Regulation of the Murine Adipocyte Fatty Acid Transporter Gene by Insulin

Michael Z. Man, To Yuen Hui, Jean E. Schaffer*, Harvey F. Lodish, and David A. Bernlohr

Whitehead Institute for Biomedical Research (J.E.S., H.F.L.) and The Massachusetts Institute of Technology Boston, Massachusetts 02142

Department of Biochemistry (M.Z.M., T.Y.H., D.A.B.) and Institute of Human Genetics (D.A.B.) University of Minnesota St. Paul, Minnesota 55108

A cDNA encoding a novel fatty acid transport protein (FATP) was identified recently using expression cloning methodologies. We have studied the expression of FATP in differentiating 3T3-L1 cells and adipose tissue in vivo. When 3T3-L1 preadipocytes are treated with a combination of methylisobutylxanthine, dexamethasone, and insulin to induce differentiation, the abundance of FATP mRNA decreases within 24 h to less than one-third that of preadipocytes and increases subsequently, such that mature adipocytes have 5–7 times more FATP mRNA than fibroblastic precursors. In fully differentiated 3T3-L1 adipocytes, insulin alone is sufficient to down-regulate FATP mRNA levels 10-fold. The concentration of insulin necessary for half-maximal repression (I_{50}) is ~1 nM and is specific for insulin; insulin-like growth factor I (IGF-I) has little effect at similar concentrations. Kinetic analysis indicates that the reduction in expression of FATP mRNA by insulin is rapid (t_1/2 = ~4 h) and reversible upon withdrawal of insulin. The half-lives of FATP mRNA are 2.9 h and 4.4 h in the absence and presence of insulin, respectively. The insulin-mediated decrease in FATP steady state mRNA level correlates with a decrease in its transcription rate as measured by nuclear run-on transcription assay. To determine whether physiological conditions that alter insulin concentration in vivo affect adipose FATP levels, feeding/fasting studies are employed. Fasting of C57BL/6J mice for 48 h results in a 11-fold up-regulation of FATP mRNA expression in adipose tissue. Refeeding of fasted animals for 72 h results in a return of FATP mRNA to basal levels. In sum, these results indicate that the expression of FATP gene is negatively regulated by insulin at the transcriptional level in cultured adipocytes and that transporter mRNA expression in murine adipose tissue is altered in a manner consistent with insulin being a negative regulator of gene activity. (Molecular Endocrinology 10: 1021-1028, 1996)

INTRODUCTION

The molecular basis for long chain fatty acid transport across biological membranes has remained enigmatic despite intense examination. Fatty acids may diffuse rapidly across membranes due to the solubility of the lipid in mixed-phospholipid bilayers (1). The cellular uptake of fatty acids may therefore be driven by mass action based upon cellular metabolism. Alternatively, a protein(s) may mediate the transfer of fatty acids across the membrane. In support of a protein-mediated mechanism, transport activity in adipocytes has been characterized as a high-affinity process (K_m of 200 nM) that is inhibited by fatty acid analogs, is sensitive to prior protease treatment of the cell surface, and is blocked by antibodies directed toward specific plasma membrane proteins (2–6).

Recently, several proteins have been proposed to function as fatty acid transporters. The first, plasma membrane fatty acid binding protein (FABPpm) is biochemically indistinguishable from the mitochondrial matrix enzyme aspartate aminotransferase (mAspAT) (7). A cDNA clone for the 43-kDa FABPpm has not been described; however, stable 3T3 cell lines overexpressing mAspAT exhibit a 10-fold increase in oleate uptake. A polyclonal antibody directed against FABPpm stains the periphery of mAspAT-expressing cell lines (10). Despite this evidence, it remains unclear how a soluble mitochondria protein may be directed to the plasma membrane and how it might function as a transporter. The second, fatty acid translocase (FAT), is an 88-kDa adipocyte plasma membrane glycoprotein with two predicted transmembrane domains. FAT is the murine adipocyte homolog of the human CD36 cell surface antigen. N-Sulfosuccinimidyld long-chain fatty acid derivatives hind FAT and inhibit fatty acid

Downloaded from mend.endojournals.org on December 29, 2005
transport by 75% (8). The third, fatty acid transport protein (FATP), is the most promising candidate for a cellular long chain fatty acid transporter because of its subcellular localization and demonstration of function. FATP is a 63-kDa adipocyte integral plasma membrane protein with six predicted membrane-spanning domains (9). Stable fibroblast cell lines expressing FATP demonstrate a 3- to 4-fold increase over baseline in specific transport of long-chain fatty acids with an apparent $K_m$ of 200 nM for oleic acid.

