Dissociation between adipose tissue fluxes and lipogenic gene expression in ob/ob mice

S. M. Turner,1,3 S. Roy,1 H. S. Sul,1 R. A. Neese,1 E. J. Murphy,2 W. Samandi,3 D. J. Roohk,1 and M. K. Hellerstein,1,2

1Department of Nutritional Sciences and Toxicology, University of California, Berkeley; 2Division of Endocrinology and Metabolism, Department of Medicine, San Francisco General Hospital, University of California, San Francisco; and 3KineMed, Emeryville, California

Submitted 8 July 2005; accepted in final form 6 November 2006

Turner SM, Roy S, Sul HS, Neese RA, Murphy EJ, Samandi W, Roohk DJ, Hellerstein MK. Dissociation between adipose tissue fluxes and lipogenic gene expression in ob/ob mice. Am J Physiol Endocrinol Metab 292: E1101–E1109, 2007. First published December 12, 2006; doi:10.1152/ajpendo.00309.2005.—Recent evidence has been presented that expression of lipogenic genes is downregulated in adipose tissue of ob/ob mice as well as in human obesity, suggesting a functionally lipoatrophic state. Using 2H2O labeling, we measured three adipose tissue biosynthetic processes concurrently: triglyceride (TG) synthesis, palmitate de novo lipogenesis (DNL), and cell proliferation (adipogenesis). To determine the effect of the ob/ob mutation (leptin deficiency) on these parameters, adipose dynamics were compared in ob/ob, leptin-treated ob/ob, food-restricted ob/ob, and lean control mice. Adipose tissue fluxes for TG synthesis, de novo lipogenesis (DNL), and adipogenesis were dramatically increased in ob/ob mice compared with lean controls. Low-dose leptin treatment (2 μg/day) via miniosmotic pump suppressed all fluxes to control levels or below. Food restriction in ob/ob mice only modestly reduced DNL, with no change in TG synthesis or adipogenesis. Measurement of mRNA levels in age-matched ob/ob mice showed generally normal expression levels for most of the selected lipid anabolic genes, and leptin treatment had, with few exceptions, only modest effects on their expression. We conclude that leptin deficiency per se results in marked elevations in flux through diverse lipid anabolic pathways in adipose tissue (DNL, TG synthesis, and cell proliferation), independent of food intake, but that gene expression fails to reflect these changes in flux.

deuterated water; lipogenesis; isotope; gene expression

THE ob/ob MOUSE EXHIBITS OBESITY, insulin resistance, hyperphagia, sterility, and other metabolic and hormonal disturbances due to a mutation in the leptin gene (23). Histological (1) and biochemical evidence (22, 25, 26, 28, 46) suggests that these disturbances include abnormal dynamics of both the lipid and cellular components of adipose tissue.

De novo lipogenesis (DNL) has been well studied in the ob/ob mouse. In vivo 3H2O labeling studies have shown increased hepatic (14, 22, 29) and adipose tissue (6, 14, 22, 29) DNL. Inhibitory effects of leptin on DNL have also been demonstrated (2).

Triglyceride (TG) synthesis and degradation (lipolysis) can also now be measured in adipose tissue by administration of 2H2O (5, 39, 44). Measurement of TG synthesis contributes another metric of adipose tissue metabolism in vivo and also allows for more accurate assessment of DNL rates (39). Generally, DNL is measured as a fraction of total lipid or palmitate present in a lipid sample. By measuring the fraction of new TG from all sources concurrently with the fraction of TG from DNL, the true contribution of DNL to newly synthesized TG can be calculated (39). We have shown elsewhere (39) that cell proliferation, DNL, and TG synthesis can be measured concurrently using 2H2O labeling.

Leptin has also been shown to inhibit differentiation of preadipocytes (2, 42) and to reverse the differentiation of mature adipocytes (47) in vitro. These effects on adipocyte differentiation have been proposed to have a role in the weight-reducing effects of leptin treatment (2, 42, 47). Changes in the rate of preadipocyte differentiation may or may not alter the proliferation rates of mature adipocytes, depending upon whether there is a change in the fate of new preadipocytes in vivo. The effect of leptin on proliferation of mature adipose cells has not been determined in vivo. The effects of leptin deficiency or calorie restriction on adipocyte proliferation have also not been directly measured.

