Inherent capacity for lipogenesis or dietary fat retention is not increased in obesity-prone rats

S. RENEE COMMERFORD,†–3 MICHAEL J. PAGLIASSOTTI,†,3 CHRISTOPHER L. MELBY,2 YUREN WEI,† AND JAMES O. HILL†
1 Department 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 3Exercise Science Research Institute, Arizona State University, Tempe, Arizona 85287


Obesity results from positive energy balance and, perhaps, abnormalities in lipid and glycogen metabolism. The purpose of this study was to determine whether differences in lipogenesis, retention of dietary fat, and/or glycogenesis influenced susceptibility to dietary obesity. After 1 wk of free access to a high-fat diet (HFD; 45% fat by energy) rats were separated on the basis of 1 wk body weight gain into obesity-prone (OP; ≥ 48 g) or obesity-resistant groups (OR; ≤ 40 g). Rats were either studied at this time (OR1, OP1) or continued on the HFD for an additional 4 wk (OR5, OP5).

Weight gain and energy intake were greater (P ≤ 0.05) in OP vs. OR at both 1 (53 ± 2 vs. 34 ± 1 g; 892 ± 27 vs. 755 ± 14 kcal) and 5 (208 ± 7 vs. 170 ± 7 g; 4,484 ± 82 vs. 4,008 ± 72 kcal) wk, respectively. Rats were injected with 3H2O and were either provided free access to an HFD meal containing labeled fatty acids (fed; n = 10 or 11/group) or were fasted (n = 10/group) overnight. The amount of food or 14C tracer eaten overnight was equivalent between OP and OR rats. In liver, the fraction of 3H retained in glycogen or lipoprotein was not significantly different between OP and OR groups. Retention of dietary fat in the liver was not increased in OP rats. In adipose tissue, retention of 3H was ~49% greater (P ≤ 0.05) in OP1 vs. OR1 and ~30% greater in OP5 vs. OR5, but retention of dietary fat was not increased in OP vs. OR. At the same time, the amount of fat retained in skeletal muscle was significantly less in the obese Zucker rats relative to lean rats (1). Taken together, these data imply that inherent differences in the ability to partition meal-derived nutrients toward storage and away from oxidation contribute to obesity susceptibility and/or are a consequence of the obese state.

We have used an HFD model of obesity development to describe processes that differ between rats either prone or resistant to obesity development (OP and OR, respectively) early in their exposure to an HFD (35% carbohydrate, 45% fat). Several observations have been made regarding energy intake, nutrient partitioning, and weight gain using this model. When fed a low-fat diet (LFD; 68% carbohydrate, 12% fat), energy intake and weight gain are not significantly different between OP and OR rats (7, 10), but during the first 2 wk of HFD feeding, OP rats eat more and gain more weight than do OR rats. With longer term HFD feeding (5 wk), the differences between OP and OR rats in energy intake and weight gain are less pronounced. After 1 or 2 wk of HFD, OP rats exhibit a metabolic profile more conducive to carbohydrate use compared with OR rats in skeletal muscle, liver, and adipose tissue, but by 5 wk of HFD, these differences are no longer apparent (7, 10). Increased carbohydrate use may promote obesity development by reducing reliance on lipogenesis.
on fat oxidation and/or partitioning carbohydrate into lipids via lipogenesis in liver or adipose tissue (6, 8, 16). Alternatively, a greater capacity for carbohydrate use may influence energy intake via effects on glycogen metabolism. For example, previous studies have suggested that whole body glycogen stores can influence energy intake (4, 5).

The present study was designed to determine whether glycosgenesis, lipogenesis, and/or retention of dietary fat differed between OP and OR rats after overnight consumption of an HFD meal. Meal energy intake was held constant to determine whether obesity susceptibility was characterized by inherent differences in the capacity for nutrient partitioning among these metabolic pathways.

