Effects of Dietary n-3 Fatty Acids on Hepatic and Peripheral Insulin Sensitivity in Insulin-Resistant Humans

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Diabetes Care 2015;38:1228–1237 | DOI: 10.2337/dc14-3101

OBJECTIVE

Dietary n-3 polyunsaturated fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), prevent insulin resistance and stimulate mitochondrial biogenesis in rodents, but the findings of translational studies in humans are thus far ambiguous. The aim of this study was to evaluate the influence of EPA and DHA on insulin sensitivity, insulin secretion, and muscle mitochondrial function in insulin-resistant, nondiabetic humans using a robust study design and gold-standard measurements.

RESEARCH DESIGN AND METHODS

Thirty-one insulin-resistant adults received 3.9 g/day EPA+DHA or placebo for 6 months in a randomized double-blind study. Hyperinsulinemic-euglycemic clamp with somatostatin was used to assess hepatic and peripheral insulin sensitivity. Postprandial glucose disposal and insulin secretion were measured after a meal. Measurements were performed at baseline and after 6 months of treatment. Abdominal fat distribution was evaluated by MRI. Muscle oxidative capacity was measured in isolated mitochondria using high-resolution respirometry and non-invasively by magnetic resonance spectroscopy.

RESULTS

Compared with placebo, EPA+DHA did not alter peripheral insulin sensitivity, postprandial glucose disposal, or insulin secretion. Hepatic insulin sensitivity, determined from the suppression of endogenous glucose production by insulin, exhibited a small but significant improvement with EPA+DHA compared with placebo. Muscle mitochondrial function was unchanged by EPA+DHA or placebo.

CONCLUSIONS

This study demonstrates that dietary EPA+DHA does not improve peripheral glucose disposal, insulin secretion, or skeletal muscle mitochondrial function in insulin-resistant nondiabetic humans. There was a modest improvement in hepatic insulin sensitivity with EPA+DHA, but this was not associated with any improvements in clinically meaningful outcomes.

Insulin resistance is an early metabolic abnormality in the progression of type 2 diabetes. Genetic predisposition (1) is often intertwined with excess calorie intake and lack of exercise (2). Positive energy balance is widely accepted as the major culprit in the development of insulin resistance and the metabolic derangements

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Received 31 December 2014 and accepted 5 March 2015.

Clinical trial reg. no. NCT01686568, clinicaltrials.gov.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc14-3101/-/DC1.

that affect insulin-sensitive tissues. Ectopic lipid accumulation in skeletal muscle (3) and liver (4), oxidative stress (5), and mitochondrial dysfunction (6) are implicated in insulin resistance. Chronic inflammation (7) and macrophage infiltration of adipose tissue, sarcopenia, as well as progressive decline in β-cell function (8), especially in the context of obesity and aging, have also been linked in the pathogenesis of insulin resistance. Although exercise is a highly effective countermeasure to insulin resistance, there is great interest in alternative or supplemental therapeutic strategies for individuals who are unable to participate in exercise at recommended levels.

n-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have emerged as a promising therapeutic strategy for their pleiotropic effects in the arena of diabetes (9,10), cardiovascular disease (11), and aging (12). The insulin-sensitizing effects of n-3 PUFAs are well documented in animal models (13–15). Leading mechanisms include repression of macrophage-induced tissue inflammation (15), improved mitochondrial function (13,16), and activation of anabolic pathways including a peroxisome proliferator–activated receptor signaling, a target of the current antidiabetic drugs thiazolidinediones. However, data from human studies have been conflicting. Some studies indicate that n-3 PUFAs improve insulin sensitivity in humans (17–21), whereas others find that n-3 PUFAs have no insulin-sensitizing effects (22–25) or may even worsen long-term glycemic control (26). Interestingly, studies in humans with a high inflammatory status such as overweight women (17), cancer cachexia (21), or hemodialysis (19) showed a beneficial effect of n-3 fatty acids. These discrepancies are at least partially attributed to the variation in the population studied, the source of n-3 PUFAs, dose, duration of treatment, underlying level of insulin resistance, and diversity of methods used to evaluate insulin sensitivity. A paucity of placebo-controlled studies of adequate treatment dose and duration using gold-standard measures of insulin sensitivity precludes a clear consensus about the efficacy of dietary n-3 PUFAs as insulin sensitizers in humans.

In this study, we determined the effects of dietary n-3 fatty acids (3.9 g/day) on insulin sensitivity in a 6-month randomized, placebo-controlled, double-blind study in insulin-resistant, nondiabetic humans. Our primary aim was to systematically evaluate whole-body insulin sensitivity using the pancreatic clamp technique and to differentiate between hepatic and peripheral insulin sensitivity using deuterated glucose. We hypothesized that n-3 PUFAs would improve insulin sensitivity and that this improvement would be associated with increased skeletal muscle mitochondrial function. The rationale for this hypothesis originates from our recent observations in mice that n-3 PUFAs protect insulin sensitivity in association with transcriptional evidence of mitochondrial biogenesis (13). Here, we used a combination of in vitro and in vivo techniques to comprehensively evaluate mitochondrial function in skeletal muscle.

RESEARCH DESIGN AND METHODS

Subjects

A total of 62 overweight (BMI >25 kg/m^2) individuals gave written informed consent as approved by the Mayo Foundation Institutional Review Board. Participants underwent screening for eligibility by standard hematologic and biochemical blood tests, resting electrocardiogram, physical examination, and medical history. As a general screening to exclude overweight participants with normal insulin sensitivity, participants were excluded if their fasting HOMA of insulin resistance (HOMA-IR) was >2.6, a threshold that was identified based on normal reference values (27). Participants were excluded if they were diagnosed with diabetes or if their fasting glucose exceeded 7 mmol/L (126 mg/dL). Additional exclusion criteria included cardiovascular disease, uncontrolled hypothyroidism, smoking and alcohol abuse, and participation in structured exercise more than 2 days per week for 30 min per day. Participants who reported using n-3 nutritional supplements and medications known to affect muscle metabolism such as β-blockers, corticosteroids, and anticoagulants were also excluded. A total of 31 participants (8 men and 23 women) were randomized.