Although many biochemical studies in ob/ob mice have reported increased lipogenesis, gene expression microarray data have painted a different picture of adipose tissue dynamics in ob/ob mice (32, 37) and obese humans (8). Expression of mRNAs for lipidogenic enzymes [e.g., fatty acid (FA) synthase (FAS), ATP citrate lyase, phosphoenolpyruvate carboxykinase (PEPCK)] and sterol response element-binding protein-1c (SREBP-1c) were reported to be reduced, not elevated, in adipose tissue of ob/ob mice (32).

Paradoxically, treatment of ob/ob and lean mice with exogenous leptin further reduced the expression of similar sets of such lipidogenic genes (32). On the basis of these gene expression results, Nadler and Attie (31) reasonably suggested that adipose tissue in ob/ob mice operates as if in a functionally replete state, with reduced capacity for lipid anabolism and storage. According to this model, adipose tissue function in ob/ob mice is analogous to a lipotrophic state rather than an avidly lipid anabolic milieu. It is important to resolve these apparently discordant models of obesity suggested by gene expression vs. flux measurements.

Reduced expression of lipogenic genes has also been reported in human obesity (8). Increased hepatic DNL fluxes were measured with stable isotopes, yet there was reduced...
adipose lipogenic gene expression, perhaps secondary to increased circulating leptin levels (8), providing further evidence of a functionally lipoatrophic state in obesity.

Here, we compare lipogenic fluxes in vivo and gene expression in the same tissue (adipose). One of our objectives was to establish the independent effects of leptin and calorie intake. Accordingly, a low dose of leptin (2 μg/day) was administered, since this dose has previously been shown (17) to reduce food intake but not plasma insulin levels. Also, caloric restriction of ob/ob mice results in gorging or a “meat” feeding pattern, followed by a prolonged fast, which may by itself affect DNL compared with ad libitum feeding. We therefore controlled the pace of food intake in the food-restricted ob/ob mice by using an automatic pellet dispenser to administer food at a fixed rate over each 24-h period.

We show here that, in the C57BL/6Jlep/lep– (ob/ob) genetic model of obesity, fluxes through lipogenic and adipogenic pathways in adipose tissue are markedly elevated and that gene expression is not necessarily reflective of flux though these lipid anabolic pathways.

METHODS

Animals

Stable isotope studies. All animal experiments were performed in compliance with the University of California Berkeley animal care and use committee. Six- to seven-week-old female, C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) were studied: C57BL/6Jn controls (ctrl), ad libitum fed C57BL/6Jlep/lep– (ob/ob), leptin treated ob/ob (ob-lep), and food restricted ob/ob (ob-r). The rationale for studying ob-lep and ob-r was to determine whether changes in adipose tissue dynamics in ob/ob are driven by excess food intake or leptin deficiency per se. Mice were housed individually in hanging wire cages and fed American Institute of Nutrition 93 purified diets (Bio Serv, Frenchtown, NJ). Mice were given 3 days to acclimate to the cages and fed American Institute of Nutrition 93 purified diets (Bio Serv, Frenchtown, NJ). Mice were given 3 days to acclimate to the environment, after which they grew normally. All treatments and interventions began 5 days prior to the start of labeling with 2H2O. Five animals were studied in each group.

Food for the ob-r group was administered in a continuous manner with automatic pellet dispensers (Coulbourn Instruments, Allentown, PA). Food intake was set at 2.8 g/day, which represents the lower range of intake for lean mice, partially adjusting for the reduced total energy expenditure in ob/ob (4, 27, 36). This ensured that sufficient caloric restriction was achieved for comparison to the effects of leptin administration (which also reduces food intake (see below)).

Ob-lep mice received murine leptin (a gift from Amgen, Thousand Oaks, CA) subcutaneously at a dose of 2 μg/day via a 28-day Alzet miniosmotic pump (Alza, Palo Alto, CA). This dose has previously been shown (17) to achieve near-physiological leptin concentrations in ob/ob mice.

Mice received 2H2O (deuterated water) as a priming dose at 0.012 ml/g body wt via intraperitoneal injection. The normal drinking water was then replaced with water enriched to 4%2H2O, which was continued for a total of 21 days. 2H2O treatment had no impact on food intake or body weight. Prior to death, mice were fasted for 4 h, anesthetized with isoflurane, and exsanguinated via cardiac puncture.

