THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 280, No. 5, Issue of February 4, pp. 3331–3337, 2005
Printed in U.S.A.

Expression of DGAT2 in White Adipose Tissue Is Regulated by Central Leptin Action*

Received for publication, September 23, 2004, and in revised form, November 18, 2004
Published, JBC Papers in Press, November 18, 2004, DOI 10.1074/jbc.M410955200

Ryo Suzuki‡§, Kazuyuki Tobe‡§, Masashi Aoyama‡, Kentaro Sakamoto‡§, Mitsuru Ohsugi‡, Nozomu Kamei‡, Shigeyuki Nemoto‡, Atsushi Inoue‡, Yusuke Ito‡, Shoko Uchida‡, Kazuo Hara‡§, Toshimasa Yamauchi‡§, Naoto Kubota‡§, Yasuo Terauchi‡§, and Takashi Kadowaki‡§¶

From the ‡Department of Metabolic Diseases, Graduate School of Medicine, University of Tokyo, Tokyo 113-8655, Japan and §CREST of Japan Science and Technology Agency, Kawaguchi 332-0012, Japan

Acyl-CoA:diacylglycerol acyltransferase (DGAT) enzymes catalyze the final step in mammalian triglyceride synthesis, and their functions are considered to be involved in the mechanisms of obesity, insulin resistance, and leptin resistance. Insulin receptor substrate-2 (IRS-2)-deficient mice exhibit obesity-associated with hypertrophic adipocytes and leptin resistance. Screening for transcripts of genes involved in fatty acid and triglyceride synthesis to investigate the mechanism of the hypertrophic change in the adipocytes showed that expression of DGAT2 mRNA was up-regulated in the white adipose tissue (WAT) of *Irs2*$^{-/-}$ mice, whereas that of DGAT1 was down-regulated. This reciprocal expression of DGAT1 and DGAT2 was also observed in WAT of leptin-deficient *ob/ob* mice. A high fat diet also resulted in increased DGAT2 and reduced DGAT1 in the WAT of C57BL/6 mice. Induction of adipocyte hypertrophy *in vitro* up-regulated both DGAT1 and DGAT2 expression in 3T3-L1 cells, suggesting that adipocyte non-autonomous mechanism *in vivo* is required for the reciprocal changes in expression of DGAT1 and DGAT2. In fact, intracerebroventricular infusion of leptin reduced DGAT2 expression in WAT of *Irs2*$^{-/-}$ mice and *ob/ob* mice, independently of DGAT1 expression. We propose the hypothesis that leptin regulates adipocyte size by altering expression patterns of DGAT via central nervous system to determine the levels of triglyceride synthesis.

We have hypothesized that the size of adipocytes is inversely correlated with insulin sensitivity; namely, that larger adipocytes are associated with insulin resistance and smaller adipocytes are associated with insulin sensitivity (1). Acyl-CoA:diacylglycerol acyltransferase (DGAT)[1] is a key enzyme that catalyzes the final step in mammalian triglyceride synthesis (2), and two DGAT enzymes have been identified (3–5). Although the genes encoding DGAT1 and DGAT2 belong to different gene families, both genes are ubiquitously expressed and the enzymes they encode have similar substrate specificity (5). *Dgat1*$^{-/-}$ mice have been reported to exhibit normal growth on a chow diet, and to be resistant to diet-induced obesity (6). Interestingly, *Dgat1*$^{-/-}$ mice exhibit increased insulin sensitivity and a leptin-sensitive phenotype associated with decreased tissue triglyceride content, suggesting that DGAT1 is somehow involved in insulin and leptin action throughout the body (7, 8). Recently, *Dgat2*$^{-/-}$ mice have been reported to exhibit marked reduction of triglyceride and fatty acids in the body, suggesting a critical role of DGAT2 in lipogenesis; however, the physiological roles of DGAT2 in adult mice are still unknown because *Dgat2*$^{-/-}$ mice die soon after birth (9).

The insulin receptor substrate (IRS) proteins play a key role in signal transduction from the insulin receptor (10, 11) and are major intracellular phosphorylation targets of activated insulin receptor tyrosine kinase. *Irs2*$^{-/-}$ mice develop diabetes because of inadequate β-cell proliferation combined with insulin resistance (12–14). Another noteworthy feature of *Irs2*$^{-/-}$ mice is that they exhibit increased adiposity associated with leptin resistance (15, 16). We previously reported that increased expression of sterol regulatory element binding protein-1 (SREBP-1) mRNA in the liver of *Irs2*$^{-/-}$ mice causes fatty liver, and we attributed its development to leptin resistance (16). We have also reported that the obese phenotype in *Irs2*$^{-/-}$ mice contributes to their insulin resistance (17).

In this study, we discovered that the adipocytes of *Irs2*$^{-/-}$ mice are hypertrophic and concluded that fatty acid and triglyceride synthesis must be activated in *Irs2*$^{-/-}$ mouse adipocytes. Screening for genes involved in the fatty acid and triglyceride synthesis pathways revealed that DGAT2 mRNA was significantly up-regulated in *Irs2*$^{-/-}$ mouse white adipose tissue (WAT) but that expression of DGAT1 was reduced. This antithetical expression of DGAT1 and DGAT2 was also observed in the WAT of *ob*/*ob* mice, however, SREBP-1 mRNA was not increased, suggesting that SREBP-1 is not the major regulator of DGAT expression. Intracerebroventricular administration of leptin reduced DGAT2 mRNA expression in *Irs2*$^{-/-}$ mice and *ob*/*ob* mice, independently of DGAT1 expression. In contrast in 3T3-L1 cells, leptin did not affect the expression of DGAT, and induction of hypertrophy increased expression of both DGAT1 and DGAT2. The relationship

* This work was supported by a grant-in-aid for the development of innovative technology and a grant-in-aid for creative basic research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to T. K. and K. T.) and by a grant for the Promotion of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research (to T. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed: Dept. of Internal Medicine, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Tel.: 81-3-5800-8818; Fax: 81-3-5689-7209; E-mail: kadowaki-3im@h.u-tokyo.ac.jp.

[1] The abbreviations used are: DGAT, acyl-CoA:diacylglycerol acyltransferase; IRS, insulin receptor substrate; SREBP, sterol regulatory element-binding protein; WAT, white adipose tissue; WT, wild-type; ER, endoplasmic reticulum.

