Trans-endothelial trafficking of metabolic substrates and its importance in cardio-metabolic disease

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Word count: 3739

Abstract

The endothelium acts as a gatekeeper, controlling the movement of biomolecules between the circulation and underlying tissues. Although conditions of metabolic stress are traditionally considered as causes of endothelial dysfunction, a principal driver of cardiovascular disease, accumulating evidence suggests that endothelial cells are also active players in maintaining local metabolic homeostasis, in part, through regulating the supply of metabolic substrates, including lipids and glucose, to energy-demanding organs. Therefore, endothelial dysfunction, in terms of altered trans-endothelial trafficking of these substrates, may in fact be an early contributor towards the establishment of metabolic dysfunction and subsequent cardiovascular disease. Understanding the molecular mechanisms that underpin substrate trafficking through the endothelium represents an important area within the vascular and metabolism fields that may offer an opportunity for identifying novel therapeutic targets. This mini-review summarises the emerging mechanisms regulating the trafficking of lipids and glucose through the endothelial barrier and how this may impact on the development of cardio-metabolic disease.

Key words: Endothelium, metabolism, trafficking, transcytosis, fatty acids, glucose, metabolic dysfunction, diabetes, cardiovascular disease

Abbreviations: CD36, cluster of differentiation 36; DOCK4, dedicator of cytokinesis 4; ERK, extracellular signal-regulated kinase; FOXO1, forkhead box O1; GSK3β, glycogen synthase kinase 3 beta; Meox2, mesenchyme Homeobox 2; PGC1α, peroxisome proliferator activated receptor gamma co-activator 1 alpha; PPAR, peroxisome proliferator activated receptor; Rac-1, ras-related C3 botulinum toxin substrate 1; Rbp-jk, recombination signal binding protein for immunoglobulin kappa J region; Tcf15, transcription factor 15; TGFβ, transforming growth factor beta.

Introduction

The endothelium regulates multiple processes including inflammation and vascular tone, while also acting as a gatekeeper, controlling the movement of nutrients, metabolites, hormones and other bioactive molecules between the circulation and underlying tissues (1, 2). Conditions of metabolic stress, including obesity, dyslipidaemia and type 1 (T1) and 2 (T2) diabetes mellitus (DM), are traditionally considered as instigators of endothelial dysfunction, a principal driver of cardiovascular disease (CVD) (3, 4). However, evidence now suggests that the endothelium may in fact be an active player in maintaining systemic and tissue metabolic homeostasis, in part, through regulating the supply of metabolic substrates including lipids and

glucose (3, 4). Alterations in the trans-endothelial delivery of substrates may, therefore, be an early contributor towards the establishment of metabolic dysfunction.

The molecular mechanisms governing trans-endothelial transport of metabolic substrates are not well-established and unravelling these represents an important area within the vascular and metabolism fields. The incidence of metabolic disease, such as T2DM, is projected to increase over the next decade and is a major risk-factor for development of CVD (5-7). Going forward, as current treatment options remain sub-optimal, the ability to adapt substate supply to target organs may have significant implications for how we approach and develop novel therapeutics to protect such patients.

This mini-review summarises the emerging mechanisms regulating the trans-endothelial delivery of lipids and glucose, the principal energy substrates for most tissues, while highlighting the gaps that still need to be addressed. For the purpose of this mini-review a focus is placed on the peripheral blood vasculature. Further information on this, alongside trans-endothelial transport at the blood-brain-barrier (BBB) and lymphatic system, can also be found elsewhere (8, 9).

Trans-endothelial transport of fatty acids

Although passive transfer was thought to be the likely means of fatty acids (FA) traversing cell membranes, it is now clear that a regulated transfer is also at play (10). This is particularly important for transporting FAs across non-fenestrated continuous endothelium of metabolically active tissues. For example, a recent study on the beating human heart indicates that the oxidation of FAs accounts for approximately 85% of energy production (11).

FAs can principally be found in the circulation bound to albumin (free fatty acids (FFAs)) or esterified within triglyceride-rich lipoproteins (TRLs) (10). These TRLs can be transported intact or undergo hydrolysis, with the resulting long-chain FAs (LC-FA) trafficked across the endothelium (10, 12) (Figure 1). Alterations in trafficking TRLs and LC-FAs across the endothelial barrier may have significant implications in the development of CVD, a concept particularly pertinent to diabetic patients who are prone to accelerated development of such complications (13-15).

