

## Endothelial Fatty Acid Transport: Role of Vascular Endothelial Growth Factor B

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Dietary lipids present in the circulation have to be transported through the vascular endothelium to be utilized by tissue cells, a vital mechanism that is still poorly understood. Vascular endothelial growth factor B (VEGF-B) regulates this process by controlling the expression of endothelial fatty acid transporter proteins (FATPs). Here, we summarize research on the role of the vascular endothelium in nutrient transport, with emphasis on VEGF-B signaling.

### Endothelial Fatty Acid Transport

Blood vessels deliver oxygen and nutrients to tissues and cells of the body. Although tissue oxygenation and glucose uptake have been extensively studied, much less is known about the transport of dietary lipids over the vascular endothelial barrier (80, 114). Metabolically active peripheral tissues, such as heart, skeletal muscle, and brown adipose tissue (BAT), rely on fatty acids (FAs) as their main source for energy production and therefore require an efficient supply system of FAs. Most dietary lipids consist of long-chain FAs (LCFAs; FAs with 12–20 carbons), whereas medium-chain (with 6–12 carbons) and short-chain FAs (with <6 carbons) are less frequently found in our diet and do not require active transport over biological membranes. The LCFAs are transported in the bloodstream in the form of triglyceride (TG)-rich lipoproteins (23). To generate nonesterified or free FAs, TGs are hydrolyzed from lipoproteins at the site of peripheral tissues by lipoprotein lipase (LPL), which is anchored at the luminal side of the endothelium (88). During periods of fasting, free FAs are generated through lipolysis of the white adipose tissue (WAT) and transported in the bloodstream bound to albumin. WAT and BAT are in general the only organs equipped to safely store large quantities of lipids (60, 66). A surplus of lipids within nonadipose tissues can be deleterious, since excessive accumulation of FAs causes metabolic dysfunction (99, 116). More specifically, intracellular FA accumulation can directly impair insulin signaling and glucose uptake, causing insulin resistance (11, 103, 104). Thus tight control of the uptake, storage, and utilization of LCFAs in peripheral tissues is essential for normal physiological functions.

Initially, the concept of active LCFA transport was questioned and, in contrast, postulated that the hydrophobic FAs would freely diffuse through the plasma membrane (PM) via a flip-flopping mechanism (47). However, mounting evidence

supports the view that active protein-mediated transport is the major route of LCFA entry into cells (41). First, the cellular transport of LCFAs is saturable and substrate selective, characteristics of an active transport mechanism (41). Second, treatment of cells with proteases, which hydrolyze all extracellular proteins, inhibits cellular FA uptake (41). Finally, tissues need to ensure lipid delivery when circulating FA levels are low and limit uptake when levels are high, processes that demand regulated transport mechanisms. Until today, research has focused on LCFA transport across the sarcolemma, leaving the transport across the endothelial cell layer incompletely understood (75, 95). However, if an active transport system is required for LCFA transport at the sarcolemma, a reminiscent transport system is likely to exist in the endothelium. This should, at least, be the case in tissues with a continuous endothelium that forms a tight physical barrier such as in muscle and heart. Furthermore, it would be advantageous for the organism to limit excessive LCFA uptake already at the vascular wall, allowing only the required metabolites to cross the endothelium. Recently, a mechanism for how vascular endothelial cells (ECs) regulate LCFA uptake to myocytes has been discovered (FIGURE 1) (44). Paracrine signaling by VEGF-B from tissue cells to the endothelium induced the expression of two LCFA transport proteins, FATP3 and FATP4 (see *The FATPs* below) (44). These endothelial FATPs were required and were adequate for LCFA transport across the vascular endothelial barrier. VEGF-B-mediated LCFA uptake represents a novel pathway in which tissue cells can regulate their own import of FAs already at the level of the vascular wall. By this, VEGF-B coordinates nutrient uptake with the energy production and the energy requirements of the tissue cells (44, 86).

### VEGF-B

VEGF-B is a member of the VEGFs, a family of growth factors and key mediators of angiogenesis and vasculogenesis both during embryonic