Study Design

This was a randomized, placebo-controlled, double-blind study of n-3 PUFAs (EPA+DHA) versus placebo (ethyl oleate). The Mayo Clinic Research Pharmacy maintained the double-blind status and randomly assigned individuals to groups based on a table prepared by a statistician. The n-3 PUFAs and placebo softgels were supplied by Sancilio & Company, Inc (Riviera Beach, FL). Each n-3 softgel contained 675 mg EPA and 300 mg DHA and an additional 75 mg of "non EPA/DHA" n-3 fatty acids. Placebo softgels contained 1,200 mg ethyl oleate. Participants were instructed to consume two softgels with their morning meal and two with their evening meal for a total of 3.9 g/day EPA+DHA for 6 months. Every 4 weeks, participants reported to the Clinical Research Unit (CRU) to pick up a new prescription and return remaining capsules, which were counted to determine compliance. Compliance was also assessed from plasma levels of EPA and DHA measured by mass spectrometry at the end of the study. Participants were monitored for liver function (alanine aminotransferase, aspartate aminotransferase, and bilirubin), coagulation (international normalized ratio and prothrombin time), blood lipid profile, glucose, insulin, and creatine. At baseline and 6 months, all participants underwent a series of tests to evaluate body composition, energy metabolism, insulin sensitivity, and mitochondrial function.

Body Composition

DEXA was used to determine whole-body fat mass, percent body fat, and fat-free mass (FFM) (Lunar DPX-L; Lunar Radiation, Madison, WI).

MRI and Spectroscopy

Participants were positioned supine in the bore of a 3.0 Tesla GE Signa MRI scanner. Serial T1-weighted axial images were acquired through the abdominal region, between the pelvis and the upper end of the diaphragm, during a breath-hold phase. Abdominal subcutaneous and visceral fat areas were measured using Analyze Software System (Mayo Clinic Biomedical Imaging Resource) by a single trained analyst. Participants then performed in-magnet exercise to measure muscle oxidative capacity using phosphorous (^{31}P)-MRS as previously described (28). A ^{31}P-tuned transmission/receive coil was placed over the tibialis anterior muscle. After a habituation session, participants were asked to maximally dorsiflex for 30 s. Phosphorous metabolites were measured for 60 s of rest, throughout the 30-s muscle contraction, and during a 10-min recovery

period. Phosphocreatine, inorganic phosphate, and ATP were analyzed using NUTS software (Acorn NMR, Livermore, CA). Oxidative capacity was measured from the rate constant of phosphocreatine recovery from a single exponential fit of the recovery data as previously described (28).

Hyperinsulinemic-Euglycemic Clamp

Subjects consumed a weight-maintaining diet (20% protein, 50% carbohydrate, 30% fat, and 10 kcal/kg body wt) for 3 days provided by the CRU metabolic kitchen after a consultation with a dietitian. Participants were admitted to the CRU on the evening of the 3rd day of the weight-maintenance phase. Participants consumed only water after 1900 h. Indirect calorimetry (TrueOne 2400; Parvo Medics) was used for measuring energy expenditure and respiratory quotient at 0600 h (baseline fasting) and 1230 h (insulin infusion). A retrograde catheter was inserted into a dorsal hand vein, and the hand was kept in a heated box (130°F) for collection of arterialized blood. Venous catheters in the contralateral arm were used for infusion of glucose and hormones. A two-stage hyperinsulinemic-euglycemic clamp was performed for 6 h. Three hours prior to the beginning of hormone infusion, a primed (6 mg/kg FFM), continuous (4 mg/kg FFM/h) infusion of [6,6-$^2H_2$]-D-glucose was initiated. Regular insulin was infused at a low dose (0.62 mU/kg FFM/min) for 3 h and then at a high dose (2.3 mU/kg FFM/min) for 3 h. Somatostatin (60 ng/kg total body wt/min), glucagon (0.65 ng/kg total body wt/min), and human growth hormone (3 ng/kg total body wt/min) were infused for the entire period to achieve a pancreatic clamp. Blood samples were taken at 10-min intervals for plasma glucose concentration measurement by glucose oxidase (Analox Instruments). Euglycemia (~5.0 mmol/L [90 mg/dL]) was maintained with titrated infusion of 40% dextrose solution containing 2% [6,6-$^2H_2$]-D-glucose (29). Peripheral insulin sensitivity was evaluated from the average glucose infusion rates required to maintain euglycemia during the last 60 min of each stage of the clamp. Hepatic insulin sensitivity was evaluated from the suppression of endogenous glucose production (EGP) during the third hour of the low-insulin stage of the clamp compared with baseline EGP (29).

Mixed-Meal Test and Muscle Mitochondrial Function

At least 1 week after the pancreatic clamp, participants were readmitted to the CRU after 3 days of standardized meals. At 0700 h, a biopsy was taken from vastus lateralis muscle under local anesthesia (2% lidocaine) using a Bergstrom needle as previously described (30). Muscle tissue was immediately prepared for measurements of mitochondrial function as described in detail previously (31). At 1230 h, participants consumed a mixed liquid meal containing 15% protein, 55% carbohydrate, and 30% fat, approximately equal to 35% of resting energy expenditure (REE) for evaluating meal glucose tolerance. Arterialized blood samples were taken from a heated hand vein for glucose and insulin measurements at intervals over 6 h after the test meal, which was consumed over 10 min.