METHODS

Experimental Animals, Diet and Feeding Protocol, and Group Classification

Male Wistar Crl(WI)BR rats (Sasco, Madison, WI), weighing between 135 and 180 g (7 wk of age) on arrival, were housed individually in a temperature-, humidity-, and light-controlled (12:12-h light-dark cycle) animal facility that met guidelines of the American Association for the Accreditation of Laboratory Animal Care. Throughout experiments, rats had free access to water. All protocols were approved by the University of Colorado Health Sciences Center Animal Care Committee. Rats were provided ad libitum access to a pelleted LFD (percent by energy: 12% fat, 20% protein, 68% carbohydrate; Research Diets, New Brunswick, NJ; Table 1) for a 2-wk baseline period, followed by 1-wk ad libitum access to a pelleted HFD (percent by energy: 45% fat, 20% protein, 35% carbohydrate; Research Diets, Table 1). Rats were then separated on the basis of body weight gain during the 1st wk of HFD into either OP (body weight gain \( \geq 48 \) g) or OR (body weight gain \( \leq 40 \) g) groups. These weight gain criteria were selected a priori to ensure 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 and were determined from previous data from our laboratory (7, 10). A total of 81 animals was studied (5 cohorts). Rats with weight gains between 41 and 47 g were not studied further (31.1% of total rats fed the HFD). Rats were studied either after 1 wk (OR1, OP1) or 5 wk of HFD feeding (OR5, OP5) and were permitted ad libitum access to food until the night before their day of study. In this model, 1 wk of HFD feeding represents a time in which obesity is developing and is not yet established; after 5 wk of HFD feeding, obesity is established. Thus groups studied at 1 and 5 wk of HFD feeding permit comparisons of processes associated with developing obesity and the established obese state. Throughout the protocol, food intake was measured three times per week (corrected for food spillage) and body weight was recorded once per week.

Study Procedures

On the morning before the day of study (between 1000 and 1100), body weight was determined and all food was removed. Water remained accessible. That evening (1700), one-half of the animals was provided an HFD meal (fed; HFD; percent energy: 45% fat, 20% protein, 35% carbohydrate), while the other one-half continued to fast (unfed). Immediately before the meal was provided, both fed and fasted rats were injected subcutaneously with 1.0 mCi \(^{3}H_{2}O\) (1700). The following morning (0800), rats were anesthetized (subcutaneous administration of pentobarbital sodium, 50 mg/kg), body weight was determined, and animals were killed by exsanguination. Blood was collected into heparinized tubes from which plasma insulin, glucose, triglycerides, and the specific activity of plasma \(^{3}H_{2}O\) were determined. The liver and fat pads (epididymal, retroperitoneal, and mesenteric) were removed within 2 min of death, weighed, and placed immediately into liquid nitrogen.

Verification of steady state for plasma \(^{3}H_{2}O\) over study day duration. Separate studies \((n = 4)\) were performed to determine whether \(^{3}H_{2}O\) in the plasma pool remained in steady state for a period of time inclusive of the 15-h study day protocol. Rats with body weights approximately equal to rats maintained on HFD for 1 and 5 wk were permitted ad libitum access to a standard chow diet. Four days before the day of study, a carotid artery was catheterized as previously described (12). On the morning of study (0900), 1.0 mCi of \(^{3}H_{2}O\) was injected subcutaneously. At 2, 4, 6, 8, and 24 h postinjection, arterial blood (100 \(\mu\)l) was collected for determination of plasma \(^{3}H_{2}O\). In these studies, the specific activity of plasma \(^{3}H_{2}O\) after subcutaneous injection of 1.0 mCi of \(^{3}H_{2}O\) reached steady state some time between 0 and 2 h and was maintained over 24 h (Fig. 1).

\(^{14}C\)-labeled HFD meal. The HFD meal was the same as the HFD rats had been consuming up to the day of study. To increase the likelihood that all food was consumed during the overnight study period and to equate energy intake/substrate delivery, the caloric value of the HFD meal (18.1 g, 95.4 kcal) was equal to 75% of the usual HFD intake of all rats during the 1st wk of HFD feeding. Because OP rats eat more during the 1st wk of HFD, OP rats were provided \(~68\%\) of their usual intake, and OR rats were provided \(~82\%\) of their usual intake. The HFD meal was labeled with equal proportions of \(1\)-\(^{14}C\)-oleate, \(1\)-\(^{14}C\)-palmitate, and \(1\)-\(^{14}C\)-linoleate (9). A combination of labeled fatty acids was used to track the dietary fat in the HFD meal [corn oil: 11% palmitate, 25% oleate, and 55% linoleate (9)]. A combination of labeled fatty acids was used to track the dietary fat provided in the HFD meal, because individual fatty acids are partitioned differently between oxidation and storage depending on chain length and degree of saturation (11). After tissue collection, individual cages in which the animals were

