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Transport of Long-Chain Fatty Acids Across the Muscular Endothelium

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Abstract

Both skeletal and cardiac muscle cells rely heavily on the oxidation of long-chain fatty acids to utilize chemically stored energy for contractile work. Under normal conditions fatty acids are continuously supplied from the microvascular compartment to the contracting myocytes. Exogenous fatty acids are transported to muscle tissue via the blood either complexed to albumin or covalently bound in triacylglycerols forming the neutral lipid core of circulating lipoproteins such as chylomicrons or very low-density lipoproteins. The first barrier met by fatty acids on their way from the vascular compartment to the myocytes is the endothelium constituting the capillary wall. After dissociation of the albumin–fatty acid complex or release from the triacylglycerol core of lipoproteins, fatty acids most likely transverse the endothelium by crossing the luminal membrane, the cytosol, and subsequently the abluminal wall of the endothelial cell. Transfer through the interendothelial clefts or lateral diffusion within the phospholipid bilayer of the endothelial plasmalemma should be considered as inconsequential. The mechanism responsible for transmembrane movement of fatty acids is incompletely understood, although recent findings suggest the involvement of a number of membrane-associated proteins. Kinetic studies have revealed that interaction of the albumin-fatty acid complex with the endothelial membrane may accelerate the dissociation of the complex, which facilitates the uptake of fatty acids by the endothelium. Albumin-binding proteins (ABP) might be instrumental in this interaction. Moreover, plasmalemmal fatty acid-binding protein (FABP_{pm}), fatty acid translocase (FAT) and fatty acid- transport protein (FATP) are putatively involved in transmembrane movement of the fatty acid molecules. Diffusion through the endothelial cytosol might be facilitated by a cytoplasmic fatty acid-binding protein, the type of which may be related to the epithelial fatty acid-binding protein (E-FABP_c).

2. Introduction

To fulfil their energy requirements for contractile activity, myocytes in both cardiac and skeletal muscle tissue oxidize lipids and carbohydrates. In skeletal muscle the contribution of lipids to overall energy conversion depends, among others, on the type of muscle cell, the

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intensity and duration of exercise, the availability of other oxidizable substrates, dietary composition, the training status of the exercising muscles and the plasma concentration of regulatory hormones³⁰. Under normal conditions, lipid oxidation contributes substantially to energy conversion in cardiac myocytes²⁸. Fatty acids are the most important source of lipids for myocytes both in skeletal muscle and cardiac tissue (note that throughout this chapter the term “fatty acids” will refer to long-chain fatty acids present in the unesterified form). Muscle cells can store relatively small amounts of fatty acids in triacylglycerols forming cytoplasmic lipid droplets. In addition fatty acyl moieties present in cellular membrane phospholipids are not readily available for oxidative degradation. Thus, under normal conditions fatty acids are supplied to muscle cells mainly via blood streaming through the capillaries. These fatty acids are either non-covalently bound to plasma albumin or covalently bound to glycerol forming the triacylglycerol core of circulating lipoproteins. Because only minor amounts of the albumin-fatty acid complex or the lipoprotein corpuscles can cross the endothelium of the capillary wall, the fatty acid moieties must be released from albumin or from the neutral lipids of the circulating lipoproteins before passing the endothelial cells and being taken up by the myocytes. Oxidative conversion of fatty acids in muscle cells occurs mainly inside mitochondria, present in between the myofibrils or located close to the sarcolemma. On their way from the microvascular compartment to the muscular mitochondria, fatty acids meet a number of barriers, the first being the endothelium. Subsequently, the interstitial compartment, the sarcolemma, the cytoplasm of the muscle cell and the mitochondrial inner-membrane are potential barriers for fatty acids or their metabolic derivatives (Fig. 1).

In the present chapter the role of endothelial cells in the overall uptake process of plasma-borne fatty acids in skeletal muscle will be discussed. Special attention will be paid to possible mechanisms underlying fatty acid transport processes across the endothelium. In selected cases, detailed information derived from studies on cardiac tissue will be included and attempts will be made to extrapolate these findings to the skeletal muscle.