Transcytosis of TRLs

Transcytosis of intact TRLs occurs via caveolae, invaginations of the apical membrane that detach as vesicles and shuttle cargo to the basolateral side and plays an important role in the development of atherosclerosis (12, 16). Indeed, silencing of caveolin-1 (CAV-1) significantly reduces uptake and transcytosis of low-density lipoprotein (LDL) across the endothelium and

reduces atherogenesis (17, 18). Furthermore, a number of receptors capable of interacting with TRLs are localised within caveolae, however, not all of these receptors are involved in transcytosis and heterogeneity between vascular beds also exists. For example, although the LDL receptor may be involved in the internalisation and trafficking of LDL across the BBB (19), within the peripheral vasculature, it facilitates the uptake and degradation of native and oxidised LDL but appears not to be involved in TRL transcytosis (20, 21). Similarly, CD36 (also known as scavenger receptor B2 (SR-B2)) is involved in LDL internalisation and has been suggested as a possible target to prevent transcytosis within arteries to limit atherogenesis (17). However, it has been argued that LDL transcytosis through arterial endothelial cells (ECs) is not mediated through CD36/SR-B2, but instead, is via the scavenger receptor B1 (SR-B1), a receptor usually associated with the binding and trafficking of HDL (20-22). Although the precise intracellular mechanisms of TRL transcytosis within ECs is not known, it was found that SR-B1 interacts with DOCK4, a guanine nucleotide exchange factor for the small GTPase, Rac1. Furthermore, mice lacking endothelial SR-B1 had reduced atherogenesis compared with controls, with reduced accumulation of LDL and VLDL within the vessel wall (20). It must be noted that other studies report an absence of an effect of SR-B1 on LDL transcytosis, with a role only seen for HDL (22). Whichever route is used, the mechanism by which TRL-containing vesicles avoid trafficking to lysosomes and instead traffic to the basolateral membrane of ECs remains unclear. Similarly, the molecular mechanisms of TRL exocytosis at the basolateral membrane also remain unknown (12, 23). However, as indicated in the transcytosis of other cargoes, such as albumin, likely involves the general endocytic pathways that utilise exocyst and SNARE complexes to facilitate docking and fusion of vesicles to the plasma membrane (23, 24).

Although dyslipidaemia is a feature of diabetes and has long been linked to increased risk of developing atherosclerosis (14), an additional compounding factor could be increased LDL transcytosis in response to hyperglycaemia. Indeed, under hyperglycaemic conditions, LDL transcytosis appears to be enhanced as a consequence of reduced LC3B-mediated autophagic degradation of CAV-1 (25). Improved understanding of the molecular consequences of the diabetic milieu on TRL transcytosis may prove useful in developing new and effective treatment options for protecting such patients.

Trafficking of LC-FAs

As mentioned above, TRLs are also hydrolysed at the cell surface or following their uptake, and this is particularly important at the level of the capillary within the heart, where alterations in LC-FA trafficking across the endothelial barrier may have significant implications in the development of diabetes-associated heart failure.

To process TRLs, capillary ECs express a lipoprotein lipase (LPL) on the apical surface. Interestingly, endogenous expression of LPL is quite low and is instead synthesised by parenchymal cells (26). Within the heart, cardiomyocytes are the main source of LPL and following its release from the cell surface, LPL binds in a 1:1 ratio to the glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1) located on the basolateral side of the endothelium (26-28). Once bound, the GPIHBP1-LPL complex is transported through the EC and presented at the apical surface (29). This process is bi-directional so GPIHBP1 can shuttle back and forth collecting more LPL as needed. Once at the apical surface, the GPIHBP1-LPL complex facilitates the capture and hydrolysis of circulating TRLs (30, 31), upon which, the released LC-FAs are taken up and rapidly made available for use by the underlying tissue (31, 32). The importance of GPIHBP1-LPL can be seen in animal models in which GPIHBP1 has been silenced. In these animals, margination of TRLs along the capillaries is significantly reduced and LPL accumulates within the interstitial space, resulting in hypertriglyceridaemia and reduced FA delivery to parenchymal cells (30, 31, 33). On the other hand, an increase in GPIHBP1 expression has been demonstrated in cardiac ECs under diabetic conditions (28). This was associated with an elevation in apical LPL, thereby having the potential to increase FA supply to cardiomyocytes.

