

# Characterization of lipoprotein lipase activity, secretion, and degradation at different sites of post-translational processing in primary cultures of rat adipocytes

Rosa B. Simsolo, John M. Ong, and Philip A. Kern<sup>1</sup>

Department of Medicine/Division of Endocrinology, Cedars-Sinai Medical Center, Los Angeles, CA 90048

**Abstract** The regulation of adipose tissue lipoprotein lipase (LPL) by feeding and fasting occurs through post-translational changes in the LPL protein. In addition, LPL activity and secretion are decreased when N-linked glycosylation is inhibited. To better understand the role of oligosaccharide processing in the development of LPL activity and in LPL secretion, primary cultures of rat adipocytes were treated with inhibitors of oligosaccharide processing. LPL catalytic activity from the heparin-releasable fraction of adipocytes was inhibited by more than 70%, with similar decreases in LPL mass, when cells were cultured for 24 h in the presence of either tunicamycin or castanospermine. On the other hand, deoxymannojirimycin (DMJ) and swainsonine had no effect on LPL activity. LPL secretion was examined after pulse-labeling cells with [<sup>35</sup>S]methionine. The appearance of <sup>35</sup>S-labeled LPL in the medium was blocked by treatment of cells with tunicamycin and castanospermine, whereas secretion was not affected by DMJ or swainsonine. To examine the effect of oligosaccharide processing on LPL intracellular degradation, adipocytes were treated with tunicamycin, castanospermine, and DMJ and then pulse-labeled with [<sup>35</sup>S]methionine, followed by a chase with unlabeled methionine for 120 min. The unglycosylated [<sup>35</sup>S]LPL that was synthesized in the presence of tunicamycin demonstrated essentially no intracellular degradation. In the presence of castanospermine and DMJ, the half-life of newly synthesized LPL was increased to 81 and 113 min, as compared to 65 min in control cells. Thus, castanospermine-treated adipocytes demonstrated a decrease in LPL activity and secretion, suggesting that the glucosidase-mediated cleavage of terminal glucose residues from oligosaccharides is a critical step in LPL maturation. In addition, the maturation of N-linked oligosaccharides on LPL was associated with an increased intracellular turnover. —Simsolo, R. B., J. M. Ong, and P. A. Kern. Characterization of lipoprotein lipase activity, secretion, and degradation at different steps of post-translational processing in primary cultures of rat adipocytes. *J. Lipid Res.* 1992. 33: 1777-1784.

**Supplementary key words** glycosylation • castanospermine • tunicamycin • deoxymannojirimycin • glucosidase • mannosidase

Lipoprotein lipase (LPL) is a central enzyme in lipid metabolism. The enzyme is synthesized and secreted by

adipocytes, and then transported to the capillary endothelium, where hydrolysis of the triglyceride core of circulating VLDL and chylomicrons takes place (1, 2). Recent studies of LPL gene expression have indicated that there are multiple sites of transcriptional and post-transcriptional regulation. Rat adipocytes demonstrate an increase in LPL mRNA in response to insulin (3, 4), and a decrease in response to dexamethasone (5). On the other hand, both rats and humans demonstrate increases in LPL activity after feeding due to post-transcriptional changes, characterized by an increase in LPL specific activity (6, 7).

The importance of LPL glycosylation in the development of LPL activity has been demonstrated in several studies. When adipocytes were treated with tunicamycin, or cultured in glucose-free medium, a deglycosylated form of LPL was synthesized that had low or absent LPL activity and was not secreted from the cell (8-10). Several studies have further examined LPL post-translational processing using various inhibitors of oligosaccharide processing. One study suggested that cells produced active and secreted LPL after inhibiting cellular mannosidases I and II, as well as after inhibition of glucosidases I and II with methyldeoxymannojirimycin (MDN) (11). These data suggested that only core glycosylation was necessary for fully developed LPL activity and secretion. On the other hand, studies with hepatic lipase, which is structurally similar to LPL and part of the same gene family, demonstrated that hepatic lipase was active and secreted

Abbreviations: LPL, lipoprotein lipase; DMJ, deoxymannojirimycin; MDN, methyldeoxymannojirimycin; HL, hepatic lipase; VLDL, very low density lipoprotein; FFA, free fatty acids; TCA, trichloroacetic acid; HR, LPL activity in adipocyte fraction released by heparin; EXT, LPL activity in fraction remaining after heparin release in cell extracts.

<sup>1</sup>To whom correspondence should be addressed at: Division of Endocrinology, Becker 131, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Los Angeles, CA 90048.

after the action of glucosidase I and II on the core oligosaccharide (12). A recent study examined LPL processing in Chinese hamster ovary (CHO) cells (13), which secrete large amounts of LPL (14), and also found that the trimming of terminal glucose residues from the oligosaccharide in the rough endoplasmic reticulum (RER) was essential for further processing and secretion.

