Role of Adipocyte-Derived Factors in Enhancing Insulin Signaling in Skeletal Muscle and White Adipose Tissue of Mice Lacking Acyl CoA:Diacylglycerol Acyltransferase 1

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Mice that lack acyl CoA:diacylglycerol acyltransferase 1 (DGAT1), a key enzyme in mammalian triglyceride synthesis, have decreased adiposity and increased insulin sensitivity. Here we show that insulin-stimulated glucose transport is increased in the skeletal muscle and white adipose tissue (WAT) of chow-fed DGAT1-deficient mice. This increase in glucose transport correlated with enhanced insulin-stimulated activities of phosphatidylinositol 3-kinase, protein kinase B (or Akt), and protein kinase Cλ (PKC-λ), three key molecules in the insulin-signaling pathway, and was associated with decreased levels of serine-phosphorylated insulin receptor substrate 1 (IRS-1), a molecule implicated in insulin resistance. Similar findings in insulin signaling were also observed in DGAT1-deficient mice fed a high-fat diet. Interestingly, the increased PKC-λ activity and decreased serine phosphorylation of IRS-1 were observed in chow-fed wild-type mice transplanted with DGAT1-deficient WAT, consistent with our previous finding that transplantation of DGAT1-deficient WAT enhances glucose disposal in wild-type recipient mice. Our findings demonstrate that DGAT1 deficiency enhances insulin signaling in the skeletal muscle and WAT, in part through altered expression of adipocyte-derived factors that modulate insulin signaling in peripheral tissues. *Diabetes* 53:1445–1451, 2004

Because adiposity is closely correlated with insulin resistance (1,2), most knockout mouse models of leanness and obesity resistance are characterized by increased insulin sensitivity (3). However, the relationship between decreased adiposity and enhanced insulin action remains incompletely understood. In-depth studies of mouse models of leanness may shed light on the underlying mechanisms.

One model of leanness and obesity resistance is mice that lack acyl CoA:diacylglycerol acyltransferase 1 (DGAT1). DGAT1 is one of two known enzymes that catalyze the final step in mammalian triglyceride synthesis (4,5). DGAT1-deficient (*Dgat1*$^{-/-}$) mice have reduced amounts of white adipose tissue (WAT) (6) and decreased levels of triglycerides in the skeletal muscle (7). *Dgat1*$^{-/-}$ mice are resistant to diet-induced obesity (6), and they are more sensitive to insulin than wild-type (*Dgat1*$^{+/+}$) mice, as demonstrated by hyperinsulinemic-euglycemic clamp studies (7). The effects of DGAT1 deficiency on energy and glucose metabolism result in part from altered secretion of adipocyte-derived factors, as shown by the obesity resistance and enhanced insulin-stimulated glucose disposal in *Dgat1*$^{+/+}$ mice transplanted with *Dgat1*$^{-/-}$ WAT (8).

In this study, we sought to determine whether DGAT1 deficiency enhances insulin action in the major sites of glucose disposal, the skeletal muscle and WAT. We measured insulin-stimulated glucose uptake and examined the activity of key molecules in the insulin-signaling pathway: phosphatidylinositol 3-kinase (PI3K), protein kinase B (PKB, or Akt), and an atypical isoform of protein kinase C (PKC-λ). We also explored potential mechanisms that may contribute to altered insulin action in DGAT1 deficiency. Our findings indicate that the enhanced insulin signaling in *Dgat1*$^{-/-}$ mice occurs in part because of altered endocrine function of *Dgat1*$^{-/-}$ WAT.

RESEARCH DESIGN AND METHODS

Dgat1$^{-/-}$ mice (~98% C57BL/6 and 2% 129/SvJae background) were generated previously (6). *Dgat1*$^{+/+}$ mice (100% C57BL/6 background) were from The Jackson Laboratory (Bar Harbor, ME). Age-matched 10- to 14-week-old male mice were used for all experiments. Mice were housed in a pathogen-free barrier facility (12-h light/12-h dark cycle) and fed rodent chow (Ralston Purina, St. Louis, MO). For high-fat diet experiments, mice were fed a Western-type diet containing 21% milk fat by weight (Harlan Teklad, Madison, WI) for 6–8 weeks unless stated otherwise. All experiments were approved by the Committee on Animal Research of the University of California, San Francisco.

Glucose uptake. Glucose uptake in skeletal muscle and WAT adipocytes was measured as described (9–11). Briefly, soleus muscles were incubated in glucose-free Krebs-Ringer phosphate medium for 30 min with 0 or 100 nmol/l insulin. The uptake of [^{3}H]2-deoxyglucose (NEN Life Science, Boston, MA) was then measured over a 5-min period. For WAT experiments, adipocytes of reproductive fat pads were incubated in glucose-free Krebs-Ringer phosphate

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DGAT1, acyl CoA:diacylglycerol acyltransferase 1; IRS, insulin receptor substrate; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B; PKC, protein kinase C; WAT, white adipose tissue.

