The regulation of adipose tissue lipoprotein lipase (LPL) was examined in rats fed or fasted overnight, and was found to be controlled posttranslationally. LPL catalytic activity decreased by 50% after fasting while LPL mRNA levels and rates of synthesis increased nearly 2-fold; enzyme mass remained unchanged. The distribution of LPL within the endoplasmic reticulum (ER) and Golgi/post-Golgi secretory pathway was assessed by differentiating between LPL high mannose and complex forms. After fasting, the majority of LPL is in the high mannose ER form (65%, 0.97 μg/g wet weight tissue), whereas the LPL complex form comprises only 35% (or 0.52 μg/g). After refeeding, however, the Golgi-derived LPL complex form predominates (65%, 1.03 μg/g) over the high mannose ER form (35%, 0.55 μg/g). Kinetic analysis suggests that high mannose LPL disappears with a half-life of t₀.5 = 40 min in both fed and fasted rats, indicating that the redistribution of I.P.L. mass during feeding/fasting does not arise by retention relative to the Golgi apparatus. Instead, the fractional catabolic rate of complex LPL within the Golgi/post-Golgi secretory compartment can be calculated to be 3.5-fold greater in fasting. In heart, changes in LPL activity in response to feeding/fasting are also not due to differences in mRNA levels or rates of synthesis. Based on these findings, a model of LPL posttranslational regulation is proposed and discussed.

Lipoprotein lipase (LPL; EC 3.1.1.34) is a N-linked glycoprotein secreted by parenchymal cells from a variety of extrahepatic tissues, principally muscle and adipose tissue (1). The secreted enzyme, bound at the surface of capillary endothelium (2) and called "functional LPL," is involved in the hydrolysis of triglycerides from circulating chylomicrons and very low density lipoproteins. Postprandially, LPL activity is elevated in adipose tissue compared with heart and muscle, resulting in the channeling of circulating triglyceride fatty acids into lipid depots. During fasting, the inverse is true; relatively high heart and muscle LPL activities redirect triglyceride fatty acids appropriately into these tissues and away from adipose stores (1, 3). Obviously, the coordinated regulation of LPL in adipose tissue and muscle during feeding/fasting is critical for maintaining triglyceride homeostasis. For example, in obesity, overexpression of adipose tissue LPL may contribute to increased triglyceride deposition (4).

In chicken (5) and guinea pig (6), a prolonged 48-h fasting period was observed to reduce, in parallel, adipose tissue LPL activity and mRNA levels. However, insulin, which is believed to be the major effector of LPL postprandially (1), increases LPL activity in 3T3-L1 adipocytes, whereas LPL synthetic rate decreases (7). Indeed, many possible mechanisms of regulating LPL at posttranslational levels have been reported: e.g. catalytic activity may depend on the acquisition and complete processing of LPL's N-linked oligosaccharide chains (8–10); newly synthesized LPL is degraded rather than secreted in the absence of heparin (11–15); and, insulin-enhanced I.P.L. secretion may depend on a phospholipase that releases LPL from a membrane bound glycosyl phosphatidylinositol anchor (16).

Thus, the role of LPL transcription and translation in regulating adipose tissue and heart LPL activity and mass levels was investigated in rats fasted or refeed over a period of 12 h. This period of time was chosen since most animals undergo a natural feeding/fasting cycle of approximately 1/2 day. In addition, to verify posttranslational changes in the cellular distribution and kinetics of adipose tissue LPL in response to feeding and fasting, glycosidase sensitivity was used to distinguish between LPL high mannose and complex forms. These two forms represent the products of a series of N-linked oligosaccharide processing events that are known to occur within the ER and Golgi apparatus, respectively (17); thus, glycosidase sensitivity was used as a means of locating LPL to these organelles (18).

