Fat oxidation, lipolysis, and free fatty acid cycling in obesity-prone and obesity-resistant rats

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1Department of Pediatrics, University of Colorado Health Sciences Center, Denver 80262; 2Department of Food Science and Human Nutrition, Colorado State University, Fort Collins, Colorado 80525; and 3Department of Exercise Science and Physical Education, Arizona State University, Tempe, Arizona 85287

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Commerford, S. Renee, Michael J. Pagliassotti, Christopher L. Melby, Yuren Wei, Ellis C. Gayles, and James O. Hill. Fat oxidation, lipolysis, and free fatty acid cycling in obesity-prone and obesity-resistant rats. Am J Physiol Endocrinol Metab 279: E875–E885, 2000.—Defects in fat metabolism may contribute to the development of obesity, but what these defects are and where they occur in the feeding/fasting cycle are unknown. In the present study, basal fat metabolism was characterized using a high-fat diet (HFD)-induced model of obesity development. Male rats consumed a HFD (45% fat, 35% carbohydrate) ad libitum for either 1 or 5 wk (HFD1 or HFD5). After 1 wk on the HFD, rats were separated on the basis of body weight gain into obesity-prone (OP, ≥48 g) or obesity-resistant (OR, ≤40 g) groups. Twenty-four-hour-fastated rats were studied either at this time (OP1, OR1) or after 5 wk (OP5, OR5). Fat pad weight (sum of epididymal, retroperitoneal, and mesenteric fat pads) at HFD1 was 26% greater and at HFD5 was 45% greater (P≤0.05) in OP vs. OR. Free fatty acid rates of appearance (FFA Ra) and oxidation were not significantly different between OP and OR at 1 or 5 wk. Glycerol Ra, when expressed in absolute terms (μmol/min), increased from 1 to 5 wk of HFD feeding in both OP and OR, but significantly so only in OP. Likewise, increased rates of intracellular FFA cycling (estimated as (3 × glycerol Ra) – FFA Ra) were observed in both OP and OR rats from 1 to 5 wk of HFD feeding, but significantly so in OP rats only. When expressed relative to fat cell volume (μmol·pl−1·min−1), neither lipolysis nor intracellular cycling was significantly different between OP and OR, regardless of time on HFD. These data suggest that 1) if low rates of fat oxidation contribute to obesity development in OP rats, the contribution does not occur at times when fat oxidation is at or near maximum rates (i.e., 24-h fasted conditions), and 2) between 1 and 5 wk of HFD feeding, basal lipolysis and reesterification may work to expand fat cell volume and increase fat pad weight in both OP and OR rats, although more so in OP rats.

fatty acid metabolism; reesterification; triglyceride cycling; high-fat diet

OBESITY IS CHARACTERIZED by increased fat stores, where energy intake exceeds energy expenditure. In rodents, a high-fat diet (HFD) promotes obesity through hyperphagia (9, 17, 18). However, even under conditions of isocaloric feeding, there exists a direct relationship between percent body fat and fat content of the diet (2). These data suggest that a HFD promotes nutrient partitioning in a way permissive to expansion of fat pad stores. Importantly, HFD-induced obesity occurs in some but not all rats. For example, after 1 wk of ad libitum HFD feeding, rats prone to obesity development (OP, top tertile of weight gain) eat more, gain more weight, and have a higher percent body fat than do rats resistant to obesity development (OR, lowest tertile of weight gain). By 5 wk of HFD, weekly weight gain in OP rats is only slightly greater than that observed in OR rats. Thus this model permits comparisons between rats that are developing obesity (i.e., OP rats after 1 wk of HFD) and rats in which the obese state is established (OP rats after 5 wks of HFD).

Data from several studies previously reported from our laboratory (10, 20) suggest that OP rats either respond differently to early exposure to the HFD relative to OR rats, or that, upon beginning the HFD feeding period, OP rats are metabolically distinct from OR rats. For instance, both lipoprotein lipase mRNA and activity were significantly greater in adipose tissue but were lower in skeletal muscle of OP rats after 1 and 2 wk of HFD feeding, but not after 5 wk of HFD (20). Likewise, skeletal muscle enzyme profiles of OP rats (e.g., the ratio between the maximal activities of phosphofructokinase to those of b-hydroxyacyl-CoA dehydrogenase) indicated an increased capacity for carbohydrate over fat oxidation at both 1 and 2 wk of HFD feeding, but not at 5 wk (10). These findings imply that, with early exposure to the HFD, OP rats would oxidize less fat under conditions of maximal fat oxidation. Although comparisons of 24-h respiratory quotient (RQ) have been made between OP and OR at 5 wk of HFD feeding (4), nothing is known regarding the ability of OP rats to oxidize fat early in their exposure to the HFD. We were therefore interested to learn...
whether, as implied by their enzymatic profiles, OP rats were characterized by a decreased capacity to oxidize fat under conditions of near-maximum fat oxidation (i.e., after a 24-h fast) at 1 wk but not at 5 wk of HFD.