While short-and medium-chain FAs do not require facilitated transport, LC-FA internalisation and transport can occur through a combination of receptors and fatty acid transport (FATP) and binding (FABP) proteins (9, 10). However, the precise role of these proteins in governing FA utilisation by, and trafficking across, the endothelium remains unclear.

Endothelial CD36/SR-B2 appears to play a part in the uptake and trafficking of LC-FAs across the capillary endothelium where its expression is greatest (34). Crystallisation studies revealed that LC-FAs bind within hydrophobic sites within a tunnel cavity of the CD36/SR-B2 receptor that spans most of the protein's length (35). The importance of CD36/SR-B2 in trans-endothelial transport of LC-FAs is seen in mice where its endothelial expression has been silenced (34). In these mice, LC-FA transfer to the heart and skeletal muscle is severely compromised and lipid droplets are significantly reduced. This is associated with greater consumption of glucose and an elevation in plasma FFAs (34), a metabolic phenotype similar to that observed in humans with CD36/SR-B2 deficiency (36, 37). However, this impairment is not universally observed (31), suggesting additional factors are in play.

As with GPIHBP1, expression of CD36/SR-B2 is increased in microvascular ECs under hyperglycaemic conditions (38) and endothelial CD36/SR-B2-deficient mice on a high-fat diet (HFD) show improved insulin-sensitivity, better glucose handling and reduced accumulation

of toxic lipid species within the myocardium (34), suggesting that inhibition of endothelial CD36/SR-B2 may have cardiac-protective effects.

Once captured by the endothelium, the mechanism by which LC-FAs are processed and trafficked to their subsequent destination remains unresolved. ECs are known to express FATP3 and FATP4, alongside FABP3, FABP4 and FABP5 (4). It has recently been shown that FATP4, located within the membrane of the endoplasmic reticulum, utilises a localised supply of mitochondrial-derived ATP to fuel its acyl-CoA synthetase activity, thereby 'activating' incoming FFAs (39). It was suggested that this reduces the concentration of intracellular FFAs allowing for further FFA uptake. When mitochondrial ATP supply, but not glycolysis (the principal source of total cellular ATP in ECs (40)), was disrupted, or when FATP4 was silenced, FFA uptake and transcellular transport was significantly diminished (39). What remains unclear is what determines the fate of the FA as to whether it is utilised internally or passed through to the underlying tissue. Whether a proportion is trafficked via the compartment of lipid droplets prior to its release into the interstitial space is also not known, although this has been shown to be a possibility under acute lipid load (41), again suggesting some form of regulation at the level of the endothelium.

Whatever the final trafficking route, trans-cellular transport of FAs across the endothelial barrier is likely to require a FABP as a chaperone, with FABP4 and FABP5 being expressed in cardiac and skeletal muscle capillaries. When Iso et al. (2013) generated FABP4 and 5 double-knockout mice, a reduction in cardiac FA uptake was observed with a compensatory increase in glucose and ketone body consumption; a response in line with a potential consequence of disrupted endothelial FA transcytosis (42). The precise role played by endothelial FABPs in enhancing FA transcytosis in the diabetic vasculature is not known. However, circulating levels of FABP4 are inversely associated with endothelial function in patients with T2DM (43) and multiple signalling molecules known to be active in the diabetic state promote FABP4 expression (see below). Furthermore, administration of a small molecule inhibitor of FABP4 has beneficial effects on improving insulin sensitivity and glucose handling in insulin-resistant obese mice (44). Generation of endothelial-specific FABP knockout mice will help to clarify this further and provide an in vivo model for advancing mechanistic understanding.

Signals regulating LC-FA transfer

Alteration in trans-endothelial transport within energy-demanding tissues is often in response to signalling from parenchymal cells to the capillary endothelium, thereby linking substrate supply to changes in local metabolic need (45-48). Recently, a number of factors have been identified as stimulators of FA trafficking (Figure 1).

