Fish Oil Feeding Improves Muscle Glucose Uptake in Tumor Necrosis Factor–Treated Rats

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This study was conducted to characterize the effects of fish oil and sunflower oil on hepatic glucose production and peripheral glucose utilization during infusion of saline or tumor necrosis factor (TNF), using the euglycemic-hyperinsulinemic clamp technique combined with a primed-constant tracer infusion of high-performance liquid chromatography–purified ^{3}H-3-glucose for estimation of whole-body glucose appearance and utilization rates. Insulin 10 mU/kg · min was infused to reach a plasma insulin level of 200 μU/mL. ^{14}C-1-deoxyglucose (^{14}C-DG) uptake was also measured in specific tissues following intravenous bolus administration. The results showed that during a hyperinsulinemic-euglycemic clamp, infusion of TNF 20 μg/kg for 3 hours resulted in a significant reduction of glucose infusion and a significant increase of hepatic glucose production in both dietary groups as compared with saline infusion, indicating a state of insulin resistance induced by TNF. The results also showed that TNF infusion significantly decreased the rate of ^{14}C-DG uptake in muscle in the sunflower oil group but not in the fish oil group, suggesting that fish oil is able to restore to normal the glucose utilization impaired by TNF. These observations suggest that in hyperinsulinemic and euglycemic conditions, prefeeding with fish oil significantly improves glucose uptake in muscle tissue, but does not alter the increase in hepatic glucose production during TNF infusion.

A NUMBER OF STUDIES have demonstrated that the fatty acid composition of membranes is one cellular factor that may influence the action of insulin. In rats, consumption of a small proportion of ω3 polyunsaturated fatty acids in a high-fat diet can prevent the development of insulin resistance.[1] In cultured cells, increasing the membrane content of polyunsaturated fatty acids increases membrane fluidity and the number of insulin receptors and improves the action of insulin[2-4]; the opposite effects occur when the concentration of saturated fatty acids in membranes is increased.[3,5] These findings provide evidence for the potential importance of ω3 fatty acids in the therapy for or prevention of insulin resistance that develops as a consequence of infection or inflammation.

A recent study from our laboratory has demonstrated that prefeeding with fish oil, enriched in ω3 fatty acids, attenuates the increased plasma glucose and insulin induced by infusion of interleukin-1 20 μg/kg for 4 hours and also improves glucose oxidation as compared with the prefeeding of soybean oil, which is rich in ω6 fatty acids.[6] These results support the hypothesis that ω3 fatty acids may benefit glucose metabolism in the host during infection or inflammation. However, these conclusions are based on the observed changes in plasma concentrations of glucose and insulin. It is still not known whether fish oil directly alters the action of insulin on peripheral glucose utilization and hepatic glucose production under the conditions of insulin resistance that developed during infection and inflammation.

Our previous studies have also demonstrated that infusion of tumor necrosis factor ([TNF] 20 μg/kg) for 3 hours can produce insulin resistance in both hepatic glucose production and peripheral glucose utilization. Thus, the present study was designed to determine the effects of fish oil and sunflower oil, enriched either in ω3 fatty acids or in ω6 fatty acids, on insulin action in normal animals and in animals receiving TNF infusion at 20 μg/kg, using the hyperinsulinemic-euglycemic clamp technique combined with a primed-constant tracer infusion of purified ^{3}H-3-glucose and bolus injection of ^{14}C-1-deoxyglucose (^{14}C-DG). In this hyperinsulinemic-euglycemic clamp model, the effects of two different families of fatty acids and the effects of administration of TNF in both dietary groups on peripheral glucose utilization and hepatic glucose production could be compared at similar levels of glucose and insulin. In addition, a bolus injection of ^{14}C-DG was used to determine glucose uptake in various tissues.

MATERIALS AND METHODS

Animal Preparation

Male Sprague-Dawley rats (weight, 45 to 50 g; Taconic Farms, Germantown, NY) were acclimatized in individual cages in a light-controlled room (12 hours on/12 hours off) at a temperature of 22° to 24°C for 4 days. During this period, animals were fed a regular rat chow diet and had access to tap water ad libitum. Animals were then randomly divided into two groups to be pair-fed with a modified AIN-76 rat diet containing either menhaden oil 14.5% (wt/wt) or sunflower oil 15% (wt/wt) for 6 weeks (Table 1). The fish oil diet also contained 0.5% sunflower oil by weight (wt/wt) to prevent essential fatty acid deficiency. These two diets were isonitrogenous and isocaloric. Body weights of the animals were recorded three times weekly to ensure similar weight gain in both groups.

