Lipase maturation factor 1 is required for endothelial lipase activity

Osnat Ben-Zeev, Maryam Hosseini, Ching-Mei Lai, Nicole Ehrhardt, Howard Wong, Angelo B. Cefalu, Davide Noto, Maurizio R. Averna, Mark H. Doolittle, and Miklos Peterfy

Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA; Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, CA; Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA; and Department of Clinical Medicine and Emerging Diseases, University of Palermo, Palermo, Italy

Abstract Lipase maturation factor 1 (Lmf1) is an endoplasmic reticulum (ER) membrane protein involved in the hydrolysis of two principal lipid substrates associated with lipoprotein metabolism: triglycerides (TG) and phospholipids. The vascular lipase family is composed of three evolutionarily related enzymes, lipoprotein lipase (LPL), hepatic lipase (HL), and endothelial lipase (EL) (1–3). Localized to the luminal face of tissue capillaries, lipases hydrolyze TGs and phospholipids into metabolically usable fatty acids and other lipids. The released fatty acids result from lipolysis are taken up by subjacent tissue and used for energy storage (adipose), oxidation, and energy production (skeletal muscle and heart). LPL is predominantly a phospholipase affecting HDL metabolism, but it also shares a redundant role with HL in the metabolism of LpBs (11, 17). Indeed, modulation of EL activity in mice leads to changes in plasma HDL levels similar to those of HL (18–20), reflecting their related substrate specificities. Consistent with their multifaceted involvement in lipoprotein metabolism, LPL, HL, and EL are strongly associated with plasma lipid levels in the general population (21).

Supplementary key words combined lipase deficiency • endoplasmic reticulum • hepatic • metabolism • phospholipases

This work was supported by National Institutes of Health Grant HL-028481; its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health or other granting agencies. Support was also received from the Cedars-Sinai Medical Center, the United States Department of Veterans Affairs, and the University of Palermo. Manuscript received 9 September 2010 and in revised form 23 March 2011. Published, JLR Papers in Press, March 28, 2011 DOI 10.1194/jlr.M011155

Abbreviations: apoB, apolipoprotein B; cld, combined lipase deficiency gene; EL, endothelial lipase; ER, endoplasmic reticulum; Lmf1, lipase maturation factor 1; LpB, apoB-containing lipoproteins; TG, triglyceride.

To whom correspondence should be addressed.

This article has been withdrawn by Osnat Ben-Zeev, Maryam Hosseini, Nicole Ehrhardt, Angelo B. Cefalu, Davide Noto, Maurizio R. Averna, Mark H. Doolittle, and Miklos Peterfy. Ching-Mei Lai and Howard Wong could not be reached. After a review of the data, the corresponding author learned that data in Figures 1B, 2B, and 3 were inappropriately presented, which raises concerns about the validity of conclusions reached. The Averna laboratory (A. B. C., D. N. and M. R. A.) was not involved in the generation or presentation of data in Figures 1B, 2B, and 3.
The lipase maturation factor 1 (Lmf1) gene has been recently identified as the gene affected by the combined lipase deficiency (cld) mutation in the mouse (22). Homozygous cld mice develop severe hypertriglyceridemia and die shortly after birth due to complications arising from massive chylomicronemia (23). Although LPL deficiency is the principal cause of elevated plasma TG levels, HL activity is also diminished in cld mice. As mRNA and protein expression of LPL and HL in cld mice are unaffected, the lack of enzymatic activity is the result of lipase misfolding, causing aggregation and retention of the inactive lipase protein in the endoplasmic reticulum (ER). Thus, Lmf1 is a critical factor in the posttranslational maturation of nascent lipase polypeptides into active enzymes (24). Lmf1 is an ER membrane protein that has been shown to interact with LPL and HL through one of its loops extending into the ER lumen (25). Expression constructs

Human LPL, HL, PL, and EL cDNAs were subcloned into the pcDNA6 expression vector (Invitrogen) containing a C-terminal c-Myc epitope tag as described previously (28). For experiments using lipase affinity purification, a tandem affinity purification (TAP) tag was synthesized for in-frame integration into an AgeI site occurring just after the V5 epitope tag of pcDNA6 (29). After transfection, the resulting expressed LPL, HL, PL, and EL proteins contained a C-terminal V5-TAP tag consisting of the V5 epitope followed by a single calmodulin-binding peptide domain, a tobacco etch virus (TEV) protease site and ending in two adjacent IgG-binding domains derived from protein A (29). Mouse Lmf1 cDNA was subcloned into the pcDNA3.1 expression vector (Invitrogen) containing an N-terminal c-Myc epitope tag (25).

