Adiponectin-Dependent and -Independent Pathways in Insulin-Sensitizing and Antidiabetic Actions of Thiazolidinediones

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The metabolic syndrome has become one of the major public health challenges worldwide (1,2) and is thought to result from obesity and obesity-linked insulin resistance, the combination of which promotes diabetes, hypertension, hyperlipidemia, and cardiovascular diseases (1,2). Obesity, defined as increased adipose tissue mass, is mainly characterized by adipocyte hypertrophy, especially in adulthood (1,2). Adipose tissue serves as the site of triglyceride storage and free fatty acid (FFA)/glycerol release in response to changing energy demands (1). Adipose tissue also participates in the regulation of energy homeostasis as an important endocrine organ that secretes a number of biologically active "adipokines," such as FFA (3), tumor necrosis factor (TNF)-α (4), resistin (5), and leptin (6). Although the association of obesity and insulin resistance has been recognized, the mechanisms by which obesity causes systemic insulin resistance largely remain unclear. One such mechanism is upregulation of insulin resistance–inducing adipokines, such as FFA, TNF-α, and resistin (Fig. 1). In contrast to such insulin resistance–causing adipokines, adiponectin, as well as leptin, is one of the adipokines that directly sensitizes the body to insulin, and its expression and serum levels are known to be upregulated by thiazolidinediones (TZDs), a group of insulin sensitizers. In this review, we describe recent progress in research into the role of adiponectin in amelioration of insulin resistance and diabetes by TZDs.

TZDs DECREASE PRODUCTION AND SECRETION OF INSULIN RESISTANCE–CAUSING ADIPOKINES VIA GENERATION OF SMALL ADIPOCYTES

TZDs have been shown to increase insulin action in skeletal muscle and liver in animal models of obesity-linked insulin resistance and diabetes, and TZDs have been widely used for the treatment of type 2 diabetes (7–10). Peroxisome proliferator–activated receptor-γ is a family of ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily and plays a critical role in the regulation of adipocyte differentiation (11–15). TZDs bind to and activate peroxisome proliferator–activated receptor-γ in adipose tissue, thereby promoting adipose tissue differentiation and increasing the number of small adipocytes that are more sensitive to insulin and decreasing the number of large adipocytes by inducing apoptosis (16–19). Because peroxisome proliferator–activated receptor-γ is predominantly expressed in adipose tissue, it is reasonable to speculate that the effect of TZDs on insulin resistance in skeletal muscle and the liver is mediated largely via the effects of TZDs on adipose tissue, including alterations of adipokine expression and secretion by adipocytes (10,17–21). Generation of small insulin-sensitive adipocytes by TZDs lowers circulating serum FFA levels and downregulates the production and secretion of TNF-α and resistin (4,5,16,20–23), subsequently ameliorating insulin resistance (20,21) (Fig. 1). However, it remains to be elucidated how these effects by TZDs participate in the amelioration of insulin resistance in skeletal muscle and the liver.

TZDs INCREASE EXPRESSION AND SECRETION OF A MAJOR INSULIN-SENSITIZING ADIPOKINE, ADIPONECTIN

Adiponectin is an adipose tissue–derived secreted protein that circulates in serum (24–27). We previously reported that replenishment of adiponectin ameliorated insulin resistance in obese mice with decreased serum adiponectin levels and that a combination of physiological doses of adiponectin and leptin reversed insulin resistance in lipoatrophic mice (28). Independently, administration of adiponectin has been reported to decrease plasma glucose levels by suppressing hepatic glucose production (29,30), and administration of globular adiponectin reportedly lowers elevated fatty acid concentrations by oxidizing fatty acids in muscle (31). In fact, adiponectin deficient ($adipo^{-/-}$) mice are insulin resistant and glucose intolerant (32–34). Previous studies have shown that adiponectin stimulates fatty acid oxidation in skeletal muscle and inhibits glucose production in the liver by activating AMP-activated protein kinase (AMPK) (35) through its specific receptors, AdipoR1 and AdipoR2 (36). As a result,

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Received for publication 2 May 2006 and accepted in revised form 24 May 2006.

This article is based on a presentation at a symposium. The symposium and the publication of this article were made possible by an unrestricted educational grant from Servier.

AMPK, AMP-activated protein kinase; EGP, endogenous glucose production; FFA, free fatty acid; GIR, glucose infusion rate; HMW, high-molecular-weight; TNF, tumor necrosis factor; TZD, thiazolidinedione.

