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Effects of Purified Eicosapentaenoic and Docosahexaenoic Acids in Nonalcoholic Fatty Liver Disease: Results From the WELCOME* Study

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There is no licensed treatment for nonalcoholic fatty liver disease (NAFLD), a condition that increases risk of chronic liver disease, type 2 diabetes, and cardiovascular disease. We tested whether 15-18 months of treatment with docosahexaenoic acid (DHA) plus eicosapentaenoic acid (EPA; Omacor/Lovaza, 4 g/day) decreased liver fat and improved two histologically validated liver fibrosis biomarker scores (primary outcomes). Patients with NAFLD were randomized in a double-blind, placebo-controlled trial (DHA+EPA, n = 51; placebo, n = 52). We quantified liver fat percentage by magnetic resonance spectroscopy in three liver zones. We measured liver fibrosis using two validated scores. We tested adherence to the intervention (Omacor group) and contamination (with DHA and EPA; placebo group) by measuring erythrocyte percentage DHA and EPA enrichment (gas chromatography). We undertook multivariable linear regression to test effects of (1) DHA+EPA treatment (intention-to-treat analyses) and (2) erythrocyte DHA and EPA enrichment (secondary analysis). Median (interquartile range) baseline and end-of-study liver fat percentage were 21.7 (19.3) and 19.7 (18.0) (placebo) and 23.0 (36.2) and 16.3 (22.0) (DHA+EPA). In the fully adjusted regression model, there was a trend toward improvement in liver fat percentage with DHA+EPA treatment ($\beta = -3.64$; 95% confidence interval [CI]: −8.0, 0.8; $P = 0.1$), but there was evidence of contamination in the placebo group and variable adherence to the intervention in the Omacor group. Further regression analysis showed that DHA enrichment was independently associated with a decrease in liver fat percentage (for each 1% enrichment: $\beta = -1.70$; 95% CI: −2.9, −0.5; $P = 0.007$). No improvement in fibrosis scores occurred. *Conclusion:* Erythrocyte DHA enrichment with DHA+EPA treatment is linearly associated with decreased liver fat percentage. Substantial decreases in liver fat percentage can be achieved with high-percentage erythrocyte DHA enrichment in NAFLD. (HEPATOLOGY 2014;60:1211-1221)

Nonalcoholic fatty liver disease (NAFLD) is associated with metabolic syndrome (MetS) and is defined by the presence of ≥5% hepatic steatosis. NAFLD increases risk of chronic liver disease, hepatocellular carcinoma, type 2 diabetes, and cardiovascular disease,[1-6] but there is no licensed treatment. Lifestyle change may ameliorate liver fat,[7-9] but is difficult to achieve, and although treatment with

Abbreviations: AEs, adverse events; CI, confidence interval; CK18, cytokeratin 18; CVR, cardiovascular risk; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GC, gas chromatography; HA, hyaluronic acid; ITT, intention to treat; MET, metabolic equivalents of task; MetS, metabolic syndrome; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PIINP, procollagen-III N-terminal propeptide; SD, standard deviation; TG, triglyceride; TIMP-1, tissue inhibitor of matrix metalloproteinase 1; WELCOME, Wessex Evaluation of fatty Liver and Cardiovascular markers in NAFLD with OMacor thErapy.

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thiazolidinediones[10,11] and vitamin E[12] has produced encouraging results in NAFLD, there are serious safety concerns about long-term use of these agents.[13-15]

Omega-3 fatty acid treatment is safe and has attracted considerable interest as a potential treatment for NAFLD.[16] A dose of 2-4 g/day of omega-3 fatty acids (eicosapentaenoic acid [EPA] plus docosahexaenoic acid [DHA]) is approved for treatment of hypertriglyceridemia.[17] However, whether DHA+EPA are effective in NAFLD is unproven.[18]

In a randomized, placebo-controlled trial, we tested the hypothesis that 15-18 months of treatment with the highest licensed dose (4 g/day) of DHA+EPA was effective in ameliorating the early stages of NAFLD,[19] with primary outcomes related to (1) liver fat measured by magnetic resonance spectroscopy (MRS) scan in three discrete liver zones and (2) two algorithmically derived, histologically validated liver fibrosis scores.[20,21] We monitored adherence to the intervention in the DHA-EPA group, and potential contamination in the placebo group, by measuring erythrocyte enrichment of DHA or EPA between baseline and end of the study.

Patients and Methods

Study Design. The design and rationale for the WELCOME study (Wessex Evaluation of fatty Liver and Cardiovascular markers in NAFLD with OMacor thErapy) have been reported previously.[19] The WELCOME study was approved by the Southampton and South West Hampshire local research ethics committee (08/H0502/165). All participants gave written informed consent. Omacor (DHA+EPA as ethyl esters), also known as Lovaza, was provided free of charge by Pronova BioPharma/Abbott (Pronova BioPharma ASA, Lysaker, Norway; Abbott Laboratories, Southampton, UK). The conduct of the trial, data analyses, and writing of the manuscript were all undertaken by the authors and were completely independent from Pronova BioPharma/Abbott. The primary endpoint was to test whether treatment with purified DHA+EPA over a maximum of 18 months and a minimum of 15 months (1) decreased liver fat percentage measured by MRS (we calculated the average liver fat percentage for three liver segments: segments 3 [inferior subsegment of the lateral segment], 5 [inferior subsegment of the anterior segment], and 8 [superior subsegment of the anterior segment]) and (2) improved two histologically validated liver fibrosis scores.[20,21]

