The lipogenic enzymes DGAT1, FAS, and LPL in adipose tissue: effects of obesity, insulin resistance, and TZD treatment

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Abstract

Acyl-coenzyme A:diacylglycerol transferase (DGAT), fatty acid synthetase (FAS), and LPL are three enzymes important in adipose tissue triglyceride accumulation. To study the relationship of DGAT1, FAS, and LPL with insulin, we examined adipose mRNA expression of these genes in subjects with a wide range of insulin sensitivity (SI).

DGAT1 and FAS (but not LPL) were measured in adipose tissue. After pioglitazone treatment, DGAT1 expression was increased by 33% (P < 0.05) and FAS expression increased by 63% (P < 0.05); however, LPL expression was not altered. DGAT1, FAS, and LPL mRNA expression were not significantly changed after metformin treatment. The treatment of mice with rosiglitazone also resulted in an increase in adipose expression of DGAT1 by 2- to 3-fold, as did the treatment of 3T3 F442A adipocytes in vitro with thiazolidinediones. These data support a more global concept suggesting that adipose lipid storage functions to prevent peripheral lipotoxicity. — Ranganathan, G., R. Unal, I. Pokrovskaya, A. Yao-Borengasser, B. Phanavanh, B. Lecka-Czernik, N. Rasouli, and P. A. Kern. The lipogenic enzymes DGAT1, FAS, and LPL in adipose tissue: effects of obesity, insulin resistance, and TZD treatment. J. Lipid Res. 2006. 47: 2444–2450.

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Although it is well known that obesity is associated with insulin resistance, this relationship is not strong and is associated with other factors (1). Much of the relationship between obesity and insulin resistance actually comes from visceral and ectopic lipid accumulation (2, 3). Indeed, the removal of subcutaneous fat by liposuction does not improve insulin resistance (4), and the improved insulin sensitivity (SI) from thiazolidinedione (TZD) treatment is associated with weight gain (5).

TZDs are insulin-sensitizing agents that act as high-affinity ligands for peroxisome proliferator-activated receptor γ, a transcription factor turned on early during adipocyte differentiation. TZDs are known to regulate genes involved in adipocyte differentiation and lipid metabolism (6, 7). TZDs regulate glucose homeostasis by improving SI, and this is known to be accompanied by an increase in lipid accumulation in subcutaneous adipose tissue (8, 9) along with a decrease in lipid in muscle and liver (10, 11). In vitro studies have demonstrated an increase in the triglyceride content of adipose tissue through stimulating triglyceride synthesis and fatty acid availability in adipocytes (12, 13).

A number of genes are involved in adipocyte lipid accumulation, including acyl-coenzyme A:diacylglycerol transferase (DGAT), fatty acid synthetase (FAS), and LPL. DGAT catalyzes the addition of the third fatty acyl-CoA moiety to diacylglycerol. Two DGAT genes have been identified: DGAT1 is a microsomal enzyme with high expression in white adipose tissue (WAT), skeletal muscle, and intestine and has sequence homology with the acyl-CoA cholesterol acyltransferase gene family, whereas DGAT2 bears no sequence homology with DGAT1 (14). DGAT1 may be important in obesity and insulin resistance, because mice overexpressing DGAT1 in adipose tissue were obese but did not become insulin-resistant (15), whereas mice deficient in DGAT1 were resistant to obesity and had enhanced SI (16). Two enzymes that provide nonesterified fatty acid substrate for triglyceride synthesis are FAS and LPL. FAS regulates de novo lipogenesis from acetyl-CoA,

Abbreviations: BMI, body mass index; DGAT, acyl-coenzyme A:diacylglycerol transferase; FAS, fatty acid synthetase; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; SI, insulin sensitivity; TZD, thiazolidinediones; WAT, white adipose tissue.

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malonyl-CoA, and NADPH and is expressed at high levels in adipose tissue, liver, and lung. Feeding increases FAS mRNA and protein levels. LPL is expressed at high levels in adipose tissue and muscle and hydrolyzes triglyceride-rich lipoproteins at the capillary endothelium to generate NEFA for uptake in peripheral tissues (17). Both FAS and LPL are increased by feeding (18–21), and systemic overexpression of LPL in rabbits results in increases in whole body S_t (22).