Both fat pad weight and percent body fat are significantly greater in OP rats by 1 wk of HFD feeding. Fat stores expand when nutrient uptake by adipocytes exceeds their release. If lipolysis is lower relative to nutrient uptake, or if rates of reesterification exceed nutrient release, fat pad mass will increase. The mechanism by which an animal on a HFD partitions nutrients toward fat storage is presently unknown, but it may relate to impairments in insulin action. Lipolysis is very sensitive to the antilipolytic effects of insulin (14), so that even under basal conditions, insulin concentrations will have a significant effect on lipolysis. Some data in OP rats suggest that they are relatively more insulin resistant than are OR rats with longer-term HFD feeding, but not at 1 wk of HFD (4, 10, 20, 22). In addition to lipolysis, the partitioning of fatty acids between reesterification and oxidation will influence fat accumulation and therefore obesity development. As such, a second aim of the present investigation was to characterize rates of lipolysis and reesterification in rats prone and resistant to HFD-induced obesity under the same conditions in which fat oxidation was measured. In conjunction with these estimates, catecholamines and corticosterone concentrations were measured, because these two hormones can regulate fat metabolism.

METHODS

Experimental Animals

Male Crl(WI)BR rats (7 wk of age) were housed at the University of Colorado Health Sciences Center Animal Resource Center for either 3 or 7 wk. Rats weighed 135–180 g on arrival. Nine cohorts of between 16 and 50 animals per cohort were studied (total number of rats studied was 92). Rats were individually caged under controlled conditions (12:12-h light-dark cycle).

Diet Protocol

On arrival, animals were provided ad libitum access to a semi-purified low-fat diet (LFD; 12% fat, 68% carbohydrate, 20% protein; Research Diets, New Brunswick, NJ; Table 1) for 2 wk (baseline period). After the baseline period, rats either remained on the LFD or were switched to an HFD (45% fat, 35% carbohydrate, 20% protein; Research Diets; Table 1). Rats on the HFD were separated on the basis of body weight gain after 1 wk of ad libitum access into either OP (body weight gain ≥48 g) or OR (body wt gain ≤40 g) groups. These weight gain criteria were selected a priori to ensure that OP and OR rats were distinct with respect to weight gain and that the weight gain criteria used to identify OP and OR remained consistent across cohorts. Rats with weight gains between 41 and 47 g were not studied further (58.7% of total rats fed the HFD). Rats were studied either after 1 wk of HFD (OR1, OP1, and LFD1) or after 5 wk of HFD feeding (OR5, OP5, and LFD5). Throughout the protocol, food intake was measured three times per week, and body weight was recorded once per week.

Table 1. Diet composition

<table>
<thead>
<tr>
<th></th>
<th>LFD</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat: corn oil, %</td>
<td>12</td>
<td>45</td>
</tr>
<tr>
<td>Carbohydrate, %</td>
<td>68</td>
<td>35</td>
</tr>
<tr>
<td>Protein, %</td>
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<td>20</td>
</tr>
<tr>
<td>Casein</td>
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<tr>
<td>DL-Methionine</td>
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<td>0.37</td>
</tr>
<tr>
<td>Cornstarch</td>
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<td>20</td>
</tr>
<tr>
<td>Maltodextrin 10</td>
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<td>18.5</td>
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<tr>
<td>Cellulose</td>
<td>5.0</td>
<td>6.15</td>
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<tr>
<td>Corn oil</td>
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<td>Salt mix</td>
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<td>4.3</td>
</tr>
<tr>
<td>Vitamin mix</td>
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<td>1.2</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Composition is given for the low-fat diet (LFD), with a metabolizable energy of 4.23 kcal/g, and the high-fat diet (HFD), with a metabolizable energy of 5.22 kcal/g. Maltodextrin 10 is enzyme-converted cornstarch with a dextrose equivalent of 10%. Salt and vitamin mix are prepared according to the American Institute of Nutrition guidelines (24).

Animal Preparation

After either 1 or 5 wk of HFD feeding and several days before an animal’s day of study, catheters were implanted into the jugular vein (tracer infusions) and carotid artery (blood sampling), with animals under general anesthesia as previously described (23). Rats were allowed to recover ≥4 days. During recovery, rats ate their respective diets ad libitum. For a rat to be studied, body weight was required to be ≥92.5% of presurgery weight. Rats not meeting this weight by 7 days postsurgery were not studied.

Experimental Protocol

At 0900 on the morning before the day of study, body weight and the previous day’s food intake were measured, and all food was removed. Studies commenced between 0900 and 1200 of the following morning (fasted conditions). Because fuel stores present within a tissue can potentially impact upon the substrate mixture used to meet energy needs, we chose to fast rats for 24 h, because this length of fast reduces liver glycogen to minimal concentrations and equates skeletal muscle glycogen in OP and OR rats (21). On the morning of the study day, extensions were placed onto exteriorized catheters to allow easy access without disturbing animals. Before the tracer infusion was initiated, an arterial blood sample was drawn, rats were placed into a metabolic chamber (airflow = 1.0 l/min), and a preinfusion respiratory gas sample was collected. An arterial infusion of normal saline (no heparin) was then initiated to maintain arterial line patency.