One hundred and three participants were randomized (Fig. 1) to either Omacor (DHA+EPA) 4 g per day (n = 51; 1 g of Omacor contains 460 mg of EPA and 380 mg of DHA as ethyl esters] or 4 g per day of placebo (olive oil; n = 52; 1 g of olive oil contains 600 mg of oleic acid plus lesser amounts of linoleic, palmitic, stearic, and α-linolenic acids]. DHA+EPA and placebo capsules were gelatine coated and of similar appearance and taste. We assessed erythrocyte EPA and DHA enrichment (between baseline and end of study) to test adherence to the intervention in the DHA+EPA group and monitor contamination with DHA and EPA in the placebo group. Compliance with the allocated medication was also monitored by recording returned unused capsules. Adverse events (AEs) were recorded.

Inclusion and Exclusion Criteria. Inclusion and exclusion criteria have been described previously.[19] Briefly, subjects were eligible (1) with histological confirmation of NAFLD or (2) imaging evidence of liver fat (ultrasound, magnetic resonance imaging [MRI], or computed tomography scan), features of MetS,[22] and exclusion of other liver conditions causing liver fat accumulation or cirrhosis. Subjects were also excluded if alcohol consumption was >35 units (1 unit is 7.9 g of alcohol) per week for women and >50 units per week for men, which is the threshold for harmful alcohol consumption.[23] Additional exclusion criteria were pregnancy, breastfeeding, and hypersensitivity to DHA+EPA, soya, or the excipients.

Biochemical Measurements, Body Composition and Energy Expenditure. All measurements (including MRS liver fat percentage) were undertaken at baseline and end of study. Fibrosis markers, including hyaluronic acid (HA), procollagen-III N-terminal propeptide (PIIINP), and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1), were measured along with cytokeratin 18 (CK18), which has been used to assess nonalcoholic steatohepatitis (NASH).[24] Energy

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Potential conflict of interest: Dr. Calder consults and is on the speakers' bureau for Vifor. He consults for Amarin and advises Pronova.

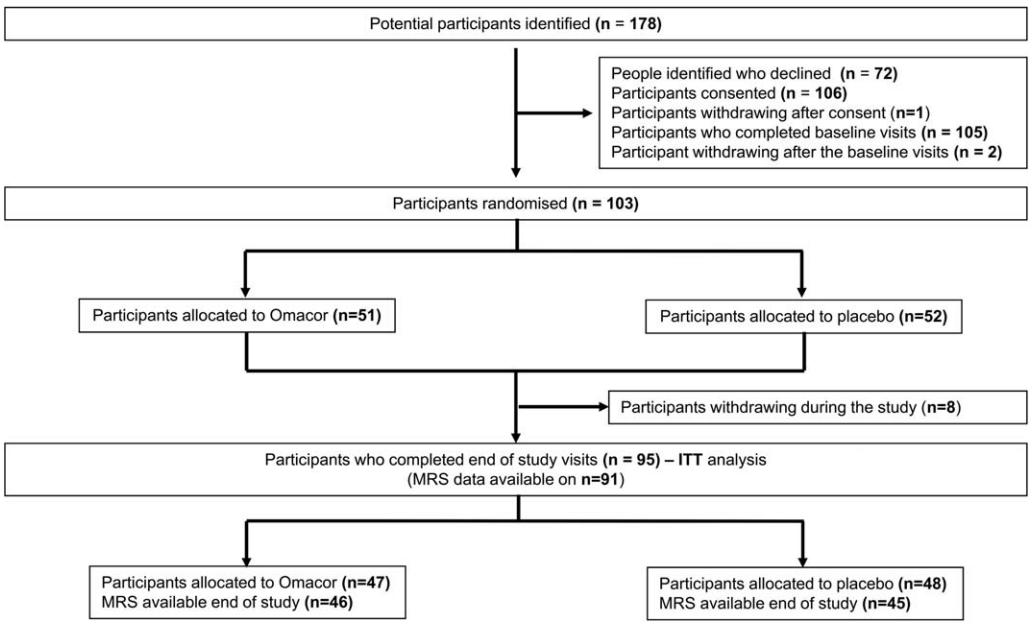

Fig. 1. Consort diagram showing recruitment to the study. For the reasons for withdrawal from the study, see a previous work.[19]

expenditure was assessed by measurement of metabolic equivalent of task (MET; SenseWear Pro 3 Armband monitor; BodyMedia, Inc., Pittsburgh, PA). Any change in diet during the study was assessed by food frequency questionnaire. We also generated a "prudent diet score" as a healthy diet index, using principal component analyses.[25]

DHA and EPA Enrichment of Erythrocyte Fatty Acids. Quantification of erythrocyte enrichment with DHA+EPA was determined by gas chromatography (GC) at baseline and end of study in all participants.[19,26]

