Original Article

Fish Oil Regulates Adiponectin Secretion by a Peroxisome Proliferator–Activated Receptor-γ–Dependent Mechanism in Mice

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Adiponectin has insulin-sensitizing, antiatherogenic, and anti-inflammatory properties, but little is known about factors that regulate its secretion. To examine the effect of fish oil on adiponectin secretion, mice were fed either a control diet or isocaloric diets containing 27% safflower oil or 27, 13.5, and 8% menhaden fish oil. Within 15 days, fish oil feeding raised plasma adiponectin concentrations two- to threefold in a dose-dependent manner, and the concentrations remained approximately twofold higher for 7 days when the fish oil diet was replaced by the safflower oil diet. Within 24 h, fish oil markedly induced transcription of the adiponectin gene in epididymal adipose tissue but not in subcutaneous fat. The increase of plasma adiponectin by fish oil was completely blocked by administration of the peroxisome proliferator–activated receptor (PPAR)γ inhibitor bisphenol-A-diglycidyl ether. In contrast, there was no effect of fish oil feeding on adiponectin secretion in PPARα-null mice. These data suggest that fish oil is a naturally occurring potent regulator of adiponectin secretion in vivo and that it does so through a PPARγ-dependent and PPARα-independent manner in epididymal fat. ***Diabetes*** **55:924–928, 2006**

Adiponectin is a factor exclusively derived from adipose tissue that has been shown to exert anti-inflammatory and antiatherogenetic effects and reverse insulin resistance in rodents (1–4) primarily by increasing hepatic insulin sensitivity (5–8). Furthermore, plasma adiponectin concentrations are diminished in obese and insulin-resistant individuals, suggesting that these insulin-sensitizing effects may extend to humans (9–12). However, despite numerous studies demonstrating important physiological effects of adiponectin, little is known about factors that regulate its secretion. Like adiponectin, dietary fish oil protects against fat-induced insulin resistance and has anti-inflammatory and antiatherogenic properties; however, the mechanism by which fish oil mediates these effects is poorly understood (13–17). Given these major parallels between fish oil and adiponectin action, we decided to examine whether fish oil might modulate adiponectin secretion in vivo.

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BADGE, bisphenol-A-diglycidyl ether; PPAR, peroxisome proliferator–activated receptor.

RESEARCH DESIGN AND METHODS

129Sv mice (wild type; Jae Substrain) and mice lacking functional peroxisome proliferator–activated receptor (PPAR)α (PPARα null; Jae Substrain) purchased from The Jackson Laboratories were bred under standard vivarium conditions. Dietary interventions were started in singly housed, male, 10- to 12-week-old, weight-matched mice. All procedures were approved by the Yale University Animal Care and Use Committee.

Chronic fish oil feeding and dose-response studies in wild-type and PPARα-null mice. Mice had unrestricted access to a standard control diet (7% fat-derived calories), a 27% (wt/wt) safflower oil diet (59% fat-derived calories; 78% C18:2n-6), or a 27% (wt/wt) menhaden fish oil diet (59% fat-derived calories; 16% C20:5n-3, 9% C22:6n-3). The diets (110700, 112245, and 112246; Dyets, Bethlehem, PA) contained mineral (210025) and vitamin (310025) supplements. For fish oil dose-response studies, 27% fish oil diet was admixed to 27% safflower oil diet to obtain isocaloric 14.5% (wt/wt) and 8% (wt/wt) fish oil diets. PPARα-null and a group of wild-type mice were fed 14.5% (wt/wt) menhaden fish oil diet admixed to powdered control diet. All diets were exchanged every 2nd day and fed for 15 days. For measurement of plasma adiponectin concentrations, 15-μl blood samples were collected from fed mice between 10:00 and 12:00 A.M. on days 0, 2, 4, 8, and 15 of dietary intervention via a tail restraining method. In one mouse batch, body fat gain was measured before and at the end of dietary interventions using in vivo ^{1}H NMR-spectroscopy (Minispec MQ10 analyzer; Bruker Optics, Billerica, MA). Epididymal fat pads were dissected and weighed at the end of dietary interventions.

