Insulin-sensitizing effects of thiazolidinediones are not linked to adiponectin receptor expression in human fat or muscle

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Li W, Tonelli J, Kishore P, Owen R, Goodman E, Scherer PE, Hawkins M. Insulin-sensitizing effects of thiazolidinediones are not linked to adiponectin receptor expression in human fat or muscle. Am J Physiol Endocrinol Metab 292: E1301–E1307, 2007. First published January 9, 2007; doi:10.1152/ajpendo.00312.2006.—Circulating adiponectin levels are increased by the thiazolidinedione (TZD) class of PPARγ agonists in concert with their insulin-sensitizing effects. Two receptors for adiponectin (AdipoR1 and AdipoR2) are widely expressed in many tissues, but their physiological significance to human insulin resistance remains to be fully elucidated. We examined the expression patterns of AdipoR1 and AdipoR2 in fat and skeletal muscle of human subjects, their relationship to insulin action, and whether they are regulated by TZDs. Expression patterns of both AdipoRs were similar in subcutaneous and omental fat depots, with higher expression in adipocytes than in stromal cells and macrophages. To determine the effects of TZDs on AdipoR expression, subcutaneous fat and quadriceps muscle were biopsied in 14 insulin-resistant subjects with type 2 diabetes mellitus after 45 mg pioglitazone or placebo for 21 days. This duration of pioglitazone improved insulin’s suppression of glucose production by 41% and enhanced stimulation of glucose uptake by 27% in concert with increased gene expression and plasma levels of adiponectin. Pioglitazone did not affect AdipoR expression in muscle, whole fat, or cellular adipose fractions, and receptor expression did not correlate with baseline or TZD-enhanced insulin action. In summary, both adiponectin receptors are expressed in cellular fractions of human fat, particularly adipocytes. TZD administration for sufficient duration to improve insulin action and increase adiponectin levels did not affect expression of AdipoR1 or AdipoR2. Although TZDs probably exert many of their effects via adiponectin, changes in these receptors do not appear to be necessary for their insulin-sensitizing effects.

Adiponectin is an adipocyte-specific protein that is strongly induced during adipocyte differentiation (2, 15, 26). Circulating levels of adiponectin correlate with insulin sensitivity in humans and rodents (17, 24) and are reduced in human obesity and type 2 diabetes mellitus (T2DM; see Ref. 39). Adiponectin enhances insulin’s suppression of hepatic glucose production in conscious mice (7). Chronically, adiponectin decreases triglyceride content in skeletal muscle and other tissues because of stimulation of AMP kinase and β-oxidation of fatty acids (17, 24). Circulating levels of adiponectin rise in concert with the insulin-sensitizing effects of the thiazolidinedione (TZD) class of peroxisome proliferator-activated receptor-γ (PPARγ) agonists (3, 23, 32). Adiponectin is found as two forms in serum, as a lower-molecular-weight (LMW) trimer-dimer and a high-molecular-weight (HMW) complex (22). Notably, increases in the active HMW complex are tightly correlated with TZD-induced improvements in hepatic insulin action (23, 32).

Screening of a human skeletal muscle cDNA library for binding with a proteolytic cleavage product of adiponectin ("globular" adiponectin) identified two gene sequences encoding transmembrane proteins, termed adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2; see Ref. 41). Expression of AdipoR1 or AdipoR2 in C2C12 myocytes enhanced the binding of bacterially expressed globular and full-length adiponectin and was associated with increased fatty acid oxidation (41). Expression of AdipoR1 and AdipoR2 has recently been reported in 3T3-L1 adipocytes (10) and in mouse adipose tissue (33), with decreased adipose expression of these receptors in insulin-resistant mouse models (33).