Sample-Size Calculations. Initially, we estimated that a 15% decrease in liver fat % would result from DHA+EPA treatment.[19,27] Subsequently, a meta-analysis of seven studies suggested the pooled effect size of omega-3 fatty acid treatment could be greater (Hedge's g pooled effect size = −0.97; 95% confidence interval [CI]: −1.35, −0.58; $P < 0.0001$).[18] With an estimated sigma of 0.3,[19] this would equal an ∼30% decrease in liver fat percentage with treatment. Adopting a more conservative estimate of the effect size (i.e., a 20% decrease in liver fat with DHA+EPA treatment), assuming a sigma of 0.3 and an alpha of 0.05 with 91 participants completing our trial, we had 86% power to detect a 20% change in liver fat (two-tailed test). At the time of the study design, the change in liver fibrosis score[20] that equates to a clinically meaningful change was uncertain, and it was difficult to undertake a satisfactory sample-size calculation to power the study sufficiently to test this primary outcome. However, based on the available evidence, we assumed that a 0.6- to 1.0-unit change in fibrosis score might be clinically significant.[20] Consequently, to detect a minimum 0.6-unit change in score (e.g., 9.0 at baseline and 8.4 at the end of the study) with a standard deviation (SD) of 1.0, 100 participants would provide >80% power at the 5% significance level, and with a 15% dropout of participants, there would also be >80% power to detect this effect. A sample-size calculation was not undertaken for the NAFLD fibrosis score[20,21] because of the paucity of available data to guide a sample-size calculation.

Statistical Analysis. All statistical analyses were performed using SPSS for Windows (version 21.0; SPSS, Inc., Chicago, IL). The primary endpoints were change in liver fat percentage and change in two histologically validated, algorithmically derived liver fibrosis biomarker scores (intention-to-treat [ITT] analyses).[20,21] Data are reported as means and 95% CIs or SDs for normally distributed variables, or as median and IQR, interquartile range for non-normally distributed variables. Multivariable linear regression was undertaken with change in liver fat percentage or change in either of the histologically validated liver fibrosis scores as the outcome variable and adjusted for baseline. The ITT analysis included all patients randomized who had complete data (i.e., having baseline and end-of-study measurements), regardless of whether they were later found to be ineligible, a

Table 1. Baseline Variables in Placebo and DHA+EPA Groups at Randomization

	Placebo	DHA+EPA	*P* Value
Variables			
Age, years	54.0 (9.6)	48.6 (11.1)	0.09
Sex, M/F	35/17	25/26	0.08
Diabetes, %	9.0	9.0	0.90
Weight, kg	93 (14.4)	97 (17)	0.20
BMI, kg/m^2	32.0 (4.3)	34.3 (5.8)	0.02
Waist circumference, cm	108.1 (11.9)	112.8 (12.0)	0.06
Diastolic blood pressure, mmHg	85.3 (8.1)	84.7 (11.8)	0.80
Systolic blood pressure, mmHg	137.7 (15·9)	138.2 (16.7)	0.90
Fasting plasma glucose*, mmol/L	6.2 (2.0)	6.2 (2.8)	0.30
Fasting plasma insulin*, μU/mL	11.3 (12.2)	13.6 (11.9)	0.80
HbA1c*, % total	6.1 (1.6)	5.9 (1.2)	0.20
Plasma TGs*, mmol/L	1.4 (0.9)	1.8 (1.2)	0.04
Plasma cholesterol, mmol/L	4.8 (1.3)	4.9 (1.1)	0.40
LDL-cholesterol, mmol/L	2.7 (0.8)	3.0 (0.9)	0.30
HDL-cholesterol, mmol/L	1.1 (0.3)	1.0 (0.2)	0.10
ALT*, IU/L	56.0 (34)	54.0 (43)	0.60
AST*, IU/L	41.5 (19)	38.0 (24)	0.20
DEXA total fat mass*, g	32,390.1 (8,140)	37,500.7 (15,109)	0.08
DEXA total lean mass, g	58,536.4 (10933.0)	58,033.9 (12370.1)	0.80
DEXA andro/gynoid (ratio)	1.2 (0.2)	1.2 (0.1)	0.10
MRI subcutaneous fat (%)	30.2 (9.5)	34.2 (9.8)	0.05
MRI visceral fat (%)	16.7 (4.5)	15.6 (5.1)	0.30
HA*, μg/L	22.5 (27.0)	18.0 (21.0)	0.04
PIIINP*, (μg/L)	5.1 (1.9)	5.4 (3)	0.60
MRS liver fat %*	21.7 (19.3)	23.0 (36.2)	0.75
NAFLD fibrosis score	−1.7 (1.3)	−1.5 (1.4)	0.60
Liver fibrosis score†	9.0 (0.8)	8.8 (0.8)	0.34
Erythrocyte DHA (%)	4.2 (1.4)	3.9 (1.2)	0.32
Erythrocyte EPA (%)	0.9 (0.4)	0.8 (0.3)	0.43

Variables that are normally distributed are expressed as mean (SD).

*Variables that are non-normally distributed are expressed as median (IQR).

†Fibrosis score calculated from PIIINP, HA, and TIMP-1.[20]

Abbreviations: M, male; F, female; BMI, body mass index; Hb1Ac, glycosylated hemoglobin; LDL, low-denisty lipoprotein; HDL, high-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DEXA, dual-energy X-ray absorptiometry.

protocol violator, given the wrong treatment allocation, or never treated). Models are also reported as adjusted for potential confounders. For potential confounding factors that may have changed during the period of the study (e.g., weight, diet, or physical activity), we calculated the difference between baseline and end-of-study measurement. As a secondary analysis, the above was repeated with DHA+EPA treatment replaced by erythrocyte DHA and EPA enrichment. ITT and secondary analyses included all participants with baseline and end-of-study measurements, regardless of whether they were later found to be noncompliant, ineligible, or protocol violators. A P value of <0.05 was considered to be statistically significant.