At the end of a 6-week feeding period, all animals underwent a surgical procedure for catheter placement under ether inhalation. A polyethylene catheter (PE 50, 0.011 × 0.024 in; Becton Dickinson, Parsippany, NJ) was placed into the right carotid artery for sampling of blood. Two other silastic catheters (0.025 × 0.04) were placed in both jugular veins for administration of infusions of tracer, TNF, insulin, and glucose. The catheters were externalized in the midscapular region and attached to a flow-through swivel to

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Table 1. Diet Compositions (g/kg)

Ingredient	Fish Oil	Sunflower Oil
Casein	200	200
Menhaden oil	145	—
Sunflower oil	5	150
D-L-Methionine	3	3
Corn starch	150	150
Sucrose	400	400
Cellulose	50	50
Salt mix no. 200000 AIN-76	35	35
Vitamin mix no. 300050 AIN-76	10	10
Choline chloride	2	2

NOTE. Salt mix no. 200000 AIN-76 includes the following (mg/kg diet): calcium 5,200; phosphorus 4,000; potassium 3,600; sodium 1,020; chloride 1,560; sulfur 337; magnesium 507; iron 35; copper 6.0; manganese 54.0; zinc 30.0; chromium 2.0; iodine 0.2; and selenium 0.1. Vitamin mix no. 300050 AIN-76 includes the following (mg/kg diet): thiamine 6; riboflavin 6; pyridoxine HCL, 7; niacin 30; calcium pantothenate 16; folic acid 2; biotin 0.2; cyanocobalamin 10; and menadione sodium bisulfite 0.8; and vitamin A 4,000 IU; vitamin E 175 IU; and vitamin D_3 1,000 IU.

permit free movement of the animals. After surgery, animals were allowed to recover in individual cages for 1 day and were fasted overnight before experimentation.

The experiment was approved by the Animal Care Committee of New England Deaconess Hospital, which follows guidelines established for the care and use of laboratory animals of the Institute of Laboratory Animal Resources of the National Research Council.

Experimental Design

On the morning of study, animals in each dietary group were further randomly divided into two subgroups to receive a 3-hour infusion of 0.1% human albumin in saline (saline as control) or 0.1% albumin in saline containing recombinant murine TNF 10 μg/kg (Genentech, San Francisco, CA) at the rate of 2.3 mL/h. For the TNF group, TNF 10 μg/kg was also given as a bolus at the beginning of the study. All saline and cytokine solutions were freshly prepared on the day of the experiment. Basal arterial blood samples (0.5 mL) were drawn for determination of blood glucose and insulin levels before initiation of the infusion. At the end of the first hour of saline or TNF infusion, arterial blood (0.5 mL) was drawn for determination of plasma glucose and insulin (60 minutes; Fig 1). Rats then received a priming dose of 5 μCi high-performance liquid chromatography–purified ^{3}H-3-glucose (Du Pont, Wilmington, DE), followed by a constant infusion of 5 μCi/h (2.3 mL/h) for 2 hours (180 minutes) combined with saline or TNF infusion to provide a steady-state level of tracer concentration, as previously described.[7] At the same time, insulin 10 mU/kg · min, which was able to completely suppress hepatic glucose production in a normal healthy animal consuming a diet with 30% nonprotein calories from fat according to our pilot study, was combined with saline or TNF solution and continuously infused through the same jugular vein for 2 hours. During this 2-hour infusion period, plasma glucose levels were monitored every 10 minutes to maintain basal glucose levels. Through another jugular vein, 20% dextrose was infused at a variable rate using a variable syringe infusion pump. At 138 minutes of the initial infusion, a bolus of 5 μCi ^{14}C-DG (Du Pont) was injected intravenously, and then arterial blood samples (0.4 mL) were drawn at 140, 150, 160, 170, and 180 minutes for measurement of ^{14}C-DG levels in plasma. Additional 0.1-mL arterial blood samples were also collected at 140, 160, and 180 minutes for measurement of plasma glucose specific activity. The data confirmed that the isotopic steady state was achieved during this period, ie, between 60 and 120 minutes of the clamp period.