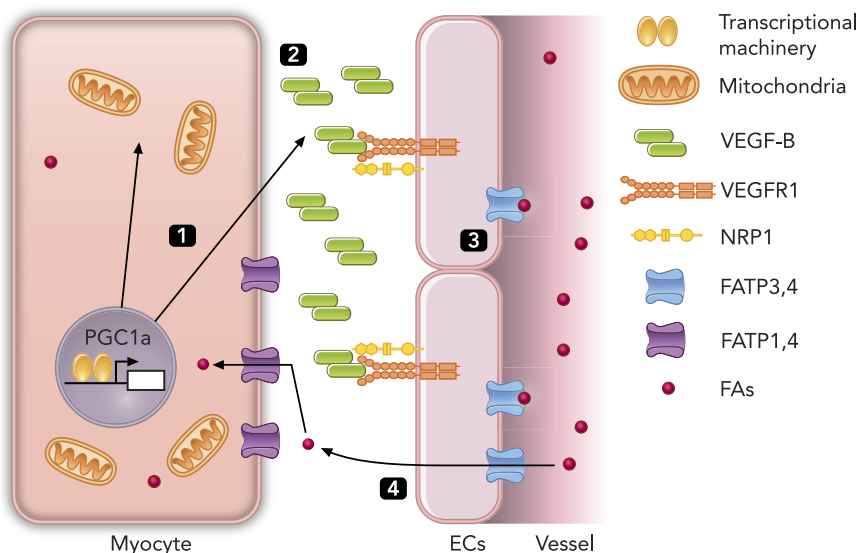
development and in adult physiology and pathology (92, 110). The family comprises five mammalian ligands, VEGF-A, -B, -C, -D, and placental growth factor (PlGF), which all occur in different splice isoforms (92). They bind in a partially overlapping pattern to three tyrosine kinase (TK) receptors, VEGF receptor-1, -2, and -3 (VEGFR1–3), that are expressed by vascular and lymph ECs and by some leukocytes (FIGURE 2) (110). The VEGF-ligands are secreted as ~40-kDa large dimeric glycoproteins and signal in a paracrine fashion to the VEGFRs (1, 13). VEGF-A is the prototypical member of the family and a strong angiogenic inducer, predominately by signaling through VEGFR2 (70). VEGF-B binds specifically to VEGFR1 and to the common co-receptor neuropilin-1 (NRP1) (78, 89). VEGF-B is secreted as two different splice isoforms, VEGF-B<sub>167</sub> and VEGF-B<sub>186</sub>, which differ in their COOH-terminal amino acid sequences and have different diffusion properties and receptor-binding abilities (78, 89–91). VEGF-B<sub>167</sub> is the prevalent isoform and binds to cell surface heparan sulphate proteoglycans (71). On the contrary, VEGF-B<sub>186</sub> is freely diffusible and requires proteolytic cleavage before binding to NRP1 (91). Both isoforms of VEGF-B induced mRNA and protein expression of FATP3 and FATP4 in ECs in vitro (44). Stimulation of cultured ECs with VEGF-B increased cellular uptake of LCFAs, which was blunted by siRNA-mediated silencing of *Fatp3* and/or *Fatp4* expression (44, 69). Furthermore, in vivo, adenoviral administration of VEGF-B<sub>186</sub> increased cardiac *Fatp3* and *Fatp4* expression (44, 69). Thus VEGF-B has an important role in the

regulation of endothelial LCFA uptake, a unique feature among the VEGFs.

*Vegfb* is expressed in most adult tissues, with the highest expression in the myocardium, skeletal muscle, and BAT (44, 71, 90). The expression of *Vegfb* is not induced by hypoxia, unlike most of the other members of the VEGF family (31, 87). Moreover, VEGF-B does not induce vascular permeability (14, 44, 72) but has been described as a survival factor for different vascular and neuronal cell types by inhibiting the expression of apoptotic genes (73, 122). Several studies have concluded the angiogenic role of VEGF-B to be restricted to the heart (62, 69, 74). However, the VEGF-B-deficient mice (*Vegfb*<sup>-/-</sup>) are healthy and fertile and do not harbor any vascular defects (2, 9). The metabolic phenotype of the *Vegfb*<sup>-/-</sup> mice is further discussed below (see section 4). Cardiac overexpression of VEGF-B induced myocardial hypertrophy, both in mice and rats, albeit through an unclear molecular mechanism (14, 56, 62, 69, 121). Increased coronary growth was also detected in cardiac-specific VEGF-B transgenic rats but not mice (14, 62). The cardiac hypertrophy in the VEGF-B transgenic mice was suggested to be dependent on *Vegfr1* expression by cardiomyocytes, although this is in contrast to previous reports (35, 56, 121). Potentially, the cardiac hypertrophy could involve paracrine signaling between ECs and cardiomyocytes by additional ligands (14, 69). Alternatively, the VEGF-B-induced hypertrophy could be due to excess delivery of nutrients to the heart, as abundant lipid accumulation has been shown to cause cardiac hypertrophy (19, 113). Mice with cardiac-specific VEGF-B overexpression accumulated ceramides in the heart, which eventually caused mitochondrial dysfunction and premature death (62). This observation correlates well with the role of VEGF-B in lipid metabolism, in particular with regard to its function in regulating LCFA transport across the vascular endothelium (44).