Acute fish oil treatment. At 0 and 12 h, mice received an oral dose (0.01 ml/g body wt) of 0.9% NaCl (vehicle), menhaden fish oil (403950; Dyets), or OmegaRx (Ultra-Refine Fish Oil Liquid; Zonelabs), the latter a commercially available n-3 fatty acid–rich preparation containing 360 mg/ml C20:5n-3 and 180 mg/ml C22:6n-3 fatty acids. Tail blood samples for adiponectin measurements were obtained before the initial gavage and at 1, 3, 7, and 24 h.

Fish oil wash-out studies. After feeding mice the 27% fish oil diet for 8 days, the fish oil diet was exchanged with an isocaloric 27% safflower oil diet that was continued until day 15. For measurement of plasma adiponectin concentrations, 15-μl blood samples were collected on days 0, 8, 10, 13, and 15.

Fish oil treatment and coadministration of the PPARγ antagonist bisphenol-A-diglycidyl ether. Wild-type mice had unrestricted access to a 27% (wt/wt) safflower oil or menhaden fish oil diet for 4 days. During dietary regimen, the PPARγ antagonist bisphenol-A-diglycidyl ether (BADGE; Cayman Chemical, Ann Arbor, MI) was subcutaneously injected once daily (1 mg/kg body wt). For measurement of plasma adiponectin concentrations, 15-μl blood samples were collected on days 0, 1, and 4 as described for dose-response studies.

Plasma assays. Total plasma adiponectin concentrations were determined via radioimmunoassay (Linco Research, St. Charles, MO). At the end of treatment, an additional blood sample from vena cava was obtained from fed,

