Mechanisms of Decreased Lipoprotein Lipase Activity in Adipocytes of Starved Rats Depend on Duration of Starvation1,2

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ABSTRACT The aim of this study was to delineate the mechanisms by which varying periods of starvation decrease lipoprotein lipase (LPL) activity in rat adipose tissue. LPL mRNA levels and rates of LPL synthesis, degradation and secretion were compared in adipocytes from male rats that had been fed or starved for 1 or 3 d. The decreased LPL activity after 3 d of starvation (~76%) was explained mainly by a 50% decrease in the relative abundance of LPL mRNA levels (P < 0.05) and a parallel 50% decrease in relative rates of LPL biosynthesis (P < 0.05). In contrast, starvation for 1 d decreased total LPL activity by 47% (P < 0.05) but did not affect LPL mRNA levels or relative rates of LPL biosynthesis. Pulse-chase studies demonstrated that 1 d of starvation increased the rate of degradation of newly synthesized LPL (P < 0.05) and markedly decreased its secretion into the medium (P < 0.05). A decrease in overall protein synthesis also contributed to the decreased LPL activity after 1 and 3 d of starvation. We conclude that the relative importance of pre- and post-translational mechanisms in regulating adipose tissue LPL activity depends on the duration of starvation. During short-term starvation, degradation of newly synthesized LPL is an important determinant to its secretion from the adipocyte and hence its functional activity at the capillary endothelium. J. Nutr. 128: 940–946, 1998.

KEY WORDS: acyl CoA synthetase  adipose tissue  lipoprotein lipase  rats  post-translational regulation

Lipoprotein lipase (LPL)6 catalyzes the hydrolysis of circulating triglycerides, allowing their utilization by peripheral tissues. In adipose tissue, LPL activity increases upon feeding and decreases with starvation. Thus LPL plays an important role in regulating rates of triglyceride deposition in adipocytes according to the energy needs of the organism. LPL regulation is quite complex because the enzyme is synthesized within the adipocyte and then secreted to its extracellular site of action, the luminal surface of the capillary endothelium (Braun and Severson 1992, Enerback and Gimble 1993). Despite much research attention, mechanisms of LPL regulation remain incompletely understood (Enerback and Gimble 1993).

The decrease in adipose tissue LPL activity during starvation in rats has been attributed to both pre- and post-translational mechanisms (Bergo et al. 1996, Doolittle et al. 1990, Ladu et al. 1991, Oliver and Rogers 1993). Discrepant results reported in the literature may have been due to the use of animals of different ages or to differences in experimental design and technique (Semb and Olivecrona 1989). Ladu et al. (1991) found a tight correlation between adipose tissue LPL activity and LPL mRNA levels in rats that were starved for 0, 1 and 6 d. In agreement with these results, Oliver and Rogers (1993) found that overnight starvation decreased relative rates of LPL synthesis by 50%, and Bergo et al. (1996) noted a 20–40% decrease in LPL mass in adipose tissue from rats starved overnight. However, Doolittle et al. (1990) used an experimental design in which adipose tissue from rats starved for 12 h was compared with that of refed controls (starved for 24 h and then refed with nonpurified diet supplemented with a 15% glucose solution for 12 h) to conclude that starvation increased adipose tissue LPL mRNA levels and rates of LPL synthesis.

The decreased adipose tissue LPL activity in the starved rats was explained partially by a more rapid degradation of newly synthesized LPL and partially by a decreased LPL specific activity (Doolittle et al. 1990). It is not clear from that experiment whether the conclusions were limited to the comparison between a starved and a refed group. No previous studies have analyzed the turnover and secretion of LPL in adipocytes from starved vs. freely feeding rats.

Although LPL is an important determinant of the availability of exogenous fatty acids, rates of triglyceride synthesis are also dependent on the activities of a number of other steps in this pathway. In particular, the activation of fatty acids by acyl CoA synthetase, BSA, bovine serum albumin; HR, heparin releasable; IgG, immunoglobulin G; KHB, Krebs Henseleit Buffer; LPL, lipoprotein lipase; MEM, minimum essential medium; PIA, phenylisopropylamine; SVF, stromal-vascular cell function; TCA, trichloroacetic acid; TE, total extractable.

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6 Abbreviations used: ACS, acyl CoA synthetase; BSA, bovine serum albumin; HR, heparin releasable; IgG, immunoglobulin G; KHB, Krebs Henseleit Buffer; LPL, lipoprotein lipase; MEM, minimum essential medium; PIA, phenylisopropylamine; SVF, stromal-vascular cell function; TCA, trichloroacetic acid; TE, total extractable.


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synthetase (ACS) may also be regulated by nutritional and hormonal status (Shimomura et al. 1996, Weiner et al. 1992).

