Reductions in Plasma Cholesterol Levels After Fenofibrate Treatment Are Negatively Correlated With Resistin Expression in Human Adipose Tissue

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The adipocyte-derived cytokine, resistin, has been proposed as the link between obesity and type 2 diabetes mellitus in murine models. In humans, resistin is identical to FIZZ3 (found in inflammatory zone 3), which belongs to a family of proteins that appears to be involved in inflammatory processes. To study the mechanisms by which fibrates improve glucose homeostasis, we determined resistin mRNA levels by using relative quantitative reverse-transcriptase-polymerase chain reaction (RT-PCR) in omental white adipose tissue samples obtained from patients treated with placebo or fenofibrate (200 mg/d) for 8 weeks before elective cholecystectomy. Fenofibrate treatment reduced total plasma cholesterol and low-density lipoprotein (LDL)-cholesterol levels by 24% and 35%, respectively. Compared with placebo values, a 2.4-fold induction in resistin mRNA levels was observed in white adipose tissue of fenofibrate-treated patients, whereas no changes were observed in the mRNA levels of the well-known perosixome proliferator-activated receptor (PPAR) target genes CD36, acyl-CoA oxidase, and carnitine palmitoyltransferase. These findings indicate that resistin changes were not related to PPAR activation by fenofibrate. Interestingly, resistin mRNA levels showed a negative correlation with plasma cholesterol levels (r^2 = .53, P = .039, n = 8), but not with triglyceride levels (r^2 = .02, P = .73, n = 8). These results suggest that cholesterol regulates resistin expression in human white adipose tissue.

RESISTIN IS AN adipocyte-derived cytokine that impairs glucose tolerance and insulin action in murine models. Based on these observations, it has been proposed that resistin may represent the long-sought link between obesity and insulin resistance.[1] This cytokine belongs to a family of proteins (resistin-like molecules; RELM) characterized by a cysteine-rich C-terminal domain. Resistin was also identified by other groups as FIZZ3 (found in inflammatory zone 3).[2] To date, 2 other members of the family have been cloned, RELMα/FIZZ1 and RELMβ/FIZZ2. The former is expressed in white adipose tissue, tongue, and lung, where it appears to be involved in inflammatory processes.[3,4] RELMβ/FIZZ2 is intestine-specific, and its biological function remains unknown.

Fenofibrate is a hypolipidemic agent whose effects are mediated by activation of a specific transcription factor called peroxisome proliferator-activated receptor alpha (PPARα).[5] This nuclear receptor is expressed primarily in tissues with a high level of fatty acid catabolism, such as liver, kidney, heart, and skeletal muscle.[6,7] In addition to their hypolipidemic effects, there is growing evidence that fibrates improve glucose homeostasis through not well-defined mechanisms (for review, see Pineda Torra et al[8]). Given the proposed role for resistin in glucose homeostasis, in this study, we focused on the effects of fenofibrate on resistin expression in human adipose tissue. We report that fenofibrate treatment resulted in upregulation of resistin mRNA levels in human white adipose tissue. The changes in resistin expression were not accompanied by changes in the expression of PPAR-target genes and showed a negative correlation with plasma cholesterol levels. These findings suggest that cholesterol is a signal that regulates resistin expression in human white adipose tissue.

MATERIALS AND METHODS

Patients

Omental white adipose tissue samples were obtained from the last 8 patients (4 placebo and 4 fenofibrate-treated patients) of a larger clinical study conducted to assess the effects of fibrates on hepatic peroxisome proliferation. Omental white adipose tissue was obtained from patients (age 61 $\pm$ 9; body-mass index, 29.5 $\pm$ 5.7 kg/m^2) with uncomplicated gall stones disease scheduled for elective cholecystectomy at the Hospital Clínic i Provincial of Barcelona, without systemic or liver disease, a plasma concentration of LDL cholesterol $\geq$ 3.36 mmol/ (130 mg/dL), and no history of intolerance to hypolipidemic agents. The protocol was approved by the local Research and Ethics Committees, and all patients provided written informed consent to participate in the study. Patients were randomly assigned to placebo or treatment groups by using a computer-generated random-number table. Fenofibrate (200 mg) or matching placebo were given as a single dose before the evening meal during 8 consecutive weeks. Patients were advised to follow their usual diet and to maintain their regular level of physical activity. At the end of the treatment period, a laparoscopic cholecystectomy was performed and an omental adipose tissue sample

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of 30 to 50 mg was obtained. Samples were frozen in liquid nitrogen until RNA isolation. Twelve-hour fasting blood samples for lipid determination were also obtained at the beginning and at the end of the study.