Affinity purification and Western blotting

Lipase affinity purification and immunoblotting were performed as described previously (25). Before Western blot analysis of EL in cell culture medium, samples were concentrated by heparin-Sepharose affinity purification in batch mode. Briefly, heparin-Sepharose slurry was added to aliquots of conditioned medium, incubated for 1 h, washed three times with 50 mM Tris-HCl, pH 7.5, and eluted with 2 M NaCl and boiling.

Lipase assays

For lipase assays from cell extracts, lysates were prepared by sonication in 20 mM Tris-HCl buffer, pH 7.5, containing 0.2% deoxycholate and 10 U/ml heparin. The activity levels of LPL and HL were measured using the respective triolein substrates prepared by sonication (30). PL was assayed with the substrate used for HL, the presence of colipase (Sigma) as described previously (31). EL activity was determined using a phospholipase immunochemistry assay involving the bichoninic acid (BCA) reagent (Pierce). Lm15, a cell line stably expressing the lipase maturation factor 1 (Lmf1) under the control of a metallic promoter (32), was used to determine secreted EL activity, cells were harvested, and before cell culture supernatants were prepared for the determination of EL activity in pre- and post-heparin plasma samples, EL, the same phospholipid substrate was used in the assay. Lm15 cells were treated with 10 U/ml heparin for 2 h before cell culture supernatants were assayed. The EL activity in pre- and post-heparin plasma samples was determined by ELISA (Pierce Life Science, Inc.) according to the manufacturer’s instructions.

RESULTS

The cld mutation affects EL activity

We have previously demonstrated that the cld mutation abrogates the posttranslational maturation and activity of two related lipases, LPL and HL (32). However, the effect of the cld mutation on EL, a third lipase family member, has yet to be evaluated. To address the dependency on Lmf1 function among various lipase family members, expression vectors encoding LPL, HL, EL, and a more distantly related lipase, PL (Fig. 1A), were transfected into fibroblasts harboring the recessive, loss-of-function cld mutation. After transfection, cells heterozygous (cld/+), and homozygous (cld/cld) for the mutation were assessed by measurements of lipase activities. As expected from previous studies (27, 32), the activity levels of LPL and HL, but not PL, were dramatically reduced in cld/cld cells (Fig. 1B). Importantly, we found that EL was as severely affected as LPL and HL, with only ~5% of the wild-type activity detected in homozygous mutant cells. These results suggest that the combined lipase deficiency phenotype associated

MATERIALS AND METHODS

Cell lines and transfection

Fibroblast cell lines derived from cld homozygous (cld/cld) and heterozygous (cld/+). These cell lines have been described previously (27). Both the cells carrying the cld homozygous and heterozygous (cld/+). These cell lines were maintained in DMEM supplemented with 10% fetal bovine serum, penicillin-streptomycin, sodium pyruvate, and nonessential amino acids (Amaxa Biosystems) according to the manufacturer’s instructions (program U-24, solution V). Electroporated cells were plated in collagen-coated 12 well plates. HEK293 cells were transfected with FuGENE6 transfection reagent (Roche) according to the manufacturer’s instructions. Cells were harvested 24–48 h after transfection.