### Metabolic Regulation of *Vegfb* Expression

The expression of *Vegfb* is strongly co-regulated with the expression of a cluster of nuclear encoded mitochondrial genes (44). This cluster consists primarily of genes coding for proteins within the oxidative phosphorylation machinery (OXPHOS proteins), and its expression does not correlate with any other VEGF family member. Furthermore, the co-regulation of *Vegfb* with mitochondrial genes has been implicated in another study, comprising a larger dataset (supplementary Table S4 in Ref. 84). Importantly, this correlation of gene expression connects nutrient uptake by the vasculature with the mitochondrial oxidative capacity of the working cell, creating a novel type of metabolic cross talk between cells (FIGURE 1). Muscles can also



**FIGURE 1.** Schematic illustration on the role of VEGF-B in trans-endothelial FA transport

1: *Vegfb* expression is coordinated with the expression of mitochondrial proteins to co-regulate lipid uptake and  $\beta$ -oxidation. 2: VEGF-B is a secreted growth factor, which signals in a paracrine fashion to the receptors VEGFR1 and NRP1 present on endothelial cells (ECs). 3: stimulation of ECs with VEGF-B upregulates the expression of vascular FATPs and induces subsequent transport of FAs across the EC layer into tissue cells (4).

regulate LCFA uptake via LPL, which is produced by the myocytes and subsequently transported through and anchored at the endothelium (24, 88). However, VEGF-B is unique in re-programming the endothelium, where LPL is merely anchored.

Mitochondrial biogenesis and the expression of OXPHOS genes are transcriptionally controlled by the metabolic co-regulator peroxisome proliferator-activated receptor gamma co-activator 1 alpha (PGC1a) (101). PGC1a also regulates the expression of *Vegfb* and several of the LCFA transporters (12, 111). Myocyte-specific overexpression of PGC1a in mice fed a high-fat diet (HFD) thus had higher intramyocellular lipid content and were insulin resistant despite increased mitochondrial biogenesis (20). Under normal conditions, muscular PGC1a activity connects lipid supply to mitochondrial copy number in cells and thereby maintains metabolic balance (FIGURE 1). Moreover, PGC1a controls the expression of *Vegfa* and *Angiopoietin 2* (5). The metabolic regulation of this pro-angiogenic program functions to increase the vascularization in tissues that experience nutrient and/or oxygen deprivation. It underlines the tight interaction between nutrient metabolism and the vasculature, and the important role of PGC1a as a major metabolic regulator (101).

### The VEGF-B Receptors: VEGFR1 and NRP1

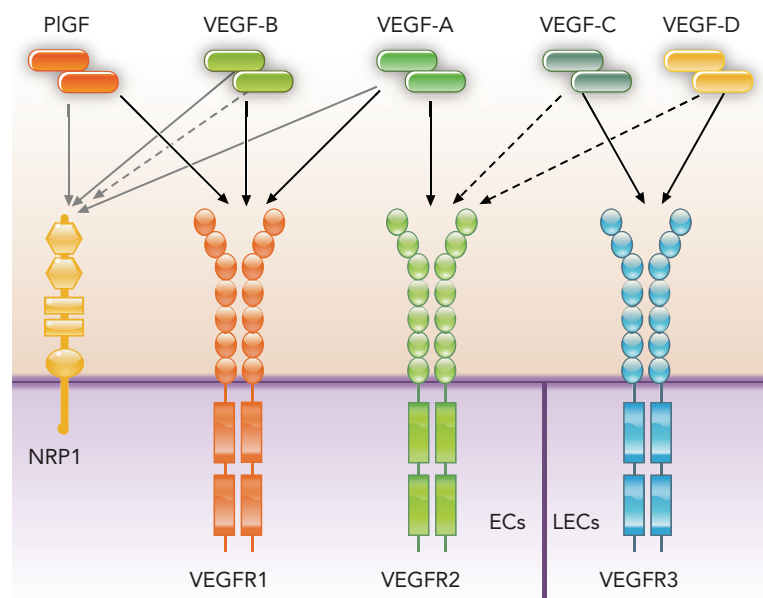
The discovery of a role for VEGF-B in lipid metabolism has opened up novel functions for VEGFR1 and NRP1 since their postdevelopmental biological roles have been partially enigmatic (34). *Vegfr1* is expressed by ECs, monocytes, and macrophages but not by tissue cells within healthy adult organs (21, 35). During development, the ligand-binding moiety of VEGFR1 seems primarily to attenuate VEGF-A/VEGFR2 signaling by trapping VEGF-A (36). *Vegfr1*<sup>-/-</sup> embryos die early during development due to VEGF-A-induced overgrowth and derangement of vessels (36). On the contrary, deletion of only the intracellular TK domain of VEGFR1 (*Vegfr1* TK<sup>-/-</sup> mice) results in healthy and fertile mice with a normal vasculature (51). Therefore, VEGFR1 was regarded to act solely as a sink, trapping VEGF-A (79). Nonetheless, *Vegfr1* TK<sup>-/-</sup> mice have reduced cardiac *Fatp3* and *Fatp4* expression, similar to the *Vegfb*<sup>-/-</sup> mice (see *Genetic Mouse Studies of VEGF-B and the LCFA Transporters* below) (44). Addition of neutralizing anti-VEGFR1 antibodies blocked the effect of VEGF-B on *Fatp3* and *Fatp4* expression in cultured ECs (44). This supports an important role for VEGFR1 in the regulation of endothelial LCFA uptake and confirms that VEGF-B signals through this receptor.