**EXPERIMENTAL PROCEDURES**

**RESULTS**

Adipose Tissue LPL mRNA Levels and Rates of Synthesis after Feeding and Fasting—Northern blots of total adipose tissue RNA (Fig. 3) hybridized to a mouse LPL cDNA clone showed a single mRNA species at 3.6 kilobases. Scanning...
3.6 kilobases. tu-Actin did not change significantly (>0.05) in heart or adipose tissue, determined on the same blot with a rat actin cDNA probe. The LPL mRNA size is under the two nutritional conditions. The rate of synthesis is expressed per g wet weight tissue. mRNA levels are expressed relative to cu-actin, determined on the same blot with a rat actin cDNA probe. under the two nutritional conditions. The rate of synthesis is expressed as a percentage of counts/min incorporated into LPL compared with counts/min incorporated into total acid-precipitable protein following a 20-min pulse of [35S]methionine. The specific activity is expressed as units/mg LPL protein, calculated from the activity and mass data in rows 1 and 4.

Table I

| LPL | Fasted | Fed | Fast/Fed |
|-----|--------|-----|---------|
| Activity (milliunits/g) | 709 ± 34.0 | 1630 ± 104* | 0.44 |
| mRNA (arbitrary units) | 95.1 ± 5.53 | 56.0 ± 6.45a | 1.70 |
| Rate of synthesis (%) | 0.318 ± 0.030 | 0.176 ± 0.011† | 1.80 |
| Mass (µg/g) | 1.50 ± 0.108 | 1.69 ± 0.074* | 0.94 |
| Specific activity (units/mg) | 29,200 | 62,000 | 0.47 |

* Differences between fasted and fed are significant at the p < 0.005 level.
† Difference between fasted and fed is not significant (p > 0.05).

densitometry of Northern blots revealed that changes in mRNA levels following feeding or fasting were parallel to changes in LPL synthetic rates, measured by incorporation of [35S]methionine (Table I). However, although LPL mRNA levels and synthetic rates increased nearly 2-fold after fasting, activity decreased by over 50%. Similarly, enzyme mass did not accompany activity loss after fasting, resulting in a 50% decrease in specific activity (units/mg LPL protein). The nature of the changes mentioned above was very reproducible and a typical response of adipose tissue LPL to 12-h feeding and fasting is shown in Table I. Since LPL mRNA levels, synthetic rates, and enzyme mass could not account for changes in activity, LPL regulation in response to 12-h feeding/fasting must be controlled posttranslationally.

Characterization of Rat Adipose Tissue LPL by Endo H Digestion—Only the high mannose form of N-linked glycoproteins is susceptible to Endo H digestion, leaving the protein intact but unglycosylated (19) and resulting in a reduction in molecular mass resolvable by SDS gel electrophoresis. Endo H sensitivity was thus utilized to distinguish between the LPL high mannose form in the ER and LPL contained within the Golgi/post-Golgi secretory pathway.