DOI: 10.2337/db06-S005

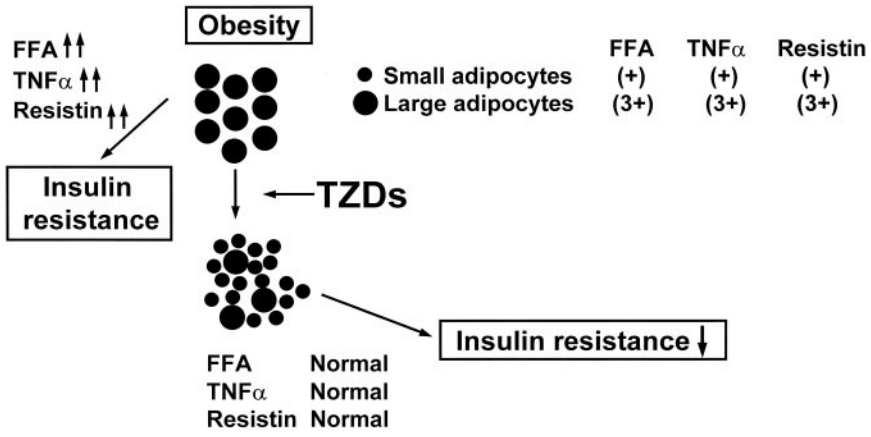

FIG. 1. TZDs decrease insulin resistance–causing adipokines via generation of small adipocytes. TZDs promote adipocyte differentiation and increase the number of small adipocytes and decrease the number of large adipocytes. Generation of small insulin-sensitive adipocytes by TZDs lowers circulating serum FFA levels and downregulates the production and secretion of TNF-α and resistin, subsequently ameliorating insulin resistance.

adiponectin has come to be recognized as a major insulin-sensitizing hormone. The expression and serum levels of adiponectin have been shown to be upregulated by TZDs (28,37–39). The expression of adiponectin was increased during adipocyte differentiation in 3T3L1 adipocytes and also increased with rosiglitazone in differentiated 3T3L1 adipocytes (28) (Fig. 2). Moreover, serum adiponectin levels in obese diabetic mice and patients with type 2 diabetes were increased after pioglitazone administration (Fig. 2) (28,37,38). These findings suggest that TZDs may upregulate adiponectin via generating small adipocytes that abundantly express and secrete adiponectin and/or directly activating adiponectin gene transcription (39).

TZDs INCREASE HIGH-MOLECULAR–WEIGHT ADIPONECTIN

Adiponectin is known to form three major characteristic multimers in serum: a trimer (low-molecular-weight), a hexamer (middle-molecular-weight), and 12–18mer (high-molecular-weight [HMW]) adiponectin (40). Several observations suggest that HMW adiponectin is the more active form of the protein and appears to have a more relevant role in improving insulin sensitivity and exerting an antidiabetes effect (41–43). Moreover, changes in the ratio of serum HMW adiponectin to total adiponectin correlate with improvement in insulin sensitivity in both mice and diabetic patients, whereas changes in total serum adiponectin levels do not show good correlations at the individual level (42). We investigated whether TZDs affect the forms of serum adiponectin in obese diabetic mice models and found that both HMW adiponectin and the ratio of HMW to total adiponectin were decreased in vehicle-treated obese and diabetic KKAy mice compared with wild-type KK mice (Fig. 3) (44). While the restriction of food intake by pair-fed mice partially restored the decrease in both HMW adiponectin and the ratio of HMW to total adiponectin in KKAy mice (Fig. 3) (44), rosiglitazone treatment dramatically increased total adiponectin and the ratio of HMW to total adiponectin (Fig. 3) (44). It is noteworthy that the ratio of serum HMW adiponectin to total adiponectin correlated more significantly with glucose tolerance or insulin levels than the total adiponectin level (43), suggesting that serum HMW adiponectin alterations may be more relevant to the prediction of insulin resistance than serum total adiponectin alterations. Consistent with this, total adiponectin, HMW adiponectin, low-molecular-weight adiponectin, and the HMW-to-total adiponectin ratio all correlated significantly with key