Downstream signaling by VEGF-B and subsequent FA transport also requires endothelial expression of NRP1 (44). *Nrp1* is expressed by the

arterial vasculature and by neurons and tissue cells such as cardiomyocytes and myocytes (92). *Nrp1*<sup>-/-</sup> mice die prenatally due to defective neuronal patterning and vessel formation (63). Inducible EC-specific *Nrp1*<sup>-/-</sup> mice (*Nrp1-EC*<sup>-/-</sup> mice) are healthy and fertile but display reduced cardiac *Fatp3* and *Fatp4* expression, and reduced lipid accumulation (3, 44). In contrast to the *Vegfb*<sup>-/-</sup> mice, the *Nrp1-EC*<sup>-/-</sup> phenotype could not be rescued by adenoviral VEGF-B administration, proving that endothelial expression of NRP1 is required for VEGF-B function. NRP1 can signal through its COOH-terminal tail by binding to the PDZ-domain of synectin (96). However, it is unclear how the VEGF-B/VEGFR1/NRP1 complex functions, and whether NRP1, or an additional yet undiscovered co-receptor, confers the ligand-specific signaling.

### LCFA Transport Proteins

Three groups of proteins have been implicated in LCFA transport into cells: the FATPs (FATP1–6), the scavenger receptor CD36, and the intracellular fatty acid binding proteins (FABPs) (41). Of these, CD36 is by far the most well studied protein (105). Despite recent advances in understanding LCFA transport, the route and detailed mechanisms of how FA transport through the endothelium is conferred remains unresolved (FIGURE 3) (114, 115). In addition to the earlier mentioned FATP3 and FATP4, also CD36, FABP3, FABP4, FABP5, and FABP<sub>PM</sub> are expressed by the vascular endothelium (4, 17, 30, 43, 82, 118).



**FIGURE 2. VEGF ligands and receptors**

The five dimeric VEGF ligands bind to three tyrosine kinase VEGF receptors (VEGFRs; black arrows) in a partially overlapping manner on endothelial cells (ECs) and lymphendothelial cells (LECs). VEGF-B, placental growth factor (PIGF), and VEGF-A also bind to the co-receptor NRP1 (gray arrows). Dashed lines indicate that proteolytic cleavage is needed before receptor binding.