Mouse LPL contains potential N-linked glycosylation sites with the consensus sequence Asn-X-Ser(Thr), where X is any amino acid but proline (21, 22), and both of these sites are utilized for glycosylation. Likewise, rat LPL contains two glycan groups/protein molecule, as ascertained by partial glycosidase digestion (18, 23) of LPL immunoprecipitated (data not shown). However, not all high mannose chains in LPL are processed to the complex form in rat adipose tissue. For example, Fig. 4A shows that a 12-h Endo H digestion of LPL immunoprecipitated from adipose tissue pulse-labeled for 10 min and chased for 40 min results in three distinct molecular mass products: the complex, Endo H-resistant form (56 kDa); the fully Endo H-sensitive high mannose form (50 kDa); and a partially Endo H-sensitive intermediate form (53 kDa). This result suggested that the intermediate form represents a “hybrid” LPL molecule, containing one high mannose and one complex chain. This was found to be the case by examining the sensitivity of the intermediate form to neuraminidase, which cleaves the terminal sialic acid residue added in the trans-Golgi. Like the complex form, the intermediate form is sensitive to neuraminidase cleavage (Fig. 4A; note the slight decrease in molecular mass of both complex and intermediate forms compared to untreated lysates). The term “end point” digestion designates these conditions of Endo H digestion, since all susceptible oligomannosyl residues are cleaved (data not shown). A, treatment of LPL immunoprecipitates with both neuraminidase and Endo H. The LPL bands referred to as “intermediate” and “high mannose” are the products of Endo H cleavage; the slight decrease in molecular mass of the complex and intermediate forms is due to neuraminidase treatment. B, fractionation of high mannose and intermediate/complex forms of LPL by β-ricin chromatography. The neuraminidase-treated lysate was fractionated as described under “Experimental Procedures.” Column samples comprising the flow-through (1–4) and galactose-eluted (5–8) fractions were subjected to LPL immunoprecipitation and end point Endo H digestion as described above. Shown is the fluorogram of the SDS polyacrylamide gel. The LPL bands referred to as “intermediate” and “high mannose” are the products of Endo H cleavage; the slight decrease in molecular mass of the complex and intermediate forms is due to neuraminidase treatment. B, fractionation of high mannose and intermediate/complex forms of LPL by β-ricin chromatography. The neuraminidase-treated lysate was fractionated as described under “Experimental Procedures.” Column samples comprising the flow-through (1–4) and galactose-eluted (5–8) fractions were subjected to LPL immunoprecipitation and end point Endo H digestion as described above. Shown is the fluorogram of the SDS polyacrylamide gel. The complex form of LPL has a molecular mass of 56 kDa.

3 C. Ailihart, personal communication.
with high mannose after neuraminidase treatment).

The nature of the Endo H cleavage products was confirmed by β-ricin affinity chromatography. β-Ricin binds the penultimate galactose residue of complex carbohydrate chains (24); like sialic acid, this galactose residue is added only in trans-Golgi. A sample from the adipose tissue lysate shown in Fig. 4A was passed through the column. The flow-through (fractions 1–4) and galactose-elicited fractions 5–8 were subjected to LPL immunoprecipitation and Endo H cleavage (Fig. 4B). Clearly, the unbound LPL present in the flow-through is fully Endo-H-sensitive, representing the high mannose form. In contrast, both the intermediate and complex forms are bound and subsequently eluted with galactose. Since processing of the oligomannosyl chain to its complex form is completed in the trans-Golgi, both LPL intermediate and complex forms in rat adipose tissue are present in, or have passed through, this organelle.

Pulse/chase analysis (Fig. 5) indicated that the high mannose form is the precursor to the intermediate and complex forms. Although a minor fraction of the LPL intermediate form may serve as a precursor to the complex form (see Fig. 5), the majority appears to be secreted, since at least 50% of radiolabeled LPL in the media of rat adipocytes is partially Endo H-sensitive (data not shown).

Distribution of Adipose Tissue LPL Mass after Feeding or Fasting—LPL high mannose, intermediate, and complex forms in adipose tissue from fed and fasted animals were determined to examine changes in the cellular distribution. Although incorporation of [35S]methionine into total adipose tissue proteins following feeding/fasting was linear for at least 120 min, incorporation of label into LPL reached steady state (i.e. when LPL radioactivity and mass are equivalent) after half that time (Fig. 6A). After 80 min, Endo H cleavage of LPL immunoprecipitates (Fig. 6B) clearly shows that changes observed in LPL activity after feeding or fasting (Table I) are accompanied by changes in the distribution of lipase mass. Scanning densitometry of Fig. 6B indicates that after fasting, this distribution is 65% high mannose and 35% intermediate/complex. Following refeeding, this 65:35 distribution inverts in favor of the intermediate/complex form. The absolute value of LPL mass represented by these forms can be calculated by multiplying the steady-state distribution percentage by the total LPL mass determined by enzyme-linked immunosorbent assay. Thus, following 12 h of fasting, the LPL mass in the high mannose and intermediate/complex forms are 0.97 and 0.52 µg/g wet weight tissue, respectively; after refeeding, the distribution becomes 0.55 and 1.03 µg/g, respectively (see Fig. 8).