Fatty acid homeostasis in adipose tissue is tightly regulated under physiological conditions; therefore, we have focused on identification of hormones and factors that may regulate the expression of FATP in fat cells. The 3T3-L1 cells provide a convenient system for study of adipose metabolism and the expression of FATP (11). These cells, which are initially fibroblastic, can be differentiated with the addition of FBS, insulin, dexamethasone (DEX), and methylisobutylxanthine (MIX) into a cell type with the morphological and biochemical properties of adipocytes (11). We report here that the FATP gene is differentially expressed during differentiation of 3T3-L1 adipocytes. In addition, in mature adipocytes, transcription of the FATP gene is down-regulated by insulin. Consistent with insulin control of FATP expression, the abundance of the FATP mRNA in vivo is regulated by conditions that alter circulating insulin levels.

**RESULTS**

**FATP mRNA Expression during 3T3-L1 Differentiation**

During adipocyte differentiation, numerous genes encoding lipogenic and lipolytic enzymes and proteins responsible for the adipocyte phenotype are transcriptionally activated and expressed (12, 13). To determine whether the expression of FATP follows a similar kinetic progression, Northern blotting was used to probe RNAs isolated at various days during the differentiation program. As shown in Fig. 1, FATP mRNA is present at low levels in the 3T3-L1 preadipocytes, decreases by two-thirds during days 1 and 2 of differentiation, and then increases 5- to 7-fold above the level at day 0 as the cells reach their fully differentiated state (day 9). The low level of FATP expression in 3T3-L1 preadipocytes is similar to that observed in nondifferentiating 3T3-C2 cells and NIH 3T3 cells (data not shown), suggesting that FATP is expressed at low levels in a number of cell types and may be involved ubiquitously in basal lipid transport. In contrast, the mRNA for the adipocyte lipid binding protein (ALBP), is expressed only in differentiated adipocytes.

During the initial stage of differentiation, the decrease in FATP mRNA occurs after exposure to insulin, MIX, and DEX. This observation suggests that one or more of the factors may control FATP gene expression. Because our interests were on the control of FATP in fat cells, we turned our attention to those factors that may regulate FATP expression in mature 3T3-L1 adipocytes. 3T3-L1 adipocytes (day 9) were incubated with all possible combinations of the factors for 24 h and the levels of FATP mRNA were assessed by Northern analysis. As shown in Fig. 2, insulin and MIX each down-regulate FATP expression and, when incubated together, their effects are additive. The inclusion of FBS had no effect on FATP expression (results not shown). While the effects of DEX tended to antagonize those of insulin, no statistical significance to the results could be assigned.

**Regulation of FATP mRNA Expression by Insulin in 3T3-L1 Adipocytes**

To explore further the effects of insulin on FATP expression in adipocytes, the concentration dependence of insulin-mediated down-regulation was investigated. The concentration of insulin necessary for 50% decrease in steady-state mRNA level is approximately 1 nM (Fig. 3). At the highest insulin concentrations (>100 nM), the FATP mRNA level decreases to 20% of the untreated control sample. In the presence of 10% FBS, higher concentrations of insulin are needed to achieve the same magnitude of repression (results not shown). IGF-1 at a concentration of 100 nM is not as effective in reducing FATP mRNA levels, which are 60% of the control untreated samples (Fig. 3).

The kinetics of insulin-mediated down-regulation of FATP expression were further examined. A time course shows that at 4 h after insulin treatment FATP mRNA decreases by 50% and at 8 h by 90% (maximal) (Fig. 4). Continued treatment with insulin to 48 h results in a small increase in FATP mRNA to 40% of control (results not shown), which may be due to the development of insulin resistance in these cells (14). To determine whether the insulin-mediated down-regulation of FATP expression was reversible, cultured 3T3-L1 adipocytes were first treated with insulin to decrease FATP mRNA level. After 12 h of insulin treatment, insulin was withdrawn and cells were maintained in the absence of insulin. Under these conditions, FATP mRNA levels increase within 2 h after insulin withdrawal and reach 70% of the control level after 8 h (Fig. 5). Therefore, in the absence of insulin, the expression of FATP mRNA returns to essentially basal levels.