Lipid Determinations

Plasma was separated within 30 minutes of blood collection. Total cholesterol and triglycerides were measured with enzymatic techniques.[9] High-density lipoprotein (HDL)-cholesterol was measured after precipitation of apolipoprotein (apo) B-containing lipoproteins.[10] and LDL-cholesterol was calculated using Friedewald's equation.[11]

RNA Preparation and Analysis

Total RNA was isolated by using the Ultraspec reagent (Biotecx Laboratories, Houston, TX). Relative levels of specific mRNAs were assessed by the reverse transcriptase-polymerase chain reaction (RT-PCR). Complementary DNA was synthesized from RNA samples by mixing 1 μg total RNA, 125 ng random hexamers as primers in the presence of 50 mmol/L Tris-HCl buffer (pH 8.3), 75 mmol/L KCl, 3 mmol/L $MgCl_2$, 10 mmol/L dithiothreitol, 200 U Moloney murine leukemia virus reverse transcriptase (Life Technologies, Paisley, Scotland), 20 U RNAsin (Life Technologies), and 0.5 mmol/L each deoxynucleotide (dNTP) (Sigma, Madrid, Spain) in a total volume of 20 μL. Samples were incubated at 37°C for 60 minutes. A 5-μL aliquot of the RT reaction was then used for subsequent PCR amplification with specific primers.

Each 25-μL PCR reaction contained 5μL of the RT reaction, 1.2 mmol/L $MgCl_2$, 200 μmol/L dNTPs, 1.25 μCi [^{32}P]-deoxyadenosine triphosphate (dATP) (3,000 Ci/mmol, Amersham), 1 U Taq polymerase (Ecogen, Barcelona, Spain), 0.5 μg of each primer, and 20 mmol/L Tris-HCl, pH 8.5. To avoid unspecific annealing, cDNA and Taq polymerase were separated from primers and dNTPs by using a layer of paraffin (reaction components contact only when paraffin fuses, at 60°C). The sequences of the sense and antisense primers used for amplification were: resistin, 5′-TCCTCCTCCTCCCTGTCCTGG-3′ and 5′-CAGTGACATGTGGTCTGGGCG-3′; acyl-CoA oxidase (ACO), 5′-GCCCAGGTGAAGCCTGATGGA-3′ and 5′-GACTGGTGCCTCACAGCGCTG-3′; carnitine palmitoyltransferase (CPT-I), 5′-CAGGCCGTGGCCTTCCAGTTC-3′ and 5′-CCATGCTGAGAAGTGCCCGGG; CD36, 5′-CTGTGACCGGAACTGTGGGCT-3′ and 5′-GAAGATGGCACCATTGGGCTG-3′; β-actin, 5′-TTGTAACCAACTGGGACGATATGG-3′ and 5′-GATCTTGATCTTCATGGTGCTAGG-3′. PCR was performed in an MJ Research Thermocycler (Waltham, MA) equipped with a peltier system and temperature probe. After an initial denaturation for 1 minute at 94°C, PCR was performed for 20 (β-actin), 23 (CD36), 25 (CPT-I), and 35 (resistin and ACO) cycles. Each cycle consisted of denaturation at 92°C for 1 minute, primer annealing at 60°C (except 58°C for ACO), and primer extension at 72°C for 1 minute and seconds. A final 5-minute extension step at 72°C was performed. Five microliters of each PCR sample was electrophoresed on a 1-mm-thick 5% polyacrylamide gel. The gels were dried and subjected to autoradiography using Kodak x-ray films (Barcelona, Spain) to show the amplified DNA products. Amplification of each gene yielded a single band of the expected size (resistin, 256 bp; ACO, 161 bp; CPT-I, 295 bp; CD36, 361 bp; β-actin, 764 bp). Preliminary experiments were performed with various amounts of cDNA to determine nonsaturating conditions of PCR amplification for all the genes studied. Thus, cDNA amplification was performed in comparative and relative quantitative conditions.[12] Radioactive bands were quantified by video-densitometric scanning (Vilber Lourmat Imaging, Torcy, France). The results for the expression of specific mRNAs are always presented relative to the expression of the control gene (β-*actin*).