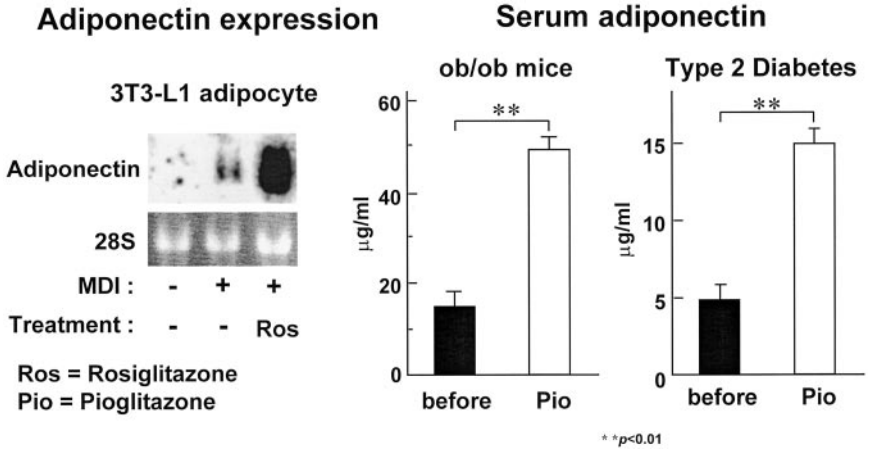

FIG. 2. TZDs increase a major insulin-sensitizing adipokine, adiponectin (28,37,38). The expression of adiponectin was increased during adipocyte differentiation in 3T3L1 adipocytes and also increased with rosiglitazone in differentiated 3T3L1 adipocytes. Moreover, serum adiponectin levels in obese diabetic mice and patients with type 2 diabetes were increased after pioglitazone administration.

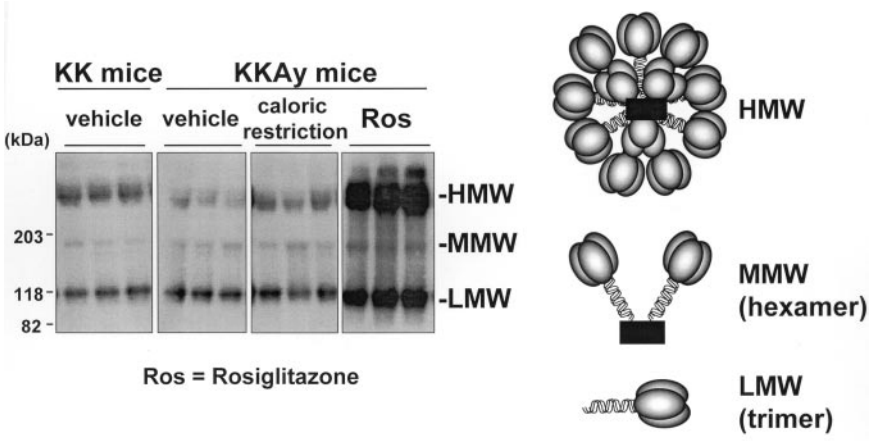

FIG. 3. TZDs increased HMW adiponectin (44). Both HMW adiponectin and the ratio of HMW to total adiponectin decreased in obese diabetic KKAy mice compared with control KK mice. While the restriction of food intake partially restored the decrease in both HMW adiponectin and the ratio of HMW to total adiponectin in KKAy mice, rosiglitazone treatment dramatically increased total adiponectin and the ratio of HMW to total adiponectin. LMH, low-molecular-weight; MMW, medium-molecular-weight.

features of central obesity and the insulin-stimulated glucose disposal rate (45). However, HMW levels, not total adiponectin levels, are primarily responsible for these relationships, suggesting that measurement of the HMW levels may be superior to measuring total adiponectin (45).

LOW DOSE OF PIOGLITAZONE IMPROVES DIABETES AND INSULIN RESISTANCE IN *OB/OB* MICE, BUT NOT IN *ADIPO*$^{-/-}$*OB/OB* MICE

