Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 58 (2009) 1753 – 1761
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Addition of *n*-3 fatty acids to a 4-hour lipid infusion does not affect insulin sensitivity, insulin secretion, or markers of oxidative stress in subjects with type 2 diabetes mellitus

Ingrid L. Mostad[a,b,*], Kristian S. Bjerve[c], Samar Basu[d], Pauline Sutton[e], Keith N. Frayn[e], Valdemar Grill[b,f]

[a]*Division of Clinical Nutrition, Department of Clinical Service, St. Olavs Hospital, Trondheim, Norway*
[b]*Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway*
[c]*Department of Medical Biochemistry, St. Olavs Hospital, Trondheim, Norway*
[d]*Department of Public Health and Caring Sciences, Faculty of Medicine, Oxidative Stress and Inflammation, Uppsala University, Uppsala Science Park, Uppsala, Sweden*
[e]*Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK*
[f]*Division of Endocrinology, Department of Medicine, St. Olavs Hospital, Trondheim, Norway*

Received 5 April 2009; accepted 16 June 2009

Abstract

Fatty acids (FA) can impair glucose metabolism to a varying degree depending on time of exposure and also of type of FA. Here we tested for acute effects of marine *n*-3 FA on insulin sensitivity, insulin secretion, energy metabolism, and oxidative stress. This was a randomized, double-blind, crossover study in 11 subjects with type 2 diabetes mellitus. A 4-hour lipid infusion (Intralipid [Fresenius Kabi, Halden, Norway], total of 384 mL) was compared with a similar lipid infusion partly replaced by Omegaven (Fresenius Kabi) that contributed a median of 0.1 g fish oil per kilogram body weight, amounting to 0.04 g/kg of marine *n*-3 FA. Insulin sensitivity was assessed by isoglycemic hyperinsulinemic clamps; insulin secretion (measured after the clamps), by C-peptide glucagon tests; and energy metabolism, by indirect calorimetry. Infusion of Omegaven increased the proportion of *n*-3 FA in plasma nonesterified fatty acids (NEFA) compared with Intralipid alone (20:5*n*-3: median, 1.5% [interquartile range, 0.6%] vs −0.2% [0.2%], $P = .001$; 22:6*n*-3: 0.8% [0.4%] vs −0.7% [0.2%], $P = .001$). However, glucose utilization was not affected; neither was insulin secretion or total energy production ($P = .966$, .210, and .423, respectively, for the differences between the lipid clamps). Omegaven tended to lower oxidation of fat ($P = .062$) compared with Intralipid only, correlating with the rise in individual *n*-3 NEFA ($r = 0.627$, $P = .039$). The effects of clamping on phospholipid FA composition, leptin, adiponectin, or F_2-isoprostane concentrations were not affected by Omegaven. Enrichment of NEFA with *n*-3 FA during a 4-hour infusion of Intralipid failed to affect insulin sensitivity, insulin secretion, or markers of oxidative stress in subjects with type 2 diabetes mellitus.

1. Introduction

Evidence indicates that *n*-3 fatty acids (FA) exert several beneficial effects. For example, *n*-3 FA of marine origin may protect against cardiovascular disease [1] and modulate immune processes [2]. Consequently, an increased intake of dietary marine *n*-3 FA has been widely recommended [3,4] also in diabetes [5] and has been proposed as part of intravenous nutrition [2].

However, the effects of marine *n*-3 FA on important metabolic parameters have not been completely elucidated. We found that a high intake of marine *n*-3 FA for 9 weeks [6] reduced insulin sensitivity and altered proportion of carbohydrate vs fat oxidation in subjects with type 2 diabetes mellitus. These results question the use of high doses of *n*-3 supplements in type 2 diabetes mellitus. On the other hand, a long-term study reported that *n*-3 FA reduce peroxidation of lipids (reducing oxidative stress) [7].

The work was done at St. Olavs Hospital, Trondheim, Norway.
The local ethics committee approved the study protocol. All participants gave written informed consent.

* Corresponding author. Division of Clinical Nutrition, Department of Clinical Service, St. Olavs Hospital, N-7006 Trondheim, Norway. Tel.: +47 72571310; fax: +47 72571203.
E-mail address: ingrid.mostad@stolav.no (I.L. Mostad).

0026-0495/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.06.003

It is not known if effects seen in long-term studies can be reproduced by intravenous administration of *n*-3 FA and/or whether short-term intravenous administration has other effects. Lipid infusions induce insulin resistance [8], both in obese men [9] and in type 2 diabetes mellitus [10,11], and reduce insulin secretion in subjects with impaired glucose tolerance [8]. However, few studies have investigated if changing the FA composition of the lipid infusion (usually Intralipid) could affect the response on insulin sensitivity, insulin secretion, or other metabolic variables. To our knowledge, effects of adding marine *n*-3 FA to a standard infusate of lipids have not been tested in subjects with type 2 diabetes mellitus.

Omegaven is an *n*-3 FA emulsion of fish oil that can be given together with Intralipid. We designed a study in subjects with type 2 diabetes mellitus to compare acute effects of infusions of Intralipid with and without added Omegaven. The primary outcome was an effect on insulin sensitivity. Secondary outcomes were effects on insulin secretion, energy metabolism, and markers of oxidative stress. Results on these variables were interpreted in relation to measurements of the distribution of *n*-3 and other FA in plasma and in phospholipids. We hypothesized that supplementing marine *n*-3 FA (Omegaven) to a standard lipid infusion (Intralipid) would increase lipid-induced insulin resistance (ie, reduce glucose uptake) and reduce oxidative stress (F_2-isoprostanes) in a setting of increased or unaltered insulin secretion.

