Published in final edited form as:
Am J Physiol Endocrinol Metab. 2006 August ; 291(2): E388–E394.

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

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Abstract

Mice lacking acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1), an enzyme that catalyzes the terminal step in triacylglycerol synthesis, have enhanced insulin sensitivity and are protected from obesity, a result of increased energy expenditure. In these mice, factors derived from white adipose tissue (WAT) contribute to the systemic changes in metabolism. One such factor, adiponectin, increases fatty acid oxidation and enhances insulin sensitivity. To test the hypothesis that adiponectin is required for the altered energy and glucose metabolism in DGAT1-deficient mice, we generated adiponectin-deficient mice and introduced adiponectin deficiency into DGAT1-deficient mice by genetic crosses. Although adiponectin-deficient mice fed a high-fat diet were heavier, exhibited worse glucose tolerance, and had more hepatic triacylglycerol accumulation than wild-type controls, mice lacking both DGAT1 and adiponectin, like DGAT1-deficient mice, were protected from diet-induced obesity, glucose intolerance, and hepatic steatosis. These findings indicate that adiponectin is required for normal energy, glucose, and lipid metabolism but that the metabolic changes induced by DGAT1-deficient WAT are independent of adiponectin and are likely due to other WAT-derived factors. Our findings also suggest that the pharmacological inhibition of DGAT1 may be useful for treating human obesity and insulin resistance associated with low circulating adiponectin levels.

Keywords

triglyceride; adipose tissue; gene knockout; acyl-coenzyme A:diacyl-glycerol acyltransferase 1

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

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GRANTS

This work was supported by the American Heart Association Western States Affiliate (postdoctoral fellowship to R. S. Streeper), the National Institute of Diabetes and Digestive and Kidney Diseases (R01-DK-56084 to R. V. Farese, Jr.), the Sandler Family Supporting Foundation, an extramural research facilities improvement program project grant (C06-RR-018928), the Yamanouchi U. S. A. Foundation, and the J. David Gladstone Institutes.

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 (A^{Y}/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 ($WAT^{Dgat1KO}$) into both wild-type and A^{Y}/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 Acdc, Acrp30, adipoQ, 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 $Dgat1^{-/-}A^{Y}/a$ mice (6). Adiponectin levels also increased twofold in medium conditioned by $Dgat1^{-/-}A^{Y}/a$ WAT, and serum adiponectin levels increased in high-fat-fed wild-type mice transplanted with $WAT^{Dgat1KO}$ and in high-fat-fed $Dgat1^{-/-}$ 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 $Dgat1^{-/-}$ mice. We then analyzed parameters of energy and glucose metabolism in $Dgat1^{-/-}Adipoq^{-/-}$ 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 *Adipoq* 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′-atttgcggccGCTGAGCAGATATGCACGGAGCATGCG-3′ and 5′-atttgcggcCGCAGGACCTGCATCATGTCCTATATAGC-3′; short-arm primers, 5′-ccgctcgagGCCTAGACACAGACACAAGAAA-GAGGC-3′ and 5′-ACTCTCGAGCTA GATACTACATCCTTGT-TCG-3′). Primer sequences (lowercase letters) were added on the primer termini to introduce *Not*I (long arm) and *Xho*I (short arm) restriction enzyme sites for cloning (the antisense short-arm primer contained the endogenous *Xho*I site in intron 2). The targeting construct was introduced into 129/SvJae murine embryonic stem cells (line RF8) (21), and clones containing targeted alleles were identified by PCR and verified by Southern blotting. Cells containing the targeted *Adipoq* allele were used to generate mice carrying the mutation (24).