Table 1. Diet composition

<table>
<thead>
<tr>
<th></th>
<th>LFD</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat: corn oil, %energy</td>
<td>12</td>
<td>45</td>
</tr>
<tr>
<td>Carbohydrate, %energy</td>
<td>68</td>
<td>35</td>
</tr>
<tr>
<td>Protein, %energy</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Casein, g/100 g</td>
<td>20</td>
<td>24.6</td>
</tr>
<tr>
<td>DL-Methionine, g/100 g</td>
<td>0.3</td>
<td>0.37</td>
</tr>
<tr>
<td>Cornstarch, g/100 g</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>Maltodextrin 10, g/100 g</td>
<td>15</td>
<td>18.5</td>
</tr>
<tr>
<td>Cellulose, g/100 g</td>
<td>5</td>
<td>6.15</td>
</tr>
<tr>
<td>Corn oil, g/100 g</td>
<td>5</td>
<td>24.6</td>
</tr>
<tr>
<td>Salt mix, g/100 g</td>
<td>3.5</td>
<td>4.3</td>
</tr>
<tr>
<td>Vitamin mix, g/100 g</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td>Choline bitartrate, g/100 g</td>
<td>0.2</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Low-fat diet (LFD) with a metabolizable energy of 4.23 kcal/g. 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 (13).
housed between the time of $^3$H$_2$O injection and death were
swiped, and a portion of the shavings were counted to estimate
losses of $^3$H and $^{14}$C through urine and the animals’
handling of the labeled meal. Any remaining meal was
collected, weighed, and counted for determination of the amount
of meal and tracer not consumed during the overnight study
period.

**Analytic Methods**

**Blood analyses.** Plasma glucose concentration was determined
using a Beckman glucose analyzer (Glucose Analyzer II,
Fullerton, CA). Plasma triglycerides were determined
spectrophotometrically (Sigma Kit No. 320-A, Sigma Chemi-
cals, St. Louis, MO). Insulin was determined by radioimmu-
nounoassay (Linco Research, St. Charles, MO).

**Plasma $^3$H$_2$O specific activity.** Plasma was deproteinized
overnight (14) with equal volumes of barium hydroxide
and zinc sulfate (0.03 N). A portion of the resulting supernatant
was counted, and the remainder was taken to dryness and
counted. The difference in counts between the two portions
represents $^3$H$_2$O counts in plasma.

**Tissue analyses.** Liver glycogen was determined by the
method of Chan and Exton (2). Liver triglyceride content and
total lipid content of liver and adipose tissues were deter-
mined according to the methods of Denton and Randle (3).

**Net lipogenesis, glycogenesis, and dietary fat retention.**
Aliquots of the extracts resulting from the determinations of
liver glycogen and liver and adipose tissue lipid content were
used to estimate net retention of both $^3$H and $^{14}$C dpm.
Aliquots were taken to dryness, reconstituted with normal
saline and 0.5 ml solvable (Sigma Chemicals), and counted
(LS 6500, Beckman, Fullerton, CA).

**Calculations**

The specific activity of plasma $^3$H$_2$O was calculated as
follows: [(dpm plasma (dpm/ml)) - (dpm remaining
after drying (dpm/ml)] = $^3$H$_2$O in plasma (dpm/ml). The
density of plasma (1.03 g/ml, a value intermediate to that of
whole blood and water) was used to convert $^3$H$_2$O disintegrations
per minute per milliliter to disintegrations per minute
per milligram.