Analytical Methods

Glucose was measured in plasma samples by glucose oxidase method (Analox Instruments). [6,6-$^2H_2$]-D-glucose enrichment of blood samples and infusions was measured by gas chromatography–mass spectrometry (32). Insulin was measured using a two-site immunometric assay using electrochemiluminescence immunoassay detection (Roche Diagnostics, Indianapolis, IN). C-peptide was measured using a two-site immunometric (sandwich) assay using electrochemiluminescence detection (Roche Diagnostics, Indianapolis, IN). Interleukin (IL)-6 was measured using the Quantikine HS IL-6 Immunoassay, a solid-phase ELISA designed to measure human IL-6 in serum, plasma, and urine. Leptin and adiponectin were measured by radioimmunoassay, and C-reactive protein was measured by particle-enhanced immunoturbidimetric assay. With the exception of D2 glucose, all blood measurements were done in the Mayo Clinic Immunochemical Core Laboratory. Plasma EPA and DHA free fatty acids were measured by high-performance liquid chromatography–mass spectrometry as previously described (33).

Statistical Analysis

All outcomes were checked for normal distribution. Variables with normal distributions are presented as means ± SEM. Two-way (treatment group and time) repeated-measures ANOVA was used to compare outcomes from baseline to follow-up in placebo compared with n-3–treated groups. For variables that were not normally distributed, data are presented as medians and interquartile range with group comparisons made using Wilcoxon rank-sum test on the Δ from baseline to follow-up. Statistical analyses were performed using JMP Software (SAS Institute, Cart, NC).

RESULTS

Thirty-one subjects underwent randomization with 16 participants assigned in the n-3 arm and 15 in the placebo arm. Four participants withdrew from the study for personal reasons. One participant was withdrawn by investigators for noncompliance with the protocol. In total, 14 participants completed the study in the n-3 group and 11 in the placebo group. Four subjects have missing insulin sensitivity data because of failed intravenous access during the clamp procedure. Four participants have missing meal tolerance test data because of failed intravenous access. Four participants did not have mitochondrial capacity measured in muscle biopsies because of technical difficulties.

Anthropometric Characteristics

Main clinical characteristics at baseline and postintervention are shown in Table 1. At baseline, the two groups were similar in age and body composition. Both groups exhibited similar increases in body weight (P = 0.036) and % body fat (P = 0.008) over the 6 months of the study. The distribution of abdominal fat between subcutaneous and visceral cavities was similar in both groups at baseline and postintervention, although there was a significant interaction (P = 0.048) reflecting a subtle increase in visceral fat in the n-3 group and subtle decrease in the placebo group.

Lipids, Glucose, Insulin, and Inflammation Profiles

Fasting blood lipids, glucose, insulin, and inflammatory markers are given in Table 1. Compliance measured from return pill counts was 90% in the placebo group and 92% in the n-3 group, with marked increases in fasting plasma EPA and DHA free fatty acid levels in the n-3 group (Table 1). Total cholesterol, HDL and LDL, did not change in either group. As expected, serum triglyceride levels decreased in the n-3 group and increased

Table 1—Subject characteristics

	Placebo (n = 11)		n-3 (n = 14)		
	Baseline	Follow-up	Baseline	Follow-up	P
Physical characteristics					
Age (years)	32.6 ± 2.5	33.2 ± 2.5	35.3 ± 2.9	35.8 ± 3.0	0.604
Height (cm)	168.1 ± 2.3	168.0 ± 2.0	172.3 ± 2.2	172.7 ± 2.3	0.174
Weight (kg)	99.6 ± 4.5	101.7 ± 4.4	105.3 ± 4.2	107.3 ± 5.1	0.724
BMI (kg/m^2)	35.2 ± 1.4	36.0 ± 1.4	35.5 ± 1.2	36.0 ± 1.3	0.412
FFM (kg)	51.8 ± 2.6	52.5 ± 2.5	56.6 ± 2.7	56.4 ± 2.8	0.191
Body fat (%)	45.1 ± 2.1	45.8 ± 2.1	43.9 ± 1.9	44.9 ± 1.6	0.791
Abdominal fat (cm^2)	46.5 (32.2–59.5)	50.2 (36.8–62.1)	46.7 (32.7–58.8)	47.1 (34.0–57.6)	0.271
Visceral fat (cm^2)	9.69 ± 1.18	8.55 ± 0.89	9.92 ± 1.04	10.50 ± 1.15	0.048
Lipids					
Total cholesterol (mg/dL)	193 ± 7	196 ± 7	170 ± 11	171 ± 9	0.810
Triglyceride (mg/dL)	120 ± 19	153 ± 35	175 ± 18	141 ± 13	0.012
HDL cholesterol (mg/dL)	48 ± 3	46 ± 3	51 ± 3	50 ± 4	0.087
LDL cholesterol (mg/dL)	99 ± 8	95 ± 9	110 ± 6	113 ± 7	0.254
Non-HDL cholesterol (mg/dL)	124 ± 9	125 ± 11	145 ± 8	141 ± 8	0.414
EPA (μmol/L)	1.61 ± 0.37	2.34 ± 0.73	1.54 ± 0.40	11.47 ± 4.30	0.076
EPA (mg/dL)	0.049 ± 0.011	0.071 ± 0.022	0.047 ± 0.012	0.347 ± 0.130	0.076
DHA (μmol/L)	1.47 ± 0.20	2.22 ± 0.61	1.62 ± 0.35	4.49 ± 0.82	0.068
DHA (mg/dL)	0.048 ± 0.006	0.073 ± 0.020	0.053 ± 0.012	0.148 ± 0.027	0.068
Glucose and hormones					
Glucose (mg/dL)	89.9 ± 1.6	95.2 ± 4.9	92.4 ± 2.8	93.4 ± 3.1	0.321
Insulin (μIU/mL)	17.3 ± 2.3	18.8 ± 2.0	16.4 ± 0.9	17.1 ± 1.8	0.934
HOMA-IR	3.83 ± 0.50	4.52 ± 0.54	3.76 ± 0.28	3.94 ± 0.52	0.715
Inflammatory markers					
Leptin (ng/mL)	41.7 ± 5.6	41.2 ± 5.6	35.2 ± 5.8	35.2 ± 4.9	0.878
Adiponectin (μg/mL)	6.37 ± 0.68	6.80 ± 0.88	5.47 ± 0.49	5.70 ± 0.53	0.672
C-reactive protein (mg/dL)	0.11 (0.06–0.26)	0.21 (0.14–0.31)	0.34 (0.09–0.46)	0.32 (0.05–0.68)	0.487
Interleukin-6 (pg/mL)	1.81 ± 0.37	1.87 ± 0.29	1.73 ± 0.20	1.67 ± 0.22	0.688
Energy expenditure					
RQ (fasting)	0.87 ± 0.01	0.86 ± 0.01	0.87 ± 0.02	0.86 ± 0.02	0.659
RQ (steady-state insulin)	0.93 ± 0.02	0.95 ± 0.01	0.92 ± 0.01	0.92 ± 0.02	0.268
REE (fasting)	1,701 ± 83	1,770 ± 89	1,862 ± 66	1,910 ± 70	0.763
REE (steady-state insulin)	1,777 ± 95	1,760 ± 51	1,846 ± 51	1,898 ± 66	0.306