Gene expression studies. To measure the expression of lipogenic genes, 8-wk-old female ob/ob (n = 5), ob-lep (n = 5), and control (n = 5) mice were studied. Leptin was administered for 7 days as described above. Mice were killed at the end of the dark cycle (7 AM) without a prior fast (to reflect maximally stimulated lipid anabolism in the circadian cycle). Inguinal adipose tissue was immediately dissected and frozen in liquid nitrogen.

Blood Measurements

At the end of the 3-wk study period, blood was collected by cardiac puncture and placed on ice until plasma was separated and frozen. Plasma glucose concentrations were measured on a YSI (Yellow Springs, OH) auto analyzer. Plasma insulin and leptin assays were performed by assay services at Linco Research (St. Charles, MO).

Changes in Fat Pad Weight

The gain in weight of each fat pad was compared with the net synthesis of TG. The weight of each fat pad at the beginning of the 2H2O labeling period was estimated by sacrificing three separate ob/ob (30 ± 5 g) and 5 control mice (18 ± 0.6 g) at the same age as the study mice at the start of 2H2O labeling period (6 wk old). The relative percentage of body weight from each pad was calculated and averaged for each group. This percentage was then applied to the starting weight of the study animals to estimate the initial weight of each depot. The average starting weight was then subtracted from the final measured weight of the isolated pads to estimate the absolute fat gain in each depot.

Adipose Tissue Preparation and Isolation of Adipocytes

Immediately following dissection, inguinal, perimetrial, retroperitoneal, and mesenteric fat pads were placed in HBSS with calcium in preweighed tubes for isolation of mature adipocytes according to the method of Rodbell (35). Inguinal and retroperitoneal pads from the left and right sides were pooled for analysis. Mice were anesthetized with isoflurane, and exsanguinated via cardiac puncture. Mice were killed at the end of the dark cycle (7 AM) without a prior fast (to reflect maximally stimulated lipid anabolism in the circadian cycle). Inguinal adipose tissue was immediately dis-

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.3 on April 8, 2017
Glycerol triacetate was analyzed using a DB-225 fused silica column, monitoring mass-to-charge ratios (m/z) 159 (parent ion, M0) and 160 (M1) or m/z 159, 160, and 161 (M0, M1, and M2, respectively). Methane chemical ionization (Cl) was used with selected ion monitoring. FA methyl ester composition was analyzed by flame ionization detection and for 2H enrichment by GC-MS, as described elsewhere (21, 24).

Tetramethylsilane (H3O) was analyzed using a DB-225 fused silica column, monitoring m/z 265 and 266 (M0 and M1 ions). Standard curves of known 2H2O enrichment were run before and after each group of samples to calculate isotope enrichment.

PTA samples (dR) were analyzed for incorporation of deuterium using methane Cl with a 30-m DB-225 column under selected ion monitoring of m/z 245–246 (M0 and M1 ions). Natural abundance (unenriched) dA samples were measured concurrently, and the excess M1 abundance in the unenriched standard was subtracted from the M1 abundance measured in the unenriched standard from the M1 abundance in the sample. Bone marrow DNA samples were run simultaneously. Bone marrow DNA represents a completely, or nearly completely, turned over tissue and is used as a reference present (33). The absolute adipose cell proliferation rate (f) was calculated as the EM1 in adipose DNA divided by the DNA enrichment achievable within each animal at the body water enrichment present (33). The absolute adipose cell proliferation rate was estimated by multiplying f times total DNA yield from the tissue and converting to cell numbers (assuming ~10 μg DNA/106 cells).

RNA Isolation and Reverse Transcription

Total RNA was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA) and purified using RNeasy (Qiagen, Valencia, CA). During purification, an on-column DNase I digestion step was carried out as described in the RNeasy Mini Protocol. Five-hundred nanograms of total RNA from each sample was reverse transcribed with random hexamers using TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA).