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medium for 30 min with the indicated concentrations of insulin. The uptake of [^{3}H]2-deoxyglucose was measured over a 1-min period.

PI3K activity assays. Insulin receptor substrate (IRS)-1–dependent PI3K activity assays were performed as described (9–11). Briefly, mice were injected intraperitoneally with saline or insulin (1 unit/kg body wt), and vastus lateralis muscle and reproductive WAT were removed after 10 min. Tissue lysates were immunoprecipitated with polyclonal antibodies that recognize IRS-1 (Upstate Biotechnology, Lake Placid, NY), and precipitates were incubated with [γ-^{32}P]ATP. Aliquots of the mixture were separated by thin-layer chromatography, and ^{32}P radioactivity of phosphatidylinositol 3-phosphate was measured.

PKB and PKC-λ activity assays. PKB and PKC-λ activity assays were performed as described (9–11). Briefly, mice were injected intraperitoneally with saline or insulin (1 unit/kg body wt), and vastus lateralis muscle and reproductive WAT were removed after 10 min. PKB activity was measured with a kit (Upstate Biotechnology). For PKC-λ studies, tissue lysates were immunoprecipitated with polyclonal antibodies that recognize specific isoforms of PKC (Santa Cruz Biotechnologies, Santa Cruz, CA). Precipitates were then incubated with [γ-^{32}P]ATP and the serine analog of PKC-ϵ pseudosubstrate (Biosource International, Camarillo, CA). Aliquots of the mixtures were spotted on p81 filter paper, and ^{32}P radioactivity was measured.

Glucose metabolism studies. For measurements of basal glucose and insulin levels, blood samples were obtained at 10:00 A.M. For the insulin tolerance test, bovine insulin (Sigma, St. Louis, MO) was injected intraperitoneally, and blood samples were collected after 15 min. Blood glucose levels were measured with a blood glucose meter (Accu-chek; Roche Diagnostics, Indianapolis, IN), and serum insulin levels were measured with an immunoassay kit (ALPCO Diagnostics, Windham, NH).

PKC-θ activity assay. Muscle samples were homogenized in a buffer containing 250 mmol/l sucrose, 20 mmol/l Tris-HCl, 1.2 mmol/l EGTA, 20 mmol/l β-mercaptoethanol, 1 mmol/l phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 20 μg/ml leupeptin, 1 mmol/l Na_3VO_4, 1 mmol/l NaF, 1 mmol/l $Na_4P_2O_7$, and 1 μmol/l L-microcystin. Homogenates were centrifuged at 700*g* for 10 min, and the supernatant was then removed and centrifuged at 100,000*g* for 60 min. After reconstituting the pellets in a buffer containing 0.15 mol/l NaCl, 1% Triton X-100, and 0.5% Nonidet P-40, PKC-θ was immunoprecipitated with a specific antiserum (Santa Cruz Biotechnologies), recovered on Sepharose AG beads, and assayed in the presence of phorbol ester (1 μmol/l) as described (12).

Immunoblots. Mice were injected intraperitoneally with saline or insulin (1 unit/kg body wt), and vastus lateralis muscle and reproductive WAT were removed after 10 min. Immunoblotting was performed as described (13) with an antibody that recognizes serine (307)-phosphorylated IRS-1 (Upstate Biotechnology). To control for equal loading and transfer of muscle proteins, membranes were stripped and reprobed with an antibody that recognizes total IRS-1 (Upstate Biotechnology). Because the anti-total IRS-1 antibody was unable to detect IRS-1 in WAT samples, membranes were treated with the Ponceau stain to verify equal loading and transfer of WAT proteins. Image density was quantified with Adobe PhotoShop (Adobe Systems, San Jose, CA).

Fat transplantation. Fat transplantation was performed as described (8). Briefly, donor and recipient mice were age- and sex-matched to minimize rejection. Reproductive fat pad (500 mg) from the donor was inserted into the subcutaneous space of the recipient. Two weeks after transplantation, mice were treated with intraperitoneal injections of saline or insulin, and muscle, WAT, and blood samples were removed for analysis as described. Fourteen mice were transplanted, and all fat grafts appeared viable at the time of death.

Statistical analysis. Values are expressed as means ± SE. Measurements were compared with the two-tailed *t* test or Mann-Whitney rank-sum test.