The primary goal of this study was to determine the effects of variable periods of starvation (1 or 3 d) on adipocyte LPL mRNA and rates of LPL biosynthesis, degradation and secretion. We also assessed the effect of starvation on the expression of acyl CoA synthetase (ACS) mRNA levels.

MATERIALS AND METHODS

Materials. All chemicals were obtained from Sigma Chemical, St. Louis, MO, unless otherwise noted.

Animals. Male Wistar rats (4–5 wk of age) were purchased from Charles River Laboratories (Kingston, NY) and kept in our animal facility until they were 2–4 mo old as specified. The light cycle was from 0700 to 1900 h. Nonpurified diet (Ratlin Purina, St. Louis, MO) and water were available at all times unless specifically noted. For starvation experiments, food was removed at 1600–1700 h, 1 or 3 d before the experiments, but water was provided. On the day of an experiment, at ~1100 h, rats were placed under light anesthesia in a CO₂-saturated chamber and killed by decapitation. Thus, the group starved for 1 d was deprived of food for ≥18 h. All procedures were approved by the Rutgers University Animal Care Committee.

Preparation of adipocytes and analysis of mRNA levels. Preliminary data showed no differences in LPL activity between the epididymal and retroperitoneal adipose tissue; thus these two depots were pooled for subsequent analysis unless otherwise noted. Tissues from rats in fed and starved groups were processed in parallel. In experiments in which RNA was extracted from tissue and isolated cells, fat depots from two rats per group were pooled. Otherwise, tissue from individual rats was used. Adipose tissue was minced into small (30–50 mg) fragments. An aliquot (1 g) was placed in a 50-mL polypropylene test tube, immediately frozen in a dry-ice/methanol bath, and stored at −80°C until further analysis. Aliquots of tissue were taken for the quantitation of adipocyte number and tissue lipid content (DiGirolamo et al. 1971). The remaining tissue was digested for 1 h with collagenase (1 g/L, Worthington Biochemicals, Freehold, NJ) in Krebs Henseleit Buffer (KHB) (Ward and Cushman 1978), containing 30 mmol/L HEPES, 5 mmol/L glucose, 100 mmol/L phenylisopropyladrenoline (PIA) and 40 g/L bovine serum albumin (BSA, CRG7, Intergen, Purchase, NY). The digest was filtered through a 250-μm nylon mesh and the fat cells were allowed to float. The infranatant was removed and centrifuged at 14,000 g for 5 min to pellet the Stromal-vascular cells. The fat cell and Stromal fractions were then washed three times in KHB with 10 g/L BSA, and resuspended in KHB with 4 g/L BSA. After removing the wash medium, aliquots (~5 × 10⁶ cells) were immediately homogenized in guanidine isothiocyanate buffer for RNA extraction (Chomczynski and Sacchi 1987). Northern blotting and slot blotting were conducted as previously described (Fried and Zechner 1989, Fried et al. 1992) utilizing cDNA probes for human LPL, mouse ACS (Weiner et al. 1992) and 28S ribosomal RNA.

Analysis of LPL biosynthesis. These experiments were conducted essentially as previously described (Fried et al. 1992 and 1993). Aliquots of the adipocytes were resuspended in minimal essential medium (MEM) lacking methionine and cysteine but containing 4 g/L BSA, 100 nmol/L PIA and 5 mmol/L glucose. Fat cells were preincubated at 37°C for 30 min in the presence of 3.5 nmol/L insulin. At the end of this period, 3.7 Qg/L of Expensive 35S membrane isolation medium (New England Nuclear, Boston, MA) was added, and the incubation was continued for 15 or 30 min as specified in individual experiments. Because the results of the starved vs. fed comparison did not depend on the labeling period, and incorporation of label into LPL was linear for 30 min (Lee, J.-J., Smith, P. J. and Fried, S. K., unpublished observation), the data using both pulse times were pooled for statistical analysis of the effects of starvation. At the end of the pulse-labeling period, adipocytes were concentrated and separated from the medium by centrifugation through 0.4 mL of silicone oil and immediately placed in lysis buffer containing proteolytic inhibitors at 4°C (Doolittle et al. 1990, Fried et al. 1992).