This study used primary cultures of rat adipocytes, and was designed to determine what steps in LPL glycosylation are necessary for LPL activity and secretion, and also to determine whether different forms of LPL differ in their rate of degradation. As with hepatic lipase (12), cleavage of the terminal glucoses from the core N-linked oligosaccharide was a critical step in the development of LPL activity and in LPL secretion from the adipocyte.

## MATERIALS AND METHODS

### Preparation of adipocytes

Adipocytes were prepared from epididymal fat pads of male Sprague-Dawley rats, as described previously (3). Cells were cultured for 24 h in Medium 199 (Irvine Scientific, Santa Ana, CA) containing 10% fetal bovine serum (Hyclone Laboratories, Logan, UT), and were then washed in the same medium, followed by the addition of the indicated concentrations of either castanospermine, 1-deoxymannojirimycin (DMJ), N-methyldeoxynojirimycin (MDN), swainsonine, or tunicamycin (Sigma, St. Louis, MO).

### Measurement of LPL activity

LPL catalytic activity was measured in the adipocyte fraction released by heparin (HR), as well as in the fraction remaining after heparin release in cell extracts, which is referred to as EXT, as described previously (8). In brief, adipocytes were incubated in phosphate-buffered saline (PBS) containing 13  $\mu\text{g/ml}$  heparin (Fisher Scientific Co.) for 30 min at 37°C. An aliquot of this buffer was then assayed as described below. The cells were then washed and the EXT fraction was prepared by homogenizing the cells in buffer containing deoxycholate and heparin, as described previously (6). LPL activity was then measured in the supernatant after centrifugation.

LPL activity was determined as described previously (15) using a [ $^3\text{H}$ ]triolein-containing substrate emulsified with lecithin, and containing normal human serum as a source of apoC-II. Activity was expressed as nEq FFA released/min per  $10^6$  cells.

### LPL immunoreactive mass

Previous studies have described the measurement of LPL immunoreactive mass by enzyme-linked im-

munosorbent assay (ELISA) (16, 17). In brief, samples for LPL immunoreactive mass were prepared as described above for LPL activity, except protease inhibitors were present in all the buffers. Affinity-purified chicken anti-bovine LPL antibodies are used as a capture antibody, and biotinylated affinity-purified anti-LPL antibody, followed by streptavidin-peroxidase, is used as the indicator antibody. The concentration of LPL was then calculated using the standard curve for bovine LPL, and expressed as ng/ $10^6$  cells.

### [ $^{35}\text{S}$ ]methionine labeling, immunoprecipitation, and Western blotting

The pulse-labeling and immunoprecipitation procedures have been described previously (3, 8). In brief, adipocytes were cultured overnight in Medium 199 containing 10% fetal bovine serum, and were then treated with the indicated concentrations of castanospermine, DMJ, or tunicamycin. Cells were pulse-labeled with 50  $\mu\text{Ci}$  of [ $^{35}\text{S}$ ]methionine for 30 min in methionine-free Medium 199, and then chased with complete medium (containing 30 mg/l methionine, as well as the same concentration of the processing inhibitory drug) for the times indicated. Previous studies have shown that incorporation of [ $^{35}\text{S}$ ]methionine into LPL is linear for at least 45 min (18). As heparin is necessary for LPL secretion from rat adipocytes (3), some experiments were performed in the presence of heparin and some were not, as described in the text. Cells were then lysed as described previously (8), and quantitative immunoprecipitation with affinity-purified anti-LPL antibody was performed, followed by analysis of the samples on a 10% polyacrylamide-SDS gel. To account for any differences in [ $^{35}\text{S}$ ]methionine labeling, an aliquot of cell lysate was TCA-precipitated, and the protein gels were loaded in proportion to the total TCA-precipitable counts in the samples.

Western blotting was performed as described previously (17), except with some minor changes. After blotting the membrane with primary antibody, biotinylated anti-chicken antibody (Sigma) was used, followed by streptavidin-peroxidase, and development with a chemoluminescent peroxidase substrate.

### Statistics

Quantitative data are expressed as a mean  $\pm$  SEM, and statistical comparisons used the Mann-Whitney rank sum test (19).

