The adipose tissue-derived hormone adiponectin improves insulin sensitivity and its circulating levels are decreased in obesity-induced insulin resistance. Here, we report the generation of a mouse line with a genomic disruption of the adiponectin locus. We aimed to identify whether these mice develop insulin resistance and which are the primary target tissues affected in this model. Using euglycemic/insulin clamp studies, we demonstrate that these mice display severe hepatic but not peripheral insulin resistance. Furthermore, we wanted to test whether the lack of adiponectin magnifies the impairments of glucose homeostasis in the context of a dietary challenge. When exposed to high fat diet, adiponectin null mice rapidly develop glucose intolerance. Specific PPARγ agonists such as thiazolidinediones (TZDs) improve insulin sensitivity by mechanisms largely unknown. Circulating adiponectin levels are significantly up-regulated in vivo upon activation of PPARγ. Both TZDs and adiponectin have been shown to activate AMP-activated protein kinase (AMPK) in the same target tissues. We wanted to address whether the ability of TZDs to improve glucose tolerance is dependent on adiponectin and whether this improvement involved AMPK activation. We demonstrate that the ability of PPARγ agonists to improve glucose tolerance in ob/ob mice lacking adiponectin is diminished. Adiponectin is required for the activation of AMPK upon TZD administration in both liver and muscle. In summary, adiponectin is an important contributor to PPARγ-mediated improvements in glucose tolerance through mechanisms that involve the activation of the AMPK pathway.

Adiponectin/ACRP30 (adipocyte complement-related protein of 30 kDa), an adipocyte-specific secretory protein, has been shown to modulate insulin sensitivity; however, the mechanism(s) by which it acts are not fully understood (1). A number of clinical studies revealed a strong link between whole body insulin sensitivity and circulating adiponectin levels (2). Furthermore, circulating adiponectin is negatively correlated with the body mass index (3). Weight reduction leads to a significant increase in adiponectin plasma levels slightly preceding improvements in insulin sensitivity, thus suggesting a causative role of adiponectin in enhancing insulin sensitivity (4). Adiponectin null mouse models were described previously, however, with somewhat varying outcomes regarding their metabolic phenotype. Kubota et al. (5) noted mild insulin resistance under basal conditions in heterozygotes (60% reduction in adiponectin serum levels) and more severe insulin resistance in adiponectin null animals. This report differed from adiponectin null mice described by Maeda et al. (6) that showed nearly normal insulin sensitivity when fed on a standard laboratory diet but developed severe insulin resistance in as few as 2 weeks on a high fat/high sucrose diet. However, a third independent report of adiponectin null mice by Ma et al. (7) described an unexpected increase in fatty acid oxidation in skeletal muscle but no effect on insulin sensitivity.

Adiponectin transcript levels are up-regulated in adipocytes upon treatment with thiazolidinediones (TZDs), which parallels increased secretion and elevated adiponectin levels in the circulation (8). TZDs are a novel class of antidiabetic agents that improve systemic insulin sensitivity by enhancing glucose disposal in skeletal muscle and insulin dependent repression of gluconeogenesis in the liver (9). In addition, they significantly reduce circulating free fatty acids and triglycerides, also known to correlate negatively with insulin sensitivity (10). Despite the widespread clinical use of these drugs (rosiglitazone and pioglitazone), the precise molecular mechanisms by which TZDs exert their insulin-sensitizing effects remain largely unknown. TZDs are specific ligands for the γ isoform of the peroxisome proliferator-activated receptor (PPARγ) family of nuclear receptors that are intimately involved in the regulation of energy homeostasis (11). Ligand-activated PPARγ heterodimerizes with the retinoid X receptor and regulates transcription by binding to specific PPARγ-responsive elements within promoters of target genes (12). A number of key glucoregulatory and lipogenic genes (glucokinase, GLUT4, lipoprotein lipase, adipocyte fatty acid transporter protein, fatty acyl-CoA synthase) and genes involved in energy expenditure (mitochondrial uncoupling proteins) as well as other nuclear encoded mitochondrial genes are responsive to TZDs, thus providing a putative mechanism for increased insulin sensitivity (12, 13). PPARγ is predominantly expressed in adipose tissue with nominal expression in muscle and liver, suggesting that adipose tissue is the primary target for TZD action, and therefore anti-diabetic effects