At the end of infusion (180 minutes), the animals were decapitated and blood was collected for determination of plasma glucose and insulin and glucose specific activity. Pieces of liver, rectus abdominus muscle, and abdominal mesenteric adipose tissues were removed and weighed for determination of ^{14}C-DG radioactivity. Another piece of liver or muscle was collected for determination of the amount of phospholipid in the tissues.

All samples were stored at −20°C until analysis.

Analytical Procedures

Plasma glucose specific activity was determined as previously described.[6] Glucose concentration was determined by the glucose oxidase method using a Beckman Glucose Analyzer II (Brea, CA). Plasma insulin was determined by radioimmunoassay using porcine insulin as a standard (Binax, South Portland, ME).

Plasma ^{14}C-DG level and accumulation of ^{14}C-DG in tissues were determined using a method described elsewhere.[7-9] In brief, blood samples were deproteinized with $Ba(OH)_2$-$ZnSO_4$ and immediately centrifuged. An aliquot of the supernatant was used for counting ^{14}C-DG radioactivity (Beckman LS6K-IC Scintillation Systems, Columbia, MD). Tissue samples were placed in 0.5 to 1 mL 1-mol/L NaOH (according to size), digested at 60°C for 1 hour, and then neutralized with 1 mol/L HCL. Separate 200-μL aliquots of digested tissues were treated with either 1 mL $HCLO_4$ (4% wt/vol) or 1 mL $Ba(OH)_2$-$ZnSO_4$ and centrifuged: The supernatants were counted for ^{14}C-DG plus ^{14}C-DG-6-phosphate ($HCLO_4$-treated fractions) and ^{14}C-DG-6-phosphate [$Ba(OH)_2$-$ZnSO_4$–treated fractions].

The components of tissue phospholipids were extracted according to the method reported by Folch et al.[10] Muscle or liver tissue (200 mg) was homogenized with 2 mL water, and then 1-mL samples of homogenate were mixed with 8 mL chloroform-methanol (2:1 by volume) solution. Phospholipid was added as internal standard before extraction. Phospholipids were separated

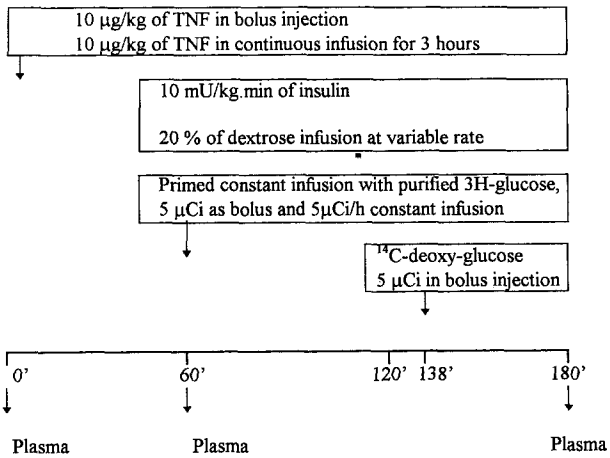

Fig 1. Study protocol for hyperinsulinemic-euglycemic clamp. Primed-constant infusion of ^{3}H-3-glucose was administered to label the glucose pool for 2 hours after 1 hour of initial infusions of saline and TNF (20 μg/kg) in animals fed either fish oil or sunflower oil. Insulin (10 mU/kg · min) was infused to reach a plasma insulin concentration of ~200 μU/mL; glucose was infused at a variable rate to maintain a fasting euglycemic level for 2 hours. In addition, 5 μCi ^{14}C-DG was injected at 138 minutes; blood samples were drawn at 140, 150, 160, 170, and 180 minutes for determination of ^{14}C-DG in plasma. Blood samples were also obtained at 140, 160, and 180 minutes to determine plasma glucose specific activity.

by thin-layer chromatography using Whatman LK 5D silica-gel plates (Whatman Chemical Separations, Clifton, NJ) and a mobile phase of petroleum ether-diethyl ether-glacial acetic acid (80:20:1 by volume). After separation, samples were methylated under nitrogen in a steam bath for 45 minutes. Methyl esters of the phospholipid fraction were then separated and quantified on a Hewlett Packard Series 5890 gas chromatograph (Hewlett Packard, Andover, MA) fitted with a fused-silica capillary column (Supelco, Bellefonte, PA). Fatty acids were identified by comparing their retention times with those of authentic standard mixtures.

