**Combined Lipase Deficiency in the Mouse**

**EVIDENCE OF IMPAIRED LIPASE PROCESSING AND SECRETION**

(Received for publication, March 1, 1990)

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Newborn combined lipase-deficient (cld) mice have severe hypertriglyceridemia associated with a marked decrease of lipoprotein lipase (LPL) and hepatic lipase (HL) activities. Since the cld mutation and lipase genes reside on separate chromosomes, combined lipase deficiency cannot result from defects occurring within the LPL or HL structural genes. To elucidate the biochemical basis of this trans-acting defect, cld mice were compared to unaffected littersmates for changes in lipase mRNA levels, rates of synthesis, and posttranslational processing and secretion. LPL and HL mRNA levels in cld liver and LPL in cld heart were comparable to controls; corresponding lipase synthetic rates were modestly decreased by about 30%. However, those reduced synthetic rates were not lipase-specific, since the rates of apolipoprotein (apo) A-I and apoA-II synthesis in cld liver were similarly decreased. Despite LPL synthetic rates that were 70% of controls, LPL mass in cld postheparin plasma was markedly reduced to only 7% of control values, suggesting that the majority of LPL is not secreted but remains intracellular. Consistent with a lipase secretory defect, neither the LPL nor HL oligomannosyl forms were converted to their respective complex forms in cld tissues, indicating that the lipases had failed to move from the endoplasmic reticulum/cis-Golgi to the medial/trans-Golgi network. In addition, the majority of intracellular LPL was catalytically inactive, since LPL specific activity (units/mg LPL protein) in cld heart, kidney, and brain was reduced 80–97%. In contrast to the severe impairment of lipase posttranslational processing and secretion, cld mouse plasma contained normal levels of another secretory N-linked glycoprotein, adipsin, with its oligosaccharide chains fully processed to the complex form. Thus, the cld mutation appears not to globally disrupt the secretion of all N-linked glycoproteins, but rather selectively impairs LPL and HL at points essential to their normal intracellular transport and secretion.

Lipoprotein lipase (LPL)\(^1\) and hepatic lipase (HL) are lipolytic enzymes involved in the hydrolysis of lipoprotein-derived triglycerides and phospholipids. LPL is synthesized and secreted by a wide variety of cell types, including myocytes, white and brown adipocytes, and fetal hepatocytes (1, 2), whereas HL is synthesized and secreted exclusively by hepatocytes (3). The secreted lipases are bound to the luminal surface of capillary endothelium, where they direct tissue influx of esterified fatty acids.

The homozygous combined lipase-deficient (cld) mouse is an excellent model of severe hypertriglyceridemia resulting from the almost total absence of both LPL and HL catalytic activities (4–6). While unaffected newborn animals incorporate circulating triglycerides rapidly into lipid droplets in parenchymal cells of liver, heart, lung, and brown adipose tissues, cld mice produce very few intracellular lipid droplets (7). Instead, capillaries of these tissues are packed with numerous chylomicron particles (7). As a consequence of the massive hypertriglyceridemia (>20,000 mg/dl), the cld animals die several days after birth. The triglycerides of these chylomicrons are readily hydrolyzed by bovine LPL without the addition of exogenous apoC-II (7), suggesting that cld hyperchylomicronemia is due mainly to diminished LPL activity. The cld mutation, located on chromosome 17 (4), is not linked to either the LPL (8) or HL structural gene loci. Thus, combined lipase deficiency in mice cannot result from the focal disruption of lipase genomic coding regions leading to the synthesis of an aberrant, inactive protein.

Expression of the gene disrupted by the cld mutation appears important only to LPL and HL, or perhaps to a small subset of proteins, since normal development of the homozygous cld fetus occurs in utero, and the phenotype becomes apparent in the neonate only after the ingestion of dietary triglyceride. This suggests that the trans-acting gene disrupted by the cld mutation has a specific function within the lipase processing pathway. In this study we further characterize the lipase deficiency and show that the cld mutation does not markedly affect LPL and HL mRNA levels or synthesis but does disrupt normal lipase processing and secretion.