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**Abbreviations used are:** TZD, thiazolidinedione; AMPK, AMP-activated protein kinase; PPAR, peroxisome proliferator-activated receptor; ACC, acetyl-CoA carboxylase; WT, wild type; KO, knock-out; TNF, tumor necrosis factor.
observed in liver and skeletal muscle may largely depend on PPARγ activation in adipocytes (14). A central role for adipose tissue in TZD action is supported by the loss of the glucose-lowering effects of TZDs in the A-ZIP/F1 lipodystrophic mouse model that lacks white adipose tissue (15). Implantation of fat pads from wild type mice into these mice completely restores responsiveness to TZDs. Similarly, we have recently described a mouse model of inducible lipolipatropathy that lost its ability to improve insulin sensitivity in response to TZD treatment in the fatless state (16). Interestingly, adipose tissue is required for the hypoglycemic effects of TZDs, whereas their efficacy as hypolipidemic agents is preserved despite the lack of fat (17).

TZDs have also been shown to activate 5’-AMP-activated protein kinase (AMPK) in both liver and muscle (18). AMPK is a critical metabolic regulator that promotes glucose uptake and fatty acid oxidation. Its activity is stimulated in response to muscle contraction or stresses such as hypoxia and starvation (19). AMPK phosphorylation rapidly inhibits acetyl-CoA carboxylase (ACC), leading to decreased tissue malonyl-CoA content. Malonyl-CoA is an inhibitor of carnitine palmitoyltransferase 1 the rate-limiting enzyme for β-oxidation (20, 21). The net result is an increase in mitochondrial fatty acid oxidation. AMPK activation in the liver has been shown to inhibit gluconeogenic pathways by down-regulating key regulatory enzymes G6Pase and phosphoenolpyruvate carboxykinase (PEPCK) (22). Interestingly, AMPK activation in the liver was found when treated with recombinant, full-length adiponectin (23), and a globular form of adiponectin was able to induce AMPK signaling in skeletal muscle (24).

Using the euglycemic clamp technique, we show that the lack of adiponectin causes primarily a defect in the insulin-dependent suppression of hepatic glucose production without altering glucose uptake and disposal in skeletal muscle. The condition is rapidly aggravated through feeding of a high fat diet. Furthermore, our data suggest that mice lacking adiponectin have an impaired response to PPARγ agonist treatment.

We show that TZD-mediated improvements in glucose tolerance are attenuated in the absence of adiponectin in a genetic model of obesity and diabetes. This defect is linked to a failure to induce AMPK upon treatment with TZDs in adiponectin null mice.

**EXPERIMENTAL PROCEDURES**

**Generation of Adiponectin Knock-out Mice**—A mouse 129/SvEv genomic library was screened, and a positive clone containing the adiponectin genomic sequence was isolated. The targeting vector was generated by inserting a 2.74-kb BamHI fragment and a 4.3-kb SalI to ClaI fragment into pKO922 (Stratagene), flanking the neomycin resistance cassette. The targeting vector was linearized with NotI digestion and transfected into AB21 embryonic stem cells by electroporation with a Bio-Rad Gene Pulser. A total of 750 clones were selected by culturing the transformed embryonic stem cells in the presence of G418 and then analyzed by mini-Southern blot. One correctly targeted clone was identified. The targeted embryonic stem cells were injected into C57BL/6 blastocysts and transferred to foster females for development to term. Germ line transmission was obtained from seven chimeric males. No phenotypic differences were observed between the lines, and all experimental cohorts consisted of wild type and knock-out littersmates.

**ob/ob and Adiponectin Double Knock-out Mice**—Obese mice deficient in adiponectin were generated by crossing adiponectin null mice (Adn KO) with mice lacking the leptin gene (ob/ob, C57/Bl6; Jackson Laboratories) to obtain littersmates heterozygous at both loci. The double heterozygous mice were intercrossed to produce an F2 cohort deficient for adiponectin and leptin (Adn KO, ob/ob). Control groups of Adn KO or ob/ob single knock-outs and wild type littersmates were sibpairs from the same cross.