Real-time PCR

Real-time PCR was performed in 96-well format using the ABI Prism 7900 HT sequence detection system and analyzed using SDS 2.0 software (Applied Biosystems). For each gene transcript, 10 ng of cDNA from each sample was analyzed as an unknown against a standard curve derived from a fivefold dilution series of cDNA reverse transcribed from RNA pooled from the control group. Each 25-μl PCR reaction was carried out using TaqMan Universal PCR Master Mix (Applied Biosystems) and primer and probe sets from Applied Biosystems Assays on demand. Relative mRNA levels for each gene were measured in arbitrary units and normalized to GAPDH levels.

Calculations

TG synthesis. The fraction (f) of TG newly synthesized during the labeling period in an adipose depot was calculated on the basis of the precursor-product relationship, as described previously (44, 20), using the measured body water enrichment to estimate the maximal or asymptotic enrichment in TG-glycerol. The absolute rate of retained TG synthesis was calculated by multiplying f times the TG mass present at the time of measurement.

Estimate of net lipolysis. Net lipolysis was calculated as described previously (44); net lipolysis (mg·kg−1·21 days−1) = absolute TG synthesized − absolute TG accumulated.

In the case of the leptin treated mice, in which TG retained was a negative value (i.e., body fat was lost), the absolute TG synthesis rate was added to the quantity of TG lost to yield the net lipolysis rate (i.e., the negative number was subtracted).

DNL. Fractional DNL was calculated from the incorporation of 2H into TG-palmitate, using mass isotopomer distribution analysis as described in detail elsewhere (39). Fractional DNL represents the fraction of FA synthesized via the DNL pathway during the labeling period, relative to all other sources of TG-FAs. However, this underestimates the contribution of DNL to newly synthesized TG to the extent that preexisting fat is present, since “non-DNL” TG could represent preexisting TG or newly synthesized TG from non-DNL pathways. This problem can be resolved by correcting DNL for the proportion of TG that was newly synthesized during the labeling period. The ratio of fractional DNL to fractional TG synthesis reveals the true contribution of DNL to new TG-palmitate (39). Data presented here are expressed as the corrected value, representing the fractional contribution from DNL to newly synthesized TG.

The absolute rate of palmitate DNL was calculated by multiplying measured corrected fractional DNL times 0.8 (the estimated fraction by weight of TG in adipose tissue) times the weight of the fat pad times the percentage of FA in the depot that is palmitate. This value represents the absolute amount (g) of palmitate synthesized during the labeling period.

Adipose cell proliferation (adipogenesis). Fractional adipose cell proliferation (f) was calculated as the EM1 in adipose DNA divided by EM1 in bone marrow DNA, as described in detail elsewhere (33). Bone marrow DNA enrichment is used to approximate the maximum DNA enrichment achievable within each animal at the body water enrichment present (33). The absolute adipose cell proliferation rate was estimated by multiplying f times total DNA yield from the tissue and converting to cell numbers (assuming ~10 μg DNA/106 cells).
Statistical Analysis

Data are presented as means \pm SD unless otherwise stated. Significance was determined by one-way ANOVA followed by Tukey’s pairwise comparisons. \( P \leq 0.05 \) was considered significant.

RESULTS

Food Intake

Food intake in the control mice was 3.1 \pm 0.4 g/day. Food intake of the food-restricted \( \text{ob/ob} \) mice (\( \text{ob-r} \)) was fixed at 2.8 g/day. \( \text{ob/ob} \) Mice consumed 5.1 \pm 1.1 g/day, and the leptin-treated \( \text{ob/ob} \) mice (\( \text{ob-lep} \)) ate 3.8 \pm 0.9 g/day. Significant differences were present between \( \text{ob/ob} \) and controls, \( \text{ob-lep} \), and \( \text{ob-r} \) (\( P < 0.05 \); Table 1).

Body Weight

The effects of leptin treatment and food restriction on body weight during the \( \text{H2} \) labeling period are shown (Fig. 1). Leptin treatment reduced body weight significantly more than food restriction despite higher food intake in the leptin-treated group.