Expression constructs

Human LPL, HL, PL, and EL cDNAs were subcloned into the pcDNA6 expression vector (Invitrogen) containing a C-terminal V5 epitope tag as described previously (28). For experiments using lipase affinity purification, a tandem affinity purification (TAP) tag was synthesized for in-frame integration into an AgeI site occurring just after the V5 epitope tag of pcDNA6 (29). After transfection, the resulting expressed LPL, HL, PL, and EL proteins contained a C-terminal V5-TAP tag consisting of the V5 epitope followed by a single calmodulin-binding peptide domain, a tobacco etch virus (TEV) protease site and ending in two adjacent IgG-binding domains derived from protein A (29). Mouse Lmf1 cDNA was subcloned into the pcDNA3.1 expression vector (Invitrogen) containing an N-terminal c-Myc epitope tag (25).
indicating that Lmf1 transfection restored the activity of these lipases to heterozygous levels. Furthermore, analysis of untransfected cells demonstrated that the low activity detected in the absence of Lmf1 (2Lmf1 in Fig. 2B) represents endogenous lipases and not exogenous EL (data not shown). Thus, EL activity is completely dependent on functional Lmf1 in our assay. Importantly, immunoblot analyses indicated that elevated lipase activities in Lmf1-expressing cells were not due to increased lipase protein mass but, rather, to higher specific activity (Fig. 2B). To determine the dependence of secreted EL activity on Lmf1, we also analyzed cell culture medium after heparin treatment. As shown in Fig. 2C, EL activity was undetectable in conditioned medium from Lmf1-deficient cells, but activity was rescued by Lmf1 expression. Interestingly, unlike LPL and HL (32), EL protein secretion was unaffected by the presence or absence of Lmf1 (Fig. 2C), indicating that inactive EL is readily secreted from cells carrying the cld mutation. In conclusion, our results demonstrate that Lmf1 transfection restored the activity of these lipases to heterozygous levels. Furthermore, analysis of untransfected cells demonstrated that the low activity detected in the absence of Lmf1 (−Lmf1 in Fig. 2B) represents endogenous lipases and not exogenous EL (data not shown). Thus, EL activity is completely dependent on functional Lmf1 in our assay. Importantly, immunoblot analyses indicated that elevated lipase activities in Lmf1-expressing cells were not due to increased lipase protein mass but, rather, to higher specific activity (Fig. 2B). To determine the dependence of secreted EL activity on Lmf1, we also analyzed cell culture medium after heparin treatment. As shown in Fig. 2C, EL activity was undetectable in conditioned medium from Lmf1-deficient cells, but activity was rescued by Lmf1 expression. Interestingly, unlike LPL and HL (32), EL protein secretion was unaffected by the presence or absence of Lmf1 (Fig. 2C), indicating that inactive EL is readily secreted from cells carrying the cld mutation. In conclusion, our results demonstrate that, similar to LPL and HL, Lmf1 is critically required for the posttranslational processing of EL into active enzyme.

Lmf1 interacts with EL

To investigate whether EL dependency on Lmf1 function involves physical interaction between these two proteins, we performed affinity purification experiments.

Fig. 1. Members of the lipase gene superfamily that are affected by the cld mutation are shown. A: The phylogenetic tree of the lipase gene family shows a group of closely related members (HL, LPL, and EL) that form homodimers compared with the more distantly related PL, which is active as a monomer. The subunit structure of the remaining members is not known. All members of the family are secreted enzymes, and thus, all mature within the ER. The members include PLA1A, phospholipase A1 member A (Q53H76); LIPH, lipase member H (Q8WWY8); PL, pancreatic triacylglycerol lipase (P16233), and the three PL-related lipases, PLR1 (P54315), PLR2 (P54317), and PLR3 (Q17RR3); HL, hepatic triacylglycerol lipase (P11150); LPL, lipoprotein lipase (P16238); and EL, endothelial lipase (Q9Y5X9). B: The four members of the lipase gene family with known subunit structures were transfected into cells homozygous for the cld mutation (cld/cld), and expression was compared with that of control cells carrying only one copy of the cld allele (+/cld). Panels show representative Western blots of total cell lysates detected using an antibody against a lipase-specific epitope tag.

WITHDRAWN
July 17, 2019

with the cld mutation extends to a third member of the triglyceride lipase family, namely EL.