**Genotyping**—The adiponectin allele was screened for by PCR from tail DNA isolated using the Qiagen DNeasy kit (Qiagen, Valencia, CA). Duplex PCR was run for 15 min at 95 °C followed by 35 cycles of 30 s at 94 °C, 30 s at 64 °C, and 1 min at 72 °C using Qiagen HotStarTaq polymerase (Qiagen, Valencia, CA) and the following primers: ACRP-forward, 5’-GGACCCCTGAACCTGTTCCAC-3’; ACRP-reverse, 5’-CACCCACAGTAATTCCAGGG-3’; and Neo-reverse, 5’-GAATTGGCGTCGACCGCTTCTCGTG-3’. The resulting PCR fragment(s) were run on a 2% agarose gel, and the mice were genotyped by the presence of a 482-bp amplicon for the disrupted allele and a 260-bp amplicon for the wild type allele. Screening for the ob allele was performed as described previously (25).

**Animal Studies**—Male mice were housed at five per cage and allowed ad libitum access either to a regular diet or high fat diet as indicated. The regular chow diet was PicoLab diet 20 (number 50548) with 21% by kilocalories from fat, 22% from protein, and 57% from carbohydrate (LabDiet, Richmond, IN). The high fat diet (number D12492) contained 60% of kilocalories from fat, 20% from protein, and 20% from carbohydrate (Research Diets, New Brunswick, NJ). For PPARγ agonist studies, the animals were weighed every day and gavaged daily with vehicle (0.25% carboxymethylcellulose) with or without rosiglitazone (Cayman Chemical, Ann Arbor, MI) at a dose of 10 mg/kg/day over a period of 10 days.

Plasma glucose and triglyceride determinations were performed from tail blood using glucose oxidase assay kits for glucose (Sigma) and glyceral kinase for triglycerides (Roche Applied Science), respectively. Insulin and adiponectin were measured by radioimmunoassay (Linco Research Inc., St. Charles, MO). At the end of the treatment period, animals were sacrificed, blood was collected by cardiac puncture, and tissues were dissected and snap frozen in liquid nitrogen for further analyses.

**Pancreatic Euglycemic/Hyperinsulinemic Clamp Procedures**—Euglycemic clamps in conscious, unrestrained, catheterized mice were as previously described (1, 26). Food was removed 5–6 h before the in vivo protocol. A solution of glucose (10%) was infused at a variable rate as required to maintain euglycemia (8 mA). The total infusion volume was ~750 μl/mouse. Mice received a constant infusion of high pressure liquid chromatography-purified [3-3H]-glucose (0.1 μCi/min; PerkinElmer Life Sciences) and insulin (3.6 milliunits/kg of body weight/min). Thereafter, plasma samples were collected to determine glucose levels at 10, 20, 30, 40, 50, 60, 70, 80, and 90 min as well as the specific activity of [3-3H]glucose at 40, 50, 60, 70, 80, and 90 min. The average volume of blood withdrawn was ~300 μl/mouse. The basal rate of glucose appearance (Ra) in wild type mice was ~32 mg/kg/min. The insulin infusion stimulated the Rd by 2-fold and suppressed glucose production by 50%. Steady-state conditions for both plasma glucose concentration and specific activity were achieved by 40 min in these studies. At the end of the in vivo studies, mice were anesthetized, the abdomen was quickly opened, portal blood was collected, and liver and hind limb muscle were freeze-clamped in situ with aluminum tongs that were cooled in liquid nitrogen. The time between the injection of anesthesia and freeze-clamping of tissue samples was less than 60 s. Tissue samples were stored at ~80 °C for further analysis. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the Albert Einstein College of Medicine.

**Glucose Tolerance Tests**—Fasted mice (5 h) were given an oral glucose load of 2.5 mg of glucose/g of body weight using a solution of 10% glucose in physiologic saline. Blood was drawn at the indicated times.
and serum glucose concentrations were measured using glucoanalyzer blood glucose strips (MediSense Precision Xtra; Abbott). Access to food was denied during the course of the study.

**Protein Determinations**—Tissues were homogenized using a Con- Torque Power Unit with Kontes glass homogenization tubes in buffer containing 30 mM Na-Hepes (pH 7.4), 2.5 mM EGTA, 3 mM EDTA, 32% glycerol, 20 mM KCl, 40 mM glycerophosphate, 40 mM NaF, 4 mM NaPP, 1 mM Na3VO4, 0.1% Nonidet P-40, 2 mM diisopropyl fluorophosphate, 2 mM phenylmethylsulfonyl fluoride, and protease inhibitors.