Nuclear run-on assays were used to estimate the relative rate of FATP gene transcription as a consequence of insulin treatment. After insulin addition to cultured 3T3-L1 adipocytes, nuclei were isolated and nascent transcripts elongated in the presence of [$\alpha$-32P]UTP. The radioactive RNA was isolated and hybridized to DNA probes affixed to nylon membranes. The relative transcription rates were calculated as a function of time after insulin addition and normalized to the hybridization of labeled RNA to total genomic DNA. As shown in Fig. 4, the rate of FATP transcription decreases within 1.5 h after insulin addi-
Fig. 1. FATP mRNA Expression during 3T3-L1 Differentiation

Panel A, 3T3-L1 preadipocytes were induced to differentiate as described in Materials and Methods. At various days after the initiation of the adipogenic program, total cellular RNA was isolated and the level of FATP mRNA determined. The Northern blot was subsequently stripped and reprobed with the cDNA corresponding to ALBP and an oligonucleotide specific for 28S rRNA [5' AACGATAGTAGGTGATTCACC3' (40)]. The size of each RNA species is shown in kilobases on the right. Panel B, the autoradiographs corresponding to FATP and 28S were scanned using laser densitometry, and the integrated intensities were represented as the ratio of FATP to ribosomal RNA.

The decrease in FATP gene transcription occurs at a rate greater than that of its cognate mRNA, suggesting that insulin down-regulates the expression of FATP at the transcriptional level.

Using 5 μg/ml actinomycin D to block transcription by RNA polymerase II, the half-life of the FATP message is determined to be 2.9 h (n = 4). Insulin does not accelerate the degradation of FATP message, but rather slightly increases its half-life to 4.4 h (n = 3). In order to study the down-regulation of the insulin-responsive glucose transporter (GLUT 4) by polyunsaturated fatty acids, Tebbey et al. (15) have used the identical conditions in 3T3-L1 cells to block RNA polymerase II action. The short half-life for FATP message is consistent with the rapid reduction in the presence of insulin. Taken together, our experiments strongly suggest that the insulin-mediated decrease in FATP mRNA results from down-regulation of FATP gene transcription.

Nutritional Control of FATP Expression

Because insulin is strongly implicated in control of FATP expression in 3T3-L1 adipocytes, we evaluated
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Insulin</th>
<th>DEX</th>
<th>MIX</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.40 g</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>1.20 k</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1.00</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>0.80</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>0.60</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>0.40</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.20</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>0.00</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 2. Regulation of FATP mRNA Level by Adipogenic Agents

Day 9 3T3-L1 adipocytes were washed with PBS and then incubated in fresh DMEM with the indicated combinations of adipogenic factors: 174 nM insulin, 0.5 mM MIX and/or 255 nM DEX. Total cellular RNA was isolated from the cells after 24 h treatment, and the relative abundance of FATP and 28S RNAs was determined by Northern analysis. The bar graph is a summary of four independent experiments, mean ± sd. A single asterisk indicates significance at a level of P < 0.05 while a double asterisk indicates significance at P < 0.001. Statistical significance was determined by two-tail paired t test and confirmed by three-factorial ANOVA.

Fig. 3. Down-Regulation of FATP mRNA Level by Insulin or IGF-I

Day 9 3T3-L1 adipocytes were washed with PBS, then incubated with the indicated concentrations of insulin or IGF-I in DMEM. RNA was isolated after 24 h, and Northern blotting was carried out to determine the relative abundance of FATP and 28S RNAs. Autoradiographs were scanned using laser densitometry and analyzed using NIH Image 1.54 software (40). Open circles represent treatment with insulin while filled circles represent treatment with IGF-I. Means and sos of FATP mRNA normalized to 28S rRNA from three independent experiments are shown.

