Dissociation of Obesity and Impaired Glucose Disposal in Mice Overexpressing Acyl Coenzyme A:Diacylglycerol Acyltransferase 1 in White Adipose Tissue

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Acyl coenzyme A:diacylglycerol acyltransferase 1 (DGAT1) is one of two DGAT enzymes known to catalyze the final step in mammalian triglyceride synthesis. Mice deficient in DGAT1 are resistant to obesity and have enhanced insulin sensitivity. To understand better the relationship between triglyceride synthesis and energy and glucose metabolism, we generated transgenic (aP2-Dgat1) mice in which expression of murine DGAT1 in the white adipose tissue (WAT) was twofold higher than normal. aP2-Dgat1 mice that were fed a regular diet had larger adipocytes and greater total fat pad weight than wild-type (WT) mice. In response to a high-fat diet, aP2-Dgat1 mice became more obese (~20% greater body weight after 15 weeks) than WT mice. However, the increase in adiposity in aP2-Dgat1 mice was not associated with impaired glucose disposal, as demonstrated by glucose and insulin tolerance tests. Correlating with this finding, triglyceride deposition in the liver and skeletal muscle, two major target tissues of insulin, was similar in aP2-Dgat1 and WT mice. Thus, DGAT1 overexpression in murine WAT provides a model in which obesity does not impair glucose disposal. Our findings support the lipotoxicity hypothesis that the deposition of triglycerides in insulin-sensitive tissues other than adipocytes causes insulin resistance. Diabetes 51:3189–3195, 2002

Although obesity is a major risk factor for the development of insulin resistance and type 2 diabetes (reviewed in 1,2), the relationship between obesity and the development of insulin resistance is incompletely understood. One mechanism that has been proposed as a cause of obesity-induced insulin resistance is an elevation of plasma free fatty acid (FFA) levels (3). Elevated plasma FFA levels are associated with a downregulation of insulin signaling in insulin-sensitive tissues (4,5). Although the mechanism is unclear, FFA itself is unlikely to cause insulin resistance. Acute changes in plasma FFA levels, for example, do not immediately affect insulin resistance; instead, there is typically a 2- to 4-h time lag (3). Furthermore, several recent studies of murine models with disruptions of genes involved in fatty acid and triglyceride metabolism have shown that elevated levels of plasma FFA do not always correlate with insulin resistance (reviewed in 6).

A more likely mechanism by which elevated plasma FFA levels cause insulin resistance is by increasing intracellular fatty acid metabolites such as fatty acyl Coenzyme A (CoA) or diacylglycerol (reviewed in 2,7). Elevated FFA levels may also increase intracellular triglyceride content, which is inversely related to insulin sensitivity (8–10) and may directly interfere with insulin action (3,11). According to this lipotoxicity hypothesis, insulin resistance develops when excess lipids are deposited in insulin-sensitive tissues other than adipocytes, such as the liver and skeletal muscle, thereby interfering with insulin signaling.

One of the key enzymes in fatty acid and triglyceride metabolism is acyl coenzyme A:diacylglycerol acyltransferase 1 (DGAT1) (12). DGAT1 is one of two known enzymes that catalyze the final step in mammalian triglyceride synthesis (13). It does so by covalently linking a fatty acyl CoA with a diacylglycerol (14). DGAT1 is expressed in all tissues examined (12), with high levels of expression in the white adipose tissue (WAT) and other tissues (e.g., skeletal muscle, small intestine) in which triglyceride metabolism is prominent. We have reported that DGAT1-deficient (Dgat1<sup>−/−</sup>) mice have increased energy expenditure and are obesity-resistant (15). Dgat1<sup>−/−</sup> mice also have decreased tissue levels of triglycerides and increased insulin sensitivity (16).

To understand better the relationship between triglyceride synthesis in WAT and systemic glucose metabolism, we generated transgenic mice that overexpress murine DGAT1 in WAT and examined the effects of DGAT1 overexpression in WAT on obesity and glucose metabolism. Our findings support the lipotoxicity hypothesis that insulin resistance occurs when excess triglycerides are deposited in insulin-sensitive tissues other than adipocytes.

**RESEARCH DESIGN AND METHODS**

**Mice and diet.** The DGAT1 transgene was made with a 5.4-kb promoter of the adipocyte fatty acid binding protein (aP2; a gift from B. Spiegelman, Dana-Farber Cancer Institute, Boston, MA), the 1.6-kb murine DGAT1 cDNA (12) with an amino-terminal FLAG epitope (Pharmingen, San Diego, CA), and a

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aP2, adipocyte fatty acid binding protein; ASP, acylation-stimulating protein; CoA, coenzyme A; DGAT, acyl coenzyme A:diacylglycerol acyltransferase; FFA, free fatty acid; TNF, tumor necrosis factor; WAT, white adipose tissue.
2.2-kb human growth hormone minigene (a gift from J. Marsh, University of California, San Diego, CA). The epitope-tagged murine DGAT1 cDNA increases DGAT activity when overexpressed in cultured cells (12). The DGAT1 transgene was injected into the pronucleus of fertilized eggs from superovulated C57BL/6J female mice. Founder animals were bred with C57BL/6J mice, and two transgenic mouse lines were established: a high-expression line (line 4.0-kb DNA fragment containing the DGAT1 transgene was injected) and a low-expression line (line 3). Unless specified, results from line 2 are shown.

Wild-type (WT), Agouti yellow (A/Y), and leptin receptor–deficient (db/db) mice (all in C57BL/6J background) were from The Jackson Laboratory (Bar Harbor, ME). A/Y mice are obese and insulin-resistant, reflecting the antagonism of melanocortin receptors in the hypothalamus (17,18). Mice were housed in a pathogen-free facility (12 h light/12 h dark cycle) and fed rodent chow (Ralston Purina Co., St. Louis, MO). For high-fat diet experiments, mice were fed a Western-type diet containing 21% fat by weight (Harlan Teklad, Madison, WI) for 4 weeks unless stated otherwise. All experiments were approved by the Committee on Animal Research of the University of California, San Francisco.

Genotyping. For Southern blotting, 10 µg of tail DNA was digested with EcoRI, fractionated by agarose gel electrophoresis, and blotted on nylon membranes. An ~4-kb DNA fragment containing the DGAT1 transgene was detected with a 32P-labeled 1.6-kb DGAT1 cDNA probe. Transgene detection was shown.

Reverse transcriptase and real-time PCR. Total RNA was extracted from tissues, and 1 µg was reverse-transcribed into cDNA (Superscript II; Invitrogen, Carlsbad, CA). The RT-PCR for FLAG-Dgat1 was performed for 30 cycles and the RT-PCR for β-actin for 25 cycles. Primers for β-actin were the same ones used for real-time PCR (Table 1). Real-time PCR was performed as described (19).

Immunoblot. Reproductive fat pads from 20-week-old male mice were homogenized, and protein samples (25 µg) were loaded on a 10% polyacrylamide gel, transferred to polyvinylidene fluoride membrane, and incubated with an anti-DGAT1 polyclonal primary antibody that recognizes the amino terminus of the protein (45 kDa). Binding was detected with enhanced chemiluminescence (Pierce Biotechnology Inc., Rockford, IL) as described (20).

Adipocyte size and mass measurements. Adipose tissue was obtained from the reproductive fat pads of 10- to 12-week-old male mice. The samples were fixed in paraformaldehyde, embedded in paraffin, sliced to 5 µm in thickness, and stained with hematoxylin and eosin. Images of the histological sections were analyzed with Adobe Photoshop 5.0.1 (Adobe Systems, San Jose, CA) and Image Processing Tool Kit (Reindeer Games, Gainesville, FL) as described (21). Triglycerides were measured with a kit (A202; Sigma Chemical Co., St. Louis, MO) as described (22). DNA was extracted with phenol/chloroform: isoamyl alcohol (24:1), precipitated with ethanol, and quantified with a spectrophotometer at 260 nm. Inguinal, reproductive, mesenteric, and perirenal fat pads were used to determine total fat pad content.

<table>
<thead>
<tr>
<th>Gene</th>
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<th>Sequence</th>
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<tbody>
<tr>
<td>Actin</td>
<td>5′</td>
<td>5′-CATCTTGCGCTCACTGTTCCA-3′</td>
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<tr>
<td></td>
<td>3′</td>
<td>5′-GGGCCGACTCATGCTACT-3′</td>
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<td></td>
<td>probe</td>
<td>5′-CTTCCCCAGCTAGTGATCCAGAACG-3′</td>
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<td>Adiponecin</td>
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<td></td>
<td>3′</td>
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<td></td>
<td>3′</td>
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<tr>
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<td>probe</td>
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<tr>
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<td>5′-TCCCGAGGCGACATCCAG-3′</td>
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<td>3′</td>
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<tr>
<td></td>
<td>3′</td>
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</tr>
<tr>
<td></td>
<td>probe</td>
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**Metabolic parameters.** Mice were fasted for 1 h, and serum samples were obtained by tail-vein bleeding at noon. Serum FFA and triglyceride levels were measured as described (15). Serum insulin and leptin levels were measured by AniLytics, Inc. (Gaithersburg, MD). For glucose metabolism studies, glucose (1 g/kg body wt) or bovine insulin (1 unit/kg body wt; Sigma Chemical Co.) was injected intraperitoneally, and plasma glucose concentrations were measured with a glucometer (Accu-chek; Roche Diagnostics, Indianapolis, IN). The relative insulin resistance index was calculated with the area under the curve from glucose tolerance tests.

Tissue triglyceride measurements were performed as described (23). The quadriceps, soleus, and gastrocnemius muscles from the lower extremities were used for skeletal muscle measurements, and total liver was used for liver measurements.

**Statistical analysis.** Data are shown as mean ± SD. Measurements were compared with the two-tailed t test or Mann-Whitney rank-sum test. Differences in body weight were compared with ANOVA and, if appropriate, post hoc Tukey-Kramer test to determine the effect of transgene expression at specific time points. Correlation was determined by linear regression.

**RESULTS**

**Restriction of DGAT1 transgene expression to WAT of aP2-Dgat1 mice.** The DGAT1 transgene was detected by both Southern blotting and PCR in two lines of mice (Fig. 1A). The transgene was expressed only in WAT (Fig. 1B). In the high-expression line, DGAT1 mRNA levels were increased twofold in WAT but not in other tissues examined (Fig. 1C). In addition, DGAT1 protein levels were increased at least twofold in WAT of aP2-Dgat1 mice (Fig. 1D). In the low-expression line, DGAT1 mRNA levels were increased ~1.5-fold in WAT (not shown).

**Increased adipocyte size and mass in aP2-Dgat1 mice.** Adipocytes from aP2-Dgat1 mice were larger than those from WT mice (Fig. 2A), as reflected in a nearly twofold increase in mean cell surface area (3,417 ± 686 vs. 1,923 ± 544 μm2; P < 0.05; n = 3 male mice per group). Moreover, reproductive fat pads of aP2-Dgat1 mice contained more triglycerides than those of WT mice despite having similar DNA contents (Fig. 2B). These findings suggest that DGAT1 overexpression in WAT resulted in adipocyte hypertrophy but not hyperplasia.

aP2-Dgat1 mice had a greater mean total fat pad weight than WT mice when fed either a chow or a high-fat diet (Fig. 2C), although the difference was less pronounced.
with high-fat feeding, aP2-Dgat1 mice from line 3 also had increased total fat pad weight compared with WT mice fed a high-fat diet (10.5 ± 0.4% vs. 9.6 ± 0.1% of total body wt; \( P < 0.05 \)). However, the difference was smaller than that observed in mice from line 2.

Increased weight gain in aP2-Dgat1 mice fed a high-fat diet. aP2-Dgat1 and WT mice had similar body weights on a chow diet (not shown). In contrast, aP2-Dgat1 mice gained more weight than WT mice in response to a Western-style high-fat diet, and the difference in weight curves became apparent after 8 weeks (Fig. 2D). aP2-Dgat1 mice from line 3 also tended to gain more weight than WT mice in response to a high-fat diet, although the difference was not statistically significant (39.1 ± 2.8 vs. 37.7 ± 1.7 g at week 15; \( P > 0.05 \)). Because of the greater obesity observed in the high-

![FIG. 1. Expression of the aP2-Dgat1 transgene. A: Detection of the transgene in aP2-Dgat1 mice. The two blots were performed in separate experiments and exposed to film for different lengths of time. B: Restriction of transgene expression to WAT of aP2-Dgat1 mice. Tissues from one male and one female transgenic mouse were used. C: Increased DGAT1 mRNA expression in WAT of aP2-Dgat1 mice; \( n = 3–4 \) per group; 6- to 8-week-old male mice fed a chow diet were used. D: Increased DGAT1 protein level in WAT of aP2-Dgat1 male mice. In addition to the increase in monomeric DGAT1 protein (\(-45\) kDa), there was increased expression of a higher molecular weight species that likely represented a multimeric form of DGAT1 (\(-39\)). Mice were fed a high-fat diet for 6 weeks. BAT, brown adipose tissue; Sk. Muscle, skeletal muscle; * \( P < 0.05 \).]