Liver homogenates or gastrocnemius muscle homogenates (50 µg) were resolved by SDS-PAGE and transferred to polyvinylidene difluoride membranes (Bio-Rad). The membranes were blocked with 5% nonfat dried milk in TBS (25 mM Tris, 135 mM NaCl, 2.5 mM KCl)/0.05% Tween 20 (TBST) for 1 h at room temperature (phospho-AMPK) or with 5% nonfat dried milk in TBST overnight at 4 °C (phospho-ACC). The membranes were assayed with pACC- or pAMPK-specific antibodies, followed by secondary antibodies conjugated to horseradish peroxidase (Amersham Biosciences or Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Bands were visualized by enhanced chemiluminescence and quantified by laser densitometry in the linear range. AMPK activity and malonyl-CoA content were determined as described before (27). Serum adiponectin concentrations were measured by immunoblotting of serum samples using a polyclonal antibody against mouse adiponectin (bottom).

**FIGURE 1. Targeted deletion of the adiponectin gene.** A, partial restriction map of the adiponectin wild type allele (top). The translation initiation site (ATG) and stop codon are indicated. The targeting construct (middle) was generated by inserting a 2.74-kb and a 4.3-kb fragment of the adiponectin gene adjacent to the neomycin resistance cassette. The disrupted allele (bottom) has a portion of the 5′ upstream region, exons 1 and 2, and the coding region of exon 3 replaced by the neomycin gene. The direction of transcription is indicated by the arrow. The diptheria toxin A fragment gene was used as a negative selection marker. Genomic DNA was cleaved with XbaI to generate Southern blot probes specific for the wild type allele (12.2 kb) or the disrupted allele (6.8 kb). B, PCR-based genotyping of genomic DNA from adiponectin knock-out mice (top). A 246-bp product was amplified for the wild type allele (wt) using specific primers for the 5′-untranslated region of the adiponectin gene. A PCR product of 482 bp was obtained for the targeted allele (ko) using primers specific for the neomycin resistance gene. Heterozygous mice (het) were identified by the presence of both bands when the PCR was analyzed on a 1% agarose gel. Adiponectin concentrations were measured by immunoblotting of serum samples using a polyclonal antibody against mouse adiponectin (bottom).
FIGURE 2. Effect of adiponectin deficiency on glucose kinetics in euglycemic clamp studies. The measurements were obtained while the plasma glucose concentration was maintained at ~8 mg/dl under steady state conditions. A, comparison of the rate of glucose infusion (GIR); B, the rate of hepatic glucose production (GP); C, rates of glucose disappearance (Rd) between WT and adiponectin KO littermates. Animal groups were n = 7, age 10 weeks, *p = 0.014, WT versus KO. D, oral glucose tolerance test of lean mice. Male adiponectin null mice (diamonds, n = 10) and their wild type littermates (squares, n = 9) were kept on a chow diet and analyzed at an age of 10 weeks. They received a dose of glucose (2.5 mg/g body weight) by oral gavage, and glucose was measured subsequently with glucose strips. Absolute fasting glucose levels before the glucose gavage were 148.3 ± 4.3 mg/dl for Adn WT mice and 138.8 ± 7.7 mg/dl for Adn KO mice, respectively. Values are expressed as mean ± S.E. Differences in glucose levels were statistically significant at 30 min. 

RESULTS

Generation of Adiponectin Null Mice—Null mice were created by replacing all three exons and the 5′-untranslated region of the adiponectin gene with a neomycin resistance cassette (Fig. 1A). Our strategy contrasts with previous descriptions of adiponectin knock-out mice, in which only exon 2 of the adiponectin gene was targeted (5, 6). We found that mating of heterozygous pairs yielded normally sized litters at the expected Mendelian ratios. Southern blotting and PCR genotyping with adiponectin-specific primers confirmed the absence of the adiponectin locus (Fig. 1B, top). In addition, we verified the expression of adiponectin protein in the circulation of knock-out mice by immunoblotting (Fig. 1B, bottom) and by radioimmunoassay. Serum adiponectin concentrations in heterozygous mice (Adn HET) were reduced by approximately 50% compared with normal male (8.0 ± 4.9 μg/ml, n = 8) and female mice (18.5 ± 3.6 μg/ml, n = 9).