**The FATPs**

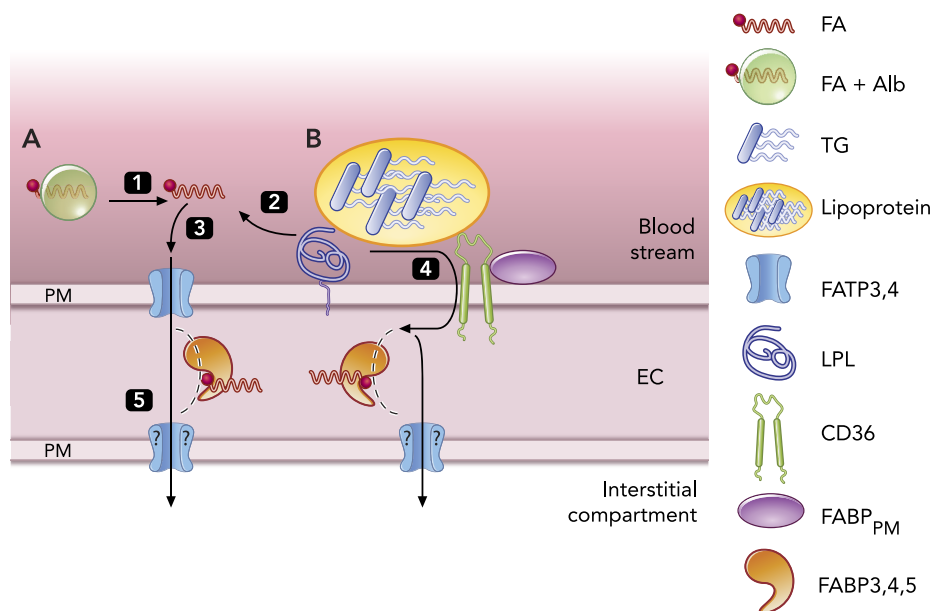
FATP3 and FATP4 were recently shown to be novel downstream targets for VEGF-B signaling in ECs (44). They belong to the FATPs (*Slc27a* family), a family of six mammalian 70- to 80-kDa-large multi-transmembrane spanning proteins, which are evolutionary conserved (28, 52, 102). The FATPs are expressed in a tissue- and/or cell type-specific manner (28). However, reports on the expression pattern of the individual FATPs have been contradictory. To clarify the matter, we designed specific primers for each FATP and analyzed the murine FATP expression pattern in heart, muscle, BAT, WAT, liver, and kidney (Hagberg H, Eriksson U, unpublished observations; Table 1). *Fatp1* is mainly expressed by tissue cells in muscle, WAT, and BAT (100). *Fatp4* has a similar expression pattern but is also expressed in the skin, in the enterocytes of the small intestine, and in the endothelium (28, 39, 44, 49). *Fatp2* is expressed in the kidney and liver, whereas *Fatp5* is exclusively detected in the liver (38, 59, 100). *Fatp6*, on the other hand, is reported to be expressed solely in the heart (40). *Fatp3* is the only FATP member that is expressed specifically in the vasculature, at least in muscular tissues (44, 93, 100).

All FATPs have been shown to enhance cellular LCFA uptake (26, 44, 83). However, there has been much debate about the localization of the FATPs and whether they are true LCFA transporters or

whether they simply drive cellular LCFA influx by intracellular acylation of FAs (58, 105). The FATPs are predominately localized to the PM, Golgi network, and the endoplasmic reticulum (25, 37, 83). In response to insulin stimulation, FATP1 and FATP4 were transported to the PM of adipocytes and myocytes (57, 108, 119). Co-expression of *Fatp3* and *Fatp4* led to the highest uptake of LCFAs in cultured ECs, suggesting synergistic effects of these two FATPs (44). Efficient LCFA uptake by FATP1 requires homodimerization, which could represent a common feature for all FATPs (97). Further studies using combinations of different FATPs could therefore be informative for detecting possible heterodimerization within the family and reveal enhanced transport activity for certain FATPs (97). Whether the FATPs are also involved in the regulation of efflux of FAs from ECs remains to be investigated.

**CD36**

CD36 [also called fatty acid translocase (FAT)] has several important biological roles as it recognizes and binds to a large variety of lipids and lipid-like molecules (6, 53, 81, 94). It potently binds LCFAs and facilitates their uptake into cells, although the detailed mechanism remains unknown and debated (8, 16). *Cd36* is expressed by platelets, monocytes, ECs, and parenchymal cells in WAT, BAT, heart muscle, and skeletal muscle, and thus has an



**FIGURE 3. FA handling proteins implicated in transendothelial lipid uptake**

FAs are transported in the blood stream bound to albumin (Alb; A) or in the form of lipoprotein-associated triglycerides (TGs; B). At the vascular wall of the target organ, the FAs dissociate from albumin (1) or are hydrolyzed by the lipoprotein lipase (LPL; 2), yielding free FAs. FATP3 and FATP4 have been shown to mediate trans-endothelial FA transport (3). CD36 facilitates FA translocation over cell membranes (4), and can also bind native lipoproteins in the blood stream. The FABP<sub>PM</sub> assists CD36 in FA handling at the plasma membrane, whereas the FABP<sub>3–5</sub> bind and manage the FAs inside ECs. 5: the efflux of FAs to the interstitial compartment could be mediated by FATP3 and FATP4 or by other transporters (5).