There is considerable evidence for direct effects of adiponectin on adipose tissue. Specifically, adiponectin enhances insulin sensitivity and modulates differentiation in 3T3-L1 adipocytes (11). Female transgenic mice with high circulating adiponectin have selective hypertrophy of interscapular and retroorbital fat depots (8). Of note, PPARγ agonists failed to improve glucose tolerance in genetically obese ob/ob mice lacking adiponectin (21). Together, these data suggest that adiponectin may mediate the effects of TZDs on adipocyte differentiation and fat pad remodeling (19) and ultimately on systemic insulin action (21). Of note, adipose tissue contains a complex array of cells and is increasingly infiltrated with macrophages with obesity (38, 40). Because AdipoR1 and AdipoR2 are expressed in human macrophages (5), their expression in adipose tissue macrophages might be of particular relevance to systemic inflammation.

Given the metabolic impact of visceral vs. subcutaneous adipose depots and certain subcellular components of adipose tissue, we examined depot- and cell-specific adipose expression patterns of AdipoR1 and AdipoR2 in nondiabetic human subjects. We show that both AdipoR1 and AdipoR2 are expressed in the main cellular components of human subcutaneous and omental fat yet not correlated with adiposity. Given the important role of PPARγ in adiponectin regulation and insulin action, we also examined the impact of TZD therapy for sufficient duration to improve insulin action, yet before confounding metabolic changes (32). Stepped hyperinsulinemic clamp studies were used to optimally quantify both hepatic and peripheral insulin action in concert with the tissue analysis in
T2DM subjects after TZD therapy. Unlike the expression of adiponectin, receptor expression was not affected by TZD therapy despite improved insulin action.

EXPERIMENTAL METHODS

Part 1: Examination of AdipoR Gene Expression Patterns in Subcutaneous and Visceral Fat Depots

The purpose of this part of the study was to examine expression patterns of adiponectin and its receptors within human visceral and subcutaneous adipose tissue depots as well as in different cell types. We studied a total of 12 nondiabetic subjects with the following characteristics: 7 female, 5 male; age 38 ± 3 yr; body mass index (BMI) 31.6 ± 1.5 kg/m² (range 23–38), with no concurrent illnesses, who were all undergoing nonemergency abdominal surgery, e.g., cholecystectomy, gastric bypass, etc. These subjects were not on any medications known to affect insulin action.

Intraoperative fat biopsies. Patients were admitted on the day of surgery. The evening before their surgery, they were instructed to drink clear liquids only. Before the induction of anesthesia, patients received one dose of a perioperative antibiotic and 5,000 units of subcutaneous heparin. Subcutaneous (1–1.5 cm) and omental (3–5 cm) fat samples were removed within 10–15 min of induction of anesthesia.

Of note, there are no significant changes in plasma insulin, glucagon, or catecholamines following this duration of anesthesia (1). Adipose tissue was either immediately homogenized in TRIzol to inhibit RNAase activity and stored at −80°C or subjected to tissue separation as described below.

Cell separation. The adipose tissue was immediately digested with collagenase type I (0.05 g/30 ml of Hanks’ balanced salt solution with 4% BSA; Worthington Biochemical, Lakewood, NJ) at 37°C with intermittent shaking. Collagenase treatment was maintained until the initial formation of miniscule lipid droplets, which usually required ~60 min. The adipocytes were subsequently separated from the stromal cells by centrifugation at 2,500 rpm for 10 min followed by filtration. Macrophages were separated from the stromal cell fraction by CD14⁺-coated antibody beads (Dynabeads; Dynal Biotech, Lake Success, NY) following the manufacturer’s recommended method.

The separated adipocytes and macrophages were washed with PBS and stored in TRIzol at −80°C for RNA extraction and analysis by RT-PCR.

Part 2: Determine TZD Effects on Adiponectin Receptor Expression and Insulin Action in T2DM Subjects

In the second part of the study, we examined the effects of TZDs on adiponectin gene expression of adiponectin receptors and on insulin action. Type 2 subjects were eligible to participate if they had HbA₁c >8% (13) and were free of macrovascular and microvascular complications. Fourteen subjects with T2DM and no concurrent illnesses were recruited to participate by local advertising (10 male, 4 female, age 46.4 ± 2.3 yr, HbA₁c 9.9 ± 0.5%, BMI 34.1 ± 1.9 kg/m², and duration of diabetes 8.0 ± 1.0 yr). Seven subjects were on a combination of metformin and a sulfonylurea, one on a combination of insulin and metformin, four on metformin alone, and two on insulin alone.