**EXPERIMENTAL PROCEDURES**

Analysis of Lipase mRNA by Northern Blot Analysis—Total RNA was isolated from liver and heart by acid guanidium thiocyanate/phenol/chloroform extraction (9). Enrichment for polyadenylated RNA (poly(A)\(^+\)) RNA was carried out using oligod(T)-cellulose (10). RNAs (10 \(\mu\)g/sample) were resolved on 0.8% agarose gels in 0.5 mM

$\text{Vol. 265, No. 29, Issue of October 15, pp. 17960–17965, 1990}$

$\text{Printed in U.S.A.}$

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\(\text{---This work was supported by National Institutes of Health Grant HL 28481, American Heart Association, Greater Los Angeles Affiliate Grant 492-IG15, and by the Veterans Administration. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.}$

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\(\text{‡ The abbreviations used are: LPL, lipoprotein lipase; HL, hepatic lipase; ER, endoplasmic reticulum; apo, apolipoprotein; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate; MOPS, morpholinopropanesulfonic acid; Endo H, endo-β-N-acetylglucosaminidase.}$

\(\text{* A. J. Lusis, personal communication.}$

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MOPS, 50 mM sodium acetate, 10 mM EDTA, 2.2 mM formaldehyde (11) and transferred by capillarity to Nytran membranes (Schleicher & Schuell). Blots were probed using full length human LPL cDNA (12) or the mouse LPL clone m5 (13), rat H1 cDNA (14), or rat α-tubulin cDNA (a generous gift from T. Kirchgessner, UCLA) labeled with [35S]dCTP by random priming (15). Following prehybridization (1 h at 65°C) and hybridization (1.5 x 10^6 cpm/ml, 24 h at 65°C) in 50% formamide, 0.1% SDS, pH 7.0, containing 1 mM EDTA, 7% SDS, and 1% BSA, the blots were washed at a final stringency of 2 x SSC (0.45 M NaCl, 0.045 M trisodium citrate), 1% SDS, at 55°C, and autoradiographed for 20-40 h with Kodak X-ray film at -80°C using Dupont-Cronex Hi Plus intensifying screens.

Tissue Labeling and Homogenization—Newborn mice were decapitated and livers and hearts excised and sliced into approximately 0.1-1 mm³ pieces at room temperature in KRP-HEPES (Krebs-Ringer bicarbonate supplemented with 10 mM HEPES, pH 7.4) previously equilibrated with O2/CO2 (95:5). The tissue pieces were incubated for 5 min with shaking in the same buffer containing 1% BSA at 37°C. Following addition of [35S]methionine (ICN Transcalb) to 400 μCi/ml, the tissues were pulse-labeled for varying times. For chase experiments, the tissue slices were transferred to Dulbecco’s modified essential medium containing 1% BSA and 1 mM unlabeled methionine equilibrated in O2/CO2 (95:5). Incorporation of label was stopped by addition of cycloheximide (10 μg/ml, 16). After labeling, tissue homogenization and fractionation were carried out at 0-4°C. The labeled tissue pieces were washed in several changes of 0.15 M NaCl containing 10 μg/ml cycloheximide. To obtain liver microsomes, homogenization was carried out in 10 volumes of isotonic sucrose buffer (0.3 M sucrose, 5 mM EDTA, 1 mM p-mercaptoethanol, 0.2% Triton X-100, pH 7.2) per g tissue. The post-mitochondrial supernatant was subjected to 100,000 x g for 60 min to obtain microsomes (16). The microsomal pellet was sonicated in lysis buffer (3% Triton X-100, 0.1% Na-lauroyl sarcosine, 0.15 M NaCl, 1 mM phenylmethylsulfonyl fluoride in 0.1 M Tris, pH 7.5), and centrifuged at 100,000 x g for 60 min. In some cases, (α-2-labeled liver and heart tissue pieces were homogenized directly in lysis buffer. Nuclei and tissue debris were removed by centrifugation (160,000 x g). All supernatants were stored at -80°C until analyzed.