Patients were randomly assigned to receive placebo (ethyl oleate) or n-3 (EPA+DHA) for 6 months. Measurements were made prior to randomization (baseline) and again after 6 months (follow-up). For normally distributed data, means ± SEM are given, and two-way (group and time) repeated-measures ANOVA was used to compare outcomes across groups. Precise P values are given for the group-by-time interaction. For data that were not normally distributed (abdominal fat and C-reactive protein), data are given as median (interquartile range), and Wilcoxon rank-sum test was used to compare the Δ from baseline to follow-up between placebo and n-3 groups.

in the placebo group (P = 0.012). Fasting plasma glucose and insulin concentrations were not altered by the intervention, and likewise there was no change in HOMA-IR. Baseline levels of leptin and adiponectin were in the higher and lower normal range, respectively, and did not change over time. Likewise, the inflammation markers C-reactive protein and IL-6 were not altered in either group.

Hepatic and Peripheral Insulin Sensitivity

Plasma glucose and insulin were similar during baseline and follow-up studies in both groups during the 6-h clamp procedure (Supplementary Fig. 1). The glucose infusion rates required to maintain euglycemia were also similar from baseline to follow-up studies in both groups (Fig. 1*A* and *B*). There were no differences between groups in the average glucose infusion rates measured during the last 60 min of each 3-h stage of the clamp (Fig. 1*C* and *D*), indicating that peripheral insulin sensitivity was unaffected by EPA+DHA supplementation. EGP was modestly suppressed during the low-dose insulin infusion (Fig. 1*E* and *F*), but the percent suppression decreased by 12% on average in the placebo group and increased by 15% on average in the n-3 group (P = 0.024) (Fig. 1*G*), indicating that hepatic insulin sensitivity improved in response to 6 months of EPA+DHA supplementation. Indirect calorimetry measurements at baseline and during steady-state high insulin infusion showed an increase in respiratory quotient (RQ) and REE in response to insulin in both groups (Table 1), with no changes in either group after the intervention.

Meal Glucose Tolerance and Insulin Secretion

Postprandial glycemia during the 6 h after a mixed meal was similar in placebo and n-3 groups at baseline and after 6 months (Fig. 2*A* and *B*). Although there was a trend for increased peak postprandial glucose after 6 months in the placebo group (Fig. 2*A*), the glucose areas above baseline were similar in