Results

Ninety-five participants completed the study (55 men and 40 women). Table 1 shows the baseline characteristics of participants according to randomization group. Table 2 shows the changes between baseline and end-of-trial measurements, stratified by randomization group, for the main anthropometric and biochemical variables. Inadequate MRS data were obtained for 4 of 95 participants, and end-of-study liver fat percentage was available on 91 participants for the primary and secondary analyses.

From capsule counts at 6 and 12 months and at the end of study, we estimated that all participants consumed >50% of their study medication and 78% consumed >75%. No serious AEs occurred that were attributed to medication. Alcohol consumption was not associated with baseline liver fat percentage ($P = 0.93$).

DHA and EPA Enrichment of Erythrocytes. We hypothesized *a priori* that DHA+EPA intervention should produce a minimum 2% increase in erythrocyte DHA and a minimum 0.7% increase in erythrocyte EPA[26,28] to produce an effect. Figure 2 shows the percentage enrichment in erythrocyte DHA and EPA

Table 2. Main Anthropometric and Biochemical Variables at Baseline and End of Study According to Randomization Group

	Placebo			DHA+EPA		
Variables	**Baseline**	**End of Study**	***P* Value**	**Baseline**	**End of Study**	***P* Value**
BMI, kg/m^2	32.0 (4.5)	30.8 (4.5)	0.31	34.4 (5.8)	33.4 (4.9)	0.3
Waist circumference, cm	108.1 (11.9)	107.7 (10.3)	0.45	112.8 (12.0)	112.3 (10.4)	0.87
Fasting plasma glucose*, mmol/L	6.2 (2.0)	6.7 (3.0)	0.12	6.2 (2.8)	6.1 (2.0)	0.81
MRI subcutaneous fat (%)	30.2 (9.5)	28.8 (9)	0.46	34.2 (9.8)	32 (9.6)	0.47
MRI visceral fat (%)	16.7 (4.5)	16.5 (5.4)	0.35	15.6 (5.1)	15.9 (4.7)	0.67
HbA1c*, % total	6.1 (1.6)	6.0 (2.0)	0.14	5.9 (1.2)	5.7 (2.0)	0.53
Plasma TGs*, mmol/L	1.4 (0.5)	1.8 (0.6)	0.08	1.8 (1.2)	1.5 (1.2)	0.018
Plasma cholesterol, mmol/L	4.8 (1.3)	4.8 (1)	0.25	4.9 (1.1)	4.7 (1.1)	0.18
LDL-cholesterol, mmol/L	2.7 (0.8)	2.8 (0.8)	0.44	3.0 (0.9)	2.8 (0.9)	0.14
HDL-cholesterol, mmol/L	1.1 (0.3)	1.1 (0.2)	0.87	1.0 (0.2)	1.1 (0.3)	<0.0001
ALT*, iU/L	56.0 (34)	48.5 (25)	0.06	54.0 (43)	44.0 (34)	0.70
AST*, iU/L	41.5 (19)	35.0 (17)	0.04	38.0 (24)	30.0 (27)	0.83

Variables that are normally distributed are expressed as mean (SD). Variables that are non-normally distributed (*) are expressed as median (IQR). There were 9 patients (4 in the placebo group and 5 in the DHA+EPA group) with high NAFLD fibrosis score[21] and 14 (8 in the placebo group and 6 in the DHA+EPA group) with high liver fibrosis score.[20]

Abbreviations: BMI, body mass index; Hb1Ac, glycosylated hemoglobin; LDL, low-denisty lipoprotein; HDL, high-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

between baseline and end of study in participants randomized to placebo or DHA+EPA. Enrichment was highly variable in the DHA+EPA group and 5 and 6 participants in the DHA+EPA group did not reach the prespecified threshold for EPA and DHA enrichment, respectively. In the placebo group, we