RESULTS

Increased insulin-stimulated glucose uptake in skeletal muscle and WAT of *Dgat1$^{-/-}$* mice. We previously showed that chow-fed *Dgat1$^{-/-}$* mice have increased insulin sensitivity (7). To identify the tissues in which DGAT1 deficiency enhances insulin-stimulated glucose disposal, we measured [^{3}H]2-deoxyglucose uptake in the soleus muscle and reproductive WAT adipocytes of chow-fed *Dgat1$^{+/+}$* and *Dgat1$^{-/-}$* mice. *Dgat1$^{+/+}$* and *Dgat1$^{-/-}$* skeletal muscle had similar levels of glucose uptake at baseline. In response to insulin treatment, glucose uptake was greater in *Dgat1$^{-/-}$* than in *Dgat1$^{+/+}$* skeletal muscle (Fig. 1*A*). Similarly, glucose uptake was comparable in *Dgat1$^{+/+}$* and *Dgat1$^{-/-}$* WAT adipocytes at baseline but was higher in *Dgat1$^{-/-}$* WAT adipocytes in response to a range of insulin concentrations (Fig. 1*B*).

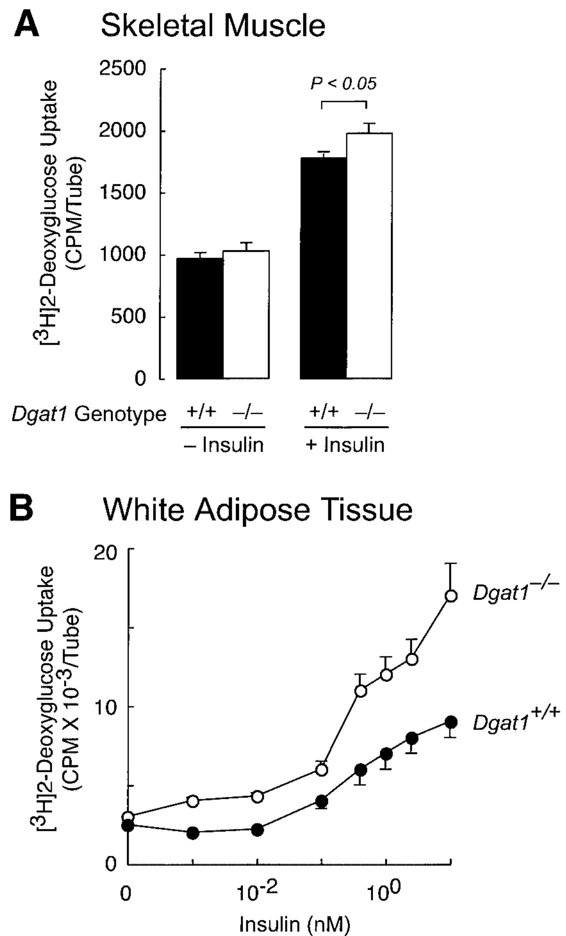

FIG. 1. Increased insulin-stimulated glucose uptake in skeletal muscle and WAT of *Dgat1$^{-/-}$* mice. *A*: Skeletal muscle. n = 9–10 per group. *B*: WAT. n = 9–10 per group. CPM, counts per million.

Increased insulin-stimulated activities of IRS-1–dependent PI3K, PKB, and PKC-λ in skeletal muscle of chow-fed *Dgat1$^{-/-}$* mice. To determine whether the increase in insulin-stimulated glucose uptake correlated with enhanced activation of the insulin-signaling pathway, we measured the activities of IRS-1–dependent PI3K, PKB, and PKC-λ (14,15) in the vastus lateralis muscle of chow-fed *Dgat1$^{+/+}$* and *Dgat1$^{-/-}$* mice. Basal activity levels for all three molecules were similar in *Dgat1$^{+/+}$* and *Dgat1$^{-/-}$* skeletal muscle. However, insulin treatment resulted in a greater increase in the activities of PI3K, PKB, and PKC-λ in *Dgat1$^{-/-}$* skeletal muscle than in *Dgat1$^{+/+}$* skeletal muscle (Fig. 2).

Increased insulin-stimulated PKC-λ activity in WAT of chow-fed *Dgat1$^{-/-}$* mice. We also investigated whether the increase in insulin-stimulated glucose uptake correlated with enhanced activation of the insulin-signaling pathway in WAT. Because of limited protein availability, we focused solely on PKC-λ activity in the reproductive WAT of chow-fed *Dgat1$^{+/+}$* and *Dgat1$^{-/-}$* mice. Although basal PKC-λ activity was similar in *Dgat1$^{+/+}$* and *Dgat1$^{-/-}$* WAT, insulin-stimulated activity was greater in *Dgat1$^{-/-}$* WAT (Fig. 3).