Since adiponectin is an insulin-sensitizing adipokine, it is reasonable to speculate that TZDs increase insulin sensitivity, at least in part, by increasing serum adiponectin. However, whether the TZD-induced increase in serum adiponectin is causally involved in TZD-mediated insulin-sensitizing effects has not been addressed experimentally. To address this issue, *adipo*$^{-/-}$*ob/ob* mice with a C57Bl/6 background were used to investigate whether the TZD pioglitazone is capable of ameliorating insulin resistance in the absence of adiponectin (37). The absence of adiponectin had no effect on either the obesity or the diabetic phenotype of *ob/ob* and *adipo*$^{-/-}$*ob/ob* mice. *Ob/ob* mice exhibited diabetic glucose tolerance, and the diabetes was significantly improved in association with significant upregulation of serum adiponectin levels with low-dose pioglitazone treatment (Fig. 4*A*). *Adipo*$^{-/-}$*ob/ob* mice showed comparable diabetic glucose tolerance to *ob/ob* mice, but the diabetes was not improved by low-dose pioglitazone treatment (Fig. 4*B*). Hyperinsulinemic-euglycemic clamp studies to measure insulin sensitivity in the liver and skeletal muscle revealed that glucose infusion rates (GIRs) were comparable in *ob/ob* and *adipo*$^{-/-}$*ob/ob* mice (Fig. 4*C*). A low dose of pioglitazone increased the

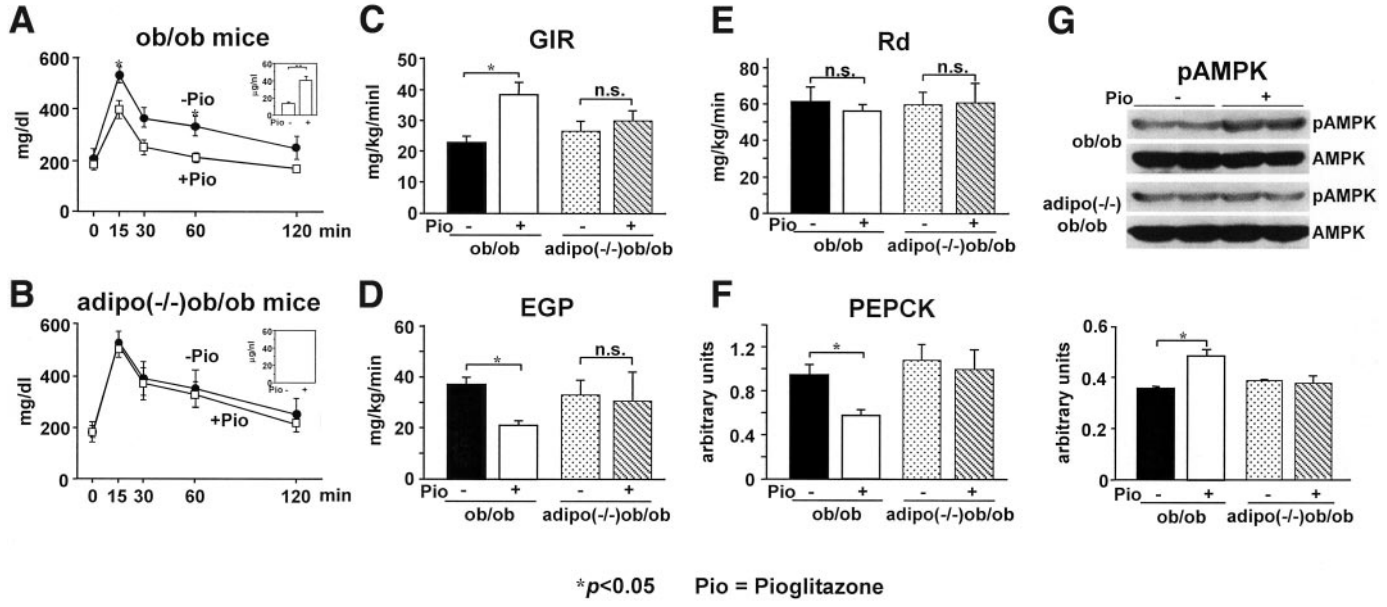

FIG. 4. Low-dose pioglitazone treatment. Low doses of pioglitazone improve diabetes and insulin resistance in *ob/ob* mice, but not in *adipo*$^{-/-}$*ob/ob* mice (37). *A* and *B*: Blood glucose levels during the oral glucose tolerance test of *ob/ob* (*A*) and *adipo*$^{-/-}$*ob/ob* (*B*) mice. Insets of *A* and *B* indicate serum adiponectin levels of *ob/ob* (*A*, inset) and *adipo*$^{-/-}$*ob/ob* (*B*, inset) mice not treated or treated with a low dose of pioglitazone (*C-E*). GIRs (*C*), EGP (*D*), and R_d values (*E*) of *ob/ob* and *adipo*$^{-/-}$*ob/ob* mice in the clamp study are shown. *F*: PEPCK expression levels in the livers of *ob/ob* and *adipo*$^{-/-}$*ob/ob* mice after the clamp studies. *G*: Phosphorylation of AMPK in the livers of *ob/ob* and *adipo*$^{-/-}$*ob/ob* mice after the clamp studies.