**Table 1. Specific primers for each FATP and analysis of the murine FATP expression pattern in heart, muscle, BAT, WAT, liver, and kidney**

FATP Member	Tissue/Cell Type-Specific Expression	Phenotype of Mice Depleted of FATPs	Refs.
FATP1 ( <i>Slc27a1</i> )	Tissue: Heart, muscle, BAT, WAT, kidney, brain Cell type: (Cardio)myocytes, brown/white adipocytes	General deletion: Protected from obesity, showed increased insulin sensitivity and reduced intramuscular FA accumulation	28, 44, 65, 119
FATP2 ( <i>Slc27a2</i> )	Tissue: Liver, kidney, BAT Cell type: Hepatocytes	General deletion: not generated Hepatocyte-specific deletion: reduced hepatosteatosis and improved liver physiology	28, 32, 44
FATP3 ( <i>Slc27a3</i> )	Tissue: Heart, muscle, BAT, WAT, lung, kidney Cell type: ECs in heart, muscle, BAT, lung	General deletion: Not generated	#, 28, 44, 93
FATP4 ( <i>Slc27a4</i> )	Tissue: Heart, muscle, BAT, liver, WAT, skin Cell type: (Cardio) myocytes, ECs, keratinocytes, brown/white adipocytes, hepatocytes	General deletion: Peri-natally lethal Inducible keratinocyte-specific deletion: disrupted epidermal barrier	#, 28, 39, 44, 48, 49, 85
FATP5 ( <i>Slc27a5</i> )	Tissue: Liver Cell type: Hepatocytes	General deletion: Defective hepatic and bile metabolism, FA-accumulation in heart and muscle	#, 27, 28, 55
FATP6 ( <i>Slc27a6</i> )	Tissue: Heart Cell type: Unknown	General deletion: Not generated	28, 40
VEGF member	Tissue/Cell Type-Specific Expression and Signalling	Phenotype of Mice Depleted of VEGF-B Signalling	Refs.
VEGF-B	Tissue: Heart, muscle, BAT, pancreas, WAT, kidney, brain Cell type: (Cardio)myocytes, brown/white adipocytes  Signalling: To ECs in heart, muscle, BAT, pancreas, WAT and kidney, as well as to neurons in brain	General VEGF-B deletion: Healthy and fertile with minor cardiac abnormalities. Lower FA-uptake and less lipid accumulation in heart and muscle. Protected from T2D. Induced deletion of endothelial NRP1 expression: Lower FATP expression and less FA-uptake in heart and muscle. General deletion of VEGFR1 TK-domain: Defective transmigration of leucocytes and lower cardiac FATP-expression.	#, 1, 2, 3, 9, 13, 44, 45, 51, 90, 122

expression pattern similar to *Fatp1* and *Fatp4* (100, 106).

To date, the biological role of CD36 in the endothelium is unresolved. *Cd36* is upregulated by the transcription factor peroxisome-proliferator activated receptor gamma (PPAR $\gamma$ ) (98, 112). Interestingly, EC-specific deletion of *Ppar $\gamma$*  in mice ablated endothelial *Cd36* expression and in many aspects phenocopied the general *Cd36*<sup>-/-</sup> mouse (see *Genetic Mouse Studies of VEGF-B and the LCFA Transporters* below), suggesting a role for CD36 in endothelial lipid transport (61). Potentially, CD36 in the endothelium could have a role in tethering TG-rich lipoproteins to the vascular wall for LPL to hydrolyze the TGs (7, 15, 29). *Cd36* was strongly induced by cold exposure in the BAT and, together with LPL, mediated increased cellular TG accumulation to sustain thermogenesis (7). On the contrary, the same study showed that *Vegfb* and *Fatp3* were downregulated in BAT in response to cold exposure, pointing to sepa-

rate roles for VEGF-B and CD36 in lipid handling and transport, at least in the BAT (7). Further research should determine whether endothelial CD36 expression can be regulated via paracrine signaling from the tissue cells and, if so, whether it is linked to the VEGF-B signaling pathway.

### The FABPs

The FABPs are a family of at least 13 soluble ~15-kDa large proteins that act as intracellular FA handling proteins (109). They are expressed in numerous tissues, with a direct positive correlation to the lipid metabolizing capacity of the tissue. To date, three FABPs have been shown to be expressed by ECs: the heart-type *Fabp3* (also known as H-FABP and Mdgi), the adipose-type *Fabp4* (also known as A-FABP and aP2), and epidermal-type *Fabp5* (also known as E-FABP and mall1) (4, 30, 82, 118). *Fabp3* is expressed by a wide range of cells, including cardiomyocytes, ECs, renal epithelial

cells, and by neurons in the brain (118). *Fabp4* is expressed in adipocytes and macrophages, as well as in cardiac and renal capillaries and veins in both humans and mice (30). VEGF-A signaling via VEGFR2 induced *Fabp4* expression in vitro, whereas the VEGFR1-specific ligand PlGF had no effect (30). This VEGF-A-mediated induction of FABP4 has been postulated to be important for EC proliferation (30). *Fabp5* is expressed in the endothelium of placenta, heart, skeletal muscle, small intestine, lung, and renal medulla, in the epidermis of lung, as well as by tissue cells of several organs such as heart, skeletal muscle, and lung (82, 107). The specific endothelial role of FABPs in FA trafficking is difficult to determine due to the overlapping expression pattern of the FABP family members (4, 30, 82, 107).