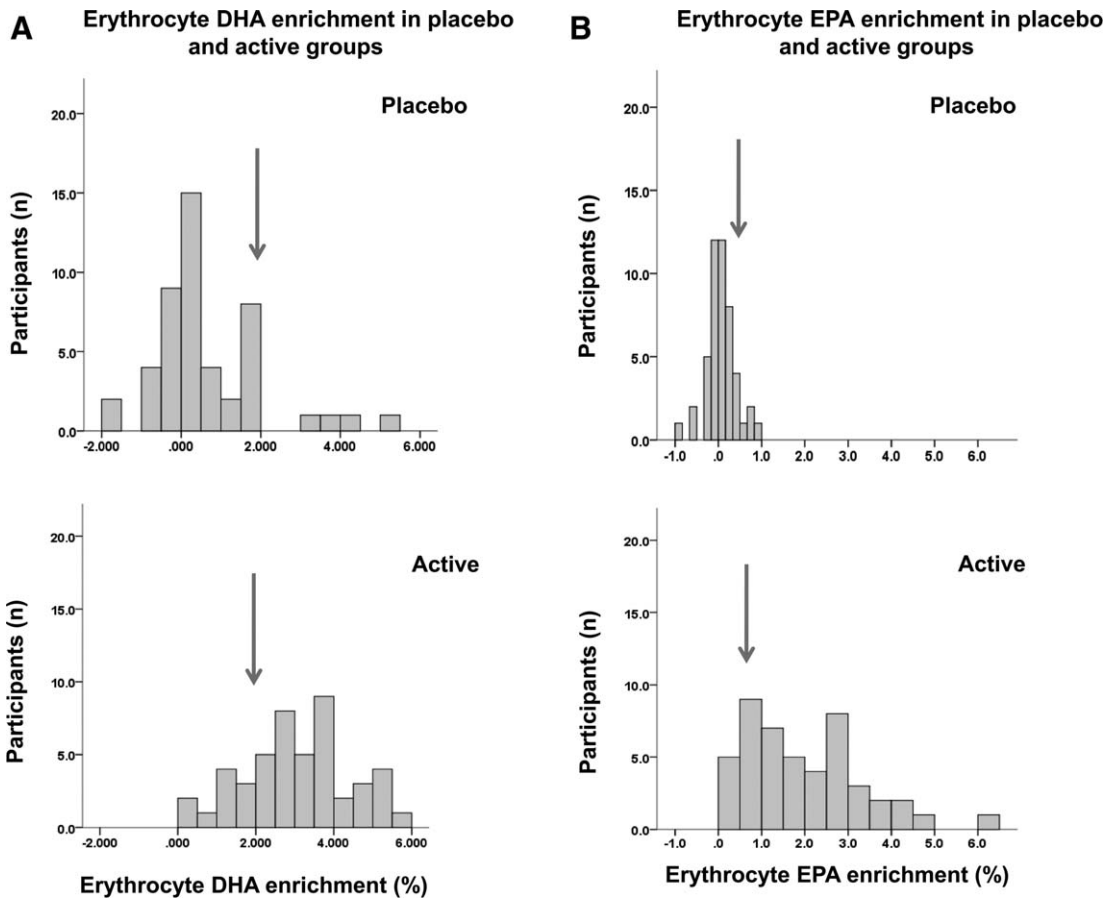

Fig. 2. Percentage change in erythrocyte DHA and EPA concentration between baseline and end of study in placebo and Omacor groups.

expected no enrichment between baseline and end of study in all participants in this group, but 3 and 4 participants reached the thresholds set for the DHA+EPA group, for EPA and DHA, respectively (Fig. 2). One participant in the placebo group admitted to taking cod liver oil during the study and another markedly increased consumption of fish.

ITT Analyses and Secondary Analyses. Table 3 shows baseline and end-of-study data for each of the primary outcomes and the results of regression models for the ITT analyses and secondary analyses. Data for change in plasma triglyceride (TG) concentration are shown for comparison (Omacor is licensed for lowering plasma TG concentrations). In the ITT analyses, in the fully adjusted model, there was a 3.64% decrease in liver fat % ($\beta = -3.64$; 95% CI: −8.0, 0.8; $P = 0.1$) with DHA+EPA treatment. We undertook secondary analyses (Table 3) to test the association between DHA or EPA enrichment and each of the primary outcomes (adjustments as per the ITT analysis). Erythrocyte DHA enrichment was independently associated with a decrease in liver fat percentage (−1.7% for each 1% DHA enrichment; $\beta = -1.7$; 95% CI: −2.9, −0.5; $P = 0.007$ in the fully adjusted model). In our cohort, there were only 9 patients with high NAFLD fibrosis score[21] and 14 with high liver fibrosis score.[20] There was no improvement in either liver fibrosis score with DHA+EPA or with DHA or EPA enrichment.

Because there was clear evidence of contamination with DHA and EPA enrichment in some participants in the placebo group, and there was also poor enrichment with DHA and EPA in some participants in the treatment group (Fig. 2), we assessed the relationships between liver fat percentage at recruitment and the change in liver fat percentage between baseline and end of study in all participants, stratified by the prespecified threshold for erythrocyte DHA enrichment (<2% and ≥2%) and regardless of randomization group (Fig. 3). For those individuals achieving a ≥2% absolute increase in DHA between baseline and end of study, there was a strong inverse association between liver fat percentage at recruitment and the change in liver fat percentage between baseline and end of study.

Magnitude of the Effect to Decrease Percentage of Liver Fat by Percentage of DHA Enrichment. We further investigated the magnitude of the effect of each 1% DHA enrichment to decrease liver fat percentage adjusting for change in physical activity during the study (available in n = 82 of 91 participants). In a regression model that included liver fat percentage difference as the outcome, and age, sex, difference in body weight (kg), difference in CK18 (as a marker of apoptosis and necrosis), difference in MET, between baseline and follow-up, and DHA percentage enrichment as the explanatory variables; each 1% DHA enrichment was associated with a 3.3% reduction in liver fat percentage ($\beta = -3.3$; 95% CI: −4.8, −1.8; $P < 0.0001$).