## RESULTS

To determine the effects of oligosaccharide processing on LPL activity, adipocytes were cultured for up to 24 h in the presence and absence of tunicamycin, castanospermine, DMJ, and swainsonine. The specificities of these

drugs have been described previously (20), and are shown in **Fig. 1**. Whereas tunicamycin inhibits all N-linked glycosylation, castanospermine and MDN inhibit the glucosidase-mediated cleavage of the terminal glucose residues from the oligosaccharides, and DMJ inhibits further processing by mannosidase I. After treatment of cells with castanospermine, MDN, and DMJ, N-linked oligosaccharides are in the high mannose form, and are therefore susceptible to cleavage by endo H. Swainsonine inhibits mannosidase II and results in the formation of hybrid oligosaccharides that contain both high mannose and complex features.

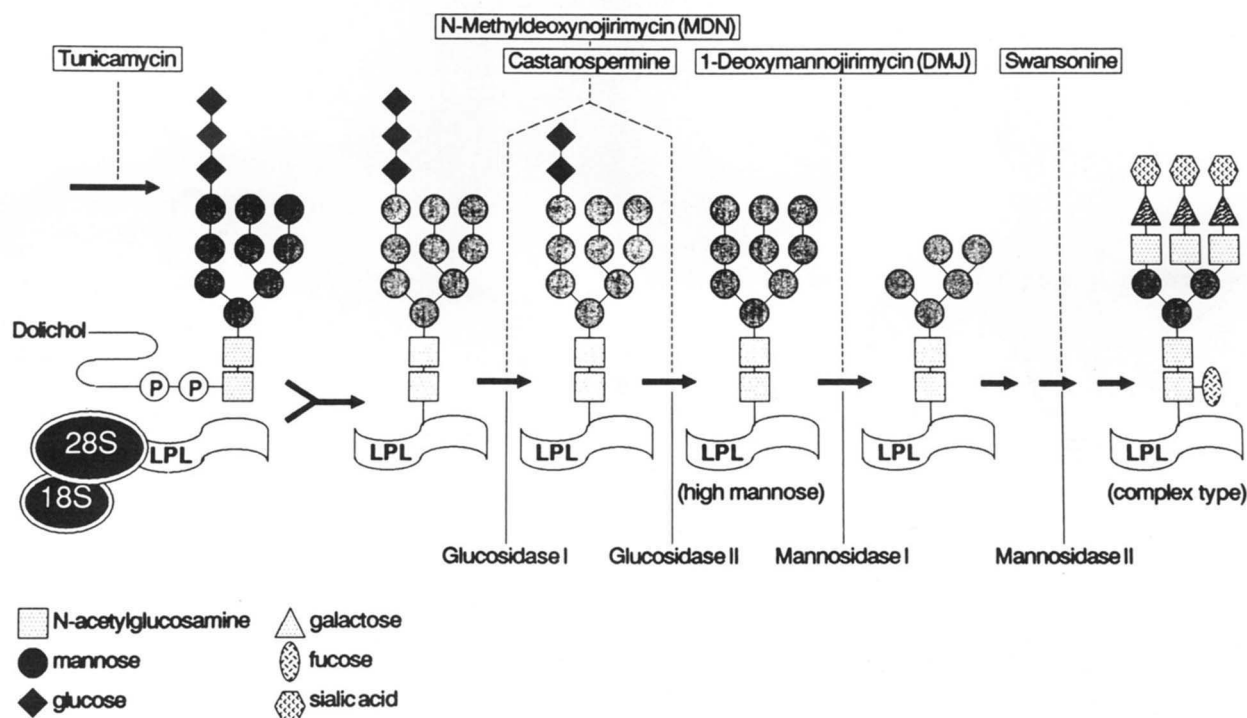
### LPL activity and mass

To examine the effects of glycosylation inhibition on LPL activity and mass, castanospermine, DMJ, and tunicamycin were added to adipocyte cultures for up to 24 h. As shown in **Fig. 2**, the addition of castanospermine and tunicamycin resulted in a gradual decrease in HR LPL activity to 25% and 28% of control activity. EXT activity was also inhibited equally by both castanospermine and tunicamycin (**Fig. 2B**). However, the addition of DMJ to adipocyte cultures resulted in no change in LPL activity, in either HR or EXT. When LPL immunoreactive mass was measured by ELISA, HR LPL activity was

38% and 45% of control in response to castanospermine and tunicamycin, and not changed in cells treated with DMJ. In the EXT fraction, LPL mass was unchanged in response to DMJ, and significantly increased after 24 h treatment with castanospermine. A small but statistically insignificant decrease in EXT mass was noted in response to tunicamycin. The addition of 1 mM swainsonine had no effect on LPL activity or mass (data not shown).

