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

Hubert C. Chen,1,2,3 Meghana Rao,1 Mini P. Sajan,4,5 Mary Standaert,4,5 Yoshinori Kanoh,4,5 Atsushi Miura,4,5 Robert V. Farese, Jr.,1,2,3 and Robert V. Farese4,5

Mice that lack acyl CoA:diacylglycerol acetyltransferase 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 (PI3K), protein kinase B (or Akt), and protein kinase Cα (PKC-α), 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-α 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 acetyltransferase 1 (DGAT1). DGAT1 is one of two known enzymes that catalyze the final step in mammalian triglyceride synthesis (4, 5). DGAT1-deficient (Dgat1<sup>−/−</sup>) mice have reduced amounts of white adipose tissue (WAT) (6) and decreased levels of triglycerides in the skeletal muscle (7). Dgat1<sup>−/−</sup> mice are resistant to diet-induced obesity (6), and they are more sensitive to insulin than wild-type (Dgat1<sup>+/+</sup>) 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<sup>−/−</sup> mice transplanted with Dgat1<sup>+/−</sup> 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-α). 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<sup>−/−</sup> mice occurs in part because of altered endocrine function of Dgat1<sup>−/−</sup> WAT.

RESEARCH DESIGN AND METHODS

Dgat1<sup>−/−</sup> mice (~88% C57BL/6 and 2% 129/SvJae background) were generated previously (6). Dgat1<sup>−/−</sup> 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 23% 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 mmol/l insulin. The uptake of [3H]-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

From the 1Gladstone Institute of Cardiovascular Disease, San Francisco, California; the 2Cardiovascular Research Institute, University of California, San Francisco, California; the 3Department of Medicine, University of California, San Francisco, California; the 4James A. Haley Veterans Hospital, Tampa, Florida; and the 5Department of Medicine, University of South Florida, Tampa, Florida.

Address correspondence and reprint requests to Robert V. Farese, James A. Haley Veterans Hospital, ACOS-151, 13000 Bruce B. Downs Blvd., Tampa, FL 33612. E-mail: rfarese@hsc.usf.edu.

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DGAT1, acyl CoA:diacylglycerol acetyltransferase 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 [3H]2-deoxyglucose was measured over a 1-min period.

**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. Tissue lysates were immunoprecipitated with polyclonal antibodies that recognize IRS-1 (Upstate Biotechnology, Lake Placid, NY), and precipitates were incubated with [γ-32P]ATP. Aliquots of the mixture were separated by thin-layer chromatography, and [32P]radioactivity of phosphatidylinositol 3-phosphate was measured.

**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 [3H]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. 1A). 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. 1B).

**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).

**Enhanced insulin-stimulated glucose disposal in Dgat1–/– mice fed a high-fat diet.** To determine
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 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-α 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-α activity trended higher in Dgat1−/− skeletal muscle, the difference was not statistically significant.

We also measured PKC-α activity in the reproductive WAT of Dgat1+/+ and Dgat1−/− mice fed a high-fat diet. Although basal PKC-α 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. 6A 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
insulin treatment increased PKC-α activity in Dgat1+/+ and Dgat1−/− skeletal muscle to similar levels.

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

Increased insulin-stimulated PKC-α 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-α activity, we transplanted Dgat1−/− WAT into Dgat1+/+ mice. We also transplanted Dgat1+/+ WAT into Dgat1+/+ mice to serve as controls. Although basal PKC-α activity levels trended higher in the skeletal muscle of mice transplanted with Dgat1−/− WAT, the difference was not statistically significant (Fig. 8A). As in nontransplanted mice, insulin treatment resulted in a greater increase in PKC-α 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. 8B).

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 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-α activity, we transplanted Dgat1−/− WAT into Dgat1+/+ mice. We also transplanted Dgat1+/+ WAT into Dgat1+/+ mice to serve as controls. Although basal PKC-α activity levels trended higher in the skeletal muscle of mice transplanted with Dgat1−/− WAT, the difference was not statistically significant (Fig. 8A). As in nontransplanted mice, insulin treatment resulted in a greater increase in PKC-α 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. 8B).

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 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-α activity, we transplanted Dgat1−/− WAT into Dgat1+/+ mice. We also transplanted Dgat1+/+ WAT into Dgat1+/+ mice to serve as controls. Although basal PKC-α activity levels trended higher in the skeletal muscle of mice transplanted with Dgat1−/− WAT, the difference was not statistically significant (Fig. 8A). As in nontransplanted mice, insulin treatment resulted in a greater increase in PKC-α 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. 8B).

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
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/H11001/ and Dgat1/H11002/ 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/H11002/ WAT than in the tissues of control mice (Fig. 9A 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 P3K, PKB, and PKC- and was associated with decreased serine phosphorylation of IRS-1. The enhanced PKC- 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 show that this transplantation-induced enhancement correlates with enhanced insulin signaling in peripheral tissues, as demonstrated by an increase in PKC- 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

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

**FIG. 8.** Increased insulin-stimulated PKC- 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.
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<sup>−/−</sup> 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<sup>+/+</sup> and Dgat1<sup>−/−</sup> mice (7), and in this study we show that PKC-θ activity was similar in Dgat1<sup>+/+</sup> and Dgat1<sup>−/−</sup> skeletal muscle. In fact, in mice fed a high-fat diet, insulin-stimulated PKC-θ/H<sub>9258</sub> activity was not due to the absence of Dgat1<sup>−/−</sup> mice.

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

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