Turnover Rates of LPL High Mannose and Complex Forms after Feeding and Fasting—During pulse/chase experiments, total adipose tissue LPL mass remained constant. Turnover rates of the LPL high mannose form measured in adipose tissue from fed or fasted rats were identical, with a t½ = 40 min or fractional turnover rate (FTR) of 0.75 (h⁻¹) (Fig. 7A). Thus, the larger mass of the high mannose form following fasting (0.97 versus 0.55 µg) can be attributed to the proportional increase in the LPL synthetic rate and is not due to selective retention within the ER (see also Fig. 8). In contrast, the kinetics of the complex form are quite different. As shown in Fig. 7B, in fed animals, LPL radioactivity (represented as a fraction of the initial 10-min chase point) peaks approximately 20–30 min after initiation of the chase, after which it disappears steadily. A different kinetic pattern, however, is observed in fasted animals: maximal LPL radioactivity appears to have been reached very rapidly, as evidenced by the lack of a peak prior to the disapppearing phase of the curve. Attainment of peak radioactivity appears to have been reached very rapidly, as evidenced by the lack of a peak prior to the disappearing phase of the curve. Turnover of peak radioactivity appears to have been reached very rapidly, as evidenced by the lack of a peak prior to the disappearing phase of the curve. Thus, the calculated fractional catabolic rate (FCR) following fasting was 1.40 (h⁻¹) compared with 0.40 (h⁻¹) after refeeding, a difference of 3.5-fold (see Fig. 8).

Evidence for Posttranslational Regulation of Heart LPL—Adipose tissue and heart LPL activities are reciprocally regulated after feeding or fasting (1, 3). Heart LPL activity increased 2-fold after fasting while activity decreased in adipose tissue (Table II). As in adipose tissue, changes in heart LPL activity following feeding/fasting were not accompanied by parallel changes in LPL mRNA levels (Fig. 3, Table II) and rates of lipase synthesis (Table II). However, in contrast to adipose tissue LPL, heart LPL mass changed proportionally with activity, maintaining a constant specific activity.

**DISCUSSION**

The decrease in rat adipose tissue LPL activity following a 12-h fast and its subsequent increase after refeeding are controlled posttranslationally. Compared with the fasted state, adipose tissue LPL activity increases over 2-fold after refeeding, whereas mRNA levels and rates of synthesis decrease almost by half; enzyme mass remains unchanged.
**FIG. 6.** The relative distribution of LPL mass in ER/cis-Golgi and Golgi/post-Golgi secretory compartments following feeding and fasting. A, the incorporation of [35S]methionine into total proteins and LPL immunoprecipitable protein as a function of time of pulse. The measurement of label incorporation into total protein and immunoprecipitable LPL is described in the legend to Fig. 3 and under “Experimental Procedures.” B, LPL was immunoprecipitated from: “FED” lane, 0.05 mg adipose protein, in which 3200 cpm of [35S]methionine were incorporated into total LPL; “FASTED” lane, 0.07 mg of adipose protein containing 5000 cpm of [35S]methionine incorporated into the total immunoprecipitable LPL. Scanning densitometry was used to quantitate the percent distribution of label into the three bands.

**FIG. 7.** The kinetics of [35S]-labeled high mannose and complex-type adipose tissue LPL after feeding/fasting. Adipose tissue from overnight-fasted or refed animals was pulse-labeled for 20 min and then chased for up to 240 min as described under “Experimental Procedures.” LPL was immunoprecipitated, treated with Endo H and then fractionated by SDS polyacrylamide gel electrophoresis. The resulting bands representing the high mannose and complex form of LPL were quantitated by scanning densitometry. The results were expressed as a percentage of the intensity of the high mannose form after 10 min of chase. A, turnover rates of the high mannose form of LPL. B, the appearance and disappearance of label into the complex form of LPL. The pattern of labeling of the intermediate form was very similar to the complex form and is not shown for the sake of clarity.

A similar finding was reported (7) in 3T3-L1 adipocytes, where insulin was shown to increase heparin-releasable LPL activity over 2-fold when enzyme synthesis decreased by about 50%.

