Effect of diet on fat cell size and hormone-sensitive lipase activity

JOSHUA J. BERGER AND R. JAMES BARNARD
Department of Physiological Science, University of California, Los Angeles, Los Angeles, California 90095-1527

Effect of diet on fat cell size and hormone-sensitive lipase activity. J. Appl. Physiol. 87(1): 227–232, 1999.—This study was designed to examine the relationship between diet-induced insulin resistance/hyperinsulinemia, fat cell hypertrophy, and hormone-sensitive lipase (HSL) to elucidate whether an attenuated HSL activity leads to obesity. Female Fischer 344 rats were fed either a low-fat, complex-carbohydrate diet or a high-fat, refined-sugar (HFS) diet for 2 wk, 2 mo, or 6 mo. Adipose tissue morphology and HSL activity as well as plasma free fatty acid and glycerol levels were determined at these times. No differences between groups were seen after 2 wk except the previously reported hyperinsulinemia in the HFS animals. At both 2 and 6 mo, the HFS animals demonstrated adipocyte hypertrophy. Basal and stimulated HSL activities and plasma glycerol were significantly elevated in the HFS group. There was a positive correlation between adipocyte size and HSL activity for both basal and stimulated states. These results demonstrate that an attenuated HSL activity is not observed with the onset of insulin resistance/hyperinsulinemia and therefore does not play a role in the development of obesity.

MATERIALS AND METHODS

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The typical Western diet is very high in fat and sucrose and is considered to be a major factor involved in the development of insulin resistance and obesity. Recently, we have demonstrated in an animal model that insulin resistance precedes the development of obesity when the animals are fed a high-fat, sucrose (HFS) diet (9). The insulin resistance occurred in as little as 2 wk. Many other investigators have demonstrated a similar time course for the development of insulin resistance/hyperinsulinemia in animals fed either a diet high in fat or a diet high in sucrose (17, 29, 57). Oscai et al. (41, 42) have demonstrated in long-term experiments that animals fed ad libitum at isocaloric amounts of a HFS diet or low-fat, starch diet and yet became obese on the HFS diet. Storlein et al. (57) demonstrated that, after ~4 wk with the caloric intake controlled, the high-fat diet group showed a significant increase in the white adipose tissue weight without a change in body weight compared with the high-carbohydrate group.

A mechanism by which an insulin-resistant/hyperinsulinemic state leads to the development of obesity has been previously reviewed by Barnard and Wen (10) and by Eckel et al. (24). Insulin stimulates and catecholamines inhibit the activity of lipoprotein lipase (LPL) in a tissue-specific fashion. It has been demonstrated in animals and humans that skeletal muscle LPL is downregulated in a hyperinsulinemic state, whereas adipose tissue LPL is upregulated (7, 24). The result of these alterations in LPL activity would be the shunting of dietary fat into adipose tissue for storage. Hormone-sensitive lipase (HSL), the rate-limiting enzyme for the breakdown of stored triglycerides, is also exquisitely controlled by both catecholamines and insulin, the former being the major stimulator of activity and the latter being the major physiological inhibitor of HSL activity. In a hyperinsulinemic state, it is possible that the normal response of adipose tissue HSL is attenuated, and this along with the increased LPL activity would favor the net deposition of dietary fat and the development of obesity. However, in established obesity the activity of HSL has been found to be increased in both animals (19, 22) and humans (3, 13). A positive correlation between adiposity and sympathetic nerve activity has been reported in humans (1, 11), which may account for part of this increase in HSL activity observed in obesity.

The present study was therefore designed to quantify the activity of HSL in a time-dependent fashion when animals were raised on a HFS vs. a low-fat, complex-carbohydrate (LFCC) diet. Studies of basal and stimulated HSL activity were conducted starting at 2 wk on the diets to see whether HSL activity was depressed before fat cell hypertrophy.