Fig. 4. Down-Regulation of the FATP mRNA, Analysis of Transcriptional Control

Day 9 3T3-L1 adipocytes were washed with PBS, then incubated with 1 μM insulin in DMEM. At the indicated times, RNA was prepared and the abundance of the FATP message and 28S RNA was determined by Northern analysis. The autoradiographs were scanned using laser densitometry and analyzed using NIH Image 1.54 software. Means and sos of FATP mRNA normalized to 28S rRNA from three independent experiments are shown in the open circles. Nuclear run-on assays were carried out in the presence of [α-32P]UTP, and the labeled RNAs were hybridized to equivalent masses of cDNA corresponding to FATP, pGEM-3Z and mouse genomic DNA immobilized on nylon membranes. Autoradiographs were scanned using laser densitometry, and the intensity of the background pGEM-3Z signal was subtracted from that of FATP and total genomic DNA. The relative amount of FATP transcription is shown in the filled circles and is presented as the mean ± so, normalized to the RNA that hybridizes to total genomic DNA.

FATP mRNA expression in vivo under conditions that alter circulating insulin levels. Fasting and refeeding experiments have been used to study the regulation of many genes involved in lipid and carbohydrate metab-
Fatty Acid Transporter Control by Insulin

Fig. 5. Reversibility of Insulin Effects on FATP Expression
Day 9 differentiated L1 adipocytes were washed with PBS twice and then incubated in 1 μM insulin for 12 h. Cells were washed again with PBS twice and incubated with fresh DMEM without insulin (designated as 0 h). One plate of cells was harvested at each time point (-12, 0, 2, 4, 8, and 24 h). The levels of FATP mRNA normalized to 28S rRNA as measured by Northern blotting are shown in the bar graph (mean ± SD, n = 6).

DISCUSSION

The molecular cloning of the cDNA encoding the adipocyte FATP has allowed for new initiatives into the biochemistry of lipid transport. Because the typical diet consists of 35–45% fats, the disposal of dietary lipids is an important process for overall body energy balance. FATP is presumed to facilitate the flux of fatty acids across the plasma membrane of several target tissues, including cardiac and skeletal muscle as well as fat. It is possible that mAspAT and FAT also contribute to fatty acid flux across the plasma membrane (8, 10), and the relationship of these proteins to FATP remains to be determined.

Fatty acid flux across the adipocyte plasma membrane is bidirectional in response to different metabolic conditions. For example, fatty acids are transported into the adipocyte during nutrient abundance where they are activated with coenzyme A and condensed with α-glycerophosphate to form the storage lipid, triacylglycerol. Insulin promotes fatty acid and triacylglycerol synthesis as well as glucose transport, all factors leading to the storage of metabolic energy in the form of the lipid droplet (20). In contrast, when nutritional supplies are diminished, adipose lipolysis is activated by a β-adrenergic receptor-coupled system that activates cAMP-dependent protein kinase, ultimately resulting in the phosphorylation of the hormone-sensitive lipase. The activated hormone-sensitive lipase hydrolyzes triacylglycerol to generate intracellular fatty acids that are transported out of the cell where they are bound by albumin and transported to other tissues for metabolism (21). Insulin is antilipolytic, i.e., it inhibits the flow of fatty acids out of the fat cell. The adipocyte FATP was cloned by Schaffer and Lodish (9) based upon its ability to internalize fatty acids. Our findings of the repression of FATP transcription by insulin and superinduction during fasting suggest that FATP may function to facilitate fatty acid efflux from the fat cell during lipolytic conditions.

The induction of FATP expression during 3T3-L1 adipocyte differentiation is similar to increases in expression of ALBP and adipin, two classical markers
for fat tissue. However, FATP is expressed at low levels in preadipocytes and cannot be considered strictly adipocyte-specific. Adipose conversion has been shown to be intimately connected to the expression of immediate early transcription factors such as CCAAT-enhancer binding protein-α (C/EBPα) and peroxisome proliferator activated receptor-γ2 (PPARγ2). Experimental models suggest that the transcription factor peroxisome proliferator activated receptor-γ2 associates with a lipophilic ligand early during the differentiation process, suggested to be the prostaglandin J3 metabolite, 15-deoxy Δ12,14-PGJ2, to activate the differentiation program (24, 25). Since FATP is expressed at low levels in preadipocytes, it may facilitate uptake of a fatty acid substrate required early during adipose conversion.

Our experiments have established insulin as a negative regulator of FATP expression. This regulation is rapid (~8 h for maximal response) and insulin-specific given the low concentration (~1 nM) of insulin but much higher concentration (~100 nM) of IGF-I needed for 50% down-regulation. Nuclear run-on transcription assays and actinomycin D experiments suggest that the decrease in FATP mRNA level is primarily due to a decrease in gene transcription. In addition, the insulin-mediated down-regulation of FATP mRNA levels is compromised by the inclusion of cycloheximide in the medium (results not shown). This suggests that new protein synthesis may be required for insulin-induced down-regulation of FATP mRNA levels although alternate explanations have not been ruled out. FATP transcription is also down regulated by cAMP but not by other hormones known to affect lipid metabolism such as T3 (results not shown).