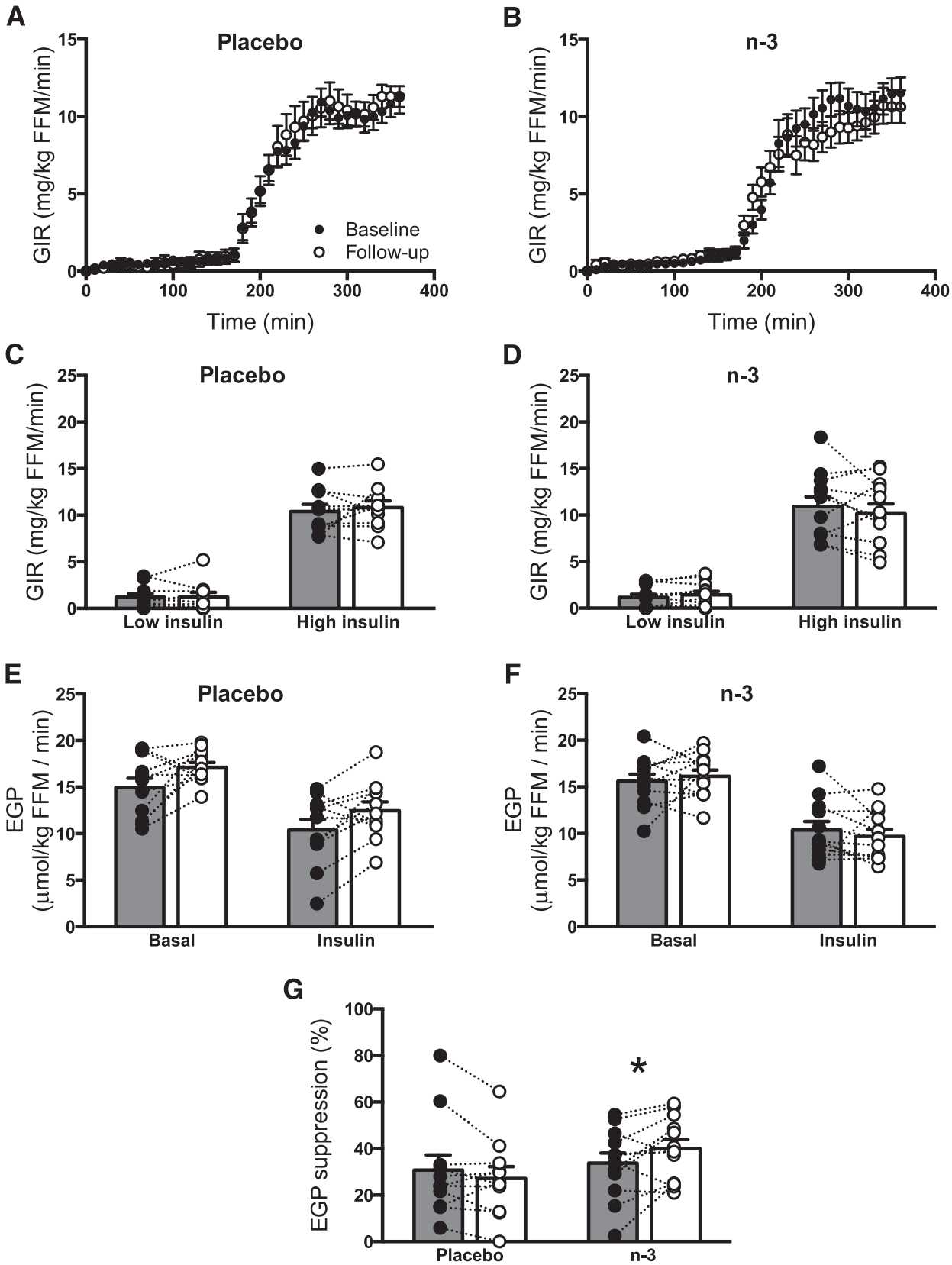

Figure 1—Hyperinsulinemic-euglycemic clamp. The glucose infusion rates (GIRs) required to maintain euglycemia in 10-min intervals during the 6-h insulin infusion in placebo (*A*) and n-3 (*B*) groups at baseline and follow-up. The average glucose infusion rate during the final 60 min of each stage of the clamp (low insulin and high insulin) in placebo (*C*) and n-3 (*D*) groups at baseline and follow-up. EGP measured in the basal fasting state and during low-dose insulin (*E* and *F*). EGP suppression was greater with n-3 (*G*). Data bars are means ± SEM. Data points represent individual subject responses with dotted lines linking baseline with follow-up observations. *$P < 0.05$ for the group × time interaction.