Subjects were African American, six were Hispanic, and two were Caucasian. Each subject underwent a clamp study after taking 45 mg pioglitazone or placebo for 21 days. The studies were randomized and double-blinded, with a washout period of at least 3 wk between each study. All comparisons are between pairs of studies in an individual subject. In a similar study design, we have previously shown that this duration of washout was sufficient to elicit changes in adipose tissue gene expression without confounding changes in glycemia or free fatty acids (FFA; see Ref. 32). Each subject was instructed to follow his or her usual weight-maintaining diet plan, maintaining consistent dietary composition (confirmed by food records) for 3 days before each study. In addition, they were instructed not to change their level of activity while participating in the studies and to refrain from vigorous activity for 3 days before each study. The subjects discontinued sulfonylureas and metformin 3 days prior, short-acting insulin the night before, and long-acting insulin 24 h before the clamp study. Before their enrollment in the study, the purpose, nature, risks, and benefits of the study were explained to all subjects, and voluntary, informed, written consents were obtained.

The following factors all reduced the heterogeneity of the subject population: selection of HbA₁c >8%, a group that displayed considerable metabolic consistency in our previous studies (13, 14, 32), a relatively short duration of T2DM and absence of complications, and withholding other antihyperglycemic medications to remove their systemic effects. Of particular note, all analyses are paired, and the end points all involve intraindividual differences on pioglitazone vs. placebo.

Fat and muscle biopsies under controlled hormonal conditions.

The subjects with T2DM all underwent a 6-h hyperinsulinemic clamp study after taking pioglitazone or placebo for 21 days. All subjects were admitted to the General Clinical Research Center 1 day before the study. Intravenous access was established. To gradually attain euglycemia, insulin infusions were begun at 3:00 A.M. and were adjusted based upon hourly plasma glucose measurements. Subjects fasted overnight but took their pills (pioglitazone or placebo) on the morning of the study. At 7:30 A.M., an additional intravenous cannula was inserted in a dorsal vein of the opposite arm for blood sampling. To obtain arterialized venous blood samples, this hand was maintained at 65°C in a thermoregulated Plexiglas box or warming blanket.

All experiments consisted of 360 min euglycemic (90 mg/dl) insulin/somatostatin (250 μg/h) infusions with replacement of glucoregulatory hormones (1 ng/kg⁻¹·min⁻¹ glucagon; 3 ng/kg⁻¹·min⁻¹ growth hormone) to maintain fixed levels of these hormones throughout. Glucose fluxes were measured with labeled isotopes (HPLC-purified [³⁵S]glucose; bolus 21.6 μCi, then 0.15 μCi/min). To reduce interstudy variability, individual basal insulin replacement rates were established from 0 to 120 min by means of variable rates of insulin infusion (prepared in albumin-containing saline, Novolin Regular; Novo-Nordisk, Princeton, NJ) to keep plasma glucose levels at ~90 mg/dl without the need for glucose infusion. Insulin infusion rates were then increased by 20 μU·m⁻²·min⁻¹ above these basal rates to reproduce physiological hyperinsulinemia from 120 to 240 min. Infusion rates were then increased further, to 150 μU·m⁻²·min⁻¹ above the basal rate, for the final 2 h of the studies. Thus two distinct levels of hyperinsulinemia were used as follows: high physiological levels (plasma insulin levels ~60 μU/ml) to suppress endogenous glucose production (EGP) and pharmacological levels (~400 μU/ml) to stimulate maximal insulin-mediated glucose uptake.

Plasma lipids and liver enzymes were measured at time 0. Plasma glucose levels were measured every 5–10 min in duplicate by a Beckman (Fullerton, CA) glucose analyzer (glucose oxidase method) and maintained at euglycemic concentrations (~90 mg/dl) by a variable infusion of 20% dextrose. From time 0 to 360 min, blood samples were obtained hourly for determination of plasma insulin, G-6-Pase, FFA, and adiponectin levels and every 15 min for [³⁵S]glucose.