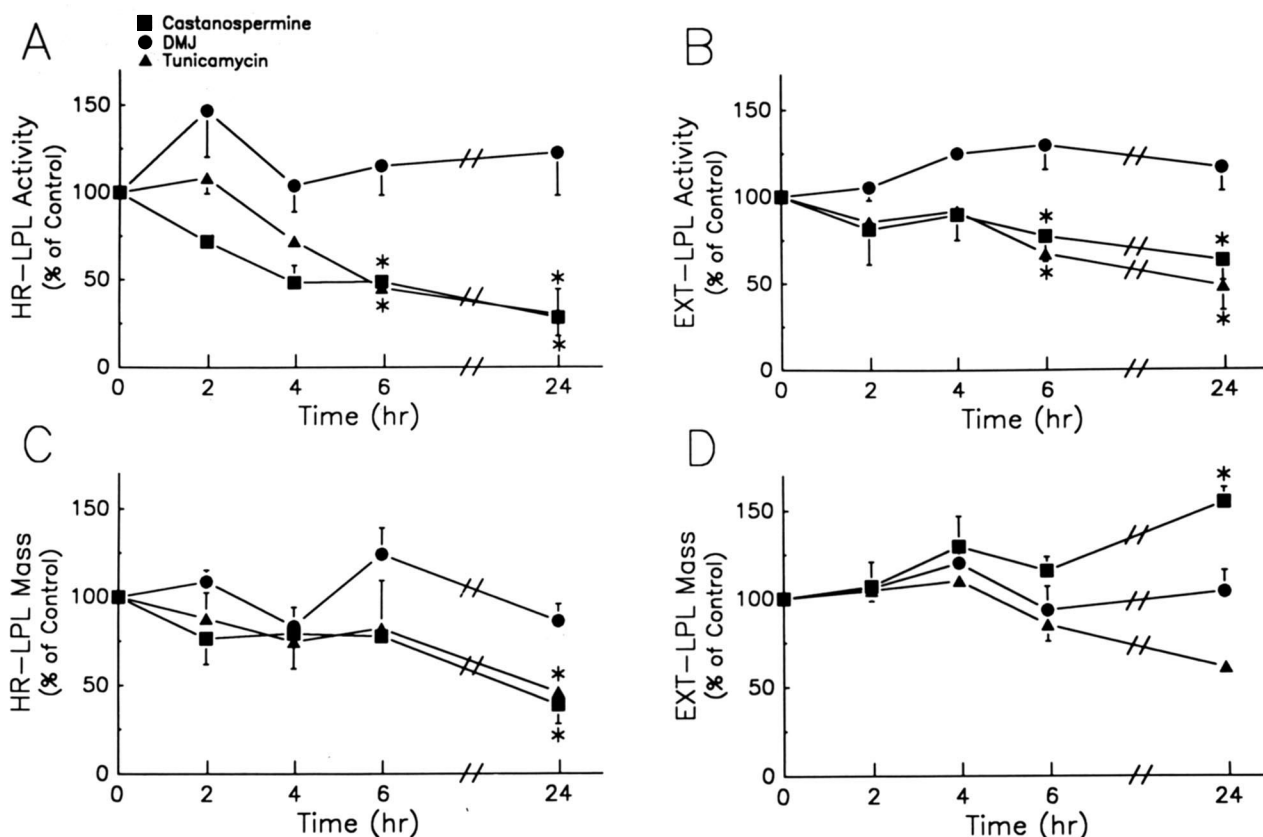
### LPL secretion

Additional studies were performed to determine whether the above processing inhibitors affected LPL secretion. Adipocytes were cultured in the presence of tunicamycin, castanospermine, and DMJ for 2 h, after which they were pulse-labeled with [<sup>35</sup>S]methionine for 30 min, and then chased for an additional 60 min. Because LPL in rat adipocytes is only secreted when heparin is present (3), the pulse-chase was carried out in the presence of 13 μg/ml heparin. As shown in **Fig. 3**, increasing concentrations of castanospermine resulted in a decrease in LPL secretion. The immunoprecipitated cellular form of LPL in the presence of castanospermine migrated perceptibly more slowly on the gel, which was likely due to the presence of the terminal glucose residues on the N-linked oligosaccharides. In contrast to the effects



**Fig. 1.** Critical steps in LPL post-translational processing. The addition of the core oligosaccharide to LPL is blocked by tunicamycin, which results in the synthesis of an unglycosylated protein. Castanospermine and N-methyldeoxymannojirimycin (MDN) inhibit the removal of the terminal glucose residues in the RER by glucosidase I and II (20). 1-Deoxymannojirimycin (DMJ) inhibits the Golgi mannosidase I, such that the terminal glucose residues are removed, but the glycoprotein is still in the high mannose form. Swainsonine inhibits mannosidase II, which results in an intermediate or complex oligosaccharide.