Fraction of glycogen and lipid retained in liver and adipose
tissue over the 15-h study period was calculated as fractional
$^3$H retention = ($^3$H dpm/mg of tissue)/($^3$H$_2$O dpm/mg in
plasma). Data for the fractional retention of $^3$H (Figs. 2B and
3B and Table 4) are presented as a percentage (fractional
retention $\times$ 100). An increase in the fractional retention of $^3$H
as calculated above represents the contribution of net glyco-
genesis or lipogenesis to the glycogen or lipid pool over the
period studied. Thus 100% retention would require that
the specific activity of glycogen be equal to the specific activity
in plasma and would suggest that the entire pool had been
replaced over the period of study. Data are also presented on
a total tissue basis (Figs. 2D and 3D), where the fractional
retention ($\times$100) was multiplied by organ weight. These data
represent the percentage of $^3$H retained in either glycogen or
lipid by the entire liver or fat pad.

Percentage of dietary fat retained in liver and adipose
tissue was calculated as ($^{14}$C dpm within a total tissue/$^{14}$C
consumed in meal) $\times$ 100. Data are also expressed as a
percentage of the $^{14}$C consumed in the meal within a gram of
tissue (Table 5).

**Data Analyses**

Data related to glycogen concentration, tritium retention,
and dietary fat retention are presented both relative to 1 g of
tissue and to total tissue weight. A difference in tritium
retention in glycogen when expressed per gram of tissue
would be interpreted as an inherent difference in the capac-
ity for glycogenesis and therefore would suggest that it may
be causally linked to obesity susceptibility. In contrast, a
difference in tritium retention in glycogen when expressed
per total liver weight would be interpreted to be a conse-
quency of the HFD, obesity, or both.

Three-way ANOVA was used to determine the significance
of group $\times$ time $\times$ fed/fasted interactions. Linear contrasts
(determined a priori) tested the significance of only certain
comparisons of interest between OP and OR group (e.g.,
difference between fed and fasted OP1 rats vs. OR1 rats).
When one-way ANOVA in combination with necessary mul-
tiple-comparison tests (15) was used to detect differences
across groups, interpretations were the same as those made
using linear contrasts. Because the results from the one-way
ANOVA and subsequent multiple comparisons offer more
information to the reader, data are reported using these
statistics. Significance was set at $P \leq 0.05$ for all compari-
sions. For fractional retention of $^3$H expressed relative to the
epididymal and retroperitoneal fat pads, an outlier (greater
than 2 standard deviations from the mean) in both the OR1
fed group and the OR1 fasted group, respectively, was ex-
cluded from the statistical analysis. These two analyses then
have one less observation than all other analyses.

**RESULTS**

**Body Weight, Energy Intake, and Body Weight Gain**

Initial body weight was not significantly different
among groups (Table 2). During the second week of
LFD, energy intake among 1 and 5 wk rats was not
significantly different. Body weight gain during the
baseline period was significantly greater in OP1 fed
rats compared with OR1 fed rats.
As anticipated from the study design, after the 1st wk of HFD, OP1 rats ate more (18%; $P < 0.05$) and gained more weight (55%; $P < 0.05$) than did OR1 rats. Over the 5-wk HFD feeding period, energy intake was 12% greater ($P < 0.05$) and weight gain was 22% greater ($P < 0.05$) in OP5 vs. OR5.

### Plasma Substrates and Hormones

Plasma glucose, insulin, and triglyceride concentrations were not significantly different between fed OP and OR rats or between fasted OP and OR rats (Table 3). Glucose and triglyceride concentrations were greater ($P < 0.05$) in the fed vs. fasted state for all groups.

#### Glycogen Concentrations and Fractional $^3$H Retention in Glycogen

Fed liver glycogen concentration (per gram liver) was lower ($P < 0.05$) in OP5 compared with OR5, OR1, and OP1 rats (Fig. 2A). Total liver glycogen content (glycogen per total liver weight) in the fed state was greater ($P < 0.05$) in fed OR5 compared with all other groups (Fig. 2C). $^3$H retention in glycogen per gram of liver was greater ($P < 0.05$) in OR1 compared with all other