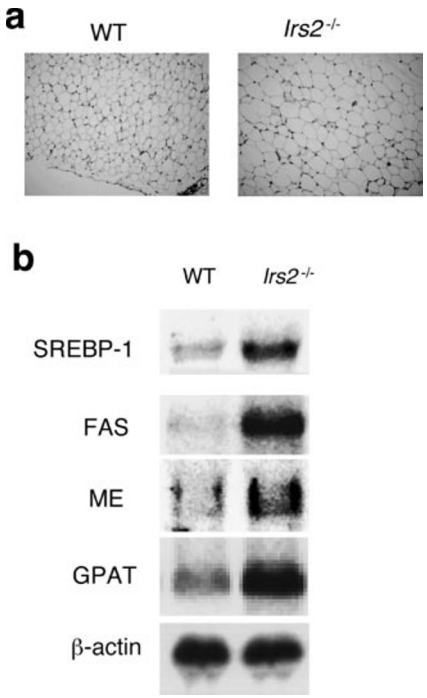
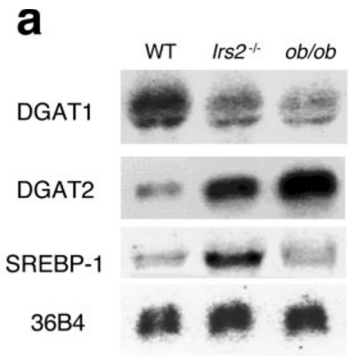


FIG. 1. **Hypertrophic change and increased SREBP-1 expression in *Irs2*$^{-/-}$ mouse adipocytes.** *a*, histological analysis of white adipose tissue from WT and *Irs2*$^{-/-}$ mice. The epididymal fat pads of 14-week-old male mice were stained with hematoxylin and eosin. Representative results are shown. *b*, Northern blots of SREBP-1 mRNA and the downstream target genes in the epididymal fat pads of WT and *Irs2*$^{-/-}$ mice. *FAS*, fatty acid synthase; *ME*, malic enzyme; *GPAT*, glycerol-3-phosphatate acyltransferase. β-Actin is shown as an internal control.



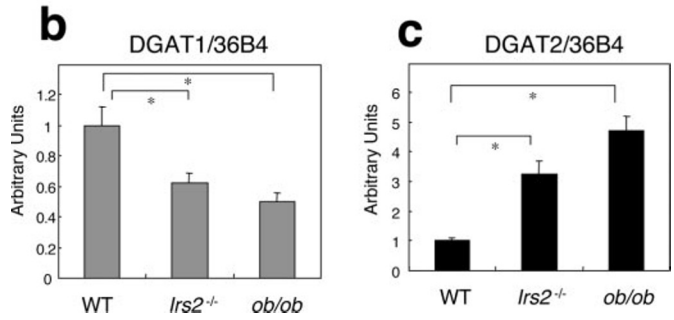


FIG. 2. **Down-regulation of DGAT1 and up-regulation DGAT2 mRNA expression in the adipose tissue of genetically obese mice.** *a*, Northern blots of DGAT1, DGAT2, and SREBP-1 in epididymal fat pads of WT, *Irs2*$^{-/-}$, and *ob/ob* mice. Expression of DGAT1 was reduced in the adipose tissue of *Irs2*$^{-/-}$ and *ob/ob* mice, and expression of DGAT2 was increased. No reciprocal pattern was observed for SREBP-1. 36B4 is shown as an internal control. *b* and *c*, quantified DGAT1 (*b*) and DGAT2 (*c*) expression levels corrected by the internal control. The bands were quantified by exposure of BAS 2000 to the filters and analysis of the images with BAStation software. n is 4–5 male mice per group. Values are means ± S.E. *, $p < 0.05$.

between hormonal action and regulation of DGATs in adipose tissue is discussed.

EXPERIMENTAL PROCEDURES

Animals—Irs2$^{-/-}$ mice were generated as described previously (14). *Irs2*$^{-/-}$ mice were originally maintained on the C57BL/6 and CBA hybrid background and backcrossed with the C57BL/6 strain for at least three generations. Wild-type (WT) mice and *Irs2*$^{-/-}$ mice were used for the following experiments when they had reached 14–18 weeks of age. *ob/ob* mice on a C57BL/6 background were purchased from the Jackson Laboratory (Bar Harbor, ME). C57BL/6 mice were purchased from CLEA Japan (Tokyo, Japan). Mice were housed on a 12 h light-dark cycle and given *ad libitum* access to regular chow MF consisting of 25% (w/w) protein, 53% carbohydrates, 6% fat, and 8% water (Oriental Yeast Co., Ltd., Osaka, Japan), except when specifically stated that a high fat diet was used. All experiments in this study were performed on male mice. The animal care and procedures of the experiments were approved by the Animal Care Committee of University of Tokyo.

Histological Analysis—An epididymal fat pad was removed from each animal, fixed in 10% formaldehyde/phosphate-buffered saline, and maintained at 4 °C until used. Fixed specimens were dehydrated and embedded in paraffin. The fat pad was then cut into 6-μm sections, and they were mounted on silanized slides. After deparaffinization, the sections were stained with hematoxylin and eosin.

RNA Preparation and Northern Blot Analysis—Mice were sacrificed after fasting for 24 h except the case especially indicated, and the epididymal fat pad was excised to obtain WAT. Total RNA was prepared from WAT or 3T3-L1 cells by using TRIzol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Northern blot analysis was performed on 10 μg of total RNA according to the standard protocol. The total RNA was loaded onto a 1.0% agarose gel then transferred to a nylon membrane (Hybond N$^+$: Amersham Biosciences). The cDNA probes for mouse DGAT1 and DGAT2 were prepared by cloning reverse transcriptase-polymerase chain reaction products from mouse WAT RNA into TA cloning vectors (Invitrogen). The polymerase chain reaction primers used to generate these probes were: DGAT1, forward primer, 5′-GTAGAAGAGGACGAGGTGCGAGAC-3′, reverse primer, 5′-GGGCTTCATGGAGTTCTGGATAGT-3′, and DGAT2, forward primer, 5′-GAGGGGTCTGGGCGATGGGGCACT-3′, reverse primer, 5′-CGACGGTGGTGATGGGCTTGGAGT-3′. The cDNA probes for mouse SREBP-1, fatty acid synthase (FAS), malic enzyme, glycerol-3-phosphate acyltransferase (GPAT), and 36B4 were prepared as described elsewhere (16, 18, 19). Probe template for mouse β-actin was purchased (DECAtemplate: Ambion, TX). The corresponding bands were quantified by exposure of BAS 2000 to the filters and measurement with BAStation software (Fuji Film, Tokyo, Japan).

High Fat Diet Experiment on C57BL/6 Mice—Seven-week-old male C57BL/6 mice were housed in individual cages and divided into two groups, a regular chow (MF) group and a high fat diet group. The high fat diet had the following composition: palm oil 32%, casein 33.1%, sucrose 17.6%, vitamin 1.4%, mineral 9.8%, cellulose 5.6%, and DL-methionine 0.5%. Palm oil was provided by Fuji Oil Co., Ltd (Osaka, Japan). All other materials were purchased from Oriental Yeast Co., Ltd. The experiments were performed after 4 weeks of feeding.

