Effects of DGAT1 deficiency on energy and glucose metabolism are independent of adiponectin

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The prevalence of obesity is increasing worldwide along with obesity-related complications, such as type 2 diabetes (25), hepatic steatosis (2), and cardiovascular disease (32). The primary defect in obesity is the accumulation of excessive triacylglycerol (TG) in white adipose tissue (WAT), skeletal muscle, liver, and other tissues. TG accumulation in WAT is associated with larger adipocytes, inflammatory responses, and changes in secreted adipokines (15, 17, 29, 33). The alterations in WAT-derived adipokines may contribute to disease processes in other tissues, such as insulin resistance in skeletal muscle and hepatic steatosis. Identifying mechanisms that limit TG accumulation and promote beneficial metabolic effects are of significant importance.

In WAT and most mammalian cells, the final step in TG biosynthesis, the joining of a fatty acyl-CoA moiety to diacylglycerol through an ester bond, is catalyzed by acyl-CoA:diacylglycerol acyltransferase (DGAT) enzymes (1). Two mammalian DGAT enzymes have been identified. DGAT2 is ubiquitously expressed in mammalian tissues (4), and the disruption of Dgat2 in mice results in severe TG depletion in homozygous offspring and early postnatal lethality (28).

DGAT1 is also ubiquitously expressed in mammalian tissues and is highly expressed in tissues that synthesize and store TG (3). However, the disruption of Dgat1 in mice results in viable mice with a pleiotropic phenotype (26). DGAT1-deficient (Dgat1−−) mice have moderate reductions in tissue TGs and are resistant to diet-induced obesity (26). The obesity resistance is due to an increase in energy expenditure (26) resulting from increases in both thermogenesis (8) and physical activity (26). Dgat1−− mice also exhibit increased sensitivity to insulin and leptin and are protected from insulin resistance caused by a high-fat diet or by genetic crosses into the Agouti yellow (Ay/a) background (7, 10). Alterations in a WAT-secreted factor or factors appear to play an important role in the DGAT1 deficiency phenotype, as transplantation of DGAT1-deficient WAT (WATDgat1KO) into both wild-type and Ay/a mice results in partial protection from obesity, reduction in the fat mass and tissue TG content of the recipient mouse, and enhanced glucose tolerance and insulin signaling (6, 9).

Which WAT-derived factors contribute to the effects of DGAT1 deficiency on glucose and energy metabolism? Previous data from our laboratory suggested that the WAT-secreted factor is not leptin but rather a leptin-sensitizing factor (6, 7). Adiponectin [also known as Acrd, Acrp30, adipQ, apM1, GBP28; http://www.gene.ucl.ac.uk/nomenclature/data (16, 31)] is a potential candidate. Present evidence indicates that adiponectin stimulates energy expenditure (23), promotes fatty acid oxidation and glucose uptake into muscle, and assists in the inhibition of hepatic glucose production by insulin (12, 14). In agreement with these functions, mice that overexpress adiponectin have increased energy expenditure and insulin sensitivity (36), whereas adiponectin-deficient (Adipoq−−) mice have impaired glucose tolerance and insulin action when fed a high-fat diet (18, 20, 22). Moreover, adiponectin and leptin activate AMP-activated protein kinase (30, 35) and work synergistically to improve glucose metabolism in lipoatrophic mice (36), consistent with a role for adiponectin as a leptin-sensitizing agent.

Several findings in Dgat1−− mice suggest that adiponectin might play a role in the phenotype. Adiponectin mRNA expression increased twofold in Dgat1−− mice fed a high-fat diet and in Dgat1−−/Ay/a mice (6). Adiponectin levels also increased twofold in medium conditioned by Dgat1−−/Ay/a cells, consistent with the finding that adiponectin stimulates energy expenditure (23) and promotes fatty acid oxidation (36). Therefore, the effects of DGAT1 deficiency on energy and glucose metabolism are independent of adiponectin.
WAT, and serum adiponectin levels increased in high-fat-fed wild-type mice transplanted with WAT\(^{Dgat1\text{KO}}\) and in high-fat-fed \(Dgat1^{\text{-/-}}\) mice when levels were adjusted for fat mass (6).