2. Methods

2.1. Participants

Eleven subjects (7 men, 4 women) between 30 and 75 years old were recruited by advertisement in the local newspaper. *Type 2 diabetes mellitus* was defined by clinical criteria and by absence of antibodies to glutamic acid decarboxylase. Eight of the participants used standard antidiabetic treatment as follows: 5 used metformin; 2, metformin and glibenclamide; and 1, metformin and glimepiride. Three participants received statins; and 4, antihypertensive treatment. Medications were not changed during the study. Participants had either never used supplement with fish oil or marine *n*-3 FA or discontinued such supplement for at least 6 months before baseline. Exclusion criteria were insulin treatment; hypertriacylglycerolemia (>2.2 mmol/L); proliferative retinopathy; pregnancy or lactation; allergy to soy bean, fish, or egg; smoking; alcoholism; or congestive heart failure or other serious diseases affecting the ability of the subject to participate. Subjects were screened on the basis of their medical history and a physical examination. We included only subjects with veins evaluated as appropriate for the study.

2.2. Ethics and registration

The local ethics committee approved the study protocol. All participants gave written informed consent. The study was registered in ClinicalTrials.gov (NCT00829569, www.clinicaltrials.gov).

2.3. General design

This was a 2-week, double-blind, crossover study. After screening, the included subjects were investigated on 3 or 4 occasions. All subjects underwent 2 Intralipid (Fresenius Kabi, Halden, Norway) infusions as isoglycemic hyperinsulinemic clamps [12], one with and one without the addition of Omegaven (Fresenius Kabi, Uppsala, Sweden), named *Omegaven respective Intralipid clamps*. In addition, 4 subjects (3 men, 1 woman) underwent an infusion of insulin and glucose to act as control clamps (named *control clamps*). The first day of investigation (day 1) included measurements of energy metabolism and body composition. The first lipid infusion took place 3 to 4 days after day 1. The second lipid infusion was carried out 2 weeks later. The order of the 2 types of infusions was randomized. Blinding was ensured by a pharmaceutical chemist preparing each lipid emulsion. A C-peptide glucagon test [13] was performed subsequent to infusions. Indirect calorimetry [14] was measured as fasting on day 1 and during each infusion.

2.4. Clinical investigations

On each day of investigation, the subjects reported to the hospital between 7:30 and 9:00 AM after fasting for 12 hours and abstaining from alcohol for 24 hours.

2.4.1. Day 1

Height (in centimeters) was measured to 1 decimal. Weight (in kilograms) was measured to 1 decimal with subjects wearing indoor clothing and without shoes. Waist circumference was measured with the subject in underwear, with shoulders relaxed and arms hanging alongside. Indirect calorimetry and body compositions were then measured (details below).

2.4.2. Isoglycemic hyperinsulinemic clamps

Before reporting to the hospital, subjects collected urine for the last 9 hours before arising in the morning. They were told not to engage in strenuous exercise on the day before any of the clamps. After measuring body weight, a cannula (Venflon; Viggo, Helsingborg, Sweden) was inserted into a vein dorsally on one hand and connected to an extension line for blood sampling. During the isoglycemic clamp [12], the arm used for blood sampling was warmed by different means (such as holding plastic gloves filled with warm water) to "arterialize" the samples. A second cannula for infusions was inserted contralaterally into a hand vein or antecubital vein. It was used for infusions of glucose and insulin in the control clamp and for infusions of glucose, insulin, and lipid emulsion in the Intralipid and Omegaven clamps. For the lipid infusions, an extension line with one 2-way and one 3-way stopcocks was connected to this cannula, with 1 and 2 back-check valves Luer lock, respectively. Infusions were started after securing fasting (0 minute) blood samples. In

clamps where Omegaven was added, 100 mL of the total volume of 20% Intralipid was removed and replaced by 100 mL 10% Omegaven as recommended by the producer of Omegaven. Heparin (0.4 U kg^{-1} min^{-1}; Leo, Ballerup, Denmark) was added to the lipid emulsions (to activate lipoprotein lipase, thereby increasing circulating nonesterified fatty acids [NEFA]). The lipid infusion rate was initially 50 mL/h the first 10 minutes. It was then increased by 10 mL/h every fifth minute until the maximal rate of 100 mL/h was reached at 30 minutes. This infusion rate was then maintained for another 210 minutes. Details of the composition of Intralipid and Intralipid + Omegaven are given in Table 1. The insulin infusion contained 3.0 mL 100 U/mL insulin Actrapid (Novo Nordisk, Bagsværd, Denmark) in 500 mL isotonic saline (ie, 0.6 U/mL) to which 2 mL of the subject's blood was added. The insulin infusion rate was 80 mU min^{-1} m^{-2} for 4 hours. Glucose (20% in the control clamps, 12% in the Intralipid and Omegaven clamps) was infused through the same 3-way stopcock as the insulin infusion, but at a variable rate. Samples for analyzing blood glucose were drawn every 5 to 10 minutes. The infusion rate of glucose was manually adjusted to maintain the concentration of fasting glucose measured at minute 0 of the first clamp for each subject. During the clamps, indirect calorimetry was measured during a 30-minute period ("Details of indirect calorimetry").

Blood samples for analyzing insulin was drawn at minute 0, 30, 60, 90, 120, 180, and 240; and for triacylglycerols (TAG), at minute 0, 90, 180, and 240 of the clamps. For all other variables, blood samples were drawn at minute 0 and 240.

Night-time (9 hours) urine was voided before each clamp. Urine was again voided after each clamp.

Table 1
Composition details[a] of Intralipid, Omegaven, and Intralipid with added Omegaven

	Intralipid[b] (g/L)	Omegaven[c] (g/L)	Intralipid + Omegaven[d] (g/L)
Soybean oil	200	–	160
Fish oil	–	100	20
14:0	Traces	4.7	0.9
16:0	19.7	10.1	17.8
18:0	7.9	1.9	6.7
16:1	Traces	8.5	1.7
18:1	43.4	13.9	37.5
20:1	Traces	1.1	0.2
22:1	–	0.4	0.1
18:2*n*-6	109	3.1	87.8
20:4*n*-6	–	2.6	0.5
18:3*n*-3	15.8	1.2	12.8
18:4*n*-3	–	3.4	0.7
20:5*n*-3	–	21.9	4.4
22:5*n*-3	–	2.6	0.5
22:6*n*-3	–	16.0	3.2
Tocopherols	190[e]	185[e]	189[e]
α-Tocopherol	12[e]	185[e]	47[e]
β-Tocopherol	3[e]	–	2[e]
γ-Tocopherol	122[e]	–	98[e]
δ-Tocopherol	44[e]	–	35[e]
Other tocopherols	9[e]	–	7[e]
TAG	198	100	178
Glycerol	21.9	24.3	22.4
NEFA	0.6[f]	1.4[f]	0.8[f]
Phospholipids	11.8	12.0	11.8
Density	987	998	989
Energy	8.4[g]	4.7[g]	7.6[g]