Plasma Insulin, Glucose, and Leptin Concentrations

Plasma insulin, glucose, and leptin levels were measured from the terminal blood collection after a 4-h fast in all groups at the end of the study. Insulin concentrations were significantly higher in all \( \text{ob/ob} \) groups, including the leptin-treated group, compared with control mice, as has been previously reported (15) (Table 1). Plasma glucose concentrations were significantly higher in the \( \text{ob/ob} \) group compared with controls (Table 1). Both food restriction and leptin treatment tended to reduce plasma glucose concentration in \( \text{ob/ob} \) mice, but only the leptin-treated group showed significant reductions compared with \( \text{ob/ob} \). Plasma leptin levels in the leptin-treated \( \text{ob/ob} \) and control mice were similar (5.7 \pm 2.9 and 4.1 \pm 1.9 ng/ml, respectively; Table 1). In the \( \text{ob/ob} \) and food-restricted \( \text{ob/ob} \) mice, plasma leptin concentrations were undetectable.

Body Fat Distribution

The untreated \( \text{ob/ob} \) animals had larger fat pads (Table 2) that represented a higher percentage of total body weight compared with controls. Food restriction significantly reduced the weight of all fat pads, and leptin treatment resulted in a significantly greater weight reduction in inguinal and mesenteric pads.

Absolute Rates of TG Synthesis (All-Source Lipogenesis)

The absolute TG synthesis rate was markedly elevated in the \( \text{ob/ob} \) and food-restricted \( \text{ob/ob} \) mice compared with control and leptin-treated mice (Fig. 2). Leptin treatment also significantly reduced the f of newly synthesized TGs (Table 3), compared with \( \text{ob/ob} \), for each fat pad.

DNL Contribution to Adipose TG-Palmitate

The contribution from DNL to newly synthesized TG-palmi- tate is shown in Table 4. In the \( \text{ob/ob} \) and control mice, the DNL fractional contribution to newly deposited adipose TG was close to 100%. The leptin-treated group exhibited a substantial reduction in the contribution from DNL to new TG.

Table 2. Individual fat pad weights

<table>
<thead>
<tr>
<th></th>
<th>Perimetral</th>
<th>Inguinal</th>
<th>Mesenteric</th>
<th>Retroperitoneal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>0.36 \pm 0.20^b,c</td>
<td>0.46 \pm 0.2^a</td>
<td>0.85 \pm 0.03^a</td>
<td>0.16 \pm 0.1^a</td>
</tr>
<tr>
<td>\text{ob/ob}</td>
<td>2.0 \pm 0.3^a</td>
<td>2.8 \pm 0.2^a</td>
<td>0.85 \pm 0.2^a</td>
<td>1.1 \pm 0.1^a</td>
</tr>
<tr>
<td>\text{ob-r}</td>
<td>1.4 \pm 0.4^a</td>
<td>2.2 \pm 0.3^a</td>
<td>0.63 \pm 0.2^a</td>
<td>0.84 \pm 0.1^a</td>
</tr>
<tr>
<td>\text{ob-lep}</td>
<td>1.6 \pm 0.6^a</td>
<td>1.5 \pm 0.5^a</td>
<td>0.34 \pm 0.1^a</td>
<td>0.66 \pm 0.3^a</td>
</tr>
</tbody>
</table>

Values are means \pm SD and in g; values not sharing a superscripted letter are significantly different within each column.

Fig. 2. Absolute rates of triglyceride (TG) synthesis over 21 days (g). Absolute amount of TG synthesized over the labeling period was calculated from fractional TG synthesis measured from \( \text{H2} \) incorporated into TG-glycerol, as described in the text. Data are means \pm SD; values not sharing a superscripted letter are significantly different within each column (40).
Food restriction also lowered the DNL contribution to newly synthesized TG compared with ob/ob and controls.

**Absolute Rate of DNL**

The absolute rate of DNL (g palmitate synthesized de novo over the 21-day labeling period) was significantly elevated in the ob/ob group compared with controls (Fig. 3). Food restriction had a small (nonsignificant) impact on absolute DNL. Leptin treatment, in contrast, reduced absolute DNL to levels equal to or below those seen in the control mice in all depots.

**Net Lipolysis**

ob/ob Mice had higher rates of net lipolysis (calculated as TG synthesis minus fat accumulated over 21 days) than the controls (Fig. 4). Food restriction (trended toward increased lipolysis) tended to increase lipolysis, and leptin treatment further increased (tended to increase) lipolytic rates (further; significant in inguinal fat pad only). The absolute magnitude of the increase in lipolysis induced by leptin treatment (range 0–0.6 g/21 days) was approximately one-half the magnitude of the reduction in TG synthesis due to leptin treatment (0.4–1.2 g/21 days; compare with Fig. 2).