[1-14C]Palmitate (0.05 μCi/min) and [2-3H]glycerol (0.10 μCi/min; NEN Life Science Products, Boston, MA), re suspended in 22% bovine serum albumin (Sigma, St. Louis, MO), were used to estimate plasma free fatty acid (FFA) turnover, plasma FFA oxidation, and whole body lipolysis. Data in a subset of rats (those fed either chow or HFD, n = 6), which compared the method described here [liquid scintillation counting (LSC) method] to estimate FFA Ra with that in which plasma palmitate was directly determined (HPLC method), indicated that the two methods were comparable (6.0 ± 1.7 mg·kg⁻¹·min⁻¹ for the LSC method vs. 6.7 ± 1.8 mg·kg⁻¹·min⁻¹ for the HPLC method; p² = 0.995). Tracers were infused via the jugular vein catheter for 140 min. At 120, 130, and 140 min of tracer infusion, arterial blood...
samples were collected concurrently with respiratory gases and expired \(^{14}\text{CO}_2\) samples. At \(t = 140\), pentobarbital sodium (50 mg/kg) was administered, and epididymal, retroperitoneal, and mesenteric fat pads were taken and immediately weighed. Portions of each pad were placed in Krebs Ringer phosphate (KRP) buffer for determination of fat cell size and number, which were determined within 6 h of tissue removal. Urine was removed from the bladder by use of a tuberculin syringe and combined with urine collected throughout the infusion period, and total urine volume was recorded.

**Determination of Acetate Correction Factor**

In separate studies, \([1-^{14}\text{C}]\) acetate was used to estimate an acetate correction factor for each group (necessary for calculation of plasma FFA oxidation; Ref. 25). \([1-^{14}\text{C}]\) acetate reconstituted with 0.9% normal saline was infused into the jugular vein at a rate of 0.05 \(\mu\text{Ci}/\text{min}\) for 140 min. Rats were maintained in metabolic chambers (airflow = 1.0 l/min) immediately before the initiation of the constant infusion and at 120, 130, and 140 min after the start of infusion. No blood samples were taken in these studies.

**Analytical Procedures**

**Blood and urine.** Blood samples were drawn into heparinized syringes. For glycerol, 50 \(\mu\)l of whole blood were deproteinized (26). The supernatant was used to determine glycerol concentrations (30) and \(^{3}\text{H}\) glycerol, after having been taken to dryness to drive off any \(^{3}\text{H}_2\text{O}\). The remaining blood was immediately centrifuged, and the plasma was used to determine circulating substrates, hormones, and \(^{14}\text{C}\)-labeled FFA. Plasma glucose concentration was determined using a Beckman glucose analyzer (Fullerton, CA). Plasma FFA was determined spectrophotometrically (Wako NEFA-C kit, Richmond, VA). Insulin (Linco Research, St. Charles, MO) and corticosterone (Coat-a-Cunt, Diagnostics Products, Los Angeles, CA) were determined by radioimmunoassay. Catecholamines were stored at \(-70^\circ\text{C}\) with reduced glutathione (5 mM) until analysis. Analysis was performed by HPLC as previously described (12). Urinary nitrogen corrected for ammonia was determined spectrophotometrically (Sigma kit no. 640-A). Determination of \(^{14}\text{C}\)-FFAs was made from extracts of plasma according to Dole (7), as modified by Trout (28). Briefly, an extraction mixture (1.0 ml of 40:10:1 isopropl alcohol-heptane-1.0 N \(\text{H}_2\text{SO}_4\)) was added to 200 \(\mu\)l of plasma and vortexed. Heptane (600 \(\mu\)l) and distilled, deionized \(\text{H}_2\text{O}\) (400 \(\mu\)l) were added, and each sample was vortexed for \(\geq 2\) min. Each sample was vortexed for 5 min and centrifuged at 1,350 rpm for 5 min, and the top layer was again removed and transferred to a glass scintillation vial. Titration mixture (100 \(\mu\)l, 0.01% thymol blue in 95% ethanol) was added, and the solution was titrated with 0.018 N \(\text{NaOH}\) (made fresh daily) to titration end point. The resulting extract was evaporated to dryness with a low-pressure stream of \(\text{N}_2\) gas and was reconstituted with 0.9% saline. Scintillation cocktail was added, the samples were allowed to sit overnight, and radioactivity was counted on the following day (LSC6500, Beckman, Fullerton, CA). Plasma standards spiked with a known quantity of \([1-^{14}\text{C}]\) palmitate were extracted in duplicate along with samples, and counts were compared with those attained from an aliquot of nonextracted spiked plasma to determine percent recovery. Percent recovery of radioactivity from plasma standards averaged 87.2%. Duplicity between labeled plasma standards (relative percent difference) never exceeded 7.2%, and the day-to-day coefficient of variation for percent recovery was 6.0%.

**Respiratory gases.** Respiratory gases were collected into modified Douglas bags for the measurement of \(\text{O}_2\) consumption (\(\text{VO}_2\)) and \(\text{CO}_2\) production (\(\text{VCO}_2\)) between 110 and 113, 120 and 123, and 130 and 133 min. Samples were drawn at a rate of 1.0 l/min through \(\text{O}_2\) and \(\text{CO}_2\) analyzers (Ametek, Pittsburg, PA), and the deviation from atmospheric air was recorded.

\(^{14}\text{CO}_2\) production was determined by trapping expired gases in a solution of 2:1 benzathionium hydroxide-methanol over a 5-min period. One-milliliter aliquots were counted (Beckman LS6500) in triplicate to determine the rate of \(^{14}\text{CO}_2\) appearance in expired air.