An early mediator shown to be involved is VEGF-B. Following its release from parenchymal cells, VEGF-B was demonstrated to act through VEGFR1 to up-regulate FATP3 and FATP4 within the endothelium, leading to increased uptake and trans-endothelial transport of LC-FAs (45). In *vegfb*$^{-/-}$ knockout mice, such localised signalling is absent and uptake of LC-FAs across the endothelium and into peripheral tissues, including the heart and skeletal muscle, is impaired, with compensation by increased glucose oxidation (45). This prompted the targeting of VEGF-B for the treatment of obesity-related T2DM. Genetic or pharmacological inhibition of VEGF-B was shown to improve ectopic lipid deposition in peripheral tissues, increase glucose uptake, and improve glucose tolerance and insulin sensitivity in *db*/*db* mice and HFD-fed rats (46, 49). However, these promising results have not been replicated by others, with VEGF-B signalling also being demonstrated to exert positive effects on adipose tissue vascularisation and systemic metabolism, as well as cardiac remodelling and protection from myocardial damage following infarction or ischaemia-reperfusion (50-52). Furthermore, in response to acute hyperglycaemia, VEGF-B signalling has been reported to increase in order to attenuate cell death pathways via activation of the ERK/GSK3β axis (53). In chronic diabetic conditions, this protective signalling is lost as a consequence of reduced VEGF-B and possible emergence of 'VEGF-B resistance' (53), indicating that intact VEGF-B signalling may be required for cardiac-protection in the chronic diabetic state.

3-hydroxyisobutyrate (3-HIB), an intermediary metabolite of valine catabolism, has also been shown to exert a similar function. In this case, increased PGC1α-driven valine catabolism within skeletal muscle leads to an accumulation and subsequent release of 3-HIB which acts on ECs to stimulate FA uptake and transcytosis (47, 54). This was dependent on the activities of FATPs 3 and 4 and elevating circulating 3-HIB concentration, through oral consumption, can cause increased FA uptake, development of glucose intolerance and insulin resistance (47). Moreover, plasma 3-HIB levels are associated with incident T2DM in humans (54), suggesting that interference of 3-HIB metabolism may be of therapeutic benefit.

More recently, a subcutaneous adipose tissue-specific regulation has been identified. Angiopoeitin-2 (ANGPT2) is highly expressed by adipocytes and can bind to the α5β1 integrin on the endothelium. Such binding leads to an increase in CD36/SR-B2 activity, together with FATP3, to stimulate an increase in LC-FA uptake into subcutaneous adipose tissue (48). Disrupting adipose tissue ANGPT2 expression leads to ectopic lipid distribution in other organs, together with glucose intolerance and insulin resistance (48), suggesting a positive action of ANGPT2 on metabolic homeostasis. However, ANGPT2's association with the progression of vascular complications in diabetes (55, 56) makes therapeutically targeting this protein complicated.

In the opposite direction, signalling mediated through apelin binding to its receptor (APLNR) can reduce LC-FA transport (57). The APLNR can be found on the endothelium of adult metabolically active tissues where upon binding of apelin, signals to inactivate the FOXO1 transcriptional regulator leading to a reduction in FABP4 expression, and ultimately, a decrease in trans-endothelial LC-FA transport (57). Such action may complement its diverse action throughout the body and targeting the apelin/APLNR pathway continues to be investigated for improving cardiovascular and metabolic outcomes in animal models of diabetes (58, 59). The current status of apelin/APLNR signalling as a druggable target in cardio-metabolic diseases has recently been reviewed (60).

Within the EC itself, a number of transcriptional regulators, alongside FOXO1 mentioned above, can influence the metabolic phenotype and, therefore, may also have the potential to affect the trans-endothelial transport of FAs.