To better understand the role of these adipose tissue lipogenic enzymes in insulin resistance, we examined their expression in obese, insulin-resistant subjects and assessed the response of these enzymes to TZDs. These data suggest that TZDs upregulate the adipocyte lipid storage genes DGAT and FAS but have no significant effect on LPL.

METHODS

Human subject recruitment

We recruited generally healthy subjects without diabetes by local advertisement. All subjects provided written, informed consent under protocols approved by the local Institutional Review Board, and studies were conducted at the University of Arkansas for Medical Sciences/Central Arkansas Veterans Health Care System General Clinical Research Center. Subjects were included if fasting glucose was < 126 mg/dl and 2 h postchallenge glucose was < 199 mg/dl. Based on an initial 75 g oral glucose tolerance test, subjects were classified as having either normal glucose tolerance (NGT) or impaired glucose tolerance (IGT). NGT subjects had fasting glucose of < 110 mg/dl and 2 h glucose of < 140 mg/dl, and IGT subjects had 2 h glucose of 140–199 mg/dl. Fifty subjects were recruited for this study (43 women and 7 men), of whom 37 had IGT and 13 had NGT. Subjects with a history of coronary artery disease were excluded. All subjects underwent a subcutaneous adipose tissue incisional biopsy from the lower abdominal wall and SI testing was performed.

Western blot analysis

The detection of LPL and DGAT1 in adipocytes was performed using Western blot analysis essentially as described (28). The tissue was minced and rinsed in cold phosphate-buffered saline, and total protein was extracted using cell lysis buffer containing 50 mM phosphate buffer, pH 7.4, 1.0% Nonidet P-40, 0.1% SDS, 20 mM PMSF, and protease inhibitor cocktail (Sigma Chemical Co., St. Louis, MO). Proteins (15 μg) were fractionated by 10% SDS-PAGE and transferred onto nitrocellulose membranes using 200 mA current for 2–3 h. Membranes were treated with phosphate-buffered saline, pH 7.6, 0.2% Tween-20, and 5% nonfat dry milk overnight at 4°C. To identify DGAT1 protein, polyclonal antibody was provided by R. V. Faresce, Jr. (Gladestone Institutes), followed by anti-rabbit HRP conjugate at 1:5,000 (Sigma). The reaction product was visualized with chemiluminescence reagents (Amer sham, Piscataway, NJ) followed by autoradiography. Densitometric analysis of the images was performed using ImageQuant software (Amer sham Biosciences, Inc.). Western blots were probed for β-
actin (Sigma), and LPL and DGAT1 protein were expressed as the ratio of β-actin to normalize for total protein.

**DGAT activity measurements**

DGAT activity was measured in cell lysates or membrane isolates according to methods described previously (29). Samples were homogenized in HEPES (20 mM, pH 7.4) with a Dounce homogenizer in the presence of protease inhibitors. 0.3 volume of 30% sucrose solution was added, followed by low-speed centrifugation to remove nuclei. To measure DGAT activity, typically 10–50 mg of cell lysate protein was incubated with oleoyl-CoA and 1,2-dioleoyl-sn-glycerol. The specific activity of oleoyl-CoA was kept constant at 0.1 μCi/500 μl. Reactions were performed for 30 min at 30°C and terminated by the addition of heptane/isopropanol/H₂SO₄. The organic phase was washed with isopropanol-water (2:3), and the incorporation of [14C]oleoyl-CoA into triglyceride was measured by scintillation counting. DGAT1 activity was expressed as nanomoles of triglyceride synthesized per 10⁶ cells.

**LPL activity measurements**

Heparin-releasable and extractable LPL activities were determined as described previously (12). To measure heparin-releasable LPL, cells were incubated in serum-free medium containing 10 U/ml heparin for 60 min at 37°C. After collecting the heparin-released fraction, the remaining LPL was extracted in 50 mM Tris, pH 7.4, containing 0.2% Triton X-114. Extracts were diluted with 3 volumes of 50 mM Tris, pH 7.4, containing 10 U/ml heparin. LPL catalytic activity was measured as described previously using a substrate containing [3H]triolein and fetal bovine serum as a source of apolipoprotein C-II (30). LPL activity was expressed as nmol free fatty acid released/h/mg protein.