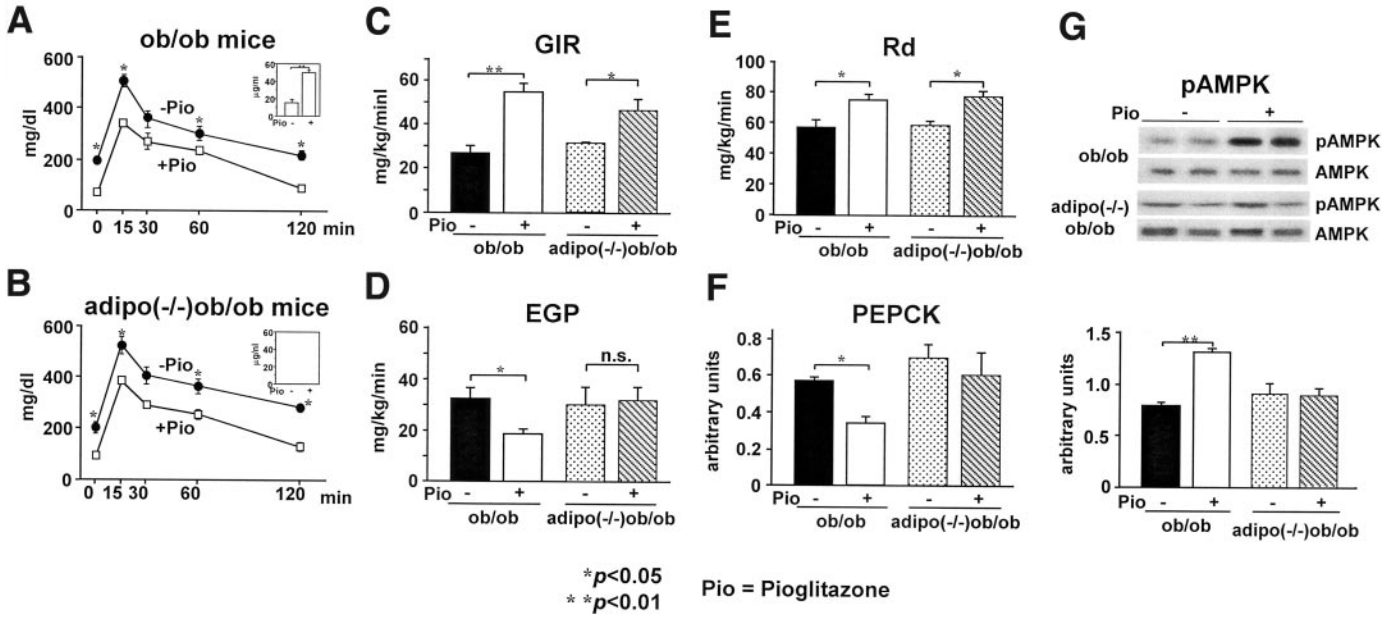

FIG. 5. High-dose pioglitazone treatment. High doses of pioglitazone improve diabetes and insulin resistance in *ob/ob* and *adipo*$^{-/-}$*ob/ob* mice (37). *A* and *B*: Blood glucose levels during an oral glucose tolerance test of *ob/ob* (*A*) and *adipo*$^{-/-}$*ob/ob* (*B*) mice. Insets of *A* and *B* indicate serum adiponectin levels of *ob/ob* (*A*, inset) and *adipo*$^{-/-}$*ob/ob* (*B*, inset) mice not treated or treated with high doses of pioglitazone. GIRs (*C*), EGP (*D*), and R_d values (*E*) of *ob/ob* and *adipo*$^{-/-}$*ob/ob* mice in the clamp study are shown. *F*: PEPCK expression levels in the livers of *ob/ob* and *adipo*$^{-/-}$*ob/ob* mice after the clamp studies. *G*: Phosphorylation of AMPK in the livers of *ob/ob* and *adipo*$^{-/-}$*ob/ob* mice after the clamp studies.