**Fat cell volume and number.** Fat cell size and number were determined by methods previously described (6). Fat cell diameter was determined under a microscope after cells had been digested for 10 min in collagenase (2 mg of collagenase/ml KRP buffer) at 37°C (9). Methylene blue was used to stain the cell membrane.

**Calculations**

**Glycerol \(R_a\) and FFA turnover.** Glycerol \(R_a\) and FFA \(R_a\) were calculated using the steady-state equation of Steele (27). FFA \(R_a\) was calculated using \([^{14}\text{C}]\) palmitate concentration (dpm/ml) relative to total FFA concentration (\(\mu\text{mol/ml}\)) as follows

\[
\text{FFA } R_a = \frac{\text{rate of } [^{14}\text{C}] \text{ palmitate infusion (dpm/min)}}{^{14}\text{C} \text{ of palmitate (dpm/ml)}/\text{FFA concentration (\(\mu\text{mol/ml}\))}}
\]

The denominator in this equation corrects for the fact that palmitate comprises some unknown fraction of the total plasma FFA pool, and thus adjusts, by direct proportion, \(R_a\) to \(R_a\). This method of determining \(R_a\) has been used by others (1). Specific activity for both glycerol and FFA was calculated using a rolling average across \(t = 120, 130,\) and 140 min. Glycerol \(R_a\) was normalized to mean fat cell number (10^6 fat cells) and mean cell volume.

**Whole body fat oxidation by indirect calorimetry.** After correction to STPD (standard temperature and pressure, dry), \(\text{VO}_2\) and \(\text{VCO}_2\) were calculated using a rolling average over the three time points collected to give a single value for each. The mean value for both \(\text{VO}_2\) and \(\text{VCO}_2\) (ml/min) was extrapolated to 140 min to determine total \(\text{VO}_2\) and \(\text{VCO}_2\) over the course of the experiment. Energy expenditure and substrate oxidation after correction for protein oxidation (6.25 x g of urinary nitrogen; see Ref. 16) were calculated from \(\text{VO}_2\), and the nonprotein RQ (npRQ) was calculated using equations previously described (8, 16).

**Fat cell volume and number.** Fat cell volume was calculated using the relationship between diameter and volume: 4.19 \((\text{diameter}^2/2)^3\). The number of fat cells per gram of tissue was calculated using cell volume and the density of lipid (1 cell/mean cell volume in pl) \(\times 0.95 \text{ ng lipid} \times (10^6)\) = cell/g lipid

Fat cell number within a given tissue was calculated using the total mass of tissue times the number of cells per gram of tissue. Fat cell number was also determined on a subset of animals from each group \((n = 5)/\text{group for each of epididymal, retroperitoneal, and mesenteric fat pads}\) after total lipid content was determined. This value was then applied to calculate fat cell number as follows
(fat pad weight in g) \cdot (\% lipid content) \cdot (10^6)

= \mu g lipid/fat pad
cell volume (pL/cell) \cdot (0.95 ng lipid/pl) \cdot (10^{-9}) = \mu g lipid/cell

Fat cell number was calculated by dividing microgram of lipid per cell by microgram of lipid per fat pad.

Acetate correction factor. An acetate correction factor was calculated in a subset of rats \((n = 4/group)\) as the \(R_s\) of expired \(^{14}\)CO\(_2\) divided by the rate of infusion of \([1-^{14}C]\)acetate. The value used for expired \(^{14}\)CO\(_2\) (dpm/min) was a rolling average of values collected at 120, 130, and 140 min of the \([1-^{14}C]\)acetate infusion.

Plasma FFA oxidation. The rate of plasma palmitate oxidation was calculated as follows

\[
\text{plasma palmitate oxidation} = \text{(expired }^{14}\text{CO}_2 \text{ dpm/min)} / \left[ \text{[1-}^{14}\text{C]palmitate infusion rate (dpm/min)} \right] \times \text{FFA } R_s
\]

Palmitate oxidation was corrected to total FFA oxidation by accounting for the proportion of the plasma FFA pool that is comprised of palmitate (44% in chow-fed rats, Ref. 11). The acetate correction factor was applied to account for the underestimate of \(^{14}\)CO\(_2\) production from the oxidation of \([1-^{14}C]\)palmitate due to label fixation (25).

Intracellular and extracellular triglyceride cycling. Triacylglyceride cycling, in which FFAs are hydrolyzed from glycerol and then reesterified to triglyceride, can occur in vivo (3, 31). Triglyceride cycling can be divided into intracellular and extracellular cycling. Intracellular cycling, defined as hydrolysis of FFAs from glycerol that are then reesterified within the adipocyte without exiting the cell, was calculated as

\[
\text{intracellular cycling} = 3(\text{glycerol } R_s) - \text{FFA } R_s
\]

Extracellular cycling, defined as the hydrolysis of FFAs from glycerol that are then released from the adipocyte and are subsequently taken back up by the fat cell and reesterified, was calculated as

\[
\text{extracellular cycling} = \text{FFA } R_s - \text{plasma FFA oxidation}
\]

Data analyses. One-way ANOVA was used to detect differences across groups. When necessary, multiple comparison tests were made using the method of Tukey (29). Two-way ANOVA was used to determine the significance of any group-by-time interactions, with linear contrasts used to determine significant differences among groups. One-way ANOVA was used to compare tracer-determined (with and without the acetate correction factor) and indirect calorimetry-determined rates of fat oxidation, with method as the dependent variable. For all comparisons, significance was set at \(P \leq 0.05\). For the LFD1 group, one animal was an outlier for all processes, as estimated from \(^{13}\)C kinetics, and thus any analyses of variables determined from \(^{14}\)C were made with only 9 animals in the LFD1 group. For all other analyses, this animal was included. Data for insulin and intracellular cycling were log-transformed to achieve homogeneity of variance.