Pulse-chase experiments. Cells were pulse-labeled for 15 min as described above except that in one of four experiments, no insulin was present. Data from all four experiments were placed for the relative difference in LPL half-life in fed vs. starved groups was similar in experiments with or without insulin present during the incubation. For chase incubations, after pulse-labeling, cells were washed four to five times with 20 mL of MEM, 40 g/L BSA, 100 nmol/L PIA containing 1.2 g/L methionine and 1.2 g/L cysteine (but no insulin). After resuspension in the same buffer at 10% v/v, cells were incubated at 37°C for up to 3 h. Aliquots of the cells were separated from the medium and homogenized in lysis buffer at specified times. The pulse and chase media were also saved at −80°C for immunoprecipitation (see below). Cell homogenates were sonicated, centrifuged at 14,000 × g (Eppendorf microtuge, Brinkman Instruments, Westbury, NY), and the infranatant below the fat cake and silicone oil was stored at −80°C for immunoprecipitation. Leakage of the cytosolic enzyme lactate dehydrogenase activity into the medium during adipocyte incubations was minimal (<5% of cellular values) in adipocytes of fed and starved rats, indicating that the appearance of newly synthesized LPL in the medium appeared to represent secretion (Fried et al. 1990).

Immunoprecipitation. After precipitation with 12% trichloroacetic acid (TCA) to determine incorporation of 35S label into total proteins, aliquots of cell homogenates from fed and starved rats containing equal counts (0.3–1 × 10⁶) were used for immunoprecipitation. LPL released from the fat cells into the medium (0.5- to 0.7-mL aliquots) were immunoprecipitated after the addition of 1% Triton-X-100. Immunoprecipitation was conducted as described by Doolittle et al. (1990) with the use of affinity-purified chicken anti-bovine LPL antibody (100 to 150 μg). The supernatant (1 mL) was pooled from individual rats, aliquots of each were separated from the medium and homogenized in lysis buffer at specified times. The pulse and chase media were stored at −80°C for immunoprecipitation (see below). Cell homogenates were sonicated, centrifuged at 14,000 × g (Eppendorf microtuge, Brinkman Instruments, Westbury, NY), and the infranatant below the fat cake and silicone oil was stored at −80°C for immunoprecipitation. Leakage of the cytosolic enzyme lactate dehydrogenase activity into the medium during adipocyte incubations was minimal (<5% of cellular values) in adipocytes of fed and starved rats, indicating that the appearance of newly synthesized LPL in the medium appeared to represent secretion (Fried et al. 1990).

Determination of fat cell size and number. The number of cells per milliliter fat cell suspension was determined by dividing the lipid content (DGirolamo et al. 1971) by the average fat cell weight (DiGirolamo et al. 1971; Fried and Zechner 1989, Fried et al. 1992) and 28S ribosomal RNA.

Analysis of LPL biosynthesis. These experiments were conducted essentially as previously described (Fried et al. 1992 and 1993). Aliquots of the adipocytes were resuspended in minimal essential medium (MEM) lacking methionine and cysteine but containing 4 g/L BSA, 100 nmol/L PIA and 5 mmol/L glucose. Fat cells were preincubated at 37°C for 30 min in the presence of 3.5 nmol/L insulin. At the end of this period, 3.7 Qg/L of Expensive 35S membrane isolation medium (New England Nuclear, Boston, MA) was added, and the incubation was continued for 15 or 30 min as specified in individual experiments. Because the results of the starved vs. fed comparison did not depend on the labeling period, and incorporation of label into LPL was linear for 30 min (Lee, J.-J., Smith, P. J. and Fried, S. K., unpublished observation), the data using both pulse times were pooled for statistical analysis of the effects of starvation. At the end of the pulse-labeling period, adipocytes were concentrated and separated from the medium by centrifugation through 0.4 mL of silicone oil and immediately placed in lysis buffer containing proteolytic inhibitors at 4°C (Doolittle et al. 1990, Fried et al. 1992).

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RESULTS

Animals and LPL activity. Rats (initially weighing 420 g, 4 mo old) lost an average of 30 g after 1 d and 58 g after 3 d of starvation. As shown in Table 1, starvation for 1 d decreased

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Effects of 1 or 3 d of starvation on total extractable (TE) and heparin-releasable (HR) lipoprotein lipase (LPL) activity in adipose tissue of 4-mo-old rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Fed</th>
<th>1 d Starved</th>
<th>3 d Starved</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR1</td>
<td>3.3 ± 0.8±</td>
<td>0.6± ± 0.1b</td>
<td>0.49 ± 0.1b</td>
</tr>
<tr>
<td>TE1</td>
<td>16.5 ± 3±</td>
<td>6± ± 0.9b</td>
<td>3.9 ± 0.4b</td>
</tr>
</tbody>
</table>

1 Data are means ± SEM (n = 6 per group). Values in each row with different superscripts are significantly different from one another (P < 0.05, Tukey’s test).
2 Heparin-releasable (HR) LPL activity was measured in media samples after incubation of tissue fragments with 5 × 10³ units/L heparin for 45 min at 24°C.
3 Total extractable (TE) LPL activities were measured in deoxycholate extracts of adipose tissue homogenates (epididymal plus retroperitoneal).