In sum, the evidence suggested that adiponectin might play a crucial role in the phenotypic effects of DGAT1 deficiency on glucose and energy metabolism. To test this hypothesis, we generated \(Adipoq^{+/+}\) mice and crossed them with \(Dgat1^{\text{-/-}}\) mice. We then analyzed parameters of energy and glucose metabolism in \(Dgat1^{\text{-/-}}\)-\(Adipoq^{+/+}\) mice to assess the requirement for adiponectin in mediating the effects of DGAT1 deficiency.

**MATERIALS AND METHODS**

Generation of \(Adipoq^{+/+}\) mice. Adiponectin genomic fragments were amplified by PCR from 129/SvJae mouse genomic DNA with primers derived from the murine \(Adipoq\) sequences. A targeting vector, designed to replace exon 2 of \(Adipox\) with neo, was constructed in pJB1 (a gift from Joachim Herz, University of Texas Southwestern, Dallas, TX) by subcloning an 8-kb long-arm fragment containing sequences located in intron 1 and a 1.3-kb short-arm fragment containing sequences located in intron 2 (long-arm primers, 5'-attgctggcGCTGAGCAGATGACCGAGATCGCCG-3' and 5'-attgcggcGCGAGCATCGATGTCCTATATAGC-3'; short-arm primers, 5'-ccgcgctagGGTCTAGACAAGAAGAAG-3' and 5'-AAGTCTCGAAGCTGACATTATAGC-3'). Primer sequences (lowercase letters) were added on the primer termini to introduce NotI (long arm) and XhoI (short arm) restriction enzyme sites for cloning (the antisense short-arm primer followed by a Newman-Keuls test.

**RESULTS**

**Generation of \(Adipoq^{+/+}\) mice.** A gene-targeting vector was designed to replace exon 2 of murine \(Adipoq\), which contains the translational start site and signal sequences for adiponectin, with \(neo\) (Fig. 1A). This vector was used to generate embryonic stem cells, and subsequently mice, with the targeted allele. Disruption of \(Adipoq\) in mice was demonstrated by Southern blotting of mouse genomic DNA (Fig. 1B). To verify the absence of the adiponectin protein, serum was analyzed with an ELISA that recognizes the full-length protein and by immunoblotting with an antiserum that recognizes the COOH-terminal globular domain. Adiponectin was undetectable in serum from \(Adipoq^{+/+}\) mice by both assays (Fig. 1, C and D).

Because of conflicting reports about the development of glucose intolerance in \(Adipoq^{+/+}\) mice (18–20, 22), we performed glucose tolerance tests on mice fed chow and high-fat diets. Male wild-type and \(Adipoq^{+/+}\) mice fed a chow diet had similar glucose tolerance (Fig. 2A). However, male \(Adipoq^{+/+}\) mice fed a high-fat diet for 15 wk had a greater impairment of glucose tolerance than wild-type mice (Fig. 2B). Glucose tolerance was not impaired in female \(Adipoq^{+/+}\) mice fed either chow or a high-fat diet (not shown). Therefore, male mice were used for ensuing genetic crosses.

**Effects of \(DGAT1\) deficiency on body weight and energy expenditure are independent of adiponectin.** To test the hypothesis that adiponectin contributes to the metabolic consequences of DGAT1 deficiency, we crossed \(Adipoq^{+/+}\) mice with \(Dgat1^{\text{-/-}}\) mice to obtain mice with the following genotypes: \(Dgat1^{+/+}/Adipoq^{+/+}\), \(Dgat1^{\text{-/-}}/Adipoq^{+/+}\), \(Dgat1^{+/+}/\).
Adipoq<sup>−/−</sup>, and Dgat1<sup>−/−</sup>Adipoq<sup>−/−</sup>. The double knockout (Dgat1<sup>−/−</sup>Adipoq<sup>−/−</sup>) mice were viable and appeared healthy. The dry fur, hair loss, and lactation defect observed in DGAT1-deficient mice (5, 11, 26) were present in Dgat1<sup>−/−</sup>Adipoq<sup>−/−</sup> mice (not shown), indicating that adiponectin is not responsible for these aspects of the DGAT1 deficiency phenotype.