The disruption of *Adipoq* was confirmed by Southern blotting of genomic DNA digested with *Xba*I and probed with a 525-bp fragment corresponding to *Adipoq* exon 3 sequence and located downstream of the vector sequences. The probe was generated by PCR with primers 5′-GTCTTCTTGGTCCTAAGGGTGAGAC-3′ and 5′-GCAGTCA-GTTGGTATCATGGTAGAG-3′. *Adipoq*$^{-/-}$ mice were also genotyped by a three-primer PCR reaction designed to distinguish homozygosity from heterozygosity, where the targeted allele gave a 570-bp fragment and the wild-type allele gave a 200-bp fragment (primers: 5′-GTGCAGGTTGGATGGCAGGCATC-3′, 5′-GCCTC-TTTCTTGTGTCTGTGTCTAGGC-3′, and 5′-CCTCGTGCTTT-ACGGTATCGCCGCTC-3). *Adipoq*$^{-/-}$ mice (50% C57BL/6J and 129/SvJae genetic background) were crossed with *Dgat1*$^{-/-}$ mice (C57BL/6J background) to generate study group mice (~75% C57BL/6J and 25% 129/SvJae genetic background). Specifically, *Dgat1*$^{+/-}$ *Adipoq*$^{-/-}$ mice were intercrossed to generate *Dgat1*$^{+/+}$ *Adipoq*$^{-/-}$ and *Dgat1*$^{-/-}$*Adipoq*$^{-/-}$ mice, and *Dgat1*$^{+/-}$ *Adipoq*$^{+/+}$ mice were intercrossed to generate *Dgat1*$^{+/+}$*Adipoq*$^{+/+}$ and *Dgat1*$^{-/-}$ *Adipoq*$^{+/+}$ mice. All mice were housed in a pathogen-free barrier-type facility (12:12-h light-dark cycle). Male mice were used for all studies, and they were fed either a standard chow diet (5053 PicoLab Diet; Purina, St. Louis, MO) or a high-fat, Western-type diet (TD. 01064; Harlan-Teklad, Madison, WI) that contained 20% anhydrous milk fat, 1% corn oil, and 0.2% cholesterol by weight. All experiments were approved by the University of California, San Francisco, Committee on Animal Research.

Protein analysis

Adiponectin levels in serum were analyzed by ELISA (MRP300; R&D Systems, Minneapolis, MN) and immunoblotting (1 μl of serum) with a polyclonal antiserum that recognizes the mouse globular head region of adiponectin (6). Purified mouse globular adiponectin (450-27; PeproTech, Rocky Hill, NJ) served as a positive control.

Serum analyses

Serum was collected from mice that were fed a high-fat diet for 20 wk and fasted for 4 h. Leptin and insulin were measured by ELISA (MOB00; R&D Systems, and EZRMI-13K; Linco Research, St. Charles, MO, respectively).

Body composition

Mice were fasted for 4 h and anesthetized with tribromoethanol (125 mg/kg), and their body composition was analyzed by dual-energy X-ray absorptiometry (DEXA) with a PixiMus2 scanner (GE Healthcare Lunar, Madison, WI).

Energy balance

Food intake and oxygen consumption ($\forall O_2$) were measured by indirect calorimetry (Oxymax Lab Animal Monitoring System; Columbus Instruments, Columbus, OH) over 3 days. Both parameters were normalized to lean body mass as measured by DEXA scanning on the day before calorimetry studies.

Glucose tolerance

After an overnight fast, mice were injected intraperitoneally with glucose (2 g/kg body wt), and blood glucose was measured at 0, 15, 30, 60, and 120 min with a One-Touch UltraSmart glucose monitoring system (Lifescan, Milpitas, CA).

Lipid analyses

Hepatic TG and cholesterol esters were analyzed as described (28). Briefly, lipids were extracted from liver homogenates in $CHCl_3$-MeOH (2:1) and separated by thin-layer chromatography with hexane-ethyl ether-acetic acid (80:20:1) on silica gel G-60 TLC plates. TG and cholesterol ester masses were quantified by the method of Snyder and Stephens (27) with triolein and cholesteryl oleate as standards.