Enhanced insulin-stimulated glucose disposal in *Dgat1$^{-/-}$* mice fed a high-fat diet. To determine

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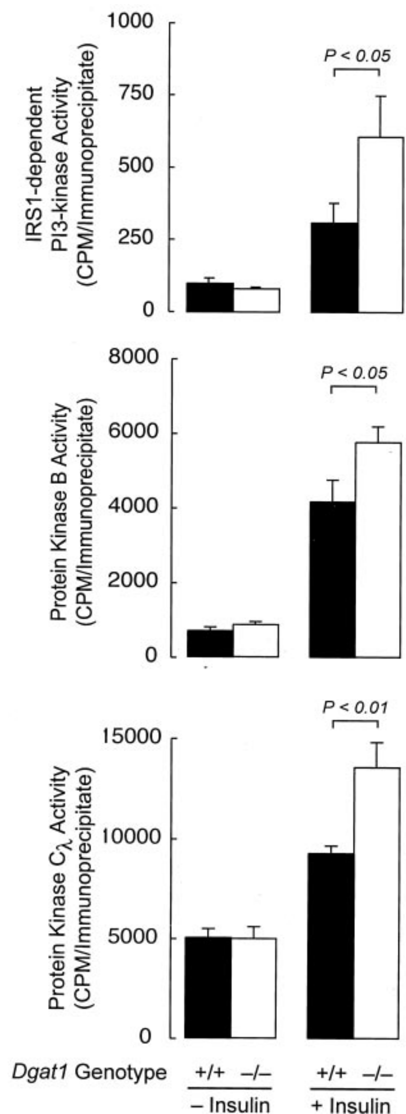

FIG. 2. Increased insulin-stimulated activities of IRS-1–dependent PI3K, PKB, and PKC-λ in skeletal muscle of chow-fed *Dgat1*$^{-/-}$ mice. *n* = 4 per group for PI3K, 10 per group for PKB, and 36–40 per group for PKC-λ. CPM, counts per million.

whether high-fat feeding affected glucose metabolism in *Dgat1*$^{-/-}$ mice, we measured serum insulin and blood glucose levels in mice fed a high-fat diet for 1 week. The mean body weights of *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ mice were similar (23.8 ± 0.5 vs. 22.4 ± 0.6 g, $n = 5$ per group, $P > 0.05$). Although basal serum insulin levels were similar in *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ mice (2.7 ± 0.9 vs. 3.3 ± 0.7 ng/ml, $n = 5$ per group, $P > 0.05$), blood glucose levels were lower in *Dgat1*$^{-/-}$ mice (157 ± 10 vs. 200 ± 11 mg/dl, $n = 5$ per group, $P < 0.05$). Treatment with insulin (1 mU/g body wt) decreased blood glucose levels in *Dgat1*$^{-/-}$ mice (157 ± 10 to 131 ± 6 mg/dl, $n = 5$ per group, $P < 0.05$), whereas it had no significant effect in *Dgat1*$^{+/+}$ mice (200 ± 11 to 190 ± 11 mg/dl, $n = 5$ per group, $P > 0.05$). Thus, DGAT1 deficiency enhances insulin-stimulated glucose disposal in mice fed either a chow or a high-fat diet.

Increased insulin-stimulated activities of IRS-1–dependent PI3K and PKB in skeletal muscle of *Dgat1*$^{-/-}$ mice fed a high-fat diet. To determine whether high-fat feeding affected activation of the insulin-signaling pathway in *Dgat1*$^{-/-}$ mice, we measured the activities of IRS-1–dependent PI3K, PKB, and PKC-λ in the vastus lateralis muscle of *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ mice fed a high-fat diet. Similar to the findings in chow-fed mice, basal activity levels for all three molecules were similar in *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ skeletal muscle (Fig. 4). Compared with chow-fed mice, the increase in activity levels after insulin treatment was blunted in both *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ mice fed a high-fat diet. Insulin treatment resulted in a greater increase in the activities of PI3K and PKB in *Dgat1*$^{-/-}$ skeletal muscle than in *Dgat1*$^{+/+}$ skeletal muscle. Although insulin-stimulated PKC-λ activity trended higher in *Dgat1*$^{-/-}$ skeletal muscle, the difference was not statistically significant.