Negative regulation of gene expression by insulin has been the focus of studies in several systems. The mRNA levels of insulin like growth factor binding protein-1 (IGFBP-1) (26) and the gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK) (26) and glucose-6-phosphatase (27), are all reduced by insulin. In addition, PEPCK (28), glucose transporter-4 (GLUT4) (15), microsomal triglyceride transfer protein (29), and IGFBP-1 (30) have all been shown to be negatively regulated by insulin at the transcription level. The negative insulin responsive element may be a GC factor binding site conserved in upstream sequences of several insulin-responsive genes (31). However, the insulin response element in the PEPCK and IGFBP-1 genes has been characterized by the sequence 5'-TGGTGCTTAG-3', which forms the core of the hepatic nuclear factor 3 binding site (30). Interestingly, in preliminary analysis of the FATP gene upstream region, the sequence 5'-TGGTGCTTAG-3', which is very similar to the consensus insulin response element, is found ~200 nucleotides upstream of the initiating methionine codon. While no experiments have been done to characterize this sequence, it is tempting to speculate that this region of DNA may be related to the insulin responsiveness of the FATP promoter.

The regulation of expression of FATP and the insulin-responsive glucose transporter, GLUT4, are strikingly similar. Transcription of each is negatively regulated in adipocytes by insulin and cAMP. The cAMP response element in the GLUT4 gene has been identified, but the negative insulin-regulatory element has yet to be characterized (32–34). MacDougald et al. (35) have suggested that down-regulation of GLUT4 by insulin may be mediated through posttranslational modification (dephosphorylation) and/or decreased expression of C/EBPα. Since FATP is also rapidly repressed by insulin (50% inhibition at 4 h, 90% at 8 h), it is reasonable to consider that C/EBPα may also play a role in regulating FATP transcription. Even though GLUT4 and FATP are regulated similarly in cultured adipocytes, differential responses are observed in fasted animals. For GLUT4, the cAMP effect is dominant over that of insulin; therefore, during fasting GLUT4 mRNA is down-regulated (16). For FATP, the effects of cAMP and insulin appear to be independent of each other; FATP mRNA is up-regulated during fasting.

In sum, we have described the expression of the fatty acid transporter mRNA during adipose conversion of 3T3-L1 preadipocytes in culture. We have demonstrated that in mature differentiated adipocytes insulin-responsive glucose transporter, GLUT4, are strikingly similar. Transcription of each is negatively regulated by insulin. Furthermore, FATP mRNA levels are increased during starvation, which is associated with low insulin levels. These results suggest insulin is a primary endocrine factor controlling expression of the murine fatty acid transporter. Identification of the mechanistic basis for these effects of insulin may provide insight into lipid metabolism in fat cells and in pathophysiological conditions such as obesity and type II diabetes.

**MATERIALS AND METHODS**

**Materials**

Insulin was purchased from Gibco BRL (Gaithersburg, MD); IGF-I from R&D System (Minneapolis, MN); cycloheximide, MIX, and DEX from Sigma (St. Louia, MO); actinomycin D from Boehringer Mannheim (Indianapolis, IN); and FBS from Whittaker (Walkersville, MD). All other materials were reagent grade.

**Cell Culture**

3T3-L1 preadipocytes were cultured as previously described (36). Briefly, cells 2 days postconfluence were differentiated by the addition of 174 nM insulin, 0.5 nM MIX, and 0.25 μM DEX in DMEM containing 10% FBS (defined as Day 0). After 48 h (day 2), the DEX and MIX were withdrawn and the cells were incubated with 174 nM insulin in DMEM containing 10% FBS. Two days later (day 4), insulin was withdrawn and cells were maintained in DMEM with 10% FBS thereafter. Day 9 cells, which display fully differentiated adipocyte phenotype, were used for experiments.
RNA Isolation and Northern Hybridization