In our studies, the use of adipose tissue *in lieu* of isolated adipocytes was a deliberate step to include in the analysis the fraction of LPL secreted and bound at the endothelial surface (functional LPL). Since the LPL mass in adipose tissue remained constant during incubation *in vitro*, there was no resulting net loss of LPL to the medium in the absence of heparin. Thus, in these studies, the intermediate and complex forms include LPL which has passed beyond cis-Golgi, including secreted, endothelial-bound LPL; the high mannose form represents LPL located intracellularly within the ER/cis-Golgi. Thus, the calculated pool sizes of the high mannose and intermediate/complex forms shown in Fig. 8 are representations of the LPL mass distribution within the adipose tissue, including the fraction of LPL bound at the endothelial surface under these experimental conditions. It should be noted that the calculated intermediate/complex pool sizes do not take into account endothelial-bound LPL that might have been lost to the medium, although in the absence of heparin, this would constitute a minor fraction (14).

A significant finding in our studies is that a major cellular redistribution of adipose tissue LPL occurs in response to feeding/fasting. Although the high mannose form comprises the majority of LPL following fasting, the intermediate/complex form predominates after refeeding (Fig. 6); this strongly suggests a shift of LPL out of ER and into the post-
FIG. 8. Summary of the kinetic parameters and pool sizes of the high mannose and complex types of adipose tissue LPL after feeding and fasting. The LPL mass of each compartment, located within the stippled boxes, is microgram LPL/g wet weight adipose tissue. The kinetic parameters are: relative rate of synthesis, defined as the percentage of \(^{[35]S}\)methionine incorporated into LPL relative to total protein synthesis; FTR, defined as the fraction of the pool of the high mannose form of LPL turned over each hour with the units (hours\(^{-1}\)); and FCR, defined as the fraction of the pool of the intermediate/complex form of LPL leaving the system by degradation each hour with the units (hours\(^{-1}\)). The relative rate of synthesis and FTR were determined experimentally; the FCR was calculated from the pool sizes of the high mannose and intermediate/complex forms of LPL (micrograms of LPL/g of tissue), and the FTR assuming the system was at steady state. For details see “Experimental Procedures” and “Results.”

![Kinetic Parameters Diagram](http://www.jbc.org/)

**TABLE II**
**Response of heart LPL following 12-h feeding and fasting**

| LPL          | Fasted | Fed   | Fast/Fed |
|--------------|--------|-------|----------|
| Activity (milliunits/g) | 1330 ± 92.0 | 580 ± 30.0* | 2.31 |
| mRNA (arbitrary units)       | 76.6 ± 4.2a | 80.7 ± 10.8a | 1.06 |
| Rate of synthesis (%)      | 0.113 ± 0.003 | 0.096 ± 0.002a | 1.18 |
| Mass (μg/g)               | 1.36 ± 0.069 | 0.71 ± 0.040a | 1.93 |
| Specific activity (units/mg) | 58,300 | 49,600 | 1.19 |

* Differences between fasted and fed are significant at the \(p < 0.005\) level.
* Differences between fasted and fed is not significant (\(p > 0.05\)).

Golgi secretary pathway. The larger pool size of the high mannose LPL form in fasting compared with refeeding arises as a result of a higher LPL synthetic rate and not by a slower turnover rate out of the ER/cis-Golgi compartment. In turn, an apparently slower turnover rate of the intermediate/complex LPL form during refeeding compared to fasting results in the increased intermediate/complex pool size, as indicated by the calculated fractional catabolic rates in Fig. 8. It will be important in the future to determine by direct measurement the fraction and turnover rate of the intermediate/complex LPL form that comprises endothelial-bound functional LPL.