[a] Details as to respective certificates of analysis and declaration of contents.
[b] Batch 1015082 of Intralipid 20% in Excel bag, 500 mL (Fresenius Kabi, Halden, Norway).
[c] Batch PE1622 from 33836031 Omegaven 10∗100 mL (Fresenius Kabi, Uppsala, Sweden).
[d] 80% Intralipid + 20% Omegaven.
[e] In milligrams per liter.
[f] In millimoles per liter.
[g] In megajoules per liter.

2.4.3. C-peptide glucagon tests

About 15 minutes after ending infusions of each clamp, samples were drawn for analyzing glucagon, C-peptide, and insulin. Afterward, 1 mg glucagon was injected intravenously. Samples taken 6 minutes later were analyzed for C-peptide and insulin. The increment from 0 to 6 minutes of C-peptide was used as the variable of stimulated insulin secretion [13].

2.4.4. Details of indirect calorimetry

In the fasting mode at day 1, indirect calorimetry (Deltatrac II metabolic monitor; Datex-Ohmeda Division, Helsinki, Finland) was performed [14,15] for 30 minutes after 20 minutes of relaxation, with the subject horizontal. Oxygen consumption (milliliters of O_2 per minute) and carbon dioxide production (milliliters of CO_2 per minute) were measured with subjects breathing in a ventilated canopy, which was coupled to the Deltatrac gas exchange monitor. Calculations of results included resting energy production rate (EPR, kilojoules per 24 hours); the respiratory quotient (RQ), that is, the ratio between produced CO_2 in milliliters per minute (VCO_2) and consumed O_2 in milliliters per minute (VO_2); the percentage distribution of carbohydrate, fat, and protein as oxidative fuels; and the nonprotein RQ (ie, RQ adjusted for the protein oxidation) based on a default value of nitrogen loss in urine of 13 g/24 h. No urine for individual nitrogen loss calculations was voided, as on clamp days (see next paragraph).

During each clamp, indirect calorimetry was performed in an identical manner for 30 minutes starting at around minute 160. We calculated nonprotein RQ manually for all subjects using each subject's urinary loss of nitrogen based on the output of urea and creatinine from the urine collected during the clamps (nonprotein RQ = $[VCO_2 - 4.89\ U_N]/[VO_2 - 6.04\ U_N]$, where U_N is the urinary loss of nitrogen (grams per 24 hours) [15]. U_N was extrapolated from actual individual collection time (5-8 hours) to 24 hours. This is because the default value is less accurate during clamp conditions than during fasting. Using the individual value of grams protein

oxidation, we were able to calculate the percentage of total energy for all fuels.

2.4.5. Details of body composition

Lean body mass (LBM) was assessed by dual-energy x-ray absorptiometry scanning (Hologic QDR 4500 A; Hologic, Bedford, MA). Scans were reviewed and analyzed using Hologic software 9.02.b (1996).

2.4.6. Details of dietary recording

Diet during the day before each clamp was recorded prospectively by household measures. The subjects recorded portions, which were afterward estimated to grams, related to standard portions, and analyzed using the diet calculation software "Mat på data" (version 4.2) to give the intake of energy and nutrients. The software system was developed by the National Association of Diet and Health and was based on the official Norwegian Food Table [16]. After the first clamp day, each subject got a copy of his or her dietary recording of the day before. The subjects were then instructed to try to eat similarly the day before the succeeding clamps to obtain comparable metabolic conditions before each of the clamps. As a corollary, the diet was recorded the day before each clamp.

2.4.7. Assays

Blood and urinary glucose were determined with a glucose analyzer (YSI; Yellow Springs Instrument, Yellow springs, OH) during the clamps. Glycosylated hemoglobin (HbA_{1c}) was measured in EDTA whole blood. Total NEFA were measured in EDTA plasma. Samples were centrifuged immediately (1500*g*, 15 minutes, 20°C) before freezing at −80°C pending later analyses by an enzymatic colormetric method (NEFA-C kit; Wako Pure Chemical Industries, Osaka, Japan) by the Department of Medical Biochemistry, St Olavs Hospital, Trondheim. To verify total NEFA results, a second set of samples was analyzed in a second laboratory (Clinical Nutrition and Metabolism, Uppsala University, Uppsala) by the same method. Glucose, TAG, total cholesterol, and high-density lipoprotein cholesterol were measured by standard laboratory techniques in serum samples obtained by allowing blood samples to clot at room temperature for 1 to 2 hours and centrifuging at 2000*g* for 12 minutes at 20°C. Urea and creatinine were measured in urine. Low-density lipoprotein cholesterol was calculated by the formula of Friedewald et al [17].

2.4.8. FA composition

To determine FA composition, total lipids were extracted from plasma and methyl esters prepared from NEFA fractions as previously described [18]. Fatty acid compositions in the fractions were determined by gas chromatography [19]. Plasma phospholipid fatty acids (PL-FA) were analyzed as previously described [20].

2.4.9. Hormones

Glucagon was measured in serum to which aprotinin (Trasylol; Bayer, Leverkusen, Germany) was added as a preservative. Samples were centrifuged (1500*g*, 20 minutes, 4°C) before freezing at −80°C. Serum insulin, C-peptide, leptin, and adiponectin were measured using human-specific radioimmunoassay kits (Linco Research, St Charles, MO).

2.4.10. Oxidative stress

As a marker of oxidative stress, free 8-*iso*-prostaglandin $F_{2\alpha}$ (8-*iso*-$PGF_{2\alpha}$), a major F_2-isoprostane, was analyzed in EDTA plasma and urine as previously described [21]. The detection limit of the assay was 8 pg/mL.