### Table 2. Energy intake, body weight gain, and body weight during baseline and HFD feeding periods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 wk Rats</th>
<th>5 wk Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OP</td>
<td>OR</td>
</tr>
<tr>
<td>Initial body wt, g</td>
<td>155.4 ± 9.8$^{a}$</td>
<td>159.5 ± 6.4$^{a}$</td>
</tr>
<tr>
<td>Baseline period EI, kcal</td>
<td>1,402 ± 36$^{a}$</td>
<td>1,339 ± 32$^{a, c}$</td>
</tr>
<tr>
<td>Body wt gain, g</td>
<td>147.2 ± 7.5$^{a, c}$</td>
<td>132.1 ± 7.8$^{a, c}$</td>
</tr>
<tr>
<td>Cumulative HFD period EI, kcal</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Body wt gain, g</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Values are means ± SE. OP, obesity prone, $n = 10$ or 11/group; OR, obesity resistant, $n = 10$/group; N/A, not applicable; EI, energy intake. Baseline period includes 2 wk of LFD feeding. Cumulative HFD period includes 5 wk of HFD. Symbols are derived from Tukey’s post hoc comparisons. Means within a row sharing common symbols are not significantly different ($P < 0.05$).

### Table 3. Tissue weights and plasma substrate and hormone concentrations after animal death

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 wk Rats</th>
<th>5 wk Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OP</td>
<td>OR</td>
</tr>
<tr>
<td>Liver</td>
<td>16.0 ± 0.4$^{a, c}$</td>
<td>10.2 ± 0.2$^{a}$</td>
</tr>
<tr>
<td>Sum of 3 fat pads</td>
<td>18.9 ± 1.0$^{b, d}$</td>
<td>16.4 ± 1.9$^{c, d}$</td>
</tr>
<tr>
<td>Epi</td>
<td>7.2 ± 0.3$^{a}$</td>
<td>5.9 ± 0.6$^{a, c}$</td>
</tr>
<tr>
<td>Ret</td>
<td>6.8 ± 0.6$^{a, c}$</td>
<td>6.2 ± 0.9$^{a, c}$</td>
</tr>
<tr>
<td>Mes</td>
<td>4.9 ± 0.3$^{a}$</td>
<td>4.3 ± 0.5$^{a, c}$</td>
</tr>
<tr>
<td>Glucose</td>
<td>10.4 ± 0.5$^{a}$</td>
<td>7.1 ± 0.3$^{a}$</td>
</tr>
<tr>
<td>Insulin</td>
<td>715 ± 135$^{a}$</td>
<td>235 ± 55$^{a, c}$</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>2.26 ± 0.26$^{a}$</td>
<td>0.58 ± 0.05$^{a}$</td>
</tr>
</tbody>
</table>

Values are means ± SE. OP, $n = 10$ or 11/group; OR, $n = 10$ group; Epi, epididymal fat pad; Ret, retroperitoneal fat pad; Mes, mesenteric fat pad. Units of measure for glucose are mmol/l, for insulin are pmol/l, and for triglyceride are mmol/l. Supercritics after means are derived from Tukey’s post hoc comparisons. Means within a row sharing common superscripts are not significantly different ($P < 0.05$). Sum of 3 fat pads refers to the summed weight of Epi, Mes, and Ret fat pads.
fed groups (Fig. 2B). Total $^3$H retention in glycogen was not significantly different among groups (Fig. 2D).

Liver Triglyceride Concentrations and Fractional $^3$H Retention in Lipid

Liver triglyceride concentrations were greater ($P \leq 0.05$) in OP5 vs. OR5 in the fasted state only (Fig. 3A). Total liver triglyceride content was higher ($P \leq 0.05$) in fasted OP5 vs. OR5 rats and in fed OP5 vs. OR5 rats (Fig. 3C). $^3$H retention in liver lipid was not significantly different among 1 wk rats (Fig. 3, B and D). At 5 wk, $^3$H retention was also not significantly different when OP and OR rats were compared in the fasted or fed state (Fig. 3, B and D).
Adipose Tissue Fractional \(^3\)H Retention in Lipid

In the fasted state, \(^3\)H fractional retention was not significantly different between OP1 and OR1 or between OP5 and OR5 whether expressed per gram or per total fat pad weight (Table 4). In the fed state, \(^3\)H fractional retention was not significantly increased in OP rats when expressed per gram of tissue weight (Table 4). In fact, \(^3\)H fractional retention was lower (\(P \leq 0.05\)) in the mesenteric fat pad in OP1 rats vs. OR1 (Table 4). \(^3\)H fractional retention relative to total fat pad mass in OP1 vs. OR1 rats was increased by 30% (\(P > 0.05\)) and 49% (\(P \leq 0.05\)) under fasted and fed conditions, respectively, and was increased (\(P > 0.05\)) by 55 and 30% in OP5 vs. OR5 under fasted and fed conditions, respectively.