Immunoprecipitation—LPL immunoprecipitation was carried out in tissue and microsomal lysates adjusted to 1 M NaCl as described previously (13). HL immunoprecipitations were carried out with rabbit anti-rat HL-antiserum (3) using a two-cycle procedure. Briefly, 1-5 μl of rabbit anti-rat HL antiserum was added per mg liver protein, and immunoprecipitation carried out as described (3), except that, after SDS dissociation, Triton X-100 was added to a concentration 5-7 times higher than SDS, and rabbit anti-rat HL antiserum was added a second time. The immunoprecipitation was repeated and the immunoprecipitation fluid from the Staph A (crude insoluble Protein A) was removed and incubated with the Protein A (from lyophilized Staphylococcus aureus cells (Cosmac strain)) by boiling for two min with 35 μl of 2% SDS, 25% glycerol, 0.1 M Tris, pH 6.8. Immunoprecipitates were resolved by SDS-polyacrylamide gel electrophoresis and visualized by fluorography (3, 13).

Endo H and N-Glycanase Digestion—Following denaturation at 95°C with 0.5% SDS, LPL and HL immunoprecipitates were incubated with 1.25 milliliters of endo-β-N-acetylglucosaminidase (Endo H) at 37°C for 16-20 h (13). The reaction was stopped by addition of 60 μl of 1% SDS, 50% glycerol, 0.15 M Tris, pH 7.5, and boiling for 2 min. For control samples, Endo H was substituted with an equal volume of buffer phosphate (6.0). For plasma Endo H experiments, 1 μl of plasma was heated to 95°C for 2 min in the presence of 100 μl of 0.1% SDS, 50 mM sodium phosphate, pH 6.0; after cooling, 2.5 units of Endo H was added and incubated for 16 h at 37°C. The reaction was terminated by heating to 95°C followed by SDS gel electrophoresis. For N-glycanase experiments, 1 μl of plasma was heated to 95°C for 2 min in the presence of 100 μl of 0.1% SDS, 100 mM Tris, pH 7.5; after cooling, Triton X-100 was added to 0.7%, and 10 units of N-glycanase (Genzyme) was added. After incubation at 37°C for 16-20 h, SDS was added to a final concentration of 2% and the sample processed for electrophoresis as described above.

Western Blotting—0.5 μl of serum or plasma was electrophoresed in a 10 or 14% polyacrylamide gradient gel (3). After electrophoretic transfer (30 volts, 16 h) onto nitrocellulose, the blot was treated with blocking buffer for at least 30 min (3% BSA, 0.15 M NaCl in 0.1 M Tris, pH 7.5). Rabbit anti-mouse apoA-I and -A-II antiserum (17), rabbit anti-mouse IgG (Cappel) or rabbit anti-mouse adipin antiseraum (R. Spielgelman, Dana-Farber Cancer Institute) were diluted 1:500 to 1:1000 with blocking buffer, and incubated with the blot at 4°C for 16-20 h. The blot was developed using the alkaline phosphatase-second antibody system (Promega) per the manufacturer’s instructions.

Lipase Activity and Mass Assays—Tissues to be assayed for activity and mass were homogenized in 50 mM ammonium buffer, pH 6.1, containing 0.125% Triton X-100, 2 μg/ml leupeptin, 2 μg/ml pepstatin, and 20 kallikrein units/ml aprotonin. LPL (13, 18) and HL (3) activities were assayed using a tri(3,10'-H)oleoylglycerol substrate. Homogenates were diluted 100-fold in the assay mixture to prevent inhibition by Triton X-100. 1 milliunit of LPL or HL enzyme activity represents the release of 1 nmol of free fatty acid/mg tissue.

Combined Lipase Deficiency

RESULTS

The cd Mutation Does Not Specifically Affect the Synthesis of HL or LPL—Since lipase deficiency could arise as a result of impaired lipase synthesis, LPL and HL mRNA levels and rates of synthesis were determined in several tissues. Northern blots of total RNA from cd mice and unaffected littermates were hybridized with LPL and HL CDNs; as an internal control, α-tubulin cDNA was hybridized to the same blots. Two separate LPL mRNA sizes (3.4 and 3.6 kilobases) are found in both mice and humans and probably arise from the use of two separate polyadenylation sites (12). Both LPL mRNA sizes in cd liver (Fig. 1A) and heart (Fig. 1B) were present at levels similar to unaffected tissues, particularly when compared to the α-tubulin control. Similarly, when compared to α-tubulin, HL mRNA levels (1.8 kilobases) in cd liver were comparable to unaffected controls (Fig. 1A).