Statistical analyses

Data are presented as means ± SE. Means were compared with a Mann-Whitney rank sum test or by analysis of variance followed by a Student-Newman-Keuls multiple comparison test. For glucose tolerance tests, the area under the curve was calculated using the trapezoid rule (Prism4; GraphPad Software, San Diego, CA). Weight curves were compared with a Kruskal-Wallis test 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. 1*A*). 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. 1*B*). 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. 2*A*). However, male *Adipoq*$^{-/-}$ mice fed a high-fat diet for 15 wk had a greater impairment of glucose tolerance than wild-type mice (Fig. 2*B*). 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*$^{-/-}$ mice to obtain mice with the following genotypes: *Dgat1*$^{+/+}$ *Adipoq*$^{+/+}$, *Dgat1*$^{-/-}$*Adipoq*$^{+/+}$, *Dgat1*$^{+/+}$ *Adipoq*$^{-/-}$, and *Dgat1*$^{-/-}$ *Adipoq*$^{-/-}$. The double knockout (*Dgat1*$^{-/-}$ *Adipoq*$^{-/-}$) 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*$^{-/-}$*Adipoq*$^{-/-}$ 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 four genotypes were weaned onto a high-fat diet, and their body weights were monitored for 20 wk (Fig. 3*A*). Body weights were lower in *Dgat1*$^{-/-}$*Adipoq*$^{+/+}$ mice and higher in *Dgat1*$^{+/+}$ *Adipoq*$^{-/-}$ mice than in wild-type controls. DGAT1 deficiency lowered body weights in adiponectin-deficient mice (*Dgat1*$^{-/-}$*Adipoq*$^{-/-}$) to levels similar to those of *Dgat1*$^{-/-}$ *Adipoq*$^{+/+}$ mice.

After 20 wk of high-fat feeding, body composition was analyzed. Mice of all four genotypes had similar lean body masses (Fig. 3*B*). However, both *Dgat1*$^{-/-}$ *Adipoq*$^{+/+}$ and *Dgat1*$^{-/-}$*Adipoq*$^{-/-}$ had similarly lower fat masses and body fat percentages than mice with a *Dgat1*$^{+/+}$ genotype (Fig. 3*B*). The fat mass and percent body fat trended higher in *Dgat1*$^{+/+}$ *Adipoq*$^{-/-}$ mice than in *Dgat1*$^{+/+}$*Adipoq*$^{+/+}$ mice, but the differences were not significant (Fig. 3*B)*. Consistent with the body composition analyses, serum leptin levels were significantly reduced in mice lacking *Dgat1*, irrespective of their *Adipoq* genotype (*Dgat1*$^{+/+}$*Adipoq*$^{+/+}$, 17.9 ± 3.0 ng/ml; *Dgat1*$^{+/+}$*Adipoq*$^{-/-}$, 17.4 ± 3.4 ng/ml; *Dgat1*$^{-/-}$*Adipoq*$^{+/+}$, 4.2 ± 2.4 ng/ml; *Dgat1*$^{-/-}$*Adipoq*$^{-/-}$, 2.5 ± 1.4 ng/ml; $P < 0.01$ for *Dgat1*$^{-/-}$ vs. *Dgat1*$^{+/+}$ mice).

Energy balance studies demonstrated that *Dgat1*$^{-/-}$ *Adipoq*$^{+/+}$ and *Dgat1*$^{-/-}$*Adipoq*$^{-/-}$ mice fed a high-fat diet for 2 wk had higher levels of $\forall O_2$ than mice with the *Dgat1*$^{+/+}$ genotype (Fig. 4*A*). Although both *Dgat1*$^{-/-}$*Adipoq*$^{+/+}$ and *Dgat1*$^{+/+}$*Adipoq*$^{-/-}$ tended to have increased food intake, the only significant increase in food intake was in *Dgat1*$^{-/-}$ *Adipoq*$^{-/-}$ mice (Fig. 4*B*). 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 *Dgat1*$^{-/-}$*Adipoq*$^{+/+}$ mice and higher in *Dgat1*$^{+/+}$*Adipoq*$^{-/-}$ mice than in *Dgat1*$^{+/+}$*Adipoq*$^{+/+}$ controls after an overnight fast (Fig. 5*A*). *Dgat1*$^{-/-}$*Adipoq*$^{-/-}$ mice were completely protected from this diet-induced increase in fasting glucose concentrations (Fig. 5*A*). Serum insulin levels were similar in mice of all four genotypes (*Dgat1*$^{+/+}$*Adipoq*$^{+/+}$, 0.54 ± 0.18 ng/ml; *Dgat1*$^{+/+}$*Adipoq*$^{-/-}$, 1.10 ± 0.24 ng/ml; *Dgat1*$^{-/-}$*Adipoq*$^{+/+}$, 0.95 ± 0.35 ng/ml; *Dgat1*$^{-/-}$*Adipoq*$^{-/-}$, 0.38 ± 0.09 ng/ml; $n = 8–10$ for each genotype).