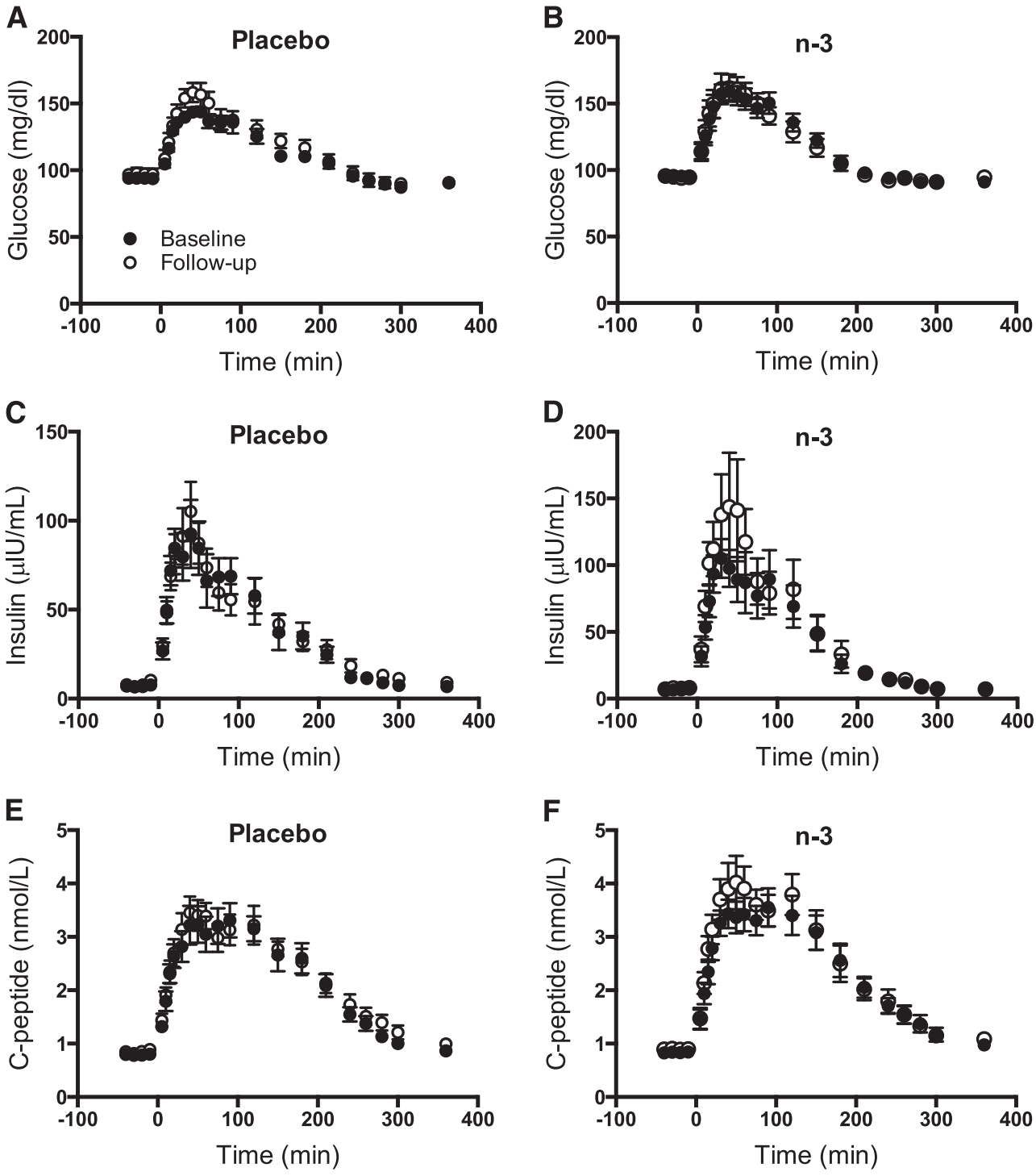

Figure 2—Mixed-meal tolerance test. Plasma glucose (*A* and *B*), insulin (*C* and *D*), and C-peptide (*E* and *F*) concentrations measured 6 h after ingestion of a liquid meal were similar in both groups and unchanged with either intervention. Data are means ± SEM.

both groups before and after the intervention (Supplementary Fig. 2). These data are in agreement with the measurements of peripheral insulin sensitivity assessed during the pancreatic clamp. Insulin secretion determined from C-peptide concentrations over 6 h after the mixed meal was also similar in both groups at both time points (Figs. 2*E* and *F*). The area above baseline for C-peptide was similar in placebo and n-3 groups and did not change with either intervention (Supplementary Fig. 2), indicating that insulin secretion did not change in response to high-dose EPA+DHA.

Skeletal Muscle Mitochondrial Function

Skeletal muscle oxidative capacity measured in mitochondria isolated from muscle biopsy tissue was unchanged by n-3 fatty acids or placebo in response to carbohydrate-based mitochondrial substrates (Fig. 3*A*) or lipid-based

