Adipose Tissue Resistin Expression Is Severely Suppressed in Obesity and Stimulated by Peroxisome Proliferator-activated Receptor γ Agonists*

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Elevated levels of the hormone resistin, which is secreted by fat cells, are proposed to cause insulin resistance and to serve as a link between obesity and type 2 diabetes. In this report we show that resistin expression is significantly decreased in the white adipose tissue of several different models of obesity including the ob/ob, db/db, tub/tub, and KKAy mice compared with their lean counterparts. Furthermore, in response to several different classes of antidiabetic peroxisome proliferator-activated receptor γ agonists, adipose tissue resistin expression is increased in both ob/ob mice and Zucker diabetic fatty rats. These data demonstrate that experimental obesity in rodents is associated with severely defective resistin expression, and decreases in resistin expression are not required for the antidiabetic actions of peroxisome proliferator-activated receptor γ agonists.

Adipocytes secrete a number of molecules such as tumor necrosis factor-α, leptin, and free fatty acids that can influence the ability of the body to respond to insulin and metabolize glucose (1–4). Recently, a novel 12.5-kDa cysteine-rich protein, termed resistin, was shown to be secreted by adipocytes (5). Resistin expression was markedly induced during the conversion of 3T3-L1 cells to mature adipocytes (5, 6). Administration of resistin to wild type mice impaired glucose tolerance and insulin action, and resistin levels were reported to be increased in genetic and diet-induced forms of obesity (5). When an antibody against resistin was administered to obese mice, an increase in systemic insulin sensitivity was noted (5). Based on these data, it was suggested that resistin serves as a hormonal link between obesity and peripheral insulin resistance in diabetes (5).

Resistin expression was also shown to be regulated by glitazones, a class of insulin-sensitizing drugs approved for the treatment of type 2 diabetes (5). Rosiglitazone and other glitazones lower glucose and lipid levels in patients with type 2 diabetes by activating the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) (7). Rosiglitazone treatment was shown to reduce resistin expression in 3T3-L1 adipocytes in vitro and in the white adipose tissue (WAT) of mice fed a high fat diet (5). These data raised the interesting possibility that decreases in resistin levels might be integral to the antidiabetic actions of PPARγ agonists.

In this report, we have examined resistin expression in several different rodent models of obesity and its regulation in response to different classes of PPARγ agonists. Surprisingly, we find that resistin expression is decreased in obese mice and increased in ob/ob mice and Zucker diabetic fatty (ZDF) rats in response to PPARγ agonists.

MATERIALS AND METHODS

Experimental Animals and Protocols—All procedures performed were in compliance with the Animal Welfare Act, United States Department of Agriculture regulations and approved by the GlaxoSmithKline and Harvard University Institutional Animal Care and Use Committee. Animals were housed at 72 °F and 50% relative humidity with a 12-h light and dark cycle and fed chow diet (Formulab Diet 5008; PMI Feeds Inc., Richmond, IN). Age (9 weeks) and glucose-matched male Zucker diabetic fatty rats (Genetic Models, Inc., Indianapolis, IN) were gavaged twice daily for 7 days with vehicle (0.05 M N-methylglucamine), GW1929 (5.0 mg/kg), or rosiglitazone (3.0 mg/kg). Glucose, triglycerides, and non-esterified fatty acids were measured as described previously (8). Insulin-treated animals received a mixture of Humulin®N and Humulin®R (Lilly) by subcutaneous injection and were sacrificed 6 h later. Genetically obese ob/ob mice were from a colony maintained at Harvard; the db/db, tub/tub, and KKAy mice were from Jackson Laboratories (Bar Harbor, ME). All mice were maintained on standard rodent diet (5% FCC-55020 mg/kg) was administered by daily gavage for 10 days. Rosiglitazone (5 mg/kg) and GW1929 (5 mg/kg) were administered by daily intraperitoneal injections. Ambient blood samples were obtained in the beginning and at the end of the treatment period, and tissues were collected for further analyses 4 h after food withdrawal.

RNA Preparation and Northern Blot Analysis—Total RNA from epididymal white adipose tissue was prepared from ZDF rats, resolved on agarose gels, and blotted as described previously (9). Filters were prehybridized at 68 °C in Express-Hyb (CLONTECH Laboratories, Inc., Palo Alto, CA) for 60 min, followed by hybridization to specific [32P]labeled cDNA probes at a concentration of 1 × 105 cpm/ml for 2 h at 68 °C. Filters were washed twice in 2× SSC/0.1% SDS for 20 min, followed by a single wash for 20 min in 0.1× SSC/0.1% SDS at 60 °C. A rat resistin cDNA clone was isolated from rat adipose tissue RNA by reverse transcriptase PCR using nucleotide sequence reported by Kim et al. (6). PCR oligonucleotide sequences used were as follows: coding strand, GATGCCGTCCTGCGACGGTACT; non-coding strand, GCCTGACACTAACCGGTC. The cDNA was subcloned into pUC18 (Amersham Pharmacia Biotech), sequenced to confirm its identity, and used to make specific radiolabeled probes. Filters were hybridized at 68 °C in Express-Hyb (CLONTECH Laboratories, Inc., Palo Alto, CA) for 60 min, followed by hybridization to specific [32P]-labeled cDNA probes at a concentration of 1 × 105 cpm/ml for 2 h at 68 °C. Filters were washed twice in 2× SSC/0.1% SDS for 20 min, followed by a single wash for 20 min in 0.1× SSC/0.1% SDS at 60 °C. A rat resistin cDNA clone was isolated from rat adipose tissue RNA by reverse transcriptase PCR using nucleotide sequence reported by Kim et al. (6). PCR oligonucleotide sequences used were as follows: coding strand, CAGGCACTTCCCTGCACGGTACT; non-coding strand, GTCCTGACTTCATCAATCGCCGTC. The cDNA was subcloned into pUC18 (Amersham Pharmacia Biotech), sequenced to confirm its identity, and used to make specific radiolabeled probes.
CA). Mouse resistin cDNA was cloned by reverse transcriptase PCR based on the published sequence, cloned and sequenced to confirm its identity, and used in Northern blot analysis as described (10).