Table 1. Changes in Lipid Variables After 8-Week Courses of Placebo and Fenofibrate Treatment

Plasma Lipids (mg/dL)	Placebo (n = 4)	Fenofibrate (n = 4)
Triglycerides	147 ± 38	112 ± 33 −24%
Total cholesterol	220 ± 35	167 ± 25* −24%
LDL cholesterol	142 ± 38	92 ± 24* −35%
HDL cholesterol	49 ± 9	49 ± 8 0%

NOTE. Results are expressed as mean ± SD. Percent changes between placebo and fenofibrate-treated patients are indicated.
*$P < .05$.

Statistical Analyses

Results are expressed as mean ± SD. Correlations between 2 variables were performed by linear regression using the GPIP computer program (Graph Pad Software, San Diego, CA).

RESULTS

Fenofibrate treatment reduced plasma triglyceride levels by 24% (Table 1). The plasma levels of total and LDL-cholesterol were reduced by 24% and 35%, respectively. In contrast, HDL-cholesterol levels were not affected by drug treatment. Compared with placebo values, a 2.4-fold induction was observed in resistin mRNA levels in omental white adipose tissue of fenofibrate-treated patients (Fig 1A). A similar induction was observed in resistin expression in subcutaneous white adipose tissue after fenofibrate treatment (data not shown). Because fenofibrate mediates its hypolipidemic effects through PPARα activation,[5] we also determined the mRNA expresion of several well-known PPAR-target genes after drug treatment. Fenofibrate treatment did not affect the mRNA levels of acyl-CoA oxidase, carnitine palmitoyltransferase I, or the fatty acid transporter CD36/FAT in adipose tissue (Fig 1B, C, and D). This finding suggests that the changes observed in resistin expression are not related to PPARα activation by fenofibrate. We next analyzed whether the changes in resistin expression after fenofibrate treatment were the result of changes in plasma lipids. A negative correlation was observed between resistin mRNA levels and plasma total cholesterol ($r^2 = .53$, $P = .039$, n = 8) (Fig 2A) and LDL cholesterol ($r^2 = .51$, $P = .046$, n = 8) (Fig 2B). The correlations obtained for total and LDL-cholesterol levels were specific, because no relationship was found between resistin and triglyceride levels ($r^2 = .02$, $P = .73$, n = 8) (Fig 2C).

DISCUSSION

The discovery by Steppan et al[1] that resistin (also known as FIZZ3 and adipose tissue-specific secretory factor) impaired glucose tolerance and insulin action led these investigators to propose that this adipocyte-specific secreted cytokine was the long-sought link between obesity and insulin resistance. In addition, the insulin-sensitizing thiazolidinedione (TZD) class of drugs, which act as agonists for the nuclear receptor PPARγ, inhibited resistin expression in murine adipocytes,[1] affording a