**Adipogenesis**

The absolute rate of adipose cell proliferation (adipogenesis) was markedly increased in the perimetric and retroperitoneal fat pads of ob/ob mice compared with controls (Fig. 5). Leptin treatment, but not food restriction, markedly reduced adipose tissue cell proliferation in all fat pads compared with ob/ob mice.

**Gene Expression**

Expression levels of mitochondrial glycerol-3-phosphate acyltransferase (GPAT), SREBP1, diacylglycerol acyltransferase (DGAT)1, glucose transporter 4 (GLUT4), FA-binding protein (FABP4), PEPCK, acetyl-CoA acyltransferase 1 (ACAT1), and peroxisome proliferator-activated receptor-γ (PPARγ) were not elevated in ob/ob compared with controls (Fig. 6), confirming previously reported microarray results (32, 37). Notably, the expression of PEPCK, ACAT1, and FABP4 were reduced, whereas only DGAT2 and FAS expression were significantly elevated in ob/ob compared with controls. Interestingly, leptin treatment increased PEPCK and GLUT4 expression, and FAS expression also trended upward with leptin treatment, whereas DGAT2 expression was no longer significantly elevated after leptin treatment. Most mRNA levels studied were not significantly altered by leptin administration.

**Table 3. Fractional TG synthesis (%) in adipose tissue**

<table>
<thead>
<tr>
<th>Component</th>
<th>Perimetric</th>
<th>Inguinal</th>
<th>Mesenteric</th>
<th>Retroperitoneal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>66±5a</td>
<td>62±4ad</td>
<td>70±7a</td>
<td>63±5a</td>
</tr>
<tr>
<td>ob/ob</td>
<td>43±6e</td>
<td>56±8ad</td>
<td>51±7a</td>
<td>47±8e</td>
</tr>
<tr>
<td>ob-r</td>
<td>66±2a</td>
<td>65±3a</td>
<td>69±5a</td>
<td>61±6a</td>
</tr>
<tr>
<td>ob-lep</td>
<td>25±3e</td>
<td>25±3e</td>
<td>26±5c</td>
<td>23±2e</td>
</tr>
</tbody>
</table>

Values are means ± SD; values not sharing a superscripted letter are significantly different within each column. TG, triglyceride.

**Table 4. Fractional DNL* synthesis (%) in adipose tissue**

<table>
<thead>
<tr>
<th>Component</th>
<th>Perimetric</th>
<th>Inguinal</th>
<th>Mesenteric</th>
<th>Retroperitoneal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>93±20a</td>
<td>92±11a</td>
<td>100±9a</td>
<td>87±13a</td>
</tr>
<tr>
<td>ob/ob</td>
<td>114±10ab</td>
<td>65±3b</td>
<td>103±10a</td>
<td>112±17b</td>
</tr>
<tr>
<td>ob-r</td>
<td>60±7b</td>
<td>37±6c</td>
<td>59±6b</td>
<td>57±6c</td>
</tr>
<tr>
<td>ob-lep</td>
<td>16±4b</td>
<td>14±2d</td>
<td>38±14c</td>
<td>24±6d</td>
</tr>
</tbody>
</table>

Values are means ± SD; values not sharing a superscript are significantly different within each column. DNL, de novo lipogenesis. *Calculated as measured fractional DNL / fractional TG synthesis.

**DISCUSSION**

Leptin provides a signal from the adipose tissue to the central nervous system and other organs that regulates energy intake, energy expenditure, and nutrient partitioning (11, 15–17). Here, we show in vivo that this endocrine signal completes a full circle back to the adipose tissue, inducing dramatic effects on adipose lipid dynamics and cell proliferation, independent of food intake or insulin concentrations. Of perhaps equal interest, the measured changes in flux through lipid anabolic pathways in ob/ob vs. lean control mice, or in response to leptin administration, were not generally reflected by adipose tissue mRNA levels.