**Fig. 2.** Effect of oligosaccharide-processing inhibitors on LPL activity and LPL mass. Cells were cultured overnight in medium containing 10% serum, and then placed into medium containing 1 mM castanospermine, 1 mM DMJ, or 4  $\mu$ g/ml tunicamycin for the indicated times. LPL activity was measured in the HR (A) and EXT (B) fractions, along with LPL immunoreactive mass (C) and (D) in the same fractions, respectively. Data are from five experiments and expressed as a percent of control. Control LPL activity at time 0 was  $0.51 \pm 0.15$  and  $2.83 \pm 0.60$  nEq/min per  $10^6$  cells (mean  $\pm$  SEM,  $n=8$ ) for HR and EXT, respectively, and control LPL mass in the same fractions was  $1.64 \pm 0.71$  and  $7.73 \pm 2.08$  ng/ $10^6$  cells; \* $P < 0.05$  versus control cells.

of castanospermine, increasing concentrations of DMJ did not affect LPL secretion (Fig. 3). Similar experiments with swainsonine demonstrated no change in LPL secretion (data not shown).

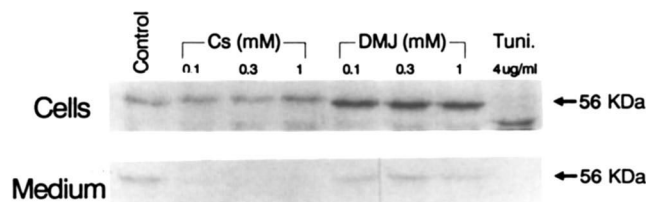
#### Effect of endo H

To verify that the treatment with DMJ was indeed inhibiting oligosaccharide processing, immunoprecipitated cells and medium from DMJ-treated cells were treated with endo H (Fig. 4). At the end of the 30-min pulse, the cellular form of LPL was endo H-sensitive. Endo H treatment of the immunoprecipitates yielded a 50 kDa LPL form, which is the molecular mass of the unglycosylated protein, and identical to the LPL form produced by tunicamycin. After 90 min of chase, however, the LPL synthesized in the presence of castanospermine and DMJ were still endo H-sensitive, demonstrating that the LPL was in the high mannose form, whereas control LPL was partially endo H-resistant. Medium (secreted) LPL was endo H-resistant in control cells, and endo H-sensitive in DMJ-treated cells. Thus, the endo H sensitivity of the

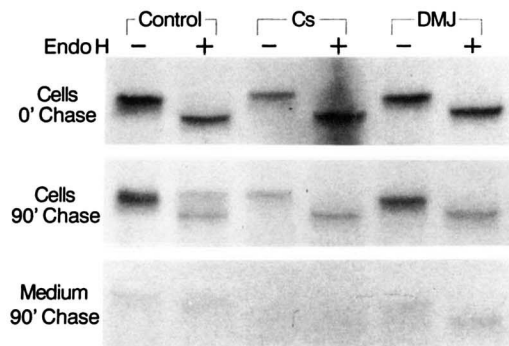
LPL secreted in the presence of DMJ demonstrates that the high mannose form of LPL can be secreted.

#### LPL degradation

Previous studies have provided evidence for the regulation of LPL degradation (7, 21). To determine whether



**Fig. 3.** Effect of glycosylation on LPL secretion. Cells were treated as described in the legend to Fig. 2 for 2 h, then pulse-labeled with [ $^{35}$ S]methionine for 30 min, and then chased for 60 min with medium containing unlabeled methionine. The pulse and chase were performed in the presence of heparin, and inhibitors were present in the same concentrations during the pulse and chase. LPL was then immunoprecipitated from the cells and medium. The concentrations of castanospermine and DMJ that were used in these cultures were 0.1, 0.3, and 1 mM, which are shown in lanes 2–4, and 5–7 for castanospermine and DMJ, respectively. The concentration of tunicamycin was 4  $\mu$ g/ml.