A hypothetical model is depicted in Figs. 9 and 10, proposing a possible mechanism of regulating the amount of endothelial-bound functional LPL in adipose tissue during feeding and fasting, taken into account the findings of this study; i.e. a change in the activity and cellular distribution of LPL without a change in total LPL mass (Fig. 9). It is the endothelial-bound fraction of LPL that is critical in determining the rate of triglyceride fatty acid influx into adipose stores during feeding and fasting. Fig. 10 proposes that the redistribution of LPL mass could arise by diverting newly synthesized LPL into pathways leading either to secretion (during feeding) or degradation (during fasting). Other investigators (11-15), using isolated adipocytes, have also shown that newly synthesized LPL is diverted into a lysosomal degradative pathway in the absence of a secretagogue (heparin). In an alternative model, shown by the dashed lines and question marks in Fig.

![Regulation of Lipoprotein Lipase](http://www.jbc.org/)
Regulation of Lipoprotein Lipase

10, secretion is constitutive, and the amount of functional LPL is regulated by turnover at the endothelial surface. However, endothelium does not appear to be the major site of LPL degradation, since exogenously added LPL is degraded much slower by endothelial cells than adipocytes (25). Nevertheless, it is still possible that LPL is removed from endothelium by triglyceride-rich lipoproteins and degraded in liver (26, 27).

The decrease in adipose tissue LPL specific activity noted after fasting (Table I) most likely arises from the presence of inactive mass. Coincidently, in fasting, the majority of LPL is within ER, which may be inactive. For example, studies utilizing tunicamycin (9–11) and glucose deprivation (28) in adipocytes indicate that LPL glycosylation is required for secretion and that lipase retained intracellularly is inactive. Carbonyl cyanide m-chlorophenylhydrazone, which blocks the energy dependent budding involved in the formation of transport vesicles from ER to Golgi, causes retention of LPL within ER and loss of activity (6, 12). Combined lipase deficiency in mice (29, 30), a genetic defect located on a chromosome separate from the LPL gene (31) and apparently affecting LPL translocation from ER to Golgi,4 again is characterized by inactive mass. However, our laboratory has recently shown by site-directed mutagenesis5 that uncleaved heparic lipase, which is structurally and functionally very similar to LPL (29, 32), is catalytically active. Thus, loss of catalytic activity within ER is probably not a consequence of "immature" glycosylation. Indeed, the high mannose form of LPL in guinea pig adipocytes is active against a trigonol substrate (18), although specific activity was not reported. Therefore, the precise intracellular location and nature of the inactive LPL mass arising in rat adipose tissue following fasting remains to be determined.

LPL activity in heart is also regulated posttranslationally. Although LPL activity is modulated several fold after feeding/fasting, LPL mRNA levels and rates of synthesis are not affected proportionally. Since pulse/chase studies in perfused hearts are technically difficult, the mechanism of LPL regulation in this tissue has not been determined. It would seem a priori that the regulation in heart would be similar to that proposed in adipose tissue; i.e., a redistribution of LPL in response to feeding/fasting. In cells isolated from newborn rat hearts (33), an increase in heparin-releasable LPL activity in response to the β-adrenergic agent isoproterenol or dibutyryl-cAMP did, in fact, coincide with an apparent redistribution of LPL from the high mannose to complex forms. However, unlike adipose tissue, LPL mass did change in parallel with activity following feeding or fasting (Table II). Whether this indicates that LPL is regulated by a posttranslational mechanism different in heart than in adipose tissue is unknown.

LPL activity levels are most likely regulated during feeding and fasting by a complex array of factors, such as insulin, glucagon, and glucocorticoid levels as well as sympathetic innervation. This study examined adipose and heart LPL regulation at only one time point (12 h) after fasting and refeeding. It is certainly possible that LPL activity levels could be regulated by different mechanisms and to different degrees at various time points after refeeding, e.g., when circulating insulin levels are maximal. Unquestionably under different conditions and possible in different species, LPL activity levels are also regulated at the level of transcription.