Several areas of adipose metabolism were studied simultaneously, using heavy water labeling techniques recently developed (33, 39, 44). Previous studies (6, 14, 22) have shown increases in DNL in adipose tissue of ob/ob mice, with labeled water (D2O or H2O18) used to measure adipose tissue TG synthesis as well as DNL. However, a comprehensive evaluation of the integrated dynamics of different adipose tissue components in ob/ob mice in response to leptin administration or food restriction had not previously been carried out.

We found that leptin administration to ob/ob mice reduced rates of total TG synthesis (Fig. 2) and stimulated lipolysis (Fig. 4) in vivo, consistent with previous studies (12, 30, 45). Our data that clearly show that leptin deficiency, independent of hyperphagia or hyperinsulinemia, results in markedly increased lipogenic fluxes in ob/ob mice, consistent with reported in vitro (30) and in vivo (47) effects.

The long-term contribution from DNL to adipose tissue TG stores could be measured accurately because 3H2O can be administered easily for an extended period of time. This method cannot distinguish between adipose DNL and hepatic DNL as the initial site of biosynthesis but measures the accumulation in adipose tissue via either pathway. The measured DNL contribution to newly synthesized adipose TG-palmitate in lean controls as well as ob/ob mice was 70–100% (Table 1), a higher value than we previously estimated based on short-term labeling studies in rodents, which measured primarily hepatic DNL (24). DNL, therefore, makes a quantitatively substantial contribution to the accrual of adipose fat in rodents on chow diets, and this pathway is stimulated in the ob/ob mouse. In humans, by contrast, DNL contributes about 20% of newly synthesized adipose TG-palmitate (39).

Of note, leptin suppressed adipose TG synthesis and DNL without significantly reducing plasma insulin concentrations. The dose of leptin (2 µg/day) was selected to alter body weight, but not plasma insulin levels, and was based on the work of Harris et al. (17). The reduction in food intake with leptin treatment was also less than was achieved by food restriction.
restriction alone, but leptin administration reduced lipogenic fluxes to a much greater extent than was observed with food restriction. Thus, leptin deficiency, independent of hyperphagia or hyperinsulinemia, results in major alterations in flux through lipid anabolic pathways in adipose tissue.

In addition to the inhibition of adipose cell proliferation rates reported here, leptin has been shown to have inhibitory or stimulatory effects on the proliferation of numerous cell types. Proliferation and differentiation of hematopoietic cells (3, 13), promotion of angiogenesis and epithelial wound repair (38), and proliferation and suppression of apoptosis in pancreatic ß-cells (34, 41) have been reported. Leptin inhibits the differentiation of marrow stromal cells into adipocytes, consistent with our observations here (42).

The mature adipocyte-enriched fraction on which we measured cell proliferation may contain residual vascular-stromal cells. The values reported for adipogenesis should therefore be interpreted as reflecting adipose tissue as an organ rather than as exclusively mature adipocytes. Previous work (9) has demonstrated, however, that the stromal-vascular compartment of adipose tissue also expands in settings of adipose tissue lipid and cell accumulation.

![Fig. 3. Absolute rates of palmitate de novo lipogenesis (DNL; g palmitate synthesized over 21-day labeling period).](http://ajpendo.physiology.org/)

![Fig. 4. Net adipose tissue lipolysis (g TG/21 days).](http://ajpendo.physiology.org/)
A result of general interest is the comparison between directly measured metabolic flux rates through complex pathways and expression levels of related genes. Three previous reports have examined the expression of lipogenic genes in adipose tissue from obese humans (8) or ob/ob mice (32, 37). These studies reported a reduction in the expression of the genes controlling expression of lipogenic enzymes, namely SREBP-1c, and genes coding lipogenic enzymes (FAS, DGAT1, DGAT2).

Fig. 5. Absolute rates of adipose cell proliferation (cells/21 days). Adipose cell proliferation was calculated from fractional cell proliferation times number of cells/fat pad. Data are means ± SD; values not sharing a superscripted letter are significantly different within each column.