2.5. Statistic analysis

Results in text and tables are given as median values and the variability as the interquartile range [IQR; the distance between the 75th and 25th percentile values]. In the figures, result values are given as mean and SEM. Results of the differences on variables from fasting, after clamps, or during clamps (absolute values and incremental effects) for the Intralipid and Omegaven clamps were analyzed by the nonparametric test paired Wilcoxon signed rank test, exact significance (2-tailed). Within-clamp analyses of the change over time of concentrations of TAG and insulin were performed by repeated-measures analysis of variance separately within the Intralipid respective Omegaven clamp after assumption of normality of these variables was checked by visual inspection of normal Q-Q plots. Spearman correlation coefficients (r) were used to evaluate bivariate correlations. A P value < .05 (2-sided) was considered significant. Statistical analyses were performed with SPSS version 15.0 (SPSS, Chicago, IL, 2008).

The last 40 minutes of each clamp were used for the calculations of glucose utilization, adjusted for urinary loss of glucose. Insulin sensitivity was calculated as the amount of glucose (milligrams per minute per kilogram LBM) needed to maintain the fasting glucose concentration of the first clamp performed in each individual. Power analysis indicated that 18 patients would be needed to detect a 20% difference in glucose utilization between the 2 lipid infusions at the .05 level of significance.

3. Results

3.1. Baseline characteristics

All subjects completed the study. Age, duration of diabetes, blood pressure, manual anthropometric variables, fasting glucose, HbA_{1c}, lipids, and hormones at baseline are given in Table 2.

3.2. Intervention

Neither weight nor the fasting variables shown in Table 2 differed between the subjects at the 2 lipid clamp days, except for fasting C-peptide (0.8 [0.5] vs 0.9 [0.6] mmol/L in the Intralipid vs Omegaven clamps, $P = .007$). The concentration of C-peptide increased and that of glucagon decreased after 240 minutes in both lipid clamps ($P < .05$, data not shown),

Table 2
Fasting variables at baseline[a]

Variables	Day of baseline (n = 11; 7 men, 4 women)	
	Median	IQR
Age (y)	57	14
Duration of diabetes (y)	4	6
Weight (kg)	79.0	21.7
BMI (kg/m^2)	28.5	3.5
LBM (kg)	58.2	21.4
Waist[b] (cm)	101	16
Waist-hip ratio[c]	1.01	0.09
HbA_{1c} (%)	7.1	2.1
Serum glucose[d] (mmol/L)	9.2	3.1
Blood glucose[e] (mmol/L)	7.8	3.2
Total cholesterol (mmol/L)	4.9	1.6
LDL cholesterol[f] (mmol/L)	3.0	1.6
HDL cholesterol (mmol/L)	1.22	0.46
TAG (mmol/L)	1.1	0.9
Insulin (pmol/L)	86	87
C-peptide (nmol/L)	0.7	0.5
Glucagon (pmol/L)	89	43
Systolic blood pressure (mm Hg)	130	20
Diastolic blood pressure (mm Hg)	78	20

BMI indicates body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

[a] Baseline signifies the first day participants met for any of the clamps.
[b] Waist: men (n = 7), 105 (18) cm; women (n = 4), 99 (10) cm.
[c] Waist-hip ratio: men (n = 7), 1.03 (0.08); women (n = 4), 0.99 (0.08).
[d] Analyzed by the hospital laboratory.
[e] Analyzed by the glucose analyzer before the clamps.
[f] Calculated by the formula of Friedewald et al [17].

with no differences between the clamps. There were no differences in the diet recorded the day before each lipid clamp (Supplementary Material, Supplemental Table 1).

The volumes of lipid emulsion infused during 4 hours were the same in both lipid clamps, that is, 384 [5] and 384 [3] mL, respectively. Replacement of 20% of the Intralipid volume by Omegaven reduced the content of 18:2*n*-6 to 34 [0] vs 42 [1] g in the Intralipid-only infusion ($P = .003$) and also the content of 18:3*n*-3 (to 4.9 [0] vs 6.1 [0] g, $P = .003$). The replacement added 3.1 (0) g long-chain marine *n*-3 FA, ie, 1.7 [0] g 20:5*n*-3 (eicosapentaenoic acid [EPA]), 0.2 [0] g 22:5*n*-3 (docosapentaenoic acid), and 1.2 g [0] g 22:6*n*-3 (docosahexaenoic acid [DHA]). As a consequence, the *n*-6/*n*-3 ratio was markedly lower in the lipid infusion with Omegaven compared with Intralipid only (4.0 vs 6.9). The fish oil contribution was 0.1 [0] g/kg body weight and amounted to 0.04 g/kg of marine *n*-3 FA. The energy supplied during the Omegaven clamp was 9% less than that in the Intralipid clamp (2929 [21] vs 3213 [42] kJ), and the amount of TAG given was 8% less (0.22 [0.06] vs 0.24 [0.07] g kg^{-1} h^{-1}) (P =.002 for both comparisons).

3.3. Hyperinsulinemic isoglycemic clamps

The fasting insulin concentrations on the day of the Intralipid clamps (78 [66] pmol/L) did not differ from concentrations on the day of the Omegaven clamps (72 [97] pmol/L) ($P = .859$). Neither did the glucose concentrations differ (7.8 [1.9] vs 8.6 [4.4] mmol/L, $P = .102$). The fasting insulin and glucose concentrations were comparable in the control clamps (79 [90] pmol/L insulin and 8.8 [6.8] mmol/L glucose). Hyperinsulinemia (Fig. 1A) and isoglycemia (Fig. 1B) were achieved in lipid, as well as control clamps.

