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Endothelial Cell Receptors in Tissue Lipid Uptake and Metabolism

Nada A. Abumrad, PhD[1], **Ainara G. Cabodevilla, PhD**[2], **Dmitri Samvoski, PhD**[1], **Terri Pietka, BS**[1], **Debapriya Basu, PhD**[2], **Ira J. Goldberg, MD**[2]

[1]Department of Medicine, Division of Nutritional Sciences, Washington University School of Medicine, Saint Louis, MO 63108

[2]Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, New York University Grossman School of Medicine, New York, New York 10016

Abstract

Lipid uptake and metabolism are central to the function of organs such as heart, skeletal muscle, and adipose tissue. Although most heart energy derives from fatty acids (FAs), excess lipid accumulation can cause cardiomyopathy. Similarly, high delivery of cholesterol can initiate coronary artery atherosclerosis. Hearts and arteries—unlike liver and adrenals—have non-fenestrated capillaries and lipid accumulation in both health and disease requires lipid movement from the circulation across the endothelial barrier.

This review summarizes recent in vitro and in vivo findings on the importance of endothelial cell (EC) receptors and uptake pathways in regulating FAs and cholesterol uptake in normal physiology and cardiovascular disease.

We highlight clinical and experimental data on the roles of ECs in lipid supply to tissues, heart and arterial wall in particular, and how this affects organ metabolism and function. Models of FA uptake into ECs suggest that receptor-mediated uptake predominates at low FA concentrations, such as during fasting, whereas FA uptake during lipolysis of chylomicrons may involve paracellular movement. Similarly, in the setting of an intact arterial endothelial layer, recent and historic data support a role for receptor-mediated processes in the movement of lipoproteins into the sub-arterial space.

We conclude with thoughts on the need to better understand endothelial lipid transfer for fuller comprehension of the pathophysiology of hyperlipidemia, and lipotoxic diseases such as some forms of cardiomyopathy, and atherosclerosis.

Keywords

triglyceride; fatty acids; CD36; SR-B1; GPIHBP1; lipoproteins; chylomicrons; lipoprotein lipase

Correspondence to the following: Nada A. Abumrad, nabumrad@wustl.edu; 660 South Euclid Avenue, CB 8051, St. Louis, MO 63110, Ira J. Goldberg, Ira.Goldberg@nyumc.org; 435 East 30th Street, SB 617, New York, NY 10016.

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Introduction

Lipids support energy production and caloric storage, processes essential for cellular structure and growth. In mammals, lipids circulate in the blood as non-esterified (NE) "free" fatty acids (FAs), but mostly (~90%) as esterified FA components of triglycerides (TGs), phospholipids and cholesteryl esters in lipoproteins. In all tissues, except for those with fenestrated capillaries like the liver and adrenals, lipid uptake requires transfer from the circulation across the endothelial cell (EC) barrier, a process coordinated by lipoprotein lipase (LpL), its binding protein glycosylphosphatidylinositol-anchored HDL binding protein 1 (GPIHBP1), cluster of differentiation 36 (CD36) and likely other molecules. This basic function of ECs normally allows optimal heart function and protects against some forms of dilated cardiomyopathy[1, 2]. This review will focus on EC transport of circulating lipids and how deletions of LpL, GPIHBP1, CD36, and SR-B1 affect EC lipid transport, tissue metabolism and organ function. The biology of metabolically driven cardiac lipotoxicity has been recently reviewed elsewhere[3] and in a special issue on cardiovascular disease[4]. We will also review recent data that implicate ECs in the arterial wall lipid accumulation that initiates atherosclerosis.

The levels of circulating lipids likely regulate the receptors and enzymes involved in their transfer across the EC barrier. During fasting when stored lipids released from adipose tissue and VLDL secreted by the liver are the primary sources of FAs, CD36 facilitates cellular FA uptake acting as a high affinity receptor[5, 6]. In a well-controlled reaction, LpL releases FAs from circulating dietary TGs. Locally, LpL actions depend on its expression, expression of its inhibitors, and EC expression of GPIHBP1. LpL facilitates FA uptake into high-energy tissues such as the heart and brown fat. During the postprandial period, LpL directs dietary lipids within chylomicrons (CMs) into adipose storage.

Lipolysis within capillaries is well established as the principal route for conversion of CM TGs into NEFAs[7] and additional details were provided by the discovery of GPIHBP1[8] and angiopoietin-like protein (ANGPTL) inhibitors. What is less defined is how NEFAs liberated from different lipoproteins leave the capillaries. Transfer of NEFAs released from TG within VLDL lipids appears partially to require EC CD36 (discussed below). Other receptors, e.g. those recently shown to mediate movement across ECs of the cholesterol-rich lipoproteins LDL and HDL might also transfer VLDL lipids. In contrast, as shown in Figure 1, FAs from CMs could enter the subendothelial space via a yet to be defined receptor, or via non-receptor movement either through or around the cells through channels created by the loosening of EC junctions. Evidence both in vitro [9] and in perfused arteries[10] suggest that active lipolysis increases permeability of the EC barrier.

This review will focus on the central processes of vascular lipid delivery to tissue, address current areas of controversy and highlight experimentally tractable findings with observations related to normal tissue function and pathophysiology. We will highlight how

new studies of LpL and GPIHBP1 regulation and of the role of EC lipid receptors add to our understanding of the processes regulating sites of tissue lipid uptake. We will conclude by considering the poorly studied biophysical processes that occur within vessels and that likely regulate interaction of lipoproteins with the luminal EC surface.

Endothelial Cell Transport of FAs

FA oxidation supplies most of the energy for chronically working muscles such as heart and diaphragm, in contrast to type 2 or fast twitch muscles that preferentially use glucose. During restricted caloric intake, the sustained energy necessary for the heart requires more FA use as glucose availability becomes limiting[11, 12]. In the postprandial period, circulating FAs are mostly derived from lipolysis of lipoprotein (CM and VLDL) associated TGs, whereas during fasting NEFAs result both from intracellular lipolysis of adipose TG stores and from lipolysis of VLDL TGs. The first step in movement of NEFAs from the circulation to myocytes or adipocytes requires their uptake by ECs or their paracellular movement between ECs. The relative importance of these pathways is likely affected by concentrations of NEFAs near the EC luminal surface (Figure 1).

Circulating NEFA levels fluctuate throughout the day in parallel with the metabolic state. Most NEFAs are bound to albumin and unbound protein-free FA level in the circulation is determined by the molar ratios of FA to albumin and is very low. Fasting FA : albumin ratios in serum range between 0.3–1.5 and associate with unbound FA levels of 5–15 nM[13]. Much higher ratios would occur in vicinity of ECs during CM lipolysis. Studies of long chain FA uptake kinetics in intact cells[14, 15], reviewed in[5, 16, 17], documented saturation of uptake at <10 nM unbound FA and competitive inhibition between different FAs[14]. The kinetic data suggest that a receptor-mediated process is used for uptake of FAs of more than eight carbons. A similar conclusion was reached in a real time fluorescence microscopy study of FA transfer that included inhibitors and long versus short chain FAs[18]. Overall, kinetic properties of cellular FA uptake identify a saturable receptor mediated process at low FA: albumin ratios and a non-saturable, presumably non-receptor mediated process at higher FA/ albumin ratios.

Receptor mediated FA uptake: Transporters mediate transmembrane movement of NEFAs while intracellular FA-binding proteins trap the FA and influence cellular lipid accumulation, the combined steps referred to as vectorial transport. Each of these steps can affect tissue concentration of tracers and lipids, but a transporter must be on the plasma membrane, bind to FAs, and lead to rapid cellular FA accumulation.

The best characterized FA transporter is CD36 (Table 1), a member of the CD36 class B scavenger receptor family that includes scavenger receptor-B1 (SR-B1), a lipoprotein binding receptor (see later sections), and the lysosomal integral membrane protein 2 (LIMP2)[19]. In addition to FAs, CD36 recognizes lipoproteins (VLDL, HDL, and LDL), bacterial lipids, and non-lipid ligands (e.g. thrombospondin-1). The ligands induce CD36 mediated signaling and consequently the protein has pleiotropic cellular effects[12, 19–21] that include, as recently reported, the regulation of insulin and AMPK actions[22, 23]. CD36 fulfills the requirements for a transporter: membrane localization, FA binding, and modulation of

cellular FA content. Its expression enhances FA oxidation and FA incorporation into lipids and it translocates to the plasma membrane, acutely in response to insulin, AMPK, leptin, or chronically with excess FA supply[24–28], as reviewed[29].