Slot-blot analysis of the relative abundance of lipoprotein lipase (LPL) and acyl CoA synthetase (ACS) mRNA in adipose tissue, isolated fat cells and stromal-vascular fraction from fed and starved rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Fed</th>
<th>1 d Starved</th>
<th>3 d Starved</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPL/28S</td>
<td>1.45 ± 0.24a</td>
<td>1.20 ± 0.12a</td>
<td>0.62 ± 0.05b</td>
</tr>
<tr>
<td>ACS/28S</td>
<td>1.31 ± 0.22</td>
<td>1.14 ± 0.19</td>
<td>0.94 ± 0.07</td>
</tr>
</tbody>
</table>

Isolated fat cells

<table>
<thead>
<tr>
<th>Group</th>
<th>Fed</th>
<th>1 d Starved</th>
<th>3 d Starved</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPL/28S</td>
<td>1.41 ± 0.24a</td>
<td>1.18 ± 0.09a</td>
<td>0.59 ± 0.08b</td>
</tr>
<tr>
<td>ACS/28S</td>
<td>0.87 ± 0.08</td>
<td>0.90 ± 0.12</td>
<td>0.65 ± 0.05</td>
</tr>
</tbody>
</table>

Stromal-vascular cells

1 Data are means ± SEM, n = 6 except for stromal vascular cells where n = 5. Values in a row bearing different letter superscripts are significantly different from one another (Tukey’s test), P < 0.02.
2 Varying quantities (1–8 µg) of total RNA from each sample were slot-blotted onto nylon filters and hybridized sequentially with 32P-labeled cDNAs for LPL, ACS and 28S ribosomal RNA. After quantitation by scanning laser densitometry, values for LPL and ACS within the linear range of each sample were used to calculate the ratio to the 28S probe.
3 ND, not detected.

In addition to adipocytes, adipose tissue contains other cell types, including preadipocytes. Therefore, LPL and ACS mRNA expression in RNA extracts of the stromal-vascular cell fraction (SVF) was also assessed. LPL mRNA was present in the SVF, but its expression did not change during starvation (Table 2). Furthermore, ACS was not expressed in the SVF fraction, suggesting that the cells expressing LPL mRNA in the SVF are mainly preadipocytes at a very early stage of differentiation (Amir et al. 1991).

LPL synthesis. As depicted in Figure 2 and Table 3, starvation for 1 d did not affect rates of LPL synthesis relative to rates of total protein synthesis, but significantly decreased adipocyte total protein synthesis to 56% of control values. Therefore the decrease in total extractable LPL activity to 36% of control values after 1 d of starvation appeared to be mainly accounted for by the decrease in total protein synthesis. However, the decrease in HR-LPL activity (19% of control) was proportionately greater than the decrease in total extractable LPL activity and could not be explained at the level of LPL synthesis.

Starvation for 3 d decreased rates of LPL synthesis to 44 ± 1% of control (n = 3, paired comparisons to control-fed levels, Table 3). Total adipocyte protein synthesis after 3 d of starvation also decreased to 44 ± 5% (n = 3) of fed controls. Thus the combined effects of decreased total protein synthesis and relative rates of LPL synthesis appeared to largely explain the decrease in total extractable and heparin-releasable LPL activity to 24 and 15% of control values, respectively (Table 1).

Effect of insulin on LPL synthesis in adipocytes of fed rats and rats starved for 1 d. Because pulse-labeling was conducted in the presence of insulin, it was of importance to determine whether insulin had an acute stimulatory effect on LPL synthesis in adipocytes of either fed rats or those starved.
nonimmune immunoglobulin G (IgG)].

The figure shows a fluorograph of one experiment representative of three independent replications of the experiment (Table 3) was made. The figure shows a fluorograph of one experiment representative of the four performed. Ctl, control [immunoprecipitation performed with nonimmune immunoglobulin G (IgG)].