RESULTS

Body Weight, Energy Intake, and Body Weight Gain

Rats entered the study with body weights that were not significantly different (Table 2). During the baseline period, energy intake, body weight gain, and body weight were significantly greater in OP than in OR (Table 2). Of note, over the 5 wk of HFD, cumulative energy intake by OP5 rats exceeded that in OR5 rats by only 11%, but body weight gain in OP5 rats was 24% greater than that observed in OR5 rats.

| Table 2. Energy intake, body weight gain, body weight, fat pad weight, fat cell size, and fat cell number |
|---------------------------------|---------------------------------|---------------------------------|
|                                | 1-wk Rats                        | 5-wk Rats                        |
|                                | OR1 \((n = 11)\)                 | OP1 \((n = 12)\)                 | LFD1 \((n = 10)\)                 |
|                                | OR5 \((n = 10)\)                 | OP5 \((n = 14)\)                 | LFD5 \((n = 11)\)                 |
| Initial body wt, g             | 167.8 ± 6.4^a                    | 171.5 ± 4.5^a                    | 158.3 ± 6.6^a                     |
| Baseline period                |                                 |                                 |                                 |
| EI, kcal                       | 1356.9 ± 39.3^a                  | 1332.9 ± 29.2^a                  | 1388.6 ± 49.1^a                   |
| Body wt gain, g                | 117.7 ± 4.0^a                    | 119.7 ± 4.9^a                    | 133.9 ± 8.4^a                     |
| Body wt, g                     | 285.5 ± 4.3^a                    | 291.2 ± 4.3^a                    | 292.2 ± 8.0^a                     |
| First week of HFD              |                                 |                                 |                                 |
| EI, kcal                       | 774.4 ± 14.3^a                   | 870.0 ± 12.2^a                   | 765.0 ± 33.8^a                    |
| Body wt gain, g                | 33.9 ± 1.2^abd                    | 53.1 ± 1.7^b                     | 43.3 ± 2.9^a                      |
| Body wt, g                     | 319.5 ± 4.0^a                    | 344.3 ± 4.2^a                    | 335.5 ± 9.6^ab                    |
| Cumulative HFD period          |                                 |                                 |                                 |
| EI, kcal                       | NA                               | NA                               | 4043.9 ± 99.0^b                   |
| Body wt gain, g                | NA                               | NA                               | 4888.0 ± 87.0^a                   |
| Body wt, g                     | NA                               | NA                               | 3651.5 ± 76.2^b                    |
| Fat pad                        |                                 |                                 |                                 |
| Sum of 3 pat pads, g           | 12.4 ± 1.1^c                     | 15.6 ± 0.8^c                     | 12.9 ± 1.3^c                      |
| Fat cell volume, pl            | 259.9 ± 43.7^a                   | 246.2 ± 25.7^a                   | 241.7 ± 36.0^a                    |
| Fat cell number, \(\times 10^6\) | 22.1 ± 2.8^ab                    | 24.6 ± 1.6^ab                    | 20.6 ± 1.5^b                      |

Values are means ± SE. OP, obesity prone; OR, obesity resistant; LFD, low fat diet control animals; NA, not applicable; EI, energy intake; sum of 3 pat pads is the combined weight of epididymal, retroperitoneal, and mesenteric fat pad weights. Baseline period includes 2 wk of LFD feeding. Cumulative HFD period includes either 1 wk of HFD for OR1 and OP1 or 5 wk of HFD for OR5 and OP5. LFD1 and LFD5 rats consumed LFD during this time. Superscripts are derived from Tukey’s post hoc comparisons. Means within a row that share common superscripts were not significantly different \((P \leq 0.05)\).
Energy Expenditure and RQ

Energy expenditure and npRQ were not significantly different across groups (pooled average for energy expenditure: 0.037 ± 0.002 kcal/min; pooled average for npRQ: 0.723 ± 0.011, respectively). Energy expenditure, normalized to body weight, was not significantly different among 1-wk rats (pooled 1-wk average: 0.12 ± 0.006 kcal·kg⁻¹·min⁻¹) or among 5-wk rats (pooled 5 wk average: 0.093 ± 0.004 kcal·kg⁻¹·min⁻¹).

Circulating Substrates, Urinary Nitrogen, and Plasma Hormones

Glycerol concentrations were significantly higher in OP5 compared with LFD5 rats (Table 3). Glucose concentrations were significantly lower in OP5 rats relative to LFD5 rats (Table 3). Norepinephrine concentrations were significantly higher in OR1 rats compared with LFD1 rats (Table 3). No other significant differences existed in substrates, hormones, or urinary nitrogen among groups (Table 3).

