A lipase fusion feasts on fat

DOI 10.1074/jbc.H120.012744

Philip M. M. Ruppert and Sander Kersten

From the Nutrition, Metabolism and Genomics Group, Division of Human Nutrition and Health, Wageningen University, Stippeneng 4, 6708 WE Wageningen, The Netherlands

Edited by Dennis R. Voelker

The enzyme lipoprotein lipase (LPL) is responsible for breaking down triglycerides in the blood. Mutations in LPL cause a rare but debilitating disorder characterized by excessive plasma triglyceride levels for which treatment options are limited. Nimonkar et al. now present a fusion protein between LPL and its physiological transporter GBIHBP1 that is highly active and largely resistant to physiological inhibitors of LPL. Injecting this fusion protein effectively lowers plasma triglycerides in mice and represents a promising new approach for lowering triglycerides in patients with familial chylomicronemia syndrome.

Fatty acids are vital for life. Not only are fatty acids a major fuel source for cells, but they also serve as important structural components in membranes. Fatty acids are transported through the blood partly as triglycerides. These triglycerides are synthesized in the intestine (from dietary fats) and liver (from endogenous sources) and are secreted as part of chylomicron and very low-density lipoprotein (VLDL) particles, respectively. As these particles are too big to be directly taken up by most tissues, clearance of triglycerides from the blood requires the action of the enzyme lipoprotein lipase (LPL). LPL is found at high levels in adipose tissue, skeletal muscle, heart, and several neuronal cell types. To reach its substrate, LPL is secreted from these cells, first binds to heparan sulfate proteoglycans, and then crosses into the capillaries with the help of the protein glycosylphosphatidylinositol-anchored HDL-binding protein 1 (GPIHBP1). There, LPL can transiently bind to circulating VLDL and chylomicron particles and degrade the triglycerides to individual fatty acids, which are then taken up by underlying tissues for storage or oxidation as fuel (Fig. 1A) (1).

The activity of LPL is tightly regulated in a tissue-specific manner in response to physiological stimuli such as fasting, physical inactivity, and cold exposure. This regulation occurs both at the transcriptional and post-translational level and serves to accommodate the tissue-specific requirement for fatty acids (2). The importance of LPL for plasma triglyceride metabolism is illustrated by the consequences of mutations in the human LPL gene. For example, carriers of the S474X gain-of-function mutation in the LPL gene have higher amounts of LPL protein sitting on the capillaries. The resultant increase in LPL activity increases clearance of triglycerides from the circulation and lowers the risk for coronary artery disease. By contrast, a deficiency in LPL leads to hypertriglyceridemia and a condition called familial chylomicronemia syndrome (FCS) (Fig. 1B). FCS patients can develop a host of comorbidities, including severe and potentially fatal pancreatitis. Affected individuals have to live what they categorize as a “very difficult” life, avoiding dietary fats and simple sugars, to reduce the triglycerides circulating in the blood. Current treatment options are not sufficient for management of many cases of FCS. One obvious solution, perhaps, would be enzyme replacement therapy. However, purified LPL is highly unstable and as such unsuitable for clinical use. A gene therapy–based drug that delivered LPL in an adeno-associated viral vector, Glybera, failed due to its transient effect and high price and was withdrawn from the market in 2015 (3).