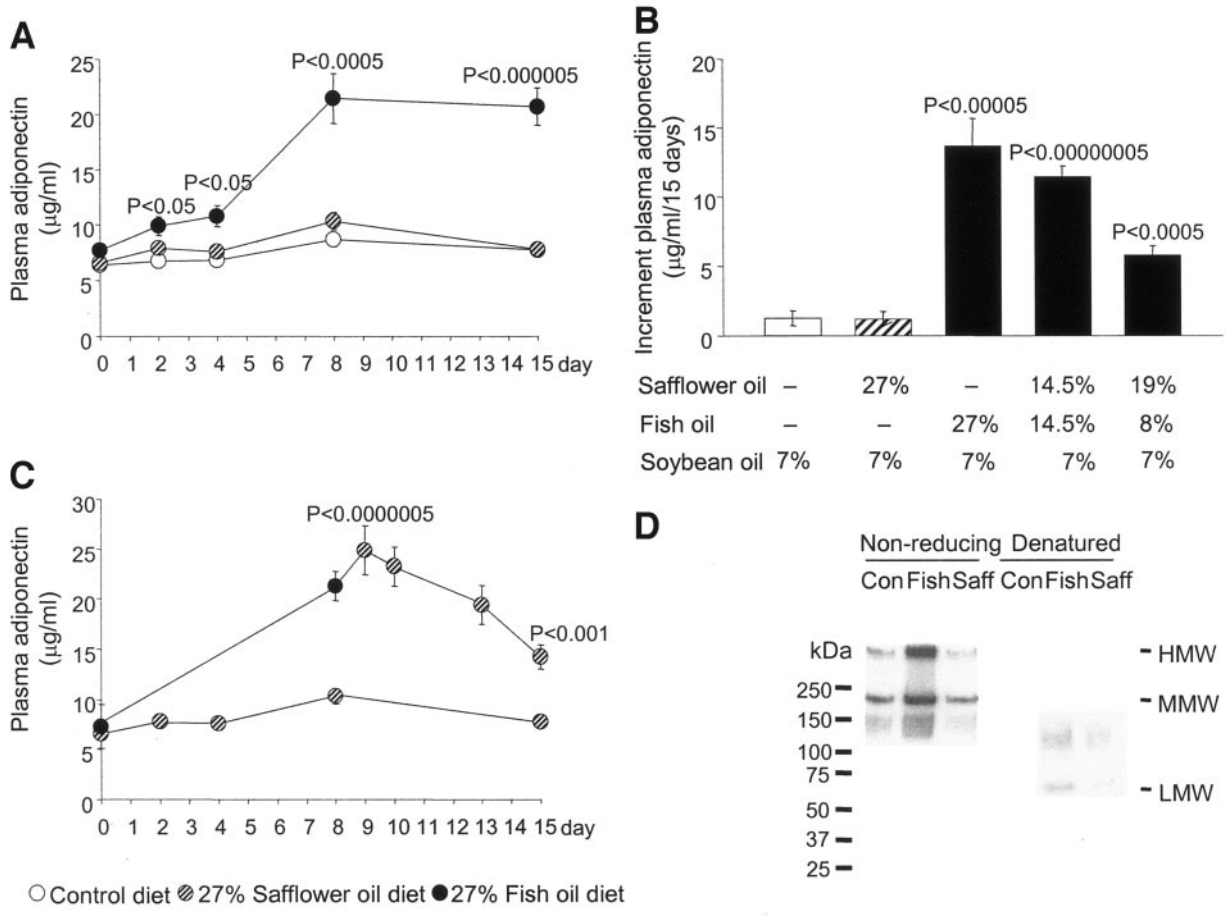

FIG. 1. Effect of fish oil on plasma adiponectin concentrations. Mice were fed a control diet or isocaloric high-fat diets containing 27% safflower oil and 27, 14.5, or 8% fish oil. Total plasma adiponectin concentrations were determined in fed mice on day 0 before and days 2, 4, 8, and 15 during dietary regimen (*A*) and show the increment in plasma adiponectin concentrations from days 0 to 15 (*B*). *C*: Mice were fed a 27% fish oil diet that was replaced by a 27% safflower oil diet on day 8, and plasma adiponectin concentrations were determined on day 0 and day 8 of fish oil diet treatment and after discontinuation of fish oil until day 15. *D*: Nonreducing and denatured plasma adiponectin was determined by Western blotting. Mice were fed a control diet, 27% safflower oil, or 27% fish oil. HMW, high molecular weight (>300 kDa); MMW, middle molecular weight (hexamer, ~167 kDa); LMW, low molecular weight (trimer, ~67 kDa). Data are means ± SE with $n = 10$/group (*A* and *B*) and $n = 6–8$/group (*C*). *P* value indicates statistical difference from safflower oil diet.

isoflurane-anesthetized mice for measurement of plasma metabolites, hormones, and additional adipocyte cytokines. Plasma glucose, triacylglycerol, total cholesterol, HDL cholesterol, nonesterified fatty acid, and β-hydroxybutyrate concentrations were measured via enzymatic methods with a Cobas Mira Analyzer (Roche Diagnostics). Plasma insulin, leptin, resistin (all Linco Research) and corticosterone concentrations (ICN, Costa Mesa, CA) were determined via radioimmunoassay.

Quantitative RT-PCR–based gene expression analysis in adipose tissue. At the end of treatment, mice were anesthetized with isoflurane, and epididymal and subcutaneous adipose tissues were rapidly dissected and snap-frozen in liquid nitrogen. RNA was isolated using a commercially available kit for lipid-rich tissue (Qiagen RNeasy kit; Qiagen, Valencia, CA) in combination with DNase digest treatment. After 1 μg of total RNA was reverse transcribed (Stratagene, La Jolla, CA) with an oligo-prime, PCR was performed with a DNA Engine Opticon 2 System (MJ Research, Boston, MA) using SYBR green QPCR dye kit (Stratagene). The following primers were used: adiponectin, 5′ACAGGAGATGTTGGAATGACAG3′ (F) and 5′-CTGC CGTCATAATGATTCTGTT-3′ (R); PPARγ, 5′-ATGCCAAAAATATCCCTG GTTTC-3′ (F) and 5′-GGAGGCCAGCATGGTGTAGA-3′ (R); CD36, 5′-GA CATGCTTATTGGGAAGACAA-3′ (F) and 5′-TAACCTTGATTTTGCTGCTG TT-3′ (R); and 18S rRNA, 5′-TTCCGATAACGAACGAGACTCT-3′ (F) and 5′-TGGCTGAACGCCACTTGTC-3′ (R). Messenger RNA levels (ΔC_T values), normalized to 18S rRNA were expressed using the comparative method. 18S rRNA levels showed no statistical differences between genotypes.