At 345 min of the clamp studies, fat and muscle biopsies were obtained from the periumbilical region and mid thigh, respectively, and processed as previously described (11). This point was chosen to study subjects under similar hormonal conditions, normoglycemia, and with maximal glucose uptake. A small 0.25-cm cutaneous incision was performed under local anesthesia (1% lidocaine), and 1–2 g of adipose tissue were obtained by needle aspiration (20). A skeletal muscle biopsy of ~50 mg was obtained with a spring-loaded biopsy needle (Bard Instruments) in the mid thigh region 1.5 cm above the knee following local anesthesia. Biopsy specimens were immediately washed at least three times with saline to remove contaminating blood, homogenized in TRIzol reagent (Invitrogen Technologies) at
the bedside, and subsequently stored at −80°C. Some fat biopsy specimens were also processed for cell separation as described above.

**Analytical Procedures**

Plasma hormones and substrates. Plasma insulin, glucose, C-peptide, FFA, glycerol, lactate, and glucose levels were measured by techniques previously described (14). Total plasma adiponectin was quantified by a Human Adiponectin RIA kit (Linco Research, St. Charles, MO). HMW and LMW adiponectin multimers were measured following separation by Velocity Sedimentation/Gel Filtration Chromatography as described by Pajvani et al. (23).

Glucose turnover. Plasma [3-3H]glucose and triitated water specific activity were measured as previously described (14). Rates of glucose appearance and glucose uptake (or glucose disappearance) were calculated using Steele’s steady-state equation (30). Rates of EGP were calculated as previously described (32).

Quantitative real-time RT-PCR. From the biopsy samples obtained as described above, total RNA was extracted with TRIzol. cDNA was made using the Superscript First Strand Synthesis System for RT-PCR (Invitrogen Technologies). Gene expression was then studied by quantitative “real-time” RT-PCR using the specific protocol for the LightCycler instrument (Roche Diagnostics, Indianapolis, IN). SYBRGreen1 dye (Roche Diagnostics) was used for fluorescent detection of double-stranded DNA. Standard curves were generated for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the genes of interest using plasmids to calculate mRNA copy number. Melting curves were used to determine the specificity of the gene products, which were subsequently confirmed by running the PCR products on agarose gels. All the reactions were performed three times. Primers used were as follows: GAPDH (“housekeeping” gene): forward TGGAGGCTACAAGGGATTTG, reverse GCATCGCCCACTTTGATG; β-actin: forward ACGTCTCCAGCCCTTCCTTCT, reverse CGTCATACCTCCTGCTTGCTGA; ribosomal protein S18: forward GAGGATGAGGTGGAACGTGT, reverse CTTCCTTCCT, reverse CGTCATACTCCTGCTTGCTGA; adiponectin: forward GGTGGGCTCCCTTACAGAACA, reverse TTCCAAAGCATCACAAGGACCA; AdipoR1: forward GGTGTGCCACTTCTAAGCAC, reverse AGCATAAAGGCGAGCCCTCA; and AdipoR2: forward ATGGCCGAGCGCAGAGAG; reverse CTTCAGTGCCTCAGGTTCT; and AdipoR2 did not differ between subcutaneous and omental fat (1.24-fold in omental fat and 1.13-fold in subcutaneous fat, AdipoR1 and AdipoR2 were expressed in both adipocytes and stromal cells (Fig. 1). AdipoR1 expression was approximately twofold higher in adipocytes than in stromal cells, both in omental fat and subcutaneous fat (degree of changes 1.94 and 1.80, respectively, \( P = 0.013 \) and \( P = 0.0007 \)). Additionally, AdipoR2 was also more highly expressed in adipocytes than in stromal cells, both in omental fat (6.3-fold higher, \( P = 0.0006 \)) and in subcutaneous fat (14.1-fold higher, \( P = 0.0004 \)). The receptors were also expressed in macrophages, with fairly comparable AdipoR1 and AdipoR2 gene expression overall in stromal cells and macrophages. Thus, although AdipoR1 was more abundantly expressed than AdipoR2 in macrophages and stromal cells, the expression of AdipoR2 closely approximated that of AdipoR1 in adipocytes and whole fat.