### FABP<sub>PM</sub>

In contrast to the cytosolic FABPs, the PM-associated FABP (FABP<sub>PM</sub>; also known as Got2 or mAspAt) belongs to a separate family of FA handling proteins (41). FABP<sub>PM</sub> is larger (~40 kDa) than the small cytosolic FABPs and is associated with biological membranes found both in mitochondria and on the extracellular side of cardiomyocytes, myocytes, hepatocytes, adipocytes, and ECs (4, 17, 41). FABP<sub>PM</sub> at the PM associates with CD36, where its primary role seems to be to assist CD36 in the binding and transport of FAs (41). The functional role of FABP<sub>PM</sub> in ECs remains unexplored.

### Genetic Mouse Studies of VEGF-B and the LCFA Transporters

Novel insight in the field of LCFA transporters has emerged as lipid research has switched from yeast to genetically engineered mice as model system. Most genetic knockout studies of LCFA transporters yield healthy viable mice, supporting the existence of several redundant pathways for cellular LCFA uptake (28, 41). Genetic deletion of LCFA transport proteins in one tissue typically redirects lipid uptake to other tissues. Interestingly, this is also true for the *Vegfb*<sup>-/-</sup> mice, which exhibited reduced muscular expression of *Fatp3* and *Fatp4*, and as a consequence had decreased lipid uptake to heart and skeletal muscle (44). Instead, lipids accumulated in the WAT depots of the *Vegfb*<sup>-/-</sup> mice, and thus the mice had larger fat pads and gained more weight. A metabolic switch to higher cardiac glucose uptake seemed to compensate for the reduced lipid uptake in the *Vegfb*<sup>-/-</sup> mice (44). The pattern of lipid shunting to other organs often generate complex mouse phenotypes in which the overall insulin sensitivity depends on both which tissues becomes depleted of lipids and which

tissues the lipids are re-routed to (66, 116). *Vegfb*<sup>-/-</sup> mice fed a HFD or crossed with the diabetic *db/db* mouse line (*db/db vegfb*<sup>-/-</sup> mice) maintained normal blood glucose levels and had increased insulin sensitivity (45). Moreover, the blood lipid profile and glucose sensitivity of the *db/db vegfb*<sup>-/-</sup> mice were also greatly enhanced, showing that lowering endothelial lipid uptake in muscular tissues could be a promising therapeutic approach (45).

Specific detection of the different FATPs has been difficult due to the high sequence similarities (Falkevall A, Eriksson U, unpublished observations). The individual roles of the LCFA transporters have been clarified by using cell type-specific genetic mouse models. HFD-fed *Fatp1*<sup>-/-</sup> mice displayed less intramuscular FAs, were protected from obesity, and had greater insulin sensitivity (65, 119). However, muscle-specific overexpression of FATP1 in HFD-fed mice increased FA uptake but did not cause insulin resistance (70). Thus the metabolic improvements seen with general FATP1-deletion are more likely to be connected to alterations in FA handling in the WAT and not in the skeletal muscle. *Fatp4* is ubiquitously expressed, and *Fatp4*<sup>-/-</sup> mice are embryonically/neonatally lethal (39, 49, 85). This lethality has been attributed to defects either in embryonic FA absorption or to a disrupted epidermal barrier with aberrant lipid content (39, 49, 85). Analysis of mice with inducible *Fatp4* deficiency, specifically in the skin keratinocytes, confirmed that FATP4 expression is required for normal epidermal structure (48). Furthermore, mutations in *Fatp4* have been shown to cause an inherited skin disease in humans (67). Due to the lethal phenotype, the importance of endothelial FATP4 expression in vivo remains unexplored. *Fatp2* and *Fatp5* are expressed in the liver (38, 100). Recently, it was shown that liver-specific deletion of *Fatp2* in mice reduced hepatic lipid levels (32). Similarly, *Fatp5*<sup>-/-</sup> mice displayed decreased hepatic lipid uptake and accumulation (27, 55). In the *Fatp5*<sup>-/-</sup> mice, lipids were as a consequence shunted away from liver into heart, muscle, and WAT, and the resulting ectopic lipid accumulation led to hyperglycaemia and hyperinsulinemia (27). FATP3 and FATP6 have been less well characterized, and the respective gene-deficient mice have not been generated yet.