3T3-L1 Cell Culture, Induction of Adipocyte Differentiation, and RNA Interference—3T3-L1 cells were cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum, and induction of adipogenic differentiation was carried out according to methods described previously (20). After induction for 8 days, the cells were starved for 8 h and then treated with murine recombinant leptin (PeproTech EC Ltd., London, UK) or human insulin (Humulin R: Lilly,) at the concentration indicated. The cells were lysed with TRIzol reagent 12 h later. In the hypertrophy-induction experiments, 3T3-L1 cells were cultured on gelatin-coated cell culture dishes (21). siRNAs were chemically synthesized, annealed, and transfected into 3T3-L1 cells at day 5 after induction with Lipofectamine (Invitrogen) and Plus Reagent (Invitrogen). The sequences of the sense siRNAs were: DGAT1, 5′-GAUUCUUUGUUCAGCUCAGACTT-3′; DGAT2, 5′-GACAUCUUCUCUGUCACCUGGTT-3′. Forty-eight hours after transfection, the cells were lysed for total RNA extraction.

Real-time Quantitative PCR—Total RNA was extracted from 3T3-L1 cells with TRIzol reagent according to the manufacturer's instructions.

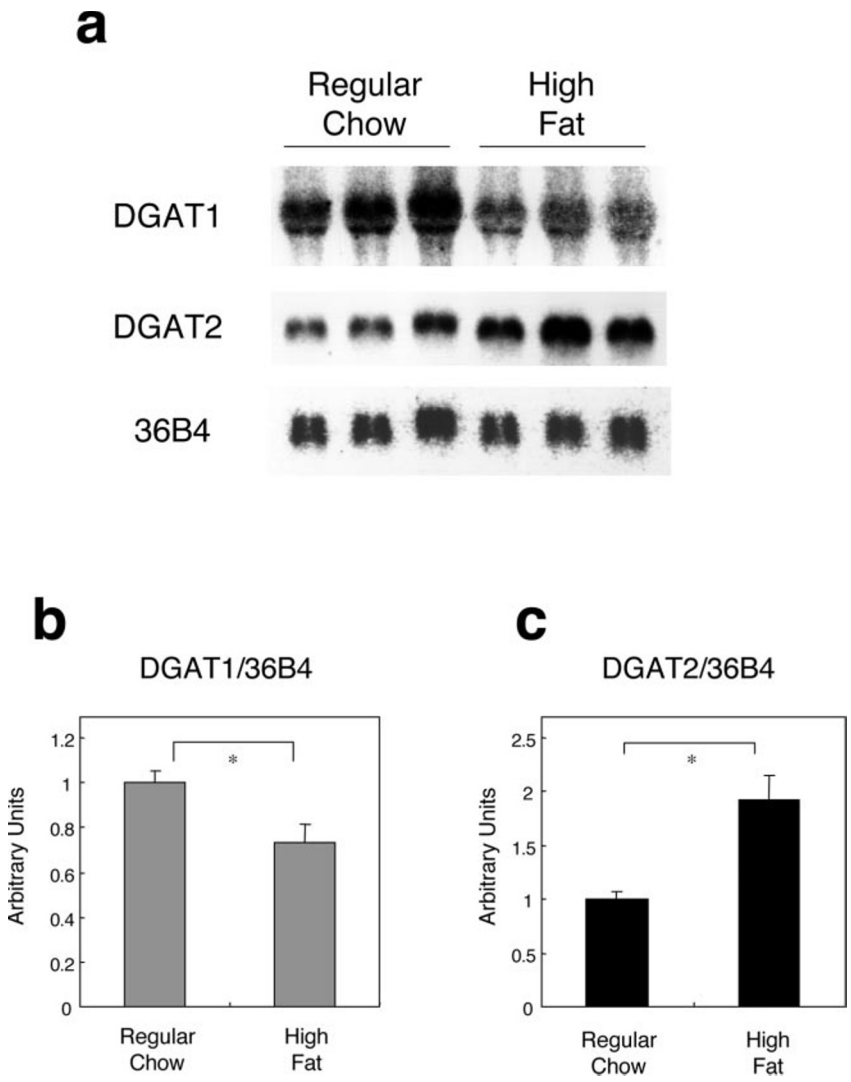


FIG. 3. **Reciprocal changes in DGAT1 and DGAT2 expression in the adipose tissue of diet-induced obese mice.** Seven-week-old C57BL/6 mice were fed regular chow or a high fat diet for 4 weeks. *a*, Northern blots of DGAT1 and DGAT2 in epididymal fat pads. Expression of DGAT1 was reduced in the adipose tissue from mice on a high fat diet, whereas expression of DGAT2 was increased. 36B4 is shown as an internal control. *b* and *c*, quantified DGAT1 (*b*) and DGAT2 (*c*) expression levels corrected by the internal control. The bands were quantified by using BAS 2000 and BAStation software. *n* is 3 male mice per group. Values are means ± S.E. *, $p < 0.05$.

After treatment with RQ1 RNase-Free DNase (Promega, Madison, WI) to remove genomic DNA, cDNA was synthesized with MultiScribe Reverse-Transcriptase (Applied Biosystems, Foster City, CA), and TaqMan quantitative PCR (50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 1 min) was then performed with the ABI Prism 7000 PCR instrument (Applied Biosystems) to amplify samples for the DGAT1, DGAT2, and cyclophilin cDNA. The primers and probes were: DGAT1 forward, TCCGCCTCTGGGCATTC; DGAT1 reverse, GAATCGGCCCACAATCCA; DGAT1 probe, CCATGATGGCTCAGGTCCCACTGG; DGAT2 forward, GCTGAGTCCCTGAGCTCCAT; DGAT2 reverse, CACAAAGCCTTTGCGGTTCT; DGAT2 probe, CCTGGCAAGAACGCAGTCACCCTG. The sequences of the cyclophilin primers and probe are described elsewhere (22).

Chronic Cannulation and Infusion Methods—Male mice at 16–18 weeks of age were used for the experiments. Before surgery, the mice were intraperitoneally anesthetized with 1 mg/kg pentobarbital. The mice used were then placed in a stereotactic device, and a 30-gauge stainless steel cannula was inserted into the right lateral cerebral ventricle: 1.0 mm posterior, 1.0 mm lateral, and 2.0 mm (*ob/ob*) or 1.5 mm ($Irs2^{-/-}$) ventral to the bregma. To prevent the cannula from becoming blocked by blood clots, a stainless steel stylet was inserted into each cannula until used. The animals were allowed to recover for 1 week after the operation. One week after insertion of the cannula, ALZET mini-osmotic pumps (DURECT Corp., Cupertino, CA) were connected to the ICV cannula, and the pumps delivered a constant infusion of 1.0 μl/h for 7 days. The mice were divided in two groups, a group infused with leptin ($Irs2^{-/-}$: 300 ng/h, *ob/ob*: 10 ng/h) and a group infused with saline as a control.