3. Plasma Fatty Acyl Moieties

Because the capacity of *de novo* synthesis of fatty acids in muscle cells is very low, ultimately all fatty acids utilized for oxidative energy conversion are supplied to the muscle cells by the blood flowing through the muscle capillaries. Fatty acids, released from their general site of storage, i.e., adipose tissue cells, circulate in blood non-covalently bound to plasma albumin. One albumin molecule can carry up to 8 molecules of fatty acids²⁴; the binding affinity for fatty acids declines with the increasing number of ligands bound to the albumin molecule. Under normal conditions the plasma concentration of fatty acids is on the order of 0.2–0.5 mmol·l⁻¹. A minor portion of fatty acids is present in the plasma in its “free” or “non-protein-bound” form. Recently, Richieri and colleagues calculated that the non-protein-bound portion of circulating fatty acids is in the lower nmol·l⁻¹ range¹⁹.

Under normal conditions about 250 nmol fatty acid (albumin-bound) is supplied to the heart per gram of tissue per minute, of which, on the average, 100 nmol·g⁻¹·min⁻¹ is extracted. In red skeletal muscle tissue the supply and uptake of fatty acids depend, among others, on the type and degree of the work performed, but recent estimations indicate that in exercising

skeletal muscles the flux of albumin-delivered fatty acids is on the same order of magnitude as found in cardiac tissue³⁰.

An alternative source of plasma-borne fatty acids are the fatty acyl moieties present in circulating lipoproteins such as chylomicrons and very-low density lipoproteins (VLDL). The release of chylomicrons from enterocytes into the blood via the lymphatic system is substantially increased after a fat-rich meal. VLDL are produced by liver cells in the post-prandial phase. The supply of lipoprotein-derived fatty acyl moieties to skeletal muscle tissue depends, among others, on the composition of the diet, the time interval between digestion of a meal and the onset of exercise, and the intensity of exercise. On the average about 90 and 1800–2700 nmol triacylglycerol-fatty acids per gram per minute, are delivered to resting and moderately exercising skeletal muscles, respectively³⁰. As discussed below, only a relatively small portion of the circulating triacylglycerol-fatty acids is extracted during one single capillary transit of blood. Experimental evidence is accumulating that neither the albumin-fatty acid complex nor the triacylglycerol-containing lipoproteins are able to pass the endothelial cells lining the muscle capillary wall in significant amounts³⁰. Thus, fatty acids have to be released from the albumin-fatty acid complex and from the neutral lipid core of lipoproteins prior to being transferred through the first barrier, i.e., the endothelium (Fig. 1).

The fatty acyl moieties esterified to glycerol in the core of circulating lipoproteins are released from the parent triacylglycerol molecule by the catalytic action of lipoprotein lipase. This hydrolytic enzyme is attached to the luminal surface of the endothelial cells. Evidence is accumulating that lipoprotein lipase is synthesized *de novo* in the striated muscle cells and transported to the endothelial cells⁵. The enzyme catalyses the hydrolysis of lipoprotein triacylglycerols most likely in the dimer configuration. Lipoprotein lipase activity is present in appreciable quantities in heart and red skeletal muscle⁴. The activity in white skeletal muscles is substantially lower than in red muscles. In skeletal muscle lipoprotein lipase activity is under the control of a variety of factors. The activity of lipoprotein lipase increases after fasting and ingestion of a high-fat diet. Endurance training results in enhanced lipoprotein lipase activity in red skeletal muscles³⁰. Insulin exerts a strong negative effect on skeletal muscle lipoprotein lipase activity. In contrast, ACTH, adrenaline, and noradrenaline elevate its activity in muscles³⁰.

There is a long-standing debate regarding the quantitative significance of lipoprotein lipase-liberated fatty acids for overall skeletal muscle fatty acid utilization²⁶. Gollnick and Saltin⁹ stated that only a minor fraction of the fatty acids present in lipoprotein-triacylglycerol is oxidized in skeletal muscles during exercise. In contrast, Terjung and colleagues²⁷ advocated that lipoprotein-fatty acyl moieties represent an important source of blood-borne substrates for the exercising muscle cells. Attempts to measure the quantity of fatty acyl residues from lipoprotein-triacylglycerols utilized for oxidative energy conversion, however, have remained inconclusive³⁰. Carefully conducted studies by Olsson and colleagues¹⁸ indicate that during fore-arm exercise the arterio-venous differences of circulating triacylglycerols are small and do not exceed the level of error of the analytical methods available to measure the triacylglycerol content in blood. Similar findings were reported by Kiens and Lithell, studying the arterio-local venous differences of VLDL across exercising

thigh muscles of trained and untrained human volunteers¹³. Interestingly, the latter investigators observed a small, but significant arterio-local venous difference of VLDL during rest. It should be kept in mind, however, that, due to the relative abundance of triacylglycerols in plasma, small arterio-venous differences of these lipids, for instance, on the order of 3% of the arterial concentration, will already result in a substantial contribution of triacylglycerol-fatty acids to overall plasma-borne lipid utilization by the exercising muscle cells.