Discussion

Our study is the first randomized, double-blind, placebo-controlled trial to test the efficacy of a high-dose DHA+EPA intervention on a quantitative measurement of liver fat in NAFLD and relate changes in erythrocyte DHA+EPA enrichment to changes in liver fat percentage. Although we were not able to prove that there was a significant effect of the intervention on the primary outcomes in the ITT analyses, our novel results show, in the secondary analyses, that erythrocyte DHA enrichment with DHA+EPA treatment is linearly associated with decreased liver fat percentage, and substantial decreases in liver fat percentage can be achieved with high-percentage DHA enrichment. The ITT analyses showed a strong trend toward a decrease in liver fat percentage with DHA-EPA treatment, but there was strong evidence for contamination with DHA and EPA enrichment in the placebo group and poor adherence to DHA+EPA intervention in the treatment arm. (There was evidence of poor adherence to the intervention, or contamination with DHA+EPA in 10 of 95 participants completing the trial.) Poor adherence to the DHA+EPA intervention (in the treatment group), or contamination with DHA and EPA (in the placebo group), would bias the result toward the null, attenuating any effect of the DHA+EPA intervention on primary outcomes. In contrast, the secondary analysis (undertaken in all participants) circumvented this problem, because randomization to Omacor (as a dichotomous exposure variable) was replaced in the regression model by either erythrocyte DHA percentage enrichment or erythrocyte EPA percentage enrichment, as a continuous exposure variable. Thus, there was no stratification by randomization group in this analysis, and so this additional analysis allowed us to test the effect of percentage DHA enrichment (or percentage EPA enrichment), regardless of randomization group on each of the primary outcomes. The data show the effect of each 1% DHA enrichment to decrease liver fat percentage (Table 3). We have presented data on all participants in Table 3 and have not excluded protocol violators. Further regression analysis was undertaken

Table 3. Baseline and End-of-Study Results and Regression Models Testing the Effects of the Intervention on the Primary Outcomes (and Plasma TG Concentrations)

	Placebo		Treatment		Difference in Change From Baseline to End of Study* (95% CI)	Adjusted Difference in Change From Baseline to End of Study†** (95% CI)	Adjusted Difference in Change From Baseline to End of Study† (95% CI)	Adjusted Difference in Change From Baseline to End of Study† (95% CI)
Primary Outcomes	Baseline	End of Study	Baseline	End of Study	Omacor Treatment Primary Analysis	Omacor Treatment Primary Analysis	% Erythrocyte EPA Enrichment (Secondary Analysis)	% Erythrocyte DHA Enrichment (Secondary Analysis)
Liver fat %	21.7 (19.3)	19.7 (18.0)	23.0 (36.2)	16.3 (22.0)	−1.7 (−6.3, 3.0) $P = 0.48$	−3.64 (−8.0, 0.8) $P = 0.1$	−1.0 (−2.7, −0.6) $P = 0.20$	−1.7 (−2.9, −0.5) $P = 0.007$
Liver fibrosis score‡	9.0 (0.8)	9.2 (0.8)	8.8 (0.8)	9.1 (0.9)	−0.001 (−0.3, 0.3) $P = 1.0$	0.14 (−0.26, 0.33) $P = 0.8$	0.01 (−0.1, 0.1) $P = 0.86$	0.03 (−0.1, 0.1) $P = 0.50$
NAFLD fibrosis score	−1.7 (1.3)	−0.8 (1.2)	−1.5 (1.4)	−0.7 (1.5)	−0.03 (−0.4, 0.3) $P = 0.9$	0.01 (−0.3, 0.3) $P = 0.9$	0.00 (−0.1, 0.1) $P = 0.9$	0.01 (−0.1, 0.1) f$P = 0.6$
Plasma TG, mmol/L	1.4 (0.5)	1.8 (0.6)	1.8 (1.2)	1.5 (1.2)	−0.2 (−0.6, 0.1) $P = 0.2$	−0.4 (−0.8, −0.1) $P = 0.02$	−0.2 (−0.3, −0.1) $P = 0.002$	−0.1 (−0.2, −0.0) $P = 0.007$

Baseline and end-of-study results for primary outcomes and for serum TG concentrations are reported either as means and SDs or median and IQRs (liver fat % and plasma TG concentration). Results of regression models testing the effects of Omacor randomization (ITT analyses) and EPA and DHA percentage enrichment (secondary analyses) between baseline and end of study are shown.

For each primary outcome (liver fat %, liver fibrosis score, and NAFLD fibrosis score), the difference in outcome represents the change in the outcome between baseline and end of study (*) adjusted for baseline measurement of the outcome variable only, and (†) adjusted for baseline measurement of the outcome plus age, sex, change in weight (kg) between baseline and end of study, and change in CK18 concentration between baseline and end of study.

TG data are presented for comparison because this dose of Omacor treatment is licensed for the treatment of high TG concentrations.

*Adjusted for baseline measurement of outcome only.

†Fully adjusted.

‡Liver fibrosis score (score derived from TIMP-1, HA, and PIIINP concentrations; see Patients and Methods).