RESULTS

Increased SREBP-1 Expression in the Hypertrophic Adipocytes of $Irs2^{-/-}$ Mice—We previously reported that $Irs2^{-/-}$ mice exhibit obesity, fatty change in the liver, and up-regulation of SREBP-1 mRNA expression, all of which are consequences of their phenotype of leptin resistance (16, 23). Impaired leptin action causes adipocyte hypertrophy as shown in obese mouse models, *e.g. ob/ob* mice, *db/db* mice, and KKA^y mice. Histological study revealed that $Irs2^{-/-}$ mice have enlarged adipocytes (Fig. 1*a*), and we examined the expression of genes involved in fatty acid and triglyceride synthesis pathway by Northern blot analyses as a means of investigating the mechanism of the hypertrophy of $Irs2^{-/-}$ mice adipocytes.

Since the Northern blots revealed increased (3.4-fold) expression of SREBP-1 mRNA in $Irs2^{-/-}$ mouse adipose tissue (Fig. 1*b*), we analyzed expression of its downstream target genes. The results showed increased expression of FAS, malic enzyme, and GPAT, all of which are downstream targets of SREBP-1, suggesting that increased expression of SREBP-1 contributed to fatty acid synthesis in enlarging $Irs2^{-/-}$ mice adipocytes (Fig. 1*b*).

Decreased DGAT1 and Increased DGAT2 Expression in the WAT of $Irs2^{-/-}$ and ob/ob mice—Next, we studied triglyceride synthesis pathway in an attempt to identify the genes responsible for enlarging the adipocytes of $Irs2^{-/-}$ mice. Two enzymes that catalyze the final step of triglyceride synthesis, have been identified and named DGAT1 and DGAT2 (5, 24). Surprisingly, expression of DGAT1 was found to be significantly reduced (0.62-fold) in $Irs2^{-/-}$ mouse adipose tissue, whereas expression of DGAT2 was clearly increased (3.3-fold, Fig. 2, *a–c*). We

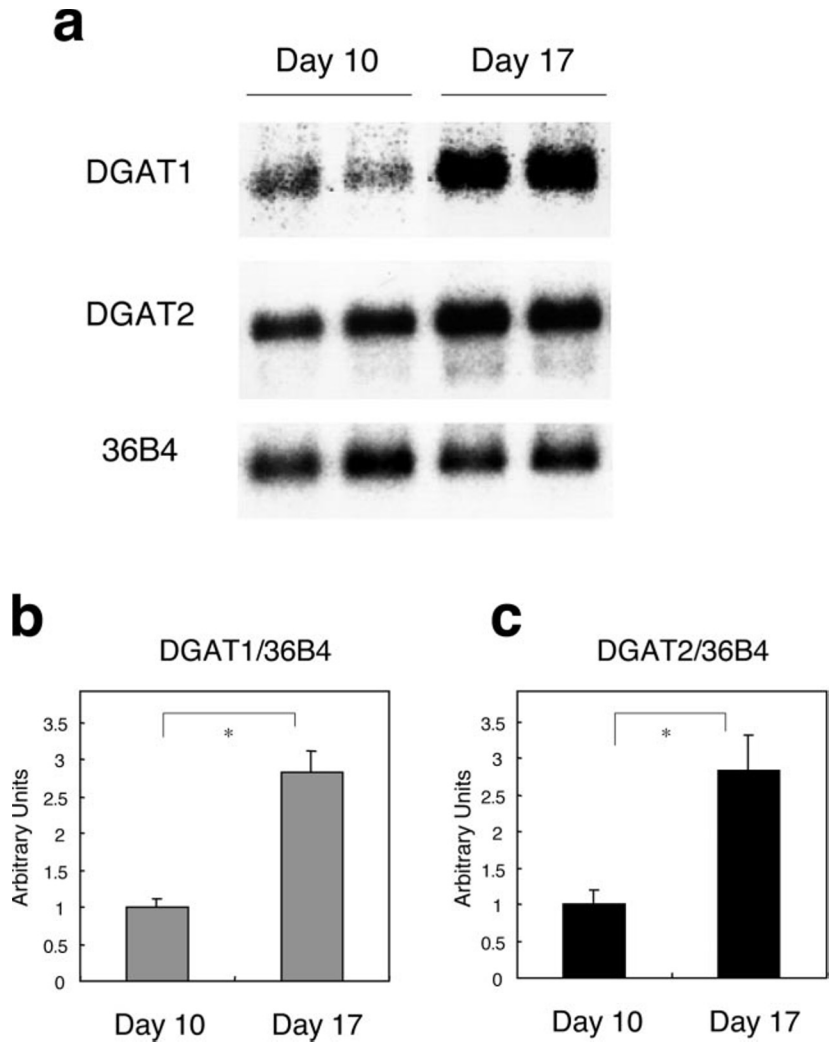


FIG. 4. **Hypertrophic 3T3-L1 adipocytes showed increased expression of both DGAT1 and DGAT2.** 3T3-L1 cells were incubated as described under "Experimental Procedures." The cells were fully differentiated at day 10, and hypertrophic change was observed at day 17. *a*, Northern blots of DGAT1 and DGAT2 in 3T3L1 cells at days 10 and 17. Expression of both DGAT1 and DGAT2 was increased in the hypertrophic cells at day 17. 36B4 is shown as an internal control. *b* and *c*, quantified DGAT1 (*b*) and DGAT2 (*c*) expression levels corrected by the internal control. The bands were quantified by exposure of BAS 2000 to the filters and analysis of the images with BAStation software. *n* is 6 per group. Values are means ± S.E. *, $p < 0.05$.

examined expression of the genes in WAT of another model of obesity, *ob/ob* mice. Both *Irs2*$^{-/-}$ mice and *ob/ob* mice are considered to show obesity because of attenuated leptin action. A similar reciprocal relationship between DGAT1 and DGAT2 expression has also been observed in *ob/ob* mice (DGAT1: 0.50-fold, DGAT2: 4.7-fold), indicating that adipocyte hypertrophy associated with attenuated leptin action may be responsible for the changes in expression, not the lack of IRS-2 *per se* (Fig. 2, *a–c*). The fact that SREBP-1 expression was not elevated in the WAT of *ob/ob* mice (Fig. 2*a*) is an important finding, because it is consistent with the findings in previous reports (25, 26) and suggests that SREBP-1 may not play a major role in the regulation of DGAT1 and DGAT2 expression in the WAT of obese animals.