As indicated above a significant amount of fatty acid molecules is non-covalently bound to plasma albumin when carried from adipose tissue to skeletal muscle. Positive arterio-local venous differences of fatty acids in resting and exercising muscles have been reported by a host of investigators, stressing the notion that albumin-bound fatty acids are important substrates for both the resting and the working muscle cell³⁰.

Because the muscular endothelium is virtually impermeable for lipoproteins and high molecular mass proteins such as albumin, fatty acids have to cross the endothelial barrier either after hydrolytic removal from the neutral lipid core of the lipoproteins or after release from albumin.

4. Transport of Fatty Acids Across the Endothelium

In general, according to thermodynamical considerations, the driving force for the flux of solutes from the vascular to the interstitial compartment is determined by the concentration of the solute in the vascular compartment. Net flux occurs when the concentration in the interstitium is (kept) lower than that in the vascular compartment. This situation holds when no active transport of the solute, i.e., transport against a concentrating gradient, takes place. In case of fatty acids, it is reasonable to assume that the mechanism underlying transendothelial transfer is diffusion, while facilitation by specific proteins may occur. Under normal circumstances the net flux of fatty acids from the capillary vascular to the interstitial compartment is guaranteed by subsequent uptake and metabolic conversion of the fatty acids in the skeletal or cardiac muscle cells, keeping the concentration of fatty acids in the interstitium low. Because fatty acids are hardly soluble in water and the bulk of fatty acids is bound to albumin (both in the vascular and interstitial compartment) the question can be posed whether the free, non-protein bound concentration of fatty acids or the albumin-bound fatty acid concentration generates the driving force for the transendothelial flux. The ultimate choice between the two possibilities depends on the mechanism(s) underlying the transport of fatty acids across the endothelial barrier.

As indicated in Fig. 2, on theoretical grounds at least four different mechanisms of transport for fatty acids across the capillary wall can be envisioned: 1, transfer of fatty acids via interendothelial clefts; 2, lateral diffusion through the plasmalemma of the endothelial cell; 3, diffusion of fatty acids across the luminal membrane, the cytoplasm and the abluminal membrane of the endothelial cell; and 4, same route as in option 3, but in option 4 a whole set of proteins is thought to be involved in facilitating the transfer of fatty acids from the vascular to the interstitial compartment.

5. Mechanisms Underlying Transendothelial Transport of Fatty Acids

5.1. Diffusion through Interendothelial Clefts

Passive fatty acid transfer (route 1, Fig. 2) as the fatty acid-albumin complex through the clefts is precluded because the albumin molecule is too large (68 kD) to diffuse through the interendothelial clefts in appreciable amounts. This leaves the possibility that non-protein bound fatty acids diffuse through the clefts. Their low concentration, however, makes the transfer of non-protein bound fatty acids through the clefts inconsequential, as is argued as follows.

By using the multiple indicator dilution technique¹⁴ Van der Vusse and colleagues²⁹ investigated the kinetics of transendothelial fatty acid transport in isolated rabbit hearts. In the absence of albumin, the endothelial permeability-surface area product (PS_{endo}) of free, non-protein bound palmitate was found to be on the order of $6 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. This value is about 3 times that for glucose³. Assuming that non-albumin bound fatty acids generate the driving force for transendothelial diffusion, the flux of palmitate through the clefts equals the PS_{endo} times the concentration of the free, non-protein bound fatty acid in the vascular compartment. In case of physiological vascular concentrations of albumin of about $600 \mu\text{M}$ and equimolar total palmitate concentrations, the vascular non-albumin bound palmitate concentration amounts to ca. 5 nM ¹⁹. Thus the maximal flux would be $5 \text{ nM} \times 6 \text{ ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1} = 0.03 \text{ nmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. This value is about 3000 times less than the normally observed flux of fatty acids from the vascular compartment to the cardiac muscle parenchymal cells, as calculated on the basis of blood flow times arteriovenous differences of fatty acids (on the order of $60\text{--}120 \text{ nmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$)²⁸. While a tracer diffusion front would be slowed by any albumin in the clefts, since these must be in equilibrium, the net transfer of fatty acids by diffusion through clefts would be unaffected, and would be almost equal to PS_{clefts} times 5 nM . Therefore, transport of fatty acids through the interendothelial clefts does not substantially contribute to the overall fatty acid transfer across the endothelium.