Adiponectin Null Mice Display Hepatic Insulin Resistance—To analyze the effects of adiponectin on insulin action, we performed euglycemic-hyperinsulinemic clamp studies. 10-week-old male wild type (Adn WT) and adiponectin knock-out (Adn KO) mice, fed regular chow ad libitum, were studied. The glucose infusion rate required to maintain euglycemia was slightly reduced in Adn KO mice compared with wild type littermates (29.8 ± 7.0 versus 40.0 ± 5.7 mg/kg/min, p = 0.142; Fig. 2A). This was primarily due to the impaired ability in Adn KO mice to suppress insulin-stimulated hepatic glucose production (29.1 ± 4.0 versus 15.9 ± 3.5 mg/kg/min, p = 0.014) (Fig. 2B); the calculated rates of peripheral glucose disposal (Rd) in the two groups were similar (58.4 ± 4.3 versus 55.9 ± 5.0 mg/kg/min, p = 0.356) (Fig. 2C). The insulin levels during the clamp were 7.6 ± 1.4 ng/ml in Adn KO and 7.2 ± 0.9 ng/ml in Adn WT mice. The specific activities were as follows: 123.6 ± 15.2 dpm/μg at 60 min, 126.6 ± 20.1 dpm/μg at 70 min, 135.1 ± 19.5 dpm/μg at 80 min, and 122.1 ± 17.1 dpm/μg at 90 min for Adn KO mice and 129.7 ± 15.4 dpm/μg at 60 min, 137.4 ± 15.9 dpm/μg at 70 min, 130.5 ± 8.5 dpm/μg at 80 min, and 130.6 ± 14.1 dpm/μg at 90 min for WT littermates.

In agreement with previous metabolic studies of adiponectin null mice (5, 6), when analyzed by standard glucose tolerance tests, differences in glucose tolerance were marginal in mice fed on a standard chow diet (Fig. 2D). However, when Adn KO mice were put on a high fat diet for a period of 10 weeks, glucose tolerance was significantly attenuated after an oral glucose challenge compared with WT mice, indicating that adiponectin-deficient mice are more susceptible to diet-induced insulin resistance (Fig. 2E). The calories in the high fat diet were derived 60% from fat, 20% from protein, and 20% from carbohydrates. After high fat feeding, the body weights in Adn KO and Adn WT mice were similar (51 ± 4.8 g versus 52 ± 6.0 g, respectively). Basal fasting insulin levels (Adn KO, 2.65 ± 1.5 ng/ml; WT, 2.72 ± 1.8 ng/ml) and insulin 20 min after the glucose challenge (Adn KO, 5.70 ± 2.6 ng/ml; WT, 6.70 ± 3.4 ng/ml) were not significantly different between the two groups.

Maeda et al. (29) have reported elevated TNFα levels in adiponectin null mice (6), and TNFα has often been correlated with reduced adiponectin levels. In our experiments, TNFα levels in the circulation were below the detection limit of a radioimmunoassay-based assay (data not shown). Interleukin-6 levels were not altered in adiponectin null mice compared with their wild type siblings, and in neither group were they affected by treatment with rosiglitazone (data not shown).

Anti-diabetic Effects of TZDs Are Decreased in Obese Adiponectin Null Mice—To analyze whether the insulin-sensitizing effects of TZDs are mediated through adiponectin, we created diabetic mice in which insulin resistance is present and comparable, independent of the status of plasma adiponectin. For this purpose, we crossed the Adn KO, Adn HET, and WT mice into the leptin-deficient, severely insulin-resistant ob/ob background. 10-week-old male mice were treated for 10 days
Adiponectin and PPARγ Agonist Response

FIGURE 3. Glucose tolerance in obese, ob/ob mice lacking adiponectin after treatment with TZDs. A, before TZD treatment (pre-TZD); B, after TZD treatment (post-TZD). Twelve-week-old male mice were treated with rosiglitazone (10 mg/kg of body weight/day) over 10 days and subjected to an oral glucose tolerance test. Blood glucose was measured at times $t = 0$ (prebleded), 30, 60, 90, and 180 min after glucose dosage. The curves for adiponectin and leptin double null mice (Adn KO/ob KO, squares), obese adiponectin wild type mice (Adn WT/ob KO, triangles), and lean control mice (Adn WT/ob WT, diamonds) are shown. Values are percentage induction from basal glucose levels. *, $p \leq 0.05$.