A High Fat Diet Reduced DGAT1 and Increased DGAT2 Expression in the White Adipose Tissue of C57BL/6 Mice—A high fat diet is known to induce leptin resistance and obesity in rodents (27, 28). We examined the effect on WAT of obesity and leptin resistance acquired as a result of consuming a high fat diet in C57BL/6 inbred mice. Seven-week-old C57BL/6 mice were fed a high fat diet or a regular chow diet for 4 weeks, and a high fat diet induced significantly more weight gain of the mice than a regular chow diet (8.5 ± 0.3 g *versus* 3.5 ± 0.3 g). Plasma leptin concentrations clearly increased in the mice fed a high fat diet (22.5 ± 3.2 ng/ml *versus* 2.6 ± 0.2 ng/ml). Plasma non-esterified fatty acid (NEFA) concentrations were reduced in high fat diet group (0.74 ± 0.05 mEq/liter *versus* 1.4 ± 0.1 mEq/liter). Northern blot analysis showed that DGAT2 expression increased (1.9-fold) but DGAT1 decreased (0.73-fold) in WAT of the diet-induced obese mice (Fig. 3, *a–c*). These changes are essentially the same as observed in genetically obese mice, such as *Irs2*$^{-/-}$ mice and *ob/ob* mice (Fig. 2, *a–c*). To inspect the possibility that change of plasma fatty acid might directly regulate DGAT expression, we intravenously infused lipid (20% w/v triglyceride emulsion, 5 ml/kg/h) and heparin (6 units/h) simultaneously for 5 h in C57BL/6 mice. Plasma NEFA concentrations were significantly increased by lipid plus heparin infusion (lipid + heparin group: 4.3 ± 0.2 mEq/liter, saline + heparin group: 1.1 ± 0.1 mEq/liter), however, DGAT1 and DGAT2 mRNA expression in WAT was not altered (data not shown).

Reciprocal Expression of DGATs Was Not Observed in Hypertrophic 3T3-L1 Cells—If the hypertrophic change *per se* is the major repressor of DGAT1 in the adipocytes, the reciprocal expression of DGAT1 and DGAT2 would be mimicked also in cultured cells. Hypertrophic change was induced in 3T3-L1 cells by culturing on gelatin-coated culture dishes for a few weeks after induction of differentiation, and the cells contained higher triglyceride levels and exhibited alterations of gene expression, such as increased resistin expression and decreased adiponectin expression, which was reminiscent of *in vivo* adipocyte hypertrophy (21). At day 17 of differentiation, hypertrophic 3T3-L1 cells showed significantly increased (5.1-fold) triglyceride content and increased DGAT1 and DGAT2 expression, compared with the fully differentiated cells at day 10 (DGAT1: 2.8-fold, DGAT2: 2.8-fold, Fig. 4, *a–c*), suggesting

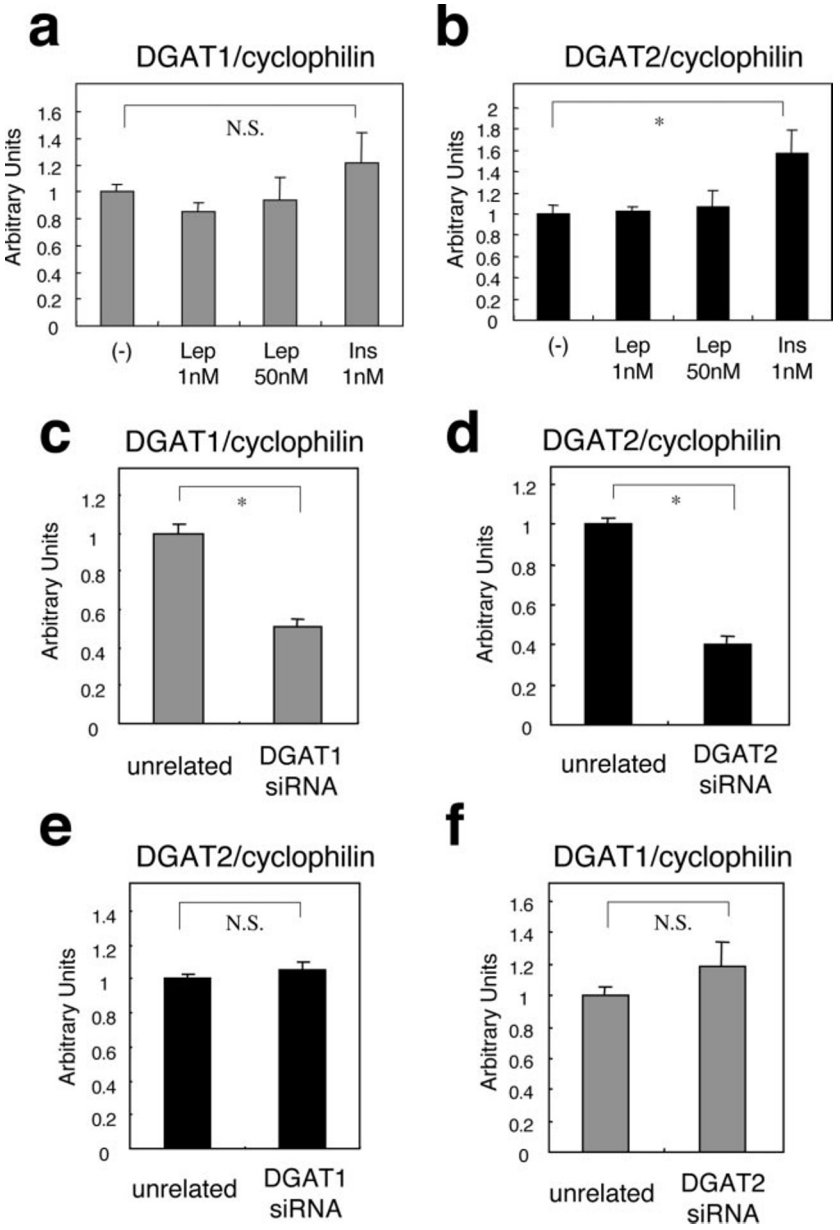


FIG. 5. **DGAT expression in 3T3-L1 cells was unaffected by leptin or by inhibition of either isozyme.** *a* and *b*, effect of leptin and insulin in 3T3-L1 cells. DGAT1 mRNA expression (*a*) and DGAT2 mRNA expression (*b*) were measured by TaqMan real-time PCR in differentiated 3T3-L1 cells. Leptin did not affect the expression of either DGAT1 or DGAT2 but physiological concentrations of insulin significantly increased DGAT2 expression. Cyclophilin was used as an internal control. *n* is 6 per group. *c–f*, results of RNA interference experiments. DGAT1 (*c* and *e*) or DGAT2 (*d* and *f*) mRNA was knocked down by siRNA oligonucleotides in 3T3-L1 cells as described under "Experimental Procedures." Luciferase siRNA was used as an unrelated control. Expression of DGAT1 and DGAT2 was measured by TaqMan real-time PCR. *n* is 8 per group. Values are means ± S.E. *, $p < 0.05$. *N.S.*, difference not significant.

that adipocyte non-autonomous mechanism *in vivo* should be required for the reciprocal regulation of DGAT1 and DGAT2.