5.2. Transport of Fatty Acids through the Endothelial Cells

Because diffusion of fatty acids through the interendothelial clefts is unlikely, the sole option left is transport of these lipid moieties through the endothelial cells. As diagrammed in Fig. 2 at least three different mechanisms of transendothelial transport (and a variety of combinations thereof) can be envisioned. The first option is delivery of fatty acids to the luminal membrane followed by lateral diffusion of fatty acids within the phospholipid bilayer, and release of fatty acids from the abluminal side of the endothelium to the interstitial compartment (route 2). This type of transport is part of the route originally proposed by Scow and Blanchette-Mackie²². Previously, Basingthwaite and colleagues³ have reasoned that this mechanism is too slow to be effective. Their arguments, however, are based on calculation rather than experimental observations.

The effective flux of fatty acids from a point of the luminal side of the endothelial cell to the abluminal side through lateral diffusion within the phospholipid bilayer is given in equation 1.

$$\text{Flux} = \frac{D}{L} \times \frac{\text{bilayer length}}{\text{g of tissue}} \times \Delta C \quad (1)$$

where: D = coefficient of diffusion of fatty acids within a phospholipid bilayer
 L = the average diffusion distance
 C = the concentration difference of fatty acids in the membrane on the two sides

According to Brenner⁶ the value of D for unimpeded diffusion of fatty acids in an artificial membrane composed of a phospholipid bilayer amounts to $10^{-10} \text{ cm}^2 \cdot \text{s}^{-1}$. The average diffusion distance (L) amounts to $15 \mu\text{m}$. The total bilayer length of the opposed edges (per gram of tissue) depends on the size and number of endothelial cells in muscle tissue. For ca. $20 \mu\text{m}$ diameter cells the circumference is approximately $0.166 \mu\text{m}^{-1}$ length per unit surface area. Given a total endothelial capillary surface area in cardiac tissue of $500 \text{ cm}^2 \cdot \text{g}^{-1}$, the bilayer length per gram of tissue amounts to approx. $12 \text{ cm} \cdot \text{g}^{-1}$.

From these values it follows that the palmitate flux equals $4.8 \times 10^{-5} \text{ C mole} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$.

For adequate substrate delivery the translateral flux should be on the order of $100 \text{ nmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$. To reach this flux would require C inside the membrane to be unphysiologically high, i.e., approx. 2 mmol/cm^2 surface area. Taking into account that the plasmalemma is constituted of phospholipids spaced at approx. $4.2 \text{ \AA}$, the phospholipid concentration is approx. $10^{-6} \text{ mmol/cm}^2$. This would imply that the fatty acid content in the plasmalemma would have to be about 10^6 times the phospholipid content to provide adequate flux³. From these calculations it can be concluded that this route will not significantly contribute to transendothelial transfer of fatty acids from the vascular to the interstitial compartment.

Since both transfer via the interendothelial clefts and lateral movement of fatty acids within the plasmalemmal phospholipid bilayer have been ruled out as potential routes for fatty acids to cross the endothelial barrier³, these lipid moieties have to pass the endothelium by (facilitated) diffusion through the luminal membrane, the cytoplasm and the abluminal membrane of the endothelial cells. The two extremes (Fig. 2, routes 3 and 4) are either passive diffusion of fatty acids from the vascular compartment through the endothelial cells or facilitated diffusion, implicating the presence of a set of specific binding/transport proteins in the plasmalemma and cytoplasm of the endothelial cell.

Passive diffusion of non-protein bound fatty acids from the microvascular compartment to (and through) the luminal membrane of the endothelial cells implicates that the concentration of non-protein bound fatty acids in the plasma is the driving force for transendothelial transport. As discussed above this condition is highly unlikely. The corollary is that the concentration of albumin-bound fatty acids is the driving force. Taking into account that the dissociation rate of fatty acids from the albumin complex is too slow to account for the actual flux of fatty acids in muscular tissue³, the albumin-fatty acid complex is thought to interact with the luminal membrane whereby the release of fatty acids from albumin is accelerated and fatty acids are immediately transferred to the membrane. Studies from several laboratories^{2,21} have shown the existence of membrane albumin-binding