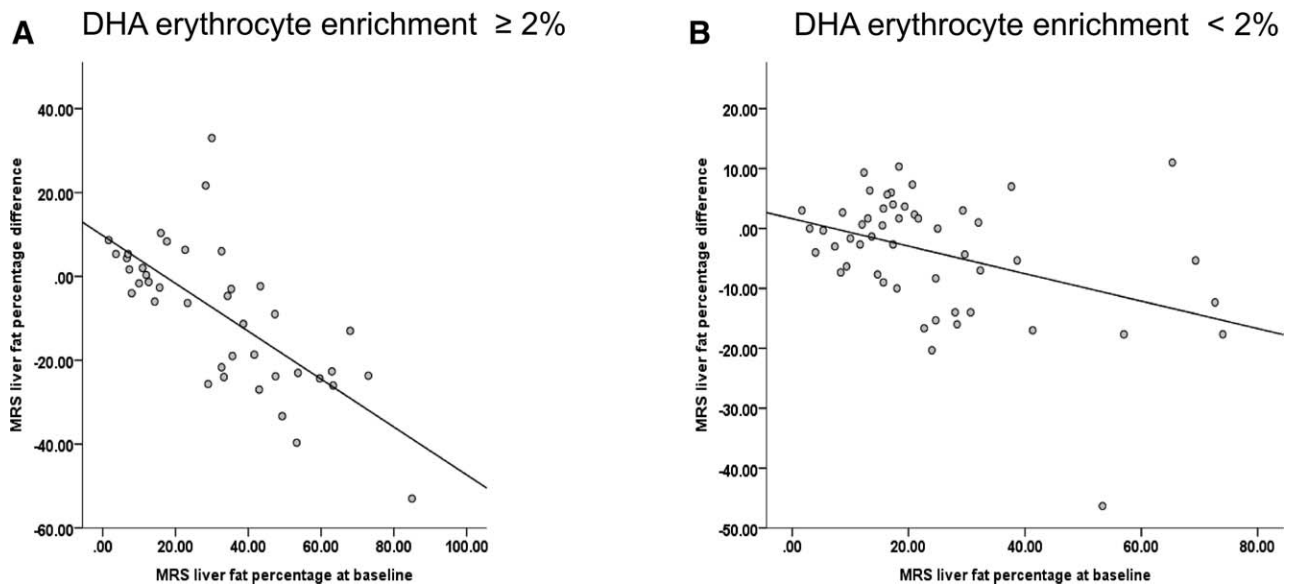

Fig. 3. Scatter plots showing the relationships between baseline liver fat % at recruitment and the change in liver fat % between baseline and end of study for all participants stratified by erythrocyte DHA enrichment (A) ≥2% DHA enrichment (between baseline and end of study) or (B) <2% erythrocyte DHA enrichment.

after excluding the 2 participants who admitted to being protocol violators. With these 2 participants excluded, there was improvement in the effect of DHA+EPA treatment to decrease liver fat percentage (−4.2%; $\beta = -4.2$; 95% CI: −8.6, 0.1; $P = 0.057$).

The omega-3 index is the sum of DHA and EPA in erythrocyte membranes and is expressed as a percentage of total erythrocyte fatty acids.[29] We anticipated an erythrocyte sum of DHA and EPA (omega-3 index)[29] of between 4 and 5 at baseline (which is associated with an intermediate level of cardiovascular risk [CVR]) and found this to be the case. Furthermore, we considered that a 0.7% increase in EPA and a 2% increase in DHA would be sufficient to improve the index to a value proposed to result in significantly decreased CVR.[29]

We did not specifically select participants with high liver fat % at baseline, but patients with high liver fat percentage would likely derive most benefit from achieving good DHA enrichment, because we show that, for a reasonably large (6%) enrichment in DHA, there was a $(6 \times 3.3\%) = \sim 20\%$ decrease in liver fat percentage. Consequently, for individuals with >80% liver fat, such an absolute reduction in liver fat would be considerable. This benefit, and greater, was noted in many of the individuals treated with DHA+EPA.

We found no suggestion of benefit of DHA+EPA treatment on the two liver fibrosis scores that might have indicated an effect of treatment in NASH, but we suggest that treatment with DHA+EPA needs to be tested for a longer period than 18 months if any improvement in liver fibrosis is secondary to improvements in liver fat. Importantly, we did not observe any deterioration in either fibrosis score during the period of the study.

Very recently, Dasarathy et al. investigated the effects of omega-3 fatty acids in 37 patients with diabetes and NAFLD.[30] These investigators found no effect of omega-3 fatty acids on liver fat, but there was no estimation of tissue enrichment of omega-3 fatty acids in this trial. By contrast, Nobili et al. tested the effect of 18 months of treatment with DHA in children with NAFLD. Liver biopsy was undertaken before and after treatment. In this study, the investigators found that DHA improved hepatic steatosis, ballooning, and inflammation.[31]

Previous studies have investigated the effect of omega-3 fatty acids in NAFLD.[18,32-34] These studies differed from ours with regard to (1) duration of the treatment, (2) dosage of omega-3 fatty acids, (3) composition of the omega-3 fatty acid treatment, and (4) lack of testing for both adherence to the omega-3 intervention and for contamination with omega-3 fatty acids obtained for other readily available sources, using DHA and EPA measurements. Notably, the limitations of these studies were the nonblinding of participants and investigators, the lack of a placebo for the control group, the semiquantitative measures for assessing

changes in liver fat percentage, and the short periods of the intervention.

We selected the highest licensed dose of purified DHA+EPA, because 4 g/day of Omacor has been shown to be effective in hypertriglyceridemia.[17] By using such a pharmaceutical-grade preparation of omega-3 fatty acids, we also avoided the fat-soluble vitamins, A and D, contained within fish oil preparations.

The main limitation of our study is the small sample size. However, ours was a proof-of-concept study to test efficacy of the DHA+EPA intervention and DHA and EPA enrichment to decrease liver fat percentage and liver fibrosis scores. We tested whether the intervention changed both liver fibrosis scores, analyzing each of the fibrosis score as continuous variables, and did not attempt to treat the variables as categorical variables to define more-severe forms of liver fibrosis.