MATERIALS AND METHODS

Animals and diets. Inbred female Fischer 344 rats were obtained from Harlan Sprague Dawley (San Diego, CA) at 2 mo of age. Female rats were used because we have previously reported that they develop insulin resistance, hyperinsulinemia, and obesity in response to a high-fat, refined-sugar diet.
The rats were allowed to acclimatize to their new environment for 1 wk before being assigned to either a LFCC diet or a HFS diet. Both diets contained a standard vitamin and mineral mix as described previously (8, 9). Briefly, the percent distribution of calories and caloric density of the LFCC and HFS diets were as follows: 23% protein, 9% fat, 68% starch, 0.0% sucrose, and 13.8 kJ/g for the LFCC vs. 21% protein, 39% fat, 0.0% starch, 40% sucrose, and 19.7 kJ/g for the HFS. The food was prepared in powder form by Purina Mills and was provided ad libitum along with water to the animals, which were housed four per cage with a 12:12-h light-dark cycle at 24–25°C. The University of California at Los Angeles Animal Research Committee approved this protocol.

Adipose tissue histology. A portion of the omental fat pad was rinsed in 0.85% NaCl solution and then placed into a solution of 10% phosphate-buffered Formalin. These adipose tissue samples were then prepared for sectioning and staining with hematoxylin and eosin by transferring the adipose tissue into cassettes for dehydration, infiltration, and embedding in paraffin (53). Sections were sliced at a thickness of 4 μm at three different depths (at least 200 μm apart) within the same tissue sample and fixed to slides. Samples were then observed under the microscope, and video prints were taken for the determination of cell size and number. Direct microscopic observations were made into video prints by using a CODONICS VP-3500 Video Printer attached to a Perceptive Systems Image Analysis System, which uses an Olympus BH2 Microscope as its base apparatus. From the video images taken of the slides, the number of cells within a known area were used to calculate the mean cell volume and mean cell number per gram of tissue. Lemonnier (37) and Ashwell et al. (5) both describe this technique in detail and have determined that a constant correction factor of 1.15 could be used to adjust the apparent cell diameter of fixed cells.

Blood chemistry. After an overnight fast, the animals were anesthetized with chloral hydrate (7%, 0.5 ml/100 g body wt), and a blood sample was taken via cardiac puncture into a syringe containing EDTA as an anticoagulant. The sample was then centrifuged for 20 min, the plasma was separated, and the sample was frozen and stored at −70°C until further analysis. Plasma free fatty acid (FFA) concentrations were determined enzymatically (Wako Chemicals) from the samples at 2 wk, 2 mo, and 6 mo in both groups. The samples were used to calculate the mean cell volume and mean cell number per gram of tissue. Lemonnier (37) and Ashwell et al. (5) both describe this technique in detail and have determined that a constant correction factor of 1.15 could be used to adjust the apparent cell diameter of fixed cells.

Adipocyte HSL activity and glycerol assay. Samples of omental fat were taken from the abdominal cavity fat depots in both the HFS and LFCC diet group. The fat pads were rinsed first in 0.85% NaCl solution and then were rinsed three more times in medium 199 solution (Sigma Chemical). The adipose tissue was then patted dry and weighed so that ñ500 mg were added to 2.0 ml of prewarmed incubation medium (medium 199) and incubated at 37°C for 1 h. There was a time-dependent increase in lipolysis for >60 min; therefore, this time period was chosen for the incubations.

For the stimulation of lipolysis, isoproterenol was used, as opposed to epinephrine or other catecholamines, because it is a pure β-agonist and as such it illicit the greatest lipolytic response. Isoproterenol produced a dose-dependent increase in lipolysis, with the maximal response being at 10−5 M. To measure HSL activity, glycerol release was measured over 1 h in both the basal state and in the presence of isoproterenol (10−5 M) so that maximal HSL responsiveness could be examined, as described by Rodbell (49). Glycerol was determined enzymatically from the supernatant samples by using a Sigma Diagnostic Kit. Because adipose tissue has a very low level of glycerol kinase, only a very small fraction of the glycerol produced by intracellular lipolysis can be reutilized and converted to α-glycerophosphate for use in triglyceride synthesis (14, 32, 38). Glycerol release is therefore a valid index of lipolysis and thus of HSL activity. At the end of the incubation, three aliquots of infranatant (100 μl each) were removed from each incubation mixture for the measurement of glycerol.