Calculations

The rate of glucose appearance (R_a) was calculated using the infusion rate of ^{3}H-labeled tracer (I) and the steady-state plasma ^{3}H-glucose specific activity, as follows: R_a = I/plasma ^{3}H-glucose specific activity. The rate of endogenous glucose production was calculated from the difference between the determined total rate of glucose appearance (isotope dilution) and the exogenous glucose infusion rate during the clamp.[10] The apparent glucose uptake is determined as

$$\frac{^{14}\text{C-DG}_{\text{tissue}}}{\int_{i=0}^{42} {}^{14}\text{C-DG}_{\text{plasma}}(t) \cdot dt} \cdot G_{\text{plasma}},$$

where G is plasma glucose concentration at steady state. The quantity $\int_{i=0}^{42} {}^{14}\text{C-DG}_{\text{plasma}}(t) \cdot dt$ was determined by numerical integration (trapezoid rule) of ^{14}C-DG blood measurements using a computer program.

Statistical Analysis

Data are presented as the mean ± SEM. Group means were compared by two-way ANOVA using the SYSTAT statistical software package (SYSTAT, Evanston, IL). Significance was defined as P less than .05. Comparison of multiple groups was performed according to the Tukey test when ANOVA was found to be significant at the 95% confidence level.

RESULTS

Initial body weight of the animals was not significantly different among groups. After 6 weeks of pair-feeding with sunflower oil and fish oil diets, final body weight ranged from 348 to 355 g and was not significantly different between the two diets.

Table 2 lists changes in plasma levels of glucose and insulin. Basal plasma glucose concentration (0 minutes) was not significantly different among animals. Unlike plasma glucose concentrations, basal plasma insulin levels were significantly higher in fish oil–fed groups as compared with sunflower oil–fed groups. After 1 hour of infusion with saline or TNF (60 minutes), plasma glucose concentrations were not significantly different between fish oil or sunflower oil feeding or between the two treatments, saline or TNF. However, TNF infusion significantly increased plasma insulin levels from 53 ± 4 to 71 ± 7 μU/mL in both dietary groups as compared with saline infusion. During the clamp, plasma glucose in each group was monitored and controlled at the basal level, but plasma insulin levels rapidly increased to 205 to 245 μU/mL without significant differences among groups.

Table 3 lists glucose kinetic data during the insulin clamp. The glucose infusion rate necessary to maintain plasma glucose levels at similar hyperinsulinemic conditions varied significantly. Animals receiving TNF infusion required less exogenous glucose (30% less) to maintain normoglycemia as compared with those receiving saline infusion. There was a trend toward a decrease in the amount of required glucose in sunflower oil–fed groups (P = .08), but this change did not reach statistical significance. The overall results showed that the lowest amount of glucose required was found in animals fed the sunflower oil diet and treated with TNF (3.8 ± 0.4 mmol glucose/kg · h). The highest amount of glucose was infused in animals fed fish oil and receiving saline infusion (6.5 ± 0.5 mmol/kg · h).

During the clamp, plasma glucose appearance as measured by isotope dilution was not significantly different among groups. The calculated hepatic glucose production with saline infusion was 0.3 ± 0.4 and 0.5 ± 0.3 mmol/kg · h in the fish oil diet and sunflower oil diet, respectively. Neither was significantly different from zero. However, with TNF infusion, the rate of hepatic glucose production significantly increased to 1.8 ± 0.3 mmol/kg · h in the fish oil–fed group and 0.9 ± 0.5 mmol/kg · h in the sunflower oil–fed group (P < .05). Both were significantly different from zero. No significance was found between the two diets.

Figure 2 shows glucose uptake in the liver, rectus abdominus muscle (a fast-twitch skeletal muscle with mostly type I fibers), and abdominal mesenteric adipose tissues. In the muscle, a higher rate of ^{14}C-DG uptake was found in fish oil–fed groups as compared with sunflower oil–fed groups (P = .05). TNF significantly decreased ^{14}C-DG uptake in muscle in animals fed sunflower oil but not in animals fed fish oil. In the liver, a higher rate of ^{14}C-DG uptake was found in the fish oil group. However, TNF increased the rate in the sunflower oil group but not in the

Table 2. Changes in Concentrations of Plasma Glucose and Insulin

Group	Glucose (mg/dL)			Insulin (μU/mL)		
	0 min	60 min	180 min	0 min*	60 min†	180 min
FT (n = 9)	120 ± 4	112 ± 2	113 ± 3	50 ± 3	71 ± 6	246 ± 33
FS (n = 8)	120 ± 4	113 ± 3	110 ± 2	52 ± 3	53 ± 4	210 ± 30
ST (n = 9)	117 ± 4	113 ± 3	116 ± 5	44 ± 3	71 ± 7	206 ± 25
SS (n = 9)	116 ± 4	114 ± 2	116 ± 4	41 ± 3	53 ± 4	228 ± 45

NOTE. Results are the mean ± SEM.