**Oil Red O staining and quantitation**

3T3 F442A adipocytes were differentiated as described above. Oil Red O staining and quantitation were done as described previously using the Chemicon Adipogenesis Assay kit (31). Briefly, cells were fixed in 3.7% formaldehyde for 20 min at room temperature and rinsed with distilled water. Cells were stained for 2 h at room temperature, according to the manufacturer’s instructions. At the end of staining, the dye was aspirated and the plates were rinsed in distilled water. The dye was extracted for 30 min in 0.5 ml of dye extraction solution, and the absorbance was measured in a spectrophotometer at 540 nm.

**Statistical analysis**

Student’s two-sample t-test was used to compare groups with respect to continuous variables, and a paired t-test was used to compare baseline and posttreatment measurements. Pearson’s correlation coefficients were used to determine linear associations between variables. All data from human samples are expressed as means ± SEM.

**RESULTS**

**DGAT1 and FAS mRNA positively correlate with S_I**

In previous studies in mice, overexpression of DGAT1 in adipose tissue yielded obesity without insulin resistance (16). To determine whether the expression of lipogenic enzymes in subcutaneous adipose tissue of humans was associated with S_I, we measured the expression of DGAT1 in human adipose tissue of 27 subjects (25 female, 2 male; age, 45 ± 5 years) divided among 14 NGT and 13 IGT subjects; FAS mRNA was measured in 23 subjects (12 NGT and 11 IGT). The relationship between DGAT1 mRNA and S_I is illustrated in Fig. 1, and the relationship of FAS and S_I is also shown in Fig. 1. Both DGAT1 and FAS demonstrated significant positive relationships with S_I ($S_I$ vs. DGAT1, $r = 0.54$, $P < 0.004$; $S_I$ vs. FAS, $r = 0.54$, $P < 0.008$). In contrast, however, there was no significant correlation between LPL mRNA and $S_I$ (data not shown).

Insulin-resistant subjects tend to be more obese, and as expected, the subjects chosen for this study also demonstrated a significant negative relationship between body mass index (BMI) and $S_I$ ($r = -0.65$, $P < 0.001$). DGAT1 expression was inversely correlated with BMI ($r = -0.40$, $P < 0.05$); however, there was no significant association between BMI and the expression of FAS or LPL. To better understand the relationships between DGAT1, FAS, and LPL with $S_I$, we examined the expression of these lipogenic enzymes in adipose tissue of NGT and IGT subjects who were matched for BMI. To limit variability between subjects, female Caucasian subjects were selected who were between the ages of 31 and 59 years with BMI between 28 and 43 kg/m². The subject characteristics are described in Table 1. As expected, IGT and NGT subjects differed in their $S_I$, 2 h glucose measurement, and serum
triglyceride. As shown in Fig. 2, the expression levels of DGAT1 were significantly higher in NGT subjects compared with IGT subjects of the same gender, race, and BMI [NGT, 1.24 ± 0.08; IGT, 0.74 ± 0.05 (P < 0.001)]. FAS expression was also significantly higher in NGT subjects compared with IGT subjects [NGT, 0.446 ± 0.04 (P < 0.01)], but levels of LPL expression were not different between NGT and IGT subjects [NGT, 0.810 ± 0.07; IGT, 0.873 ± 0.06 (P < 0.38)].

Changes in expression of lipogenic enzymes after treatment with insulin sensitizers

As described in Methods, 37 IGT subjects were randomized to receive either metformin or pioglitazone, and adipose tissue biopsies were performed before and after 10 weeks of treatment. The expression of the lipogenic enzymes DGAT1, FAS, and LPL was then measured in subcutaneous adipose tissue. Pioglitazone but not metformin treatment resulted in a significant increase in SI, and no change in DGAT1 activity after metformin treatment (Fig. 3B). LPL activity was also measured in the same tissues; LPL activity increased from 103 ± 10 to 135 ± 17 nmol/10⁶ cells, but this increase was not statistically significant.

TZDs yield large increases in DGAT1 mRNA in mice

TZD treatment of humans yielded small but consistent increases in DGAT1 mRNA levels. To assess the effects of TZDs in mice, nondiabetic C57BL/6 mice were treated with rosiglitazone as described in Methods. DGAT1 expression in WAT increased ≥2-fold, from 0.78 ± 0.1 to 1.98 ± 0.1 (P < 0.05), and DGAT1 protein expression increased ~5-fold from 20.4 to 116.2 arbitrary units (ARU) after rosiglitazone treatment (Fig. 4). LPL mRNA expression and protein were also measured in the same tissues; LPL mRNA increased modestly after rosiglitazone treatment, from 0.93 ± 0.43 to 1.37 ± 0.6, but this increase was not statistically significant, and there was no change in LPL protein in adipose tissue (data not shown).