Expression of DGATs Was Unaffected by Leptin or Inhibition of Either Isozyme in 3T3-L1 Cells—We hypothesized that systemic interaction among the organs involved with obesity and leptin action *in vivo* is required for the antithetical regulation of DGAT1 and DGAT2. This hypothesis includes the possibility that leptin may directly increase DGAT1 and suppress DGAT2 expression in the adipose tissue. However, DGAT1 and DGAT2 showed similar patterns of expression in WAT either from *Irs2*$^{-/-}$ mice with hyperleptinemia (16) or from *ob/ob* mice lacking secretion of leptin, which led us to conclude that the reciprocal expression of DGATs is not caused by a direct effect of leptin on white adipocytes. In fact, exposure to leptin did not affect either DGAT1 or DGAT2 expression in 3T3-L1 cells, but 1 nM insulin significantly increased (1.6-fold) expression of DGAT2 (Fig. 5, *a* and *b*).

To investigate the reciprocity of DGAT1 and DGAT2 expression, we performed RNA interference experiments. DGAT1 siRNA and DGAT2 siRNA reduced the expression of each isozyme by half, but expression of the other isozyme was unaffected (Fig. 5, *c–f*).

Intracerebroventricular Infusion of Leptin Reduced DGAT2 Expression in WAT Independently of DGAT1 Expression—Finally, we hypothesized that DGAT1 and DGAT2 expression in WAT is regulated by leptin action throughout the body. To verify the hypothesis, we investigated the gene expression in the WAT of *Irs2*$^{-/-}$ mice and *ob/ob* mice after continuous intracerebroventricular infusion of leptin. Leptin infusion at a rate of 300 ng/h yielded lean *Irs2*$^{-/-}$ mice and overcame their leptin resistance as previously reported (17), and it reduced DGAT2 mRNA expression by about 60% in the WAT of *Irs2*$^{-/-}$ mice whereas it significantly increased DGAT1 expression (Fig. 6, *a–c*). Smaller amount of leptin infusion (10 ng/h) was enough to reduce body weight of *ob/ob* mice, and it clearly reduced DGAT2 expression by about 40% (saline group:leptin group = 1:0.59) whereas DGAT1 expression was unaltered (saline group:leptin group = 1:0.96) (Fig. 6*d*). These results suggested the hypothesis that central leptin action *in vivo* is responsible for the expression of DGAT2 in WAT.

DISCUSSION

We have found that adipocyte size is inversely correlated with systemic insulin sensitivity: that larger adipocytes are

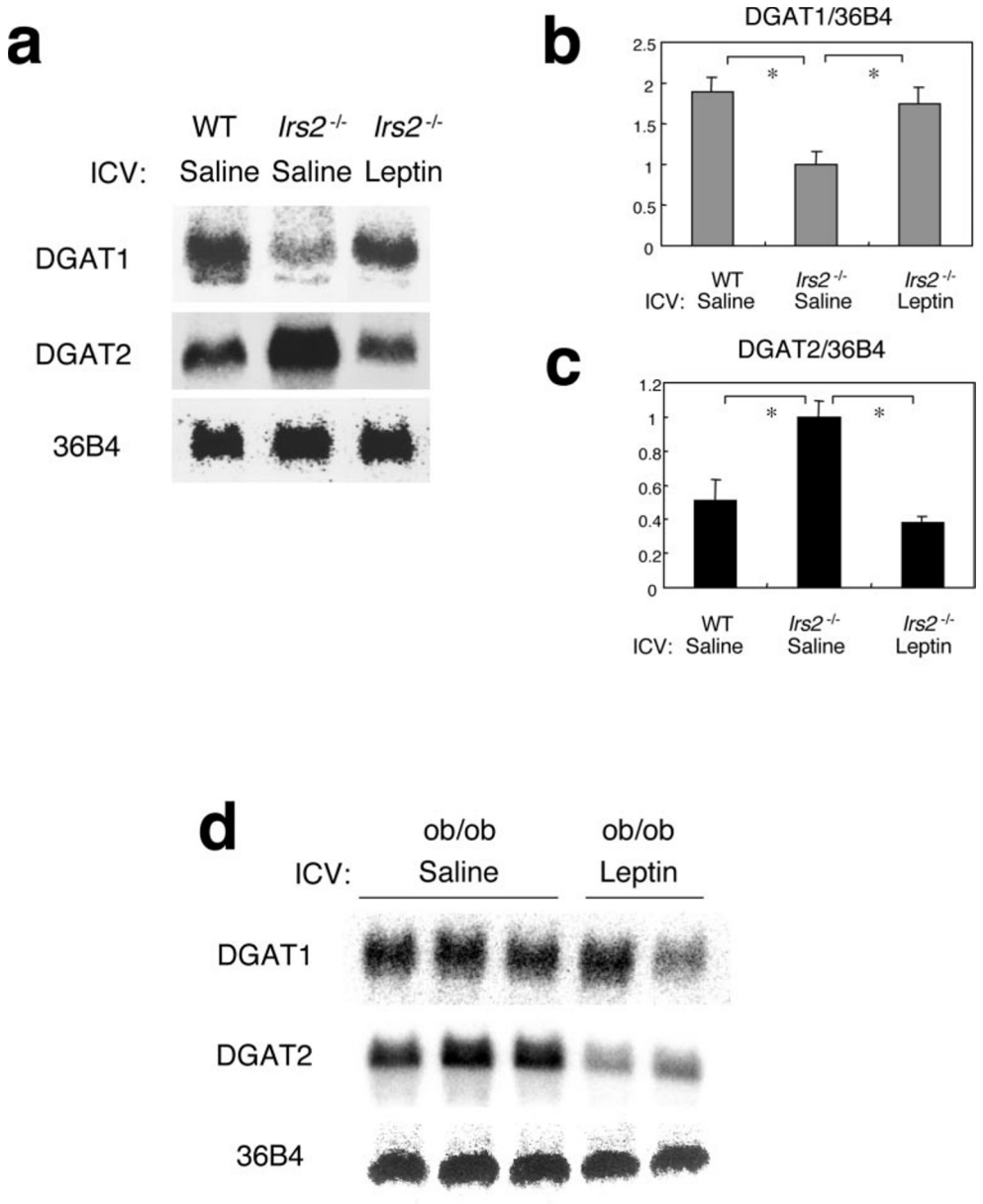


FIG. 6. **Intracerebroventricular leptin infusion reduced DGAT2 expression in WAT.** Mice were treated with intracerebroventricular infusion of saline or leptin for 7 days as described under "Experimental Procedures." Intracerebroventricular infusion of leptin always decreased DGAT2 expression in the epididymal fat pads independently of DGAT1. *a*, Northern blots of DGAT1 and DGAT2 in epididymal fat pads of *Irs2*$^{-/-}$ mice. *b* and *c*, quantified DGAT1 (*b*) and DGAT2 (*c*) expression levels corrected by the internal control. *n* is 3–6 male mice per group. *d*, Northern blots of DGAT1 and DGAT2 in epididymal fat pads of *ob*/*ob* mice. Mice were sacrificed after fasting for 12 h, and fat pads were excised. 36B4 is shown as an internal control.

associated with insulin resistance, and smaller adipocytes are associated with insulin sensitivity (1, 20). We reported that obesity associated with leptin resistance contributes to insulin resistance of *Irs2*$^{-/-}$ mice as well as insulin signaling defects in the liver (17). Because histological analysis revealed larger adipocytes in leptin-resistant *Irs2*$^{-/-}$ mice, we concluded that attenuated leptin action may be the link between insulin resistance and adipocyte hypertrophy.