To determine whether DGAT1-deficient mice require adiponectin for protection against diet-induced obesity, mice of all

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**Fig. 1.** Generation of adiponectin-deficient mice. A: gene-targeting strategy. Homologous recombination of the targeting vector with the Adipoq allele replaces exon 2 with neo. TK, thymidine kinase. B: Southern blot demonstrating disruption of the Adipoq locus. Targeted allele is identified by an ~4-kb decrease in an Xbal restriction fragment that is detected with a 525-bp probe located downstream of the targeting vector sequences. Absence of adiponectin in the serum of adiponectin-deficient (Adipoq<sup>−/−</sup>) mice demonstrated by ELISA (C) and immunoblotting (D). ND, not detectable; Adipo, full-length adiponectin; gAdipo, purified globular head domain of murine adiponectin.

**Fig. 2.** Impaired glucose tolerance in high-fat-fed Adipoq<sup>−/−</sup> mice. A: similar glucose tolerance in chow-fed wild-type and Adipoq<sup>−/−</sup> mice; n = 4 male mice/genotype. B: impaired glucose tolerance in high-fat-fed Adipoq<sup>−/−</sup> mice. At 3 mo of age, mice were fed a high-fat diet for 15 wk, and glucose tolerance tests were performed after an overnight fast; n = 7 male mice/genotype. Areas under the curves (A and B, bottom) were calculated using the trapezoid rule, and the means were compared with a one-way ANOVA followed by a Newman-Keuls multiple comparison test. *P < 0.05 vs. high-fat Adipoq<sup>+/+</sup> mice.
four genotypes were weaned onto a high-fat diet, and their body weights were monitored for 20 wk (Fig. 3A). Body weights were lower in Dgatl+/−/Adipoq+/+ mice and higher in Dgatl+/−/Adipoq−/− mice than in wild-type controls. DGAT1 deficiency lowered body weights in adiponectin-deficient mice (Dgatl−/−/Adipoq−/−) to levels similar to those of Dgatl−/−/ Adipoq+/+ mice.

After 20 wk of high-fat feeding, body composition was analyzed. Mice of all four genotypes had similar lean body masses (Fig. 3B). However, both Dgatl−/−/Adipoq+/+ and Dgatl−/−/Adipoq−/− had similarly lower fat masses and body fat percentages than mice with a Dgatl+/+ genotype (Fig. 3B). The fat mass and percent body fat tended higher in Dgatl+/−/ Adipoq−/− mice than in Dgatl+/−/Adipoq+/+ mice, but the differences were not significant (Fig. 3B). Consistent with the body composition analyses, serum leptin levels were significantly reduced in mice lacking Dgatl, irrespective of their Adipoq genotype (Dgatl+/−/Adipoq+/+, 17.9 ± 3.0 ng/ml; Dgatl+/−/Adipoq−/−, 17.4 ± 3.4 ng/ml; Dgatl−/−/Adipoq+/+, 4.2 ± 2.4 ng/ml; Dgatl−/−/Adipoq−/−, 2.5 ± 1.4 ng/ml; P < 0.01 for Dgatl−/− vs. Dgatl+/+ mice).

Energy balance studies demonstrated that Dgatl−/−/ Adipoq+/+ and Dgatl−/−/Adipoq−/− mice fed a high-fat diet for 2 wk had higher levels of Vo2 than mice with the Dgatl+/+ genotype (Fig. 4A). Although both Dgatl−/−/Adipoq+/+ and Dgatl−/−/Adipoq−/− tended to have increased food intake, the only significant increase in food intake was in Dgatl−/−/ Adipoq−/− mice (Fig. 4B). These data indicate that the increased energy expenditure in DGAT1-deficient mice does not require adiponectin.

