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Molecular processes that handle — and mishandle — dietary lipids

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Overconsumption of lipid-rich diets, in conjunction with physical inactivity, disables and kills staggering numbers of people worldwide. Recent advances in our molecular understanding of cholesterol and triglyceride transport from the small intestine to the rest of the body provide a detailed picture of the fed/fasted and active/sedentary states. Key surprises include the unexpected nature of many pivotal molecular mediators, as well as their dysregulation — but possible reversibility — in obesity, diabetes, inactivity, and related conditions. These mechanistic insights provide new opportunities to correct dyslipoproteinemia, accelerated atherosclerosis, insulin resistance, and other deadly sequelae of overnutrition and underexertion.

Introduction
Why has intestinally absorbed lipid remained the focus of clinical and basic efforts spanning at least a century (1)? Because absorption of dietary essential fatty acids and lipid micronutrients by the small intestine sustains human life, but at the same time, the capacity of this organ to absorb our flagrantly lipid-rich modern diet maims and kills people. It does so in at least 3 major ways. First, intestinally derived lipoproteins, chiefly chylomicrons (CMs), transport calorie-dense triglycerides to adipose depots (2, 3), accelerating the development of obesity (2) and its many harmful sequelae. Second, while delivering triglycerides to peripheral tissues, CMs become processed into so-called CM “remnants” (CMs), transporting calorie-dense triglycerides to adipose depots (2, 3), accelerating the development of obesity (2) and its many harmful sequelae. Second, while delivering triglycerides to peripheral tissues, CMs become processed into so-called CM “remnants” (CMs), transporting calorie-dense triglycerides to adipose depots (2, 3), accelerating the development of obesity (2) and its many harmful sequelae. Third, CMs and their remnants of cholesterol-rich diets suppress Npc1l1 expression, consistent with the phenotype (21).

Facilitated absorption of cholesterol: from drug to target
The major recent breakthrough in understanding cholesterol absorption by the small intestine arose from the discovery of ezetimibe, a medicine that blocks this pathway (16). Notably, the medicine was found via low-throughput screens in whole animals; moreover, it was FDA approved and on the market for years before its molecular target was identified. Ezetimibe provides a compelling counterexample against the prevailing postgenomic paradigm for drug discovery, dubbed conveyor belts, in which high-throughput screens of compounds against known protein targets lead to FDA-approved medicines.

Despite some initial controversy (17, 18), it is clear that the target of ezetimibe is the Niemann-Pick C1–like 1 protein (NPC1L1; refs. 17, 19). The NPC1L1 protein is highly expressed in small intestine (19) and in human liver (20), precisely where the drug acts. Ezetimibe glucuronide binds to a single, saturable site on wild-type enterocyte brush border membranes and to cells expressing recombinant NPC1L1 (17). Enterocyte membranes from Npc1l1–/– mice show no ezetimibe binding (17), and Npc1l1-deficient mice exhibit an approximately 70% reduction in cholesterol absorption that cannot be lowered further by administration of ezetimibe (19).

In humans, NPC1L1 gene variants correlate with sterol absorption (21), baseline plasma LDL concentrations (21), and responsiveness of LDL levels to ezetimibe administration (18). Moreover, most human NPC1L1 variants associated with inefficient intestinal cholesterol absorption exhibit substantial impairments in protein expression, consistent with the phenotype (21).

Physiologic regulation of NPC1L1 alters cholesterol absorption in response to availability, apparently through a sterol-regulatory element in the promoter and a sterol-sensing domain in the protein. Cholesterol-rich diets suppress Npc1l1 expres-
The NPC1L1 protein exerts major effects on the transport of dietary and nondietary cholesterol within the gastrointestinal system. Even in individuals consuming a lipid-rich modern diet, more than two-thirds of the cholesterol that enters the gut lumen comes from bile (Figure 1 and ref. 21). Thus, by blocking NPC1L1 in the intestine, ezetimibe not only impairs absorption of dietary cholesterol, but also depletes cholesterol from the enterohepatic circulation. Liver cholesterol content drops, an effect augmented clinically by coadministration of statins. Consequences include a slight impairment in hepatic VLDL secretion and a substantial induction of sterol-responsive genes in the liver, particularly that encoding the LDL receptor (18). These effects lower plasma LDL levels by 15%–20%.
Table 1
Dysregulated mediators of lipid and lipoprotein trafficking in diabetes, obesity, and related conditions

<table>
<thead>
<tr>
<th>Molecule/process</th>
<th>Dysregulation</th>
<th>Likely consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPC1L1, ABCG5/G8</td>
<td>In diabetes, increased intestinal and hepatic expression of Npc1l1 (28) and decreased intestinal expression of ABCG5/G8 (28–30)</td>
<td>Increased intestinal cholesterol absorption, decreased hepatic biliary cholesterol excretion, and increased cholesterol content of secreted CMs and VLDL</td>
</tr>
<tr>
<td>MTP</td>
<td>Intestinal overexpression in intestinal insulin resistance (15) and diabetes (28, 30)</td>
<td>Substantially increased intestinal secretion of apolipoprotein B48, hence an increased number of secreted particles</td>
</tr>
<tr>
<td>Intestinal lipid synthesis</td>
<td>Increased synthesis of fatty acids, triglycerides, and cholesteryl esters by enterocytes in intestinal insulin resistance (15)</td>
<td>Increased CM secretion in insulin resistance and overt diabetes (67)</td>
</tr>
<tr>
<td>LpL</td>
<td>Increased activity in adipose tissue, but decreased activity in striated muscle, in the fed and sedentary states (3, 78, 79)</td>
<td>Diversion of CM and VLDL calories into adipose depots for storage and away from combustion in muscle</td>
</tr>
<tr>
<td>Angiopoietin-like proteins</td>
<td>Suppressed expression in adipose tissue in the fed state (84)</td>
<td>Accumulation of triglycerides and fatty acid metabolites, leading to insulin resistance in muscle</td>
</tr>
<tr>
<td>Mitochondrial β-oxidation</td>
<td>Reduced capacity in sedentary skeletal muscle (86)</td>
<td>Diversion of CM and VLDL calories away from combustion in muscle</td>
</tr>
<tr>
<td>Gpihbp1</td>
<td>Suppression of its mRNA in muscle in the fed state (90)</td>
<td>Diversion of CM and VLDL calories away from combustion in muscle</td>
</tr>
<tr>
<td>CD36 fatty acid translocase</td>
<td>Increased expression in adipose tissue in diabetes (103)</td>
<td>Enhanced adipose uptake of calories in the form of NEFAs</td>
</tr>
<tr>
<td>Ndst1</td>
<td>Suppressed mRNA, protein, and enzymatic activity in diabetic liver (122)</td>
<td>Defective hepatic assembly of HSPGs, hence abnormal persistence of atherogenic postprandial remnant lipoproteins in plasma</td>
</tr>
</tbody>
</table>

Ndst1, heparan sulfate N-deacetylase/N-sulfotransferase–1.

In transgenic mice, ezetimibe also blocks NPC1L1 within the liver, thereby allowing endogenous cholesterol to exit into bile rather than being taken back up into hepatocytes for secretion into plasma (25). Because human liver abundantly expresses NPC1L1 (20), clinical administration of ezetimibe might also increase cholesterol elimination into bile (25). Thus, blockage of NPC1L1 augments the ability of the gastrointestinal system to dispose of dietary and nondietary cholesterol. For example, reverse transport of cholesterol, from lipid-loaded peripheral macrophages to HDL to the liver, then into bile and out into the feces, becomes more efficient in ezetimibe-treated mice, owing to inhibition of intestinal reabsorption of HDL-derived biliary cholesterol, but without any apparent effects on macrophages or on HDL particles (26, 27).

Regarding dyslipidemias, type 1 diabetic rats show increased Npc1l1 expression in both intestine and liver, and the levels correlate, respectively, with CM and VLDL cholesterol content (Table 1 and ref. 28). This finding may contribute to cholesterol hyperabsorption in human type 1 diabetes (29). Type 2 diabetes also increases intestinal NPC1L1 mRNA in humans (30), and among type 2 diabetic patients, those with coronary artery disease absorb cholesterol more efficiently than those without (31). Compared with nondiabetic controls, however, type 2 diabetic subjects exhibit increased intestinal cholesterol synthesis, not absorption (29). In the other direction, genetic deficiency of Npc1l1 in the apoe knockout mouse, an animal model that accumulates remnant-like lipoproteins in its plasma, lowers the cholesterol content of atherogenic lipoproteins by 80%–90% and blocks the development of vascular disease (32). Regarding human atherosclerosis, long-term studies to examine the effectiveness of ezetimibe on cardiovascular outcomes must still be completed, even though nearly every strategy to lower plasma LDL levels has resulted in essentially the same degree of long-term cardiovascular protection (11). A 2-year study in familial hypercholesterolemic subjects failed to find a significant effect of this medicine on carotid intimal-medial thickness (33).

Surprisingly, the expression of NPC1L1 by enterocytes also facilitates uptake of toxic, noncholesterol sterols from plants, shellfish, and other dietary sources (17, 18). The ability of the normal intestine to selectively prevent these molecules from moving into the rest of the body was recognized decades ago with the discovery of β-sitosterolemia, a rare recessive disease in which plant sterols accumulate systemically. The responsible mutations reside in either of 2 ATP-binding cassette transporters (ABCs), ABCG5 and ABCG8 (34). These proteins are actually half-transporters that form heterodimers (35) that pump nearly all noncholesterol sterols out of the enterocyte, back into the gut lumen for excretion in feces (Figure 2 and ref. 34). The ABCG5/G8 transporter also pumps some cholesterol out of enterocytes (34), thereby explaining cholesterol hyperabsorption in β-sitosterolemia. Suppression of ABCG5/G8 in type 1 diabetic intestine may also contribute to cholesterol hyperabsorption (Table 1 and refs. 28, 29).

Dimers of Abcg5/g8 are also expressed in liver, where they cooperate with Abcb4 (also known as Mdr2) to pump UC and phospholipid into bile (34), while hepatic NPC1L1 transports UC from bile back into the hepatocyte (25). The combined effects of these 4 proteins regulate biliary cholesterol content before its release into the intestinal lumen. Both Abcg5 and Abcg8 are liver X receptor (Lxr) targets and are induced by high-cholesterol diets. Lxr agonists enhance intestinal cholesterol excretion through these transporters (36); but, as side effects, these agonists also induce lipid biosynthesis and steatosis in the liver as well as apoptosis of pancreatic β cells.
Intestinal packaging of absorbed lipids into apoB<sub>48</sub>-containing lipoproteins

**Similarities to hepatic production of apoB<sub>100</sub>-containing lipoproteins**

A key structural component of CMs is the huge, hydrophobic, nonexchangeable protein apoB, which exists in 2 forms in the body. Human liver makes full-length apoB<sub>100</sub>, the major protein of hepatically derived atherogenic lipoproteins (e.g., VLDL and LDL). The C terminus of apoB<sub>100</sub> contains the domain recognized by the LDL receptor, thereby mediating high-affinity clearance of these particles from plasma. In contrast, essentially all of the apoB from human intestine appears as a truncated form, apoB<sub>48</sub>, that is missing the C-terminal 52% (37), and so CMs and CM remnants rely on exchangeable proteins, particularly lipoprotein lipase (LpL; refs. 38–41), hepatic lipase (42, 43), and apoE (44–46), to serve as ligands for cell surface receptors. Polymorphisms of each of these exchangeable proteins affect fasting lipoprotein concentrations (47) and the postprandial response (43). Intestinal apoB<sub>48</sub> comes from the same gene as apoB<sub>100</sub> through the action of the APOB mRNA editing complex–1 (APOBEC1), which deaminates a single cytidine into a uridine, thereby creating a nongenomic in-frame stop codon (UAA) roughly in the middle of the RNA coding region (Figure 2 and ref. 48).

Far more details are known about the cellular processes in the liver that allow this huge, hydrophobic protein to become cotranslationally and posttranslationally assembled with lipids (49) and what regulates its subsequent secretion (50). Both intestine and liver depend on acyl-CoA:diacylglycerol acyltransferases (DGATs) and acyl-CoA:cholesterol acyltransferase–2 (ACAT2) for intracellular synthesis of triglycerides and cholesteryl esters that will be assembled into the hydrophobic cores of nascent apoB-containing lipoproteins (Figure 2). Both organs require the microsomal triglyceride transfer protein (MTP) to shuttle small amounts of triglyceride onto each newly forming apoB molecule as it translocates into the ER lumen, thereby allowing proper folding of the protein. A second lipidation step later in the secretory
pathway occurs in enterocytes (51) and in hepatocytes (52, 53) to generate large CMs and VLDL, respectively. In hepatocytes, extensive lipolysis of nascent apoB lipoproteins requires a motif that is present in both apoB100 and apoB100 (53), but a role for this motif has not yet been reported in enterocytes. Fully assembled apoB lipoproteins are released by exocytosis of secretory vesicles from the basolateral membrane of enterocytes (Figure 2).

Genetic defects or pharmacologic inhibition of MTP allows continued synthesis of apoB (49, 54), but the misfolded protein becomes degraded by ER-associated degradation (ERAD), a process mediated in cultured hepatocytes by the proteasome (49). The same mechanism is presumably used in MTP-deficient enterocytes. Genetic lack of MTP causes abetalipoproteinemia, a heritable condition characterized by the absence of apoB48 and apoB100 lipoproteins from plasma, as well as the development of intestinal and hepatic steatosis, malabsorption, and deficiencies of fat-soluble vitamins (55). Unfortunately, the side effects of fatty intestine and liver hamper the use of pharmacologic inhibitors of MTP to lower plasma apoB lipoprotein levels in humans (56). However, in unmanipulated intestinal cells (54) and in normal primary hepatocytes (57), ERAD appears to have little or no role in the physiologic regulation of apoB secretion. Fatty meals induce Mtp expression in normal enterocytes, presumably to facilitate lipid absorption and packaging (58).

In collaboration with the Fisher laboratory, my colleagues and I identified 2 new processes that regulate apoB secretion from cultured hepatocytes and from liver in vivo: post-ER presecretory proteolysis (50, 57, 59) and reuptake (39, 50, 60), both of which are of particular interest, given our finding that polyunsaturated fatty acids blunt postprandial hypertriglyceridemia in humans via decreased CM production (61). Genetic deficiency or pharmacologic inhibition of MTP allows continued synthesis of apoB (49, 54), but the misfolded protein becomes degraded by ER-associated degradation (ERAD), a process mediated in cultured hepatocytes by the proteasome (49). The same mechanism is presumably used in MTP-deficient enterocytes. Genetic lack of MTP causes abetalipoproteinemia, a heritable condition characterized by the absence of apoB48 and apoB100 lipoproteins from plasma, as well as the development of intestinal and hepatic steatosis, malabsorption, and deficiencies of fat-soluble vitamins (55). Unfortunately, the side effects of fatty intestine and liver hamper the use of pharmacologic inhibitors of MTP to lower plasma apoB lipoprotein levels in humans (56). However, in unmanipulated intestinal cells (54) and in normal primary hepatocytes (57), ERAD appears to have little or no role in the physiologic regulation of apoB secretion. Fatty meals induce Mtp expression in normal enterocytes, presumably to facilitate lipid absorption and packaging (58).

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Unique features of intestinal production of apoB48-containing lipoproteins

The intestine has a unique need to rapidly assemble and secrete gigantic CM particles to carry large amounts of newly absorbed triglycerides. Several adaptations have been identified, the first of which is apoB48 itself. Comparisons of wild-type and apoBec1–/– mice, which cannot edit intestinal Apob mRNA, revealed that the short form of apoB assembles more efficiently into CMs than does apoB100, particularly after a lipid-rich meal (48). To quickly export triglycerides after dietary absorption, the intestinal mucosa appears to keep a preformed supply of apoB48 in reserve that becomes lipilated into large CMs as needed (62). Recruitment of apoB48 from this preformed pool may involve MTP. Accordingly, a substantial increase in apoB48 secretion occurs in conditions with abnormally high intestinal MTP expression. Examples include the –493T human MTP promoter polymorphism (43), intestinal insulin resistance (15), and overt diabetes (28, 30).

Fat feeding also provokes marked intestinal expression of an amphilastic protein, apoA-IV, that serves as a surface component for the rapidly inflating apoB48 particles within the enterocyte (Figure 2 and refs. 63, 64). Once CMs reach the bloodstream, apoA-IV becomes displaced by apoCs and apoE as a prelude to lipolysis and clearance. A common apoA-IV variant that exhibits higher lipid affinity, Q360H, has been associated with increased postprandial hypertriglyceridemia, presumably by impeding this transfer of apoCs and apoE onto CMs (63). CMs vastly exceed the size of canonical ER-to-Golgi transport vesicles, which are only 60–70 nm in diameter (65). In hepatocytes, 2 adaptations have been reported: slightly larger transport vesicles of about 100 nm and the release of consistently small, dense apoB100 particles from the ER regardless of lipid availability (52). Several lines of evidence, however, indicate that intestinal adaptations possess distinct features. First, the enterocyte performs considerable lipolysis of apoB48 particles in the ER, and as a consequence, the ER-to-Golgi transport vesicles have been reported to average about 250 nm in diameter (64), over 50 times the volume of canonical transport vesicles (Figure 2). Second, to create and then target these unusual transport vesicles, the enterocyte reportedly uses a unique set of proteins. Instead of depending on coat proteins (COPII) for ER budding, the enterocyte apparently relies on intracellular fatty acid–binding protein-1 (Fabp1), and perhaps Fabp2, to create apoB48 transport vesicles (64). Of note, the A54T polymorphism of FABP2 associates with increased postprandial lipemia (43). Third, a rare inherited syndrome, CM retention disease, preferentially affects the intracellular transport and secretion of apoB100 lipoproteins from enterocytes while still permitting secretion of apoB100 particles from liver. Patients exhibit malabsorption, fat-filled enterocytes, no plasma apoB48, no postprandial CMs, present but decreased plasma levels of apoB100 lipoproteins, and low HDL levels. Jones et al. (65) and others (66) linked this syndrome with several mutations in the SAR1B protein, a GTPase involved in the budding of canonical COPII-coated vesicles from the ER. In hepatocytes, Sar1 proteins are required for these apoB-containing vesicles to bud off the ER (52). In an enterocyte system, however, blockage of Sar1 still allowed the generation of apoB48 transport vesicles, presumably in the context of continued activity of intracellular Fabp1 and Fabp2, but these abnormal vesicles failed to fuse with the Golgi complex (64). Based on these differences, it is possible that SAR1B mutations in CM retention disease allow budding, transport, and Golgi fusion of apoB-containing vesicles in hepatocytes but interfere with these processes, particularly Golgi fusion, in enterocytes. This overall cellular and molecular understanding has led to several strategies to inhibit apoB-dependent absorption of lipids from the gut lumen, some under exploration in vivo and others already in clinical use (Table 2).

Because human liver makes no apoB48 and human intestine synthesizes no detectable apoB100 (37), the type of apoB identifies the origin of an atherogenic human lipoprotein. Each of these lipoproteins contains exactly 1 molecule of apoB, and so the mass of apoB48 or apoB100 is used to estimate particle number. After a fatty meal, roughly 80% of the rise in plasma triglyceride concentration in humans is carried by apoB48 lipoproteins, chiefly large CMs, but about 80% of the increase in particle count comes from apoB100 lipoproteins (8). The comparatively low number of apoB48 particles has led some authors to propose that the effects of intestinal apoB48 lipoproteins on vascular disease are largely indirect, brought about by their ability to increase the concentration and atherogenicity of apoB100 lipoproteins and to lower plasma HDL levels (7, 8). In intestinal insulin resistance and overt diabetes, the increase in apoB48 secretion noted above implies a higher particle number. Insulin resistance also enhances endogenous intestinal synthesis of fatty acids, triglycerides, and cholesterol esters that contribute to CM overproduction (15, 67) and downstream atherogenic effects (Table 1 and ref. 7).

The intestine also mediates several pathways for lipid absorption that do not depend on apoB. For example, some cholesterol absorption occurs via HDL and its major protein, apoA-I (Figure 2 (68).
Chain triglycerides also occurs independently of apoB. The lipids are hydrolyzed in the gut lumen and absorbed directly into perireceptor expression, and thereby lowers plasma LDL levels. (See ref. 74). Mutations in LpL and its cofactor, apoC-II, are responsible for certain rare familial chylomicronemias, confirming in humans that these molecules are rate limiting in CM catabolism (73, 75). Human polymorphisms of 2 LpL inhibitors, apoC-III and apoA-II, correlate with altered postprandial lipoprotein metabolism (43). Capillary endothelium does not make LpL, but instead picks it up from underlying adipocytes and striated muscle cells (73). Studies in vitro indicate that transcytosis of the enzyme for display on the luminal side is mediated by basolateral HSPGs acting in concert with the VLDL receptor (Figure 3 and ref. 76).

Because LpL is rate limiting, this system provides a crucial metabolic branch point between storage of CM calories in adipose tissue and combustion in striated muscle (Figures 1 and 3). Targeted overexpression of LpL in muscle prevents diet-induced obesity in mice, apparently by diverting CM lipolysis away from fat and into muscle (77). Likewise, a targeted deficiency of LpL in adipose tissue ameliorates obesity in hyperphagic mice (2). Formal energy balance studies in these mice would be of interest. Similar phenomena occur physiologically in humans: differential regulation of LpL activity in fat versus striated muscle diverts triglycerides to adipose tissue in the fed and sedentary states, but to muscle during fasting (3, 78) or exercise (79) (Figure 1). These molecular processes likely contribute to the strong correlation between obesity and the tendency to sit (79, 80) and overeat (81) (Table 1). Published data on the relevant points of control show considerable complexity (3) and include regulation of LpL mRNA transcription, LpL mRNA stability, LpL protein synthesis, maturation in the ER (82), glycosylation, posttranslational degradation of LpL via its binding to HSPGs on the surface of adipocytes (83) and myocytes, and posttranslational disruption of enzymatically active LpL dimers by angiopoietin-like proteins (47, 84), which are themselves regulated by feeding, fasting, nuclear receptor ligands, and gut microbiota (Table 1 and refs. 84, 85). Despite the regulation of LpL, NEFAs can be delivered to skeletal muscle in excess of its capacity for β-oxidation, particularly in the context of obesity and physical inactivity (86). Subsequent accumulation of triglycerides within myocytes may cause insulin resistance (86, 87), a situation that can be corrected in animals by exercise (Table 1 and ref. 88).
lipoproteins? In fact, the heparan sulfate chains in liver exhibit extreme structures of high flexibility and negative charge (89), and hence show far stronger ligand affinity than does heparan sulfate in other tissues, including peripheral endothelium. Thus, injection of LpL or other heparin-binding proteins results in primarily hepatic clearance, with little if any delivery into fat or muscle.

Direct hepatic uptake of CMs may be diminished by their secretion from the intestine into lymph, which bypasses the portal circulation (Figure 1). A common explanation holds that hepatic uptake of CMs can occur only after these particles are shrunk by extrahepatic lipolysis into remnant lipoproteins that then become small enough to squeeze through the approximately 100-nm fenestrae in the endothelial lining of liver sinusoids. Yet most of the triglyceride-rich lipoproteins in plasma from hyperchylomicronemic mice were reported to be less than 100 nm in diameter (90). Thus, there must be a molecular, not just a physical, explanation for the robust initial targeting of these particles to adipose tissue and striated muscle under normal conditions.

Figure 3
Integrated model of CM binding and hydrolysis on peripheral capillary endothelium. Adipose tissue and striated muscle each synthesize LpL, regulated by the fasted/fed and active/sedentary metabolic states. HSPGs on the surfaces of these cells capture and internalize LpL for degradation. LpL that escapes degradation will be picked up by HSPGs and VLDL receptors on the basal surface of overlying endothelial cells for transcytosis to the luminal surface of capillaries (orange arrows). Heparan sulfate side-chains of syndecan and glypican are denoted by chains of small spheres. The major HSPGs of endothelium, syndecans and glypicans, move into detergent-insoluble membrane microdomains (rafts) rich in caveolin-1 (CAV1) upon clustering. On the apical surface, they encounter GPIHBP1, which should also move into rafts upon clustering.

The highly negatively charged N-terminal domain of GPIHBP1 binds LpL with approximately 10-fold greater affinity than do endothelial HSPGs. Thus, after transcytosis, LpL should be torn away from syndecans and glypicans onto GPIHBP1 (pink arrows). Dimers of GPIHBP1 bind LpL and CMs, thereby providing a platform for CM docking and triglyceride lipolysis. These processes are facilitated by apoC-II and apoA-V. Lipolysis generates NEFAs that are transported by another raft molecule, CD36, across the endothelium and into adipocytes for energy storage (blue arrows) or into striated myocytes for combustion (green arrows). After hydrolysis of CM triglycerides, the endothelium releases apoB₄₈ remnant lipoproteins that are rich in LpL, apoE, and cholesteryl ester back into the circulation (red arrow). Under normal circumstances, these remnant particles undergo safe, swift uptake by the liver.
The answer appears to reside in the recent discoveries of 2 unsuspected participants in peripheral lipolysis: apoA-V on lipoproteins and the glycosylphosphatidylinositol-anchored (GPI-anchored) HDL-binding protein 1 (GPHBP1) on peripheral endothelium. Adipocyte-specific knockout of a third molecule, LDL receptor–related protein–1 (Lrp1), revealed a role in lipid uptake from model CM remnants into brown fat, but not into white fat (91), and so its relevance to human physiology remains unclear at this point.

APOA5. In 2001, 2 laboratories discovered a new apoprotein gene (dubbed APOA5, within the well-known APOA1/APOC3/APOA4 gene cluster) using a computational search for conserved genomic regions and a survey of upregulated genes during liver injury; the protein product is referred to as apoA-V or APOA5. To demonstrate its relevance to lipoprotein physiology, Pennacchio et al. reported that mice transgenic for human apoA-V showed a two-thirds reduction in plasma triglyceride levels; that apoA-V protein potently facilitates LpL-mediated triglyceride hydrolysis in animals (94) and in humans (93). In addition, apoA-V facilitates hydrolysis of triglyceride-rich lipoproteins by LpL in solution or bound to HSPGs on the surface of cultured cells (94). This last finding requires revisiting based on recent results concerning the second molecule, GPHBP1 (Figure 3).

GPHBP1. During a screen of hundreds of knockout mice at Genentech, overly milky lipemia was unexpectedly noted in mice lacking Gphb1 (90, 95), a GPI-anchored membrane protein originally identified by its ability to bind HDL (96). Subsequent characterization of adult Gphb1–/– mice by Beigneux et al. indicated high plasma concentrations of large, triglyceride-rich apoB48 lipoproteins in the CM/VLDL size range, low plasma levels of HDL, and impaired CM removal (apparently owing to defective lipolysis in peripheral tissues), but only about 40% reduction in postheparin plasma LpL levels (90).

Mechanistic investigations of this phenotype led to the hypothesis that Gphb1, not HSPGs, provides the key platform for LpL-mediated lipolysis of CM triglycerides on microvascular endothelium (see "Integrated model for CM hydrolysis and local NEFA uptake"; Figure 3; and refs. 90, 95). Expression of Gphb1 in vivo was found exclusively on the luminal surface of endothelial cells, with particularly high levels in the capillaries of adipose tissue and striated muscle (90). Consistent with a role in caloric delivery, Gphb1 mRNA levels in muscle were doubled by fasting (90), reminiscent of the metabolic control of LpL discussed above (Table 1). Levels of Gphb1 in liver are low, which likely contributes to poor hepatic uptake of CMs until triglyceride hydrolysis transforms them into remnant lipoproteins that display different binding epitopes.

In vitro, the transfection of HSPG-deficient cells with a Gphb1 expression vector increased their binding to artificial apoA-V/phosphatidylcholine disks and to CMs harvested from Gphb1–/– mice. In each case, the interactions appear to be electrostatic: LpL, apoA-V, and other CM apoproteins contain positively charged domains, and Gphb1 contains a highly negatively charged region toward its N terminus, far from the plasma membrane. By binding both LpL and CMs, Gphb1 may bring them physically together to facilitate lipolysis. If LpL and CMs occupy the same negatively charged site on Gphb1, efficient lipolysis could require dimerization of Gphb1, a process that may be facilitated by its Ly-6 domain (90).

The Gphb1–/– mouse is the second of only 2 examples of chylomicronemia related to a defect in the endothelium of adipose tissue and muscle (90, 97). Interestingly, a rare mutation in GPHBP1 has been associated with human chylomicronemia, although this variant appears to function normally in vitro (98). Further studies are needed in order to definitively establish the role of GPHBP1 in human physiology, although it presumably serves functions similar to those of its murine homolog.

Integrated model for CM hydrolysis and local NEFA uptake

The known properties of these molecular participants suggest that CM hydrolysis and local NEFA uptake are facilitated by binding and postprandial hypertriglyceridemia (43, 47, 75, 93). Despite its substantial effects, apoA-V exhibits surprisingly low levels in normal plasma compared with other apoproteins (approximately 180 ng/ml, about 0.02% the level of apoA-I), which is why it was missed in earlier compositional studies of lipoproteins. The normal apoA-V protein potently facilitates LpL-mediated triglyceride hydrolysis in animals (94) and in humans (93). In addition, apoA-V facilitates hydrolysis of triglyceride-rich lipoproteins by LpL in solution or bound to HSPGs on the surface of cultured cells (94). This last finding requires revisiting based on recent results concerning the second molecule, GPHBP1 (Figure 3).

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During a screen of hundreds of knockout mice at Genentech, overly milky lipemia was unexpectedly noted in mice lacking Gphb1 (90, 95), a GPI-anchored membrane protein originally identified by its ability to bind HDL (96). Subsequent characterization of adult Gphb1–/– mice by Beigneux et al. indicated high plasma concentrations of large, triglyceride-rich apoB48 lipoproteins in the CM/VLDL size range, low plasma levels of HDL, and impaired CM removal (apparently owing to defective lipolysis in peripheral tissues), but only about 40% reduction in postheparin plasma LpL levels (90).

Mechanistic investigations of this phenotype led to the hypothesis that Gphb1, not HSPGs, provides the key platform for LpL-mediated lipolysis of CM triglycerides on microvascular endothelium (see "Integrated model for CM hydrolysis and local NEFA uptake"; Figure 3; and refs. 90, 95). Expression of Gphb1 in vivo was found exclusively on the luminal surface of endothelial cells, with particularly high levels in the capillaries of adipose tissue and striated muscle (90). Consistent with a role in caloric delivery, Gphb1 mRNA levels in muscle were doubled by fasting (90), reminiscent of the metabolic control of LpL discussed above (Table 1). Levels of Gphb1 in liver are low, which likely contributes to poor hepatic uptake of CMs until triglyceride hydrolysis transforms them into remnant lipoproteins that display different binding epitopes.

In vitro, the transfection of HSPG-deficient cells with a Gphb1 expression vector increased their binding to artificial apoA-V/phosphatidylcholine disks and to CMs harvested from Gphb1–/– mice. In each case, the interactions appear to be electrostatic: LpL, apoA-V, and other CM apoproteins contain positively charged domains, and Gphb1 contains a highly negatively charged region toward its N terminus, far from the plasma membrane. By binding both LpL and CMs, Gphb1 may bring them physically together to facilitate lipolysis. If LpL and CMs occupy the same negatively charged site on Gphb1, efficient lipolysis could require dimerization of Gphb1, a process that may be facilitated by its Ly-6 domain (90).
obesity (97). Likewise, mutation of human caveolin-1 is a cause of dyslipidemia with lipodystrophy, which suggests that the same processes occur in people (105).

CM catabolism: rapid hepatic uptake of apoB48 remnants

Over a quarter century ago, astute observations in vivo revealed that efficient hepatic uptake of remnant lipoproteins from plasma does not require LDL receptors (46, 106–108). This insight launched a long, difficult search for the molecules responsible for this crucial pathway. Over many years, several protein candidates were identified, most notably LRPI (109), but disablement of hepatic LRPI in vivo fails to result in poor clearance of remnant lipoproteins, particularly when LDL receptors are present, as they are in normal human physiology (109–112). Instead, work from our laboratory (39) and others (44, 45, 111, 113, 114) implicated HSPGs, contradicting a common view that these molecules play only passive, structural roles (Figure 4 and refs. 40, 74). Extreme structural features of hepatic HSPGs that enhance ligand affinity (89), noted above, direct remnants into the liver. Moreover, strong evidence now exists for a physiologic role for HSPGs in remnant lipoprotein clearance in humans (44, 45).

Because many HSPG core proteins are highly conserved, yet dissimilar from each other, we hypothesized that different classes of HSPGs would mediate distinct endocytic pathways (Figure 4 and refs. 40, 74). To date, we have demonstrated a specific endocytic pathway mediated by syndecan HSPGs when they become clustered (40, 99) and a separate pathway with distinct subcellular trafficking mediated by the perlecan HSPG when it is adherent to the cell surface (74, 115, 116). Endocytosis via glypicans has also been reported (117).

Normal liver expresses several syndecans, the perlecan HSPG, glypicans, the long variant of collagen XVIII, and agrin (Figure 4 and refs. 118–120). The contribution of each of these HSPGs to remnant lipoprotein uptake in vivo needs to be evaluated. Because syndecan-1 is particularly abundant on the sinusoidal surface of hepatic parenchymal cells (118), it is a strong candidate to participate in remnant lipoprotein clearance in vivo (40). In this context, we proposed that hepatic HSPGs can be divided into 2 functional subsets (39): those that cells readily internalize, especially the integral plasma membrane syndecans (40) and glypicans (117), and those that cells might not readily internalize, meaning extracellular HSPGs of the basement membrane and other matrix, such as collagen XVIII, agrin, and

Figure 4
Efficient hepatic uptake of CM remnant lipoproteins. Hepatic HSPGs exhibit extreme structural features of their carbohydrate side-chains that enhance ligand binding. Thus, HSPGs in the liver rapidly pull apoB48 remnant lipoproteins out of plasma via interactions with positively charged proteins on the particles, chiefly LpL and apoE. Once in the liver, the particles encounter hepatic lipase (HL), which serves as an additional bridging molecule between HSPGs and lipoproteins. Shown are 2 pathways for particle clearance. The first is direct receptor-mediated uptake, in which cholesteryl ester–rich apoB48 remnant lipoproteins pass from the hepatic sinusoid through the fenestrated endothelium and then bind directly to integral plasma membrane receptors (red arrows; shown are the LDL receptor, which binds apoE, and the syndecan and glypican HSPGs, which bind LpL, hepatic lipase, and apoE). The second clearance mechanism is a cooperative pathway, in which apoB48 remnant lipoproteins from the hepatic sinusoid are first sequestered by matrix HSPGs within the space of Disse and then taken up in cooperation with the integral plasma membrane receptors (blue arrows; shown are the collagen XVIII and perlecan HSPGs). Also shown is one of several secreted enzymes (Enz), such as heparanase or the heparan 6-O-endosulfatases, that are expressed by liver, degrade HSPGs, and may dampen these processes.
most of the perlecain within the liver. Thus, in our model, cell surface syndecan and glypicans bind, then directly carry, remnant lipoproteins and other ligands into the cells, whereas matrix HSPGs bind and sequester their ligands; however, endocytosis requires cooperation with cell surface receptors, particularly syndecan-1, the LDL receptor (115), and possibly glypicans (Figure 4). Because syndecan-mediated endocytosis proceeds less rapidly (t1/2, about 1 h; ref. 40) than uptake via LDL receptors (t1/2, about 10 min), this model may account for quick hepatic uptake of remnants from plasma into the space of Disse, followed by continued internalization into hepatocytes even when LDL receptors are absent, although the internalization occurs at a reduced speed (Figure 4 and refs. 46, 108).

Diabetes mellitus and related conditions have been reported to cause CM overproduction (15, 67), delayed hydrolysis of CM triglyceride in the periphery, and—perhaps most important to the development of vascular disease—substantial impairment of hepatic clearance of atherogenic CM remnants (4, 13, 67, 113, 121). Persistent remnants, unlike CMs themselves, readily enter the arterial wall and are retained there (4), initiating and accelerating atherosclerosis (6, 11, 12, 122). Impaired hepatic clearance of apoB48 remnants from plasma arises in large part from the failure of diabetic liver to properly assemble HSPGs (113, 122–124). Specific molecular mediators of defective hepatic HPSGP assembly in diabetes include suppression of heparan sulfate N-deacetylase/N-sulfotransferase-1 (Ndst1) (122), a key enzyme in the biosynthesis of highly charged, flexible heparan sulfate chains that avidly bind ligands, and suppression of Fxr (125), an upstream regulator of syndecan-1 expression (126). Liver-specific knockout of the same heparan sulfate assembly enzyme, Ndst1, produces hypertriglyceridemia and a robust delay in the clearance of postprandial remnant lipoproteins (111), an important observation that confirms the role of HSPGs as remnant receptors (39) and the identification of Ndst1 as a key regulatory factor (Table 1 and ref. 122). Regarding reversibility, moderate caloric restriction of obese, type 2 diabetic mice corrects their defect in remnant lipoprotein clearance (127), and similar effects occur in human diabetic subjects after short-term weight loss (128). This body of work provides tremendous explanatory power, elucidating the normal physiologic uptake of remnant lipoproteins by the liver as well as the unhealthy persistence of these particles in diabetes and related syndromes (Figure 4).

Finally, it has been hypothesized that molecules other than HSPG and LDL receptor family members may also contribute to hepatic clearance of remnants, but their nature remains a matter of speculation (112). One candidate is scavenger receptor class B type I (Srb1), proposed as a docking molecule in the liver for CM remnants (129). Because conflicting literature indicates that the hepatic expression of Srb1 may be up- or downregulated in diabetes, its contribution to poor removal of remnants in that condition remains unknown.

Conclusions and future directions

The central question facing the field is how this explosion in knowledge might benefit human health. Despite our extensive molecular understanding, major health problems related to intestinal absorption of lipids and other overly abundant macronutrients—coupled with physical inactivity—continue to grow worldwide, particularly atherosclerosis, obesity, and type 2 diabetes. Moreover, as noted throughout this review, many derangements in lipid absorption and trafficking can be reversed by well-known lifestyle changes and available pharmaceutical agents.

The situation could be improved by 2 general approaches. The first is better implementation of recognized strategies. Improvements in lifestyle can be facilitated by education and counseling, but also by pancreatic lipase inhibitors (Table 2), novel appetite suppressants, and surgical techniques that reduce food intake and body weight. New methods of lowering plasma concentrations of atherogenic apoB-containing lipoproteins are also under development (11, 12). Public policy, which is extensively reviewed elsewhere (80, 81), remains crucial to promoting active living and the cultivation and consumption of affordable, healthy foods.

The second approach is the exploration of new therapeutic targets. Despite its importance, postprandial dyslipidemia has often been overlooked because it remains inconvenient to accurately assess. Improved apoB-specific assays will likely help. Moreover, nonfasting plasma triglyceride levels, an easy yet nonstandardized measurement related to postprandial dyslipidemia, correlate with future cardiovascular events and could serve as a therapeutic target (4, 13). Novel points of control could be found through additional molecular characterizations of fed/sedentary states as well as characterization of the beneficial changes that occur during caloric restriction and exercise. For example, reversal of intestinal insulin resistance has not been explored; the molecular basis for ezetimibe-resistant cholesterol absorption needs elucidation; metabolic regulation of GIPHBP1 and its role in human biology require further study; and improvements in hepatic clearance of remnant lipoproteins upon weight loss remain completely uncharacterized on a molecular level.

There is no substitute for a combined approach. The exciting opportunities outlined above for fundamental and translational advances in the biology of lipid absorption and transport will require an aggressive mix of new basic, clinical, and public policy efforts.

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