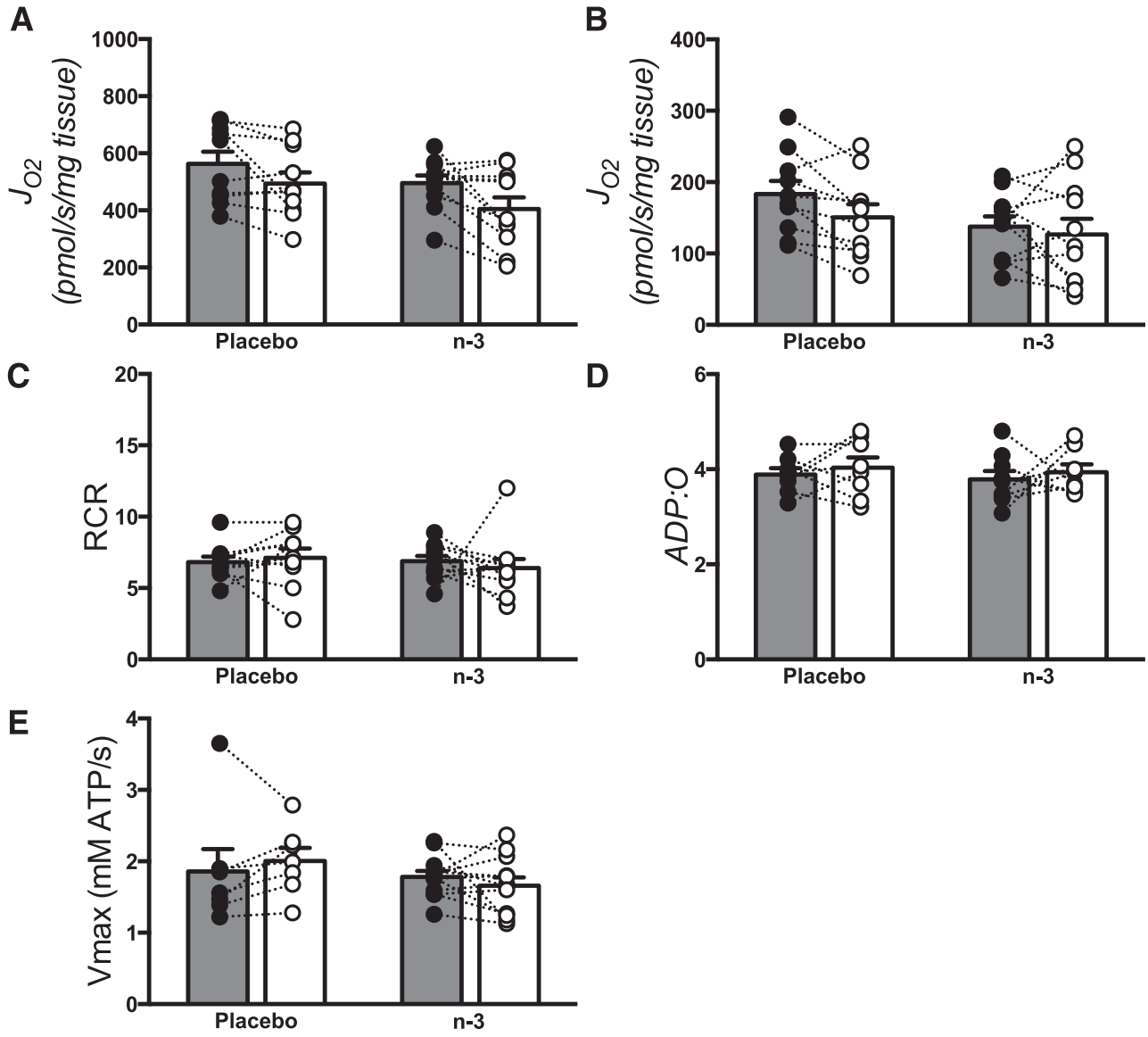

Figure 3—Mitochondrial function in skeletal muscle. Mitochondrial oxygen consumption rates (J_{O2}) were measured under state 3 respiration conditions with carbohydrate-based mitochondrial substrates (*A*) and lipid substrates (*B*). Mitochondrial coupling was assessed from the respiratory control ratio (RCR) (*C*) and ADP:O ratio (*D*). Oxidative capacity measured in vivo by magnetic resonance spectroscopy (*E*). Data bars are means ± SEM. Data points represent individual subject responses with dotted lines linking baseline (●) with follow-up (○) observations.

substrates (Fig. 3*B*). Mitochondrial efficiency as evaluated by the respiratory control ratio, the index of mitochondrial proton leak, and ADP:O ratio, the index of phosphorylation efficiency, was also similar across groups (Fig. 3*C* and *D*). The absence of any effect of EPA+DHA on mitochondrial capacity was corroborated with in vivo measurements of muscle oxidative capacity using ^{31}P-MRS, which also demonstrated no effect of n-3 or placebo (Fig. 3*E*).

CONCLUSIONS

This study demonstrates that 6 months of dietary EPA and DHA at 3.9 g/day does not alter insulin-stimulated peripheral glucose disposal, postprandial glucose disposal, or insulin secretion in overweight insulin-resistant nondiabetic individuals. We found that EPA+DHA treatment resulted in a small but significant increase in the suppression of EGP, although this was not accompanied by any changes in clinically meaningful outcomes related to hepatic insulin sensitivity. Furthermore, skeletal muscle mitochondrial function was unaffected by EPA+DHA. The results from this study provide some resolution to the current ambiguity concerning the therapeutic potential of dietary n-3 PUFAs in the context of human insulin resistance.

Precedent literature consistently shows that n-3 PUFAs prevent insulin resistance in rodents (13,14,34). The leading mechanisms include activation of peroxisome proliferator–activated receptor-α (34), suppression of inflammation (15), and attenuation of ectopic lipid accumulation in insulin-sensitive tissues (13,34). Furthermore, there is early evidence that n-3 PUFAs may stimulate mitochondrial biogenesis (13) and improve skeletal muscle mitochondrial function (35). Since mitochondrial dysfunction has been implicated in the

etiology of insulin resistance (1), it is possible that n-3 PUFAs may attenuate insulin resistance through their influence on mitochondria in insulin-sensitive tissues.

In spite of promising evidence from animal studies, there is currently no clear consensus on the effects of n-3 PUFAs on insulin sensitivity in humans. Intervention studies in humans have shown some biological effects of n-3 PUFAs similar to what is observed in rodents such as repression of macrophage-induced inflammatory response (25), improved mitochondrial function (35), and decreased circulating triglycerides and small dense LDL particles (23). Although these factors are all believed to be important determinants of insulin resistance, none of the studies found that insulin sensitivity was affected by n-3 PUFAs. A recent meta-analysis including 11 randomized clinical trials concluded that there is no overall association between intake of n-3 PUFAs and insulin sensitivity (36). A closer examination of these studies revealed substantial variability in the daily dose of n-3 PUFAs (0.14–11 g/day), duration of the intervention (2–6 months), and subject characteristics or disease states (lean, obese, diabetes, fatty liver disease, and hypertension). The vast majority of these studies evaluated insulin sensitivity from fasting glucose and insulin or oral/intravenous glucose tolerance tests, which may lack sensitivity needed to distinguish between hepatic and peripheral insulin sensitivity. We are unaware of any randomized, placebo-controlled, double-blind studies of n-3 PUFAs at pharmaceutical doses and durations in insulin-resistant nondiabetic individuals where insulin sensitivity was evaluated using gold-standard techniques. In the absence of such studies, it is possible that real therapeutic potential of n-3 PUFAs in insulin-resistant humans may be overlooked. The current study was designed to fill this gap. We recruited overweight men and women who were insulin resistant but otherwise healthy. A pharmaceutical grade EPA+DHA preparation was given at the highest dose that the FDA generally regards as safe (4 g/day) for 6 months. All of the participants demonstrated low DHA and EPA levels in the fasting plasma free fatty acid pool (Table 1), indicating low n-3 consumption in their background diets based on values reported based on tertiles of fatty fish consumption (37). The n-3 group exhibited approximately a sevenfold increase in fasting EPA levels and a fourfold increase in DHA levels in plasma free fatty acids after 6 months, indicating significant enrichment of adipose tissue n-3 content. Thus, absorption and bioavailability of n-3 fatty acids were high. An appropriate placebo group was included in a randomized double-blind fashion. Participants were carefully characterized for body composition and clinical blood parameters, and insulin sensitivity was evaluated using the pancreatic clamp technique, which is the gold standard for evaluating insulin sensitivity and offers the additional advantage of distinguishing between hepatic and peripheral insulin sensitivity. With this robust study design, we find that dietary EPA+DHA at 3.9 g/day for 6 months did not improve peripheral insulin sensitivity or postprandial glucose disposal. Although this conclusion corroborates previous meta-analyses, it does so with a high level of confidence because many of the methodological shortcomings of previous negative reports have been addressed. In addition to the pancreatic clamp, we also followed postprandial glycemia for 6 h after a mixed meal as a physiologically relevant parameter of peripheral glucose disposal. Similar to the clamp, the mixed-meal glucose disposal was comparable in both groups and although a slight worsening was observed in the placebo group, this was not statistically significant. Postprandial insulin concentrations and insulin secretion were also similar in both groups and were unchanged with either intervention.