Fed 1 day 3 day Ctl fast fast

FIGURE 2 Lipoprotein lipase (LPL) synthesis in adipocytes of fed rats and rats starved for 1 or 3 d. Adipocytes were isolated from 4-mo-old (~420 g) rats that had been starved overnight (1 d), or controls that had had free access to food (fed) until killing at 1100 h. After a 30-min preincubation with 3.5 mmol/L insulin, cells were pulse-labeled with 35S-Express label (35S-met and cys) for 30 min and immediately homogenized. Equal trichloroacetic acid (TCA)-precipitable counts were used for immunoprecipitation of cellular LPL from fed and starved rat adipocytes, followed by SDS-PAGE. A fluorograph representative of at least three independent replications of the experiment (Table 3) was made. The figure shows a fluorograph of one experiment representative of the four performed. Ctl, control [immunoprecipitation performed with nonimmune immunoglobulin G (IgG)].

for 1 d. In these experiments, adipocytes from 6- to 8-wk-old rats were used because they are more responsive to insulin than adipocytes of 4-mo-old rats (Fried et al. 1990). As shown in Table 4 and Figure 3, relative rates of LPL synthesis were similar in adipocytes isolated from younger rats that were fed or starved for 1 d, regardless of whether incubations were conducted in the absence or presence of insulin. Insulin produced a small but significant stimulation of relative rates of LPL synthesis (+38 ± 15%). In these experiments, insulin also stimulated overall protein synthesis (1.6 ± 0.05-fold and 1.6 ± 0.1-fold in adipocytes from starved and fed groups, respectively). The combination of specific and nonspecific effects of insulin resulted in an overall stimulation of LPL synthesis by 2.3 ± 0.3 (fed) and 2.3 ± 0.4-fold (starved). Thus, the inclusion of insulin during the pulse-labeling period in experiments comparing rates of LPL synthesis in fed rats and those starved for 1 d (Table 3) is unlikely to have influenced the result.

TABLE 3
Effect of starvation on adipocyte total protein synthesis and lipoprotein lipase (LPL) synthesis in 4-mo-old rats that were fed or starved for 1 or 3 d1

<table>
<thead>
<tr>
<th></th>
<th>Total protein synthesis2</th>
<th>Relative LPL synthesis3,4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n mBq/(106 cells·15 min)</td>
<td>Arbitrary units</td>
</tr>
<tr>
<td>Fed (1 d)</td>
<td>7</td>
<td>175 ± 32</td>
</tr>
<tr>
<td>Starved (1 d)</td>
<td>7</td>
<td>99 ± 29*</td>
</tr>
<tr>
<td>Fed (3 d)</td>
<td>3</td>
<td>143 ± 46</td>
</tr>
<tr>
<td>Starved (3 d)</td>
<td>3</td>
<td>51 ± 14</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM; n = 3 or 7.
2 Prepared adipocytes were pulse-labeled with 3.7 GBq/L 35S-met and cys (Express35S35S label, Dupont NEN) and incorporation into total proteins was measured by trichloroacetic acid (TCA) precipitation. Comparisons between rats starved for 1 or 3 d and controls were conducted in a paired fashion [one fed control and one starved (1 or 3 d) animal were processed in parallel on each experimental day].
3 LPL was immunoprecipitated from aliquots of adipocyte homogenates containing 106 CPM of TCA-precipitable protein. Relative rates of LPL synthesis were determined by densitometric scanning of fluorographs of SDS-PAGE gels of LPL immunoprecipitates.
4 Samples for all rats starved for 1 d and their controls were analyzed on one gel, and samples from all rats starved for 3 d and their controls were analyzed on another. Thus, the absolute values for the controls used in separate experiments cannot be compared with each other.
5 Significantly different from control by paired t test (P < 0.05).

LPL turnover. The marked decrease in HR LPL activity after 1 d of starvation was not fully explained at the level of LPL synthesis. Therefore we investigated rates of adipocyte LPL degradation using a pulse-chase protocol. To enable comparison of the rates of LPL degradation relative to total protein degradation, LPL was immunoprecipitated from aliquots of fat cell homogenates containing equal counts of TCA-precipitable protein at each time point. Total protein degradation did not differ between adipocytes of fed and starved rats (data not shown). However, specific LPL degradation was faster in adipocytes of starved compared with fed rats (Fig. 4). The sum of the rates of cell and secreted 35S-LPL remaining after a 2-h chase period in adipocytes of fed rats was 85 ± 7% (n = 5) vs. 48 ± 8% (n = 4) in adipocytes of starved rats (P < 0.01, independent t test, Fig. 4). The estimated half-life of LPL was 2 h in adipocytes of starved and 5 h in those of fed rats. Even more strikingly, only 2 ± 1% (n = 4) compared with 12 ± 4% (n = 5, P < 0.04 vs. starved) of the newly synthesized LPL was spontaneously released into the medium from adipocytes of rats starved for 1 d versus fed rats (Fig. 5). Thus, LPL degradation is more rapid in adipocytes of starved than fed rats, and LPL secretion is markedly decreased.