The PPAR nuclear receptors are lipid sensors that respond by regulating metabolic genes, particularly in relation to FA metabolism (61), and activation of PPARγ in diabetes has long been established with the use of thiazolidinediones. However, the use of some (such as rosiglitazone), but not all, have been associated with increased risk of adverse cardiovascular events (62-64). PPARγ activation leads to increased expression of GPIHBP1, FABP4 and CD36/SR-B2 within the endothelium which may promote trans-endothelial FA trafficking into organs such as the heart (65). It is tempting to speculate that this may be a contributing factor to the potential development of adverse cardiovascular effects. In contrast, silencing PPARγ expression within the endothelium causes a significant reduction in LC-FA trafficking (65-67). Whether other PPAR isoforms also play a role has not been investigated. PPARβ/δ can regulate EC metabolism in a context-dependent manner (68) and its endothelial-selective overexpression causes cardiac dysfunction (69). If this is in part related to excessive LC-FA delivery to cardiomyocytes is worth investigating.

More recently, both Notch signalling and the Meox2/Tcf15 transcriptional regulator have been shown to regulate expression of a number of genes involved in lipid metabolism within ECs (70, 71). Meox2/Tcf15 regulates the expression of GPIHBP1 and CD36/SR-B2, and mice haplodeficient for Meox2/Tcf15 present with reduced FA uptake into the heart leading to the development of systolic dysfunction and fibrosis in older mice (71). In vitro investigation in isolated ECs revealed reduced processing of TRLs and decreased trans-endothelial transfer of FAs. Under hyperglycaemic conditions, elevated TGF-β signalling stimulates endothelial Meox2 nuclear translocation and thereby enhances target gene expression, including GPIHBP1, which may contribute to the elevated delivery of FAs to peripheral tissues (28).

Similarly, diminished endothelial Notch signalling, through silencing of the Notch transducer, Rbp-jk, also limits trans-endothelial transfer of LC-FAs through reducing the expression of CD36/SR-B2 and FABP4, while allowing for increased angiopoietin-like 4, a well-characterised inhibitor of LPL (70). In this case, mice developed heart failure which was preceded by a metabolic shift away from FAs and towards glucose. The impact of diabetes on Notch signalling-induced trans-endothelial transport of FAs is not known but is worthy of investigation.

Trans-endothelial transport of glucose

Endothelial heterogeneity is also influential in the transport of glucose. The highly developed tight-junctional complexes of the BBB make it a significant barrier to paracellular diffusion and instead requires the transport of glucose through the endothelium via facilitated glucose transporters (GLUT) (8, 72-74). The importance of such trafficking can be seen in patients with GLUT1 deficiency syndrome who present with increased propensity for seizures as a consequence of a reduction in brain glucose availability (75). In contrast, the endothelium has not traditionally been viewed as a major regulator of peripheral glucose availability, with paracellular diffusion considered the main delivery route (8). However, recent studies suggest that glucose transcytosis may also be a significant mechanism within the heart (76, 77) (Figure 2), thereby questioning this long-held assumption.

A number of the GLUT transporters have been found to be expressed in various EC types but the GLUT1 isoform is by far the most abundant (78-80). With a Km of 1-2 mM, GLUT1 has a relatively high affinity for glucose (81) and in contact-inhibited EC monolayers, Notch signalling drives GLUT1 expression while simultaneously reducing PFKFB3 expression and glycolysis, suggesting that GLUT1 may become uncoupled from glycolysis in mature vessels (72). Analysis of GLUT1 in intact vessels show a possible asymmetric distribution, with greater levels in the basolateral versus the apical membrane (78, 79, 82). Such asymmetrical distribution is suggested to facilitate the efflux of glucose from the cell into the interstitial space (78, 79), although strong experimental evidence is still lacking. Even so, alterations in GLUT1 expression and/or its membrane localisation and stability would be thought to have rate-limiting effects on glucose transcytosis.

Hypoxia-inducible factor 1-alpha (HIF1α) is a strong inducer of GLUT1 expression and mice with endothelial-specific deletion of HIF1α (EC-*HIF1α*$^{-/-}$) provided early indication of a possible role in peripheral glucose transcytosis (76). These mice had disturbed systemic glucose homeostasis but normal insulin levels, and glucose uptake into the brain and heart were reduced by over 30%. While the molecular mechanism is still incomplete, EC-*HIF1α*$^{-/-}$ mice had a decreased level of GLUT1 expression, indicating a possible disruption in endothelial

glucose transport within the vascular network of the energy-demanding heart in addition to the BBB (76).