Abbreviations: FT, fish oil + TNF; FS, fish oil + saline; ST, sunflower oil + TNF; SS, sunflower oil + saline.

*P < .05 fish oil *v* sunflower oil.

†P < .05 saline *v* TNF.

Table 3. Glucose Kinetics (mmol/kg · h) During the Euglycemic-Hyperinsulinemic Clamp

Group	Plasma Glucose Appearance	Glucose Infusion (120 to 180 min)*	Endogenous Glucose Production†
FT (n = 9)	5.8 ± 0.4	4.5 ± 0.4	1.8 ± 0.3
FS (n = 8)	6.9 ± 0.8	6.5 ± 0.5	0.3 ± 0.4
ST (n = 9)	4.7 ± 0.4	3.8 ± 0.4	0.9 ± 0.5
SS (n = 9)	6.3 ± 1.1	5.4 ± 0.7	0.5 ± 0.3

NOTE. Results are the mean ± SE.

Abbreviations: FT, fish oil + TNF; FS, fish oil + saline; ST, sunflower oil + TNF; SS, sunflower oil + saline.

*$P < .001$, TNF *v* saline.

†$P < .05$, TNF *v* saline.

fish oil group. In fat tissue, there were no significant differences produced by either diet or treatment.

Tables 4 and 5 list the phospholipid components of muscle and liver. Fish oil feeding significantly increased the phospholipid composition of muscle in saturated fatty acids (C16:0), monounsaturated fatty acids (C18:1ω9), and ω3 polyunsaturated fatty acids (C20:5ω3, C22:5ω3, and C22:6ω3) and significantly decreased the components of ω6 polyunsaturated fatty acids (C18:2ω6, C20:3ω6, and C20:4ω6). Thus, the ratio of ω3/ω6 and polyunsaturated to saturated fatty acids in muscle phospholipids was significantly higher in fish oil groups. Furthermore, the degree of unsaturation, ie, the total number of double bonds, was also greater in fish oil groups. There also was an apparent competition between ω3 and ω6 fatty acids for desaturation and chain-elongation steps from 20:3ω6 to 20:4ω6 in this tissue. The same changes were observed in phospholipid composition of the liver. These diet-induced changes in fatty acid composition of tissue phospholipids have been shown by others and ourselves.[11-13] However, TNF (20 μg/kg) infusion for 3 hours did not produce any effects on the phospholipid profile in muscle or liver in either dietary group.

DISCUSSION

The present results showed that under hyperinsulinemic conditions, the glucose infusion rate necessary to maintain euglycemia was 30% less in TNF-infused rats in both fish oil and sunflower oil groups as compared with saline infusion. Because all experiments were conducted at a similar hyperinsulinemic-euglycemic condition (plasma insulin levels ranged from 204 ± 25 to 246 ± 33 μU/mL and plasma glucose levels from 110 ± 2 to 116 ± 4 mg/dL), these results indicated that a large dose of exogenous insulin (10 mU/kg · min) was unable to maintain glucose utilization in peripheral tissues after TNF 20 μg/kg was administered to animals over 3 hours. Moreover, this dose of exogenous insulin also was not able to suppress hepatic glucose production in both fish oil and sunflower oil groups, as indicated by the enhanced hepatic glucose production after TNF infusion. Thus, during infusion of TNF, insulin is less effective in both stimulation of glucose utilization by certain peripheral tissues and suppression of hepatic glucose production. The present results are consistent with our previous findings[7] and other reports.[14] All these findings indicate that TNF, as a principal component of the systemic inflammatory response, is an important mediator of glucose metabolic responses to infection or stress.