**Part 2: Examination of TZD Effects on Adiponectin Receptor Expression and Insulin Action in T2DM Subjects**

Clamp studies in T2DM subjects. Following 21 days pioglitazone (P+) there were no significant differences in admission (evening before study) glucose levels, lipid levels, or liver enzymes, or in overnight insulin requirements, relative to placebo (P−). Plasma FFA levels showed considerable variability among subjects and were not significantly affected by pioglitazone under fasting (\( P+ = 126.0 \pm 38.4 \) vs. \( P− = 196.06 \pm 30.0 \mu M, P = 0.11 \)), low insulin (\( P+ = 69.2 \pm 22.2 \) vs. \( P− = 93.4 \pm 14.0 \mu M, P = 0.14 \)), or high insulin (\( P+ = 29.9 \pm 11.5 \) vs. \( P− = 36.8 \pm 8.8 \mu M, P = 0.29 \)) conditions. Plasma glucose levels were maintained at 90.8 ± 1.4 mg/dl during the clamp studies with placebo and 90.8 ± 0.6 mg/dl during the clamp studies with pioglitazone (\( P = 0.77 \)). Insulin levels did not differ with pioglitazone vs. placebo at the beginning of the clamp studies (\( P+ = 23.3 \pm 4.4 \) vs. \( P− = 31.7 \pm 7.0 \mu U/ml, P = 0.10 \)) or during either the low-insulin (\( P+ = 65.5 \pm 11.5 \) vs. \( P− = 74.8 \pm 7.9 \mu U/ml, P = 0.3 \)) or high-insulin (\( P+ = 407.1 \pm 35.1 \) vs. \( P− = 425.5 \pm 37.3 \mu U/ml, P = 0.4 \)) phases of the clamp studies.

Pioglitazone enhanced the ability of insulin to suppress EGP during the low-plasma-insulin phase of the clamp studies, with a decrement in EGP from 1.47 ± 0.18 mg·kg\(^{-1}\)·min\(^{-1}\) (placebo) to 0.91 ± 0.13 mg·kg\(^{-1}\)·min\(^{-1}\) (pioglitazone, \( P = 0.008 \)) at 180–240 min (Fig. 2A). EGP was comparably and nearly completely suppressed in response to high insulin concentrations in both P− and P+ (\( P− = 0.42 \pm 0.14 \) mg·kg\(^{-1}\)·min\(^{-1}\) vs. \( P+ = 0.41 \pm 0.19 \) mg·kg\(^{-1}\)·min\(^{-1}\), \( P = 0.78 \)), indicating that these levels of insulin already maximally suppressed EGP in these subjects. Total body glucose uptake in response to low plasma insulin levels was not significantly improved by pioglitazone (\( P+ = 3.33 \pm 0.32 \) vs. \( P− = 3.96 \pm 0.48 \) mg·kg\(^{-1}\)·min\(^{-1}, P = 0.1 \)), whereas in contrast, it was significantly improved at high insulin levels (\( P+ = 10.20 \pm 0.64 \) vs. \( P− = 8.41 \pm 0.70 \) mg·kg\(^{-1}\)·min\(^{-1}, P = 0.005 \); Fig. 2B). Additionally, pioglitazone administration increased total plasma adiponectin levels (\( P+ = 11.49 \pm 2.04 \) vs. \( P− = 5.73 \pm 0.91 \) mg/ml, \( P = 0.014 \)) and the ratio of

**RESULTS**

**Part 1: Gene Expression Patterns of AdipoR1 and AdipoR2 in Subcutaneous and Omental Fat in Nondiabetic Subjects**

The relative level of gene expression was quantified by real-time RT-PCR in subcutaneous and omental adipose tissue from 12 nondiabetic subjects. mRNA copy numbers of AdipoR1 were not significantly higher than AdipoR2 in whole fat (1.24-fold in omental fat and 1.13-fold in subcutaneous fat, \( P = 0.2 \) and 0.3, respectively). The expression of AdipoR1 and AdipoR2 did not differ between subcutaneous and omental depots. No relationship was demonstrated between BMI and gene expression of either receptor.