GIR of *ob/ob* mice but not of *adipo*$^{-/-}$*ob/ob* mice (Fig. 4*C*). The amelioration of insulin resistance in *ob/ob* mice was, at least in part, due to decreased endogenous glucose production (EGP) (Fig. 4*D*). Rate of glucose disappearance (R_d) values were indistinguishable in all mice groups (Fig. 4*E*). Because EGP was decreased, hepatic PEPCK expression and AMPK activity were examined. Low doses of pioglitazone significantly decreased PEPCK expression in *ob/ob*, but not *adipo*$^{-/-}$*ob/ob*, mice (Fig. 4*F*). In addition, AMPK phosphorylation in *ob/ob* mice was significantly increased by low doses of pioglitazone, but was unchanged in *adipo*$^{-/-}$*ob/ob* mice (Fig. 4*G*). These findings

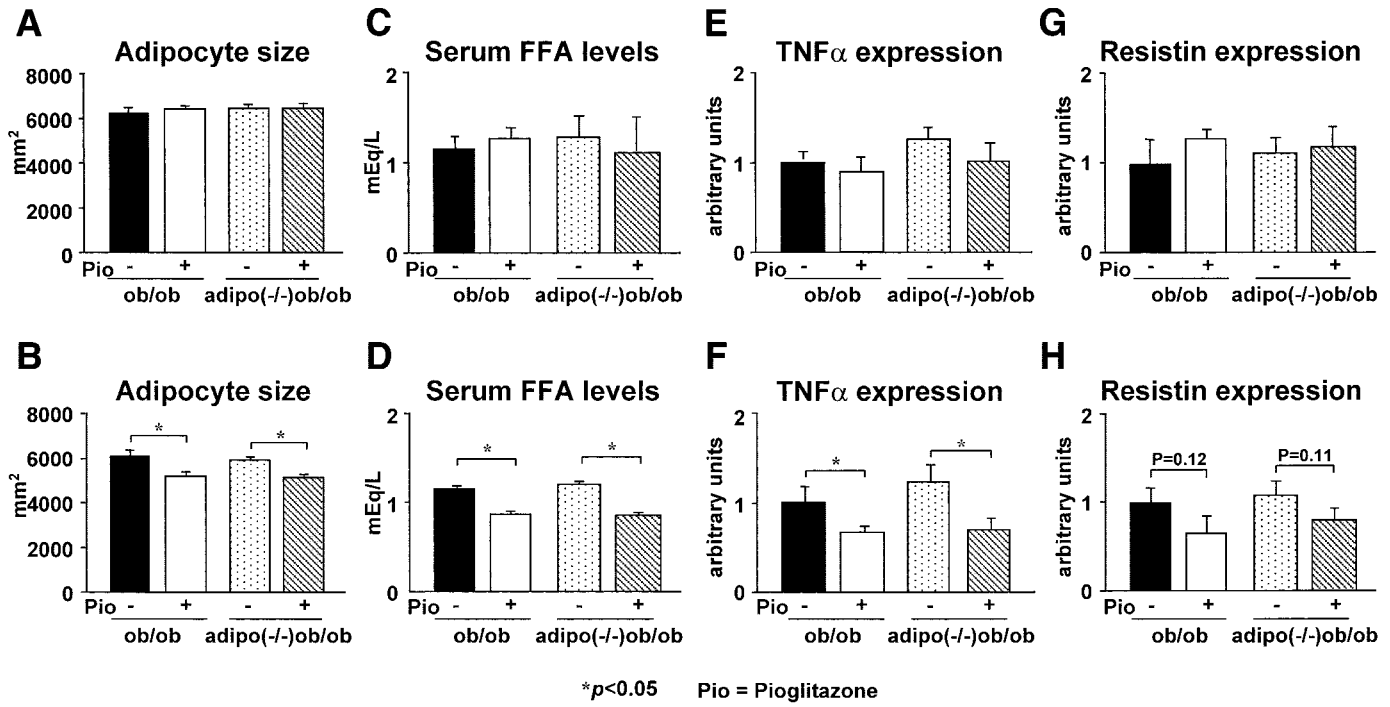

FIG. 6. High-dose but not low-dose pioglitazone decreased adipocyte size, serum FFA levels, and expression levels of TNF-α and resistin in *ob/ob* and *adipo*$^{-/-}$*ob/ob* mice (37). The adipocyte sizes (*A* and *B*) as well as serum FFA levels (*C* and *D*) in *ob/ob* and *adipo*$^{-/-}$*ob/ob* mice were unchanged after low-dose pioglitazone treatment (*A* and *C*), but were significantly reduced to a similar degree after high-dose pioglitazone treatment (*B* and *D*). Moreover, the expressions of TNF-α (*E* and *F*) and resistin (*G* and *H*) in white adipose tissues of *ob/ob* and *adipo*$^{-/-}$*ob/ob* mice were unchanged after low-dose pioglitazone (*E* and *G*), but decreased after high-dose pioglitazone (*F* and *H*).