A recent study also suggests a novel mechanism of regulation through the action of VEGF-B. It was found that VEGF-B signalling reduced recycling and cell surface expression of the LDL receptor, leading to reduced uptake and membrane loading of non-esterified cholesterol (77). In turn, this impacted membrane GLUT1 activity and decreased glucose uptake. Importantly, VEGF-B signalling had no effect on the ECs own substrate utilisation but instead reduced glucose transcytosis (77). Furthermore, this effect appeared to be independent of the action of VEGF-B signalling on increasing FA uptake reported previously by the same group (45, 46, 77). This response may contribute to the beneficial effects reported for VEGF-B inhibition in dyslipidaemia or diabetes. Indeed, reduced levels of non-esterified cholesterol was a characteristic observed in the hearts of HFD-fed and *db*/*db* mice, while inhibition of VEGF-B by neutralising antibodies or *vegf-b* knock-down increased cardiac glucose consumption and restored non-esterified cholesterol content (77). As discussed above, it is important to remember that such beneficial metabolic effects have not always been seen with VEGF-B inhibition (50-52) and so more research is needed.

While glucose uptake by ECs is not insulin-dependent, the trans-endothelial transport of insulin alongside glucose must occur to allow for glucose consumption by the underlying tissue. A number of transport mechanisms have been proposed and are disrupted in the obese insulin-resistant state, including fluid-phase, clathrin vesicle and caveolae-mediated transport (83-86) (Figure 2). The molecular mechanisms are discussed in greater detail elsewhere (8, 9), however, a role for endothelial Notch signalling in regulating caveolae-mediated transcytosis of insulin has recently been described (83). Sustained Notch signalling was associated with reduced insulin transcytosis as a consequence of reduced caveolae-related gene expression. Importantly, mice with HFD-induced insulin resistance displayed elevated endothelial Notch activity, that when inhibited, led to a restoration in caveolae-mediated insulin transport and improved glucose tolerance (83).

Although at an early stage, as the intracellular mechanisms regulating glucose transport across the endothelium are revealed and physiological relevance demonstrated, this will provide novel therapeutic targets that could help with regulating glucose availability to target organs to prevent or slow the development of metabolic disease and its complications.

Outstanding questions

In addition to those mentioned above, a number of outstanding questions remain to be answered. To what extent, and how quickly, do ECs adapt transcytosis of substrates in

response to alterations in availability, and is there a reciprocal regulation between transcytosis of FAs and glucose? From a therapeutic perspective, understanding to what extent changes in trans-endothelial transport contributes to the metabolic inflexibility of target organs often observed in diabetes will inform the potential impact of any intervention. Finally, defining the role of other vascular cells in regulating endothelial transcytosis of substrates will also be important. For example, pericytes are in close contact with the endothelium and influence multiple aspects of EC behaviour, including barrier function (87). At the BBB, pericytes were found to regulate EC gene expression and the restriction of vesicular transcytosis (88, 89). Despite the variation between tissues in terms of pericyte/EC ratio (90), it is likely that a similar function exists within the peripheral vasculature. Moreover, a disruption in pericyte-EC communication/interaction is a feature of diabetic vasculopathy that could be targeted for therapeutic benefit (90, 91).

Perspective

- Alterations in metabolic substrate delivery to energy-demanding tissues, including the heart, has implications for the establishment of metabolic dysfunction and development/progression of cardiovascular complications.
- Recent work demonstrates that delivery of metabolic substrates to target organs is regulated, at least in part, at the endothelium. While evidence is accumulating for regulated transcytosis of FAs, its role in regulating glucose availability is less clear.
- As the molecular mechanisms governing trans-endothelial delivery of metabolic substrates to peripheral tissues continue to be identified, it will be important to ascertain their role in metabolic diseases, as they may go on to underpin our future therapeutic approach for managing tissue substrate delivery in multiple patient groups.