In response to a glucose challenge, *Dgat1*$^{-/-}$*Adipoq*$^{+/+}$ mice, as expected, had improved glucose tolerance and *Dgat1*$^{+/+}$*Adipoq*$^{-/-}$ mice had impaired glucose tolerance compared with control *Dgat1*$^{+/+}$*Adipoq*$^{+/+}$ mice (Fig. 5, *B* and *C*). Glucose tolerance was better in *Dgat1*$^{-/-}$*Adipoq*$^{-/-}$ mice than in *Dgat1*$^{+/+}$*Adipoq*$^{-/-}$ mice but was slightly worse than in *Dgat1*$^{-/-}$*Adipoq*$^{+/+}$ mice (Fig. 5, *B* and *C*). In mice with either *Adipoq* genotype, DGAT1 deficiency improved glucose tolerance by ~30 –35% (Fig. 5*C*). 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 $Adipoq^{-/-}$ mice fed high-fat diets (18–20,22). Maeda et al. (20), Kubota et al. (18), and Nawrocki et al. (22) found that $Adipoq^{-/-}$ 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 $Adipoq^{-/-}$ 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, $Adipoq^{-/-}$ mice fed a high-fat diet developed glucose intolerance, supporting the conclusion that adiponectin deficiency adversely affects glucose metabolism. We also found that $Adipoq^{-/-}$ 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*. Furthermore, the serum in our $Adipoq^{-/-}$ 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*$^{-/-}$ WAT may secrete a factor that enhances leptin action. In this study, we effectively excluded adiponectin as a candidate. Further study of *Dgat1*$^{-/-}$ 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.

Acknowledgements

We thank R. Bituin for technical assistance, H. Myers for mouse embryonic stem cell culture, Q. Walker for blastocyst injections, A. Bradford for manuscript preparation, S. Ordway and G. Howard for editorial assistance, and H. Chen, T. Walther, and members of the Farese laboratory for helpful comments on the manuscript.

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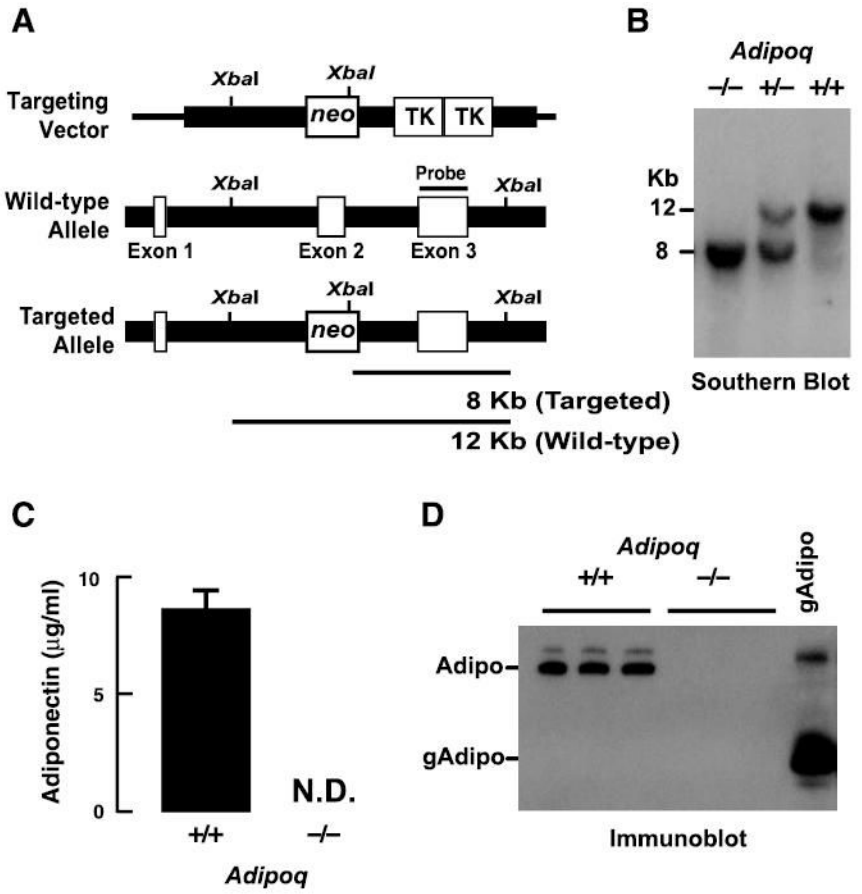