Glycerol $R_a$

By 120 min of infusion, glycerol specific activity had reached steady state in both 1-wk and 5-wk rats (Fig. 1). Glycerol $R_a$ (μmol/min) was not significantly different among 1-wk rats (Fig. 2A) but was significantly greater in both OP5 and OR5 relative to LFD5. Glycerol $R_a$ was increased twofold in OP5 vs. OR1 (7.3 ± 0.4 vs. 3.6 ± 0.4 μmol/min), whereas it was only 38% greater in OR5 compared with OR1 (5.8 ± 0.8 vs. 4.2 ± 0.4 μmol/min). Similar results were obtained when glycerol $R_a$ was expressed relative to fat cell number (μmol·10⁶ cells⁻¹·min⁻¹; Fig. 2B). When glycerol $R_a$ was expressed relative to fat cell volume (Fig. 2C), no significant differences were detected among groups.

Acetate Correction Factor

Initial body weight, energy intake, body weight gain, body weight, and fat pad weights were not different for rats used to determine an acetate correction factor from values for rats used in studies in which fat oxi-
dation was determined (data not shown). The rate of
$^{14}$CO$_2$ production (dpm/min) for both 1-wk and 5-wk
animals was in steady state by 120 min of the
[1-$^{14}$C]acetate infusion (Fig. 3). No significant differ-
ences in the acetate correction factor were found
among groups (Table 4).

**Plasma FFA Kinetics and Plasma FFA Oxidation**

Steady state was reached for plasma FFA specific
activity by 120 min of infusion in both 1-wk and 5-wk
rats (Fig. 4). When expressed in absolute terms
($\mu$mol/min, Fig. 5A) or relative to body weight
($\mu$mol·kg$^{-1}$·min$^{-1}$, Fig. 5B), FFA $R_a$ was not signifi-
cantly different between 1-wk and 5-wk rats.

The rate of $^{14}$CO$_2$ production (dpm/min) for both
1-wk and 5-wk animals was in steady state by 120 min
(Fig. 4B). The only significant differences in rates of
plasma FFA oxidation were observed when expressed
relative to body weight ($\mu$mol·kg$^{-1}$·min$^{-1}$). Rates were
significantly lower in OP5 and LFD5 vs. OP1 and
LFD1, respectively (Fig. 5B).

**FFA Reesterification and Triglyceride Cycling**

Reesterification (ratio of FFA $R_a$ to glycerol $R_a$) was
significantly greater in OP5 vs. LFD5 only ($P \leq 0.05$).

Intracellular cycling ($\mu$mol/min, $\mu$mol·$10^6$ cell$^{-1}$·min$^{-1}$
and $\mu$mol·pl$^{-1}$·min$^{-1}$) was not significantly different
among 1-wk rats but was significantly higher in both
OP5 and OR5 compared with LFD5 rats when expressed
as $\mu$mol/min (Table 5). When normalized to
either fat cell number or fat cell volume, however, only
OP5 rats demonstrated significantly higher rates of
intracellular cycling relative to LFD5 ($P \leq 0.05$). Ex-
tracellular cycling rates were negative in both OP1 and
LFD1 groups (significantly different from zero, $P = 0.0007$
and $P = 0.0018$, respectively) but were not for
the remaining groups. Because the negative extracel-
lular cycling rates were the result of either an under-
estimation of FFA $R_a$ or an overestimation of plasma
FFA oxidation, and thus not representative of a true
rate, statistical comparisons are not reported.

**Comparison of Tracer-Determined and Indirect
Calorimetry-Determined Rates of Fat Oxidation**

With no application of the acetate correction factor,
and with the assumption that palmitate comprises 44%
of the total FFA pool (11), tracer-estimated rates of fat
oxidation were significantly lower than rates deter-
mined by indirect calorimetry, regardless of group (Ta-
ble 6). With the application of the acetate correction

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**Fig. 2. Glycerol rate of appearance ($R_a$)
expressed in $\mu$mol/min (A), relative to
fat cell number (B), and relative to fat
cell volume (C). Values reported are
means ± SE of samples taken at 120,
130, and 140 min of infusion. Letters
above bars are derived from Tukey’s
post hoc comparisons; values of bars
sharing common letters are not signifi-
cantly different ($P \leq 0.05$). *Glycerol $R_a$
expressed relative to $10^6$ cells was log-
arithmically transformed. Data are
means and SE without transformation.
factor (Table 4), rates of fat oxidation were no longer significantly different from rates determined by indirect calorimetry in some (OR1 and LFD1) but not all groups. When data were pooled, tracer-determined rates of fat oxidation left uncorrected were significantly lower than both corrected rates and rates determined by indirect calorimetry. Likewise, with the data pooled, corrected rates determined by tracer methods were significantly lower than rates estimated by indirect calorimetry.

DISCUSSION

General Findings

Previous data from our laboratory suggested that, compared with OR rats, the 24-h RQ was higher in OP rats fed a HFD for 4–8 wk (4). More recent data demonstrated the “daytime RQ,” measured at a time when rats eat very little and thus representative of a fasted RQ, was also significantly higher in OP rats (19). However, no comparisons of in vivo plasma FFA oxidation have been made between rats earlier in their exposure to a HFD, a time when obesity development in this model is at its most accelerated rate. Thus the primary purpose of this investigation was to determine whether fasted rates of fat oxidation, lipolysis, or reesterification contributed to the development of obesity.

Fat Oxidation

In the 24-h-fasted state, OP1 rats oxidized fat at rates no different from OR1 rats (Fig. 5). It can be argued that the 24-h fast used in the present study maximized fat oxidation in both OP and OR rats.