Although the cd mutation does not affect lipase messenger levels, a small, tissue-specific mRNA size difference was detected. Both LPL mRNA species in cd liver (but not heart) were slightly larger than the unaffected sizes of 3.4 and 3.6 kilobases, whereas the cd HL mRNA was slightly smaller.
Combined Lipase Deficiency

A

unaffected

LPL

α tubulin

HL

unaffected
cld/cld

LPL

α tubulin

HL

B

FIG. 1. Northern blot analysis of LPL and HL mRNA. A, 1 μg of liver poly(A)+-selected RNA was electrophoresed and blotted to Nytran. Blots were hybridized first with mouse LPL and rat α-tubulin cDNA clones (left panel) and then stripped and rehybridized with a rat HL cDNA clone (right lane). HL and α-tubulin are similar in size (1.8 kilobases), and co-migrate with 18S rRNA. B, 10 μg of total heart RNA was electrophoresed and blotted as in A, followed by hybridization with a mouse LPL cDNA clone. The positions of 28S and 18S rRNAs are indicated.

than the normal 1.8 kilobases. These size polymorphisms have been noted on two separate occasions using pooled RNA samples isolated from offspring derived from separate matings of parental heterozygotes. However, no corresponding size difference was detected in the lipase proteins.

To ascertain if LPL and HL synthetic rates were comparable to mRNA levels, cld and unaffected liver and heart were pulse-labeled in vivo. Rates of [35S]methionine incorporation into total and lipase protein throughout the 16 min of labeling were linear (Fig. 2). Although rates of label incorporation into HL (Fig. 2A) and LPL (Fig. 2, B and C) per mg protein were lower in the cld animals, incorporation rates into total protein were also depressed (Fig. 2). These lower rates of overall protein synthesis in the cld neonate are probably due, at least in part, to the impaired ability of these animals to utilize dietary triglycerides, a major nutrient during suckling. Alternatively, it is also possible that the unlabeled methionine pool in cld neonates is increased compared to unaffected controls. To eliminate these various possibilities, the results in Table I were expressed as "relative" rates of synthesis (see "Experimental Procedures"). The relative rates of HL and LPL synthesis were still found to be decreased by about 30% from control values. However, this 30% decrease in synthesis was not specific to the lipases, since the relative synthetic rates for apoA-I and apoA-II were similarly decreased in cld liver (Table I).

As shown by others (4-6) and confirmed by us, LPL and

HL catalytic activities in cld tissues are nearly nonexistent, although LPL-like protein levels are elevated in cld diaphragm muscle, heart, and brown adipose tissue (6), suggesting the presence of inactive mass intracellularly. We have extended

FIG. 2. Incorporation rates of [35S]methionine into total proteins, HL, and LPL in liver and heart. Heart or liver fragments were pulse-labeled, homogenized, and total liver microsomes isolated. Total proteins were precipitated by trichloroacetic acid and the lipases immunoprecipitated from lysates and quantitated as described under "Experimental Procedures." O, unaffected mice; △, cld mice. Regression lines are indicated and have correlation coefficients >0.95. A, incorporation of label into total proteins and HL from liver microsomes. B, incorporation of label into total proteins and LPL from liver microsomes. C, incorporation of label into total proteins and LPL from heart.