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Affinity-purified chicken anti-LPL, in bovine casein medium, and purified bovine milk lipase were generous gifts from J. Gara, San Luis Obispo, CA. Affinity-purified rabbit anti-chicken IgG and hen-antibody polyclonal sera were purchased from Bethesda Research Laboratory, Endo H and neuraminidases from Genzyme, Protein A packed from New England Nuclear, ura-6 GSE-18640 strain, L-phenylalanine and glycyglycin were purchased from Sigma, Sigma strain E. coli (plasmid pUC 19), Philadelphia 88 (plasmid pUC 19), and N-lauroyl sarcosine and crude plasmid DNA (strain E. coli) were obtained from Sigma. The D5 medium of Rhizopus commune was prepared as a 4% agar gel matrix from a generous gift from Dr. C. Fred Fox.

Methods

Animals

Male Sprague-Dawley rats (Simonsen and Hilltop) weighing between 180-200 g were utilized. The test meal given was devoid of low-density lipoprotein (RBA-68). The test fast used for this study was fasted for 24 h and watered for 12 h. Heparin was given from Purina Laboratory Chow supplemented with 10% glucose in the drinking water. After decapitation, hearts and adipose tissue extracts were mixed, rinsed in saline and either used immediately for labeling experiments, or promptly frozen and stored in liquid Nz until

RNA Analysis

Nuclear, and adipose tissue extracts were isolated by SDS-phenol extraction. Three pools of RNA from heart, and adipose tissue was isolated by SDS-phenol extraction (35). Three pools of RNA from heart, and adipose tissue were isolated by SDS-phenol extraction (35). The total RNA was fractionated on 1.0% agarose gel, and detected in ethidium bromide.

Antibodies

Antibodies against lipoprotein lipase (LPL), its biotin conjugate, and purified bovine milk LPL were from the chicken LPL immunoglobulin (see also Fig. 2). and processed in a similar manner. The Staph A antibody for Staph A was used as a control for each step as described previously (23) utilizing 10% polyacrylamide, 0.25% bis-acrylamide in the separating gel. The antibody specificity was determined by immunoprecipitation of a 56 kDa band from 300 ml of 3H-labeled adipose tissue LPL, expressed as the proportion of total protein synthesized following a 30 min pulse. LPL was immunoprecipitated from pulse-labeled homogenates and electrophoresed in a 10% SDS polyacrylamide gel. After fluorography, LPL migrating in the 56 kDa region of the gel was excised and counted by liquid scintillation. The levels of A and B were monitored as the area of the corresponding protein bands.

LPL immunoprecipitation

Staph A was washed extensively and utilized as a 10% slurry. In some experiments, tissue lysates were preincubated with 40% (wt/vol) Staph for 30 min at 4°C. LPL was immunoprecipitated from tissue extracts supplemented with NaCl to total concentrations of 3.0 M Triton X-100, 0.1% N-lauroyl sarcosine, 1.0 M NaCl, 40 μCi/ml [3H]labeled chicken anti-bovine LPL antibody (see Fig. 1A and 1B) in 0.1 M Tris, pH 7.5. Utilizing an antibody concentration of 4 μg (24 μg IgG, Staph A was solubilized and electrophoresed in a 10% SDS polyacrylamide gel. Following incubation, the resulting supernatant was subjected to immunoprecipitation by utilizing the preimmune supernatant as a control for each step as described previously (23) utilizing 10% polyacrylamide, 0.25% bis-acrylamide in the separating gel.
Regulation of Lipoprotein Lipase

Endo H Digestion of LPL Immunoprecipitates

Enzyme-Linked Immunosorbent Assay

Other Methods

Calculations

Statistical significance was determined by the two-tailed Student's t-test.

The fractional catabolic rate (FCR) was calculated based on the equation:

\[ \text{FCR}(x) \times \text{FCR}(y) \]

where FTR is equal to the fractional turnover rate of LPL from ER to Golgi, and \( x \) and \( y \) are equal to the post sizes of LPL within the ER and Golgi/Golgi complex, respectively, defined as \( \mu \)LPL per g wet weight tissue. Thus, the product (FTR(x)) is the influx of LPL mass into the Golgi/Golgi compartment, whereas (FCR(y)) equals the efflux or out. By definition, the influx and efflux must be equal at steady state.
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