FIGURE 3 Effect of insulin on lipoprotein lipase (LPL) synthesis in adipocytes of rats that were fed or starved for 1 d. Adipocytes were isolated from an 8-wk-old rat that had been fed or starved for 1 d and preincubated for 30 min in the presence or absence of 3.5 mmol/L insulin; 35S-Express label was then added and the incubation continued for 15 min. The figure shows a fluorograph of the immunoprecipitated LPL typical of the data summarized in Table 3. Ctl, control immunoprecipitation.
TABLE 4

Effect of insulin on total protein and lipoprotein lipase (LPL) synthesis in rat adipocytes¹,²

<table>
<thead>
<tr>
<th></th>
<th>Total protein synthesis (kBq/g lipid)</th>
<th>LPL synthesis³ (% of total protein synthesis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Basal</td>
</tr>
<tr>
<td>Fed</td>
<td>5</td>
<td>1088 ± 102</td>
</tr>
<tr>
<td>Starved 1 d</td>
<td>3</td>
<td>633 ± 136</td>
</tr>
</tbody>
</table>

¹ Values are means ± SEM, n = 5 or 3. *P < 0.05 vs. basal, paired t test.
² Epididymal adipocytes prepared from 6- to 8-wk-old rats were incubated in MEM-albumin containing 35S-met and 35S-cysteine, in the absence or presence of 3.5 nmol/L insulin. Incorporation of label into total protein was determined by trichloroacetic acid (TCA) precipitation and expressed per gram adipocyte lipid.
³ LPL synthesis was determined by immunoprecipitation. After visualization by Fluorography, the LPL band was excised from SDS-PAGE gels and radioactivity determined by scintillation counting.

DISCUSSION

Our data demonstrate that the mechanisms regulating rat adipose tissue LPL activity vary with the duration of starvation. After a long period of starvation, alterations in LPL mRNA expression contribute importantly to the marked decreases in total and heparin-releasable adipose tissue LPL activity. In contrast, after 1 d of starvation, adipocyte LPL mRNA levels and relative rates of LPL synthesis do not change, and the decline in heparin-releasable LPL activity is partially accounted for by a decrease in total protein synthesis. Additionally, in adipocytes of rats starved for 1 d vs. those of fed rats, more newly synthesized LPL is degraded, resulting in a dramatic decrease in the amount of newly synthesized LPL that is secreted. Thus with short-term starvation, LPL degradation is an important determinant of the amount of LPL available for secretion. With more severe starvation, pretranslational mechanisms become the predominant mechanism regulating the appearance of LPL activity in an HR pool.

Pretranslational regulation of LPL activity during prolonged starvation. Our finding that LPL mRNA levels decrease with prolonged starvation is in good agreement with that of Ladu et al. (1991), who also used 4-mo-old male Wistar rats and found that starvation-induced changes in LPL mRNA were tightly correlated with the changes in LPL activity. It is apparent in the data from that study, as well as our own, that the correlation between LPL activity and mRNA levels is stronger after longer periods of starvation (3 or 6 d). Ladu et al. (1991) found a small (~20%) significant decrease in adipose tissue LPL mRNA levels after 1 d of starvation, similar to the trend in this study (~17%). It may be difficult to document a small decline in adipocyte LPL mRNA levels after overnight starvation because of the variability among rats in the timing of the last meal eaten and hence the actual length of the overnight (~1 d) starvation period.

![FIGURE 4](image-url) Degradation of newly synthesized biosynthetically labeled lipoprotein lipase (LPL) in adipocytes of rats that were fed or starved for 1 d. The figure shows a semilog plot of the disappearance of 35S-LPL (sum of immunoprecipitated cellular and secreted 35S-LPL) in pulse-chase experiments. After pulse-labeling for 15 or 30 min as described in the legend to Figure 2, aliquots of cells were immediately homogenized (pulse) or washed, then incubated in the presence of excess methionine and cysteine, but without insulin, for up to 135 min. Labeled LPL was immunoprecipitated from 0.5-mL aliquots of media samples or quantities of cell homogenates containing 10⁶ counts per minute of 35S. Samples were processed by SDS-PAGE and fluorography and the intensity of the bands was quantified by densitometry. The values for cells and media were summed to calculate the rate of disappearance of the label. Data are means ± SEM of 2–4 independent values per time point. Lines show linear fit to the mean data.

![FIGURE 5](image-url) Secretion of 35S-lipoprotein lipase (LPL) from adipocytes of rats that were fed or starved for 1 d. The percentage of the pulse-labeled LPL that was secreted into the medium at each time point of chase is plotted. To compare rates of LPL secretion during the chase period as a percentage of initial cellular values, densitometric values for 35S-LPL in the cells and medium were normalized to the number of mast cells per milliliter in the original incubation. Data were calculated from the experiments described in the legend to Figure 4. Values are means ± SEM of the indicated numbers of independent experiments.
There was a trend toward a decrease in adipocyte ACS mRNA with starvation. This effect was significant in an independent experiment with 6-mo-old rats (Lee, J.-J., Smith, P. J. and Fried, S. K., unpublished observation). ACS gene expression is increased by insulin at the transcriptional level in 3T3-L1 adipocytes (Weiner et al. 1992) but is also increased by fatty acids (Amir et al. 1991). It is possible that during starvation, increases in fatty acids minimize the decline in ACS mRNA expression despite the fall in the plasma insulin. Further experiments will be required to assess whether the activity of ACS is an important determinant of rates of esterification of exogenous fatty acids in adipocytes during starvation.