Western blot analysis. Five-microliter plasma samples were diluted with 20 μl PBS and 50 μl native sample buffer (62.5 mmol/l Tris-HCl, pH 6.8, 40% glycerol, and 0.01% bromophenol blue; Bio-Rad). Ten-microliter samples were separated on a 4–15% Tris-HCl gel (25 mmol/l Tris, pH 8.3, and 192 mmol/l glycine; Bio-Rad) and electrotransferred onto polyvinylidine fluoride membranes (Millipore, MA). Five-microliter plasma samples from the same animals were diluted with 20μl PBS and 25μl Laemmli buffer containing β-mercaptoethanol and 4-μl samples were loaded to visualize the denatured adiponectin complex. Membranes were immunoblotted with an anti-adiponectin antibody (1:500; Abcam, Cambridge, MA).

Statistical analysis. Results are expressed as means ± SE. For phenotypic comparisons between two groups, unpaired *t* tests with a significance threshold of 0.05 were conducted.

RESULTS

Chronic fish oil feeding raises plasma adiponectin concentrations. Feeding mice a diet containing 27% dietary fish oil, rich in n-3 fatty acids, raised plasma adiponectin concentrations 2.7-fold within 15 days compared with mice fed the isocaloric 27% safflower oil diet in a dose-dependent manner (Fig. 1*A* and *B*). Furthermore, when the 27% fish oil diet was replaced with an isocaloric 27% safflower oil diet, plasma adiponectin concentrations remained ~twofold elevated over baseline concentrations for 7 days after the fish oil diet was discontinued (Fig. 1*C*). Gel electrophoresis of nonreducing plasma samples outlined that fish oil increased both high molecular weight and hexameric adiponectin species (middle molecular weight; Fig. 1*D*).

Compared with mice fed the control diet, mice on the

TABLE 1
Physiological and plasma parameters in mice fed a control diet or isocaloric high-fat diets containing 27% safflower oil or 27, 14.5, or 8% fish oil for 15 days

	CONT	27% SAFF	27% FISH	14.5% FISH	8% FISH
Physiologic parameters					
Body wt initial (g)	22.8 ± 0.7	22.7 ± 0.8	22.9 ± 0.6	22.5 ± 0.3	22.6 ± 0.3
Body wt gain (g until day 15)	2.9 ± 0.4	6.2 ± 0.4*	5.1 ± 0.7†	6.9 ± 0.5*	6.6 ± 0.2*
Body fat content initial (% body wt)	11.8 ± 0.4	11.0 ± 0.7	11.3 ± 0.6	11.2 ± 0.6	11.5 ± 0.9
Body fat gain (% body wt until day 15)	10.8 ± 0.9	18.1 ± 2.2†	17.0 ± 2.2†	18.1 ± 1.3†	16.2 ± 1.2†
Epididymal fat (% body wt)	3.0 ± 0.3	4.1 ± 0.0*	4.0 ± 0.2†	4.0 ± 0.1†	4.1 ± 0.3†
Plasma metabolites					
Glucose (mg/dl)	178 ± 9	171 ± 5	171 ± 4	171 ± 12	176 ± 6
Total cholesterol (mg/dl)	133 ± 7	152 ± 4†	117 ± 2‡	140 ± 3§	134 ± 5§
HDL cholesterol (mg/dl)	134 ± 6	148 ± 5	110 ± 1†‡	136 ± 3	142 ± 2
Triacylglycerol (mmol/l)	80 ± 3	81 ± 4	50 ± 4¶‖	62 ± 4†§	77 ± 7
Nonesterified fatty acid (meq/l)	1.22 ± 0.15	1.27 ± 0.03	0.87 ± 0.05†‖	0.97 ± 0.06‖	1.14 ± 0.05
β-Hydroxybutyrate (mmol/l)	0.13 ± 0.02	0.18 ± 0.02	0.23 ± 0.02†	0.20 ± 0.03	0.17 ± 0.02
Plasma hormones and adipocyte cytokines					
Insulin (μU/ml)	31 ± 4	31 ± 4	23 ± 2	27 ± 2	31 ± 4
Corticosterone (ng/ml)	176 ± 23	147 ± 19	153 ± 30	180 ± 24	162 ± 31
Resistin (ng/ml)	4.8 ± 0.3	4.3 ± 0.4	4.7 ± 0.3	4.5 ± 0.4	4.3 ± 0.3
Leptin (ng/ml)	17 ± 3	42 ± 4¶	26 ± 5§	46.7 ± 3.1¶	39 ± 3¶