CD36's function in FA uptake by rat adipocytes was identified through its binding of a reactive ester of oleic acid, sulfo-N-succinimidyl oleate (SSO) followed by its isolation and cloning[30–32]. Because SSO is currently commercially available as a specific inhibitor of CD36, it is useful to mention some of its properties. SSO is specific in that it blocks FA uptake without directly affecting other substrates or the metabolic fate of FAs[33] and labeled [^{3}H]-SSO or the palmitate ester [^{3}H]-SSP specifically bind CD36 in plasma membranes[34,35]. However, SSO specificity, due to its long hydrocarbon chain, is dependent on its membrane impermeability. Although we initially used high inhibitor concentrations, as albumin which scavenges SSO was included in the medium, SSO is effective and recommended at low concentrations (10–25uM) [22, 36–38]. When using high SSO levels, if membrane integrity is not monitored, the inhibitor will enter cells and interact with proteins other than CD36[39].

The crystal structure of the CD36 family member LIMP2 reported in 2013[19] identified an internal lipid transport tunnel that traverses the length of the proteins in this family and empties in the bilayer's proximity. This is discussed in more detail in the section where we compare CD36 and SR-B1. SSO binds to lysine 164 in a hydrophobic pocket in the CD36 ectodomain[37] that connects with the transport tunnel[40]. Long chain FAs were associated with crystal CD36 and localized to the intramolecular tunnel[41].

CD36 enhances FA uptake and FA oxidation in mice models[42–45] and human CD36 polymorphisms associate with defects of lipid handling and metabolic disease (e.g., hyperlipidemia, metabolic syndrome, type 2 diabetes)[22, 46–49]. Total CD36 deficiency in 9–10% of African Americans and Africans[46, 50–52] and in 1%–3% of Asians[53, 54] cause's defective FA uptake by heart, muscle and adipose tissues[55–57]. Common CD36 variants increase blood FAs[58–59] and DNA methylation sites that reduce CD36 expression increase CM remnants[60, 61]. Genome wide association studies[52, 62] relate CD36 expression inversely to HDL and positively to VLDL levels. The latter could reflect a hepatic effect of CD36 deletion, which inhibits liver VLDL secretion in mice[63].

The FA transporter protein (FATP) 1 (Table 1) was identified through a functional screening for proteins that facilitate FA accumulation in cells[64, 65] and belongs to the SLC27 protein family that activate FAs by CoA modification[66]. There are at least 6 family members; FATP2–5 are found primarily inside the cell, but FATP1 and FATP6 are detected on the plasma membrane (Cell Atlas and REACTOME databases) and FATP1 translocates to the membrane after insulin[67]. The FATPs share with the yeast ortholog Fatp1 two motifs required for binding ATP and FAs, and might function, like Fatp1, in vectorial coupling of FA uptake and FA activation, as the CoA-ligase activity traps the FA inside the cell and influences its metabolic partitioning[68–70]. The effect of gain and loss of function studies in animal models (Table 2) might reflect FATP isoform tissue expression, cellular localization and FA specificity, as reviewed[67]. FATP1 is most highly expressed in the heart and its overexpression increases heart TG content and produces cardiomyopathy, [71] while overexpression of FATP4 in skeletal muscle increases FA uptake and oxidation[72]. Deletion

of FATP2 in the liver appears to be beneficial by activating PPARα remodeling of lipid metabolism[73] (Table 2). FATP3 and FATP4 are expressed in ECs and have been implicated in endothelial FA accumulation, as will be discussed in the following section (endothelial regulation of FA transport). In summary, the FATP family of proteins play an important role in cellular FA uptake, but the nature of this role remains unclear; it might involve interaction with the FA in the plasma membrane, trapping the FA through its activation or interacting with membrane transporters providing vectorial transfer of FA to specific metabolic sites.

In addition to the receptor-mediated FA uptake, there is evidence for nonsaturable receptor independent uptake. Long chain FAs can cross from the outer to the inner leaflet of the plasma membrane via a process termed 'flip-flop', which has been modeled using protein-free lipid vesicles[74, 75]. These studies initially suggested that FA transfer does not need protein facilitation. However, the flip-flop rates measured across cellular membranes are slower. In a series of studies[76–78] with some monitoring FA transfer using the fluorescent FA binding protein ADIFAB injected inside cells, Kleinfeld and colleagues concluded that flip-flop rates are too slow for FA uptake by adipocytes and cardiomyocytes and protein facilitation is required. Slow FA flip-flop rates are consistent with the need for cells to regulate FA uptake. As FA availability is variable (i.e. in fasting, feeding, exercise, stress, etc.), protein transporters allow cells to regulate FA entry and provide the organism with a mechanism for optimizing FA biodistribution. When unbound FA concentrations are in the low nanomolar range, e.g. during fasting, a high affinity cellular receptor allows FA sparing for cells and tissues with high FA requirements such as heart, muscle and brown fat. In contrast, at high FA concentrations such as during feeding, cellular downregulation of FA receptors would mitigate excess intake. In addition, it is likely that receptor independent FA uptake, which would occur at high FA levels, is also physiologically regulated as proposed in Figure 1.

Endothelial Regulation of FA Transport

Uptake of FAs from the circulation into tissues occurs primarily in capillaries where, unlike in most arteries, the ECs express both CD36 and GPIHBP1. However, we should note this generalization overlooks a newly described subpopulation of aortic ECs that can express these genes[79]. The effects of myocyte lipid uptake on cellular concentrations of ceramides, diacylglycerols and likely other signaling lipids and the insulin-signaling pathway have been reviewed by others[80, 81]. Channeling of these lipids via esterification and storage as TG leads to lipid droplets (LDs), which are thought to be non-toxic. This is illustrated by the effect of diacylglycerol acyl transferase (DGAT) overexpression. In muscle, it creates a phenotype similar to that of the athlete paradox, greater myocyte TG and greater insulin sensitivity[82]. Similarly, TG accumulation in the heart and macrophages due to DGAT overexpression reduces lipid-driven cellular toxicity[83, 84]. Thus, in addition to uptake, the ability to store the lipid affects how excess supply alters cell function.

Evidence that the ECs affect parenchymal cell lipid accumulation comes from a number of genetic studies where specific EC genes were altered. One of the early reports highlighting importance of the endothelium in regulating tissue FA uptake and metabolism involved EC deletion of the master transcriptional regulator and FA sensor PPARγ. The EC-knockout

mice had increased circulating FAs but were protected from high fat diet induced adiposity and insulin resistance, compared to control mice[85] (Table 2). Expression of PPARγ gene targets, including CD36, was reduced as expected.

The PPARγ coactivator PGC1α stimulates mitochondrial biogenesis and FA β-oxidation and in addition, co-activates VEGF expression and angiogenesis. Jang et al. tested the possibility that PGC1α might coordinate both pathways by regulating FA flux by ECs.[86]. Conditioned medium from myotubes overexpressing PGC1α increased FA uptake by human umbilical vein ECs (HUVECs). The active medium factor was identified as 3 hydroxyisobutyrate (3-HIB) generated through PGC1α upregulation of the enzymes of valine catabolism. In vivo, 3-HIB stimulated muscle tissue FA uptake and lipid accumulation and reduced insulin sensitivity[86]. Although the 3-HIB increase in FA flux was blocked by the CD36 inhibitor SSO it was more sensitive to inhibition by knockdown of FATP4 as compared to that of CD36. In a follow up study the same group reported that EC FATP4 resides in the ER juxtaposed to mitochondria and can use mitochondrial ATP to activate FA and influence EC FA uptake and oxidation[87]. This study demonstrates the importance of FA activation in EC FA uptake. Other regulators of EC FA transport are the Meox2/Tcf15 heterodimer transcription factors identified using microarray profiling, which determines a microvascular EC signature enriched in genes related to FA uptake including CD36 and LpL. The Meox2/Tcf15 in cardiac ECs upregulate uptake of FAs supplied by albumin or derived from LpL hydrolysis of triglycerides[88]. The above studies illustrated how altering EC gene expression influences tissue FA uptake and insulin sensitivity; they also highlighted the importance of a specific microvascular EC gene signature that includes LpL, GPIHBP1 and CD36 for FA uptake function[79, 88].