*Cd36*<sup>-/-</sup> mice are viable and fertile but have reduced FA uptake to muscles and WAT, which was initially thought to be beneficial (22, 33). However, in a subsequent study, *Cd36*<sup>-/-</sup> mice fed a high fructose diet developed severe insulin resistance (46). Most likely, global *Cd36* deficiency results in decreased uptake of nutrients into WAT, leading to pathological lipid accumulation in non-CD36-dependent organs such as the liver (42). This is further

supported since several *Cd36*-deficient patients with symptoms ranging from Type 2 diabetes (T2D) to cardiomyopathy have been identified (50, 117).

Mouse models lacking specific FABPs have been generated (10, 54, 77). *Fabp3*<sup>-/-</sup> mice exhibited a similar phenotype as the *Vegfb*<sup>-/-</sup> mice, with lower muscular lipid accumulation and a compensatory increase in glucose utilization, resulting in reduced blood glucose levels (10). However, in contrast to the *Vegfb*<sup>-/-</sup> mice, *Fabp3*<sup>-/-</sup> mice showed acute exercise intolerance due to their lower lipid usage (10, 74). *Fabp4*<sup>-/-</sup> and *Fabp5*<sup>-/-</sup> mice fed a HFD showed slightly enhanced insulin sensitivity (54, 77). *Fabp5* expression was increased in the WAT of the *Fabp4*<sup>-/-</sup> mice, suggesting compensatory expressions of the two FABPs (54). In line with this, HFD-fed mice deficient of both *Fabp4* and *Fabp5* were insulin sensitive and protected against the development of liver steatosis and T2D (76). The improved diabetic phenotypes of the *Fabp3*<sup>-/-</sup>, *Fabp4*<sup>-/-</sup>, and *Fabp5*<sup>-/-</sup>-deficient mice could be due to decreased endothelial FA transport and thus lowered tissue lipotoxicity. Hence, the functional importance of FABPs expressed in ECs vs. tissue cells remains to be pinpointed.

In conclusion, genetic studies of the LCFA transporters have shown that altered tissue lipid distribution has major impacts on systemic metabolism. Therefore, it is vital to examine all major metabolic organs, including the endothelium, before drawing conclusions of the suitability to activate or inhibit the function of a particular LCFA transporter.

## Future Perspectives

Pathological tissue lipid accumulation has been shown to impair insulin signaling and cause insulin resistance, which ultimately can develop into T2D (99). Several other metabolic and cardiovascular diseases such as the metabolic syndrome, non-alcoholic fatty liver disease, atherosclerosis, and myocardial infarction are characterized by pathological lipid deposition within organs (99). Tight control of lipid uptake and storage in non-adipose tissues is therefore of vital importance.

Most metabolic diseases are still best treated with lifestyle changes, such as diet restriction and increased exercise. To date, pharmacological intervention of T2D has focused on reducing hyperglycaemia and not on targeting the mechanisms underlying insulin resistance. However, insulin resistance is the core pathogenic defect of T2D, preceding pancreatic deterioration, and is present in over 90% of all patients. An exception to the “glucocentric” drugs is the thiazolidinediones, since they act by increasing lipid disposal in the WAT and thereby ameliorating peripheral lipid deposition and insulin resistance (120). Several of the thiazolidinedio-

nes have now been revoked from the market due to severe side effects. Nevertheless, the use of thiazolidinediones provides proof of concept that lowering peripheral lipid deposition can ameliorate insulin resistance and hence prevent the progression of T2D.

The endothelium represents a novel control point for regulating LCFA uptake, thus limiting excessive tissue lipid accumulation and consequently insulin resistance. A recent study showed the importance of the endothelium in the insulin-signaling cascade and its ability to thereby control muscular glucose uptake (68). Similarly, it was proven that ECs themselves can develop insulin resistance, which was shown to precede the insulin resistance in myocytes (64). Recently, we showed that administration of neutralizing anti-VEGF-B antibodies to diabetic *db/db* mice reduced muscular lipid accumulation and increased glucose tolerance and insulin sensitivity (45). Thus it will be interesting to explore, also in patients, whether inhibiting VEGF-B, and thereby limiting trans-endothelial transport of dietary lipids, will have similar protective effects on organ steatosis, atherosclerosis, insulin resistance, and T2D. Hence, VEGF-B signaling in the endothelium represents a crucial regulatory site for FA uptake and is a promising novel target for the treatment of metabolic and cardiovascular diseases. ■

U. Eriksson is a consultant of CSL Melbourne Australia. CSL has certain commercial interests in VEGF-B and is role in regulating endothelial transcytosis of fatty acids.

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