Data are means ± SE (n = 8/group except whole-body fat measurements n = 4–5/group). Whole-body fat content was determined before and at the end of dietary regimen. Epididymal fat pads were dissected on day 15 of dietary treatment and weighed. *$P < 0.0001$, ¶$P < 0.001$, and †$P < 0.05$ indicate statistical difference from control diet; ‡$P < 0.00001$, ‖$P < 0.001$, §$P < 0.05$ indicate statistical difference from 27% safflower oil diet. CONT, control diet; FISH, isocaloric high-fat diets containing 27, 14.5, or 8% fish oil; SAFF, isocaloric high-fat diet containing 27% safflower oil.

isocaloric high-fat diets markedly gained body weight paralleled by pronounced increases in whole-body fat content and epididymal fat pad weight (Table 1). Plasma concentrations of glucose, corticosterone, and the adipocyte cytokine resistin were not altered by any of the high-fat diets (Table 1).

Feeding mice the 27% fish oil diet markedly lowered plasma cholesterol, HDL cholesterol, triacylglycerol, and nonesterified fatty acid concentrations but increased β-hydroxybutyrate concentrations compared with 27% safflower oil diet–fed mice (Table 1). The majority of effects on plasma lipid metabolites observed with chronic fish oil treatment were dose dependent (Table 1). Compared with mice fed the 27% safflower oil diet, plasma leptin concentrations declined in mice when fed the 27% fish oil diet but remained unaltered when either the 14.5% or the 8% fish oil diet was provided (Table 1).

Fish oil treatment induces transcription of the adiponectin and CD36 gene in epididymal but not subcutaneous adipose tissue. We next investigated whether orally administered fish oil would acutely increase plasma adiponectin concentrations but found plasma adiponectin concentrations unaltered at 24 h after gavage (3.6 ± 0.4 μg/ml, n = 3 vs. 3.6 ± 0.3 μg/ml, n = 3 plasma adiponectin in vehicle-gavaged mice). Compared with vehicle-gavaged mice, the transcription of the adiponectin gene was markedly induced 24 h after fish oil exposure in epididymal adipose tissue, whereas this response was completely blunted in subcutaneous adipose tissue (Fig. 2*A* and *B*). A similar pattern was observed after chronic 27% fish oil feeding for 15 days, which resulted in 1.7- and 1.6-fold transcriptional induction of the adiponectin and CD36/Fatty acid transporter gene in epididymal but not in subcutaneous white adipose tissue (Fig. 3*A*–*F*). Neither the control nor safflower oil diet had a marked impact on adiponectin and CD36 gene transcription in white adipose tissue (Fig. 3*A*–*F*).

To test the direct effect of fish oil on adiponectin gene expression in vitro, we treated 3T3L1 adipocytes with C20:5n-3 (abundant fatty acid in fish oil) and OmegaRX (n-3 fatty acid rich preparation). After a 12-h exposure, we did not observe any direct stimulation in adiponectin mRNA expression compared with adipocytes incubated with fatty acid free BSA alone while troglitazone induced and dexamethasone decreased adiponectin expression (data not shown).

Fish oil treatment raises plasma adiponectin concentrations independent from functional PPARα. To elucidate whether fish oil–mediated increases in plasma adiponectin require functional PPARα, PPARα-null mice were fed a 14.5% fish oil diet for 15 days. Fish oil feeding increased plasma adiponectin concentrations comparably in PPARα-null and wild-type mice resulting in a similar

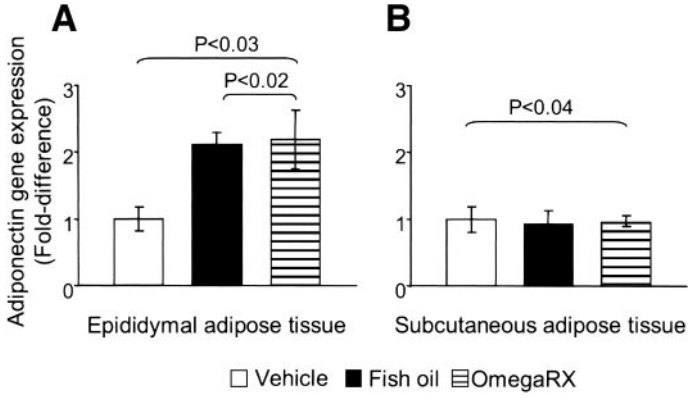

FIG. 2. Acute fish oil administration mediated the transcriptional induction of the adiponectin gene exclusively in intra-abdominal white adipose tissue. Mice received two oral doses of vehicle (0.9% NaCl), menhaden fish oil, or OmegaRX, which served as an n-3 fatty acid–rich positive control, within 12 h. Expression of mRNA in epididymal (*A*) and subcutaneous (*B*) adipose tissue was measured 24 h after the initial dose via quantitative RT-PCR. Data are means normalized to 18S rRNA ± SE with n = 3–7/group. *P* value indicates statistical difference from vehicle.