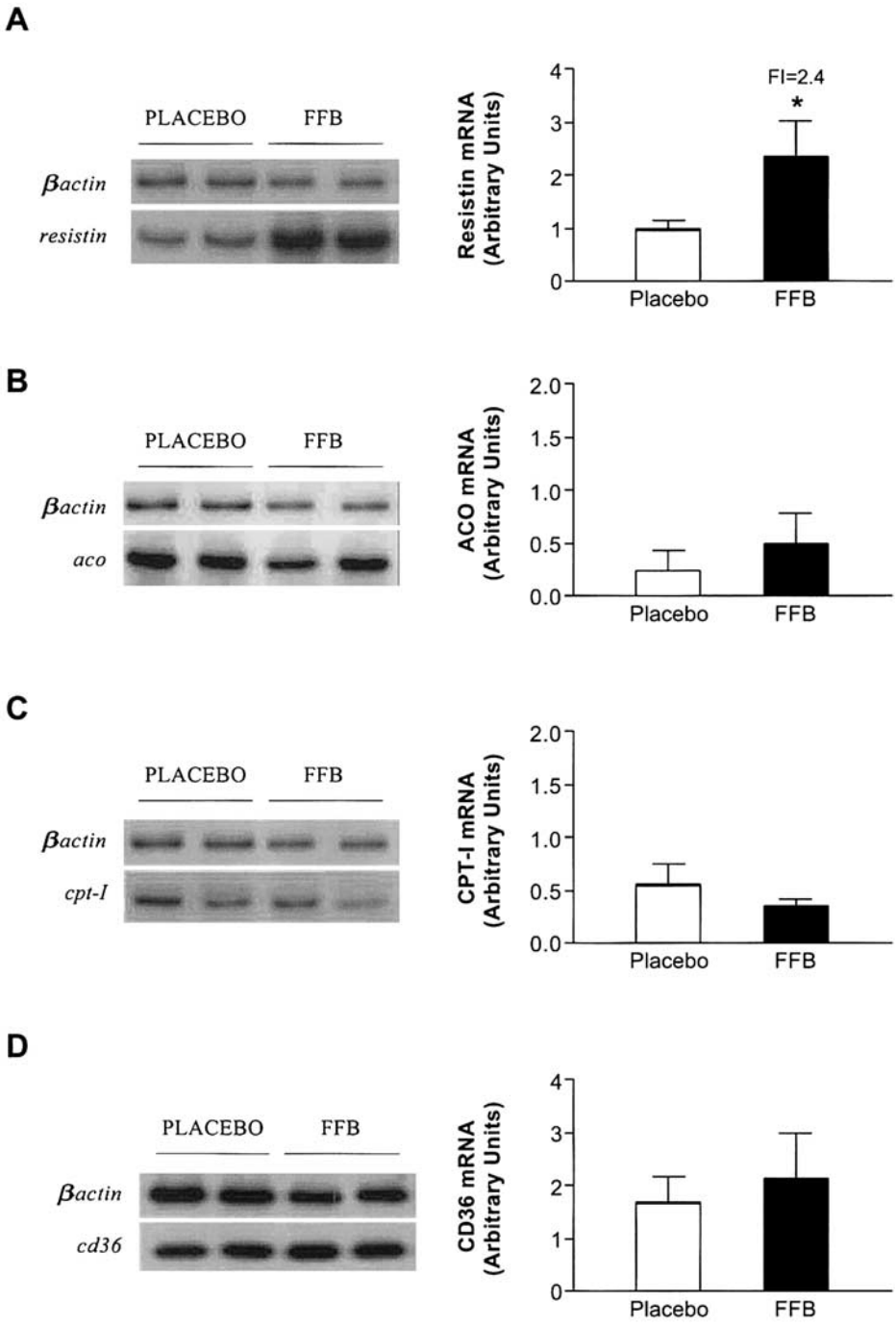

Fig 1. (A) Fenofibrate effects on resisitin, (B) acyl-CoA oxidase, (C) carnitine palmitoyltransferase I, and (D) CD36/FAT mRNA levels in human omental white adipose tissue. Total RNA (1 μg) was isolated from omental white adipose tissue and subjected to RT-PCR. A representative autoradiogram and the quantification of the β-actin-normalized mRNA levels are shown. Data are expressed as the mean ± SD of 4 samples. FI, fold induction. *$P < .05$.

potential explanation for the mode of action of these drugs. However, the role of resistin needs further research because conflicting results were reported by Way et al,[13] who found reduced mRNA levels of resistin in white adipose tissue of several murine models of obesity. Furthermore, treatment with TZD resulted in increased resistin mRNA levels in white adipose tissue.[13]

Given the potential role of resistin in the control of glucose metabolism, we performed this study to investigate whether the improvement in glucose homeostasis afforded by fibrates was mediated through changes in resistin expression in white adipose tissue. The results here presented show that fenofibrate treatment for 8 weeks upregulates resistin mRNA levels in white adipose tissue. The changes observed in resistin expression after fenofibrate treatment seem not to involve PPAR activation, because no changes were observed in well-known PPAR target genes. The lack of direct effects of fenofibrate in white adipose tissue reported in this study