Table 4. Acetate correction factor

<table>
<thead>
<tr>
<th></th>
<th>1 wk Rats</th>
<th>5 wk Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR</td>
<td>0.72 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.73 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>OP</td>
<td>0.72 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.73 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LFD control</td>
<td>0.66 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.75 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SE; <i>n</i> = 4 for all groups. Acetate correction factor reflects the fraction of infused [1-<sup>14</sup>C]acetate recovered as expired <sup>14</sup>CO<sub>2</sub>. Superscripts are derived from Tukey’s post hoc comparisons. Means that share common superscripts are not significantly different (<i>P</i> ≤ 0.05).
masking any differences between them. However, we have previously demonstrated that the ratio between the maximum activities of phosphofructokinase and \( \beta \)-hydroxyacyl-CoA dehydrogenase in skeletal muscle was significantly higher in OP compared with OR rats after 1 and 2 wk of HFD feeding, suggesting that OP rats might have a lower capacity to oxidize fat relative to carbohydrate compared with OR rats. Data presented here suggest that fat oxidation in the fasted state does not contribute to differences in weight gain or fat gain between OP and OR rats.

Two independent methods were used to estimate fat oxidation. Comparison of the results from these two methods yielded several important findings (Table 6). First, as previously reported (25), when corrected for \( ^{14} \text{C} \) label fixation, tracer methods of plasma FFA oxidation better matched indirect calorimetry determinations of whole body fat oxidation [rates of fat oxidation

### Table 5. Plasma FFA kinetics

<table>
<thead>
<tr>
<th></th>
<th>1-wk Rats</th>
<th>5-wk Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR1, ( n = 11 )</td>
<td>OP1, ( n = 12 )</td>
</tr>
<tr>
<td>FFA ( R_a )/glycerol ( R_g )</td>
<td>( 2.53 \pm 0.29^{ac} )</td>
<td>( 2.38 \pm 0.22^{ac} )</td>
</tr>
<tr>
<td>TG cycling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intracellular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \mu \text{mol/min} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \mu \text{mol\cdot10^6 \text{ cells}^{-1} \cdot \text{min}^{-1}} )</td>
<td>( 2.37 \pm 1.1^{bc} )</td>
<td>( 2.81 \pm 0.96^{bc} )</td>
</tr>
<tr>
<td>Extracellular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \mu \text{mol/min} )</td>
<td>0.21 \pm 1.0</td>
<td>( -2.97 \pm 0.43 )</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE. FFA \( R_a \)/glycerol \( R_g \), ratio of FFA appearance rate (\( R_a \)) to glycerol \( R_g \); TG, triglyceride. Superscripts are derived from Tukey’s post hoc comparisons. Means within a row that share common superscripts are not significantly different (\( P \geq 0.05 \)). \( ^a \)Because of one outlier in regard to plasma \( ^{14} \text{C} \) kinetics, \( n = 9 \) for LFD1 group. \( ^{bc} \)Intracellular cycling rates expressed relative to \( 10^6 \) cells were logarithmically transformed. Data are means and SE without transformation. \( ^{ac} \)Intracellular cycling rates expressed as \( \mu \text{mol\cdotpl}^{-1} \cdot \text{min}^{-1} \) have had a factor of \( 10^{-2} \) applied.
Comparison of tracer- and indirect calorimetry-determined fat oxidation

|                  | 1-wk Rats | | | 5-wk Rats | | | Total | | |
|------------------|-----------| | |-----------| | |-------| | |
|                  | OR1, n = 11 | OR1, n = 12 | LFD1, n = 9 | OR5, n = 10 | OR5, n = 14 | LFD5, n = 11 | n = 67 |
| Tracer determined | | | | | | | |
| No correction    | 1.7 ± 0.17† | 1.9 ± 0.13‡ | 1.7 ± 0.10†‡ | 1.7 ± 0.19† | 1.8 ± 0.17†‡ | 1.4 ± 0.15† | 1.7 ± 0.06‡ |
| With acetate correction | 2.4 ± 0.24 | 2.7 ± 0.18‡ | 2.5 ± 0.15 | 2.3 ± 0.26† | 2.5 ± 0.23† | 1.9 ± 0.21† | 2.4 ± 0.09† |
| Indirect calorimetry determined | 3.0 ± 0.27 | 3.5 ± 0.20 | 2.9 ± 0.18 | 3.4 ± 0.35 | 3.5 ± 0.19 | 3.2 ± 0.21 | 3.2 ± 0.09 |

Values are means ± SE in mg/min. Superscripts are derived from Tukey’s post hoc comparisons. Means within a row that share common superscripts are not significantly different (P ≤ 0.05). Because of one outlier in regard to plasma 14C kinetics, *n = 9 for LFD1 group. †Significantly different from rates determined by indirect calorimetry; ‡significantly different from tracer-determined method with the acetate correction factor applied.

It has been previously reported in humans that tracer estimates of fat oxidation remain slightly lower than values measured by indirect calorimetry, even when corrected for label fixation as performed here (25). The difference between the two methods could be due to oxidation of intratissue triglyceride stores, which would be included in indirect calorimetry measures but not captured by tracer methods. Conversely, in this study, we assumed that palmitate comprised 44% of the total FFA pool (11). It may be that, in our animals, palmitate comprised some lesser percentage of the total FFA pool. To equate the two methods, palmitate would have had to have occupied 33% of the total FFA pool, with no oxidation of intratissue triglyceride stores assumed.