RNA was isolated from tissues or cultured cells using the one-step guanidium isocyanate-phenol-chloroform extraction method (37). To avoid degradation, RNA was stored in 1:1 (vol/vol) isopropyl alcohol and 4°C guanidium isocyanate at −70°C until use. Agarose gel electrophoresis of glyoxal-denatured RNA was performed in circulating 10 mM diethyl pyrocarbonate-treated sodium phosphate buffer (pH 7.4). RNA was transferred onto Magna nylon membrane (Micron Separations, Inc., Westborough, MA) by overnight capillary action in 10×SSC (1×SSC = 0.1 M sodium chloride, 15 mM sodium citrate, pH 7.0) and cross-linked using a Stratagene UV Crosslinker (Stratagene, La Jolla, CA). Blots were baked in a vacuum oven at 80°C for 30 min. Prehybridization and hybridization were done in 7% solution (7% SDS, 50% formamide, 0.25 M sodium phosphate buffer, pH 7.4) at 42°C for 2–4 h and 18–24 h, respectively. 32P-Labeled probe was prepared using a Random Primed DNA Labeling Kit according to the manufacturer's instructions (Boehringer Mannheim). After hybridization, the blots were washed in 2×SSPE (1×SSPE = 0.15 M NaCl, 10 mM NaH2PO4, 1 mM EDTA) at 42°C once for 10 min, and in 2×SSPE and 0.1% SDS at 65°C twice for 20 min. The blots were exposed to Reflection x-ray film (DuPont, Boston, MA) at −70°C. Autoradiographs were scanned using a laser densitometer (Molecular Dynamics, Sunnyvale, CA 94086) and analyzed using NIH Image 1.54 software (38).

Nuclei Isolation and Run-on Transcription

Nuclei from 3T3-L1 adipocytes were isolated and run-on transcription assays were carried out as described (39). Hybridization was carried out on slot-blot to either cDNA fragments or genomic DNA that had been affixed to Magna nylon membrane. The extent of hybridization was quantified by laser densitometry and normalized to the hybridization of total genomic DNA as an internal standard.

Experimental Animals

C57BL/6J mice (7–9 weeks old, mixed sexes) from Jackson Laboratory (Bar Harbor, ME) were used. One group of mice (three animals) was subjected to a 48-h solid-food fast while a second group (three animals) was fasted 48 h followed by a 72-h refeeding period with rodent laboratory chow (4.5% fat, 23.4% protein, 5.8% fiber). A third group of mice (three animals) was fed ad libitum and used as a control. All mice were housed in identical conditions and were taken in the morning to avoid introducing diurnal variations. Mice (fasted, refeeding) were killed by cervical dislocation and tissues were immediately harvested. All tissues were snap-frozen in liquid nitrogen and stored at −70°C until RNA was isolated. All use of animals was approved by the institutional animal care committee.

Acknowledgments

We would like to thank the members of the Lodish and Bernlohr laboratories for several helpful suggestions and in particular Ms. Anne Smith for technical assistance and Mr. David Zhiyong Zhou for help in statistical analysis.

Received February 1, 1996. Revision received April 17, 1996. Accepted April 24, 1996.

Address requests for reprints to: Dr. David A. Bernlohr, Department of Biochemistry, University of Minnesota, St. Paul, Minnesota 55108.

This work was supported in part by funds from the NIH (Grant DK-49807) and the American Heart Association, Minnesota Affiliate, to D.A.B., M.Z.M., was supported by Chemical Basis of Cell and Molecular Biology Training Grant, National Institute of Health. J.E.S. is supported by a NIH Physician Scientist Award (HL-02696). Support for this work was also provided by a Program of Excellence in Molecular Biology grant from the National Heart Lung and Blood Institute (HL-41484) to H.F.L.

Present address: School of Medicine, Washington University, St. Louis, Missouri 63110.

REFERENCES

34. Ezaki O, Flores-Riversos JR, Kaestner KH, Gearhart J, Lane MD 1993 Regulated expression of an insulin-responsive glucose transporter (GLUT4) minigene in 3T3-L1 adipocytes and transgenic mice. Proc Natl Acad Sci USA 90:3348–3352
36. Student AK, Hsu FY, Lane MD 1980 Induction of fatty acid synthase synthesis in differentiating 3T3-L1 preadipocytes. J Biol Chem 255:4745–4750
40. Barbu V, Dautry F 1989 Northern blot normalization with a 28S rRNA oligonucleotide probe. Nucleic Acid Res 17:7115