with the PPARγ agonist rosiglitazone (10 mg/kg of body weight/day) or vehicle by daily gavage. The treatment induced circulating adiponectin levels in wild type mice by $\sim$4-fold from 7.1 ± 2.8 μg/ml before treatment to 29.4 ± 17.1 μg/ml after the treatment ($p = 0.005$). Oral glucose tolerance tests (2.5 mg glucose/g of body weight) performed after an overnight fast revealed that ob/ob mice, independent of the presence of adiponectin (Adn KO/ob KO, $n = 11$; Adn WT/ob KO, $n = 11$), were glucose-intolerant when compared with lean control mice (Adn WT/ob WT, $n = 6$) prior to treatment with rosiglitazone (Fig. 3A). However, whereas the Adn WT/ob KO mice substantially improved their glucose tolerance, adiponectin-deficient mice remained severely glucose-intolerant after the treatment with TZDs (Adn KO/ob KO; Fig. 3B).

Both groups had comparable body weights at the end of the TZD treatment (Adn WT/ob KO, 80.3 ± 7.3 g; Adn KO/ob KO, 81.3 ± 10.9 g), although the net weight gain during the treatment was higher in the wild type mice. Whether or not adiponectin can be implicated directly in the TZD-mediated weight gain remains to be assessed. Insulin levels did not change significantly during the TZD treatment and were similar in adiponectin null and wild type mice in the ob/ob background (data not shown).

Activation of AMPK by TZDs Is Reduced in Adn KO Mice—We next assessed whether TZD treatment differentially affected AMPK activity in control and Adn KO mice. Liver and gastrocnemius muscle were studied from young, lean mice (10 weeks) treated with rosiglitazone or vehicle for 10 days as described above. Serum adiponectin levels increased on average 4.8-fold from 8.0 ± 4.9 μg/ml at basal to 38.9 ± 23.9 μg/ml after TZD treatment ($p = 0.003$). In liver, AMPK activity was increased more than 2-fold, and the concentration of malonyl CoA was diminished by 40% in Adn WT mice compared with vehicle-treated control mice, whereas no effect of TZDs was observed in Adn KO mice (Fig. 4, A and B). In keeping with these changes, pACC (Fig. 4C, middle) was increased only in the TZD-treated Adn WT mice; however, pAMPK was unchanged (Fig. 4C, left). Similar changes in AMPK activity and malonyl CoA in TZD-treated Adn KO and WT mice were observed in muscle (Fig. 5, A and B). However, in contrast to liver, pAMPK (Fig. 5C, left) was increased, but no change in pACC was observed (Fig. 5C, middle). In neither tissue was AMPK abundance altered by TZD treatment (Figs. 4C and 5C, right). In the absence of TZD treatment, we found no difference in AMPK activity, ACC phosphorylation, or malonyl CoA content in the livers of Adn KO and WT mice (Fig. 4, WT versus KO). Furthermore, in muscle, AMPK activity tended to be increased and malonyl-CoA decreased in Adn KO mice, although differences were not statistically significant (Fig. 4, WT versus KO).

**DISCUSSION**

Injection of a physiological dose of full-length, recombinant adiponectin acutely lowers glucose levels in mice (1, 30). On the other hand, a chronic, $\sim$3-fold increase of endogenous serum adiponectin levels in a transgenic mouse model leads to improvements in insulin sensitivity due to decreased hepatic glucose output (31). In both of these experimental models, moderate increments of adiponectin have been shown to improve hepatic insulin sensitivity. In contrast, under both conditions, glucose uptake in peripheral tissues was not altered when glucose levels were clamped in the presence of a modest hyperinsulinemia (1, 30). To better understand the physiological aspects of adiponectin function, we created a genetic mouse model lacking adiponectin. Our strategy differed slightly from that for previously described adiponectin null mice in that we deleted all three exons and the 5′-untranslated region. We aimed to further analyze the metabolic phenotype of adiponectin null mice using the euglycemic clamp procedure. Our clamp data from adiponectin null mice substantiate observations made previously with mice overexpressing adiponectin, highlighting the liver as the main target of adiponectin function. The data presented here clarify some of the variations reported previously on the insulin sensitivity in adiponectin null mice. As suggested in previous studies, we observe a rapid deterioration of glucose tolerance in our adiponectin null mice when challenged metabolically with a high fat diet that can be measured easily by standard glucose tolerance tests. This is, however, the first study to provide detailed information on insulin sensitivity of mice deficient in adiponectin under euglycemic clamp conditions. The hyperinsulinemic clamps reveal significant impairments in hepatic glucose production already at a young age and even when fed a standard chow diet. This observation was not previously made in Adn KO mice with glucose tolerance tests and probably reflects an increased sensitivity of the euglycemic clamp technique.