Lmf1 is required for the posttranslational maturation of EL

The cld mutation in the Lmf1 gene arose on a rare variant form of chromosome 17, called the haplotype (33). Due to several large inversions, the haplotype region is subject to recombination suppression with wild-type chromosomes and, as a consequence, has accumulated a variety of deleterious mutations during its evolutionary history (34). To investigate whether diminished EL activity in cld/cld cells is the consequence of the cld mutation affecting Lmf1 function or the result of other linked variants associated with the haplotype, we complemented cld/cld cells with wild-type (22). In these experiments, mutant cld/cld cells were cotransfected with the Lmf1 wild type along with the various lipase expression constructs, and lipase activities were determined (Fig. 2A). As PL is not dependent on Lmf1 function, coexpression of wild-type Lmf1 did not increase the activity of this enzyme (Fig. 2B). In fact, PL activity is apparently reduced in the presence of Lmf1, an effect currently not fully understood. In contrast, Lmf1 expression elevated the activities of cell-associated HL, LPL, and EL several fold. The fold increases in LPL and EL activities are similar to those between the cld and heterozygous cells transfected with lipases only (Fig. 1B),
Fig. 2. Reconstitution of Lmf1 expression in cld/cld cells rescues lipase activity. A: Schematic diagram shows the reconstitution assay. Lmf1-deficient cells are cotransfected with Lmf1 and lipase expression vectors. At 1–2 days after transfection, lipase activity and mass were assessed in cell lysates. B: Upper panels show cell-associated lipase activities (n = 3) after cotransfection with Lmf1 (black bars) or empty vector (gray bars) and the respective lipases. Lower panels show representative Western blots of total cell lysates visualized using an antibody against a lipase-specific epitope tag. C: EL activity released into cell culture medium after heparin treatment of cells transfected with EL ± Lmf1 is shown. Lower panel shows Western blot of cell culture medium. ND, not detectable.
Epitope-tagged Lmf1 and affinity-tagged lipase proteins were coexpressed in HEK293 cells. Lipase proteins were then affinity purified from cell extracts, with Lmf1 copurification assessed by immunodetection. As demonstrated previously (25), Lmf1 could be detected in association with LPL and HL but not with PL (Fig. 3). Furthermore, and consistent with its functional effect on EL, Lmf1 was readily detected together with affinity-purified EL, indicating that the proteins physically interact. To exclude the possibility that the interaction is due to abnormal lipase structure resulting from the C-terminal affinity tag, we confirmed that affinity-tagged EL was fully active (see supplementary Fig. 1).

**LMF1 deficiency diminishes post-heparin phospholipase activity**

In order to evaluate whether Lmf1 is required for the expression of active EL in vivo, we initially attempted to analyze plasma from *cld* homozygous mice. However, as these mice die within 1–2 days after birth, we were unable to collect sufficient amounts of plasma for the reliable detection of EL activity. In contrast to mice, LMF1 deficiency in humans is not lethal, permitting the use of plasma from an LMF1-deficient patient we previously characterized (26). This individual carries a homozygous nonsense mutation in LMF1 (W464X) and exhibits diminished post-heparin activity levels for both LPL and HL associated with massive hypertriglyceridemia. Although a specific method for measuring the activity of EL has not been developed, it has been demonstrated that the majority of heparin-releasable plasma phospholipase activity can be attributed to EL (27) in mice (19). Moreover, the activity of heparin-releasable phospholipase, from that of EL based on their differential sensitivities to high salt concentrations (31). Thus, we hypothesized that if LMF1 deficiency caused diminished EL activity in vivo, it would result in reduced heparin-releasable and salt-inhibitable phospholipase activity in plasma of the patient carrying the W464X mutation. Indeed, the proband exhibited over 95% reduction in phospholipase activity compared with that of controls (Fig. 4). Furthermore, EL protein mass in post-heparin W464X plasma was similar to control levels (134.6 ng/ml vs. control median of 133.55 ng/ml). In conclusion, these data are consistent with those from our in vitro studies indicating that Lmf1 is required for EL to attain enzymatic activity.

**DISCUSSION**

We report in this study that in addition to LPL and HL, EL also requires Lmf1 to attain enzymatic activity. Multiple lines of evidence support this conclusion, including the inability of Lmf1-deficient cells to produce active EL, physical interaction between EL and Lmf1, and the virtual absence of EL activity in an LMF1-deficient mouse model. To probe whether the combined lipase deficiency results in a functional block of functional Lmf1 to the lipase gene family, EL.