Percentage of \(^14\)C From HFD Meal Retained in Liver and Adipose Tissue

The amount of \(^14\)C tracer consumed was not significantly different among fed animals (Table 5). In both liver and adipose tissue, the percent of \(^14\)C retained (per gram or total tissue weight) was lower in OP1 vs. OR1 (\(P \leq 0.05\), but was not significantly different between OP5 and OR5 rats (Table 5).

**DISCUSSION**

**General Findings**

In the present study, the overnight postprandial response to an HFD meal was determined in OP and OR rats. To evaluate whether susceptibility to dietary obesity involved differences in the capacity to partition dietary nutrients into storage pathways, energy intake was equated. Under these conditions, estimated net lipogenesis and retention of dietary fat per gram of adipose tissue or liver were not increased in OP rats. However, when expressed relative to total fat pad mass, adipose tissue lipogenesis was increased in OP rats. These data imply that OP rats are not characterized by an increased capacity for lipogenesis or retention of dietary fat. Net liver glycogenesis and the ability to restore the liver glycogen pool to fed levels were not significantly different between OP and OR groups after 1 wk on the HFD. However, the ability to restore the liver glycogen pool was significantly reduced in OP rats after 5 wk on the HFD. These results suggest that changes in liver glycogen metabolism may be the result

**Table 4. Percent of plasma \(^3\)H retained by lipid in adipose tissue after 1 and 5 wk of HFD**

<table>
<thead>
<tr>
<th>Fat Pad</th>
<th>Fed</th>
<th>Fasted</th>
<th>Fed</th>
<th>Fasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epi per g</td>
<td>0.10 ± 0.014&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.07 ± 0.007&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.10 ± 0.013&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.12 ± 0.039&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>total</td>
<td>0.74 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.43 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.39 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ret per g</td>
<td>0.20 ± 0.031&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09 ± 0.019&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.26 ± 0.042&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.06 ± 0.003&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>total</td>
<td>1.39 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.52 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.86 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.25 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mes per g</td>
<td>0.14 ± 0.014&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.09 ± 0.008&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.21 ± 0.032&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.11 ± 0.007&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>total</td>
<td>0.66 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.36 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.76 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.33 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total fat</td>
<td>2.80 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.26 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.82 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.97 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SE. OP, \(n = 10\) or 11/group; OR, \(n = 10\)/group. Superscripts after means are derived from Tukey’s post hoc comparisons. Means within a row sharing common superscripts are not significantly different (\(P \geq 0.05\)). 1 wk OR fed group, \(n = 9\) for Ret, total; 1 wk OR fasted group, \(n = 9\) for Epi, total. Total fat is the sum of Epi, Ret, and Mes data and for 1 wk OR fed and fasted group represents \(n = 9\).

**Table 5. Percent of meal derived \(^14\)C retained in liver and adipose tissue lipid of fed animals at 1 and 5 wk of HFD**

<table>
<thead>
<tr>
<th>1 wk Rats</th>
<th>5 wk Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>OP</td>
<td>OR</td>
</tr>
</tbody>
</table>

\(^{14}\)C dpm consumed: 2.13 ± 0.04<sup>a</sup> | 1.96 ± 0.14<sup>a</sup> | 1.94 ± 0.11<sup>a</sup> | 1.98 ± 0.10<sup>a</sup> |

Liver per g: 0.17 ± 0.02<sup>a</sup> | 0.37 ± 0.03<sup>a</sup> | 0.22 ± 0.03<sup>a</sup> | 0.32 ± 0.03<sup>a</sup> |
| total | 2.76 ± 0.44<sup>a</sup> | 5.08 ± 0.42<sup>a</sup> | 4.16 ± 0.64<sup>a</sup> | 5.72 ± 0.50<sup>a</sup> |