Vascular endothelial growth factors (VEGF A-D) and their receptors (VEGFR 1–3) control angiogenesis and maintenance of the vasculature and consequently, can influence nutrient exchange in tissues. Overexpression of VEGF-A in adipose tissue increased vascularization, activated thermogenesis and energy expenditure, and conferred protection from obesity and insulin resistance[89, 90]. This protection is associated with enhanced clearance of circulating FAs and TGs and the upregulation of LpL in heart and adipose tissues[90]. Deletion of VEGF-B in mice reduced heart FA uptake and increased glucose uptake. Moreover, VEGF-B was proposed to function in endothelial FA uptake through transcriptional upregulation of vascular-specific FATP3 and FATP4[91]. As deletion of VEGF-B in diabetic mice reversed tissue lipid accumulation and glucose intolerance (Table 2), the authors suggested that targeting VEGF-B could improve muscle insulin sensitivity by impairing endothelial FA transport[92]. However, other studies did not observe an effect of VEGF-B on FA uptake by ECs[86] or with VEGF-B overexpression in heart[93].

Microvascular ECs robustly express CD36, while it is sparse in macrovascular cells. This suggests that there is a specific role for this molecule in capillaries. EC-specific CD36 deletion reduced FA uptake into heart and skeletal muscle, assessed in vivo using both tracer kinetics and PET scanning. EC CD36 deficiency in ECs also downregulated expression of PPAR target genes[94]. Brown fat FA uptake was reduced which might have implications for thermogenesis; both LpL and CD36 affect brown adipose uptake of FAs during cold exposure[95].

Capillary ECs from tissues which most actively uptake circulating FAs have high levels of CD36 expression[96].These ECs also express LpL and its EC-anchoring protein, GPIHBP1. During fasting, adipose tissue lipases (ATGL and HSL) are activated, which increases blood FA levels and leads to LD accumulation in the heart, muscle and liver. Hearts of EC-*Cd36*$^{-/-}$ mice, unlike hearts of control mice, do not accumulate LDs during fasting[94]. Cardiomyocyte CD36 deletion also reduces LD formation, which suggests that this protein affects lipid uptake both in ECs and cardiomyocytes (reviewed in[29]). EC-*Cd36*$^{-/-}$ mice have slow clearance of postprandial TGs, likely reflecting LpL inhibition by increased local FAs. In CD36-deficient humans, heart FA uptake was reduced at all FA levels and only at lower FA levels in adipose and muscle tissues[57].

The EC-*Cd36*$^{-/-}$ mice, like the germline *Cd36*$^{-/-}$ mice, are more insulin sensitive and RNA-Seq data of heart tissue documented increased expression of genes of insulin signaling and glucose metabolism (Table 2). These phenotypes were not observed when CD36 was deleted from cardiomyocytes[94], where in contrast, to the effects of EC CD36 loss, inducible CD36 deletion in myocytes resulted in muscle insulin resistance. This effect is likely exclusive of the role of CD36 in FA transport and involves its action to maintain Src phosphorylation of the insulin receptor (IR). As a result, CD36 deletion prevents full activation of IR[22]. Since CD36 regulation of IR is prevented by saturated FAs, this could be related to FA-induced muscle insulin resistance. Overall, these findings indicate that EC CD36 acts as a gatekeeper of tissue FA delivery and its metabolic effects integrate both its transport and signaling functions.

Tissue Specific Effects due to EC FA Transport

Capillaries and ECs are heterogeneous across organs and it is likely that the effects mentioned above, where targeting of EC FA transport enhanced systemic insulin sensitivity, reflect changes in muscle tissues. Skeletal muscle and heart are the major FA consumers and increasing or decreasing FA uptake would enhance or reduce muscle insulin stimulated glucose disposal. This interpretation is consistent with data suggesting that enhancing EC FA uptake targeted to adipose tissue improves systemic metabolism. Increasing adipose tissue vascularity by overexpression of VEGF-A improved insulin sensitivity while enhancing clearance of circulating FAs and TGs[90]. A recent study more directly supports this concept. Adipocyte-specific knockout of Angiopoietin-2 (Angpt2) identified it as a regulator of EC-FA uptake in subcutaneous adipose tissue[97]. Angpt2 interaction with ECs stimulates integrin α5β1 signaling and EC CD36 internalization. The Angpt2 stimulated uptake process was inhibited by knockdown of CD36 and FATP3 but not of FATP4 (unlike in muscle). Inhibition of Angpt2-stimulated EC FA uptake in adipose tissue caused ectopic fat accumulation and promoted insulin resistance, supporting the benefit of upregulating EC FA uptake in subcutaneous fat[97]. Other data suggested that adipose tissue has a specific EC FA uptake process mediated by a prohibitin–annexin A2 complex that increases cell surface CD36 and FA uptake by ECs and adipocytes[98].

In summary, EC uptake of FAs is a highly regulated process that influences tissue metabolism and targets FAs to specific tissues with high nutrient requirements, such as the heart and brown fat. Several important regulators of EC FA uptake (Table 1) work either at

the level of gene transcription or acutely through EC receptors. Much remains to be learned about the effects of angiogenic factors on the regulation of the endothelial barrier and how this might affect FA transfer. The role of the EC FATPs, notably FATP3 and FATP4, will need to be investigated further in animal models with targeted EC deletion of these proteins, and information on their functional protein interactions is needed. Understanding the molecular mechanism by which CD36 facilitates FA transfer and how its function might be regulated by interaction with membrane integrins or integrin ligands[99] and possibly the FATPs are needed. CD36 contributes to uptake of albumin-bound FAs and of FAs released from VLDL by LpL as shown for heart[100] and brown adipose tissue, [95] but it does not contribute to heart CM FA uptake in vivo[100]. The regulation of lipoprotein lipid uptake by ECs and the receptors likely to be involved are discussed in the following section.

Regulation of Lipoprotein Lipolysis at the EC Luminal Surface

The major sources of cellular lipids other than NEFAs are the circulating lipoproteins that contain TGs, cholesterol, cholesteryl esters and phospholipids. LpL is the rate-limiting enzyme for hydrolysis of TG-rich lipoproteins (TRLs), VLDL and CMs and it associates with the luminal surface of capillary ECs where it generates NEFAs, monoacylglycerols and remnant lipoproteins. LpL is synthesized in parenchymal cells of metabolic tissues like heart, skeletal muscle and adipose tissue and secreted into the sub-endothelial space. The endothelial anchoring protein GPIHBP1, present on the basolateral side of ECs, captures LpL from the interstitial space and transports it across ECs to the capillary lumen, which is the site of LpL's catalytic action[101]. Other proteins such as the VLDL receptor might provide a secondary transport system[102] and account for the reduced TG levels after heparin injection with GPIBHP1 deficiency. GPIHBP1 may also anchor TG-rich lipoproteins to the endothelial surface and protect LpL from endogenous inhibitors[120, 121]. Deficiency of LpL in humans causes massive chylomicronemia, low levels of HDL-cholesterol and recurrent pancreatitis. Unlike humans, LpL deficiency is neonatally lethal in mice, likely from hypoglycemia[103] and mice at equally high plasma TGs do not develop pancreatitis.

Mice lacking GPIHBP1 develop severe chylomicronemia due to decreased ability of LpL to metabolize circulating TGs[8]. Surprisingly, on a high fat diet, these mice have lower TG levels, perhaps due to better hepatic TG-rich lipoprotein uptake. Patients with GPIHBP1 deficiency have less functional LpL in the capillary lumen, diminished TG hydrolysis and severe chylomicronemia. Moreover, chylomicronemic patients have been identified who have GPIHBP1 autoantibodies[104] that block GPIHBP1's binding and LpL transport, thus interfering with LpL mediated TG hydrolysis.

Olivecrona[105] and others[106] had noted many years ago that monomeric LpL was unstable. During enzyme purification from either post-heparin plasma or bovine milk, active LpL aggregated into dimers or multimers; this aggregation likely was aided by the presence of heparin used to stabilize LpL activity. These aggregates are more stable at 37°C, leading to the assumption by the research community that *in vivo* active LpL required dimers. Beigneux et al.[107] reinvestigated this question and showed that active LpL is indeed present as a monomer. They added purified GPIHBP1 to LpL and eluted active and monomeric LpL. They also co-expressed both inactive but GPIHBP1-binding LpL together with active LpL

that did not bind GPIHBP1. Dimerization would have been expected to produce double tagged, more active enzyme. However, very little of this mixed dimer was found, and what was found was in the inactive fraction. It is plausible that non-GPIHBP1 binding LpL is less stable, and native LpL has its activity stabilized by GPIHBP1. Additionally, Birrane et al.[108] and Arora et al.[109] independently solved the crystal structure of LpL bound to GPIHBP1. The derived structures were very similar and will likely provide more in depth structural view of the lipolysis process.