It should be noted that the so-called “VA-mode” of infusion/sampling was used to estimate rates of appearance and oxidation. This method will result in lower rates compared with the “AV-mode” of infusion/sampling. It is unlikely, however, that the choice of infusion/sampling sites will significantly impact comparisons between groups (15). There were two groups (OP1 and LFD1) in which rates of plasma FFA oxidation exceeded plasma FFA Ra, because, in these two groups, either plasma FFA oxidation was overestimated or plasma FFA Ra was underestimated. It is worth noting that, when compared in total (n = 67), rates of FFA appearance were nearly equal to rates of plasma FFA oxidation (9.5 ± 0.5 vs. 9.9 ± 0.4 μmol/min).

Basal Lipolysis and Reesterification

The greater fat pad weight in OP5 rats was due largely to increased fat cell volume (Table 2) and did not appear to relate to differences in basal concentration of catecholamines, corticosterone, or insulin (Table 3). This would predict that reesterification was in excess of lipolysis in OP rats relative to OR rats. Regardless of how expressed, lipolysis (estimated using glycerol Ra) was not significantly different between OP and OR rats (Fig. 2, A-C). Thus reduced basal lipolysis, per se, does not contribute to the expansion of fat cell volume and fat pad weight in OP rats. However, whereas glycerol Ra increased in both OP and OR rats from 1 to 5 wk (significantly so in OP), FFA Ra did not (Fig. 5A). Taken together, these data suggest that, relative to the 1-wk rats, a greater proportion of the FFAs derived from lipolysis subsequently underwent reesterification within the adipocyte in the 5-wk rats (measured either as the ratio between FFA Ra and glycerol Ra or as intracellular cycling). Indeed, the significantly greater increase in lipolysis from 1 to 5 wk in OP rats relative to OR rats was accompanied by a significantly greater increase in intracellular triglyceride cycling in OP rats (Fig. 2A and Table 5).

Recent data suggest that glycerol Ra may not accurately represent adipose tissue lipolysis (5, 13). If so, we have overestimated adipose tissue lipolysis in both OP and OR rats. However, our conclusion that a greater mismatch between fasted adipose tissue lipolysis and reesterification contributed to or was a consequence of obesity development in OP rats is still reasonable, because it was based on a greater increase in glycerol Ra from 1 to 5 wk in OP rats relative to OR rats. Only if the excess glycerol Ra observed in OP5 rats originated from tissues other than adipose tissue or from circulating very low-density lipoprotein triglyceride would this conclusion be in error.

The mechanism(s) that might permit rates of reesterification to increase to a greater extent in OP rats relative to OR rats from 1 to 5 wk of HF diet cannot be delineated from the data presented here. The greater increase in absolute rates of lipolysis between 1 and 5 wk in OP rats would be a likely source for intracellular FFAs. The greater increase in intracellular reesterification implies that within adipocytes of OP rats, there is either greater availability of 1) glycerol-3-phosphate resulting from an increased glucose uptake and subsequent metabolism to glycerol-3-phosphate, or 2) mono- or diacylglycerides for free fatty acid reesterification. Future work is required to establish the
physiological role of increased reesterification in this model of obesity development. The differences described in this study between OP and OR rats were measured under 24-h-fasted conditions and do not reflect a typical metabolic setting in either group of rats. Knowing whether or not lipolysis and reesterification differ between OP and OR rats under insulin-stimulated conditions requires additional work, although fasting insulin concentrations measured here suggest that whole body insulin action was not significantly different between OP and OR rats (Table 3). Adipose tissue metabolism and insulin action have not been well characterized in this model. If a mismatch between lipolysis and reesterification under insulin-stimulated conditions is contributing to obesity development, we would predict that adipose tissue of OP1 rats would be more sensitive to insulin action than would the adipose tissue of OR1 rats.

The rates of extracellular reesterification reported in Table 5 for OP1 and LFD1 rats were significantly less than zero ($P = 0.0007$ for OP1 and $P = 0.0018$ for LFD1). Because extracellular reesterification was calculated as the difference between FFA $R_a$ and plasma FFA oxidation, the negative rates were the result of either an underestimation of FFA $R_a$ or an overestimation of plasma FFA oxidation in these two groups. As such, we presented the means ± SE for extracellular cycling, but because the values could not be reflective of any physiological process, we chose not to make any statistical comparisons. If anything, these data suggest that whole body extracellular reesterification is essentially zero under fasted conditions. In conclusion, there are several important findings from this study. First, fasted rates of fat oxidation were not impaired in OP rats. Thus the higher 24-h RQ (4) and higher daytime RQ (19) reported previously for OP rats must be driven by processes other than low fasted rates of fat oxidation. Second, basal rates of lipolysis were not significantly different between OP and OR rats, and with 5 wk of HFD, lipolytic rates actually increased in both OP and OR rats, although significantly so only in OP rats. At the same time, intracellular reesterification increased as well. Taken together, these findings implicate an earlier or greater increase in intracellular reesterification relative to lipolysis with HFD feeding to be one process contributing to the larger fat cell volume, and thus fat pad mass, in OP rats.

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