Effects of DGAT1 deficiency on fasting blood glucose and glucose tolerance are independent of adiponectin. We next assessed the contribution of adiponectin to the enhanced insulin sensitivity in DGAT1-deficient mice. Mice of all four genotypes were fed a high-fat diet for 8 wk. Blood glucose concentrations were lower in Dgatl−/−/Adipoq+/+ mice and higher in Dgatl+/−/Adipoq−/− mice than in Dgatl+/−/Adipoq+/+ controls after an overnight fast (Fig. 5A). Dgatl−/−/Adipoq−/− mice were completely protected from this diet-induced increase in fasting glucose concentrations (Fig. 5A). Serum insulin levels were similar in mice of all four genotypes (Dgatl+/+/Adipoq+/+, 0.54 ± 0.18 ng/ml; Dgatl+/−/Adipoq−/−, 1.10 ± 0.24 ng/ml; Dgatl−/−/Adipoq+/+, 0.95 ± 0.35 ng/ml; Dgatl−/−/Adipoq−/−, 0.38 ± 0.09 ng/ml; n = 8–10 for each genotype).

In response to a glucose challenge, Dgatl+/−/Adipoq+/+ mice, as expected, had improved glucose tolerance and Dgatl+/−/Adipoq−/− mice had impaired glucose tolerance compared with control Dgatl+/+/Adipoq+/+ mice (Fig. 5, B and C). Glucose tolerance was better in Dgatl+/−/Adipoq−/− mice than in Dgatl+/−/Adipoq−/− mice but was slightly worse than in Dgatl−/−/Adipoq+/+ mice (Fig. 5, B and C).
with either Adipoq genotype, DGAT1 deficiency improved glucose tolerance by \(\sim 30-35\%\) (Fig. 5C). Conversely, adiponectin deficiency impaired glucose tolerance by \(-25\%\) for mice with either Dgat1 genotype. These data indicate that DGAT1 deficiency and adiponectin deficiency have opposite and independent effects on glucose tolerance.

**DGAT1-deficient mice lacking adiponectin are protected against diet-induced hepatic steatosis.** DGAT1-deficient mice are protected against hepatic steatosis induced by a high-fat diet (26), and adiponectin protects against hepatic steatosis (34). We therefore examined whether this protection from diet-induced hepatic steatosis in DGAT1-deficient mice requires adiponectin. To induce steatosis, mice were fed a high-fat, Western-style diet for 20 wk, and their livers were harvested and assayed for neutral lipid content (Fig. 6, A and B). As expected, wild-type mice accumulated considerable amounts of TG and cholesterol esters. The hepatic content of TG in Dgat1\(^{+/+}\) Adipoq\(^{-/-}\) mice was significantly increased compared with controls. In contrast, hepatic TG and cholesterol ester levels were markedly reduced in both Dgat1\(^{-/-}\) Adipoq\(^{-/-}\) and Dgat1\(^{+/+}\) Adipoq\(^{-/-}\) mice, indicating that protection against diet-induced steatosis in DGAT1 deficiency does not require adiponectin. These data also indicate that the increased hepatic steatosis associated with adiponectin deficiency requires DGAT1.