Cellular separation of adipose tissue revealed that adiponectin gene expression was specific for adipocytes, whereas AdipoR1 and AdipoR2 were expressed in both adipocytes and stromal cells (Fig. 1). AdipoR1 expression was approximately twofold higher in adipocytes than in stromal cells, both in omental fat and subcutaneous fat (degree of changes 1.94 and 1.80, respectively, \( P = 0.013 \) and \( P = 0.0007 \)). Additionally, AdipoR2 was also more highly expressed in adipocytes than in stromal cells, both in omental fat (6.3-fold higher, \( P = 0.0006 \)) and in subcutaneous fat (14.1-fold higher, \( P = 0.0004 \)). The receptors were also expressed in macrophages, with fairly comparable AdipoR1 and AdipoR2 gene expression overall in stromal cells and macrophages. Thus, although AdipoR1 was more abundantly expressed than AdipoR2 in macrophages and stromal cells, the expression of AdipoR2 closely approximated that of AdipoR1 in adipocytes and whole fat.
plasma HMW to total adiponectin ($P = 0.38 \pm 0.04$ vs. $P = 0.19 \pm 0.03$ mg/ml, $P = 0.030$).

Muscle AdipoR1 and AdipoR2 gene expression in T2DM subjects. Both adiponectin receptor subtypes were abundantly expressed in human muscle, with AdipoR1 being $4.5$-fold more abundant than AdipoR2. We did not observe significant differences in muscle gene expression of AdipoR1 ($0.99$-fold change, $95\%$ confidence interval (CI) 0.84–1.15) or AdipoR2 ($0.89$-fold change, $95\%$ CI $0.29$ to 2.08) after pioglitazone therapy (Fig. 3A).

Subcutaneous Fat AdipoR1 and AdipoR2 Gene Expression in T2DM Subjects

Gene expression of adiponectin, AdipoR1, and AdipoR2 from subcutaneous adipose tissue biopsies performed at the end of the clamp studies following 21 days of placebo vs. pioglitazone was analyzed by real-time RT-PCR. Although pioglitazone increased adiponectin gene expression by $1.70$-fold ($P < 0.05$), there were no significant changes in AdipoR1 ($1.33$-fold change, $P = 0.46$) or AdipoR2 expression ($0.92$-fold change, $P = 0.64$; Fig. 3B) expression. Indeed, although there was an upward trend in AdipoR1, its gene expression rose with pioglitazone in two subjects while two subjects showed minimal increase. Gene expression was reduced or unchanged in the remaining subjects such that there was no significant change. Similarly, pioglitazone did not affect adiponectin receptor expression in any of the adipose cellular fractions, including adipose tissue macrophages (Fig. 3C). Although we cannot exclude the possibility that prolonged effects of pioglitazone on gene expression could undermine the results, we found significant effects on insulin action regardless of the order in which pioglitazone was given, indicating that the washout was sufficient to return insulin action to baseline.

Correlation of AdipoR1 and AdipoR2 expression with in vivo measurements. No association was found between BMI and receptor expression levels in adipose tissue in T2DM subjects. We examined a number of correlations between measures of in vivo insulin action and tissue AdipoR expression. In the placebo studies, there was a lack of correlation between baseline rates of glucose uptake and baseline expression of AdipoR1 and AdipoR2 in muscle and between the percent change in glucose uptake with pioglitazone vs. changes in AdipoR1 and AdipoR2. Furthermore, there were no significant relationships between the percent change (i.e., improved suppression) in EGP and the changes in AdipoR1 or AdipoR2 in adipose tissue in response to pioglitazone. By contrast, there was a strong correlation between percent change in EGP with pioglitazone vs. percent change in the ratio of circulating HMW to total adiponectin ($r^2 = 0.8837$), similar to what we previously reported (32).