Fig. 6. Quantitative RT-PCR of lipogenic genes in C57BL/6 mice (control, n = 5), ob/ob mice (n = 5), and ob/ob mice administered leptin (n = 5). Total RNA was isolated from inguinal adipose tissue and reverse transcribed. Ten nanograms of cDNA were used in each PCR reaction. Relative mRNA levels for each gene were normalized to an internal standard, GAPDH, and are expressed as this ratio. Data are expressed as means ± SE. For each gene, 1-way repeated-measures ANOVA was used to test for significant differences between the 3 groups. If groups differed significantly, pairwise comparisons were performed and P values calculated using the Holm-Sidak method. Statistical analysis was done using SigmaStat 3.0. *P < 0.05 compared with control; †P < 0.01 compared with control; ‡P < 0.05 compared with ob/ob. GPAT, mitochondrial glycerol-3-phosphate acyltransferase; SREBP1, sterol regulatory element-binding protein-1; FAS, fatty acid synthase; DGAT1, diacylglycerol O-acyltransferase 1; DGAT2, diacylglycerol O-acyltransferase 2; GLUT4, solute carrier family 2 (facilitated glucose transporter), member 4; FABP4, adipocyte fatty acid-binding protein-4; PEPCK, cytosolic phosphoenolpyruvate carboxykinase 1; ACAT1, acetyl-CoA acetyltransferase 1; PPARγ, peroxisome proliferator-activated receptor-γ.
PEPCK, ATP citrate-lyase, pyruvate carboxylase). We surveyed genes involved in several aspects of lipid storage in adipose tissue. GPAT, DGAT1, DGAT2, and PEPCK are involved in the synthesis of glycerol phosphate and triacylglycerols; GLUT4 and FAS are essential in the uptake of glucose and conversion to TG; SREBP1 and PPARγ act as signals regulating several aspects of lipid biosynthesis and the differentiation of preadipocytes. We confirm that low or normal levels of mRNAs were present for most of these enzymes in ob/ob adipose tissue, and few effects were observed with leptin treatment (Fig. 6). The mRNA levels of FAS were significantly elevated in ob/ob mice but, paradoxically, were not suppressed by leptin treatment; in fact, message levels were further increased by leptin. Only expression of DGAT2 was elevated in ob/ob mice and reduced by leptin treatment, consistent with its recently described role in obesity and adipose metabolism (40). Thus, with some exceptions, the use of gene expression results by themselves to understand adipose tissue fluxes or to identify therapeutic targets would not have been reliable. Indeed, mRNA expression could be quite misleading if considered independent of flux analysis. The effects of leptin treatment, for example, might appear to be absent or even prolipogenic, whereas, in fact, leptin potently reduced measured flux rates through all the lipid anabolic pathways studied.

Straightforward interpretation of previous gene expression results led to the suggestion (31) that lipogenic and lipid storage capacities are reduced in ob/ob mice. Our direct measurements of fluxes clearly show that this is not the case. Essentially identical results were obtained from four different depots, and the metabolic alterations were normalized by administration of leptin but not by caloric restriction, as expected from previous literature concerning the effects of leptin on other metabolic alterations (27).

This disparity between mRNA levels and the flow of molecules in vivo though complex pathways is instructive (18, 19, 43). Considerable work from the field of metabolic engineering has led to the conclusion that, even in systems as simple as bacteria, manipulating gene expression does not typically alter metabolic flux distributions in a predictable manner within complex pathways and metabolic networks. Fischer and Sauer (10) recently reported, for example, that the vast majority of gene deletions have no effect on flux through central metabolic pathways in Bacillus subtilis, whereas those genes that altered one flux tended to alter many fluxes. Thus, a simple relationship between gene expression and metabolic flux should not be assumed in evolved metabolic networks, and caution must be used in extrapolating from gene expression to fluxes through the pathways for which they code.

The specific explanation for dissociation between mRNA levels and metabolic fluxes in the ob/ob mouse is not clear. For example, although mRNA levels are reduced, enzyme concentration or activity may not be decreased because of altered translation controls or changes in the catabolic rates of the enzymes. Alternatively, lipogenic enzymes may not be rate limiting but may be present in excess in vivo. Cofactors or the availability of substrates could be responsible for higher rates of synthesis through these pathways, independent of enzyme levels, or enzyme activities could be regulated allosterically. In a general sense, the multiple levels of potential flux control that exist beyond gene transcription give mRNA levels low predictive power for lipogenic and adipogenic fluxes in this model of obesity.

In summary, these results emphasize the general rule, often ignored, that gene expression data should be interpreted cautiously with regard to functional outputs of complex pathways (10, 18, 19, 43).

REFERENCES