Fig. 3. HL, LPL, and EL, but not PL, copurify with the Lmf1 protein. HEK293 cells were cotransfected with Lmf1 and each of four affinity-labeled lipase constructs (HL, LPL, PL, and EL), including an empty vector control (vect). The top and middle panels represent Western blots of total cell lysates visualized using antibody (Ab) to either lipase-specific (VS) or Lmf1-specific (c-myc) epitope tags. Each expressed lipase construct was then affinity (affi) purified from total cell lysates under mild conditions that favor retention of protein-protein interactions. The bottom panel represents a Western blot of the affinity-purified lipases probed using antibody directed to the Lmf1-specific epitope tag.
analysis of lipases indicates that PL diverged from an ancestral lipase prior to the evolutionary emergence of LPL, HL, and EL, which share a more recent root (43). Similar to PL, bacterial and fungal lipases also function as monomers (44), suggesting that the homodimeric subunit structure is a more recent evolutionary development. Interestingly, the evolutionary emergence of Lmf1 homologs coincides with that of homodimeric lipases (M. Doolittle, unpublished data), raising the possibility that Lmf1 coevolved with the LPL/HL/EL branch of lipases to facilitate attainment of their unique structure. Further mechanistic studies will be required to address this hypothesis.

Studies of naturally occurring polymorphisms and experimental models (4, 16) have identified a central role for secreted lipases in fat metabolism. These lipases regulate lipoprotein metabolism through their bifunctional lipolytic activities (i.e., triacylglycerol and phospholipid lipase) and interactions with multiple classes of lipoprotein particles. Although the metabolic consequences of deficiencies in individual lipases have been extensively studied, metabolic interactions among the lipases remain poorly characterized. Considerable redundant enzymatic activities and substrate specificities and the distinct, yet overlapping expression patterns of lipases, such interactions may have important contributions to dyslipidemia and atherosclerotic cardiovascular disease. For example, an association analysis of LPL, HL, and EL variants revealed genetic interaction in the determination of plasma TG levels in a normal population (47). To investigate the interaction between HL and EL directly, Brown et al. (17) recently generated HL/EL double-knock-out mice representing the first engineered mouse model of combined lipase deficiency. These mice exhibit phenotypes that neither of the single-lipase knock-outs do, including increased neonatal lethality and the accumulation of small LDL particles, which revealed a redundant role for HL and EL in the metabolism of apoB-containing lipoproteins. LMF1 deficiency represents a unique metabolic scenario where the activities of three lipases are simultaneously diminished and offers further insights into lipase interactions. For example, hypertriglyceridemia is typically associated with low HDL levels, a consequence of enhanced clearance resulting from the lipolytic actions of HL, and perhaps EL, on TG-enriched HDL particles (48). Yet, despite severely elevated TG levels due to LPL deficiency, HDL-cholesterol in the LMF1<sup>W464X</sup> patient is within normal range (26), likely reflecting reduced activities of HL and EL. Although the lack of HDL-lowering effect is predictable based on known activities of the individual lipases, more detailed characterization of the combined lipase deficiency phenotype will no doubt uncover further insights about lipase interactions and their effects on lipoprotein metabolism.

In addition to plasma lipoprotein remodeling, lipases are also critical components in tissue lipid homeostasis. Through the liberation of FA from lipoprotein-bound glycerolipids, these enzymes provide substrates for energy storage and generation in adipose and muscle, respectively (49), and FA-derived signaling molecules involved in transcriptional regulation in various tissues (50). Nonetheless, fat cell processes are likely to be affected by the lipase deficiency in LMF1 deficiency, as adipose tissue mass is largely unaffected by LMF1 deficiency in human subjects (52, 53) and animals (54, 55), which has been attributed to adipocyte metabolism. First, de novo synthesis of TG in adipose tissue is substantially upregulated in the absence of LPL activity (5, 56). Further, EL expression is substantially upregulated in lipodystrophic adipose tissue, providing an alternative pathway for FA uptake (57). However, the latter compensatory mechanism is expected to be ineffective in LMF1-deficient human subject due to impaired posttranslational maturation of EL. Thus, lipid homeostasis is likely to be more severely affected in LMF1-deficient than LPL-deficient adipose tissue, especially in humans where lipogenic capacity is lower than in mice (58). Consistent with this hypothesis, an LMF1-deficient human subject exhibits lipodystrophy (22). Similar to adipose tissue, reciprocal regulation of lipase expression also occurs in other tissues. Namely, LPL and HL are upregulated in EL-deficient muscle and liver, respectively, further highlighting the functional redundancy and interrelatedness of lipase family members (20).