proteins². Although these binding proteins were originally thought to be involved in transendothelial transport of albumin by pinocytosis, it cannot be excluded that they also play a role in the delivery of fatty acids to the endothelial luminal membrane (Fig. 3). It should be noted that transendothelial transport of albumin, most likely vesicle-mediated, is too slow to account for the observed rate of fatty acid transport. Multiple indicator dilution experiments²⁹ have shown that in the isolated rabbit heart the effective PS_{endo} for tracer palmitate declines 6-fold from 1.8 to 0.3 $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ with increasing concentrations of the albumin-palmitate complex, i.e. from 0.044 to 0.88 mM, keeping the protein/fatty acid ratio constant at 0.9. This implies that the process of transendothelial fatty acid transport is saturable. Interestingly, PS_{endo} for palmitate did not change when the albumin concentration was kept constant at 0.44 mM, while the palmitate concentration varied from 0.044 mM to 2.0 mM, indicating that the unidirectional flux of tracer palmitate across the endothelium is linearly proportional to the palmitate content of the albumin-fatty acid complex. These combined findings strongly suggest that on the luminal side of the endothelial cell specific interactions occur between the albumin-fatty acid complex and (protein components of) the membrane.

The transport is diminished when the plasma concentration of albumin is increased. Hence, it is tempting to think that the albumin-binding proteins on the luminal endothelial surface are involved in the putative interaction of the albumin-fatty acid complex with the luminal membrane. Michaelis-Menten analysis of the data obtained in the multiple indicator dilution experiments reveals a K_m value for albumin of less than 0.2 mM. Because under normal physiological conditions the plasma albumin concentration is approximately 0.6 mM (i.e., far above the calculated K_m) the actual unidirectional flux of fatty acids across the endothelium is mainly dictated by the fatty acid/albumin ratio or, in other words, by the total concentration of fatty acids in blood plasma. Up to 2.0 mM palmitate no saturation of the unidirectional palmitate flux across the endothelium could be observed in the multiple indicator dilution experiments on isolated rabbit hearts²⁹.

After release of fatty acids from the albumin carrier a membrane fatty acid-binding protein (FABP_{pm}) is most likely involved in the overall transfer of fatty acids through the luminal membrane (Fig. 3)^{7,25}. This notion is based on studies performed by Goresky and colleagues¹⁰ on isolated rat hearts making use of the earlier mentioned multiple indicator dilution technique. As indicated in Fig. 4, preperfusion of the isolated hearts with an antibody raised against liver FABP_{pm} significantly hampered the extraction of labelled palmitate from the perfusion buffer when flowing through the coronary vascular bed. The PS_{endo} for tracer palmitate decreased from 1.62 to 0.33 $\text{ml}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ in the antibody preperfused hearts¹⁰.

Recent studies have provided circumstantial evidence that in addition to the peripheral 40-kD FABP_{pm} integral membrane proteins are involved in the transfer of fatty acids across the luminal membrane^{1,16,20}. Likely candidates are fatty acid translocase (FAT, 88kD) and fatty acid-transport protein (FATP, 63 kD). Greenwalt and co-workers¹¹ established the presence of FAT in cardiac endothelial cells. Recent investigations in our laboratory also revealed the presence of FATP in endothelial cells (Van Nieuwenhoven, unpublished results). It is of interest to note that, for instance in contrast to liver, in cardiac tissue all three

membrane associated proteins, putatively involved in fatty acid transport, are present. This also holds for skeletal muscle although it is not definitively proven that all three proteins are present in the plasma membrane of skeletal muscle endothelial cells. The mechanism(s) by which the integral membrane proteins facilitate transmembrane fatty acid transport is (are) incompletely understood. The integral proteins may act as true fatty acid transporters analogous to ion channels. The proteins, however, may also enhance the rate of flip-flop of the fatty acids moieties from the outer to the inner leaflet of the plasma membrane. Alternatively, after binding of the albumin-fatty acid complex to the albumin-binding protein and delivery of the fatty acids to the peripheral FABP_{pm}, the integral proteins may facilitate the uptake and/or release of fatty acids by the membrane. At present it is not yet known whether FAT and FATP serve a similar purpose in transmembrane fatty acid transport or have entirely different functions. Also their mechanism of action remains to be elucidated. Because in vitro studies have shown that fatty acids can also cross artificial membranes¹², it should be expected that some of the fatty acids are transferred through the luminal membrane by passive diffusion.