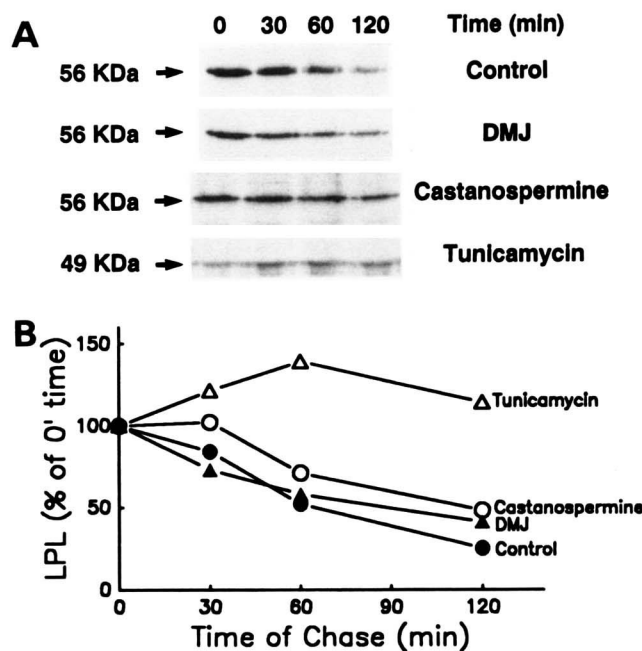
**Fig. 4.** Endo H sensitivity of LPL forms. To demonstrate that LPL from DMJ- and castanospermine-treated cells were still in the high mannose form, immunoprecipitated cell extracts and medium were treated with endo H, followed by analysis by SDS-PAGE. In the first two panels are cell extracts from control, castanospermine- (Cs), and DMJ-treated cells. The first panel represents cells from the end of the pulse, or time 0 of the chase, and the second panel includes cells after 90 min of chase. Endo H-treated medium samples are shown in the third panel.

different cellular forms of LPL were degraded at different rates, rat adipocytes were labeled with [<sup>35</sup>S]methionine and chased in the presence of castanospermine, DMJ, and tunicamycin, and the disappearance of the immunoprecipitated cellular LPL was measured. The disappearance of cellular LPL is dependent on both intracellular degradation as well as secretion, and LPL is only secreted from rat adipocytes when heparin is present (3). Because we wished to study only intracellular degradation in the absence of secretion, a pulse-chase was carried out in the absence of heparin. As shown in Fig. 5, the degradation of intracellular LPL was greatly prolonged in the cells treated with tunicamycin. In cells treated with castanospermine and DMJ, LPL degradation was more similar to that of control adipocytes. The  $t_{1/2}$  values of the LPL from control, DMJ, and castanospermine-treated cells were 65 min, 81 min, and 113 min, respectively.

#### Changes in LPL synthesis

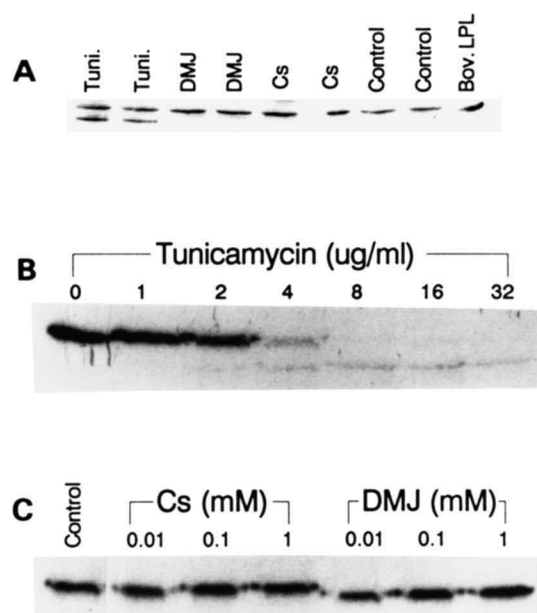
Castanospermine inhibited LPL secretion and also inhibited degradation slightly. These changes in LPL processing were therefore likely responsible for the observed increase in EXT LPL mass in castanospermine-treated cells (Fig. 2). On the other hand, cells treated with tunicamycin demonstrated no secretion and a greatly reduced rate of degradation, which would also be expected to yield an increase in EXT mass. However, as shown in Fig. 2, there was no increase in EXT LPL mass after treatment with tunicamycin. To examine these data further, the EXT samples of adipocytes were analyzed by Western blot to verify the results of the ELISA. As shown in Fig. 6A, the Western-blotted image corresponded well with the quantitative results of the ELISA. Even after 24 h of treatment with tunicamycin, adipocytes contained

some fully glycosylated (56 kDa) LPL, suggesting that there may be a slow turnover of some component of the cellular LPL that was present before the addition of tunicamycin, or that the inhibition of glycosylation by tunicamycin may become less complete after 24 h in culture. One possible explanation for the lack of accumulation of LPL mass in tunicamycin-treated cells is a decrease in LPL synthetic rate. In previous experiments (8), the effects of tunicamycin were examined, and the concentrations that were required to inhibit glycosylation fully also had some inhibitory effects on protein synthesis. To examine this more carefully, the effects of tunicamycin, castanospermine, and DMJ on LPL synthetic rate were examined. As shown in Fig. 6B, the addition of tunicamycin to adipocytes in concentrations sufficient to inhibit glycosylation also inhibited LPL synthetic rate. When the gels were loaded with equal total TCA counts, tunicamycin at 4  $\mu$ g/ml inhibited LPL synthesis by 54%. In addition, tunicamycin inhibited total protein synthesis by an additional 30%. On the other hand, castanospermine and DMJ had no effect on LPL or total protein synthesis. Therefore, the lack of change in EXT LPL mass in response to tunicamycin was likely due to the simultaneous inhibition of LPL synthesis, secretion, and degradation.