3.4. Plasma NEFA

Fasting concentrations of total NEFA were comparable on the Intralipid and Omegaven clamp days (0.54 [0.24] and 0.52 [0.36] mmol/L, respectively). Plasma NEFA composition changed markedly from 0 to 240 minutes of Intralipid infusion, as saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) decreased and *n*-6 and *n*-3 FA increased (Supplementary Material, Supplemental Table 2) ($P < .001$ for all changes). Replacement of 20% of the Intralipid volume by Omegaven brought significant changes to MUFA (a blunted decrease), *n*-6 FA (a blunted increase), and *n*-3 FA (a marked increase) (Fig. 2A and Supplemental Table 2). The individual *n*-3 FA EPA (20:5) and DHA (22:6) increased during the Omegaven clamp, in contrast to decrements of the same FA by infusion with Intralipid alone

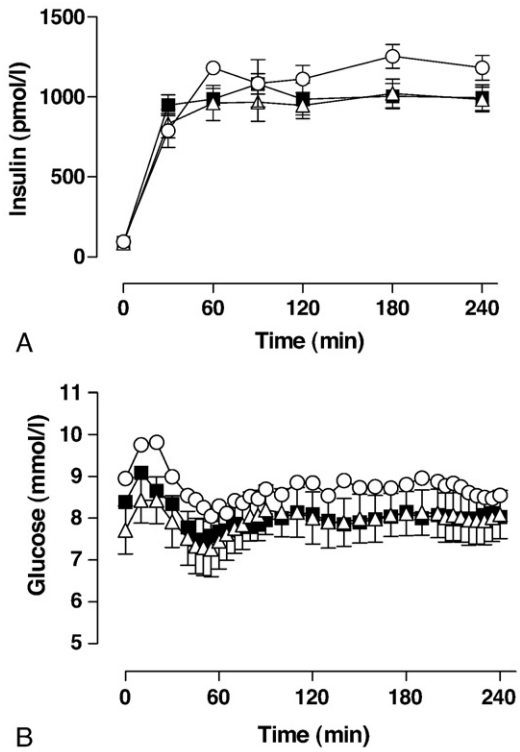

Fig. 1. Hyperinsulinemic isoglycemic clamps. A, Concentrations of insulin during control (no lipid infusion, open circles, n = 4), Intralipid (open triangles, n = 11), and Omegaven clamps (filled squares, n = 11). B, Concentrations of glucose during control (no lipid infusion, open circles, n = 4), Intralipid (open triangles, n = 11), and Omegaven clamps (filled squares, n = 11). Results are means ± SEM. Error bars for the control clamps have been omitted for typographical reasons.

(Fig. 2B). In addition, the *n*-6/*n*-3 ratio was significantly reduced after 240-minute infusion of Omegaven vs Intralipid alone (Supplemental Table 2).

3.5. Plasma PL-FA

Fasting concentrations of total PL-FA were comparable on both lipid clamp days and increased similarly during the clamps (Supplemental Table 2). Notably, Omegaven failed to increase the concentration of any of the *n*-3 FA (Supplemental Table 2).

3.6. Triacylglycerols

The fasting concentrations of TAG were 1.4 [0.9] and 1.3 [1.1] mmol/L in the Intralipid and Omegaven clamps, respectively. The concentrations increased significantly and successively throughout both clamps when measured regularly, with no differences between the clamps (data not shown). At 240 minutes, the concentration was 5.6 [5.3] mmol/L in the Intralipid clamp, compared with 6.3 [5.0] mmol/L in the Omegaven clamp ($P = .919$).

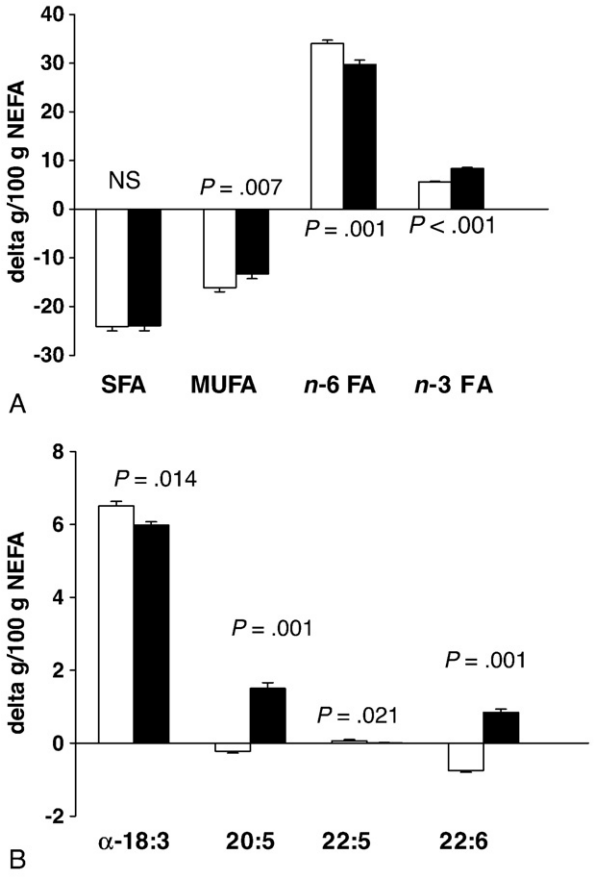

Fig. 2. Changes in plasma NEFA during lipid clamps. A, Depicted are changes in NEFA categories from 0 to 240 minutes of clamps. Open bars represent results with Intralipid; filled bars, with Omegaven. *P* values are for differences of Omegaven vs Intralipid. B, Depicted are changes in individual *n*-3 FA from 0 to 240 minutes of clamps. Open bars represent results with Intralipid; filled bars, with Omegaven. Results are means ± SEM. *P* values are for differences of Omegaven vs Intralipid.

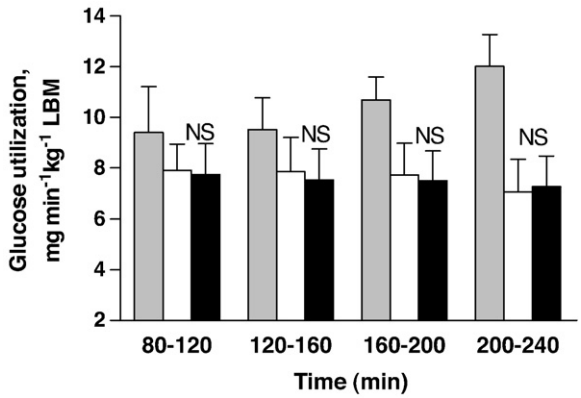

Fig. 3. Glucose utilization. Glucose utilization was calculated as milligrams per minute per kilogram LBM during successive 40-minute periods of clamping. Gray bars indicate control (no lipid infusion, n = 4); open bars, Intralipid (n = 11); and filled bars, Omegaven (n = 11) clamps. Results are means ± SEM. *P* values (NS) are for differences of Omegaven vs Intralipid.