Figure 1: Trans-endothelial transport of fatty acids. TRLs can be taken up and transported across the endothelial barrier through receptor-mediated transcytosis and is likely to be important in the development of atherosclerosis within the macrovasculature. Fatty acids are taken up from the apical side and transported to the basolateral side for use by underlying tissue. This likely involves the interaction of fatty acids with FATP and FABPs that are involved in the capture and trafficking of fatty acids. The molecular mechanisms governing the intracellular trafficking of fatty acids are not well-defined. Many proteins implicated in fatty acid transcytosis are regulated through transcriptional and non-transcriptional mechanisms in response to multiple exogenous signals derived from parenchymal cells, thereby allowing for substrate delivery to be matched to local need. Abbreviations: ANGPT2, angiopoietin 2; ANGPTL4, angiopoietin-like 4; APLNR, apelin receptor; ATP, adenosine triphosphate; CAV-

1, caveolin 1; CD36/SR-B2, cluster of differentiation 36 / scavenger receptor B2; DOCK4, dedicator of cytokinesis 4; FABP, fatty acid binding protein; FA-CoA, fatty acyl co-enzyme A; FATP, fatty acid transport protein; FFA, free fatty acid; FOXO1, forkhead box O1; GPIHBP1, glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1; 3-HIB, 3-hydroxyisobutyrate; LPL, lipoprotein lipase; PPARγ, peroxisome proliferator activated receptor γ; Rac-1, ras-related C3 botulinum toxin substrate 1; SR-B1, scavenger receptor B1; TRL, triglyceride-rich lipoprotein; VEGF-B, vascular endothelial growth factor B; VEGFR1, vascular endothelial growth factor receptor 1.

Figure 2: Trans-endothelial transport of glucose and insulin. Glucose may cross the endothelial barrier by paracellular diffusion and also through transcytosis. Transcytosis requires the uptake and subsequent release of glucose via glucose transporters. VEGF-B signalling reduces GLUT1 activity through altering non-esterified cholesterol levels, as a consequence of reduced LDL recycling, thereby down-regulating trans-endothelial glucose delivery. Modulation of glucose transcytosis may also involve HIF1α and/or Notch as regulators of GLUT1 transcription. Trans-endothelial transport of insulin can occur though fluid-phase trafficking or insulin receptor-mediated trafficking via clathrin and/or caveolae-mediated transcytosis. The latter may be regulated through Notch-driven expression of caveolae-related genes, including CAV-1. Abbreviations: CAV-1, caveolin 1; GLUT1, glucose transporter 1; HIF1α, hypoxia-inducible factor 1 alpha; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; VEGF-B, vascular endothelial growth factor B; VEGFR1, vascular endothelial growth factor receptor 1.

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Author contributions

AF conceived the study and wrote the manuscript.

Declaration of Interests

The author declares that there are no relationships or activities that might bias, or be perceived to bias, their work.

Acknowledgements

The author apologises to those whose work could not be cited due to space limitations. Figures, in part, were created using images from Servier Medical Art (https://smart.servier.com/) under a Creative Commons Attribution 3.0 Unported Licence. The author would like to acknowledge the support and mentorship of Prof. Harry Mellor and the support of members of the Mellor lab.

Funding

This work received no direct funding.