TABLE I

Relative rates of synthesis in cld/cld and unaffected mice

Relative rates of synthesis are defined as the rate of [35S]methionine incorporation into LPL and HL represented as a percentage of the rate of incorporation into total proteins. This rate was calculated from the slopes of the linear regression lines shown in Fig. 2 and as described under "Experimental Procedures." Liver HL and LPL were immunoprecipitated from total liver microsomes and thus represent the relative rates of synthesis compared to protein synthesis in microsomes; heart HL and liver apoA-I and apoA-II were immunoprecipitated from total tissue lysates and represent the relative rate of synthesis compared to total tissue protein synthesis.

| Tissue          | Protein | % of total protein synthesis | Synthesis ratio control/cld |
|-----------------|---------|------------------------------|-----------------------------|
| Liver microsomes| HL      | 0.031                        | 1.4                         |
|                 | LPL     | 0.029                        | 1.5                         |
|                 | LPL     | 0.046                        | 1.2                         |
| Heart           | LPL     | 0.037                        | 1.5                         |
|                 | ApoA-I  | 0.454                        | 1.5                         |
|                 | ApoA-II | 0.191                        | 1.5                         |

As shown by others (4-6) and confirmed by us, LPL and
these studies to two other cld tissues (brain and kidney) and have found that LPL specific activities are also dramatically reduced (Fig. 3). Thus, while the cld mutation does not markedly affect LPL synthesis and mass, it severely disrupts catalytic activity in all LPL-producing cell types.

The cld Mutation Affects the Processing and Secretion of LPL and HL—The intracellular processing of LPL and HL was assessed by examining the Endo H digestion of N-linked oligosaccharide moieties (21). Mouse LPL (22) and rat HL (14) possess two potential N-linked glycosylation sites, both of which are utilized (3, 23). Within the secretory pathway, transport of N-linked glycoproteins from the endoplasmic reticulum (ER)/cis-Golgi to the medial/trans-Golgi is marked by the transformation of the oligomannosyl group to the complex type by outer chain carbohydrate addition. The high mannose (oligomannosyl) moiety of N-linked glycoproteins found within the ER/cis-Golgi is sensitive to Endo H digestion; conversion of high mannose oligosaccharides to the complex form, which occurs within the medial/trans-Golgi, renders these glycoproteins resistant to Endo H cleavage (24).

LPL oligosaccharide processing was interrupted in cld liver (Fig. 4A). As described previously (13) and shown here (Fig. 4A), the Endo H-resistant LPL species in rat has a molecular mass of 56,000 daltons, whereas the Endo H-sensitive form has a decreased molecular mass of 51,000 daltons resulting from removal of the oligomannosyl group. In cld liver, a 20-min pulse followed by a chase period as long as 60 min revealed that little of the Endo H-sensitive LPL had been processed to the complex (Golgi) form, a result in striking contrast to the normal processing of LPL in unaffected liver (Fig. 4A). LPL processing in cld heart was also shown to be disrupted; even after a 40-min pulse-labeling period, the immunoprecipitated LPL was predominately Endo H-sensitive (Fig. 4B).

To confirm that the cld mutation had similar effects on the intracellular processing pathway of both lipases, HL oligosaccharide processing in cld liver was also examined. HL immunoprecipitated from liver and not treated with Endo H has a fully glycosylated molecular weight of 53,000 daltons (3); the 53,000-dalton species has been shown to represent the HL complex form, while the 51,000-dalton form contains only unprocessed high mannose oligosaccharides (3). Following Endo H treatment, the 51,000-dalton form decreases in molecular mass to 48,000, due to removal of the oligomannosyl residues (see lanes 2 and 4, Fig. 5). Again, only the 51,000-dalton form, sensitive to Endo H, was detected in cld liver even after a 60-min chase period (lane 2, Fig. 5). In contrast, a similar chase length in unaffected liver showed only the 53,000-dalton Endo H-resistant species (lane 5, Fig. 5). The reduced amount of this species seen in the liver of unaffected mice chased for 60 min most likely reflects secretion of the mature protein into the incubation medium, since it is known (25) that in vivo, mouse HL is mostly secreted to plasma rather than remaining anchored to the liver endothelium.