DISCUSSION

These studies examined the expression of AdipoR1 and AdipoR2 in human adipose tissue, including their expression patterns in specific depots and cell types, and potential relationships between AdipoRs and systemic insulin sensitivity. Unlike the adipocyte-specific expression of adiponectin, these data suggest that AdipoR1 and AdipoR2 are expressed in adipocytes and in adipose tissue macrophages and stromal cells. Expression of the AdipoRs in adipocytes is consistent with the concept of autocrine and/or paracrine effects of this fat-derived hormone. Indeed, hypertrophy of certain fat depots...
in adiponectin transgenic mice may be consistent with direct
adiponectin effects on adipocytes (8). Indeed, TZD-induced
rises in circulating adiponectin may contribute to the known
effects of TZDs on fat redistribution (27). Because adipocyte
differentiation affects adipocyte insulin action and adipokine
production, the changes in fat distribution may be vital to the
insulin-sensitizing effects of TZDs.

Because expression of AdipoRs cannot prove that they are
the only, or even the most functionally significant, adiponectin
receptors in these tissues, additional studies were designed to
examine expression patterns of these receptors in settings of
physiological significance to insulin action and T2DM. Stepped clamp studies in T2DM subjects were performed with
progressive rises in insulin levels designed to quantify both
hepatic and peripheral insulin sensitivity. Of note, expression
levels of AdipoRs in skeletal muscle and adipose tissue were
not correlated with BMI or baseline insulin action in these
subjects with T2DM.

Given the important connections between PPARγ and adi-
onectin, we also examined the impact of a TZD on AdipoR
expression in relation to insulin action. Improved insulin action
with 21 days of pioglitazone has been shown to precede
potentially confounding effects on glucose or FFA levels (32).
Indeed, this short duration of pioglitazone caused approxi-
mately twofold elevations in adiponectin levels and signifi-
cantly enhanced both hepatic and peripheral insulin action in
T2DM subjects. Although there was a small trend toward
increased adipose tissue AdipoR expression with pioglitazone,
this effect was not significant and did not correlate with improved
insulin action. Pioglitazone also did not affect AdipoR expression
in skeletal muscle, and there was no correlation between
changes in muscle AdipoR expression and improved insulin
action in individual subjects. These observations were in
marked contrast to the tight correlation between pioglitazone-
induced increases in HMW adiponectin and improved hepatic
insulin action. It is possible that, with longer duration of
treatment, one may observe changes in AdipoR expression, yet
such changes would be complicated by effects on metabolism
such as changes in glycemia or FFA that could have independent
effects on these receptors and thus complicate the analysis.

Several other studies have examined possible connections
between insulin resistance and AdipoRs in skeletal muscle or
cellular models. AdipoR1/R2 expression in skeletal muscle
was significantly decreased in genetically obese mice, in con-
cert with decreased AMP kinase activation by adiponectin
(33). Mexican Americans with a family history of T2DM
manifested lower skeletal muscle expression levels of AdipoR1
and AdipoR2, in association with reduced insulin sensitivity
(6). However, this finding was not replicated in individuals
without a family history of T2DM (6). AdipoR1 mRNA levels
were significantly lower among transformed lymphocytes from
a small number of diabetic African-American individuals than
among control cell lines from nondiabetic African-American
individuals, but no such difference was observed between cell
lines from diabetic vs. nondiabetic Caucasian individuals (37).
AdipoR expression did not differ between nondiabetic and type
2 diabetic subjects in a French Caucasian population despite
dramatic differences in insulin action (9). Furthermore, mRNA
expression of AdipoRs in myotubes from 40 metabolically
characterized donors was not correlated with insulin sensitivity
(29), and hypocaloric diet did not seem to regulate expression

Fig. 3. AdipoR1 and AdipoR2 expression after 21 days of pioglitazone
therapy or placebo in subjects with poorly controlled type 2 diabetes mellitus
in the following tissues: muscle (A), adipose tissue (B), and adipose tissue
macrophages (C). There was no change in expression of adiponectin receptors
after pioglitazone therapy when compared with placebo.
of these receptors, although insulin sensitivity changed significantly (36). By contrast, 4 wk of exercise training in a group of subjects with varying glucose tolerance appeared to increase circulating levels of adiponectin and AdipoR1/AdipoR2 expression in muscle (4). Finally, genetic analysis of T2DM subjects for candidate polymorphisms in AdipoR1 and AdipoR2 revealed no association of T2DM with both AdipoR1 and AdipoR2 single nuclear polymorphisms in a Japanese population (12) and AdipoR1 with a modest contribution of AdipoR2 variants in a French Caucasian population (35). Thus the association between these receptors and insulin resistance is highly variable and dependent on the population studied.