Although insulin resistance is presumed to exist during many infections, the reasons for this state are unclear. Increased levels of stress or counterregulatory hormones that are stimulated by TNF, such as cortisone, glucagon, and catecholamines,[10,15,16] have been shown to impair principally insulin-mediated glucose uptake. TNF also stimulates whole-body free fatty acid turnover and oxidation.[17] An enhanced rate of lipid oxidation is known to impair both oxidative and nonoxidative pathways of glucose metabolism[18]; therefore, alterations in the glucose–fatty acid cycle could also contribute to the etiology of TNF-induced insulin resistance. Recent study has further suggested that TNF can directly reduce the number or activity of GLUT4 glucose transporters and decrease translocation of GLUT4 from an intracellular location to the cell membrane,[19,20] or can exert its antiinsulin effect by interrupting the early insulin-stimulated events of tyrosine phosphorylation, which are crucial to insulin transmembrane signaling.[21,22] In addition, TNF may combine with other cytokines to have direct effects on glucose metabolism.

The results also showed that during hyperinsulinemia,

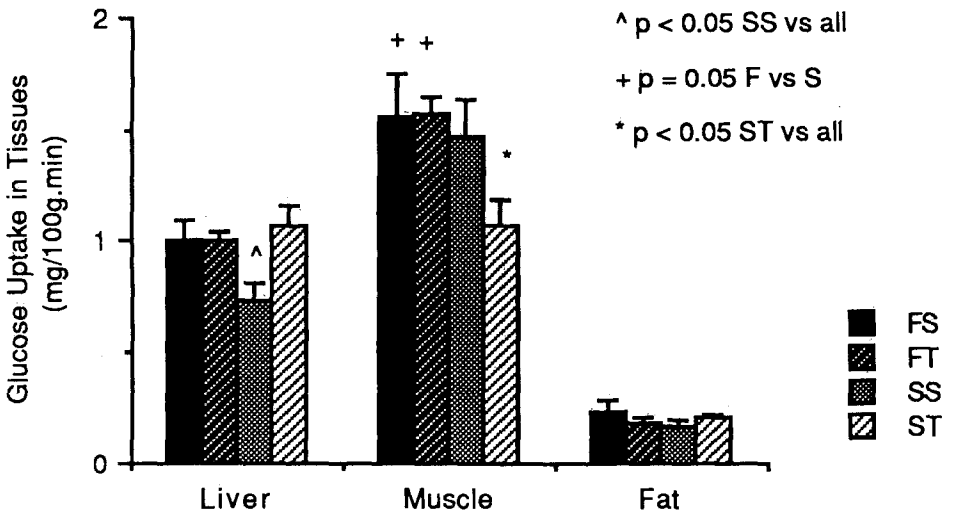

Fig 2. Rate of glucose uptake of tissues in different dietary groups, derived from ^{14}C-DG data. FT, fish oil + TNF; FS, fish oil + saline; ST, sunflower oil + TNF; SS, sunflower oil + saline.

Table 4. Profile of Fatty Acids in the Phospholipid Fraction of Muscle (μmol/mg)

Variable	FT (n = 9)	FS (n = 9)	ST (n = 10)	SS (n = 8)
C16:0	0.60 ± 0.12	0.65 ± 0.08	0.35 ± 0.02*	0.49 ± 0.06*
C18:0	0.31 ± 0.04	0.24 ± 0.03	0.26 ± 0.02	0.30 ± 0.03
C18:1ω9	0.12 ± 0.02	0.11 ± 0.01	0.06 ± 0.00†	0.08 ± 0.01†
C18:2ω6	0.21 ± 0.02	0.19 ± 0.00	0.38 ± 0.03†	0.45 ± 0.04†
C20:3ω6	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00†	0.01 ± 0.00†
C20:4ω6	0.12 ± 0.01	0.10 ± 0.02	0.29 ± 0.02†	0.36 ± 0.03†
C20:5ω3	0.07 ± 0.01	0.07 ± 0.01	0.00 ± 0.00†	0.00 ± 0.00†
C22:5ω3	0.07 ± 0.01	0.06 ± 0.01	0.01 ± 0.00†	0.03 ± 0.01†
C22:6ω3	0.52 ± 0.05	0.46 ± 0.09	0.07 ± 0.00†	0.08 ± 0.01†
Total ω3	0.65 ± 0.07	0.59 ± 0.11	0.08 ± 0.01†	0.10 ± 0.01†
Total ω6	0.35 ± 0.04	0.31 ± 0.04	0.67 ± 0.05†	0.82 ± 0.06†
ω3/ω6	1.88 ± 0.08	1.89 ± 0.19	0.12 ± 0.01†	0.11 ± 0.01†

NOTE. Results are the mean ± SEM.