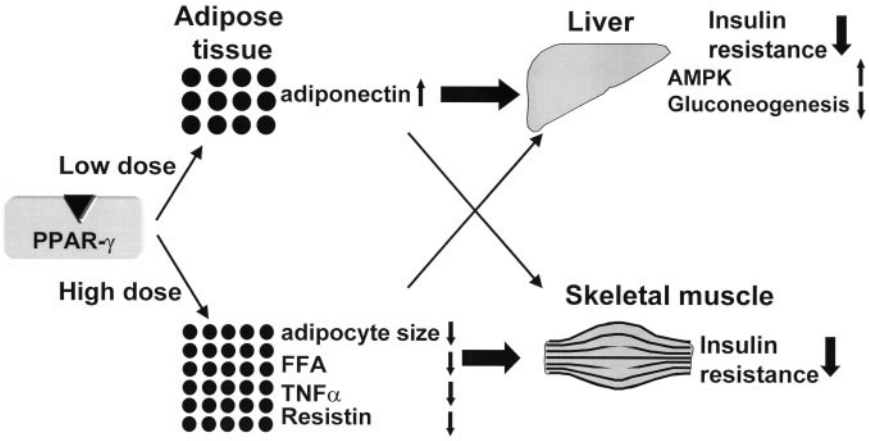

FIG. 7. Adiponectin-dependent and -independent pathways in insulin-sensitizing and antidiabetic actions of TZDs (hypothesis). We hypothesize two distinct pathways in the amelioration of insulin resistance induced by TZDs, namely the adiponectin-dependent pathway and adiponectin-independent pathway. Low doses of TZDs increase adiponectin levels, without promoting adipocyte differentiation, causing amelioration of insulin resistance, increasing AMPK activation, and decreasing gluconeogenesis in the liver. On the other hand, despite the absence of adiponectin, high doses of TZDs decrease adipocyte size, serum FFA levels, and TNF-α and resistin expression, causing amelioration of insulin resistance in skeletal muscle.

indicate that low doses of pioglitazone ameliorate diabetes and hepatic, but not muscle, insulin resistance in mice with an *ob/ob* background in an adiponectin-dependent manner via, at least in part, decreased gluconeogenesis and increased AMPK activation.

HIGH DOSES OF PIOGLITAZONE IMPROVE DIABETES AND INSULIN RESISTANCE IN *OB/OB* AND *ADIPO*$^{-/-}$*OB/OB* MICE

Increased adiponectin levels induced by high doses of pioglitazone were indistinguishable from those by low doses of pioglitazone in the *ob/ob* mice (Fig. 4*A*, inset; Fig. 5*A*, inset). The diabetes of *ob/ob* mice was again significantly ameliorated by high doses of pioglitazone (Fig. 5*A*). Interestingly, *adipo*$^{-/-}$*ob/ob* mice also displayed significant amelioration of diabetes, being similar to the levels seen in *ob/ob* mice (Fig. 5*B*). Hyperinsulinemic-euglycemic clamp studies showed that the GIR of *ob/ob* mice again significantly increased after high doses of pioglitazone (Fig. 5*C*). Interestingly, the GIR of *adipo*$^{-/-}$*ob/ob* mice also increased, indicating that insulin resistance in *adipo*$^{-/-}$*ob/ob* mice had improved (Fig. 5*C*). The EGP decreased only in *ob/ob* mice as seen after low doses of pioglitazone treatment (Fig. 5*D*), but the R_d increased in *ob/ob* and *adipo*$^{-/-}$*ob/ob* mice to a similar degree after high doses of pioglitazone (Fig. 5*E*). High doses of pioglitazone decreased PEPCK expression (Fig. 5*F*) and increased AMPK phosphorylation (Fig. 5*G*) in *ob/ob* mice, but not in *adipo*$^{-/-}$*ob/ob* mice. These findings suggest that the amelioration of diabetes and insulin resistance in *adipo*$^{-/-}$*ob/ob* mice was, at least in part, due to increased glucose uptake in skeletal muscle.