It is therefore likely that LMF1 deficiency in these tissues leads to metabolic defects not observed in the absence of individual lipases. Testing of this hypothesis awaits the generation of viable adult and tissue-specific Lmf1-deficient mouse models.***

We thank Dawn Marchadier and Daniel J. Rader for measurements of EL protein levels in human plasma.

REFERENCES

1. Wang, H., and R. H. Eckel. 2009. Lipoprotein lipase: from gene to obesity. Am. J. Physiol. Endocrinol. Metab. 297:E271–E288.
2. Perret, B., L. Mobile, L. Martinez, F. Terce, R. Barbaras, and X. Collet. 2002. Hepatic lipase: structure/function relationship, synthesis, and regulation. J. Lipid Res. 43: 1165–1169.

3. Broedl, U. C., W. Jin, and D. J. Rader. 2004. Endothelial lipase: a modulator of lipoprotein metabolism upregulated by inflammation. Trends Cardiovasc. Med. 14: 292–296.

4. Weinstock, P. H., C. L. Bisgaier, K. Aalto-Seetala, H. Radner, R. Ramakrishnan, S. Levak-Frank, A. D. Essenburg, R. Zechner, and J. L. Breslow. 1995. Severe hypertriglyceridemia, reduced high-density lipoprotein, and neonatal death in lipoprotein lipase knockout mice. Mild hypertriglyceridemia with impaired very low density lipoprotein clearance in heterozygotes. J. Clin. Invest. 96: 2555–2568.

5. Weinstock, P. H., S. Levak-Frank, L. C. HUDGINS, H. Radner, J. M. Friedman, R. Zechner, and J. L. Breslow. 1997. Lipoprotein lipase controls fatty acid entry into adipose tissue, but fat mass is preserved by endogenous synthesis in mice deficient in adipose tissue lipoprotein lipase. Proc. Natl. Acad. Sci. U.S.A. 94: 10261–10266.

6. Levak-Frank, S., H. Radner, A. Walsh, R. Stollberger, G. Knipping, G. Hoeller, W. Satler, P. H. Weinstock, J. L. Breslow, and R. Zechner. 1995. Muscle-specific overexpression of lipoprotein lipase causes a severe myopathy characterized by proliferation of mitochondria and peroxisomes in transgenic mice. J. Clin. Invest. 96: 976–986.

7. Merkel, M., P. H. Weinstock, T. Chajak-Shaul, H. Radner, B. Yin, J. L. Breslow, and I. J. Goldberg. 1998. Lipoprotein lipase expression exclusively in the mouse model for metabolism in the neonatal period and during cachexia. J. Clin. Invest. 102: 893–901.

8. Pulawa, L. K., and R. H. Eckel. 2002. Overexpression of muscle lipoprotein lipase and insulin sensitivity. Curr. Opin. Clin. Nutr. Metab. Care. 5: 569–574.

9. Ferreira, I. D. M. C., L. K. Pulawa, D. R. Jensen, and R. H. Eckel. 2001. Overexpressing human lipoprotein lipase in mouse skeletal muscle is associated with insulin resistance. Diabetes. 50: 1061–1068.

10. Kim, J. K., J. J. Fillmore, Y. Chen, C. Yu, I. K. Moore, M. Pypaert, E. P. Lutz, Y. Kako, W. Velez-Carrasco, I. J. Goldberg, et al. 2001. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. Proc. Natl. Acad. Sci. U.S.A. 98: 7522–7527.

11. Jin, W., D. Marchadour, and D. J. Rader. 2002. Liposome- and diacylglycerol-induced lipoprotein lipase overexpression increases apolipoprotein B-containing lipoprotein levels. Biochim. Biophys. Acta. 1592: 276–285.