DGAT1 expression in 3T3 F442A adipocytes during differentiation

We studied the effects of TZD treatment in vitro on 3T3 F442A adipocytes during differentiation. Cells were differentiated as described in Methods. Both insulin and rosiglitazone yielded essentially comparable adipocyte differentiation, as indicated by cellular lipid accumulation. Oil Red O staining of adipocytes was quantitated as described in Methods. There was no significant difference in Oil Red O staining of insulin-differentiated cells versus rosiglitazone-treated cells [insulin, 0.065 ± 0.025; rosiglitazone, 0.045 ± 0.015 optical density (n = 5; P = 0.11)]. Compared with cells induced to differentiate with insulin, however, rosiglitazone resulted in a much greater increase in DGAT1 expression. DGAT1 mRNA expression was 4.12 ± 0.05 with insulin treatment and 26.6 ± 0.08 with rosiglitazone treatment (Fig. 5). DGAT1 protein was in-

| TABLE 1. Characteristics of NGT and IGT subjects (BMI-matched) |
|-----------------------------|-----------------------------|
| Characteristic | NGT (n = 14) | IGT (n = 15) |
| BMI (kg/m²) | 33.6 ± 1.1 | 35.2 ± 0.6 |
| S₃ (10⁻⁵ × min⁻¹/pM) | 3.7 ± 0.5 | 1.8 ± 0.2 |
| Fasting glucose (mM) | 4.6 ± 0.13 | 5.0 ± 0.14 |
| 2 h glucose (mM) | 5.9 ± 0.3 | 10.0 ± 0.4 |
| Triglyceride (mM) | 3.0 ± 0.2 | 1.91 ± 0.2 |
| LDL (mM) | 1.5 ± 0.1 | 1.3 ± 0.1 |

BMI, body mass index; NGT, impaired glucose tolerance; NGT, normal glucose tolerance; S₃, insulin sensitivity.

*P < 0.05 versus IGT.

| TABLE 2. Characteristics of IGT subjects |
|-----------------------------|-----------------------------|
| Characteristic | Pioglitazone (n = 17) | Metformin (n = 20) |
| Age (years) | 48.37 ± 1.72 | 48.3 ± 1.76 |
| BMI (kg/m²) | 32.58 ± 0.87 | 33.55 ± 0.67 |
| S₃ before (10⁻⁵ × min⁻¹/pM) | 1.44 ± 0.20 | 1.42 ± 0.15 |
| S₃ after (10⁻⁵ × min⁻¹/pM) | 2.19 ± 0.20 | 2.11 ± 0.27 |
| Triglyceride (mM) | 1.64 ± 0.20 | 1.91 ± 0.25 |
| HDL (mM/l) | 2.75 ± 0.19 | 2.91 ± 0.25 |
| LDL (mM/l) | 1.28 ± 0.08 | 1.54 ± 0.05 |
| Gender (female/male) | 14/3 | 16/4 |
| Body fat (% body weight) | 39.6 ± 6.40 | 40 ± 6.35 |

*P < 0.005 versus S₃ before.
increased in a similar manner (4- to 5-fold compared with insulin-differentiated cells), although DGAT1 activity was higher by 43\% in cells differentiated with rosiglitazone compared with cells differentiated with insulin (Fig. 5). LPL mRNA was not significantly different between cells differentiated with insulin or rosiglitazone (data not shown).

**DISCUSSION**

Insulin resistance and diabetes are associated with increasing body weight and adipose tissue mass in both humans and rodents. However, the precise relationship between adipose tissue mass and insulin resistance is unclear, and there are a number of examples of discordance between adipose tissue mass and insulin resistance. For example, lipodystrophy of both rodents and humans results in no adipose tissue yet extreme insulin resistance (32). Many different genetic manipulations of mice, such as the knockout of tumor necrosis factor-α receptors or overexpression of the adipose tissue lipin, result in obese, insulin-sensitive mice (33, 34). TZD drugs are agonists for peroxisome proliferator-activated receptor γ, which promotes adipocyte differentiation. Treatment of patients...
with these drugs results in weight gain and an increase in fat mass accompanied by a shift of fat from the visceral to the subcutaneous depot, resulting in improved $S_I$ (35).