3.7. Insulin sensitivity

The Intralipid and Omegaven clamps similarly decreased insulin-mediated glucose utilization compared with the control clamp (Fig. 3). Glucose utilization during the last 40 minutes of the Intralipid and Omegaven clamps was 6.0 [3.7] and 6.3 [2.9] mg min^{-1} kg^{-1} LBM, respectively (n = 11, $P = .966$), compared with 11.7 [4.8] mg min^{-1} kg^{-1} LBM (n = 4) in the control clamp with glucose alone (Fig. 3).

3.8. Insulin secretion

The glucagon-stimulated C-peptide response was 2-fold increased after the Intralipid clamps compared with the control (glucose alone) clamps (Fig. 4). This enhancing

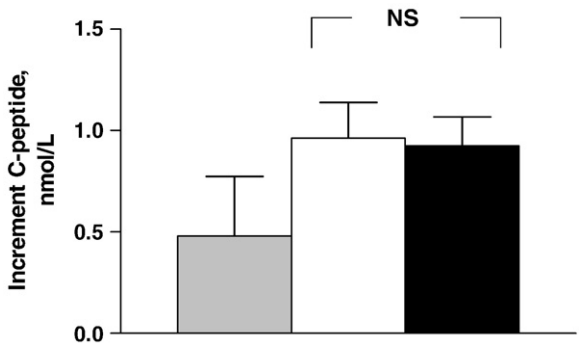

Fig. 4. Insulin secretion. Insulin secretion was calculated as 6-minute C-peptide responses to glucagon. Glucagon was injected 15 minutes after end of control (gray bars, n = 4), Intralipid (open bars, n = 11), and Omegaven (filled bars, n = 11) clamps. Results are means ± SEM. *P* values (NS) are for differences of Omegaven vs Intralipid.

Table 3
Energy metabolism

Variables	Fasting[a] (n = 11)		During the control clamps (n = 4)		During the Intralipid clamps (n = 11)		During the Omegaven clamps (n = 11)		P^b
	Median	IQR	Median	IQR	Median	IQR	Median	IQR	
EPR (kJ/24 h)	7014	2982	6741	2407	7728†	1823	7560†	1554	.423
EPR/kg LBM	122	8	118	13	134†	25	139†	21	.286
RQ	0.81	0.07	0.82	0.16	0.72†	0.04	0.74†	0.05	.167
Nonprotein[c] RQ	0.81	0.09	0.82	0.22	0.69†	0.06	0.73†	0.06	.131
Carbohydrate (% of energy)	28.0	28.5	32.1	56.2	-2.3†	17.1	8.1†	17.2	.110
Fat (% of energy)	52.5	19.4	52.1	48.4	89.1†	17.2	75.7†	15.5	.062
Protein (% of energy)	21.0	8.4	15.9	7.8	13.9†	4.4	16.2*	5.3	.790

EPR indicates energy production rate; LBM, lean body mass; RQ, respiratory quotient.
[a] Measured 3 to 4 days before the first clamp day of each individual subject.
[b] P for the difference between Intralipid and Omegaven clamps.
[c] Respiratory quotient adjusted for protein oxidation calculated from urinary loss of nitrogen in all clamp values. Default nitrogen values (13 g/24 h) were used in fasting.
* Significantly different from the fasting value, n = 11, $P < .05$.
† Significantly different from the fasting value, n = 11, $P < .01$.

effect was not changed by Omegaven (P = .210 for difference in response to Omegaven vs Intralipid alone).

3.9. Energy metabolism

The 2 types of lipid clamps increased EPR similarly (Table 3). Furthermore, both lipid clamps reduced RQ significantly compared with fasting; that is, there was a switch from carbohydrate to fat oxidation. Nonprotein RQ was similarly reduced in both clamps. There was a tendency (P = .062) for lower fat oxidation during the Omegaven clamp compared with the Intralipid clamp (Table 3). This effect correlated with the Omegaven-induced increase of 22:6*n*-3 FA in individual patients (r = 0.627, P = .039).

3.10. F_2-isoprostanes

Concentrations of 8-*iso*-$PGF_{2\alpha}$ in plasma increased markedly as a result of the Intralipid infusion (P = .033), with a similar increase when Omegaven was added (P = .003) and with no difference between the lipid clamps (P = .859, Fig. 5). The effects of lipid infusions were in contrast to the control clamps (glucose alone), which did not affect F_2-isoprostanes (Fig. 5).

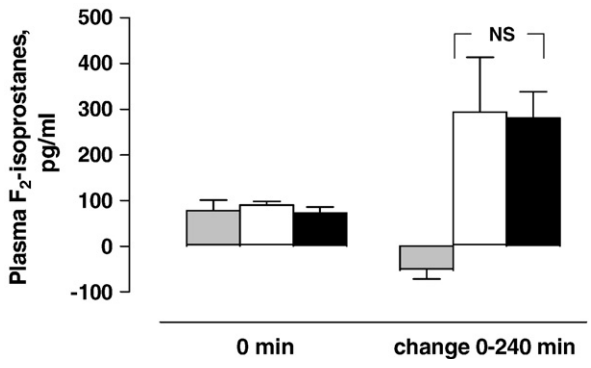

Fig. 5. Plasma F_2-isoprostanes. Depicted are changes in plasma F_2-isoprostanes (8-*iso*-$PGF_{2\alpha}$) from 0 to 240 minutes of clamps. Gray bars indicate control (no lipid infusion, n = 4); open bars, Intralipid (n = 11); and filled bars, Omegaven (n = 11) clamps. Results are means ± SEM. P values (NS) are for differences of Omegaven vs Intralipid.