Adiponectin might be required to maintain a "basal tone" of insulin responsiveness, and therefore small variations in adiponectin levels may have considerable effects on insulin sensitivity. Although adiponectin is relatively abundant (1–20 μg/ml circulating levels) and has a relatively short half-life (28), the serum levels of adiponectin remain remarkably constant, with only limited diurnal fluctuations. Changes occur over weeks to months in the clinical setting, and therefore small changes over time are very meaningful. A notable aspect of many animal models carrying chromosomal deletions of adipocyte-specific secretory proteins, such as resistin or leptin, is the relatively mild phenotype for the loss of function mutation, whereas gain of function models display much more dramatic effects. This is true for resistin and adiponectin, and even in the case of leptin there is a spread of the intensity of the ob/ob phenotype, depending on the genetic background of the mice. In contrast to mice overexpressing adiponectin, the phenotype of mice...
lacking adiponectin is subtle and therefore more apparent under clamped conditions. However, our observations are consistent with a number of studies that have implicated adiponectin functionally in increasing insulin sensitivity rather than simply as a marker of obesity and insulin resistance (2). In the present study, we demonstrate that improvements in glucose tolerance after treatment with PPARγ agonists are significantly dependent on the presence of adiponectin in a significant manner. We show that induction of adiponectin secretion from adipocytes by TZDs plays an important role in the antihyperglycemic action of these compounds. Treatment with an intermediate dose of rosiglitazone for 10 days causes a robust (~4-fold) induction of adiponectin in serum of ob/ob mice as well as mice fed a high fat diet. As shown previously, such an induction of adiponectin slightly precedes improvements in fasting serum glucose and triglyceride levels (8). TZD treatment of obese and insulin-resistant mice devoid of adiponectin does not lead to an improvement of glucose tolerance, as seen in mice that are able to induce the release of adiponectin from adipose tissue. In keeping with this observation, we found that rosiglitazone treatment for 10 days leads to a clear cut activation of the AMPK-ACC axis, ultimately resulting in significant reduction of both hepatic and muscular malonyl-CoA content. The failure of TZDs to activate AMPK pathways in adiponectin null animals supports the view that the phosphorylation of AMPK is a critical downstream signal of adiponectin action in liver and skeletal muscle. Nevertheless, our experiments cannot exclude an adiponectin-independent component of TZD action on glucose tolerance. TZD-mediated improvements in whole body insulin sensitivity are the sum of multiple direct and indirect factors, some of which affect pathways that do not involve the induction of AMPK in the liver or muscle nor the release of adiponectin from fat. For instance, TZDs promote adipocyte differentiation and thereby divert the flux of free fatty acids from liver and muscle to adipose tissue (32). It has been suggested that this accounts for the ability of TZDs to lower plasma free fatty acid levels.
and correct the excessive lipid accumulation in liver and muscle, which correlates closely with insulin resistance (33). In addition, TZDs have been shown to decrease serum levels of resistin, an insulin-desensitizing factor released by adipocytes (34). The contribution of this adipokine to TZD-related glycemic control remains to be evaluated. The question of whether TNFα expression in adipose tissue is relevant for TZD action was addressed recently by Wellen et al. (35). Using TNFα/ob/ob double knock-outs, they examined the role of TNFα in the lowering of serum triglycerides, free fatty acids, and glucose by TZD treatment and concluded that TNFα is not involved in mediating these effects.

In summary, our data suggest that adiponectin is an important mediator of the improvements of TZD therapy on whole body glucose tolerance. TZD-induced improvement in glucose tolerance is attenuated in adiponectin null mice. Furthermore, our studies suggest that the ability of TZDs to activate AMPK in liver and skeletal muscle is adiponectin-dependent. It remains to be determined whether selective means to induce adiponectin or AMPK have a positive impact on insulin sensitivity in patients with obesity and type 2 diabetes.

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