12. Zambon, A., S. Bertocco, N. Vitturi, V. Polentarut, and G. Cremaldi. 2003. Relevance of hepatic lipase in the metabolism of triacylglycerol-rich lipoproteins. Biochim. Biophys. Acta. 1642: 1070–1074.

13. Busch, S. J., R. L. BARNHART, G. A. Markert, S. J. Yates, S. J. Mao, C. E. Thomas, and S. J.当たり. 1998. Hepatic triglyceride lipase expression in protein and aortic cholesterol J. Biol. Chem. 273: 16376–16383.

14. Dichek, H. L., W. Breaugh, B. Akefe, L. Conzo, A. S. et al. 1998. Overexpression of angiotensin II increases apolipoprotein B-containing lipoprotein levels in mice. Biochim. Biophys. Acta. 1392: 276–285.

15. Homanics, G. E., H. V. de Silva, J. Osada, S. H. Zhang, H. Wong, J. Borenzstajn, and N. Maeda. 1995. Mild dyslipidemia in mice following targeted inactivation of the hepatic lipase gene. J. Biol. Chem. 270: 2974–2980.

16. Brown, R. J. W., R. J. Lagor, S. Sankaranarayanan, T. Yasuda, T. Qureshio, M. G. Rothblat, and D. J. Rader. 2010. Impact of combined deficiency of hepatic lipase and endothelial lipase on the metabolism of both high-density lipoproteins and apolipoprotein B-containing lipoproteins. Circ. Res. 107: 357–364.

17. JAYE, M. K., J. Lynch, J. Krawiec, D. Marchadour, C. Maugeais, K. Doan, V. South, D. Amin, M. Perrone, and D. J. Rader. 1999. A novel endothelium-derived lipase that modulates HDL metabolism. Nat. Genet. 21: 423–428.

18. Ishida, T., S. Choi, R. K. Kundu, K. Hirata, E. M. Rubin, A. D. Cooper, and T. Quertermous. 2003. Endothelial lipase is a major determinant of HDL level. J. Clin. Invest. 111: 347–355.

19. Ma, K., M. Cilingiroglu, J. D. Otvos, C. M. Ballantyne, A. J. Marian, and L. Chan. 2003. Endothelial lipase is a major genetic determinant for high-density lipoprotein concentration, structure, and metabolism. Proc. Natl. Acad. Sci. U.S.A. 100: 2748–2753.

20. Teslovich, T. M., K. Musunuru, A. V. Smith, A. C. Edmondson, I. M. Stylianou, M. Koseki, J. P. Pirruccello, S. Ripatti, D. I. Chasman, C. J. Willer, et al. 2010. Biological, clinical and population relevance of 95 loci for blood lipids. Nature. 466: 707–713.

21. Peterfy, M., O. Ben-Zeev, H. Z. Mao, D. Weissglas-Volkov, B. E. Aouizerat, C. R. Pullinger, P. H. Frost, J. P. Kane, M. J. Malloy, K. Reue, et al. 2007. Mutations in LMF1 cause combined lipase deficiency and severe hypertriglyceridemia. Nat. Genet. 39: 1483–1487.

22. Paterniti, J. R., Jr., W. V. Brown, H. N. Ginsberg, and K. Artzt. 1983. Combined lipase deficiency (cld): a lethal mutation on chromosome 17 of the mouse. Science. 221: 167–169.

23. Doolittle, M. H., N. Ehrt hardt, and M. Peterfy. 2010. Lipase maturation factor I: structure and role in lipid folding and assembly. Curr. Opin. Lipidol. 21: 198–203.