Fig. 1.

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 *Xba*I 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*$^{-/-}$) mice demonstrated by ELISA (*C*) and immunoblotting (*D*). ND, not detectable; Adipo, full-length adiponectin; gAdipo, purified globular head domain of murine adiponectin.

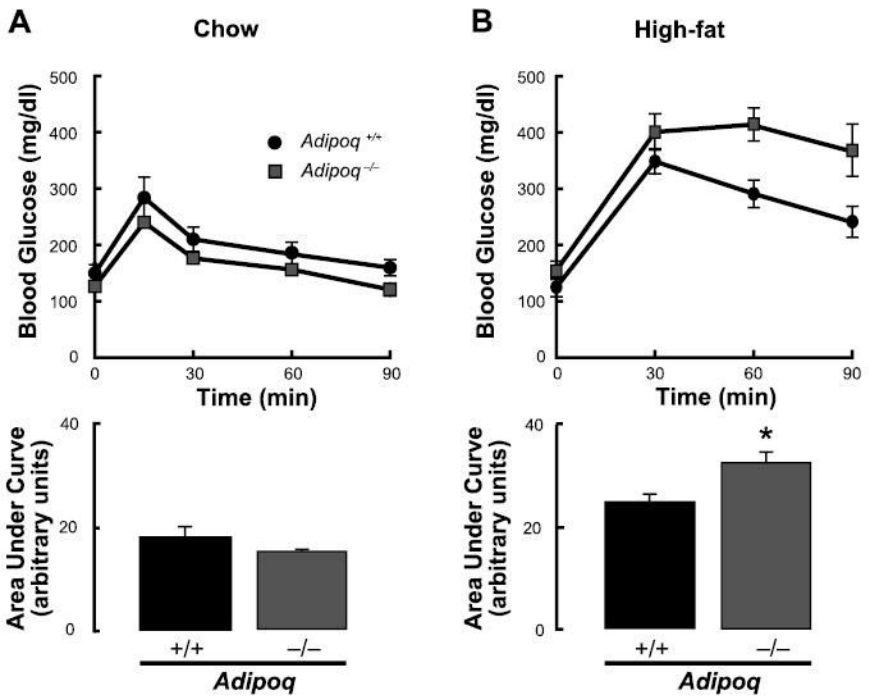

Fig. 2.

Impaired glucose tolerance in high-fat-fed *Adipoq*$^{-/-}$ mice. *A*: similar glucose tolerance in chow-fed wild-type and *Adipoq*$^{-/-}$ mice; $n = 4$ male mice/ genotype. *B*: impaired glucose tolerance in high-fat-fed *Adipoq*$^{-/-}$ 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*$^{+/+}$ mice.