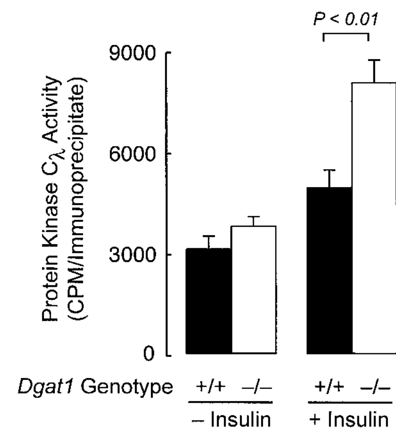

FIG. 3. Increased insulin-stimulated PKC-λ activity in WAT of chow-fed *Dgat1*$^{-/-}$ mice. *n* = 8 per group. CPM, counts per million.

We also measured PKC-λ activity in the reproductive WAT of *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ mice fed a high-fat diet. Although basal PKC-λ activity was similar in *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ WAT, insulin-stimulated activity was greater in *Dgat1*$^{-/-}$ WAT (Fig. 5).

Decreased serine phosphorylation of IRS-1 in skeletal muscle and WAT of insulin-treated *Dgat1*$^{-/-}$ mice. Because decreased serine phosphorylation of IRS-1 may increase insulin sensitivity and enhance insulin signaling in models of leanness and obesity resistance (16,17), we measured the level of serine (307)-phosphorylated IRS-1 in the vastus lateralis muscle and reproductive WAT of chow-fed *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ mice by immunoblotting. Significant variability was observed in the basal state for both *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ tissues, and major differences were not apparent (not shown). However, after insulin treatment, the level of serine (307)-phosphorylated IRS-1 was consistently 40–50% lower in *Dgat1*$^{-/-}$ mice than in *Dgat1*$^{+/+}$ mice (Fig. 6*A* and *B*).

Lack of difference in PKC-θ activity in skeletal muscle of *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ mice. Decreased activation of PKC-θ has been proposed as a potential mechanism by which decreased adiposity is associated with decreased serine phosphorylation of IRS-1 and enhanced insulin signaling (16,18). We therefore examined the activity of membrane-associated (or activated) PKC-θ in the vastus lateralis muscle of *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ mice. In mice fed a chow diet, basal PKC-θ activity was comparable in *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ skeletal muscle (Fig. 7), and

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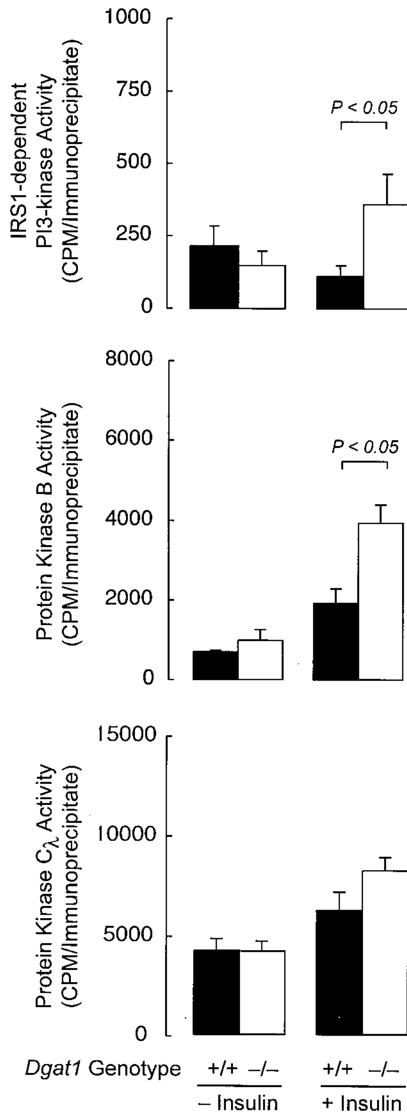

FIG. 4. Increased insulin-stimulated activities of IRS-1–dependent PI3K and PKB in skeletal muscle of *Dgat1*$^{-/-}$ mice fed a high-fat diet. n = 4 per group for PI3K, 10 per group for PKB, and 36–40 per group for PKC-λ. CPM, counts per million.

insulin treatment increased PKC-θ activity in *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ skeletal muscle to similar levels.

High-fat feeding, as anticipated, increased basal and insulin-stimulated PKC-θ activity levels in both *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ skeletal muscle. Similar to the findings in chow-fed mice, basal PKC-θ activity levels were comparable in *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ skeletal muscle. Although insulin treatment resulted in a slightly greater increase in PKC-θ activity in *Dgat1*$^{-/-}$ skeletal muscle than in *Dgat1*$^{+/+}$ skeletal muscle, this difference was not statistically significant.