The current work by Nimonkar et al. (4) revives hope in an enzyme replacement strategy by providing an astute extension to two recent publications, one from the same group, that revisited the dogma that LPL is catalytically active as a homodimer. The notion that LPL is only active as a dimer has been thoroughly embraced by the lipid community and has been a key feature of many mechanistic models. In the two publications, density gradient centrifugation and size-exclusion chromatography were used to show that LPL instead exists in a stable 1:1 monomeric state with GPIHBP1 in vivo and that this complex is catalytically active (5, 6). Not requiring a homodimer configuration for LPL to be catalytically active dramatically simplifies the molecular design and the artificial expression of this enzyme. Capitalizing on these findings, and with the crystal structure at hand (6), Nimonkar et al. conceptualized a potential novel treatment for FCS. Using a short peptide linker, they fused LPL to GPIHBP1 in a 1:1 ratio. The authors first set up an expression system in HEK293 cells, enabling the purification of recombinant LPL–GPIHBP1 fusion protein. They confirmed that the complex stabilizes LPL, increases enzyme activity, and protects against the endogenous LPL inhibitors ANGPTL3 and ANGPTL4. The LPL–GPIHBP1 fusion protein showed high catalytic activity toward VLDL and chylomicron particles in vitro and maintained this feature over several days when stored at 4 °C. Further testing with ELISAs and surface plasmon resonance revealed that the LPL moiety of the fusion protein is more resistant to inactivation by the endogenous LPL inhibitors ANGPTL3 and ANGPTL4 than the physiological, unfused complex. With these favorable in vitro data, the scientists went...
on to study the efficacy of the newly created fusion protein in vivo. Subcutaneous injections in C57BL/6 mice acutely and dose-dependently lowered plasma triglyceride levels by up to 80% following intravenous injection of the fat emulsion Intralipid to raise their intrinsically low triglyceride levels. Subsequently, these findings were confirmed in two other mouse models with higher baseline triglyceride levels, as well as hyperlipidemic mice on a high-fat/high-sucrose diet, with triglyceride levels in the same range as FCS patients. Acute and repeated subcutaneous and intravenous injections of the fusion protein dose-dependently lowered plasma triglycerides by 70–90% compared with control injections.

One potential danger of treatment with LPL is that the resultant free fatty acids could remain in the plasma and promote inflammation. Interestingly, the enhanced triglyceride hydrolysis following injection of the fusion protein was not accompanied by an elevation in plasma nonesterified fatty acids levels in the tested mice, nor were there any signs of elevated fat storage in several organs. This result led the authors to conclude that the excess fatty acids must be oxidized, although they note further investigation of the metabolic fate of the released fatty acids is needed.

These data nicely illustrate that fusing LPL to its physiological transporter GBIHBP1 in a 1:1 monomeric complex stabilizes LPL, thereby creating a feasible therapeutic strategy to correct the LPL deficiency in FCS (Fig. 1C). Moreover, the treatment may benefit people with the more common polygenic chylomicronemia, which has a late onset and is aggravated by an unhealthy lifestyle. The appeal of the HEK293-based expression system is that it produces high yields of catalytically active and nonaggregated LPL that is less sensitive to the endogenous LPL inhibitors. With this approach, Nimonkar et al. circumvent all major roadblocks that explained the lack of LPL-based drugs for the treatment of FCS to date.

References
1. Mead, J. R., Irvine, S. A., and Ramji, D. P. (2002) Lipoprotein lipase: Structure, function, regulation, and role in disease. J. Mol. Med. 80, 753–769 CrossRef Medline
2. Kersten, S. (2014) Physiological regulation of lipoprotein lipase, Biochim. Biophys. Acta 1841, 919–933 CrossRef Medline
3. Morrison, C. (2015) $1-million price tag set for Glybera gene therapy. Nat. Biotechnol. 33, 217–218 CrossRef Medline
4. Nimonkar, A. V., et al. (2020) A lipoprotein lipase–GPI-anchored high density lipoprotein binding protein 1 fusion lowers triglycerides in mice: implications for managing familial chylomicronemia syndrome. J. Biol. Chem. 295, 2900–2912 CrossRef Medline
5. Beigneux, A. P., Allan, C. M., Sandoval, N. P., Cho, G. W., Heizer, P. J., Jung, R. S., Stanhope, K. L., Havel, P. J., Birrane, G., Meiyappan, M., Gill, J. E., 4th, Murakami, M., Miyashita, K., Nakajima, K., Ploug, M., et al. (2019) Lipoprotein lipase is active as a monomer. Proc. Natl. Acad. Sci. U.S.A. 116, 6319–6328 CrossRef Medline
6. Arora, R., Nimonkar, A. V., Baird, D., Wang, C., Chiu, C. H., Horton, P. A., Hanrahan, S., Cubbon, R., Weldon, S., Tschantz, W. R., Mueller, S., Brunner, R., Lehr, P., Meier, P., Ott, J., et al. (2019) Structure of lipoprotein lipase in complex with GPIHBPI. Proc. Natl. Acad. Sci. U.S.A. 116, 10360–10365 CrossRef Medline