RESULTS

Adipose tissue morphology. Table 1 reveals the effects of diet on omental adipose tissue characteristics. As can be seen in Table 1, the HFS animals at 2 wk did not differ significantly in mean cell volume from the LFCC rats. Accordingly, the difference in mean cell number (expressed per gram of tissue) did not reach statistical significance. The adipocytes of the HFS rats at 2 mo were 83.8% more voluminous than their LFCC counterparts. The mean cell number per gram of tissue for the LFCC rats was 75.3% greater than that for the HFS rats at 2 mo. Table 1 reveals that the volume of the HFS adipocytes continued to increase after 2 mo, reaching 149.8% of the LFCC rats by 6 mo. The mean cell number per gram of tissue for the LFCC rats at 6 mo decreased again due to the increase in mean cell volume, falling in number to 40.87% of the LFCC rats. It is interesting to note that the adipocytes from the LFCC rats did not increase in volume or decrease in mean cell number per gram of tissue during the 6-mo time period.

Table 1. Effect of diet on adipocyte size and number

<table>
<thead>
<tr>
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<th>Mean Cell Volume, μm³ x 10⁶</th>
<th>Mean Cell Number/g tissue, 1 x 10⁸</th>
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<tr>
<td></td>
<td>2 wk</td>
<td>6 mo</td>
</tr>
<tr>
<td>LFCC</td>
<td>2.5 ± 0.2</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>HFS</td>
<td>2.85 ± 0.2</td>
<td>4.26 ± 0.3†</td>
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*P < 0.01, LFCC vs. HFS. †P < 0.01, HFS vs. HFS.
Plasma glycerol and FFA levels. As can be seen in Table 2, the plasma FFA levels were not statistically different between the groups at any time period. The plasma glycerol levels were not significantly different at 2 wk, but by 2 mo the values in the HFS group were 47.3% greater than those of the LFCC group. By 6 mo the HFS group had 91.4% higher glycerol levels than those of the LFCC animals.

HSL activity. In all experiments performed with isoproterenol, the stimulated rate of lipolysis was significantly greater than the basal rate ($P < 0.01$). As shown in Fig. 1, the basal activity for HSL in the HFS adipocytes at 2 wk did not differ from the activity in the LFCC adipocytes. The stimulated activity for HSL in the cells at 2 wk, although significantly increased from basal values, was not significantly elevated in the HFS group compared with the LFCC group. At 2 mo, the basal activity of HSL was nearly two times as great in the HFS adipocytes compared with the LFCC adipocytes. The stimulated rate for HSL activity in the HFS adipocytes at 2 mo was 1.72 times the rate of the LFCC adipocytes. The animals at 6 mo showed the same trends as the animals at 2 mo, but the basal rate of HSL activity in the HFS cells at 6 mo rose to nearly triple the level found in the LFCC cells at 6 mo. The stimulated activity of HSL in the HFS cells at 6 mo was 1.71 times greater than the stimulated activity of HSL in the LFCC cells. Basal and stimulated HSL activities remained unchanged throughout the 6-mo period for the LFCC rats. HSL activity was highly correlated with fat cell size for both basal and stimulated conditions (Fig. 2).

Propranolol study. Propranolol was used to determine whether the increased basal rate of lipolysis in the HFS rats was the result of increased sympathetic nervous system activity. In the HFS rats at 2 mo, in which HSL activity had increased, propranolol injection had no effect on the elevated basal activity of HSL ($2.0 \pm 0.2$ vs. $1.9 \pm 0.2 \mu\text{mol glycerol} \cdot 10^6 \text{cells}^{-1} \cdot \text{h}^{-1}$ for control vs. propranolol, respectively). The effectiveness of the propranolol was demonstrated because it totally blocked the isoproterenol-stimulated glycerol release ($2.2 \pm 0.2$ vs. $3.4 \pm 0.3 \mu\text{mol glycerol} \cdot 10^6 \text{cells}^{-1} \cdot \text{h}^{-1}$ for propranolol vs. control, respectively).

**DISCUSSION**

The primary purpose of this investigation was to determine the effect of a HFS diet on adipose tissue morphology and HSL activity. In the HFS model of diet-induced obesity, we (10) hypothesized that hyperin-