The present study was undertaken to clarify the molecular mechanism of the adipocyte enlargement in leptin-resistant *Irs2*$^{-/-}$ mice. We suspected that triglyceride synthesis was increased in *Irs2*$^{-/-}$ mice, and screening for expression of enzymes involved in the triglyceride synthesis pathway revealed increased expression of DGAT2 in *Irs2*$^{-/-}$ mice adipocytes. We further demonstrated that DGAT2 expression was increased when leptin action was attenuated, and that the expression decreased when leptin was administered intracerebroventricularly to downsize the adipocytes, suggesting that DGAT2 is involved in the physiological process of enlarging adipocytes in adult animals. The lipoatrophic phenotypes of *Dgat2*$^{-/-}$ mice are striking whereas those of *Dgat1*$^{-/-}$ mice are relatively mild (6, 9). Surprisingly, the lack of DGAT2 causes marked reduction of fatty acid, which is an essential component of triglyceride synthesis (9). Major lipogenic enzymes involved in fatty acid synthesis are localized on endoplasmic reticulum (ER). Human DGAT2 is reported to be expressed on ER (29) and overexpression of mouse DGAT2 induces large cytosolic lipid droplets accumulation whereas overexpression of mouse DGAT1 results in small lipid droplets around the cell periphery (9). In fact, an online program to predict subcellular localization of protein by its amino acid sequence (PSORT II Prediction, psort.nibb.ac.jp/form2.html) calculated the probabilities of mouse DGAT1 and DGAT2 localization as: DGAT1, plasma membrane 52.2%, ER 17.4%, vacuolar 13.0%; DGAT2, ER

55.6%, mitochondrial 11.1%, Golgi 11.1%, nuclear 11.1%, cytoplasmic 11.1%. Proximity of DGAT2 to lipogenic enzymes on ER may influence its accessibility to fatty acid, and increase of DGAT2 expression in enlarged adipocytes may be advantageous to store fatty acid *de novo* synthesized around ER when leptin action is attenuated.

We previously demonstrated that the *SREBP-1* gene and its downstream target genes expression is up-regulated in the liver of $Irs2^{-/-}$ mice and results in fatty liver (16). We attributed this to leptin resistance, which is consistent with the blunted PI 3-kinase activation in the hypothalamus of the mice (17). SREBP-1 mRNA expression is also increased in the WAT of $Irs2^{-/-}$ mice, however, it is reduced in WAT of *ob/ob* mice, suggesting that SREBP-1 does not contribute much to the adipocyte enlargement in *ob/ob* mice. Recent studies have shown a strong correlation between increased tissue triglyceride content and insulin resistance. Since both leptin resistance in $Irs2^{-/-}$ mice and leptin deficiency in *ob/ob* mice result in fatty liver in an SREBP-1-dependent manner and adipocyte hypertrophy in a DGAT2-dependent manner, loss of leptin action is considered to be a common mechanism of increased tissue triglyceride content, causing insulin resistance.

Our hypothesis is that leptin resistance provides a link between larger adipocytes and insulin resistance. In fact, mice with various levels of leptin activity can be arrayed according to adipocyte size, *e.g. ob/ob* mice, diet-induced obese (DIO) mice, WT mice, and transgenic skinny mice overexpressing leptin (30). As leptin activity increases, adipocyte size, which is large in leptin-deficient *ob/ob* mice, gradually becomes smaller, and ultimately it becomes too small to be seen, as in the leptin transgenic mice. As adipocyte size decreases in association with increased leptin activity, insulin resistance improves. Leptin transgenic skinny mice show insulin-sensitive phenotypes, quite unlike other insulin-resistant lipoatrophic mice with insufficient leptin activity (30). Thus leptin action provides a link between adipocyte size and insulin resistance.

The results of the present study suggest that DGAT2 may provide a molecular link between the loss of leptin action and larger adipocytes in adult mammals. Loss of leptin action or leptin resistance in human obesity may also explain the increased tissue triglyceride contents, as exemplified by fatty liver and larger adipocytes, that is frequently associated with type 2 diabetes.

Acknowledgments—We thank Dr. Kohjiro Ueki, Dr. Junji Kamon, Dr. Junji Matsui, Dr. Atsushi Tsuchida, Dr. Mitsuhiko Noda, and Dr. Kazuhiro Eto for helpful discussion. We thank N. Kowatari-Otsuka, C. Kokaki-Kaizuka, A. Nagano, and H. Chiyonobu for excellent technical assistance and mouse husbandry.