In adipose tissue, LPL mRNA is expressed predominantly in adipocytes but also in the SVF, within preadipocytes. To facilitate comparisons of rates of adipocyte LPL biosynthesis with adipocyte mRNA levels, we assessed mRNA levels in RNA extracted from isolated adipocyte and SVF cell fractions as well as total adipose tissue. Unlike adipocyte LPL mRNA levels, SVF LPL mRNA levels were not influenced by starvation. However, the quantitative contribution of the SVF LPL mRNA to total tissue expression is probably minor. Thus measurement of total adipose tissue LPL mRNA levels closely reflects adipocyte levels and is adequate for most studies.

The hormonal/substrate mediators of starvation-induced downregulation of LPL mRNA expression remain to be elucidated. Insulin stabilizes LPL mRNA levels; thus the decrease in plasma insulin levels may be one important factor (Raynolds et al. 1990). Catecholamines, which are elevated in starvation, downregulate LPL mRNA expression (Raynolds et al. 1990), although reports are conflicting (Ong et al. 1992). Fatty acids may also play a role (Kirkland et al. 1994, Montalto and Bensa-doun 1993).

Potential influences of starvation on total RNA levels and rates of protein synthesis must be considered in attempting to determine whether pretranslational mechanisms fully account for the observed changes in LPL activity. Kahn et al. (1989) estimated that RNA content per cell was decreased after starvation and that this mechanism explained the alterations in glucose transporter expression in fat cells during starvation and feeding. It is likely that alterations in total RNA content also contribute to the effects of starvation on overall protein synthesis (per cell) reported in this study. However, the nonquantitative nature of the RNA extraction procedure precludes drawing a firm conclusion on this issue. Additionally, it is possible that a decrease in the transport of amino acids into the fat cells from starved rats contributes to the decreased protein synthesis observed.

Effect of starvation on LPL synthesis. Changes in the rate of LPL synthesis per fat cell during starvation can be due to nonspecific effects on total protein synthesis as well as to alterations in relative rates of LPL synthesis (as a percentage of total protein synthesis). In agreement with earlier studies (Lyons et al. 1980, Semb and Olivecrona 1989), we found that total protein synthesis is decreased by starvation and contributes to the decreases in LPL activity. In this study, the marked decrease (70% to 90%) in total extractable adipose tissue LPL activity (expressed per adipocyte) that occurred after a severe nutritional intervention (3 d of starvation) was due to the following combination of events: 1) a specific decrease in LPL mRNA levels (50% to 60% relative to total RNA), which accounted for the decreased rates of LPL biosynthesis (50% relative to total protein synthesis) and 2) a general decrease in adipocyte protein synthesis with starvation (50% on a per cell basis). However, after a less severe period of starvation (1 d), the relative abundance of LPL mRNA and relative rates of LPL synthesis were unchanged, but there was decrease in total protein synthesis per cell (40%). In contrast to these results, Oliver and Rogers (1993) found a 50% decrease in relative LPL synthesis as well as a 19% decrease in total protein synthesis in the adipose tissue of rats starved for 1 d compared with those that were fed (age not specified).

The use of intact fragments of adipose tissue in that study (Oliver and Rogers 1993), compared with the isolated adipocytes in this study, may explain this discrepancy. Semb and Olivecrona (1989) found that adipocytes account for only 17% of total protein synthesis in guinea pig adipose tissue. If starvation has a greater effect on general protein synthesis in adipocytes vs. nonadipocytes, calculated rates of LPL synthesis relative to total tissue protein synthesis would be decreased, whereas rates of LPL synthesis relative to adipocyte protein synthesis would be unchanged. These difficulties in data analysis underscore the importance of using isolated adipocytes for studies of the nutritional regulation of LPL metabolism.

