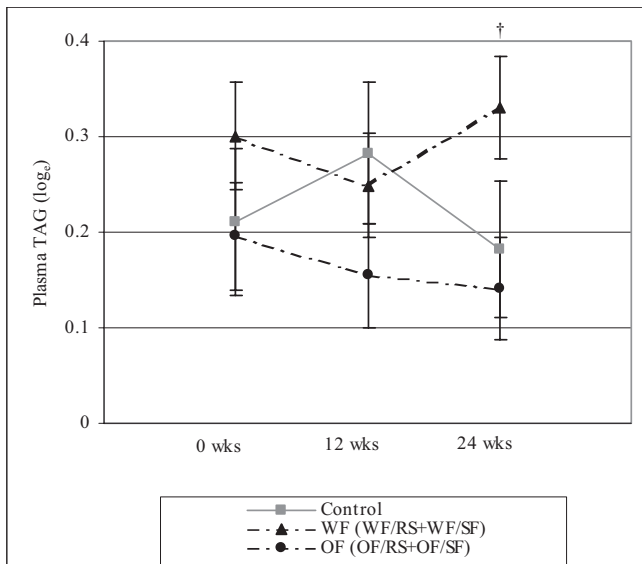


Fig. 4. Plasma TAG during the intervention. Data are presented as log_e-transformed mean values (SE) and were analyzed using random effects models of change in outcome, adjusted for baseline values, gender and weight. There was a significant time \times group interaction, † indicates OF < WF ($P < 0.05$). LA, linoleic acid; LNA, α -linolenic acid; OF, oily fish; RS, rapeseed; SF, sunflower; TAG, triacylglycerol; WF, white fish.

detrimental effect of LA on fibrinolytic factors has been shown at moderate intakes [70].

The results of this study showed no effect on insulin sensitivity with changes in LC ω -3 PUFA or manipulation of LA:LNA achieved by dietary intervention. Reported effects of LC ω -3 PUFA on insulin sensitivity are equivocal. This study used AUC insulin from an OGTT as a primary marker of insulin sensitivity. A criticism of the OGTT is that it demonstrates poor reproducibility [71] and therefore large intraindividual differences in indices of insulin sensitivity. Measurements from an intravenous glucose tolerance test show greater repeatability and the use of this test may increase the likelihood of detecting clinically significant results. Further, an isotopically labeled intravenous glucose tolerance test would allow the differential effects on hepatic and peripheral insulin sensitivity to be investigated.

Evidence relating to the anti-inflammatory effects of ω -3 PUFA is inconsistent. Significant reductions in the cytokine IL-6 and the acute-phase proteins CRP and serum amyloid A have been shown at high doses (4% E) of LNA [54]; however, at lower doses (1.6% E) no effects were demonstrated on immune markers and monocyte functional activity [72]. The literature for LC ω -3 PUFA is similarly conflicting. Suppression of *in ex vivo* cytokine and eicosanoid production has been shown in a number of studies [47–52] but different doses of LC ω -3 PUFA have generally shown no effect on circulating inflammatory markers [73–75], with the exception of one study showing decreases in fibrinogen [53]. The inconsistency of these findings may reflect the different response of different population groups according to inflammatory genotype [76] and phenotype [13], or sim-

ply that there are difficulties associated with the measurement of circulating inflammatory markers that are susceptible to large biological variation in response to infection and injury. The finding of no effect, of ω -3 PUFA on inflammation, in this study is comparable to most other studies examining circulating inflammatory markers in healthy subjects.

The lack of metabolic effects, other than changes in TAG, may be due to the relatively small magnitude of the change in fatty acid status. Although significant changes in plasma LC ω -3 PUFA concentrations were shown in the groups consuming oily fish, levels at 12 wk (1.5% EPA and 3% DHA) were substantially lower than those shown in most other intervention studies that have used high-dose fish oil capsules. For example, in a recent study at MRC HNR, plasma EPA and DHA were increased by approximately 3.5% and 5% after a 12-wk intervention of 5g/d of fish oil (1.3 g of EPA and 2.9 g of DHA). There may be a threshold for beneficial health effects of EPA and DHA and the dose-response effects need clarification. In addition, the duration of intervention may be too short to observe the full health effect. Epidemiologic studies have shown beneficial effects of habitual consumption of LC ω -3-rich diets on health outcomes [23,77], presumably reflecting lifelong dietary habits. In comparison, most intervention studies are relatively short. Other studies have shown that even with high-dose LC ω -3 PUFA capsules the proportion of EPA and DHA in adipose tissue continues to increase for at least 6 mo of intervention [78].

In addition, the recruitment of a higher-risk group may have yielded a more pronounced effect of the intervention. The inclusion of middle-aged, overweight subjects was designed to increase the chances of seeing an effect of ω -3 PUFA on CVD risks and provide results relevant to public health recommendations. Although average BMI and waist circumference measurements in this study population were indicative of increased metabolic risk, this was not the case for other clinically relevant health outcome measurements. In this study men were shown to have higher blood pressure, TAG, and glucose and lower HDL than women. Reductions in the ω -6: ω -3 ratio may have a greater effect on reducing CVD risk in men, or other population subgroups, demonstrating a less favorable health profile.

Nonetheless, there are data from epidemiologic and clinical trials to support the effects of consuming at least two servings of fish (particularly oily fish) per week [79]. In this study the substitution of white fish for oily fish, compared with increases in oily fish (from less than one portion per week at baseline to just over two portions per week), was shown to have a significant effect on TAG levels. This finding reinforces the public health messages to increase oily fish intake, which in the United Kingdom is currently only one-third of a portion a week in adults [64] and well below the two portions per week delivered in this study. The potential for added benefits through substitution of LA for LNA in habitual fats and oils warrants further investigation.

Conclusion

Two portions of oily fish per week led to significant reductions in TAG relative to consumption of two portions of white fish per week. There was a trend for greater reductions in TAG when increases oily fish intake were combined with a lower LA:LNA.

Acknowledgments

The authors are grateful to Matthew Foods Plc (Ossett, West Yorkshire, UK) for supplying the fat spreads for the intervention. They thank the HNR staff in the Volunteer Suite (with special thanks to Sarah Jones and Jenny Cooke); the Nutritional Biochemistry Laboratory (with special thanks to Ian Halsall, Maria O'Connell, and Neil Matthews); and the Nutrition Epidemiology Section (with special thanks to Dr. Sarah McNaughton for the analysis of the diet diaries).

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