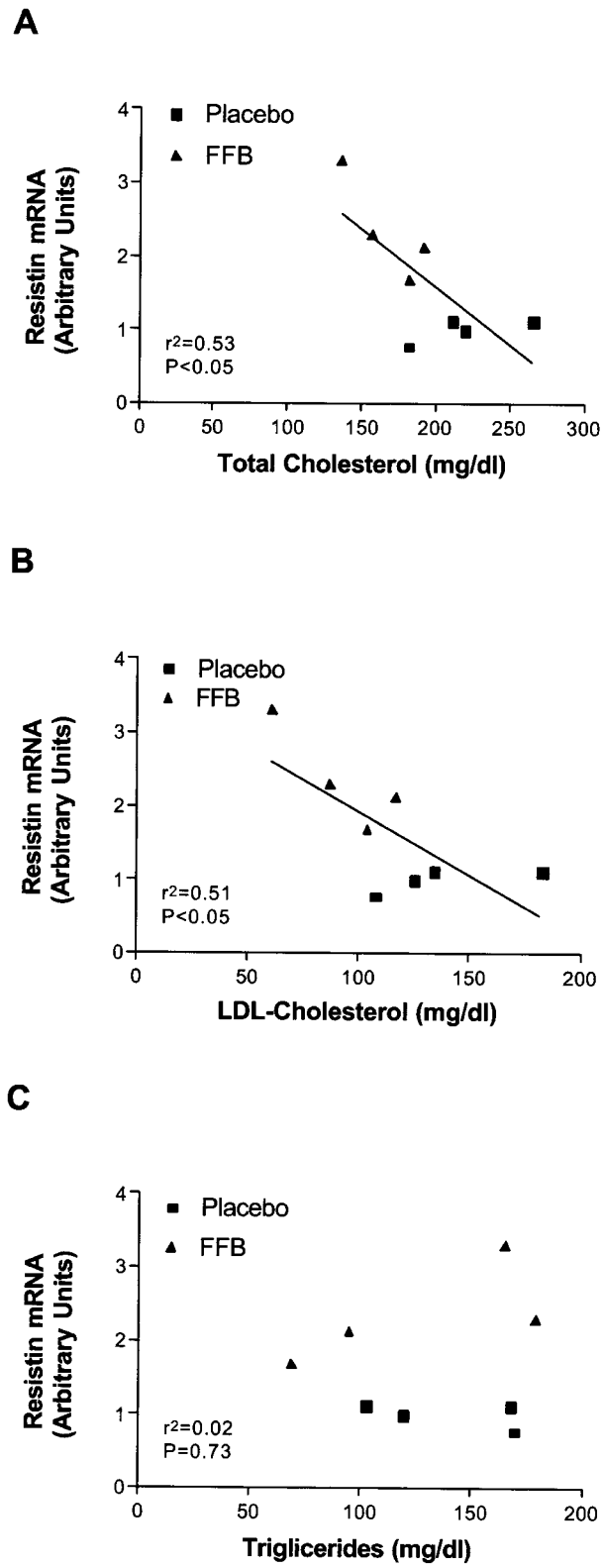

Fig 2. Correlation among resistin mRNA levels and (A) plasma total cholesterol, (B) plasma LDL-cholesterol, and (C) triglycerides. The number of pairs of data analyzed was 8 (4 placebo and 4 fenofibrate-treated patients).

are in agreement with previous results[14] showing and induction in the expression of CD36/FAT in mouse liver, but not in white adipose tissue after fenofibrate treatment. These investigators concluded that at the doses used in their study, fibrates did not appear to have major effects on white adipose tissue.

It has been reported[15] that the pattern of expression and the few biologic functions described for RELM/FIZZ proteins resemble those of other well-known proinflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα), both involved in respiratory and cardiovascular obesity-related problems. In fact, recent reports have shown that resistin expression in human adipose tissue is unrelated to obesity or insulin resistance,[2,16] suggesting that in humans, resistin may have a different biologic function. The negative correlation between resistin expression and cholesterol levels reported in this study indicates that a reduction in cholesterol levels is associated with an upregulation of resistin expression. Interestingly, cholesterol depletion upregulates the expression of TNFα and IL-6 in adipocytes.[17] The similarity between the regulation by cholesterol of TNF and IL-6 by one side and resistin by the other argue in favor of the potential involvement of resistin in inflammatory diseases.

Moreover, the plasma levels of adiponectin, a specifically adipocyte-secreted protein with anti-atherosclerotic effects, are also regulated by changes in plasma lipids. The plasma levels of adiponectin have been negatively correlated with plasma triglycerides and positively correlated with HDL-cholesterol.[18,19] Overall, these results point to a role for the plasma lipid profile in the regulation of adipocyte-derived proteins.

In summary, we show here results suggesting that cholesterol may be a signal that regulates resistin expression in human white adipose tissue. Further studies are required to confirm the biologic actions of resistin and to examine serum resistin levels in patients with chronic inflammatory diseases and dyslipidemia.

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