Increased insulin-stimulated PKC-λ activity in skeletal muscle and WAT of *Dgat1*$^{+/+}$ mice transplanted with *Dgat1*$^{-/-}$ WAT. Altered secretion of adipocyte-derived factors has been proposed as another potential mechanism by which decreased adiposity leads to enhanced insulin signaling (19,20). We previously demonstrated that DGAT1 deficiency alters the expression of several adipocyte-derived factors, including adiponectin and leptin, and that this alteration contributes to enhanced insulin-stimulated glucose disposal in *Dgat1*$^{+/+}$ mice transplanted with *Dgat1*$^{-/-}$ WAT (8). To determine whether altered expression of adipocyte-derived factors by *Dgat1*$^{-/-}$ WAT affects PKC-λ activity, we transplanted *Dgat1*$^{-/-}$ WAT into *Dgat1*$^{+/+}$ mice. We also transplanted *Dgat1*$^{+/+}$ WAT into *Dgat1*$^{+/+}$ mice to serve as controls. Although basal PKC-λ activity levels trended higher in the skeletal muscle of mice transplanted with *Dgat1*$^{-/-}$ WAT, the difference was not statistically significant (Fig. 8*A*). As in nontransplanted mice, insulin treatment resulted in a greater increase in PKC-λ activity in the skeletal muscle of mice transplanted with *Dgat1*$^{-/-}$ WAT than in the skeletal muscle of control mice. Similar results were observed in WAT (Fig. 8*B*).

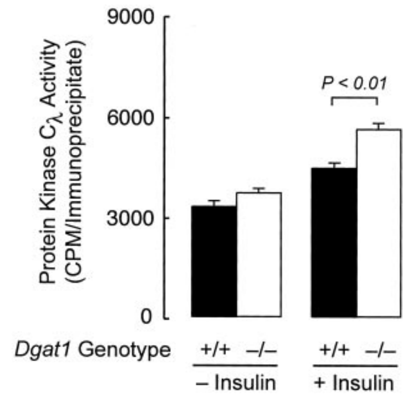

FIG. 5. Increased insulin-stimulated PKC-λ activity in WAT of *Dgat1*$^{-/-}$ mice fed a high-fat diet. n = 8 per group. CPM, counts per million.

Decreased serine phosphorylation of IRS-1 in skeletal muscle and WAT of insulin-treated *Dgat1*$^{+/+}$ mice transplanted with *Dgat1*$^{-/-}$ WAT. To further characterize the effects of transplanted *Dgat1*$^{-/-}$ WAT on the

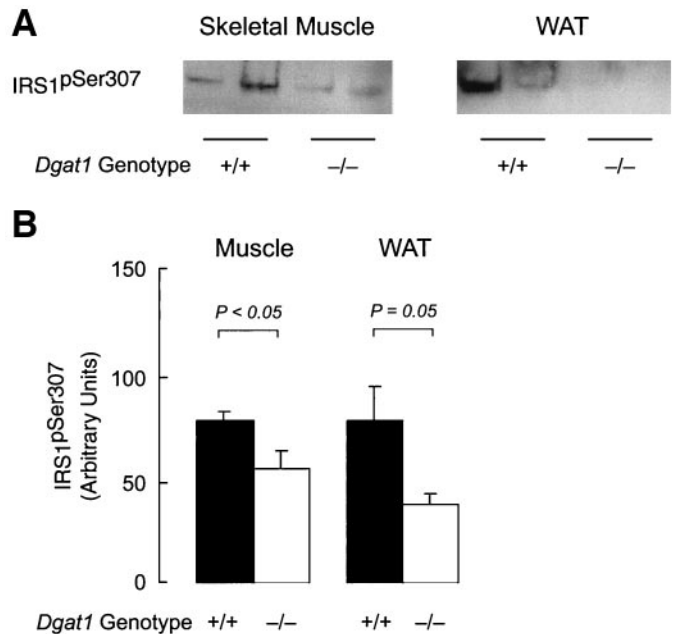

FIG. 6. Decreased serine phosphorylation of IRS-1 in skeletal muscle and WAT of insulin-treated *Dgat1*$^{-/-}$ mice. *A*: Immunoblot. Each lane represents a sample from one mouse. The experiment was performed three times, and representative results are shown. *B*: Quantification of immunoblots. n = 5–6 per group.

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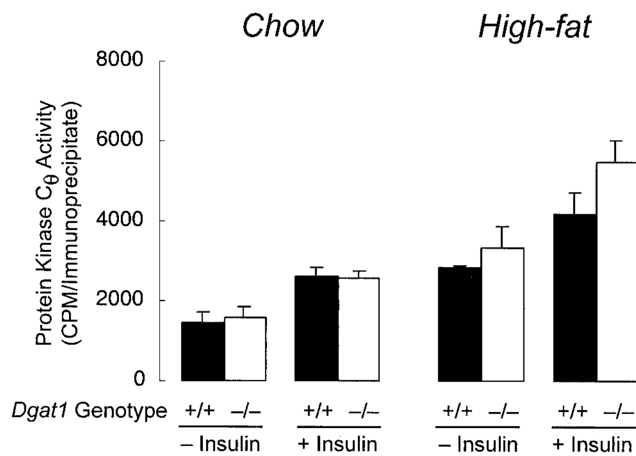