**Fig. 5.** Post-translational processing and LPL turnover. Effects of oligosaccharide-processing inhibitors on LPL degradation. Cells were treated as described in the legend to Fig. 4. A pulse with [<sup>35</sup>S]methionine was performed, followed by a chase with unlabeled methionine for the indicated times. Heparin was not present, such that LPL was not secreted (data not shown). A: Immunoprecipitated cell extracts during the chase. B: Densitometric quantitation of the immunoprecipitated LPL from A.





**Fig. 6.** Effects of processing inhibitors on LPL synthesis. **A:** Western blot of adipocyte extracts after 24 h of treatment with the indicated inhibitor of LPL processing. Castanospermine (Cs) and DMJ concentrations were 1 mM, and tunicamycin was added at 4  $\mu$ g/ml. **B:** Effect of tunicamycin on LPL synthetic rate. The indicated concentrations of tunicamycin were added to adipocyte cultures for 2 h, followed by pulse-labeling for 30 min and immunoprecipitation. Each lane was loaded with equal total TCA-precipitable counts. **C:** Same experiment as in B, except the indicated concentrations of castanospermine (Cs) and DMJ were used.

## DISCUSSION

Previous studies have indicated that much important regulation of adipose tissue LPL occurs post-translationally. When either human or rat adipose tissue was studied after feeding, there was an increase in LPL activity that was not accompanied by an increase in LPL protein or mRNA levels (6, 7). In other studies, guinea pig LPL activity was about 10-fold higher in the fed versus the fasted state (22). Although this difference in LPL activity was accompanied by a 2-fold change in LPL mRNA levels, the very large increase in LPL activity suggested that post-translational mechanisms were also part of the response to feeding. In adipocytes from fasted rats, the predominant LPL species present was sensitive to endo H, suggesting the presence of high mannose oligosaccharides that rendered the LPL protein less active (7).

To examine post-translational processing in rat adipocytes, we cultured cells in the presence of tunicamycin, castanospermine, DMJ, and swainsonine followed by the measurement of LPL activity, mass, secretion, and degradation. Both castanospermine and tunicamycin inhibited LPL activity and mass to a similar degree and eliminated

LPL secretion. In contrast, cells treated with DMJ and swainsonine synthesized and secreted active LPL. After removal of the terminal glucose residues, LPL was fully active and secretable, although it is possible that there are additional post-translational functional changes in the protein that were not detected in these studies. For example, it is possible that the LPL formed as a result of castanospermine treatment was secreted, but then rapidly degraded, and therefore not detected in the pulse-chase experiments. As illustrated in Fig. 1, castanospermine inhibits cellular glucosidases I and II (20), which permits core glycosylation, but inhibits cleavage of the terminal glucose residues. Previous studies on hepatic  $\alpha$ -1 antitrypsin and  $\alpha$ -1 antichymotrypsin demonstrated that these proteins were dependent on cleavage of terminal glucose residues for transport from the RER to the Golgi (23). Thus, it is possible that the inhibition of LPL secretion by castanospermine is due to the retention of the lipase within the RER. The inhibition of LPL activity could also be related to retention within the RER, leading to lack of proper folding or dimerization.

Other studies have examined LPL post-translational processing in cultured cells. When preadipocytes were treated with tunicamycin, a deglycosylated form of LPL was synthesized which had low or absent LPL activity and was not secreted (8–10). However, Ob17 cells secreted LPL even when glycosylation was blocked with tunicamycin (24), suggesting that LPL post-translational processing may be different in the Ob17 cell line. The amino acid sequence of LPL is highly conserved between different species, and contains two likely sites of N-linked glycosylation, which are at positions 43 and 359 in human LPL (2). When in vitro mutagenesis was used to study LPL activity and secretion, substitution at Asn<sup>43</sup> (but not at other asparagines) resulted in synthesis of an inactive LPL protein that was not secreted (25), and thus demonstrated the importance of this site in LPL processing. On the other hand, hepatic lipase (HL) had different requirements for activity and secretion. When site-directed mutagenesis was used to alter the N-linked glycosylation sites of HL, followed by transfection into *Xenopus* oocytes, glycosylation was found to be important for HL secretion, but not for HL activity (26).