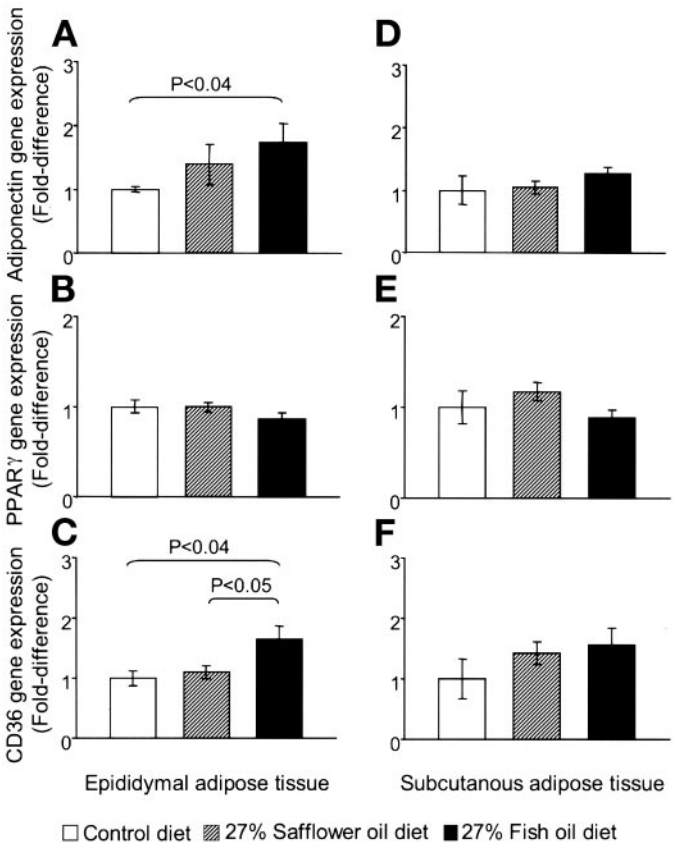

FIG. 3. Chronic fish oil feeding mediated the transcriptional induction of the adiponectin and PPARγ-responsive gene CD36 in intra-abdominal white adipose tissue. Mice were fed a control diet or isocaloric high-fat diets containing 27% safflower oil or 27% fish oil. Expression of mRNA in epididymal (*A–C*) and subcutaneous (*D–F*) adipose tissue was measured after 15 days of dietary treatment via quantitative RT-PCR analysis. Data normalized to 18S rRNA are means ± SE with n = 7/group. *P* value indicates statistical difference from either control or safflower oil diet.

plasma adiponectin concentration on day 15 (31.0 ± 2.8, n = 4 vs. 25.6 ± 3.5, n = 4 μg/ml plasma adiponectin in 14.5% fish oil–fed mice).

The PPARγ antagonist BADGE blocked fish oil–induced increases in plasma adiponectin concentrations. We next tested whether inhibition of PPARγ would affect fish oil–induced increases in plasma adiponectin concentrations in vivo and coadministered the PPARγ antagonist BADGE while mice were treated with a 27% fish oil diet. PPARγ inhibition completely blunted fish oil–mediated increases in plasma adiponectin concentrations throughout the 4 days but lacked any impact on plasma adiponectin concentrations when coadministered in safflower oil diet–fed mice (Fig. 4). The fish oil–mediated transcriptional induction of the PPARγ-responsive gene CD36 was also blocked in epididymal adipose tissue when BADGE was coadministered with fish oil (1.0 ± 0.3 in control diet, n = 3 vs. 2.6 ± 0.1 in fish oil, n = 5 vs. 1.8 ± 0.2 in fish oil + BADGE, n = 8, $P < 0.01$, fish oil vs. fish oil + BADGE)