The predominance of the lipase high mannose forms suggests that newly synthesized LPL and HL are retained within the ER, and thus prevented from normal Golgi processing and secretion. This hypothesis was tested by examining the level of LPL mass in the plasma of 1-day-old cld pups following the injection of heparin (Fig. 6). LPL is normally secreted by tissue parenchyma and binds to the luminal surface of capillary endothelium through interaction with heparan sulfate proteoglycans (26). Heparin releases endothelium-bound LPL directly into plasma. In Fig. 6, A and B, it can be seen that the postheparin plasma of unaffected mice has abundant lipase activity compared to cld littermates. LPL mass in cld
postheparin plasma is decreased by at least an order of magnitude, equivalent to mass levels found in preheparin plasma of unaffected controls and an unrelated mouse strain (CSH). Thus, while tissue LPL levels in cld neonates are at least as high as unaffected controls (6), postheparin plasma levels are dramatically reduced. This indicates that the tissues of cld mice, although capable of nearly normal LPL synthesis, are unable to secrete LPL to any appreciable level. Since intracellular processing of HL is affected in an analogous manner to LPL, it is most likely that HL secretion is similarly impaired.

**The cld Mutation Does Not Affect All N-Linked Secretory Glycoproteins**—It was of interest to determine whether the defective secretion and intracellular processing of the lipases result from a global failure in the glycosylation pathway causing all N-linked glycoproteins to either not be secreted, or to be secreted as incompletely processed (Endo H-sensitive) forms. Since plasma contains the secreted forms of N-linked glycoproteins, which have necessarily passed through Golgi processing, the level and Endo H sensitivity of an N-linked glycoprotein other than the lipases was examined. Thus, adipsin was chosen as an alternate N-linked glycoprotein, since it is synthesized and secreted principally by adipose tissue into plasma. This protein acts as a serine protease within the alternative pathway of complement (27-29). As shown in Fig. 7, unlike LPL, plasma adipsin levels are as high in cld neonates as in unaffected littermates (compare lanes 1 and 3, 5 and 7), and the N-linked glycan moieties are resistant to Endo H digestion in both groups (lanes 2 and 4). The molecular weight of unglycosylated adipsin (lanes 6 and 8, Fig. 7) was determined after N-glycanase cleavage, which unlike Endo H does not distinguish between high mannose and complex oligosaccharides but removes both types of carbohydrate chains from N-linked glycoproteins with the same efficiency. To rule out the possibility that the cld serum inhibited Endo H activity, LPL immunoprecipitated from rat adipose tissue was subjected to Endo H digestion in the absence of cld serum; the LPL high mannose oligosaccharides were completely susceptible to Endo H cleavage (data not shown).

Since in cld neonates adipsin plasma levels, unlike LPL, were comparable to unaffected littermates, it appears that this protein is actively secreted by tissues synthesizing adipin (e.g. adipose) in the presence of the cld mutation. Furthermore, the secreted form of adipisin contained fully processed oligosaccharide residues. This indicates that the observed failure of the oligomannosyl forms of LPL and HL to be converted to their respective complex forms in cld tissues is not a global defect, since another N-linked glycoylated protein, adipisin, is probably not affected.

**DISCUSSION**

Combined lipase deficiency in mice is characterized by the almost complete absence of LPL and HL activity in both tissues and postheparin plasma (4, 5). It is an autosomal recessive mutation which acts postnatally, and is linked to the tailless locus on mouse chromosome 17. Recently, the LPL (8) and HL* genes have been shown to reside on mouse chromosomes 8 and 9, respectively. Thus, the joint absence of LPL and HL activities in cld mice cannot be the result of a mutation simply affecting the lipase structural genes.

In the present study, we show that the cld mutation does not affect either LPL or HL mRNA levels, although a modest but nonspecific decrease in relative lipase synthesis was observed. This small decrease in lipase synthesis cannot account for the cld phenotype, particularly since tissue LPL mass in the cld mouse is equal to or exceeds control values (6). However, the cld mutation does dramatically lower LPL specific activity (Fig. 3) and, as we have shown here, lipase secretion. Newly synthesized LPL and HL in cld liver and heart was inactive and probably remained intracellurally within the ER, as evidenced by the predominance of the high mannose forms of both enzymes. The extremely low LPL mass measurements in cld postheparin plasma also indicated that the cld mutation somehow prevents the normal medial/trans-Golgi processing and secretion of lipase enzyme.
Recently, results consistent with ours were reported for LPL in cultured brown adipocytes isolated from cld neonates (30). These cells also failed to secrete LPL, leading to the accumulation of inactive LPL mass within the ER which was completely sensitive to Endo H digestion. Our studies of cld liver and heart show that both LPL and HL encounter a similar defect in intracellular processing and secretion. Further, we find that the defect appears to be selective to the lipases and does not extend to all secretory N-linked glycoproteins.