Additional studies have examined oligosaccharide processing using glycosidases and drugs that inhibit processing. Using guinea pig adipocytes, Semb and Olivecrona (11) demonstrated that cells produced active and secretable LPL even when the high-mannose form predominated. LPL was catalytically active and secreted after inhibition of cellular mannosidases I and II with DMJ, as well as after inhibition of glucosidases I and II with MDN (11), suggesting that only core glycosylation was necessary for fully developed LPL activity and secretion. On the other hand, hepatic lipase was inactive and was not secreted after inhibition of glucosidases with

castanospermine or MDN (12), suggesting that elimination of terminal glucose residues is essential for hepatic lipase activity or for transport from the RER to the Golgi. A recent study has described similar results with LPL expression in Chinese hamster ovary (CHO) cells (13). The development of LPL activity occurred when LPL was retained within the RER, but not when the trimming of terminal glucose residues was blocked with castanospermine or MDN. Although the data of Ben-Zeev et al. (13) in CHO cells were similar to those described herein in adipocytes, there were several interesting differences. In spite of treatment with castanospermine, CHO cells continued to secrete some LPL that was essentially endo H-resistant, suggesting that the formation of complex oligosaccharides could occur even without removal of the terminal glucose residues in the RER by glucosidases. Although rat adipocytes do not synthesize and secrete as much LPL as do CHO cells, we could detect no LPL in the media after pulse-labeling, suggesting that removal of terminal glucose residues from oligosaccharides in adipocytes may be essential for secretion.

In this study, the LPL formed as a result of tunicamycin inhibition of glycosylation was very slowly degraded. Other studies have also observed changes in LPL degradation. Doolittle et al. (7) observed a difference in the fractional catabolic rate of intermediate and complex forms of LPL when comparing fed and fasted rat adipose tissue. Another study observed a decreased rate of LPL degradation after treatment of cells with epinephrine (21). To study intracellular turnover, it was essential to account for the effects of heparin. Previous studies have demonstrated that rat adipocytes release LPL from cells only in the presence of heparin (3). In addition, studies in avian adipocytes by Cisar et al. (27) demonstrated that much degradation of LPL occurs when LPL is bound to surface glycosaminoglycans, and then taken back up by the cell. According to this paradigm, heparin decreases degradation by releasing LPL from the surface glycosaminoglycans, and thus prevents reuptake. We wished to study intracellular degradation of the various LPL forms. Because castanospermine and tunicamycin prevent LPL from reaching a heparin-releasable pool, we examined degradation in the absence of heparin. The unglycosylated form of LPL produced by tunicamycin treatment demonstrated virtually no turnover, suggesting that the addition of the initial core oligosaccharide is necessary for LPL degradation. The LPL forms generated by castanospermine and DMJ demonstrated turnover that was closer to that of control cells, although still slightly delayed. Because the castanospermine-inhibited form of LPL demonstrated some degradation, yet did not reach the membrane glycosaminoglycans (as indicated by the decrease in HR), these data demonstrate that some degradation of LPL occurs intracellularly in rat adipocytes. It is interesting to speculate that the apparent regulation of LPL

degradation demonstrated previously (7, 21) may be due to hormonal regulation of the persistence of these incompletely processed LPL forms.

Additional insight into LPL post-translational regulation can be found in the *cld/cld* mouse, which is deficient in both LPL and HL activity due to a recessive mutation not involving the structural genes for LPL or HL (28). Two recent studies (29, 30) examined LPL synthesis and processing in brown adipocytes from *cld/cld* mice and found that a large amount of inactive LPL protein was synthesized by these cells, but not secreted. In addition, the LPL synthesized by *cld/cld* cells contained mostly high mannose forms of oligosaccharides. Pulse-chase studies of *cld/cld* liver disclosed similar high mannose oligosaccharides on HL, and there was little lipase activity or LPL mass in postheparin plasma (30). These data suggest that the defect in *cld/cld* mice permits core glycosylation, but prevents acquisition of activity and secretion. Thus, the *cld* mutation appears to either interfere with LPL and HL oligosaccharide processing or with transport from the RER to the Golgi. As some other adipocyte glycoproteins are not affected (30), it appears that the *cld* mutation may operate with some degree of specificity and may be of importance in understanding the physiologic regulation of LPL and HL.

In summary, the cleavage of the terminal glucose residues from N-linked oligosaccharide(s) and transport from the RER to the Golgi is an important step in LPL maturation in adipocytes. After core glycosylation, failure to remove the terminal glucose residues results in an LPL form that is catalytically inactive, not secreted, and more slowly degraded. The regulation at this step in LPL processing may be of considerable importance in the physiologic regulation of LPL. ■

We wish to thank Dr. John Goers for the anti-LPL antibodies, and Mehrnoosh Ghiam for technical assistance.

*Manuscript received 6 January 1992 and in revised form 26 June 1992.*

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