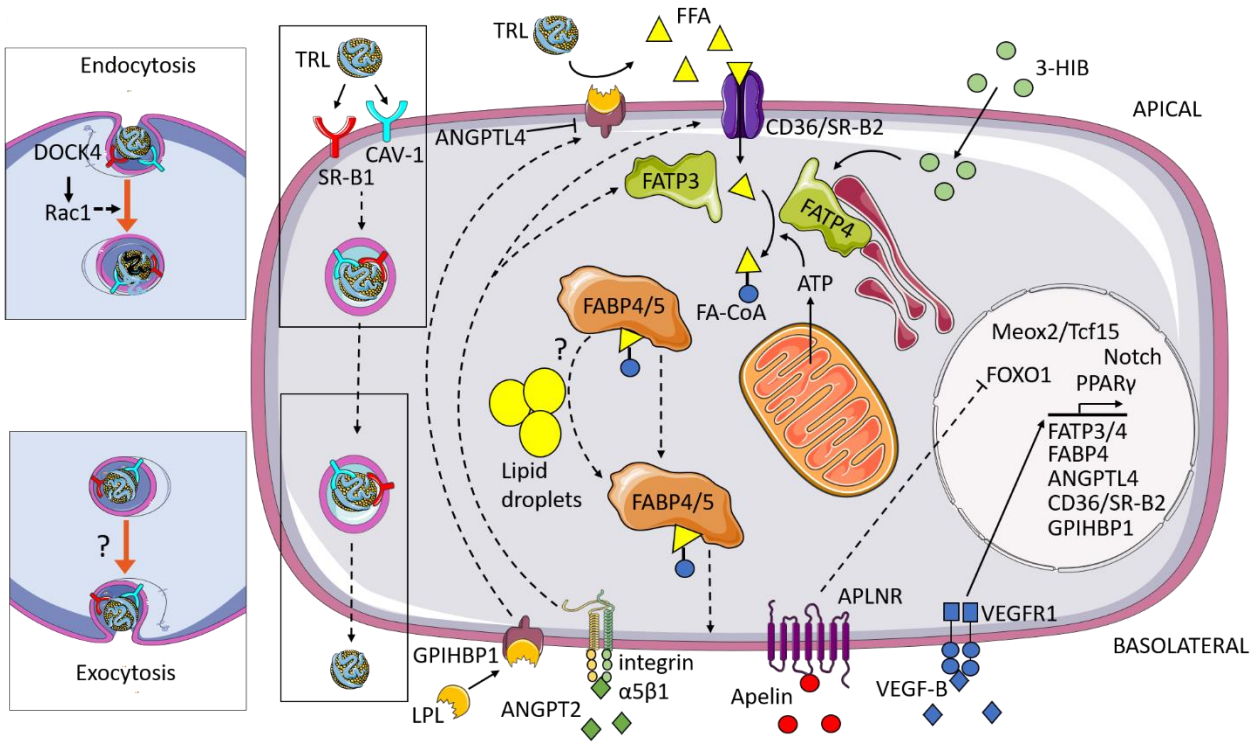

Figure 1

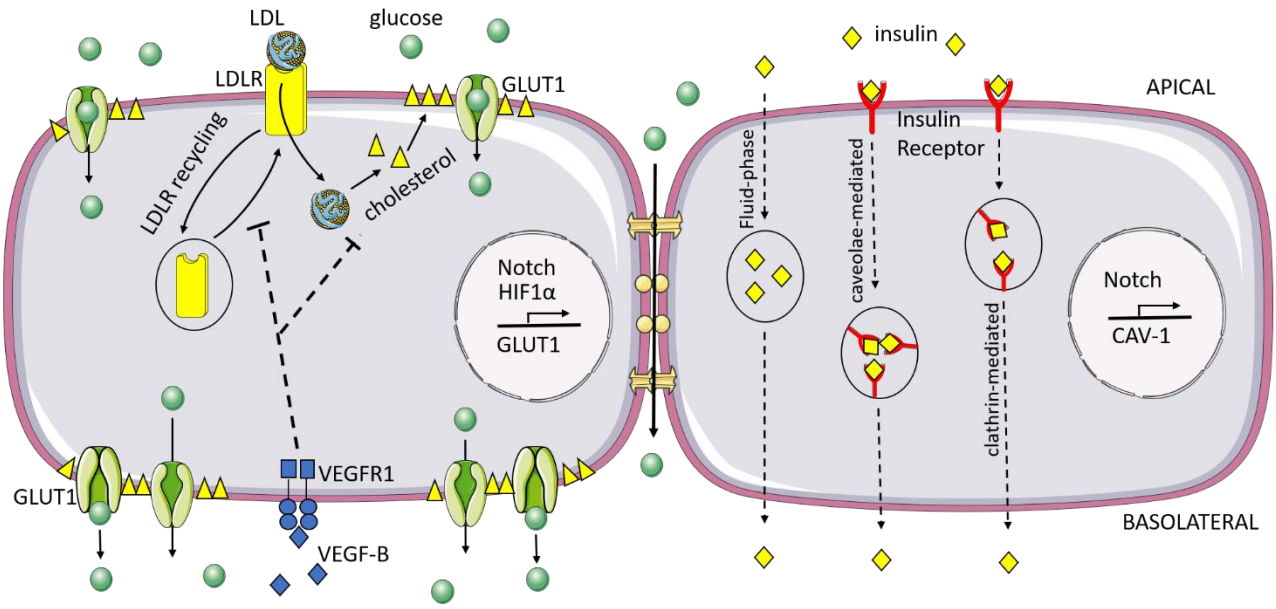

Figure 2