HIGH-DOSE, BUT NOT LOW-DOSE, PIOGLITAZONE DECREASES ADIPOCYTE SIZE, SERUM FFA LEVELS, AND EXPRESSION LEVELS OF TNF-α AND RESISTIN IN *OB/OB* AND *ADIPO*$^{-/-}$*OB/OB* MICE

As described above, TZDs increased the number of small adipocytes and decreased the number of large adipocytes, thereby ameliorating insulin resistance (16). To determine whether the presence of adiponectin is required for the occurrence of TZD-induced reduction of average adipocyte size, measurement of the adipocyte sizes in epididymal fat pads was performed. The adipocyte sizes of *ob/ob* and *adipo*$^{-/-}$*ob/ob* mice were indistinguishable and remained unchanged after low doses of pioglitazone (Fig. 6*A*). High doses of pioglitazone, however, significantly reduced the adipocyte sizes of *ob/ob* and *adipo*$^{-/-}$*ob/ob* mice to a similar degree (Fig. 6*B*). These results suggest that pioglitazone can induce a reduction in adipocyte size in the absence of adiponectin or leptin, or the absence of both. In addition, the serum FFA levels in *ob/ob* and *adipo*$^{-/-}$*ob/ob* mice were unchanged after low-dose pioglitazone treatment (Fig. 6*C*), but were significantly reduced to a similar degree after high-dose pioglitazone treatment (Fig. 6*D*). Moreover, the expressions of TNF-α and resistin in adipose tissues of *ob/ob* and *adipo*$^{-/-}$*ob/ob* mice were unchanged after low-dose pioglitazone (Fig. 6*E* and *G*), but were decreased after high-dose pioglitazone (Fig. 6*F* and *H*).

ADIPONECTIN-DEPENDENT AND -INDEPENDENT PATHWAYS IN INSULIN SENSITIZING AND ANTI-DIABETIC ACTIONS OF TZDs (HYPOTHESIS)

Although both high and low doses of pioglitazone ameliorated insulin resistance and diabetes, the underlying mechanisms may be distinct, albeit overlapped (37). We propose that there are two distinct pathways in the amelioration of insulin resistance induced by TZDs such as pioglitazone. One involves an adiponectin-dependent pathway and the other an adiponectin-independent pathway (Fig. 7) (46). Low doses of TZDs increase adiponectin concentrations at the transcriptional levels (39) without promoting adipocyte differentiation (16), causing amelioration of insulin resistance, increasing AMPK activation, and decreasing gluconeogenesis in the liver. On the other hand, independent of adiponectin, high doses of TZDs decrease adipocyte size, associated with decreased serum FFA levels and TNF-α and resistin expression, causing amelioration of insulin resistance in skeletal muscle (37). It seems likely that the increased adiponectin levels by TZDs also contribute to amelioration of skeletal muscle insulin resistance, and the decreased FFAs, TNF-α, and resistin by TZDs also contribute to amelioration of liver insulin resistance.

Scherer's group reported that rosiglitazone also improved glucose tolerance in *ob/ob* mice, but only partial improvement was achieved in *adipo*$^{-/-}$*ob/ob* mice (34). Moreover, rosiglitazone significantly increased AMPK activity in the livers of wild-type mice, whereas it had no effect on *adipo*$^{-/-}$ mice. In skeletal muscle, AMPK activity also significantly increased in wild-type mice, whereas no increase was detectable in *adipo*$^{-/-}$ mice. These data are in complete agreement with our data, suggesting that rosiglitazone also ameliorated glucose intolerance both via adiponectin-dependent and -independent pathways.

ACKNOWLEDGMENTS

This work was supported by the Program for Promotion of Fundamental Studies in Health Sciences of the Organization for Pharmaceutical Safety and Research of Japan; a grant from the Human Science Foundation (to T.K.); a Grant-in-Aid for the Development of Innovative Technology from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to T.K.); a Grant-in-Aid for Creative Scientific Research (10NP0201) from the Japan Society for the Promotion of Science (to T.K.); and by Health Science Research Grants (Research on Human Genome and Gene Therapy) from the Ministry of Health, Labor and Welfare of Japan (to T.K.).

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