FIG. 7. Similar levels of PKC-θ activity in skeletal muscle of *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ mice. *n* = 7–9 per group. CPM, counts per million.

insulin-signaling pathway, we measured levels of serine (307)-phosphorylated IRS-1 in the vastus lateralis muscle and reproductive WAT. Similar to the findings in nontransplanted *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ mice, the level of serine (307)-phosphorylated IRS-1 in the basal state varied considerably, and major differences were not apparent (not shown). However, after insulin treatment, the level of serine (307)-phosphorylated IRS-1 was significantly lower in the skeletal muscle and WAT of mice transplanted with *Dgat1*$^{-/-}$ WAT than in the tissues of control mice (Fig. 9*A* and *B*).

DISCUSSION

We previously reported that *Dgat1*$^{-/-}$ mice have increased insulin sensitivity as demonstrated by an increased glucose infusion rate during hyperinsulinemic-euglycemic clamp studies (7). In this study, we show that insulin-stimulated glucose transport was increased in the skeletal muscle and WAT of *Dgat1*$^{-/-}$ mice. This increase in glucose transport correlated with enhanced insulin-stimulated activities of IRS-1–dependent PI3K, PKB, and PKC-λ and was associated with decreased serine phosphorylation of IRS-1. The enhanced PKC-λ activity and decreased serine phosphorylation of IRS-1 were also observed in *Dgat1*$^{+/+}$ mice transplanted with *Dgat1*$^{-/-}$ WAT, suggesting that DGAT1 deficiency enhances insulin signaling in peripheral tissues in part by altering the endocrine function of WAT.

Insulin-stimulated glucose uptake in the skeletal muscle and WAT plays a critical role in determining systemic insulin sensitivity (21). For example, mice that lack GLUT4, the major mediator of insulin-stimulated glucose uptake, in either the skeletal muscle (22) or WAT (23) have increased insulin resistance. Our results demonstrate that DGAT1 deficiency significantly enhances insulin-stimulated glucose uptake and insulin signaling in the skeletal muscle and WAT and suggest that this is an important mechanism by which DGAT1 deficiency increases systemic insulin sensitivity in mice. These findings, however, do not exclude the possibility that DGAT1 deficiency also enhances sensitivity to insulin in the liver or other tissues.

We previously demonstrated that the transplantation of ~500 mg *Dgat1*$^{-/-}$ WAT is sufficient to enhance insulin-stimulated glucose disposal in wild-type mice (8). We now

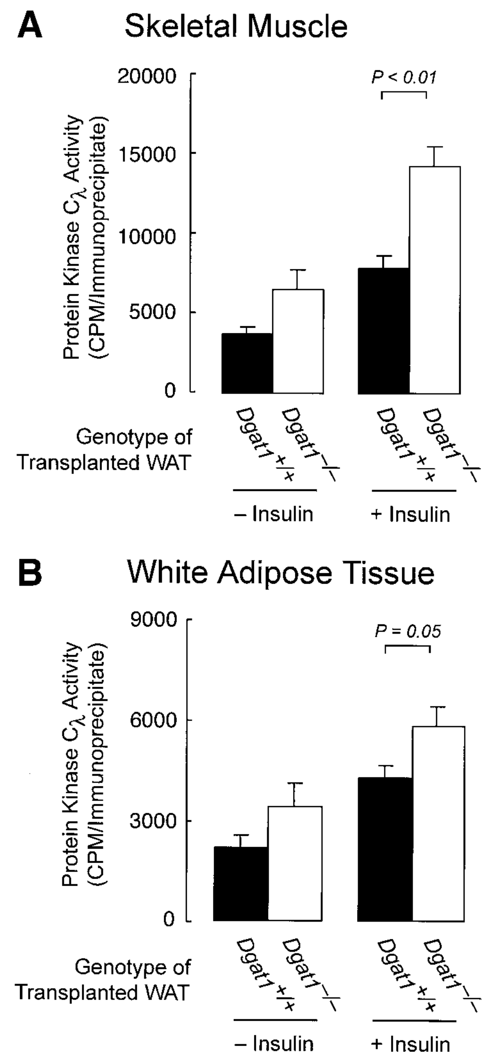

FIG. 8. Increased insulin-stimulated PKC-λ activity in skeletal muscle and WAT of *Dgat1*$^{+/+}$ mice transplanted with *Dgat1*$^{-/-}$ WAT. *A*: Skeletal muscle. *n* = 3–5 per group. *B*: WAT. *n* = 3–5 per group. CPM, counts per million.

show that this transplantation-induced enhancement correlates with enhanced insulin signaling in peripheral tissues, as demonstrated by an increase in PKC-λ activity and a decrease in serine phosphorylation of IRS-1. Thus, our findings suggest that DGAT1 deficiency enhances insulin action in the skeletal muscle and WAT by altering the secretion of adipocyte-derived factors.