Epididymal per g: 0.16 ± 0.02<sup>a</sup> | 0.43 ± 0.06<sup>a</sup> | 0.07 ± 0.02<sup>a</sup> | 0.11 ± 0.02<sup>a</sup> |
| total | 1.16 ± 0.16<sup>a</sup> | 1.73 ± 0.19<sup>a</sup> | 0.95 ± 0.20<sup>a</sup> | 0.95 ± 0.16<sup>a</sup> |

Retroperitoneal per g: 0.22 ± 0.04<sup>a</sup> | 0.78 ± 0.10<sup>a</sup> | 0.09 ± 0.02<sup>a</sup> | 0.18 ± 0.03<sup>a</sup> |
| total | 1.47 ± 0.21<sup>a</sup> | 3.10 ± 0.45<sup>a</sup> | 1.32 ± 0.27<sup>a</sup> | 1.55 ± 0.20<sup>a</sup> |

Mesenteric per g: 0.20 ± 0.03<sup>a</sup> | 0.82 ± 0.13<sup>a</sup> | 0.10 ± 0.02<sup>a</sup> | 0.20 ± 0.04<sup>a</sup> |
| total | 0.85 ± 0.13<sup>a</sup> | 2.52 ± 0.33<sup>a</sup> | 0.96 ± 0.21<sup>a</sup> | 1.23 ± 0.22<sup>a</sup> |

Values are means ± SE. OP, \(n = 10\) or 11/group; OR, \(n = 10\)/group. For lines denoted as total, percentages were calculated from the number of dpm within an entire tissue relative to the no. of dpm consumed by each rat. For lines denoted as per gram, percentages were calculated from the number of dpm within 1 g of tissue relative to the number of dpm consumed by each rat. Symbols after means are derived from Tukey’s post hoc comparisons. Means within a row sharing common symbols are not significantly different (\(P \geq 0.05\)). A factor of 10<sup>−7</sup> has been applied to the \(^{14}\)C dpm consumed data. Sum of 3 fat pads refers to the summed percentages of Epi, Ret, and Mes fat pads.
of the greater adiposity and/or energy intake in OP rats.

**Hepatic and Adipose Tissue De Novo Lipogenesis and Retention of Dietary Fat**

Although liver triglyceride concentration was elevated in OP rats after 5 but not 1 wk of HFD feeding, retention of tritium in the hepatic lipid pool (estimate of net lipogenesis) was not significantly different between OP and OR rats at either 1 or 5 wk. In adipose tissue, although the summed fat pad weight in OP1 rats was not higher statistically, it weighed 49% more tissue, although the summed fat pad weight in OP1 rats was not higher statistically, it weighed 49% more than in OR1 rats (Table 3; average of fed and fasted). By 5 wk, the summed fat pad weight was 65% greater \((P \leq 0.05)\) in OP5 rats vs. OR5 rats (Table 3). However, retention of tritium in adipose tissue (estimate of net lipogenesis) when expressed per gram of tissue was not increased in OP rats at either 1 or 5 wk. Thus OP rats are not characterized by an increased capacity for net lipogenesis in liver or adipose tissue in response to a single HFD meal. The fact that estimated net lipogenesis was significantly greater in OP rats only if expressed relative to total fat pad mass provides further support for the notion that differences in the capacity of partitioning nutrients toward storage via lipogenesis do not contribute to susceptibility to obesity.

Similar to lipogenesis, there was no evidence for increased retention of dietary fat in liver and adipose tissue of OP rats. If anything, OP rats retained less dietary fat in these tissues. We previously reported that adipose tissue lipoprotein lipase mRNA and activity was elevated in OP rats after 1 and 2 wk of HFD feeding \((10)\). Taken together, these data suggest that the increased lipoprotein lipase does not impart a greater capacity to retain dietary lipid in adipose tissue in response to a single meal.