24. Doolittle, M. H., S. B. Neher, O. Ben-Zeev, J. Ling-Liao, C. M. Gallagher, M. Hossei, F. Yin, H. Wong, P. Walter, and M. Peterfy. 2009. Lipase maturation factor LMF1, membrane topology and interaction with lipase proteins in the endoplasmic reticulum. J. Biol. Chem. 284: 39365–39363.
43. Hide, W. A., L. Chan, and W. H. Li. 1992. Structure and evolution of the lipase superfamily. J. Lipid Res. 33: 167–178.
44. Wong, H., and M. C. Schotz. 2002. The lipase gene family. J. Lipid Res. 43: 993–999.
45. Willer, C. J., S. Sanna, A. U. Jackson, A. Suteri, L. L. Bonnycastle, R. Clarke, S. C. Heath, N. J. Timpson, S. S. Najjar, H. M. Stringham, et al. 2008. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat. Genet. 40: 161–169.
46. Kathiresan, S., C. J. Willer, G. M. Peloso, S. Demissie, K. Musumuru, E. E. Schadt, L. Kaplan, D. Bennett, Y. Li, T. Tanaka, et al. 2009. Common variants at 30 loci contribute to polygenic dyslipidemia. Nat. Genet. 41: 56–65.
47. Reilly, M. P., A. S. Foulkes, M. L. Wolfe, and D. J. Rader. 2005. Higher order lipase gene association with plasma triglycerides. J. Lipid Res. 46: 1914–1922.
48. Rashid, S., K. D. Uffelman, and G. F. Lewis. 2002. The mechanism of HDL lowering in hypertriglyceridemic, insulin-resistant states. J. Diabetes Complications. 16: 24–28.
49. Zechner, R. 1997. The tissue-specific expression of lipoprotein lipase: implications for energy and lipoprotein metabolism. Curr. Opin. Lipidol. 8: 77–88.
50. Ahmed, W., O. Ziouzenkova, J. Brown, P. Devchand, S. Francis, M. Kadakia, T. Kanda, G. Orasanu, M. Sharlach, F. Zandbergen, et al. 2007. PPARs and their metabolic modulation: new mechanisms for transcriptional regulation? J. Intern. Med. 262: 184–198.
51. Bickerton, A. S., R. Roberts, B. A. Fielding, L. Hodson, E. E. Blaak, A. J. Wagenmakers, M. Gilbert, F. Karpe, and K. N. Frayn. 2007. Preferential uptake of dietary fatty acids in adipose tissue and muscle in the postprandial period. Diabetes. 56: 168–176.
52. Peeva, E., L. D. Brun, M. R. Ven Murthy, J. P. Despres, T. Normand, C. Gagne, P. J. Lupien, and P. Julien. 1992. Adipose cell size and distribution in familial lipoprotein lipase deficiency. Int. J. Obes. Relat. Metab. Disord. 16: 737–744.
53. Brun, L. D., C. Gagne, P. Julien, A. Tremblay, S. Moorjani, C. Bouchard, and P. J. Lupien. 1989. Familial lipoprotein lipase activity deficiency: study of total body fatness and subcutaneous fat tissue distribution. Metabolism. 38: 1005–1009.
54. Ginzing, D. G., S. M. Clee, J. Dallongeville, M. E. Lewis, H. E. Henderson, E. Bauge, Q. R. Rogers, D. R. Jensen, R. H. Eckel, R. Dyer, et al. 1999. Lipid and lipoprotein analysis of cats with lipoprotein lipase deficiency. Eur. J. Clin. Invest. 29: 17–26.
55. Savonen, R., K. Nordstoga, B. Christophersen, A. Lindberg, Y. Shen, M. Hultin, T. Olivecrona, and G. Olivecrona. 1999. Chylomicron metabolism in an animal model for hyperlipoproteinemia type I. J. Lipid Res. 40: 1356–1346.
56. Ullrich, N. F., J. Q. Purnell, and J. D. Brunzell. 2001. Adipose tissue fatty acid composition in humans with lipoprotein lipase deficiency. J. Investig. Med. 49: 273–275.
57. Kratky, D., R. Zimmermann, E. M. Wagner, J. G. Strauss, W. Jin, G. M. Kostner, G. Haenmerle, D. J. Rader, and R. Zechner. 2005. Endothelial lipase provides an alternative pathway for FFA uptake in lipoprotein lipase-deficient mouse adipose tissue. J. Clin. Invest. 115: 2103–2112.
58. Orban, P., C. D. Kingsley, and P. P. Lee. 2008. Fat talks, liver and muscle listen. Cell. 134: