Down-regulation of Adipose Tissue Lipoprotein Lipase during Fasting Requires That a Gene, Separate from the Lipase Gene, Is Switched On*

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During short term fasting, lipoprotein lipase (LPL) activity in rat adipose tissue is rapidly down-regulated. This down-regulation occurs on a posttranslational level; it is not accompanied by changes in LPL mRNA or protein levels. The LPL activity can be restored within 4 h by refeeding. Previously, we showed that during fasting there is a shift in the distribution of lipase protein toward an inactive form with low heparin affinity. To study the nature of the regulatory mechanism, we determined the in vivo turnover of LPL activity, protein mass, and mRNA in rat adipose tissue. When protein synthesis was inhibited with cycloheximide, LPL activity and protein mass decreased rapidly and in parallel with half-lives of around 2 h, and the effect of refeeding was blocked. This indicates that maintaining high levels of LPL activity requires continuous synthesis of new enzyme protein. When transcription was inhibited by actinomycin, LPL mRNA decreased with half-lives of 13.3 and 16.8 h in the fasted and fasted states, respectively, demonstrating slow turnover of the LPL transcript. Surprisingly, when actinomycin was given to fed rats, LPL activity was not down-regulated during fasting, indicating that actinomycin interferes with the transcription of a gene that blocks the activation of newly synthesized LPL protein. When actinomycin was given to fasted rats, LPL activity increased 4-fold within 6 h, even in the absence of refeeding. The same effect was seen with α-amanitin, another inhibitor of transcription. The response to actinomycin was much less pronounced in aging rats, which are obese and insulin-resistant. These data suggest a default state where LPL protein is synthesized on a relatively stable mRNA and is processed into its active form. During fasting, a gene is switched on whose product prevents the enzyme from becoming active even though synthesis of LPL protein continues unabated.

Lipoprotein lipase (LPL)1 plays an important physiological role in regulating the release of fatty acids from triglyceride-rich lipoproteins (1–4). The enzyme is synthesized in several extrahepatic tissues, but the highest activities are found in tissues that oxidize (e.g. heart and red skeletal muscle) or store (e.g. adipose tissue) fatty acids. After glycosylation, dimerization, and activation in the endoplasmic reticulum, the enzyme is secreted, transported through the extracellular matrix, and anchored to heparan sulfate proteoglycans on the intraluminal surface of nearby capillaries (3, 4).

LPL is regulated at the level of gene expression in several physiological states: during fetal and early postnatal life, the enzyme is absent in the liver but is then suppressed (5); in the mammary gland, the enzyme is switched on during lactation (6); in macrophages, it is switched on when the cells are activated (7); and in brown adipose tissue, the enzyme is switched on during cold adaptation (8). In white adipose tissue, LPL activity changes during the day according to the nutritional state. This appears to be mediated by posttranscriptional mechanisms. During short term fasting, LPL activity in rat adipose tissue decreases without corresponding changes in the levels of mRNA and protein mass, thereby reducing the specific activity (activity/protein mass ratio) (18, 20–22). We have found that this occurs because the distribution of lipase protein shifts toward an inactive form (9) and that refeeding for 4 h can restore the suppressed activity (10). The signal and mechanism for these changes in the activity status of the enzyme are unknown.

To delineate the regulatory mechanism, we determined the turnover rates for LPL mRNA, mass, and activity in rat adipose tissue in the fed and fasted states and explored whether the regulation during feeding, fasting, and refeeding requires synthesis of new mRNA or protein. The results point to a novel mechanism and a new perspective on the nutritional regulation of LPL in adipose tissue.

MATERIALS AND METHODS

Animals—Male Sprague-Dawley rats (23 days old) weighing around 60 g were bought from Mollegaard Breeding Center (Ejby, Denmark). After transport to Umeå they were allowed to acclimatize for 7–10 days; during this time, they reached a weight of ~120 g. The rats were kept in a well ventilated room at 21 °C and 40–50% humidity and had free access to standard laboratory chow (Laktamin AB, Stockholm, Sweden) and tap water. The light in the room was on between 6 a.m. and 6 p.m. In the fasting experiments, food was withdrawn from the cages at 6 a.m., and a grid was placed at the bottom of the cages to prevent coprophagia. In the refeeding experiments, the rats were put back on standard chow at 6 a.m. after a 24 h fast, and were killed at 12 noon by cervical dislocation. The adipose depot used in all experiments was peripéridydiad lipoprotein adipose tissue. The animal ethics committee in Umeå approved all animal experiments.

Materials—Actinomycin-D, cycloheximide, bovine serum albumin, aprotonin (TrasyloI), and HEPES were from Sigma. Leupeptin and pepstatin were from the Peptide Institute (Osaka, Japan). Heparin was from Lövens (Malmö, Sweden). The substrate for the LPL activity assay was 3H-labeled triolein in Intralipid (10%) from Amersham Biosciences, Inc. Parker medium (Parker 199) was from SBL (Stockholm, Sweden). All other reagents were of the highest commercial grade possible. Ac-


**TABLE I**

| Treatment | LPL activity (mU/μg DNA) | LPL mass (ng/μg DNA) |
|-----------|--------------------------|----------------------|
| Fed       | 4.67 ± 0.20              | 14.81 ± 0.66         |
| Fasted    | 1.17 ± 0.04              | 12.33 ± 0.47         |
| Fed + 50 μg cytochalasin D | 5.00 ± 0.62 | 12.60 ± 1.37 |
| Fasted + 50 μg cytochalasin D | 0.16 ± 0.03 | 1.54 ± 0.19 |
| Fed + 100 μg cytochalasin D | 4.67 ± 0.60 | 12.60 ± 1.49 |
| Fasted + 100 μg cytochalasin D | 4.56 ± 0.13 | 10.11 ± 0.42 |
| Fasted + 100 μg cytochalasin D | 0.13 ± 0.06 | 1.38 ± 0.28 |

**NUTRITIONAL REGULATION OF LIPOPROTEIN LIPASE**

**RESULTS**

**Up-regulation of LPL Activity on Refeeding Requires Synthesis of New Protein**—Fasting for 24 h reduced LPL activity to 25% of fed control (p < 0.001), whereas LPL protein mass decreased only to 83% (p < 0.05) (Table I). After refeeding for 6 h, LPL activity had increased 4.3-fold to 107% of fed control, but LPL protein mass remained unchanged. In rats that had been given cycloheximide to inhibit protein synthesis, both LPL activity and protein mass dropped sharply despite refeeding (Table I). These data indicate that LPL protein is turned over rapidly and that the up-regulation of LPL during refeeding requires synthesis of new protein.

To further analyze the turnover of LPL we studied fed rats, which have high LPL activity in their adipose tissue (Fig. 1). LPL activity and protein mass dropped to 40–50% of control within 2 h after the administration of cycloheximide and then decreased more slowly, reaching 20% of control at 6 h. These data demonstrate that after the block of protein synthesis by cycloheximide, the half-lives of LPL activity and protein mass in adipose tissue are less than 2 h and that LPL activity decreases as a result of protein turnover and not because the enzyme protein loses its catalytic activity.

**Down-regulation of LPL Activity on Fasting, but Not Up-regulation on Refeeding, Requires Synthesis of New mRNA**—In these experiments, we used actinomycin-D, which inhibits DNA-dependent mRNA synthesis. When 24-h fasted rats were injected with actinomycin and then refeed, LPL activity increased to the same level as in fed rats (Table I). LPL protein mass did not change significantly. Hence, up-regulation of LPL activity during refeeding does not require synthesis of new mRNA. Next, we determined whether down-regulation of LPL activity during fasting requires synthesis of new mRNA. Fed rats were injected with actinomycin or saline, and food was removed from the cages (Fig. 2). Eighteen h later, LPL-specific activity in adipose tissue from control rats (saline-injected) had decreased, as expected, to 30% of fed control. Surprisingly, in adipose tissue from actinomycin-injected rats, LPL-specific activity was slightly increased (140% of fed, p < 0.05). These findings show that the down-regulation of LPL-specific activity during fasting requires synthesis of new mRNA and suggest that, during fasting, a gene is switched on whose product prevents LPL from becoming active even though synthesis of LPL protein continues unabated. We predicted that if we injected actinomycin into 24-h fasted rats with low adipose tissue LPL activity, we would turn off this proposed inhibitory gene, allowing active LPL to be formed again. Our prediction was upheld; 6 h after injection of actinomycin into fasted rats, LPL activity in adipose tissue had increased almost 4-fold without refeeding (Table I), with no statistically significant change in LPL protein mass. These data indicate that turning off transcription with actinomycin could reproduce the signal that causes up-regulation of LPL activity on refeeding.

To test the possibility that the increase in LPL activity by actinomycin might be caused by a paradoxical effect on the LPL protein by the actinomycin molecule itself, we performed two control experiments. First, we determined whether actinomycin affected LPL activity when added to tissue homogenates *in vitro*. For this, actinomycin was added at concentrations of 5, 10, 50, and 100 μg/ml either to homogenates of adipose tissue from fasted rats before assay of LPL activity or directly to the activity assay medium. *In vitro* addition of actinomycin had no...
The Effect of Actinomycin on LPL Activity in Adipose Tissue—Previously, we showed that in adipose tissue from fed rats were 4.51 mg) in adipose tissue increased from 0.14 in the fasted group to later, the rats were killed, and adipose tissue LPL activity and one was injected with saline only (fasted controls). Six h /H11006 and 13.9 p (0.35 milliunit/mg, [50x188]p
[50x156]tion,
[50x146]shown). These results indicate that the effect of actinomycin on
[50x230]effect on LPL activity (data not shown). Second, we performed experiments with another inhibitor of DNA-dependent RNA synthesis, α-amanitin. Three groups of rats were fasted for 24 h. Then, one group was given free access to food (refed), one was injected with α-amanitin (3 mg/kg body weight in saline), and one was injected with saline only (fasted controls). Six h later, the rats were killed, and adipose tissue LPL activity and protein mass were determined. LPL-specific activity (milliunit/mg) in adipose tissue increased from 0.14 in the fasted group to 0.40 in the refed group as expected on refeeding. In the α-amanitin-treated group, LPL specific activity also increased (0.35 milliunit/mg, p < 0.001 versus fasted controls, data not shown). These results indicate that the effect of actinomycin on LPL activity in adipose tissue was not a spurious finding; it could be reproduced with an unrelated inhibitor of transcription, α-amanitin.

The Effect of Actinomycin on LPL Activity in Adipose Tissue Is Blunted after Prolonged Fasting—Previously, we showed that after 24 h of fasting, LPL activity in adipose tissue is reduced, but the level of LPL mRNA remains the same as in the fed state; however, after 60 h of fasting, the mRNA level decreases significantly to less than 50% of fed control (10). At this point, the effect of refeeding is blunted. In the following experiment, we tested whether the effect of actinomycin was simi-
injection. LPL activity in preheparin plasma increased 2-fold \( (p < 0.001) \). There were no statistically significant changes in hepatic lipase activity as measured in liver homogenate or in postheparin plasma (data not shown).

**Actinomycin Changes the Ratio between Inactive and Active Forms of LPL**—Previously, we showed that there are two forms of LPL protein in tissues that can be separated by heparin-Sepharose columns. In the fasted state, the inactive form with low heparin affinity predominates. Upon refeeding, the distribution of lipase protein shifts from the inactive toward the active form \((9)\), which has a high affinity for heparin. To further test whether actinomycin had an effect similar to that of refeeding, three rats were fasted for 24 h and then injected with actinomycin. Six h later, epididymal adipose tissue was taken and prepared as described under “Materials and Methods.” The homogenates were pooled and applied to a heparin-Sepharose column \((A)\). For comparison, pooled homogenates from three 24-h fasted rats were also separated on heparin-Sepharose \((B)\). LPL protein was eluted with a salt gradient, and the fractions were assayed for LPL activity \((\mathbb{A})\) and mass \((\mathbb{B})\).

The Effect of Actinomycin Is Blunted in Aging Rats—We have previously shown that the mechanism that channels LPL toward the inactive form on fasting is blunted in adipose tissue from aging, obese rats \((14)\). If actinomycin interferes with the same mechanism, its effect should also be blunted in aging rats. To test this hypothesis, rats weighing 505 \( \pm \) 6 g were fasted for 24 h and then injected with actinomycin \((24)\). In response to actinomycin, LPL activity increased to 149\% of saline-injected control \((p < 0.01)\). This was in sharp contrast to the young rats \((126 \pm 2 \text{ g})\), where LPL activity was up-regulated to 485\% of control \((p < 0.001)\). These data show that the response of adipose tissue LPL to actinomycin is blunted in aging rats.

**DISCUSSION**

This study shows that in fasting rats, a gene is switched on in adipose tissue that makes the tissue produce an inactive form of LPL. Inhibition of mRNA synthesis by actinomycin completely blocked the down-regulation of LPL activity in adipose tissue during fasting. Administration of actinomycin or \(\alpha\)-amanitin, another inhibitor of transcription, to fasted rats completely restored LPL activity within 6 h.

One potential explanation for our results is that actinomycin administered \textit{in vivo} changes the levels of a circulating hormone or changes nerve signaling activity to the adipose tissue and that the effect on LPL activity is secondary. Studies from the 1960s indicate that this is not the case. In these studies,
actinomycin caused a dramatic increase in LPL activity in whole fat pads from fasted rats incubated ex vivo (15, 16). This clearly indicates that the effect of actinomycin is a direct action in the adipose tissue, rather than secondary to changes in nerve signaling or in circulating hormones.

Several studies have shown that N-linked glycosylation and proper processing of the glycan chains are important steps in the formation of active, secretable LPL with high heparin affinity (1, 17–21). Specifically, the trimming of glucose residues by glucosidases in the endoplasmic reticulum seems to be a crucial step for the attainment of activity (19–21). Glycosylated proteins that have been processed in the cis-Golgi have glycan chains that are resistant to cleavage by the enzyme endoglycosidase-H (Endo-H). Doolittle et al. showed that the Endo-H-resistant form of the LPL protein predominates in adipose tissue of fed rats, whereas the Endo-H-sensitive form predominates in adipose tissue of fasted rats (18). We have found that the catalytically active form of LPL, purified on heparin-Sepharose, is mainly resistant to Endo-H, whereas the inactive form is Endo-H-sensitive. These data suggest that the mechanism for down-regulation of LPL during fasting affects the intracellular processing or trafficking of newly synthesized LPL, probably in the endoplasmic reticulum or early Golgi. Further evidence for a specific mechanism for processing of the enzyme comes from studies on mice affected by the combined lipase deficiency mutation. LPL in adipocytes from these mice is retained in the endoplasmic reticulum in an inactive form with glycan chains that are sensitive to Endo-H (22, 23). An interesting possibility is that this genetic defect is related to the mechanism that down-regulates LPL on fasting.

By injecting cycloheximide to inhibit protein synthesis, we could follow the turnover of the existing LPL molecules. LPL activity and mass in adipose tissue from both fed and fasted rats decreased rapidly and in parallel, with a half-life of less than 2 h. This means that turnover of LPL activity is directly associated with turnover of LPL protein. There are two possible pathways for this turnover: LPL protein may be degraded within the adipose tissue or the enzyme may be transported with blood for degradation in the liver (24, 25).

The turnover of LPL mRNA seems to occur slowly under most conditions. During long term fasting the half-life has been reported to be more than 24 h in white adipose tissue (10) and 40 h in brown adipose tissue (26). During weight loss in obese Zucker rats (27) and during improved diabetes control in humans (28), LPL mRNA turnover was also found to be slow. In the present study, the half-life of LPL mRNA was 13.3 h in the fed state. During cold adaptation in rat brown adipose tissue, LPL activity increases significantly, mediated by increased transcription of the LPL gene; upon return to warm conditions, a specific RNA-degrading enzyme is induced that accelerates the turnover of LPL mRNA (26). Most likely this mechanism exists also in white adipose tissue since tumor necrosis factor α can accelerate the breakdown of LPL mRNA (29). However, this mechanism does not seem to act during fasting since the turnover of LPL mRNA did not change (half-life = 16.8 h). For this reason, mRNA was available for continuous synthesis of LPL protein even after the animals had been given actinomycin. With such a stable mRNA, it is not possible for the cell to rapidly regulate LPL activity on a transcriptional level. Instead, the regulation must be mediated on the translational or posttranslational level.