It has been proposed that the glycosylation of LPL and HL is a requirement for the expression of enzyme activity, based on studies utilizing tunicamycin (31-34) and glucose deprivation (35). In turn, it has been postulated that LPL and HL are inactive in combined lipase deficiency as a consequence of improper glycosylation due to a defect within the general pathway of N-linked glycosylation (2). In support of this viewpoint, other genes affecting protein glycosylation are found in the same chromosomal region containing the cld mutation (36). However, we found no evidence of a global defect in N-linked glycosylation that decreased the secretion of all N-linked glycoproteins. On the contrary, the N-linked glycoprotein, adipsin, which like LPL is synthesized and secreted by adipose tissue, was examined in the plasma and was found not only to be present at a level as high as unaffected controls, but also to possess Endo H-resistant oligosaccharide chains. However, the analysis of serum adipin levels and glycan processing do not necessarily preclude the possibility that increased levels of Endo H-sensitive adipsin are present in adipin-producing tissues. It remains to be determined if the processing of other N-linked glycoproteins in the tissues synthesizing LPL and HL are similarly affected by the cld mutation.

What remains an enigma is how a single gene defect in the cld mouse can simultaneously affect LPL and HL activities and intracellular processing. Is the primary defect a disruption of lipase translocation from the ER to Golgi, leading to the retention of incompletely processed, and thus inactive, lipase within the ER? Or is the primary defect at the level of lipase mRNA posttranscriptional processing, resulting in the synthesis of an aberrant, inactive protein that remains within the ER until its degradation? Our results, and those of several other laboratories preclude a number of potential mechanisms for this intriguing defect. Since the genes for LPL, HL and the gene affected by the cld mutation are each present on separate chromosomes, the cld mutation cannot directly disrupt either lipase gene. Furthermore, heart and liver of cld neonates express nearly normal lipase mRNA concentrations, lipase synthetic rates and LPL protein levels (6). Thus, the cld mutation does not appear to act by decreasing stability or synthetic rates for lipase mRNAs or proteins. However, as shown by us and Maasno et al. (90), lipase processing and secretion are disrupted, leading to the intracellular retention of LPL and HL. Consequently, the cld mutation must exert its effect either posttranscriptionally or posttranslationally.

It is possible that the cld mutation disrupts a trans-acting gene on chromosome 17 necessary for proper processing of the lipase mRNAs. In such a posttranscriptional scheme, the cld mutation would interrupt the normal processing of the LPL and HL message, possibly by improper intron removal and exon splicing events, resulting in a mRNA coding for an aberrant, inactive protein. The inactive lipases would be degraded within the ER, since it is known that incorrectly folded or processed secretory proteins are often retained by ER luminal “binding” proteins (37). Another possibility is that the cld mutation somehow specifically inhibits the translocalization of the lipases from the ER to Golgi, leading to an accumulation of the oligomannosyl form of LPL and HL. In this case, newly synthesized LPL and HL would be structurally normal with respect to protein sequence, but would be inactive due to either incomplete posttranslational processing or some factor within the ER microenvironment. Indeed, retention of LPL within the ER of 3T3-422A mouse adipocytes by treatment with an ER/cis-Golgi translocation inhibitor is accompanied by a concomitant loss of enzyme activity (38, 39).

In summary, the cld mutation affects a trans-acting gene that appears to be essential and specific for the normal processing and secretion of LPL and HL. The identification of the gene containing the cld mutation and its possible involvement in lipase regulation remains to be elucidated.

Acknowledgments—We wish to acknowledge with particular gratitude Robert Scow and Karen Artzt for generous contributions of breeding pairs of heterozygous cld mice, Bruce Spiegelman and Barry Rosen for donation of adipsin antiserum, and Michael C. Schotz for critical review of the manuscript.

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