This study was intended to better understand the relationship between the expression of several adipogenic genes, DGAT1, FAS, and LPL, in adipose tissue and insulin resistance. The expression of these genes was studied in nondiabetic insulin-sensitive and insulin-resistant subjects covering a range of $S_I$ and BMI. Although obese subjects were generally more insulin-resistant, there was a positive relationship between the expression of DGAT1 and $S_I$ and between FAS and $S_I$. To confirm this finding, and to separate the effects of obesity, we compared the more insulin-resistant IGT subjects with NGT subjects. IGT and NGT subjects were matched for BMI, gender, and age, and NGT subjects expressed significantly higher levels of both DGAT1 and FAS compared with IGT subjects. Thus, increased $S_I$ is accompanied by increased DGAT1 and FAS expression in subcutaneous adipose tissue in humans. In addition, the improved peripheral $S_I$ that accompanied pioglitazone treatment was also associated with an increased expression of DGAT1 and FAS. Metformin, which improves hepatic $S_I$, had no effect on subcutaneous adipose tissue gene expression or $S_I$. In mouse adipose tissue and in 3T3 F442A mouse adipocytes, the increase in DGAT1 expression after TZD treatment was more pronounced than in humans.

DGAT1 catalyzes the addition of fatty acyl-CoA to a 1,2-diacylglycerol to yield triglyceride; hence, it is important in lipogenesis in many tissues. Mice with a genetic knockout of DGAT1 tended to resist obesity and were more insulin-sensitive (36), and transgenic mice overexpressing DGAT1 demonstrated increased adiposity, although there was no deterioration in $S_I$ (37). In a recent review of studies of DGAT1, Chen et al. hypothesized that inhibition of DGAT1 may be a useful target for antiobesity drugs (37). However, our data, which demonstrated a positive association between DGAT1 and $S_I$ and an increase in DGAT1 with TZD treatment, suggest that inhibition of DGAT1 may not be beneficial to $S_I$.

Other studies have noted a relationship between adipose tissue gene expression and $S_I$. Treatment with the antiretroviral agent ritonavir has been shown to induce lipodystrophy and insulin resistance, and this drug also inhibited preadipocytes and adipocyte differentiation along with the expression of GLUT4, FAS, and adiponectin mRNA (38). TZDs are known to promote lipogenesis in human adipose tissue (39). Another study examined the role of pioglitazone on lipid metabolism in subcutaneous human adipose tissue of type 2 diabetic subjects (40) and found that the expression of both LPL and FAS was higher after pioglitazone treatment. This study did not observe any change in LPL expression but confirmed the increase in FAS. This study was different from others in the inclusion of nondiabetic subjects. Also, our studies did not find any relationship between LPL mRNA or LPL activity and $S_I$ or in response to TZD treatment, whereas two enzymes involved in adipocyte triglyceride synthesis, DGAT1 and FAS, both were associated with $S_I$ and TZD treatment.

Because obesity is associated with insulin resistance, one might expect that genes that promote lipid accumulation would also promote insulin resistance. However, considerable evidence suggests that factors that promote adipose tissue lipid accumulation prevent the toxic action of lipid in insulin-responsive organs such as skeletal muscle and liver. In addition to the overexpression of DGAT1 (37), the overexpression of lipin in adipose tissue yields a mouse that is more obese but also more insulin-sensitive (34). Lipodystrophy in both animals and humans is associated with the absence of adipose tissue, leading to extreme ectopic lipid accumulation and insulin resistance (32). In addition, treatment with TZDs increases adipose tissue lipid accumulation in subcutaneous adipose tissue, leading to a reduction in muscle and liver lipid and improved $S_I$ (9–11, 41). These data support a concept whereby lipid accumulates in nonadipose tissue and also in ectopic sites, leading to lipotoxicity (42, 43). Furthermore, the deposition of triglycerides in adipose tissue, or the diversion of lipid to adipose tissue, may prevent lipotoxicity in muscle and liver.

In summary, these studies demonstrate the association between two adipose lipogenic genes, DGAT1 and FAS, with $S_I$ and an increase in DGAT1 and FAS gene expression after treatment of humans, mice, and cultured adipocytes with TZDs. These data are consistent with a role of the adipocyte in the appropriate storage of lipid and perhaps with another role in the diversion of lipid into adipose tissue, away from ectopic sites that are susceptible to lipotoxicity.

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