Catecholamines exert a strong effect on lipid homeostasis in adipose tissue by increasing hormone-sensitive lipase activity and by decreasing LPL activity. In an elegant study, Ranganathan et al. showed that the effect of epinephrine on LPL in cultured adipocytes is mediated by a protein that binds to the LPL transcript and effectively blocks its translation (30). This raises the question whether the same RNA-binding protein causes the down-regulation of LPL activity on fasting. If this were true, the positive effect of actinomycin on LPL activity could be to block the transcription of this protein. Three lines of evidence indicate that this is not the case. First, most studies indicate that the mechanism for down-regulation of LPL during short term fasting is posttranslational and that the levels of LPL protein change very little (9, 18, 20, 22). If a block in LPL synthesis mediated the decrease in LPL activity on fasting there would have to be an associated block in LPL degradation. The present study shows that this is clearly not the case; the turnover of LPL protein was the same in the fed and in the fasted states. Second, Wing et al. observed an increase in LPL activity in rat fat pads incubated in the presence of actinomycin (16). This effect was blocked by epinephrine, most likely by inducing the LPL mRNA-binding protein. We have preliminary evidence that this occurs also in vivo. The increase in LPL activity in adipose tissue after actinomycin administration was attenuated when rats were given epinephrine, which also caused a reduction in LPL protein mass. This indicates that the RNA-binding protein is activated posttranscriptionally by epinephrine, since actinomycin would inhibit synthesis of its mRNA. Third, it is unlikely that a translational inhibition of LPL followed by a decreased degradation would lead to a change in the structure of glycan chains in the remaining LPL molecules.

The nutritional regulation of LPL in adipose tissue has been viewed as having a default state characterized by a low, basal LPL activity that can be temporarily up-regulated after meals. Our findings in this study suggest another perspective on the regulation: the default (fed) state is characterized by a high LPL activity and mass synthesized from a stable mRNA where the newly synthesized LPL is processed mainly into the active form. The enzyme is then secreted and transported to nearby capillaries where it acts on lipoproteins. This pathway seems to predominate in most tissues that synthesize LPL (e.g., heart and skeletal muscle). During periods of caloric restriction (i.e., between meals or during fasting), LPL mRNA and protein mass remain high; however, LPL activity in adipose tissue can be suppressed by the induction of a short-lived gene product.

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2 M. Bergö, G. Wu, T. Ruge, and T. Olivecrona, unpublished observations.
that causes newly synthesized LPL to be channeled into an inactive form. Refeeding or administration of actinomycin inhibits the expression of this putative factor, allowing active LPL to be formed.

Why is there a need to down-regulate LPL during fasting? The action of adipose LPL on circulating triglycerides results in the generation of fatty acids that can either be taken up by the tissue or be released into the blood in albumin-bound form. The ratio between these two pathways varies greatly in human adipose tissue (31), from predominantly storage after a large meal to predominantly release into the circulating blood during fasting. Irrespective of the fate of the generated fatty acids, the down-regulation of LPL during fasting will decrease the hydrolysis of circulating triglycerides that pass through the adipose tissue. Failure to down-regulate LPL could contribute to the excessive generation of albumin-bound fatty acids seen in individuals with insulin resistance, or it could contribute to an increased deposition of fat in adipose tissue. Consistent with this reasoning, Ong et al. found that LPL-specific activity in adipose tissue during fasting was higher in obese subjects than in lean controls (32). Furthermore, in obese subjects, LPL-specific activity was unresponsive to a meal (refeeding). Our findings suggest that this apparent unresponsiveness to a meal may in fact reflect that LPL activity in these subjects was not down-regulated on fasting period. If so, this would be similar to what we have found in aging, obese rats where LPL-specific activity failed to be down-regulated on fasting (14). This raises the intriguing possibility that switching on and off the gene that regulates LPL activity in adipose tissue may be deranged in obesity, insulin resistance, and related metabolic disorders.

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