RESULTS AND DISCUSSION

Resistin Expression in Obese Mice—Because resistin is identified as a gene negatively regulated by the insulin-sensitizing drug rosiglitazone, and its protein level is increased in the circulation of ob/ob and db/db mice relative to wild type controls (5), it is reasonable to postulate that its expression in adipose tissue would also be increased in obesity. To address this, we examined resistin mRNA expression in several different genetic models of obesity/diabetes including the ob/ob, db/db, tub/tub, and KKAy mice compared with their age-matched lean littermates. Northern blot analysis under high stringency conditions revealed a single, 0.8-kilobase pair resistin mRNA as reported previously (Fig. 1) (5). The resistin mRNA was readily detectable in the adipose tissue of all lean mice (Fig. 1). Unexpectedly, resistin levels were severely decreased in the epididymal WAT of all models of obese mice relative to lean controls (Fig. 1). This suppression was most dramatic in the tub/tub (35-fold) and KKAy (50-fold) mice and was also very substantial in the ob/ob (20-fold) and db/db (15-fold) animals. A similar suppression in adipose tissue resistin mRNA expression was also observed in mice with diet-induced obesity (data not shown). Thus, obesity correlated with severely decreased WAT expression of resistin in these mouse models.

Resistin Expression Is Stimulated by PPARγ Agonists—We next examined the regulation of resistin expression in the WAT of male ob/ob mice treated with different PPARγ agonists including the thiazolidinediones rosiglitazone and MCC-555 and the tyrosine derivative GW1929. Rosiglitazone and GW1929 are full PPARγ agonists (8, 11). MCC-555 profiles as a low affinity full PPARγ agonist in cell-based assays but acts as a potent antidiabetic agent in vivo (12). Treatment with rosiglitazone, GW1929, or MCC-555 resulted in 50, 50, and 30% reductions in serum glucose levels, respectively, relative to treatment with vehicle alone and significant increases in insulin sensitivity (data not shown). As expected, Northern blot analysis demonstrated that each of these compounds stimulated the expression of the PPARγ target genes fatty acid transport protein (FATP) and phosphoenolpyruvate carboxykinase (PEPCK) (Fig. 2A) in WAT. Surprisingly, treatment with each compound also resulted in an increase in resistin expression in WAT (Fig. 2A and B). MCC-555 resulted in a greater increase (8.4-fold) in resistin expression compared with either rosiglitazone (3.4-fold increase) or GW1929 (2.2-fold increase). These data indicate that decreases in resistin expression are not required for the antidiabetic actions of three
different PPARγ agonists in a standard genetic model of insulin resistance.

We next examined the regulation of resistin in the WAT of ZDF rats treated with either rosiglitazone or GW1929. Treatment with rosiglitazone or GW1929 resulted in 46 and 74% decreases in glucose levels, respectively, relative to vehicle-treated animals (data not shown). Northern blot analysis with a resistin-specific probe revealed two transcripts ~0.8 and 1.4 kilobase pairs in length (Fig. 3A). These transcripts are the same size as those reported previously for rat resistin (6). Similar to the ob/ob mice, Northern blot analysis revealed that rosiglitazone or GW1929 treatment resulted in an increase in resistin expression in WAT of ZDF rats (Fig. 3, A and B). In agreement with a previous report (6), resistin expression was also induced by insulin treatment (Fig. 3A). Thus both insulin itself and insulin sensitizers stimulate the expression of resistin in WAT.

Resistin has been proposed to serve as a link between obesity and diabetes, with elevated levels of resistin promoting insulin resistance (5). Moreover, PPARγ agonists have been proposed to enhance insulin sensitivity by decreasing resistin expression (5). Our data do not support either of these proposals. We show that insulin resistance in several common rodent genetic models is associated with decreases in resistin expression. In addition, we demonstrate that different PPARγ agonists all stimulate resistin expression in two standard rodent models of type 2 diabetes. Although we were unable to determine resistin protein levels, it is unlikely that post-transcriptional regulation could account for the magnitude of differences observed in our study. Further studies are needed to determine the mode of regulation and biological functions of resistin and whether it is an effector of insulin resistance in obesity.

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