A priori, we set an alcohol threshold to exclude harmful alcohol intake, which, we acknowledge, is higher than the usual threshold to define NAFLD. However, only 1 man and 1 woman had previously consumed alcohol above UK Governmental Guidelines (21 and 14 units of alcohol per week for men and women, respectively) preceding enrollment. Exclusion of these 2 subjects did not affect the results (data not shown), and inclusion of baseline alcohol consumption as a continuous exposure variable in the regression model shown in Table 3 did not affect the association between DHA and liver fat percentage. Furthermore, in this model, alcohol intake was not associated with liver fat percentage ($\beta = 0.005$; 95% CI: -0.304, 0.314; $P = 0.98$). However, we documented alcohol consumption only at baseline and at end of study; therefore, there is the possibility that, during the study, alcohol consumption may have changed. The issue of histological monitoring for NAFLD in this trial is discussed in detail in the design and rationale of the study article.[19] Although many participants who were recruited had a baseline diagnostic liver biopsy, a further biopsy was not feasible in this trial at the end of the study (see previous work[19]). That said, MRS is currently considered the noninvasive gold-standard technique for assessing liver fat percentage and has excellent reproducibility and sensitivity,[35,36] with a coefficient of variance of only 8%,[35] and liver fat signals of only 0.2% are clearly evident above the noise level.[36] Furthermore, liver biopsy is invasive, expensive, and subject to sampling variability, and many investigators currently consider it a high-risk procedure that is unacceptable as a research test for monitoring NAFLD. Also, liver biopsy evaluates only a tiny portion (0.05 cm^3) of the liver (800-1,000 cm^3), and NAFLD is often a patchy disease.

In conclusion, erythrocyte DHA enrichment with DHA+EPA treatment is linearly associated with decreased mean liver fat percentage, calculated from liver fat percentage in three discrete liver regions, in patients with NAFLD. These data suggest that substantial decreases in liver fat percentage can be achieved with high levels of erythrocyte DHA enrichment in patients with NAFLD.

Putting Research in Context. The study design and protocol of the WELCOME study have previously been published (Design and rationale of the WELCOME trial: a randomised, placebo controlled study to test the efficacy of purified long chain omega-3 fatty treatment in non-alcoholic fatty liver disease. Contemp Clin Trials 2014;37:301-311.).

PubMed was searched for all English-language papers mentioning omega-3 fatty acids, fatty liver, and nonalcoholic fatty liver disease. A recent systematic review of omega-3 fatty acid supplementation and nonalcoholic fatty liver disease was published that has been cited

(Parker HM, Johnson NA, Burdon CA, Cohn JS, O'Connor HT, George J. Omega-3 supplementation and non-alcoholic fatty liver disease: a systematic review and meta-analysis. J Hepatol 2012;56:944-951.).

Panel Explaining "How Authors Arrived at Bottom-Line Message." One hundred and three patients with NAFLD were randomized to Omacor containing two purified omega-3 fatty acids (EPA+DHA; n = 51) or placebo (containing, principally, olive oil; n = 52) for a maximum of 18 months and a minimum of 15 months to test the effects of the intervention to decrease liver fat percentage and improve two histologically validated liver fibrosis biomarker scores. Because trials of nutritional supplements that are readily available from health food shops are susceptible to contamination effects in the placebo arm of the trial, we tested for potential contamination in the placebo group by measuring erythrocyte percentage DHA and EPA enrichment by GC. A similar analysis in the DHA+EPA group allowed us to test adherence to the DHA+EPA intervention in the Omacor group. We undertook ITT analysis and secondary analyses with multivariable linear regression modeling to test the effects of the intervention. These analyses included ITT analyses to test the effects of (1) DHA+EPA treatment (Omacor) and (2) erythrocyte percentage DHA and EPA enrichment (secondary analyses).

In the ITT analysis, there was a trend toward improvement in liver fat percentage with DHA+EPA treatment (−3.64; 95% CI: −8.0, 0.8; $P = 0.1$). However, importantly, our erythrocyte percentage DHA and EPA enrichment data showed that there was evidence of considerable contamination with high levels of erythrocyte percentage DHA and EPA enrichment in the placebo group. Also, there was evidence of poor adherence to DHA+EPA intervention in the Omacor group with low levels of erythrocyte percentage DHA and EPA enrichment. In secondary ITT analyses, we showed that each 1% DHA enrichment was associated with a 1.7% ($\beta = -1.7$; 95% CI: −2.9, −0.5; $P = 0.007$) decrease in liver fat percentage (adjusting for all measured confounders). We showed no improvement in both liver fibrosis biomarker scores. Further adjustment for change in physical activity during the trial (available in n = 82 participants showed that each 1% DHA enrichment was associated with a 3.3% decrease in liver fat percentage ($\beta = -3.3$; 95% CI: −4.8, −1.8; $P < 0.0001$). Thus, for a reasonably large (6%) enrichment in DHA, there was a ∼20% decrease in liver fat percentage. For individuals with >80% liver fat, such an absolute reduction in liver fat would be considerable. This benefit, and greater, was noted in many of the individuals randomized to the DHA+EPA intervention.

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Appendix

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