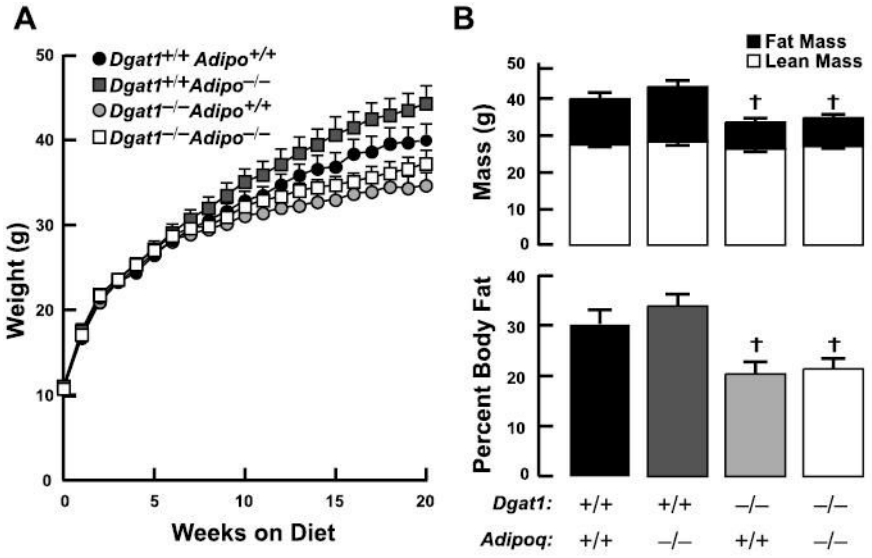

Fig. 3.

Effects of acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1) deficiency on body weight and composition are independent of adiponectin. *A*: body weights of high-fat-fed mice. Mice (n = 10 –15 male mice/ genotype) were weaned onto a high-fat diet and weighed weekly in the afternoon. Differences in weight curves are described in RESULTS. *B*: body composition was determined by dual-energy X-ray absorptiometry at the end of the 20 wk of high-fat feeding of mice in *A*. †$P < 0.05$ vs. mice with the same *Adipoq* genotype.

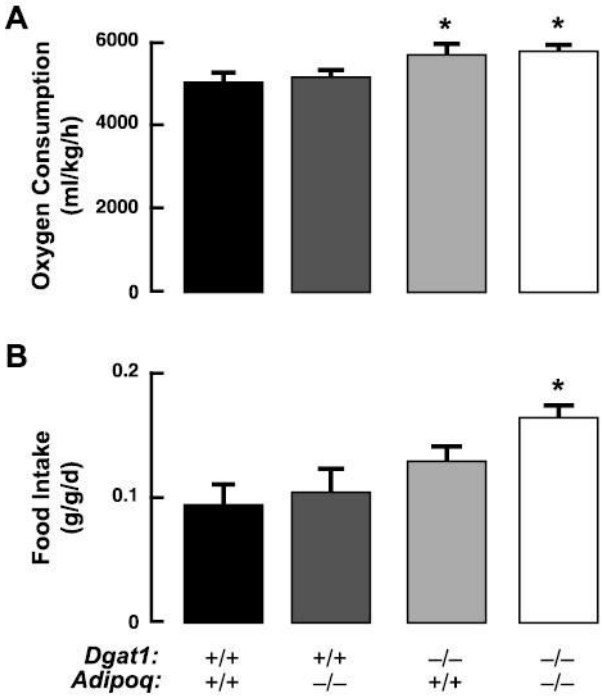

Fig. 4.

Energy balance studies. Mice of all 4 genotypes were fed a high-fat diet for 2 wk, and oxygen consumption (*A*) and food intake (*B*) were determined in metabolic cages. Oxygen consumption and food intake were normalized to lean body mass; $n = 4$ male mice/genotype. *$P < 0.05$ vs. *Dgat1*$^{+/+}$ mice.

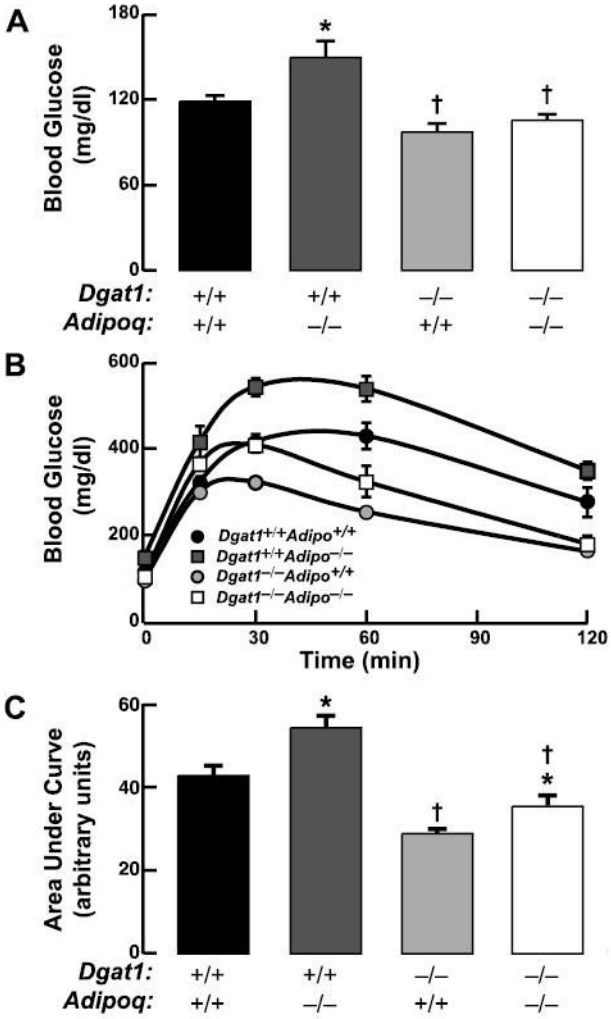

Fig. 5.

Adiponectin is not required for the improved glucose tolerance of mice lacking DGAT1. *A*: DGAT1 deficiency protects against impairment in fasting glucose associated with high-fat feeding, even in the absence of adiponectin. *B* and *C*: DGAT1 deficiency protects against diet-induced glucose intolerance in mice regardless of the presence or absence of adiponectin. Mice (n = 5–12 male mice/genotype) were fed a high-fat diet for 8 wk. After an overnight fast, blood glucose was measured (*A*). Glucose tolerance tests were performed in *B. C*: area under the curves was calculated as described in Fig. 2. *$P < 0.05$ vs. mice with the same *Dgat1* genotype; †$P < 0.05$ vs. mice with the same *Adipoq* genotype.

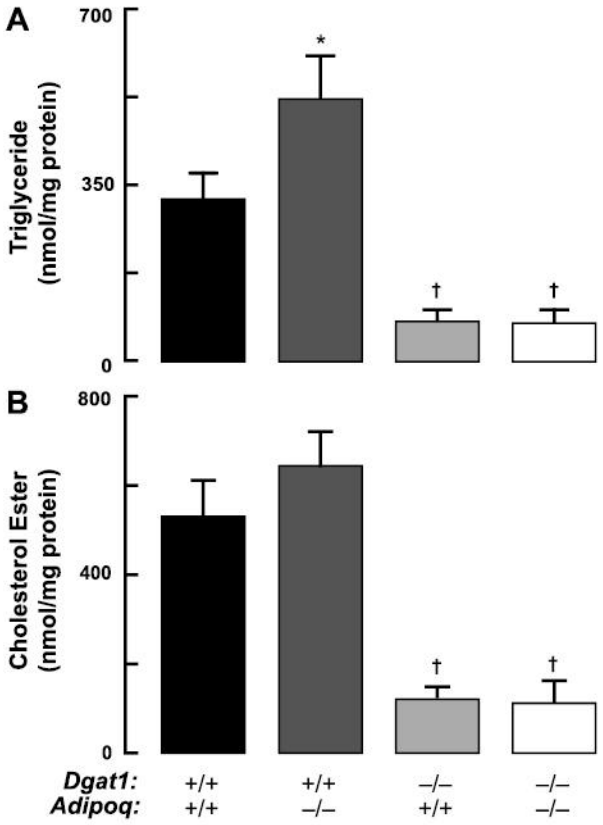

Fig. 6.

DGAT1 deficiency does not require adiponectin for protection against diet-induced hepatic steatosis. Decreased accumulation of hepatic triacylglycerol (TG; *A*) and cholesterol esters (*B*) in mice lacking DGAT1 is independent of adiponectin. Mice were fed a high-fat diet for 20 wk, and livers were removed and assayed for TG and cholesterol esters; $n = 4 – 8$ male mice/ genotype. *$P < 0.05$ vs. mice with the same *Dgat1* genotype. †$P < 0.05$ vs. mice with the same *Adipoq* genotype.