Although our data clearly show that 6 months of EPA+DHA supplementation has no measurable effect on peripheral glucose disposal, we found a small but significant improvement in hepatic insulin sensitivity measured by the suppression of EGP during insulin infusion. By using somatostatin to suppress endogenous insulin secretion, we are confident that portal insulin levels are the same in all participants, thereby eliminating a source of variability in prevailing endogenous insulin levels that could confound EGP measurements. To our knowledge, this is the first study where hepatic and peripheral insulin sensitivities were evaluated separately in response to n-3 PUFA supplementation. It is interesting to note that a meta-analysis from 2011 concluded that n-3 PUFAs do not affect insulin sensitivity; however, subgroup analysis of studies using HOMA-IR, which primarily reflects hepatic insulin sensitivity, found improvements in insulin sensitivity with n-3 PUFAs (36). The notion that n-3 PUFAs may specifically act in liver is supported by evidence that n-3 PUFAs may improve hepatic steatosis, inflammation, and fibrosis in patients with nonalcoholic fatty liver disease (38). In contrast, others found that EPA at 2.7 g/day for 1 year did not improve HOMA-IR or histological features of nonalcoholic steatohepatitis (39). Nevertheless, the data from the current study suggest that the effects of EPA+DHA may be specific to liver. It is important to consider that although the effects of EPA+DHA on hepatic insulin sensitivity were statistically significant compared with the placebo group, the improvement was subtle and was not accompanied by any changes in fasting plasma glucose, HOMA-IR, or any other clinically relevant outcomes.

The current study also examined the effect of EPA+DHA on skeletal muscle mitochondrial function, since these organelles have been linked to insulin sensitivity (1). We recently found that the insulin-sensitizing effects of n-3 PUFAs were associated with transcriptional evidence of mitochondrial biogenesis (13). Since mitochondrial dysfunction is a hallmark of insulin resistance and potentially involved in the etiology and progression of type 2 diabetes (1), we determined whether muscle mitochondrial function could be improved by n-3 PUFAs in insulin-resistant individuals. Measurements of oxidative capacity and efficiency in mitochondria isolated from muscle biopsy material showed no change with n-3 PUFAs. These in vitro findings were also verified in vivo by ^{31}P-MRS. Recent work by Herbst et al. (35) found that fish oil supplementation in healthy young men increased EPA and DHA content of mitochondrial membranes but did not change mitochondrial capacity. These investigators did, however, report an increase in ADP sensitivity of mitochondria. Although we did not specifically evaluate ADP sensitivity, we did measure phosphorylation efficiency, which was unchanged. There are two important clinical implications

of the mitochondrial measurements in skeletal muscle. There is widespread interest in strategies to improve mitochondrial function in skeletal muscle of insulin-resistant individuals because these organelles are responsible for lipid oxidation and sustaining the metabolic demands of skeletal muscle activity. In contrast to this widespread belief, we find that skeletal muscle mitochondrial dysfunction is not always a necessary feature of insulin resistance, since the participants in this study exhibited normal mitochondrial function in spite of marked insulin resistance. Nevertheless, although EPA+DHA had no effect on mitochondrial function in this population of insulin-resistant participants, it will be important for future studies to determine whether n-3 PUFAs influence mitochondrial physiology in patient populations where mitochondrial dysfunction is evident.

In summary, the current study provides strong evidence against the notion that n-3 PUFAs improve peripheral insulin sensitivity in insulin-resistant nondiabetic individuals. It is difficult to reconcile these data with rodent studies where n-3 PUFAs clearly protect against insulin resistance. A key consideration is that the majority of the rodent studies have been preventive studies, while human studies are designed to determine whether n-3 PUFAs can reverse prevalent insulin resistance. Some insight into this issue comes from a recent study where n-3 PUFAs were found to prevent insulin resistance during an acute infusion of a lipid emulsion in healthy young men (40). Thus, although dietary n-3 PUFAs do not appear to be effective in reversing peripheral insulin resistance, additional studies are required to determine whether these bioactive lipids can be part of long-term preventive strategies. Although the subtle improvements in hepatic insulin sensitivity with EPA+DHA do not appear to be clinically meaningful in the current study, there is impetus for similar investigations in other insulin-resistant populations such as patients with fatty liver disease.

Acknowledgments. The authors are grateful for the expert technical assistance from Roberta Soderberg, Katherine Klaus, Daniel Jakaitis, Dawn Morse, and Jill Schimke, Mayo Clinic Endocrine Research Unit, and Peg Helwig, Mayo Clinic Metabolomics Core. This publication was supported by Clinical and Translational Science Award KL2 TR-000136 from the National Center for Advancing Translational Sciences. Additional funding was provided by the Strickland Career Development Award, Minnesota Obesity Center grant DK50456, and U24DK100469 from the National Institute of Diabetes and Digestive and Kidney Diseases and originates from the National Institutes of Health Director's Common Fund. The contents of this work are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

Duality of Interest. I.R.L. is a member of the Scientific Advisory Board of Sancilio and Company, Inc., which supplied materials used in this study. I.R.L. receives no personal financial compensation, stock, or speaking fees from Sancilio and Company, Inc. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. A.Z.L. wrote the manuscript and analyzed data. M.L.J. collected data and contributed to the design of the study. M.D.J. analyzed data and contributed to the study design. K.C.H. analyzed data. J.D.P. contributed to the study design and data collection and analysis. I.R.L. designed the study, collected data, analyzed data, and wrote the manuscript. I.R.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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