**DISCUSSION**

Here, we tested the hypothesis that adiponectin is required for the beneficial changes in energy and glucose metabolism in DGAT1-deficient mice. By studying mutant mice that lacked both DGAT1 and adiponectin, we found that adiponectin was not required for any aspects of the DGAT1 deficiency phenotype that we tested. Under conditions of high-fat feeding, DGAT1 deficiency promoted obesity resistance, enhanced glucose tolerance, and conferred protection from hepatic steatosis in the absence of adiponectin. These data indicate that DGAT1 deficiency modulates energy and glucose metabolism through mechanisms that are largely independent of adiponectin and that metabolic changes induced by DGAT1-deficient WAT are likely due to other WAT-derived factors.
Several conclusions can be drawn from our studies, the first relating to adiponectin deficiency in mice. There have been conflicting reports regarding the development of insulin resistance in Adipoq<sup>−/−</sup> mice fed high-fat diets (18–20, 22). Maeda et al. (20), Kubota et al. (18), and Nawrocki et al. (22) found that Adipoq<sup>−/−</sup> mice fed a high-fat diet for 2 or 10 wk developed increased fasting blood glucose concentrations and impaired glucose tolerance, respectively. In contrast, Ma et al. (19) found that Adipoq<sup>−/−</sup> mice fed a high-fat, high-sucrose diet for 7 mo had normal fasting glucose concentrations, normal glucose tolerance, and glucose infusion rates similar to those of wild-type mice during a hyperinsulinemic euglycemic clamp. Similar to Kubota et al. and Maeda et al., we found that male, but not female, Adipoq<sup>−/−</sup> mice fed a high-fat diet developed glucose intolerance, supporting the conclusion that adiponectin deficiency adversely affects glucose metabolism. We also found that Adipoq<sup>−/−</sup> mice fed a high-fat, Western-type diet for 20 wk were heavier than wild-type controls, although the differences in body composition were not statistically significant. Finally, we show that adiponectin deficiency promotes hepatic steatosis in mice fed a high-fat diet.

We are confident that our gene disruption generated a null allele for Adipoq. Our targeting strategy was similar to that used by Ma et al. (19) and Maeda et al. (20), in which exon 2 of Adipoq, which contains the translational start site and signal sequence for adiponectin secretion (13), was replaced by neo<sup>+</sup>. Furthermore, the serum in our Adipoq<sup>−/−</sup> mice lacked detectable adiponectin by two methods that assay for different regions of the adiponectin protein.

The second major conclusion from these studies is that adiponectin is not a major downstream mediator of the effects of DGAT1 deficiency on systemic energy and glucose metabolism. The effects of DGAT1 deficiency on metabolism of high-fat-fed mice were present even in the absence of adiponectin and, therefore, occur independently of adiponectin. DGAT1 deficiency lowered body weight by similar percentages and increased energy expenditure irrespective of the Adipoq genotype. With respect to glucose tolerance, the genotypes had clearly independent effects. DGAT1 deficiency proportionally improved glucose tolerance irrespective of Adipoq genotype, and adiponectin deficiency proportionally impaired glucose tolerance irrespective of Dgat1 genotype. DGAT1 deficiency also afforded complete protection against hepatic steatosis irrespective of Adipoq genotype, but adiponectin deficiency only promoted hepatic steatosis when DGAT1 was present, indicating a dependency on DGAT1 for this phenotypic outcome.

These studies indicate that the improved metabolic effects conferred by DGAT1-deficient WAT are not due to adiponectin. Instead, both DGAT1 deficiency and adiponectin promoted obesity resistance, enhanced insulin sensitivity, and protected against hepatic steatosis in high-fat-fed mice, but they did so through independent, and possibly parallel, pathways. Previous studies (7) indicate that the effects of DGAT1 deficiency require leptin and suggest that DGAT1 deficiency promotes leptin sensitivity, suggesting that Dgat1<sup>−/−</sup> WAT may secrete a factor that enhances leptin action. In this study, we effectively excluded adiponectin as a candidate. Further study of Dgat1<sup>−/−</sup> WAT provides the opportunity to identify such factors.

Finally, our results may have implications for human obesity accompanied by adiponectin deficiency. Circulating adiponectin levels are inversely correlated to fat mass and directly proportional to insulin sensitivity (16). Indeed, hypoadiponectinemia appears to be a valuable biomarker of the metabolic syndrome (31). From this perspective, we conclude that DGAT1 deficiency can exert potentially beneficial metabolic effects in the absence of adiponectin. Therefore, pharmacological inhibition of DGAT1 represents a possible strategy for treating human obesity and insulin resistance associated with low circulating adiponectin levels.

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