The rise of plasma F_2-isoprostanes in individual subjects did not correlate with the increase in total NEFA (r = 0.082, P = .811 for the Intralipid clamps; r = 0.318, P = .340 for the Omegaven clamps).

3.11. Adipocyte hormones

Concentrations of leptin and adiponectin were not affected by any of the clamp conditions (data not shown).

4. Discussion

To our knowledge, this is the first study that examines acute effects (ie, by a 4-hour infusion) of marine *n*-3 FA on insulin sensitivity (the primary end point) and on insulin secretion, energy metabolism, and oxidative stress in subjects with type 2 diabetes mellitus. Our results fail to demonstrate effects on these variables, except for a possible effect on FA oxidation.

In the present study, we found, as others did [10,22,23], that a lipid infusion (Intralipid) reduced insulin sensitivity (glucose utilization). The novel finding is that the addition of marine *n-3* FA (Omegaven) did not attenuate this effect. Indeed, there was not even a tendency. Hence, a larger number of participants (desirable from a formal power analysis) would not have changed the conclusion of no effect. As to insulin secretion, we found a larger C-peptide response to glucagon after lipid infusions compared with control clamps without lipid infusion. This we attribute to the fact that short-term elevated NEFA are known to stimulate secretion [24]. However, insulin secretion was not affected by marine *n*-3 FA.

Because Omegaven markedly increased circulating non-esterified *n*-3 FA, it is unlikely that insufficient elevation of these FA explains the failure to affect insulin sensitivity and insulin secretion. Rather, insufficient time of exposure could explain the lack of insulin resistance effects that were previously seen in our long-term intervention study [6]. This notion is in line with the findings of Wendel et al [25] who found that Omegaven given for 5 days postoperatively to major surgery patients decreased glucose utilization.

As to plasma phospholipids, our results (Supplemental Table 2) demonstrate that 4-hour latency is insufficient time for an increase in the *n*-3 FA from the EPA- and DHA-containing TAG fraction of Omegaven. Our findings of delayed increase in plasma phospholipids are in line with a recent publication in humans in which ingestion of selected isotopically labeled FA failed to increase the total amount of any of the ingested FA in plasma phospholipids after 4 hours [26]. Interestingly, the same study showed that the incorporation of tracer FA into phospholipids was greatly enhanced after 7 hours (and by interpolation also after 4 hours). Hence, the process of incorporation into plasma phospholipids seems to start early, whereas the impact of a load of an individual fatty acid on the total amount of that fatty acid in phospholipids is not apparent until after a considerable time delay [26].

As expected from previous studies [27], we find that the lipid infusions markedly decreased RQ. Such an effect could reflect prompt replacement of carbohydrate as fuel for oxidation in muscle as reported in subjects without diabetes [28]. Albeit not significant, we found a tendency for lesser increase in fat oxidation with Omegaven; and the correlation with individual increments of the longest marine *n*-3 FA (22:6*n*-3) adds weight to this finding. We speculate that lesser oxidation of FA in the present setting could be related to the initial and passing decrease in RQ that was measured after 1 week of fish oil intervention in our previous study [6]. Perhaps, the enzymes responsible for oxidation by mitochondria and/or peroxisomes of *n*-3 FA are only induced after a significant time lag.

It should be mentioned that a published study in which *n*-3 FA were given during 4 days as part of total parenteral nutrition found no effects on glucose production, a tendency for lower glucose oxidation, and a normal lipid metabolism [29]. However, valid comparisons with our study are limited because the mentioned study [29] was performed in patients without diabetes who were critically ill.

As to lipid peroxidation, our results confirm, in a qualitative sense, that an Intralipid infusion increases plasma F_2-isoprostanes [30,31]. Notably, we observed a greater increase of F_2-isoprostanes in plasma than in previous studies. A similar increase was observed after Omegaven addition. Hence, we could not reproduce the decrease of F_2-isoprostanes reported after long-time supplementation of *n*-3 FA [7]. Given that the rise of isoprostanes is a marker of oxidative stress [32], our results indicate no acute influence of *n*-3 FA, which is different from that of other polyunsaturated fatty acids (PUFA) on oxidative stress, at least not in subjects with type 2 diabetes mellitus.

Because we replaced 20% Intralipid with 10% Omegaven, the amount of TAG given in the Omegaven clamps was 8% less than that in the Intralipid clamps. We do not believe that this has had a significant impact on our findings because the degree of triacylglycerolemia was the same in both situations. A slightly lower clearance of the *n*-3–enriched lipid infusion might have been masked by the present experimental conditions. It might be argued that the tendency for lower fat oxidation during the Omegaven compared with the Intralipid clamps reflects the smaller amount of fat infused. However, the effect on fat oxidation correlated with the concentration of the *n*-3 FA in individual experiments. This finding supports the notion of a specific metabolic action by the *n*-3 FA.

In summary, contrary to our hypotheses, acute enrichment of plasma with *n*-3 FA (Omegaven supplement) did not increase lipid-mediated insulin resistance; neither was oxidative stress reduced. An inhibitory effect on FA oxidation is possible. From a clinical perspective, replacement of some of the Intralipid volume by Omegaven would not, at least in the short term, induce any negative effects compared with Intralipid alone in subjects with type 2 diabetes mellitus.

Acknowledgment

The authors are grateful to the study participants and thank V Wist, S Salater, SL Lund, I Thyve, K Solem, M Mack, and E Sejby for technical assistance.

This study was funded by the aid of EXTRA funds from the Norwegian Foundation for Health and Rehabilitation, Novo Nordisk, and the Norwegian Diabetes Association.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2009.06.003.