The percentage of tritium and labeled dietary lipids retained in liver and adipose tissue lipids was not significantly increased between 1 and 5 wk, despite the fact that both liver and adipose tissue weights were increased. Thus either meal-stimulated synthesis/storage was reduced or lipid mobilization was increased between 1 and 5 wk of HFD feeding in both groups. The extent to which this observation involves changes in insulin action requires further study.

The meal feeding period in the present study encompassed 15 h. Over the 15-h period, animals from both dietary groups ate a similar amount of energy and, thus, a similar nutrient mixture. This procedure was chosen so that net nutrient storage could be assessed under usual feeding conditions (i.e., free access to the diet throughout the night) but without differences in energy intake. However, free access to the diet throughout the night did not allow us to control or equate the timing of food intake between groups. Thus it is possible that food consumption occurred over different time courses between dietary groups. The impact of this is difficult to determine but should be considered when comparing these results with other studies. We chose to make measurements at only one time point after the end of the HFD meal feeding period. Data reported by others have indicated that by 2.2 h postmeal, obese Zucker rats retained significantly more dietary fat in liver and adipose tissue than lean rats; by 6 h postmeal, the differences between obese and lean rats were even more striking \((1)\). Thus it is likely that the choice of a single 15-h time point was not the reason for the lack of differences between OP and OR rats. In addition, comparison of the results from the present study with those of the study involving Zucker rats suggests that the contribution of nutrient partitioning may be greater in genetic vs. dietary obesity.

**Liver Glycogen and Net Glycogenesis**

The only observed difference between OP and OR rats after 1 wk on the HFD was in the retention of tritium when expressed per gram of liver. Less retention of tritium suggests that net glycogenesis was reduced in OP vs. OR rats. Because liver glycogen concentration was not significantly different, these data imply that glycogen turnover was reduced in OP rats. The impact of a reduced glycogen turnover to obesity development in this model is difficult to assess; however, because it was observed early in the course of HFD feeding, it may represent an inherent difference between OP and OR rats.

By 5 wk of HFD feeding, retention of tritium in glycogen was not different between OP and OR rats, but liver glycogen concentration (expressed per gram or per total liver) was significantly lower in OP rats, suggesting that OP rats mobilize more glycogen over the course of the 15-h meal feeding period. It is likely that this impairment results from the greater adiposity and, perhaps, greater insulin resistance at the liver in OP rats.

**Importance of Energy Intake to the OP Phenotype**

In the present study, energy intake was equated between OP and OR rats to evaluate the inherent capacities for postprandial lipogenesis, retention of dietary lipid, and glycogenesis. Under these conditions, OP rats do not display a greater capacity for accumulation of lipid via lipogenesis or retention of dietary lipid. These data, therefore, underscore the significance of the increased energy intake to the OP phenotype. In fact, these data suggest that the presence of a positive energy balance, brought about by increased energy intake, represents the critical stimulus to increased body weight gain in OP rats.

**Perspectives**

The data presented here indicate that with equicaloric feeding, OP rats neither store more dietary fat nor undergo greater rates of lipogenesis than do OR rats, and, therefore, underscore the central role of energy intake in diet-induced obesity. At present, it is unclear what mechanism(s) drive the greater intake by OP rats during their early exposure to an HFD. Concentrations of certain metabolites and hormones such as β-
droxybutyrate, corticosterone, insulin, or leptin, each of which can impact food intake, do not seem to account for the increased intake by OP rats with HFD feeding. However, differences in sensitivity to any of these substrates or hormones (or others not yet investigated) may exist between OP and OR rats. Importantly, differences in energy intake between OP and OR rats are only manifest when dietary fat content is high. As such, it can be hypothesized that OP rats increase energy intake in response to an HFD in an attempt to acquire adequate carbohydrate.

We gratefully acknowledge the metabolic core of the Colorado Clinical Nutrition Research Unit (P30-DK-48520–01) for assistance with insulin measurements. We also thank Dr. Gary Grunwald (University of Colorado Health Sciences Center) for assistance with the statistical analyses of these data.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-55386 (to M. J. Pagliassotti) and DK-38088 (to J. O. Hill), and the Colorado Agricultural Experiment Station Project 616 (to C. L. Melby).

REFERENCES