Abbreviations: FT, fish oil + TNF; FS, fish oil + saline; ST, sunflower oil + TNF; SS, fish oil + saline.

*P < .05, †P < .001: fish oil *v* sunflower oil by 2-way ANOVA.

Table 5. Profile of Fatty Acids in the Phospholipid Fraction of Liver (μmol/mg)

Variable	FT (n = 9)	FS (n = 8)	ST (n = 10)	SS (n = 9)
C16:0	1.58 ± 0.08	1.38 ± 0.07	0.96 ± 0.06*	0.94 ± 0.05*
C18:0	0.94 ± 0.05	0.83 ± 0.07	1.11 ± 0.04*	1.17 ± 0.07*
C18:1ω9	0.25 ± 0.03	0.20 ± 0.01	0.07 ± 0.01*	0.07 ± 0.00*
C18:2ω6	0.20 ± 0.02	0.17 ± 0.02	0.43 ± 0.06*	0.43 ± 0.02*
C20:3ω6	0.41 ± 0.00	0.45 ± 0.03	0.01 ± 0.00*	0.01 ± 0.00*
C20:4ω6	0.50 ± 0.05	0.45 ± 0.05	1.09 ± 0.09*	1.16 ± 0.07*
C20:5ω3	0.35 ± 0.03	0.34 ± 0.04	0.00 ± 0.00*	0.01 ± 0.01*
C22:5ω3	0.11 ± 0.01	0.09 ± 0.01	0.02 ± 0.00*	0.02 ± 0.00*
C22:6ω3	0.83 ± 0.04	0.70 ± 0.01	0.17 ± 0.01*	0.16 ± 0.02*
Total ω3	1.28 ± 0.69	1.13 ± 0.12	0.19 ± 0.02*	0.18 ± 0.02*
Total ω6	0.74 ± 0.07	0.65 ± 0.07	1.39 ± 0.17*	1.61 ± 0.08*
ω3/ω6	1.82 ± 0.16	1.74 ± 0.11	0.12 ± 0.01*	0.11 ± 0.01*

NOTE. Results are the mean ± SEM.

Abbreviations: FT, fish oil + TNF; FS, fish oil + saline; ST, sunflower oil + TNF; SS, sunflower oil + saline.

*P < .005, fish oil *v* sunflower oil by 2-way ANOVA.

fish oil feeding stimulated glucose uptake in skeletal muscle. This conclusion was reached based on the following evidence. First, plasma concentrations of insulin and glucose did not differ among the groups, but glucose uptake was significantly higher in the fish oil group than in the sunflower oil group. Second, at similar hyperinsulinemic-euglycemic conditions, there was no difference in glucose uptake in muscle in the fish oil group between TNF and saline infusions. Quantitatively, TNF infusion resulted in a 30% decrease in glucose uptake of muscle tissue in sunflower oil–fed animals. These findings imply that either fish oil feeding improves insulin sensitivity in the muscle or fish oil overcomes the insulin resistance induced by TNF in this tissue. Studies have demonstrated that incorporation of ω3 fatty acids into the membrane after 6 weeks of fish oil feeding can increase membrane fluidity, and the increased fluidity can increase or facilitate the penetration of glucose transporters into the membrane[14,23,24] and result in conformational changes in the quaternary structure of the transporter,[25] which would increase responsiveness to the action of insulin. On the other hand, fish oil feeding can reduce the responsiveness to glucose,[26] endotoxin,[27,28] and cytokines.[29] This may account for the higher insulin levels at baseline in fish oil–fed animals as compared with sunflower oil–fed animals. Thus, whether the observed improvements in the present study were due to the effects of fish oil in reducing TNF effects and/or to the increased insulin sensitivity cannot be defined yet. Nevertheless, the present results do provide direct evidence that prefeeding with fish oil enriched in ω3 fatty acids could benefit glucose utilization in skeletal muscle during TNF infusion, which may have considerable clinical implications under conditions of infection or inflammation.

In summary, results from the present study add to the existing evidence that dietary fatty acids, particularly ω3 fatty acids, can rapidly modify fatty acid composition of membranes and that as a consequence insulin action is altered. Prefeeding with fish oil significantly improved glucose uptake in the abdominus muscle, but did not affect the increase in hepatic glucose production during TNF infusion. The different response to fish oil feeding may suggest that glucose uptake in muscle is modulated by membrane lipids, but insulin suppression of hepatic glucose output may be independent of this factor.

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