DISCUSSION

In this study, we show that fish oil progressively raised plasma adiponectin concentrations in mice in a dose-dependent fashion. Furthermore, these effects were long lasting in that they persisted for several days after the discontinuation of the fish oil. N-3 fatty acids are abundant in fish oil and have been shown to serve as PPARα ligands, leading to PPARα activation and subsequent transcriptional upregulation of an array of genes encoding enzymes involved in mitochondrial and peroxisomal and microsomal fatty acid oxidation (16,18–22). To examine whether fish oil might stimulate adiponectin secretion in a PPARα-dependent fashion we examined adiponectin secretion in PPARα-null mice and found that fish oil treatment still resulted in an increase in plasma adiponectin concentrations. These data demonstrate that fish oil stimulates adiponectin secretion in a PPARα-independent manner.

We also examined whether fish oil might promote adiponectin secretion through activation of PPARγ. Thiazolidinediones are synthetic ligands of PPARγ and have been shown to induce expression of the adiponectin gene and increase adiponectin levels both in vivo and in vitro (23–25). We found that acute and chronic fish oil treatment resulted in an approximately twofold increase in the expression of the adiponectin gene in epididymal fat, which was paralleled by an approximately two- to threefold increase in the expression of the PPARγ-responsive gene CD36. Furthermore, coadministration of the PPARγ antagonist, BADGE, completely abrogated the fish oil–mediated increase in plasma adiponectin concentrations paralleled by a blunted expression of the CD36 gene in epididymal adipose tissue, demonstrating that fish oil mediates induction of the adiponectin gene expression in adipose tissue through a PPARγ-dependent mechanism. Taken together these studies suggest that fish oil is a naturally occurring dual activator of PPARα and PPARγ.

Despite any differences in total body fat content between the fish oil– and safflower oil–fed groups, we found distinctly different adiponectin gene expression responsiveness to fish oil in subcutaneous versus intra-abdominal epididymal adipose tissue. Whereas transcriptional activation of adiponectin and CD36 gene expression by fish oil was observed in epididymal adipose tissue, there was no effect of fish oil treatment on adiponectin or CD36 mRNA expression in subcutaneous adipose tissue. It has recently been shown that the transcription factor C/EBPα regulates adiponectin gene expression via response elements in the intronic enhancer in an adipose tissue-specific manner in humans (26). In the current study, we did not observe any differences in the expression of C/EBPα mRNA transcrip-

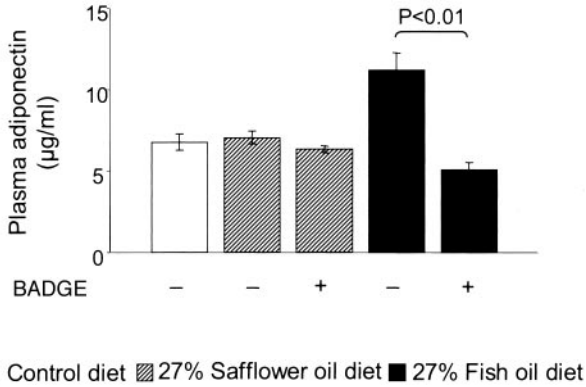

FIG. 4. PPARγ inactivation blunted fish oil–mediated increases in plasma adiponectin concentrations in vivo. Mice were either fed an isocaloric 27% fish oil or 27% safflower oil diet for 4 days with or without coadministration of the PPARγ inhibitor BADGE. Plasma adiponectin concentrations were determined on day 4. Data are means ± SE and n = 4–10/group. *P* value indicates statistical difference from 27% fish oil.

tion in subcutaneous versus epididymal fat (data not shown), suggesting that other unknown factors are involved in the differential responsiveness of the fat pads to fish oil.

The molecular mechanism of fish oil–induced PPARγ activation is still unclear. Although n-3 fatty acids are potentially PPARγ ligands (27,28), fish oil and n-3 fatty acid species (C18:3n3, C20:5n-3, and C22:6n3) failed to stimulate adiponectin mRNA expression in 3T3L1 adipocytes, suggesting that n-3 fatty acids stimulate adiponectin secretion by an indirect mechanism or that they require in vivo metabolic processing to do so.

We conclude that fish oil stimulates adiponectin secretion in epididymal fat in a PPARγ-dependent and PPARα-independent manner and that part of its anti-inflammatory, antiatherogenic, and antidiabetic effects may be mediated by this mechanism.

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