The conclusion of this study, that relative rates of LPL synthesis are not affected by starvation for 1 d, contrasts with the conclusion of Doolittle et al. (1990) that starvation increases relative rates of LPL synthesis. However, it is difficult to compare the two studies. The earlier study compared LPL expression in adipose tissue of rats starved for 12 h with that of rats starved for 24 h and refed for 12 h (Doolittle et al. 1990). Their conclusion that starvation increased LPL mRNA and synthesis may be due to the use of intact adipose tissue fragments versus isolated fat cells, as discussed above, as well as the specific refeeding paradigm used (Doolittle et al. 1990). However, using a similar refeeding protocol, Oliver and Rogers (1993) did not find differences in relative rates of LPL synthesis in adipose tissue of fed and refed rats. Further studies should include assessment of the time course of the changes in adipocyte LPL expression during starvation and refeeding to reconcile these discrepancies.

We also considered the possibility that including insulin during the adipocyte pulse-labeling had influenced the results. We used young rats in the study to maximize our chance of detecting a significant insulin effect (Fried et al. 1990). We found that insulin produced a small (~30%) but consistent stimulation of LPL synthesis in freshly isolated adipocytes of both fed and starved rats. These data confirm and extend results in primary cultures of adipocytes (Ong et al. 1988), but contrast with results in 3T3-L1 adipocytes in which insulin effects on LPL activity were not associated with changes in LPL synthetic rate (Semenkovich et al. 1989).

Post-translational regulation of adipocyte LPL during starvation. There is only one previous paper in the literature examining posttranslational LPL degradation as a function of nutritional state (Doolittle et al. 1990). They showed that, compared with refeeding, 12 h of starvation was associated with an increase in LPL degradation measured in adipose tissue fragments (Doolittle et al. 1990). Our studies show that the rate of LPL degradation is increased in starved compared with fed controls. Furthermore, by using isolated adipocytes rather than intact adipose tissue fragments, we could directly assess rates of LPL secretion. Measurement of LPL secretion is not possible with studies of adipose tissue fragments because the intact vasculature retains LPL released from the adipocyte. This work is the first to establish that alterations in the rate of spontaneous LPL secretion occur in concert with alterations in LPL degradation in rat adipocytes.

The half-life of LPL in this study of freshly isolated adipocytes (~2 h in starved rats, ~5 h in fed rats) is longer than that reported by others using isolated adipocytes that had been placed in primary culture overnight (~1 h) (Ong et al. 1992, Simsolo et al. 1992). The reason for this discrepancy is un-
known, but it will be important to investigate the hormonal and metabolic factors regulating LPL turnover in fresh compared with cultured adipocytes.

It is also as yet unclear which hormones or substrates mediate the effect of starvation of LPL synthesis, degradation/secretion. In the fed state, LPL degradation may be slowed by the combination of insulin and glucocorticoid, similar to results from studies of human adipose tissue in vitro (Appel and Fried 1992). Catecholamines and insulin are also likely to play roles, but the literature is still inconsistent with regard to specific effects of these hormones on different steps in LPL synthesis and secretion (Enerback and Gumble 1993). It does not appear that starvation affects the translation of LPL mRNA, as reported for treatment with epinephrine (Ong et al. 1992, Ranganathan et al. 1997) because, in our studies, there was a parallel decline in LPL mRNA levels and rates of LPL biosynthesis.

Alterations in LPL specific activity have been reported to be at least a partial explanation for the changes in LPL activity that occur after starvation (Bergo et al. 1996, Doolittle et al. 1990). Bergo et al. (1996) recently reported that LPL activity decreased by 70–80% and LPL mass decreased by 20–40% in the adipose tissue of rats starved for 1 d compared with fed rats (Bergo et al. 1996). The decreased LPL specific activity in adipose tissue of starved rats was associated with an increase in monomeric (inactive) LPL. We did not directly measure LPL specific activity in this study; thus we cannot rule out its contribution to the decreased LPL activity. However, the alterations in LPL activity after both 1 and 3 d of starvation appear to be explained largely by the combination of specific alterations in LPL synthesis (associated with changes in LPL mRNA expression), alterations in LPL degradation (after 1 d of starvation) and a generalized decrease in adipocyte protein synthesis.

In summary, this work clarifies some discrepancies in the literature concerning whether starvation alters LPL mRNA levels and rates of LPL synthesis at the level of the adipocytes. After longer periods of starvation (3 d), the decrease in LPL activity results from a combination of a specific decrease in LPL mRNA levels and LPL synthesis and a decrease in overall protein synthesis. After relatively short periods of starvation (overnight), the decrease in extracellular, HR LPL activity appears to be accounted for by an increase in the rate of LPL degradation that leads to a decrease in the LPL activity secreted. Our results underline the utility of using freshly isolated adipocytes for studies of the regulation of LPL biosynthesis and secretion. Furthermore, these data provide a physiologically important example supporting the hypothesis that degradation is an important point in the regulation of LPL activity (Cisar et al. 1989, Semb and Oliveira 1989).

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LITERATURE CITED