The adipose tissue plays a vital role in regulating glucose homeostasis, in part by secreting factors such as leptin, adiponectin, resistin, and tumor necrosis factor-α (24,25). Which adipocyte-derived factor may mediate the insulin-sensitizing effect of *Dgat1*$^{-/-}$ WAT? Adiponectin is one possible candidate. Adiponectin enhances insulin sensitivity and insulin signaling in part by increasing insulin-stimulated tyrosine phosphorylation of insulin receptors (26,27), IRS-1 (26), and PKB (26), and we previously demonstrated that adiponectin expression is increased in *Dgat1*$^{-/-}$ WAT in two obesity models (8). However, adipocyte-derived factors other than adiponectin may be involved. Whichever factor(s) are responsible, decreasing serine phosphorylation of IRS-1 may be an

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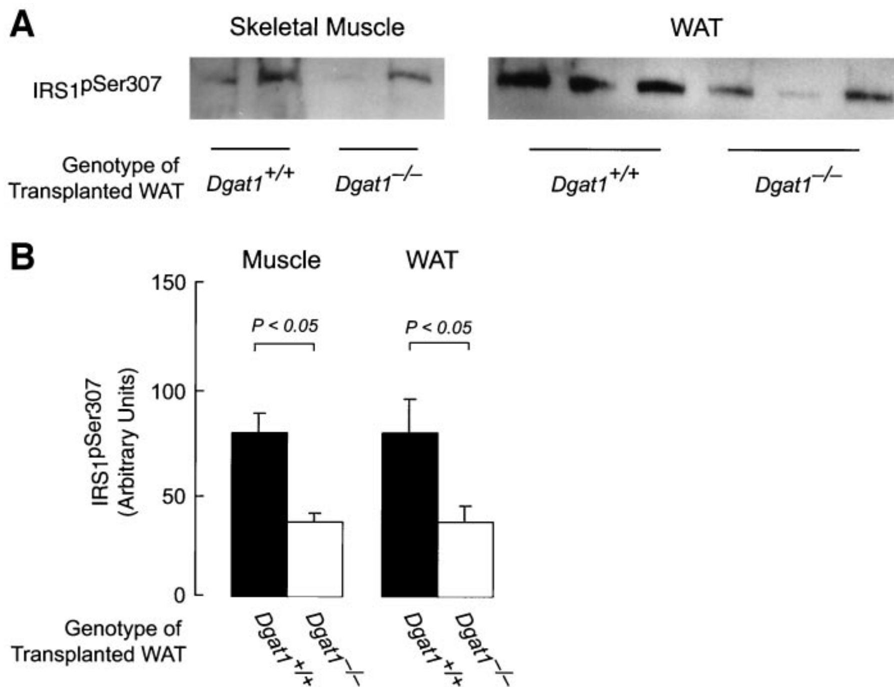

FIG. 9. Decreased insulin-stimulated serine phosphorylation of IRS-1 in skeletal muscle and WAT of *Dgat1*$^{+/+}$ mice transplanted with *Dgat1*$^{-/-}$ WAT. *A*: Immunoblot. Each lane represents a sample from one mouse. *B*: Quantification of immunoblots. *n* = 3–5 per group.

important mechanism by which these factors enhance insulin signaling.

Finally, our results suggest that alterations in intracellular lipid content or changes in PKC-θ activity are unlikely to play a major role in modulating insulin action in *Dgat1*$^{-/-}$ skeletal muscle. According to one lipotoxicity hypothesis (16,28,29), an accumulation of lipids, such as diacylglycerol and fatty acyl CoA, can activate PKC-θ and is associated with diminished insulin action. We previously reported that the levels of diacylglycerol are similar in the skeletal muscle and WAT of *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ mice (7), and in this study we show that PKC-θ activity was similar in *Dgat1*$^{+/+}$ and *Dgat1*$^{-/-}$ skeletal muscle. In fact, in mice fed a high-fat diet, insulin-stimulated PKC-θ activity trended higher in *Dgat1*$^{-/-}$ mice. Thus, our findings suggest that the enhanced insulin signaling in *Dgat1*$^{-/-}$ mice is not due to the absence of DGAT1 in skeletal muscle.

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