Consistent with the current findings, neither troglitazone nor rosiglitazone altered AdipoR expression in differentiated human myotubes (18). Additionally, PPARγ activation with rosiglitazone failed to increase expression of either adiponectin receptor in fat of obese mice despite improved insulin sensitivity (34). Rosiglitazone had variable effects on AdipoR expression in humans with T2DM; AdipoR1 expression increased in fat but decreased in muscle, and AdipoR2 was not affected in either fat or muscle (31). Because the treatment was of sufficient duration (12 wk) to impact glucose and insulin levels, effects of these alterations in metabolic status cannot be excluded. However, in the current studies, AdipoR expression did not change significantly with pioglitazone of sufficient duration to change insulin action, and changes in individual subjects are not correlated with changes in insulin action. This suggests that subsequent increases in AdipoR1 expression in fat following a longer duration of TZD treatment would not be likely to contribute to the insulin-sensitizing effects of TZDs.

Adiponectin is a relatively abundant serum protein (circulating in the µg/ml range) with a fairly short half-life (a few hours), suggesting a high level of production of this hormone in adipocytes. Its circulating levels are tightly regulated within a narrow range (22). However, the feedback loop responsible for this tight regulation may be indirect and may also involve other tissues. Given the very high local concentrations of adiponectin in adipose tissue, direct local ligand/receptor interactions within this tissue may not be particularly relevant. The discordant expression patterns of AdipoR and adiponectin observed in response to TZD treatment may be consistent with an indirect mode of adiponectin regulation by the receptors. In addition, mRNA levels of the receptors may or may not be proportional to overall receptor protein levels in the adipocyte and may not necessarily reflect actual levels of the receptors at the plasma membrane.

Indeed, the physiological significance of AdipoR1 and AdipoR2 in mediating systemic effects of adiponectin is subject to ongoing investigation. Although the AdipoR1s were noted to bind the full-length (though bacterially expressed) circulating form of adiponectin, the affinity of binding to this form was manifold lower than the binding affinity with the globular form of adiponectin, which was used in the initial search for potential adiponectin receptors (41). Of note, the globular form is not known to be produced in vivo and has not been detected in the circulation. Indeed, a subsequent search for expressed proteins that specifically bound the mammalian-generated, full-length form of adiponectin revealed a distinct protein, T-cadherin, that is expressed in endothelial and smooth muscle cells (16). Although this receptor binds the hexameric and HMW forms of adiponectin, there is no binding to the trimeric or globular forms of adiponectin.

Another intriguing conundrum about these receptors is the fact that they bear distant homology to the Class A G protein-coupled family of hormone receptors. However, in contrast with all other G protein-coupled receptors, the amino terminus is internal and the carboxy terminus is external in AdipoRs (25). Furthermore, the putative hormone-binding region of the receptor is in an intracellular location. Given the expression of these receptors at the main site of production of their ligand, their particular affinity for a noncirculating form of adiponectin, and the intracellular location of the binding site, it is conceivable that intracellular adiponectin might exert autocrine effects on AdipoRs in adipose tissue. Indeed, based on the structure of the globular domain of adiponectin, one would predict a trimeric receptor with homology to the tumor necrosis factor-R superfamily (28).

In conclusion, these studies are the first to describe the expression patterns of AdipoR1 and AdipoR2 in various depots and cell types of human adipose tissue. Although these receptors are expressed in all of the cell types examined, further study will be required to determine what in vivo role they might play in those tissues. Furthermore, expression of these receptors in skeletal muscle and adipose tissue was not affected by pioglitazone therapy and not correlated with improvements in insulin sensitivity in T2DM subjects. Thus the physiological significance of AdipoR1 and AdipoR2 in mediating systemic effects of adiponectin remains to be further elucidated.

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