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<th>Glycerol, mg/dl</th>
<th>FFA, meq/l</th>
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<tr>
<td></td>
<td>LFCC</td>
<td>HFS</td>
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<tr>
<td>2 wk</td>
<td>21.36 ± 0.69</td>
<td>23.07 ± 3.48</td>
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<tr>
<td>2 mo</td>
<td>26.75 ± 1.94</td>
<td>39.40 ± 3.33*</td>
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<tr>
<td>6 mo</td>
<td>20.67 ± 1.52</td>
<td>39.56 ± 2.58†</td>
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Values are means ± SE. Glycerol: 2 wk, $n = 7$ rats/group; 2 mo, $n = 8$ rats/group; 6 mo, $n = 10$ rats/group. Free fatty acid (FFA): 2 wk, $n = 6$ rats/group; 2 mo, $n = 6$ rats/group; 6 mo, $n = 8$ rats/group. *$P < 0.05$. †$P < 0.01$. 
sulinemia resulting from the HFS diet would initially downregulate HSL activity and ultimately lead to the development of obesity. We and others have reported that insulin resistance/hyperinsulinemia develops within a few weeks after the start of a high-fat and/or refined-sugar diet (9, 28, 45, 47). The results of the present study, however, indicate that basal or maximal-stimulated HSL activities are not attenuated due to hyperinsulinemia. In this study, plasma glycerol levels were not elevated at the 2-wk time point, but the levels were elevated at both 2 and 6 mo in the HFS group compared with the LFCC group. This indicates that there was no change in the rate of lipolysis in vivo after 2 wk despite documented hyperinsulinemia (9). The elevated plasma glycerol levels at 2 and 6 mo in the HFS group indicate increased lipolytic activity, which agrees with the in vitro HSL studies. Furthermore, the results suggest that HSL activity is determined by the size of the adipocyte, because both basal and stimulated rates of lipolysis, expressed per cell, were highly correlated with cell size.

The enlargement of adipose tissue mass seen in obesity may be the result of adipocyte hypertrophy, hyperplasia, or a combination of the two (31, 37, 50, 59). The increase in adipose tissue mass found in the HFS animals at 2 and 6 mo in the present study may have been due to both the documented hypertrophy of existing adipocytes and undocumented hyperplasia because hyperinsulinemia has been shown to cause adipocyte hyperplasia (33). Unfortunately the weight of the fat pads themselves could not be measured, and hence total cell number could not be calculated, because the omental fat pad became too enlarged and undefined to isolate as a distinct depot. Adipocyte size in the animals at 2 wk was not statistically different between the groups; however, by 2 mo on the HFS diets the adipocytes were increased in size by 70%. Thus hypertrophy of the fat mass definitely occurred. We (9) have shown previously that at these time points, 2 wk and 2 mo, there was no detectable difference in whole body fat content or weight even though fat cell hypertrophy was observed at 2 mo in the present study. This observation is in agreement with the 4-wk data of Storlien et al. (57), who used a high-fat diet. In the HFS animals at 6 mo, the omental adipocytes grew to be 150% of the size of the LFCC adipocytes. At this point in time, there was a significant difference in whole body fat (17.6 ± 0.6% LFCC vs. 22.4 ± 1.5% HFS) and body weight (188 ± 1.4 g LFCC vs. 214 ± 3.4 g HFS) between the groups (9).

In agreement with the elevation in plasma glycerol levels are the data that show increased basal and stimulated rates of lipolysis in vitro in the adipose tissue from both the HFS animals at 2 and 6 mo compared with the LFCC animals. The increments in both the basal and stimulated rates of HSL activity were highly correlated to the increases in adipocyte cell volumes found at these time periods, which agrees with earlier studies in both rats (13, 19, 55) and humans (4, 12, 15, 39, 48). It has also been demonstrated that larger fat cells incorporate more glucose label into triglycerides, thus demonstrating that fatty acid reesterification is considerably higher in larger fat cells (12, 13, 54). This could explain why fat cell hypertrophy occurred in the HFS rats despite the increase in HSL activity.

The early development of hyperinsulinemia that occurs as a consequence of the HFS diet would be expected to inhibit the rate of lipolysis in vivo. Our experiments do not support this hypothesis because at 2 wk the plasma glycerol and FFA levels were not significantly reduced in the HFS compared with the LFCC animals. These in vivo findings are in agreement with our in vitro fat depot experiments that showed no reduction in basal or stimulated HSL activity at 2 wk. As for the other time points investigated, the increased rates of lipolysis in vitro are congruent with the increased plasma glycerol levels seen at 2 and 6 mo in the HFS rats.

The fact that we did not find parallel increases in plasma FFA and glycerol levels could have different
in HSL activity with an acute exposure to insulin does not lead to chronic suppression of HSL and the development of obesity. The increase in basal and hormone-stimulated activity was not due to an increase in sympathetic nervous system activity but was highly correlated to fat cell size.

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