References

[1] Psota TL, Gebauer SK, Kris-Etherton P. Dietary omega-3 fatty acid intake and cardiovascular risk. Am J Cardiol 2006;98:3i-18i.
[2] Furst P, Kuhn KS. Fish oil emulsions: what benefits can they bring? Clin Nutr 2000;19:7-14.
[3] Nordic Council of Ministers. Nordic nutrition recommendations: NNR 2004: integrating nutrition and physical activity. Copenhagen: Nordic Council of Ministers; 2005.
[4] Gebauer SK, Psota TL, Harris WS, Kris-Etherton PM. *n*-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. Am J Clin Nutr 2006;83(6 Suppl):1526S-35S.
[5] Mann JI, De Leeuw I, Hermansen K, Karamanos B, Karlstrom B, Katsilambros N, et al. Evidence-based nutritional approaches to the treatment and prevention of diabetes mellitus. Nutr Metab Cardiovasc Dis 2004;14:373-94.
[6] Mostad IL, Bjerve KS, Bjorgaas MR, Lydersen S, Grill V. Effects of *n*-3 fatty acids in subjects with type 2 diabetes: reduction of insulin

sensitivity and time-dependent alteration from carbohydrate to fat oxidation. Am J Clin Nutr 2006;84:540-50.
[7] Nalsen C, Vessby B, Berglund L, Uusitupa M, Hermansen K, Riccardi G, et al. Dietary (*n*-3) fatty acids reduce plasma F2-isoprostanes but not prostaglandin F2alpha in healthy humans. J Nutr 2006;136:1222-8.
[8] Storgaard H, Jensen CB, Vaag AA, Volund A, Madsbad S. Insulin secretion after short- and long-term low-grade free fatty acid infusion in men with increased risk of developing type 2 diabetes. Metabolism 2003;52:885-94.
[9] Leung N, Sakaue T, Carpentier A, Uffelman K, Giacca A, Lewis GF. Prolonged increase of plasma non-esterified fatty acids fully abolishes the stimulatory effect of 24 hours of moderate hyperglycaemia on insulin sensitivity and pancreatic beta-cell function in obese men. Diabetologia 2004;47:204-13.
[10] Boden G, Chen X. Effects of fat on glucose uptake and utilization in patients with non–insulin-dependent diabetes. J Clin Invest 1995;96: 1261-8.
[11] Boden G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 1997;46:3-10.
[12] Bergman RN, Finegood DT, Ader M. Assessment of insulin sensitivity in vivo. Endocr Rev 1985;6:45-86.
[13] Faber OK, Binder C. C-peptide response to glucagon. A test for the residual beta-cell function in diabetes mellitus. Diabetes 1977;26: 605-10.
[14] Ferrannini E. The theoretical bases of indirect calorimetry: a review. Metabolism 1988;37:287-301.
[15] Takala J, Meriläinen P. Handbook of gas exchange and indirect calorimetry. Helsinki: Datex-Engström Division; 2001.
[16] Rimestad AH, Borgenjordet Å, Vesterhus KN. Den store matvaretabellen (The Norwegian food table). Oslo: Universitetsforlaget; 2001.
[17] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18: 499-502.
[18] Burdge GC, Wright P, Jones AE, Wootton SA. A method for separation of phosphatidylcholine, triacylglycerol, non-esterified fatty acids and cholesterol esters from plasma by solid-phase extraction. Br J Nutr 2000;84:781-7.
[19] Riserus U, Sprecher D, Johnson T, Olson E, Hirschberg S, Liu A, et al. Activation of peroxisome proliferator–activated receptor (PPAR)delta promotes reversal of multiple metabolic abnormalities, reduces oxidative stress, and increases fatty acid oxidation in moderately obese men. Diabetes 2008;57:332-9.
[20] Bonaa KH, Bjerve KS, Straume B, Gram IT, Thelle D. Effect of eicosapentaenoic and docosahexaenoic acids on blood pressure in hypertension. A population-based intervention trial from the Tromso study. N Engl J Med 1990;322:795-801.
[21] Basu S. Radioimmunoassay of 8-*iso*-prostaglandin F2alpha: an index for oxidative injury via free radical catalysed lipid peroxidation. Prostaglandins Leukot Essent Fatty Acids 1998;58:319-25.
[22] Lee KU, Lee HK, Koh CS, Min HK. Artificial induction of intravascular lipolysis by lipid-heparin infusion leads to insulin resistance in man. Diabetologia 1988;31:285-90.
[23] Boden G, Chen X, Rosner J, Barton M. Effects of a 48-h fat infusion on insulin secretion and glucose utilization. Diabetes 1995;44: 1239-42.
[24] Boden G. Free fatty acids and insulin secretion in humans. Curr Diab Rep 2005;5:167-70.
[25] Wendel M, Rössel T, Bergmann S, Otto S, Ragaller M, Zimmermann T, et al. Impact of total parenteral nutrition including omega-3 fatty acids on the regulation of plasma lipoproteins and glycemic control after major abdominal surgery. e-SPEN, Eur e-J Clin Nutr Metab 2007;2: e103-10.
[26] Hodson L, McQuaid SE, Karpe F, Frayn KN, Fielding BA. Differences in partitioning of meal fatty acids into blood lipid fractions: a comparison of linoleate, oleate, and palmitate. Am J Physiol Endocrinol Metab 2009;296:E64-71.
[27] Jequier E, Felber JP. Indirect calorimetry. Baillieres Clin Endocrinol Metab 1987;1:911-35.
[28] Boden G, Jadali F, White J, Liang Y, Mozzoli M, Chen X, et al. Effects of fat on insulin-stimulated carbohydrate metabolism in normal men. J Clin Invest 1991;88:960-6.
[29] Tappy L, Berger MM, Schwarz JM, Schneiter P, Kim S, Revelly JP, et al. Metabolic effects of parenteral nutrition enriched with *n*-3 polyunsaturated fatty acids in critically ill patients. Clin Nutr 2006; 25:588-95.
[30] Stojiljkovic MP, Lopes HF, Zhang D, Morrow JD, Goodfriend TL, Egan BM. Increasing plasma fatty acids elevates F2-isoprostanes in humans: implications for the cardiovascular risk factor cluster. J Hypertens 2002;20:1215-21.
[31] Lopes HF, Morrow JD, Stojiljkovic MP, Goodfriend TL, Egan BM. Acute hyperlipidemia increases oxidative stress more in African Americans than in white Americans. Am J Hypertens 2003;16(5 Pt 1): 331-6.
[32] Basu S. Isoprostanes: novel bioactive products of lipid peroxidation. Free Radic Res 2004;38:105-22.