REFERENCES

1. Okuno, A., Tamemoto, H., Tobe, K., Ueki, K., Iwamoto, K., Mori, Y., Umesono, K., Akanuma, Y., Fujiwara, T., Horikoshi, H., Yazaki, Y., and Kadowaki, T. (1998) *J. Clin. Investig.* **101,** 1354–1361
2. Chen, H. C., Smith, S. J., Tow, B., Elias, P. M., and Farese, R. V., Jr. (2002) *J. Clin. Investig.* **109,** 175–181
3. Cases, S., Smith, S. J., Zheng, Y. W., Myers, H. M., Lear, S. R., Sande, E., Novak, S., Collins, C., Welch, C. B., Lusis, A. J., Erickson, S. K., and Farese, R. V., Jr. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 13018–13023
4. Lardizabal, K. D., Mai, J. T., Wagner, N. W., Wyrick, A., Voelker, T., and Hawkins, D. J. (2001) *J. Biol. Chem.* **276,** 38862–38869
5. Cases, S., Stone, S. J., Zhou, P., Yen, E., Tow, B., Lardizabal, K. D., Voelker, T., and Farese, R. V., Jr. (2001) *J. Biol. Chem.* **276,** 38870–38876
6. Smith, S. J., Cases, S., Jensen, D. R., Chen, H. C., Sande, E., Tow, B., Sanan, D. A., Raber, J., Eckel, R. H., and Farese, R. V., Jr. (2000) *Nat. Genet.* **25,** 87–90
7. Chen, H. C., Smith, S. J., Ladha, Z., Jensen, D. R., Ferreira, L. D., Pulawa, L. K., McGuire, J. G., Pitas, R. E., Eckel, R. H., and Farese, R. V., Jr. (2001) *J. Clin. Investig.* **109,** 1049–1055
8. Chen, H. C., Rao, M., Sajan, M. P., Standaert, M., Kanoh, Y., Miura, A., Farese, R. V., Jr., and Farese, R. V. (2004) *Diabetes* **53,** 1445–1451
9. Stone, S. J., Myers, H. M., Watkins, S. M., Brown, B. E., Feingold, K. R., Elias, P. M., and Farese, R. V., Jr. (2004) *J. Biol. Chem.* **279,** 11767–11776
10. White, M. F. (1998) *Mol. Cell. Biochem.* **182,** 3–11
11. Virkamaki, A., Ueki, K., and Kahn, C. R. (1999) *J. Clin. Investig.* **103,** 931–943
12. Withers, D. J., Gutierrez, J. S., Towery, H., Burks, D. J., Ren, J. M., Previs, S., Zhang, Y., Bernal, D., Pons, S., Shulman, G. I., Bonner-Weir, S., and White, M. F. (1998) *Nature* **391,** 900–904
13. Withers, D. J., Burks, D. J., Towery, H. H., Altamuro, S. L., Flint, C. L., and White, M. F. (1999) *Nat. Genet.* **23,** 32–40
14. Kubota, N., Tobe, K., Terauchi, Y., Eto, K., Yamauchi, T., Suzuki, R., Tsubamoto, Y., Komeda, K., Nakano, R., Miki, H., Satoh, S., Sekihara, H., Sciacchitano, S., Lesniak, M., Aizawa, S., Nagai, R., Kimura, S., Akanuma, Y., Taylor, S. I., and Kadowaki, T. (2000) *Diabetes* **49,** 1880–1889
15. Burks, D. J., de Mora, J. F., Schubert, M., Withers, D. J., Myers, M. G., Jr., Towery, H. H., Altamuro, S. L., Flint, C. L., and White, M. F. (2000) *Nature* **407,** 377–382
16. Tobe, K., Suzuki, R., Aoyama, M., Yamauchi, T., Kamon, J., Kubota, N., Terauchi, Y., Matsui, J., Akanuma, Y., Kimura, S., Tanaka, J., Abe, M., Ohsumi, J., Nagai, R., and Kadowaki, T. (2001) *J. Biol. Chem.* **276,** 38337–38340
17. Suzuki, R., Tobe, K., Aoyama, M., Inoue, A., Sakamoto, K., Yamauchi, T., Kamon, J., Kubota, N., Terauchi, Y., Yoshimatsu, H., Matsuhisa, M., Nagasaka S., Ogata, H., Tokuyama, K., Nagai, R., and Kadowaki, T. (2004) *J. Biol. Chem.* **279,** 25039–25049
18. Shimano, H., Yahagi, N., Amemiya-Kudo, M., Hasty, A.H., Osuga, J., Tamura, Y., Shionoiri, F., Iizuka, Y., Ohashi, K., Harada, K., Gotoda, T., Ishibashi, S., and Yamada, N. (1999) *J. Biol. Chem.* **274,** 35832–35839
19. Miki, H., Yamauchi, T., Suzuki, R., Komeda, K., Tsuchida, A., Kubota, N., Terauchi, Y., Kamon, J., Kaburagi, Y., Matsui, J., Akanuma, Y., Nagai, R., Kimura, S., Tobe, K., and Kadowaki, T. (2001) *Mol. Cell. Biol.* **21,** 2521–2532
20. Kubota, N., Terauchi, Y., Miki, H., Tamemoto, H., Yamauchi, T., Komeda, K., Satoh, S., Nakano, R., Ishii, C., Sugiyama, T., Eto, K., Tsubamoto, Y., Okuno, A., Murakami, K., Sekihara, H., Hasegawa, G., Naito, M., Toyoshima, Y., Fujita, T., Ezaki, O., Aizawa, S., Nagai, R., Tobe, K., Kimura, S., and Kadowaki, T. (1999) *Mol. Cell* **4,** 597–609
21. Uchida, S., Yamauchi, T., Kamon, J., Hara, K., Waki, H., Kimura, S., and Kadowaki, T. (2002) *Diabetes* **51** (Suppl. 2), A465 (abstr.)
22. Suzuki, R., Tobe, K., Terauchi, Y., Komeda, K., Kubota, N., Eto, K., Yamauchi, T., Azuma, K., Kaneto, H., Taguchi, T., Koga, T., German, M. S., Watada, H., Kawamori, R., Wright, C. V. E., Kajimoto, Y., Kimura, S., Nagai, R., and Kadowaki, T. (2003) *J. Biol. Chem.* **278,** 43691–43698
23. Masaki, T., Chiba, S., Noguchi, H., Yasuda, T., Tobe, K., Suzuki, R., Kadowaki, T., and Yoshimatsu, H. (2004) *Obes. Res.* **12,** 878–885
24. Buhman, K. K., Smith, S. J., Stone, S. J., Repa, J. J., Wong, J. S., Knapp, F. F., Jr., Burri, B. J., Hamilton, R. L., Abumrad, N. A., and Farese, R. V., Jr. (2002) *J. Biol. Chem.* **277,** 25474–25479
25. Soukas, A. S., Cohen, P., Socci, N. D., and Friedman, J. M. (2000) *Genes Dev.* **14,** 963–980
26. Yahagi, N., Shimano, H., Hasty, A. H., Matsuzaka, T., Ide, T., Yoshikawa, T., Amemiya-Kudo, M., Tomita, S., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., Osuga, J., Harada, K., Gotoda, T., Nagai, R., Ishibashi, S., and Yamada, N. (2002) *J. Biol. Chem.* **277,** 19353–19357
27. Van Heek, M., Compton, D. S., France, C. F., Tedesco, R. P., Fawzi, A. B., Graziano, M. P., Sybertz, E. J., Strader, C. D., and Davis, H. R., Jr. (1997) *J. Clin. Investig.* **99,** 385–390
28. El-Haschimi, K., Pierroz, D. D., Hileman, S. M., Bjørbæk, C., and Flier, J. S. (2000) *J. Clin. Investig.* **105,** 1827–1832
29. Wakimoto, K., Chiba, H., Michibata, H., Seishima, M., Kawasaki, S., Okubo, K., Mitsui, H., Torii, H., and Imai, Y. (2003) *Biochem. Biophys. Res. Commun.* **310,** 296–302
30. Ogawa, Y., Masuzaki, H., Hosoda, K., Aizawa-Abe, M., Suga, J., Suda, M., Ebihara, K., Iwai, H., Matsuoka, N., Satoh, N., Odaka, H., Kasuga, H., Fujisawa, Y., Inoue, G., Nishimura, H., Yoshimasa, Y., and Nakao, K. (1999) *Diabetes* **48,** 1822–1829