In the last decade, LpL and its posttranslational regulators have attracted attention due to a host of genetic studies showing clear association of plasma TGs with risk of cardiovascular disease (CVD). APOC3, produced by the liver and to a small extent by the intestine, inhibits LpL and is a key regulator of plasma TG metabolism (Table 1). APOC3 associates with ApoB-containing lipoproteins and can be acquired by HDL during lipolysis. APOC3 inhibits LpL activity and also hepatic uptake of remnants[110], and its overexpression in mice increased TG levels. In contrast, APOC3-deficient mice have reduced TGs in both wild type and *Ldlr*$^{-/-}$ and *Apoe*$^{-/-}$ backgrounds[111, 112] (Table 2). The link of APOC3 to CVD risk prompted pharmaceutical development of inhibitors in the form of antisense oligonucleotides (ASOs), interference RNAs and monoclonal antibodies[113, 114].

Apolipoprotein C2 (APOC2) is required for LpL activation, as it binds LpL and guides the TG substrate into the active site[115, 116]. APOC2 mimetic peptides directly activate LpL and inhibit APOC3[115]. Loss-of-function mutations in APOC2 in humans increase plasma TG levels and can provoke acute pancreatitis[117]. Mice expressing a homozygous loss-of-function *Apoc2* mutant[118] have increased plasma TGs, consistent with its role as an LpL cofactor (Table 2).

APOA5 also increases lipolysis but its mode of action is incompletely understood. Mice lacking APOA5 develop hypertriglyceridemia[119] (Table 2) and APOA5 deficiency associates with hyperchylomicronemia in humans [120].

Three angiopoietin-like proteins also modulate lipolysis (Table 1). ANGPTL3, primarily synthesized in the liver, inhibits endothelial lipase (EL) and LpL. Its activity requires its association with a second circulating protein, ANGPTL8[121]. Mutations in *ANGPTL3* cause hypolipoproteinemia without steatosis[122]. ANGPTL4 inhibits LpL primarily in adipose tissue[123–125] while the ANGPTL3/8 complex is more effective in muscle[126].

EC Uptake and Transcytosis of Lipoprotein Lipids

Differences in gene expression between capillary ECs and most large vessel ECs likely relate to their distinctive functions. As previously mentioned, there is a gene expression signature specific for microvascular ECs that is marked by CD36 and enriched in genes (GPIHBP1, LpL, PPARγ) for TG lipolysis and fatty acid uptake[86, 77]. In large vessels such as the aorta, the gene expression signature of macrovascular ECs, marked by VCAM1 (vascular cell adhesion molecule 1), is enriched in genes for extracellular matrix organization and integrin cell surface signaling pathways[77]. However, heterogeneity of ECs is also observed within individual vessels. For example, in the aorta a subset of ECs was found to express the gene signature typical of microvascular ECs, including CD36, LpL and

GPIHBP1. These ECs show a distinctive distribution, being more abundant in the greater curvature area of the aortic arch, a non-atherosclerosis prone region, where the Vcam1/CD36 ratio is lower. In contrast, the Vcam1/CD36 ratio increases in areas of lesser curvature, [77] which are more atherosclerosis prone regions and have higher expression of SR-B1[170]. These findings suggest that although lipid lipolysis is thought to only occur in capillaries, some lipolysis and fatty acid uptake likely occur in the greater curvature areas of the aorta, while more LDL transcytosis occurs in areas of lower curvature. Such a conclusion would not support a role of CD36 in atherosclerosis or the long-held hypothesis that local lipolysis creates either remnants or toxic lipids that promote atherosclerosis[127].

Capillary EC uptake of circulating lipids might occur via caveolae-mediated transcytosis. Caveolae are small membrane invaginations (50–80 nm in diameter) present in many cell types and particularly abundant in capillary ECs as well as in adipocytes and smooth muscle cells. Caveolin-1 (Cav-1) is a structural protein required for formation of caveolae in ECs. Mice deficient in Cav-1 (*Cav-1*$^{-/-}$) show caveolae loss[128], are lean, resistant to high fat diet-induced obesity, but have high blood FA and TG levels[129], a phenotype similar to that of the *Cd36*$^{-/-}$ mouse[42, 130] (Table 2). One key function of caveolae (and Cav-1) is regulating transcytosis across the endothelial barrier[131]. *Cav-1*$^{-/-}$ mice have impaired transcytosis[132] of albumin, which carries FAs and other lipids. Likewise, the function of caveolae in transendothelial LDL transport is supported by the finding that Cav-1 deletion suppressed atherosclerosis development[133, 134], while rescue of Cav-1 expression in ECs reversed the phenotype[134] (see below). Several pathways link Cav-1 to lipid metabolism[135]. Cav-1 facilitates assembly of membrane lipid domains (caveolae)[136–138] through interaction with cholesterol[139] and FAs[140]. It also translocates to LDs during lipolysis[141–144]. Sessa and colleagues[145] reported that ECs lacking Cav-1 have reduced LD formation that is reversed by rescue of EC Cav-1. They noted that the decrease in LDs associated with a decrease in CD36, consistent with Cav-1 promoting caveolae CD36 localization in the plasma membrane[146, 147].

Aside from FAs, the heart, like most tissues, requires a number of other lipoprotein-associated lipids, including cholesterol and retinoids. However, hearts obtain little cholesterol from circulating LDL[148] and also do not synthesize it[149]. Using perfused hearts obtained without the use of heparin, which dissociates LpL from the EC surface, Fielding[150] showed that during lipolysis, CMs could supply the hearts with cholesterol. Because uptake of the APOB core protein component of these particles is very low[100], it is likely that lipids —including cholesterol, phospholipids and retinoids—liberated during lipolysis move across the EC barrier. Another option is uptake of these lipids via the VLDL receptor, which is expressed by ECs and was reported to mediate greater lipid storage and injury during cardiac ischemia[151].

While TG lipolysis accounts for most tissue uptake of lipoprotein-derived FAs, some transfer of lipoprotein TGs across the EC barrier likely occurs in the absence of LpL mediated lipolysis. An example of this is the development of skin eruptive xanthomas with lipid-enriched macrophages associated with genetic LpL deficiency and high plasma TG levels. For more than 50 years, the process underlying this pathology has remained unclear. Electron microscopy studies of skin xanthomas in patients with hyperchylomicronemia and

diabetes showed no evidence of CMs within ECs nor defective EC junctions[152]. Although a similar study in LpL deficient patients has not been reported, it is likely that the pathology is the same. The similarity of the TGs, but not cholesteryl esters, and FAs within CMs and skin xanthomas suggested that CM-derived FAs crossed the EC barrier, but not necessarily as intact CMs. Studies acutely tracking labeled nascent CMs did not find these particles within ECs[153, 154]. In contrast, Guyton and Klemp[155] noted lipid droplets adhering to the luminal membrane and within ECs in rabbits fed a cholesterol and butter diet. Presence of CM-derived lipoproteins within the artery[156] also suggests that CM remnants penetrate the artery as or more efficiently than LDL.

The reason for the inconsistent LD findings in ECs remains unclear and suggests the possibility that transfer of lipoproteins into and through ECs is extremely rapid and often completed during the time required to obtain and process tissues for microscopy. A recent study from Guo et al. using confocal microscopy confirmed the presence of EC lipid droplets in the aorta of mice after an oil gavage[157]. Although they equated these lipid droplets with those found when ECs were incubated with FAs *in vitro*, aortic ECs are unlikely to have significant local lipolysis, as most aortic ECs do not express GPIHBP1 and fail to approximate CMs and LpL[153]. However, since a subpopulation of aortic ECs that can express these genes has been recently described[79] revisiting the above studies is needed to determine the contribution of this subpopulation to the observed LDs. The uptake pathway for CMs and CM remnants remains unclear (Figure 2). Possibly, CMs non-specifically interact with the EC surface and are internalized by macro-pinocytosis after association with cell surface proteoglycans. Alternatively, specific lipoprotein receptors mediate their uptake possibly by transcytosis, as discussed below for LDL and HDL.

Most tissues obtain cholesterol from LDL and HDL after their transport through or around the endothelial barrier. Movement of both LDL and HDL into tissues has been most intensely studied in the context of atherosclerosis development. HDL must cross the endothelium to mediate athero-protective efflux of cholesterol from lipid-laden macrophages back to the liver[158]. How LDL and HDL traverse the endothelial barrier remains poorly understood. Early atherosclerotic lesions are overlaid by an intact endothelium, which would preclude the paracellular passage of molecules exceeding 6 nm in diameter[159]. Electron microscopic studies of rat aorta perfused with LDL clearly show its internalization into cellular vesicles and LDL targeting to the basolateral membrane[160]. Support for a specific aortic EC LDL transporter is provided by the atheroprotective effect of Cav-1 deletion in APO E knockout mice[161], with its rescue in ECs being sufficient to promote lesion progression and LDL infiltration of the aortic wall[162,163]. These findings strongly suggest that cholesterol-carrying lipoproteins cross the aortic endothelium via a transcytotic route.

Scavenger Receptor-B1.—SR-B1 identified by expression cloning as a CD36-related protein n binds HDL and mediates selective uptake of HDL cholesteryl esters into liver cells[164, 165]. SR-B1 also functions in peripheral tissues to facilitate efflux of cholesterol to HDL and back to the liver[166, 167] (Table 1). To access cholesterol in the intima, HDL and its main protein component, APO A-I, must cross the EC barrier. In vitro, HDL transcytosis across EC monolayers requires SR-B1 and also ABCG1[168], while transcytosis of APOA-I alone is mediated by the ATP-binding cassette transporter (ABC)A1. Endothelial-specific

overexpression of SR-B1 is athero-protective in high-fat/high-cholesterol fed C57BL/6 mice, as well as in *ApoE*$^{-/-}$ mice fed a chow diet[169]. SR-B1 in the lymphatic endothelium helps remove cholesterol by HDL transcytosis[170].

SR-B1 also binds modified, as well as native, LDL[164] and has recently been implicated in aortic uptake and transcytosis of LDL. Total internal reflection fluorescence (TIRF) microscopy demonstrated that SR-B1 mediates LDL transcytosis in human coronary ECs and HDL competition inhibited transcytosis[171]. Treatment with physiologic concentrations of estrogen reduces SR-B1 mRNA and protein expression in primary human ECs from men and postmenopausal women and inhibits LDL transcytosis[172]. The role of endothelial SR-B1 in LDL transcytosis was recently confirmed in vivo. Mice with EC-specific deletion of the receptor exhibit significantly diminished uptake of DiI-labeled LDL into the aorta, as well as reduced uptake of administered LDL by aortic wall macrophages. In culture, LDL transcytosis involves its direct binding to SR-B1 and recruitment by the receptor of the guanine nucleotide exchange factor dedicator of cytokinesis 4 (DOCK4). Endothelial-specific deletion of SR-B1 markedly reduces atherosclerosis in *ApoE*$^{-/-}$ mice, as well as in mice with genetic or PCSK9-induced LDLR deficiency[173]. How can SR-B1 deletion and overexpression in the endothelium both be protective? One possibility could entail an effect of overexpression in altering receptor dimerization. Although the functional role of SR-B1 dimerization is not well understood, it could be important for binding specific ligands and, as recently shown, for stabilizing SR-B1 at the plasma membrane[174] (see following section).

Other EC receptors have been studied in lipoprotein transcytosis. Systemic endothelial LDL transcytosis is largely independent of the LDL receptor (LDLR), made evident by the lack of effect of its genetic deletion or its PCSK9-mediated degradation on non-hepatic tissue metabolism[170, 171]. However, LDLR has been reported to participate in LDL transcytosis across the blood brain barrier[175]. In 2015, a genome-wide iRNA screen identified activin receptor-like kinase 1 (ALK1) as a novel low-affinity, high-capacity LDL receptor, which mediates LDL transcytosis in vitro independent of its kinase activity. Importantly, endothelial-specific deletion of ALK1 reduced DiI-LDL uptake into the aortic endothelium of *Ldlr*$^{-/-}$ mice[176].

CD36 and SR-B1 Structures and Interaction with Lipoproteins

Greater SR-B1 expression in more atherosclerosis prone segments of the aorta[170] could underlie the reason some vascular regions associate with more with atherosclerosis, whereas ECs with greater CD36 expression function to augment FA uptake. For this reason, a comparison of their functional structure would be informative. Overall, the two receptors overlap in ligand specificity, although efficiency of binding or transfer might differ; FAs[40, 177], modified and native lipoproteins[40, 178], phospholipids[40, 179], apoptotic cells[180, 181], pathogens[182–185] and advanced glycation end products[186, 187] are ligands for both receptors. Most aortic ECs have low CD36 and Cav1 expression and are not surrounded by cells with LpL expression (myocytes and adipocytes). Thus, TRL lipolysis is unlikely on aortic EC surfaces. In contrast, cardiac and aortic ECs express SR-B1, which could function in lipid uptake by both capillaries and large vessels

The parallel functions of SR-B1 and CD36 in lipid transport reflect structural similarities (Figure 3). Structural modeling identified a cationic enriched apex region in both SR-B1 and CD36 that is absent in LIMP-2[188] and mutation of key cationic residues inhibited binding of HDL, oxidized-LDL, acetyl-LDL[188] and silica[189] to SR-B1 and of oxidized-LDL and NEFAs to CD36[37, 188]. The electrostatic similarity between the apex regions of SR-B1 and CD36 is in line with the ligand overlap.

Lipid transport by both SR-B1 and CD36 is thought to occur through an internal tunnel. Existence of a hydrophobic “channel” within SR-B1 that facilitates cholesterol ester uptake from HDL particles to the plasma membrane was first proposed by the group of David Williams[190]. Direct evidence for tunnel existence was provided by crystallography of the ectodomain of the third CD36 family member LIMP-2[188]. Critical residues for tunnel formation are largely conserved across family members, as are the two cysteine-cysteine bridges that stabilize the structure and the dimensions of the tunnel cavity, which accommodates cholesterol esters and FAs. Crystallography of the ectodomain of CD36 confirmed presence of a hydrophobic internal tunnel as in LIMP-2[41] and identified long chain FAs within the tunnel (Figure 3). It is postulated that the FA binds to a hydrophobic pocket on the CD36 surface and accesses the internal tunnel, which delivers it to the bilayer[40]

Scavenger receptor B family members have two transmembrane domains. Both SR-B1[191, 192] and CD36[193, 194] form dimeric and higher order oligomeric forms. The GxxGxxxAxxG motif in the N-terminal transmembrane domain of both proteins is important for dimerization of both SR-B1[195] and CD36[196]. A leucine zipper motif in the C-terminal transmembrane domain of SR-BI (absent in CD36) is also required[197, 198]. Oligomerization retains SR-B1 at the plasma membrane; its disruption after mutating the C-terminal leucine zipper motif, but not the N-terminal glycine motif, induces SR-B1 endocytosis and degradation[174]. The functional role of receptor oligomerization is unclear, but it could regulate interaction with specific ligands, in addition to playing a role in receptor/ligand internalization.

Both SR-B1 and CD36 have short cytoplasmic C-terminal tails that mediate intracellular signaling in response to lipid ligands (Figure 3). Binding of HDL to SR-B1 activates endothelial nitric oxide synthase (eNOS), [199, 200] a process involving Src[201, 202] and LKB1[203] kinases with downstream activation of AMPK[203], Akt[201, 203] and MAPK[201]. The C-tail 47 amino acids of SR-B1 contain a PDZ domain sequence and the adapter protein PDZK1 facilitates SR-B1 interaction with Src[200, 202]. CD36 also relies on the Src family[23, 204–206] and LKB1 kinases[23, 207] for downstream activation of AMPK[23, 207], Akt[22, 208] and MAPK, [204–206] but its smaller C-tail of 13 amino acids has no PDZ binding domain. In macrophages, the adapter protein FcRγ facilitates CD36-Src interaction[209].

In summary, structural similarities in the ectodomains of SR-B1 and CD36 may explain the overlap in ligand specificity, and oligomerization may influence ligand interaction and handling. For example, oligomerization mediated retention of SR-B1 at the plasma membrane could facilitate cholesteryl ester uptake from HDL and at the same time prevent LDL transcytosis. If CD36 ligand uptake is mediated by endocytosis, oligomerization could

lead to plasma membrane retention. This might allow C-tail association with adapters and the activation of kinases, which like Src, could affect insulin signaling and other cellular functions.

Influence of Lipoprotein Size

In capillaries, TRLs interact with the EC surface for lipolysis to occur. A number of factors, some not fully defined, regulate this process. The radius of the lipoprotein markedly influences its interaction with ECs. Depending on its size, a chylomicron, as compared to a VLDL, is likely to have a 10–100-fold greater chance of interacting with LpL on the EC luminal surface. The size difference, rather than differences in LpL substrate preference, likely determines the more rapid lipolysis of larger lipoproteins. In rapidly flowing blood, it is likely that large CM size particles >200 nm, but not smaller lipoproteins marginate[210]. Margination would increase their effective concentration along the arterial surface where they can interact with either EC receptors or LpL (Figure 4). Other biochemical processes, exclusive of the physiology of fluid flow, modulate lipolysis, including apolipoproteins and their interaction with LpL, GPIHBP1, proteoglycans or other luminal membrane proteins.

Summary

Great advances over the past decade have provided insight into the regulation of intravascular lipolysis. The discoveries of ANGPTLs and GPIHBP1, and new findings on the importance of APOC3 as well as insight into the structure of LpL have led to the development of clinically relevant TG-reducing therapies. We can expect additional information on molecular regulation of LpL function. However, despite more than 5 decades of research, a number of basic physiologic and pathological processes central to lipid metabolism in ECs are still unclear and under investigation. Recent studies have emphasized the important role of endothelial receptors in regulating tissue lipid uptake and the tissue's metabolic phenotype. However, the pathways involved in EC interaction with FAs and lipoproteins, important in muscle energy metabolism and insulin sensitivity, adipose lipid storage, vascular function and atherogenesis, remain poorly understood. It is apparent that NEFAs derived from different sources exit the circulation by different routes. The importance of receptors versus possibly paracellular pathways depends on the FA concentration or other circulating factors that could alter EC junctions. SR-B1 and CD36, members of the same gene family, are emerging as central molecules in EC lipid metabolism and vascular biology. We need a better understanding of their interactions with different lipids and lipoproteins and how each receptor regulates transit of its ligands from the lumen. The handling of LDL and HDL by SR-B1 appears to regulate movement of atherogenic lipids into the artery. A second receptor, Alk1, also mediates EC LDL uptake. As global rather than EC-specific SR-B1 deficiency leads to vascular disease, additional information is required to understand the pro-atherogenic processes derived from SR-B1 loss in cells other than ECs. SR-B1 is expressed in both micro- and macro-ECs, whereas CD36 expression along with GPIHBP1 is relatively restricted to capillary ECs. The molecular processes controlling this differentiation are not understood although they promote the distribution of FAs required for normal physiology and are central to tissue energy and growth. Finally, human clinical observations illustrated a lipolysis-independent route that mediates exit of

CM lipids from the circulation leading to development of eruptive xanthomas in patients with LpL deficiency. The molecular details of this pathway and its importance for normal physiology are still unclear.

A final note should be included related to the role of factors that affect EC uptake and could not be addressed by this review. Multiple physiologic parameters affect lipoprotein interactions with ECs, including the rate of blood flow, turbulence, viscosity, ionic interactions, and the diameter of the vessel. In vitro studies of EC-lipoproteins interaction often use media that mimics lipoprotein concentrations found in circulating rodent or human blood. These studies do not account for the more than 50-fold difference in rate of blood flow between arteries and capillaries. In rapidly flowing blood, it is likely that large CM size particles >>200 nm, but not smaller lipoproteins marginate[210], which increases their effective concentration along the arterial surface where they can either interact with EC receptors or LpL (Figure 4). Smaller lipoproteins and RBCs likely reduce luminal EC surface exposure to HDL and LDL. The effects of these physical influences on lipoprotein/EC interactions have been poorly studied and deserve further investigation.

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Non-standard Acronyms and Abbreviations

Angpt2	Angiopoietin-2
ANGPTL	Angiopoietin like protein
APOC	Apolipoprotein C
Cav-1	Caveolin-1
CD36	Cluster of differentiation 36
EC	Endothelial cell
FA	Fatty acid
FATP	FA transporter protein
GPIHBP1	Glycosylphosphatidylinositol-anchored HDL binding protein 1
HDL	High density lipoprotein
LD	Lipid droplet
LDL	Low density lipoprotein
LIMP2	Lysosomal integral membrane protein 2

LpL	Lipoprotein lipase
NEFAs	Non-esterified free fatty acids
PPAR	Peroxisome proliferator-activated receptor
SR-B1	Scavenger receptor-B1
SSO	Sulfo-N-succinimidyl oleate
TGs	Triglycerides
VEGF	Vascular endothelial growth factors
VLDL	Very low density lipoprotein

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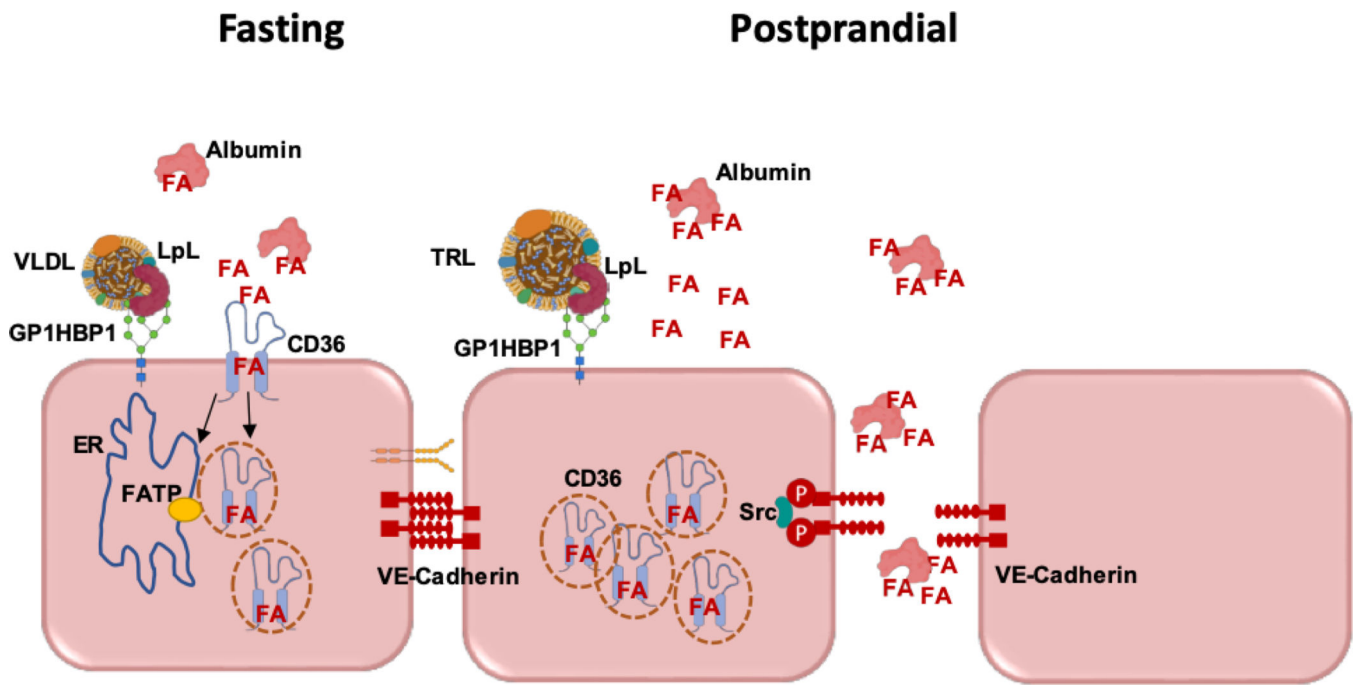

Figure 1. Pathways of FA transfer across ECs.

Left: The concentration of FAs is likely to modulate the pathway mediating their transit from the circulation to subendothelial cells. At low FA levels, as found during fasting, transfer is receptor-mediated involving cell surface CD36. FAs are internalized together with CD36, most likely in vesicles that might deliver the FA to the ER for activation by FATP3 or FATP4, for oxidation or incorporation into cell lipids. How and in what form the FA exits the basolateral EC surface to the subendothelial space is not known and is under investigation. **Right:** At higher FA concentrations such as locally occurs during lipolysis of triglyceride-rich lipoproteins (TRLs), EC CD36 will be saturated, being mostly internalized and cellular FA uptake would mainly occur via another pathway that we hypothesize involves paracellular flux between ECs. Activation of paracellular flux might occur by promoting loosening of EC junctions through phosphorylation of VE-cadherin by Src, protein kinase C or other kinases influenced by FAs. These processes are likely restricted to capillaries, as ECs of large arteries mostly do not express CD36, GPIHBP1 and do not have associated LpL.

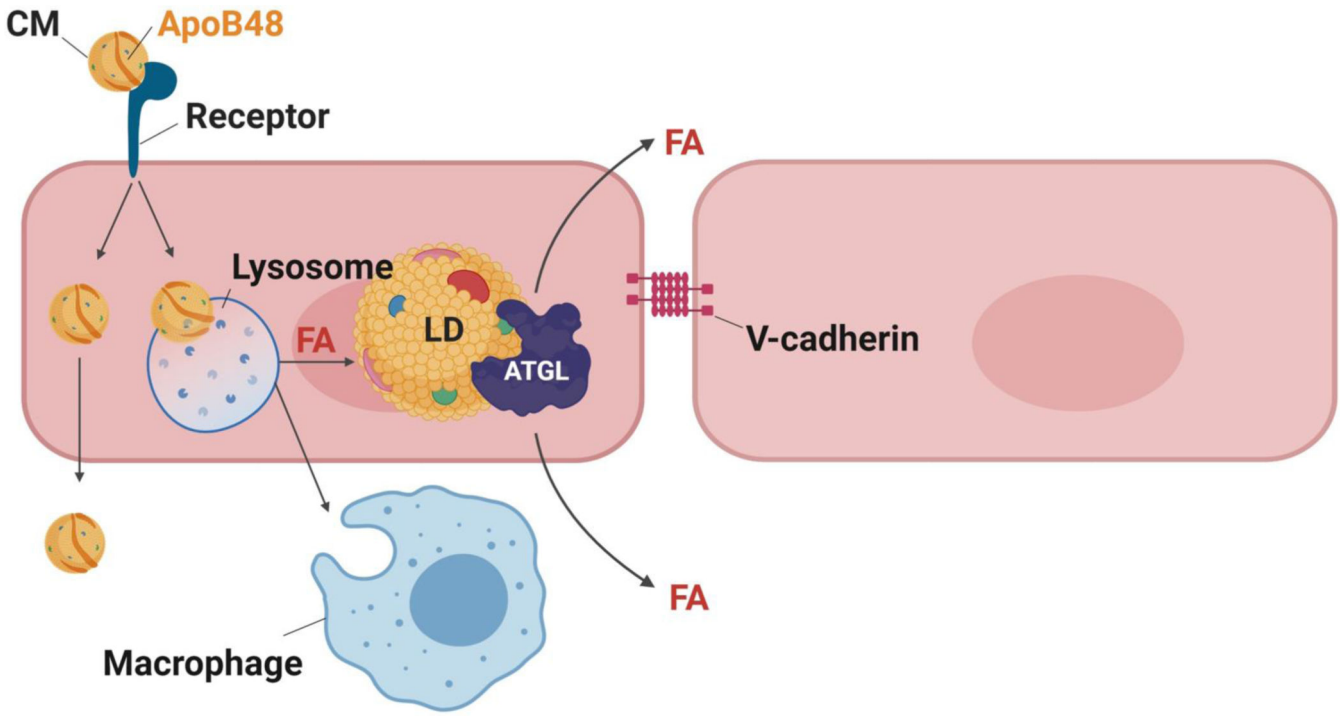

Figure 2. Non-LpL mediated uptake of CMs and VLDL by ECs.

In capillaries, most FAs from TG are liberated by LpL, but with LpL deficiency or in arteries a novel pathway for CM uptake is likely to be present that does not involve CD36. In arteries, this additional pathway leads to uptake of lipids or chylomicrons to form intracellular lipid droplets.

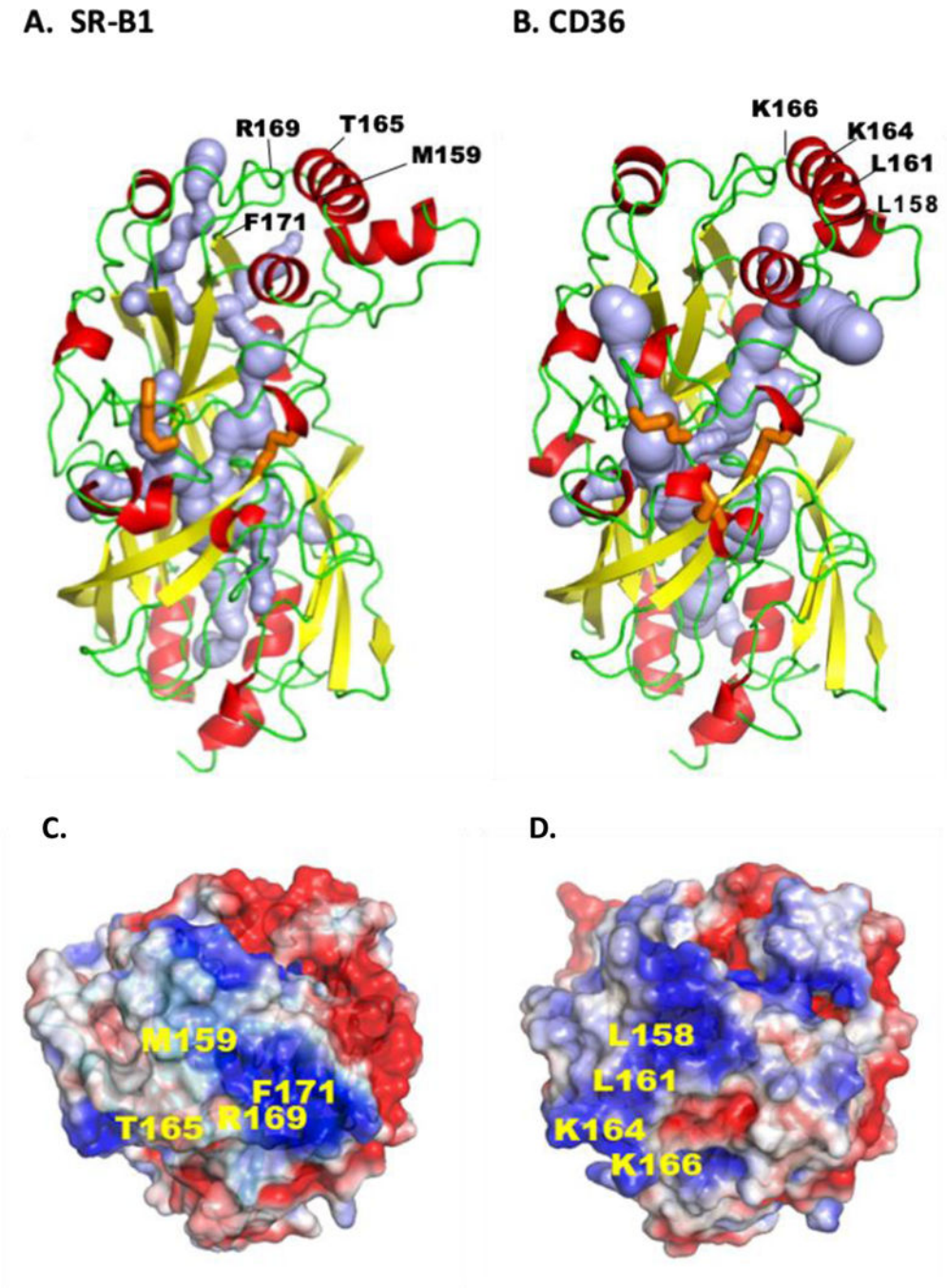

Figure 3. Tertiary structures of CD36 and SR-B1.

Both receptors are members of the CD36 family that also includes LIMP2, and both play important roles in endothelial lipid transport. The comparison is based on the crystal structures. The ectodomain of human CD36 (A) is derived from its crystallography and homology modeling of human SR-BI (B). For both proteins the α-helices are in red, the β-sheets in yellow and the loops in green. Crystal structure identified a hydrophobic internal lipid transport tunnel (light blue) that traverses the length of these proteins ending at the bilayer proximity. This tunnel can be accessed from the apex region and residues identified

to be important for ligand binding are highlighted. C and D show the apex regions' electrostatic surface potential for SR-B1 (C) and CD36 (D) and residues implicated in binding of LDL and HDL (SR-B1), and FA and oxidized-LDL (CD36) are indicated. The tunnel is surrounded by short α-helices and the disulfide bridges (orange) would stabilize structure and dimensions of the cavity for it to accommodate the lipid ligands. E shows linear sequence of the C-terminal domains for SR-B1 and CD36 highlighting the PDZ binding sequence (red) of SR-B1 and the palmitoylated (green) and ubiquitinated (orange) residues of CD36 important for signaling. The tertiary structures were rendered in PyMol[211], cavity prediction used CaverWeb 1.0[212] and the electrostatic surface potential map used APBS[212] with color gradient of −2 (red) to +2 (blue) kT/e.

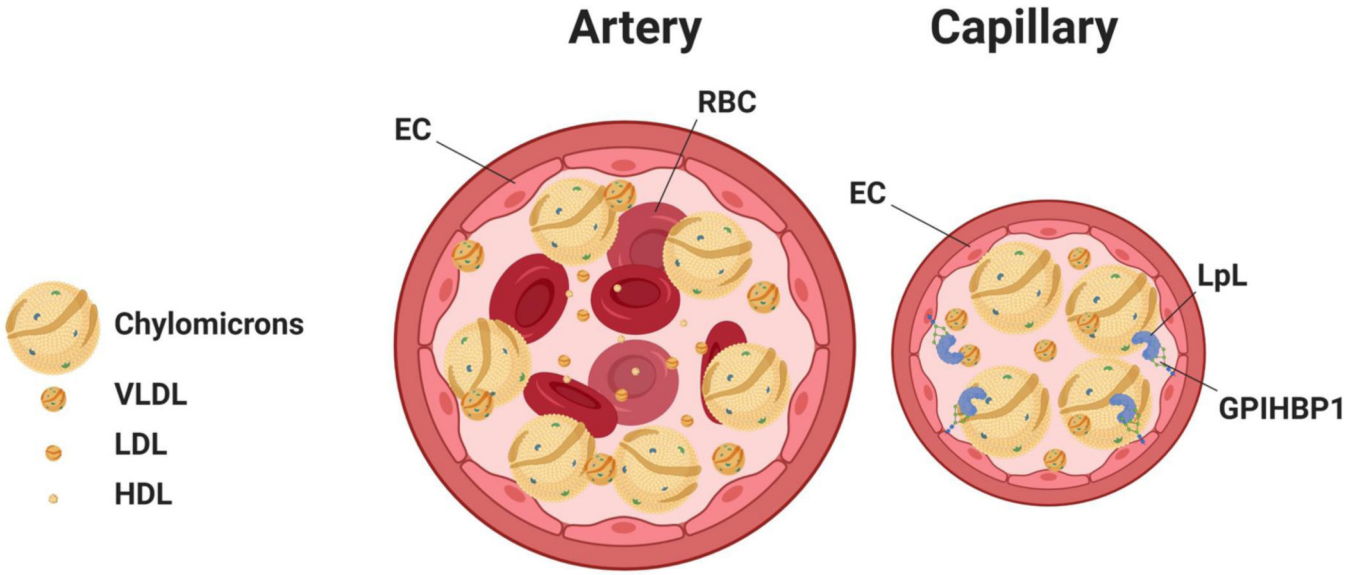

Figure 4. Lipoprotein interaction with endothelial cells differs in arteries and capillaries.

A. In an artery, the rapid blood flow likely causes margination of larger particles, leading to a gradient with more chylomicrons in proximity of the arterial wall. B. This gradient is less likely in capillaries, still chylomicrons would still interact more with lipoprotein lipase (LpL) than the smaller VLDL.

Table 1.

EC surface proteins implicated in uptake of long chain FAs	CD36, Cav-1, α5β1/CD36 complex (adipose tissue specific) Prohibitin–annexin A2-CD36 complex (adipose tissue specific),
EC surface proteins implicated in uptake of LDL, SR-B1 also mediates HDL uptake	SR-B1, ALK1,
EC FA Uptake regulators	PPARg, PGC1α, VEGF-A, VEGF-B, FATP3, FATP4, Angiopoietin-2 (adipose tissue specific), Meox2/Tcf15, 3 hydroxyisobutyrate
Proteins active in lipolysis at the EC surface	LpL, GPIHBP1
Lipolysis regulators	APOC3, APOC2, APOA5, ANGPTL3, ANGPTL4, ANGPTL8, GPIHBP1

Table 2.

Mouse model	Phenotype
FATP1 knockout *FATP*$^{-/-}$	- Reduced skeletal muscle lipid accumulation - Improved insulin sensitivity after lipid challenges
FATP1 overexpression	- Increased lipid accumulation in the heart - Cardiomyopathy
Skeletal muscle FATP4 overexpression (rats)	- Increased FA uptake/oxidation in skeletal muscle
Liver-FATP2 knockdown	- Protection from hepatosteatosis - Improved glucose levels and insulin sensitivity following high-fat diet
Knockout of PPARγ in EC (γEC/BM-KO)	- Increased circulating FAs - Protected from high fat diet induced adiposity and insulin resistance
Adipose tissue VEGF-A overexpression	- Increased vascularization - Increased thermogenesis and energy expenditure - Protection from obesity and insulin resistance
VEGF-B Knockout *Vegfb*$^{-/-}$	- Less ^{14}C FA uptake and more ^{18}F-FDG uptake by heart and brown adipose tissue (BAT), and less LDs in heart, oxidative muscle and BAT - Higher body fat mass
Diabetic mice with VEGF-B deletion (db/db *Vegfb*$^{-/-}$)	- Prevented ectopic lipid deposition - Increased muscle glucose uptake - Maintained normoglycemia
Endothelial cell CD36 knockout, EC-*Cd36*$^{-/-}$	- Postprandial hypertriglyceridemia - No cardiac accumulation of lipid droplets during fasting - Increased insulin sensitivity - Increased expression of insulin signaling and glucose metabolism genes
Gpihbp1Knockout *Gpihbp*$^{-/-}$	- Severe hyperchylomicronemia - Reduced post-heparin lipoprotein lipase activity
Adipocyte-Angpt2 knockout, *Angpt2*ΔAd)	- Markedly reduced fatty acid uptake and storage in subcutaneous adipose tissue (SAT - Ectopic lipid accumulation in glucose-consuming organs including skeletal muscle and liver - Systemic insulin resistance
ApoC3 *knockout ApoC3*$^{-/-}$	- Hypotriglyceridemia
APOC3 overexpression	- Increased TG levels
Homozygous loss-of-function a*poC2* mutant	- Hypertriglyceridemia
ApoA5 *knockout ApoA5*$^{-/-}$	- Hypertriglyceridemia
Cav-1 *knockout Cav-1*$^{-/-}$	- Lean - Resistant to high fat diet-induced obesity - High blood FA and TG levels - Impaired albumin transcytosis - Suppressed development of atherosclerosis
Endothelial cell- SR-B1 knockout SR-B1$^{\Delta EC}$	- Diminished uptake of LDL into the aorta - Reduced uptake of LDL by aortic wall macrophages - Markedly reduced atherosclerosis in ApoE$^{-/-}$ mice, and mice with genetic or PCSK9-induced LDLR deficiency
Endothelial cell SR-B1 overexpression	- Atheroprotective in high fat/high-cholesterol fed C57BL/6 mice and chow diet fed *ApoE*$^{-/-}$ mice
skeletal muscle DGAT1 Overexpression	- Increased myocyte TG - Increased insulin sensitivity
Cardiomyocyte DGAT1 overexpression (MHC-*Dgat1*)	- Increased heart TGs - Reduced lipotoxicity
Adipocyte/macrophage-DGAT1 overexpression (aP2-*Dgat1*)	- Prone to diet-induced obesity - Reduced diet-induced insulin resistance and inflammation