![FIG. 2. Increased adiposity in aP2-Dgat1 mice. A: Adipocyte hypertrophy in aP2-Dgat1 mice fed a chow diet. Representative results from 12-week-old male mice are shown. Similar findings were observed in female mice (not shown). Bar = 100 \( \mu \)m. B: Increased triglyceride content in reproductive fat pads of aP2-Dgat1 mice; \( n = 3 \) per group; 12-week-old male mice were used. C: Increased total fat pad content in aP2-Dgat1 mice. For high-fat experiments, mice were fed a Western-style diet for 12 weeks; \( n = 4–6 \) per group. Age-matched 16- to 18-week-old male mice were used. Similar results were observed in female mice (not shown). D: Increased susceptibility to diet-induced obesity in male aP2-Dgat1 mice; \( n = 4–6 \) per group. Similar results were observed in female aP2-Dgat1 mice (not shown). * \( P < 0.05 \).]
expression line, we used these mice for the remainder of our studies.

On a high-fat diet, aP2-Dgat1 mice ate more than WT mice (3.0 ± 0.4 vs. 2.1 ± 0.3 g/24 h; P < 0.05). Expressed as percentage of body weight, the increase in food intake trended higher but was not statistically significant (7.5 ± 1.5 vs. 5.5 ± 0.8% of body wt/24 h; P > 0.05). Because fasting-induced weight loss provides a simple estimation of energy expenditure, these findings suggest that aP2-Dgat1 and WT mice had similar levels of energy expenditure.

Serum metabolic parameters in aP2-Dgat1 mice. Despite their difference in adiposity, aP2-Dgat1 and WT mice fed either a chow or a high-fat diet had similar serum glucose and insulin concentrations (Table 2). aP2-Dgat1 mice fed a high-fat diet had higher serum levels of FFA and lower levels of triglycerides than WT mice. aP2-Dgat1 mice tended to have higher serum leptin levels than WT mice, although the difference was not statistically significant.

Lack of impaired glucose disposal in aP2-Dgat1 mice. Because aP2-Dgat1 and WT mice had similar serum glucose and insulin concentrations, we hypothesized that the increased adiposity in aP2-Dgat1 mice did not adversely affect their glucose disposal. Indeed, aP2-Dgat1 and WT mice fed a chow diet had comparable plasma glucose concentrations after a glucose load (Fig. 3A) or an insulin injection (Fig. 3B). Similar results were observed in mice fed a high-fat diet for 4 weeks (Fig. 3C and D).

Adipocyte gene expression in aP2-Dgat1 mice. Given the finding of increased WAT mass without impaired glucose disposal in aP2-Dgat1 mice, we were interested in examining the mRNA expression of several adipocyte-derived molecules that have been implicated in regulating glucose metabolism. Adiponectin (23) (also known as Acrp30 [24], aPM1 [25], and adipQ [26]) and leptin (27,28) are adipocyte-derived molecules that enhance insulin sensitivity, whereas resistin (29) and tumor necrosis factor-α (TNF-α) (30) are molecules associated with insulin resistance. The expression of leptin was threefold higher in aP2-Dgat1 mice than in WT mice (Table 3), consistent with their increased adipocyte size and adipose mass. In contrast, the expression levels of adiponectin, resistin, and TNF-α were not affected by DGAT1 overexpression in WAT.

Similar levels of triglycerides in the liver and skeletal muscle of aP2-Dgat1 and WT mice. Increased triglyceride deposition in the liver and skeletal muscle has been proposed as a mechanism for insulin resistance in obesity (2,3,8,9). We therefore hypothesized that the increased adiposity in aP2-Dgat1 mice was limited to their adipose tissue and did not affect the liver or skeletal muscle.

![Figure 3](3192_diabetes_vol51_nov02.png)

**FIG. 3.** Lack of impaired glucose disposal in aP2-Dgat1 mice. **A and B:** Chow diet; 24-week-old male mice were used. **C and D:** After 4 weeks of high-fat diet, 12-week-old female mice were used. Similar results were observed after 8 weeks of high-fat diet (not shown); n = 5–6 per group.

<table>
<thead>
<tr>
<th>Parameters (unit)</th>
<th>No. of animals</th>
<th>Age (weeks)</th>
<th>Diet</th>
<th>WT</th>
<th>aP2-Dgat1</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/l)</td>
<td>4–5</td>
<td>6</td>
<td>Chow</td>
<td>9.5 ± 0.9</td>
<td>10.0 ± 0.4</td>
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<tr>
<td></td>
<td>3</td>
<td>12</td>
<td>High-fat</td>
<td>10.3 ± 1.5</td>
<td>10.7 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>4–5</td>
<td>14–16</td>
<td>Chow</td>
<td>0.55 ± 0.28</td>
<td>0.58 ± 0.06</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>14–16</td>
<td>High-fat</td>
<td>2.02 ± 0.85</td>
<td>2.61 ± 0.27</td>
<td>NS</td>
</tr>
<tr>
<td>FFA (mmol/l)</td>
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<td>14–16</td>
<td>Chow</td>
<td>0.16 ± 0.06</td>
<td>0.27 ± 0.11</td>
<td>0.06</td>
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<tr>
<td></td>
<td>6</td>
<td>14–16</td>
<td>High-fat</td>
<td>0.34 ± 0.11</td>
<td>0.68 ± 0.18</td>
<td>&lt;0.01</td>
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<td>Triglycerides (mg/dl)</td>
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<td>14–16</td>
<td>High-fat</td>
<td>52.7 ± 13.4</td>
<td>29.5 ± 10.1</td>
<td>0.05</td>
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<td>Leptin (ng/ml)</td>
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<td>8</td>
<td>Chow</td>
<td>1.2 ± 0.2</td>
<td>2.3 ± 1.1</td>
<td>&lt;0.06</td>
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NS, not significant.
tual muscle. Indeed, aP2-Dgat1 and WT mice fed a high-fat diet had similar levels of triglycerides in their liver and skeletal muscle (Fig. 4A). There was also no evidence of increased deposition of lipid droplets in the brown adipose tissue of aP2-Dgat1 mice fed a high-fat diet (Fig. 4B).

**Correlation of insulin resistance with liver and skeletal muscle triglyceride content in murine obesity models.** Our findings in aP2-Dgat1 mice suggested that the triglyceride content in the liver and skeletal muscle correlated better with insulin sensitivity than WAT mass. To investigate this further, we measured the triglyceride content of the liver and skeletal muscle in several additional mouse models of obesity and insulin resistance (e.g., C57BL/6J mice fed a high-fat diet, A/J/a mice, db/db mice). The results (Fig. 5) confirmed that the triglyceride content in these tissues provided a better correlation with insulin resistance than total fat pad weight in these mouse models.

**DISCUSSION**

In this study, we show that DGAT1 overexpression in WAT increased adipocyte size and adipose mass in mice and that the level of DGAT1 overexpression correlated with the degree of adiposity. aP2-Dgat1 mice also had increased susceptibility to diet-induced weight gain. Despite their increased adiposity, aP2-Dgat1 mice fed either a chow diet or a short-term high-fat diet did not have further impairment in glucose disposal. The increased adiposity in aP2-Dgat1 mice was limited to adipose tissue and did not affect the liver or skeletal muscle. These results suggest that obesity and impaired glucose disposal were dissociated in aP2-Dgat1 mice because of the selective deposition of triglycerides in WAT but not in the liver and skeletal muscle, the two major insulin-sensitive tissues.

The twofold level of DGAT1 overexpression in WAT of aP2-Dgat1 transgenic mouse most likely represents an increase that is physiologically relevant. Little is known about the regulation of DGAT1 in mice. Although DGAT1 gene expression increases markedly (~eightfold) in NIH 3T3-L1 cells during their differentiation into adipocytes (12), changes of such magnitude are unlikely to be observed for DGAT mRNA in mature adipocytes. For example, the expression of DGAT2 in WAT is only 30–40% higher in chow-fed A/J/a mice than in lean, WT mice (16). Because small changes in the expression of a metabolic enzyme such as DGAT1 may have profound effects, the twofold increase in DGAT1 mRNA levels in aP2-Dgat1 mice probably represents a change at the upper extreme of physiologic relevance.

The aP2-Dgat1 transgenic mouse is not the only mouse model in which obesity is dissociated from impaired glucose disposal. Mice that overexpress the insulin-responsive glucose transporter (GLUT4) in WAT have adipocyte hyperplasia and enhanced glucose disposal, presumably as a result of increased glucose transport into adipocytes (31). aP2-deficient mice fed a high-fat diet develop obesity without insulin resistance (32), possibly because of decreased expression of TNF-α, a molecule implicated in obesity-related insulin resistance (30). Mice that overexpress phosphoenolpyruvate carboxykinase, a key enzyme in glyceroneogenesis, in WAT also have increased adipose mass without insulin resistance (33). Triglyceride levels in the liver and skeletal muscle were not reported in these obesity models, so it is unknown whether the lack of insulin resistance correlated with the lack of triglyceride deposition in these tissues. Nonetheless, all of these mouse models provide strong evidence

**TABLE 3**

<table>
<thead>
<tr>
<th>Gene</th>
<th>No. of animals</th>
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<th>WT (arbitrary units)</th>
<th>aP2-Dgat1 (arbitrary units)</th>
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<tr>
<td>Adiponectin</td>
<td>3–5</td>
<td>8–10</td>
<td>100 ± 30</td>
<td>100 ± 70</td>
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<tr>
<td>Leptin</td>
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<td>7–8</td>
<td>100 ± 80</td>
<td>280 ± 40*</td>
</tr>
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<td>Resistin</td>
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<td>7–8</td>
<td>100 ± 50</td>
<td>70 ± 70</td>
</tr>
<tr>
<td>TNF-α</td>
<td>5–6</td>
<td>7–8</td>
<td>100 ± 30</td>
<td>80 ± 60</td>
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*P < 0.05 versus WT.
that increased adiposity per se does not necessarily cause insulin resistance.

The aP2-Dgat1 mouse is also one of few known mouse models in which elevated plasma FFA levels and impaired insulin sensitivity are dissociated. Mice that lack acylation-stimulating protein (ASP), an adipocyte-derived molecule that enhances triglyceride synthesis, are obesity-resistant and have increased postprandial plasma FFA levels on a high-fat diet (34). However, these mice have decreased plasma insulin levels after a glucose load, consistent with increased insulin sensitivity. Findings in both aP2-Dgat1 and ASP-deficient mice suggest that elevated FFA levels per se do not impair insulin sensitivity. Instead, they support the hypothesis that the intracellular accumulation of fatty acid metabolites (e.g., fatty acyl CoAs, diacylglycerol, ceramide, triglycerides) in insulin-responsive tissues causes FFA-induced insulin resistance.

The mechanism for the increased plasma FFA levels in aP2-Dgat1 mice is unclear but may simply reflect increased lipolysis from the twofold increase in WAT mass. We speculate that most of the excess FFA released from aP2-Dgat1 WAT may be taken up again by WAT and not by the liver or skeletal muscle, because triglycerides did not accumulate in these two tissues. The lack of an increase in plasma triglycerides in aP2-Dgat1 mice, despite the elevation in plasma FFA, may also reflect the channeling of FFA to WAT rather than to the liver. Thus, selective DGAT1 overexpression in WAT may protect the liver and skeletal muscle from an excess accumulation of FFA metabolites and prevent insulin resistance.

Recent studies have shown that the adipocyte acts as an endocrine cell, secreting molecules that modulate energy and glucose metabolism (reviewed in 35,36). The size of adipocytes may affect their endocrine functions. For example, smaller adipocytes are hypothesized to secrete more adiponectin (23), whereas larger adipocytes are associated with increased resistin expression (29). Despite causing a significant hypertrophy of adipocytes, DGAT1 overexpression in WAT did not alter the expression of adiponectin, TNF-α, or resistin, suggesting that there may not be a simple correlation between adipocyte size and the expression of these molecules.

In contrast, the expression of leptin in aP2-Dgat1 WAT correlated with the increase in adipocyte size and mass. Leptin, a major regulator of food intake, energy expenditure, and glucose metabolism (27,28,37), has been proposed to function as a liporegulatory hormone that prevents the accumulation of lipids in nonadipose tissue during periods of overnutrition (38). Thus, the increased expression of leptin may help to limit the accumulation of triglycerides in the liver and skeletal muscle of aP2-Dgat1 mice. The increased leptin expression in aP2-Dgat1 mice, however, did not seem to decrease food intake or increase energy expenditure. In fact, food intake trended higher in aP2-Dgat1 mice and probably contributed to their obesity. These findings suggest that DGAT1 overexpression in WAT may be associated with leptin resistance in the central nervous system. This is plausible, because DGAT1 deficiency in mice is associated with increased leptin sensitivity (16). Understanding how DGAT1 overexpression in WAT affects leptin action is an important area for further investigation.

In conclusion, DGAT1 overexpression in WAT increased adipocyte size and adipose mass without affecting the triglyceride content of the liver or skeletal muscle in mice. The increase in triglyceride deposition in WAT but not in the liver and skeletal muscle may account for the dissociation of obesity and impaired glucose disposal in aP2-Dgat1 mice. The overexpression of DGAT1 in other tissues, such as the liver, skeletal muscle, and pancreatic β-cells, may provide additional insights into the molecular basis of obesity-induced insulin resistance.

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