After transfer across the luminal membrane, fatty acids must pass the endothelial cytoplasmic compartment to reach the abluminal membrane. Such parenchymal cells as cardiomyocytes, skeletal muscle cells, hepatocytes and adipocytes, all of which are cell types with an active fatty acid metabolism, contain substantial amounts of a cytoplasmic 14-kD fatty acid-binding proteins (FABP_c)⁸. Although the fatty acid consumption in endothelial cells is negligible²³, transport through the endothelial cytosolic space may also involve the presence of FABP_c. Attempts to measure the FABP_c content of endothelial cells, however, were inconclusive with respect to the amount and nature of the FABP_c in muscle endothelial cells. Analysis of cultured cardiac endothelial cells revealed that these cells lack both the cardiac and liver type FABP_c^{15,31}. A functional assay indicated that the cytosol of cultured endothelial cells contained no measurable amounts of proteins with fatty acid binding properties³¹. This has led us to conclude that apparently only minor amounts of FABP_c, if any, are required in endothelial cells for the trans-cytoplasmic transfer of fatty acids, probably because of the relatively short diffusion distance from the luminal to abluminal membrane (i.e., on the order of 0.2 to 1.0 μm)³¹. On the basis of a computer model, however, Vork and colleagues³² concluded that FABP_c is required for unimpeded transition of fatty acids from the inner leaflet of the plasma membrane to the adjacent aqueous layer in the endothelial cell interior. Their transition is facilitated by physical interaction of cytoplasmic FABP with the membrane. In this respect it is worth noting that immunohistochemical studies of Masouye and colleagues¹⁷ have recently shown that in cardiac tissue capillary endothelial cells express the epidermal type-FABP (E-FABP). It remains to be established whether the concentration of E-FABP is sufficiently high to play a significant role in transendothelial fatty acid transport.

Delivery of fatty acids from the cytosol to the abluminal membrane, transfer through the abluminal membrane and release into the albumin-containing interstitial space are most likely occurring analogous to the transport process across the luminal membrane of the endothelial cell, but in a mirror-like fashion.

6. Concluding Remarks

Finally, we summarize our thinking on the issues of transendothelial fatty acid transport as follows. The endothelium is the first barrier in the transport route of fatty acids from the vascular compartment to the parenchymal muscle cells. Diffusion of fatty acids, either non-protein bound or complexed to albumin, through the interendothelial clefts is highly unlikely. Because the vascular concentration of albumin-bound fatty acids rather than that of non-protein bound fatty acids is the driving force for the transendothelial fatty acids flux, and the endothelium is virtually impermeable for proteins with the size of albumin, it is proposed that the albumin-fatty acid complex interacts with the luminal side of the endothelial plasmalemma. This interaction most likely accelerates dissociation of the albumin-fatty acid complex. Subsequently, the fatty acids cross the endothelium by transfer through the luminal membrane, the soluble cytoplasm and finally the abluminal membrane of the endothelium. Transmembrane transport is thought to be facilitated by a set of membrane-associated proteins including, among others, albumin-binding proteins, plasmalemmal FABP, FAT and FATP. It remains to be established whether a cytoplasmic FABP facilitates the cytosolic transfer of fatty acids from the luminal to the abluminal side of the endothelial cell.

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Figure 1.

Highly schematic representation of the transport route of fatty acids from the vascular compartment to the interior of the myocytes. TG refers to the triacylglycerol core in circulating lipoproteins; alb*FA to the albumin–fatty acid complex; FABP*FA to the fatty acid-binding protein–fatty acid complex. A question mark indicates that details of the transport route are not completely understood.

Figure 2.

Schematic representation of the mechanisms possibly involved in the transport of fatty acids across the endothelium. 1, transport through the interendothelial clefts; 2, lateral movement within the plasmalemmal phospholipid bilayer; 3 and 4, transfer across the luminal membrane, cytoplasm and abluminal membrane, respectively. Note that route #3 refers to passive diffusion of fatty acids, whereas route #4 involves a set of proteins facilitating transmembrane and transcytosolic transport. Alb*FA refers to the albumin–fatty acid complex, FA to long-chain fatty acids.

Figure 3.

Putative mechanism of fatty acid transfer across the luminal membrane of the endothelial cell. $\text{Alb} \cdot \text{FA}$, the albumin-fatty acid complex; ABP, albumin-binding protein; FABP_{pm} , plasmalemmal fatty acid-binding protein; FAT, fatty acid translocase; FATP, fatty acid-transport protein; FABP_{c} , a cytosolic fatty acid-binding protein, the nature and quantity of which remain to be established.

Figure 4.

Outflow dilution curves from experiments in a single isolated rat heart with, in sequence, perfusion with control perfusion buffer (heart 1, upper left panel) and buffer-containing specific antibody to rat hepatocyte FABP_{pm} (K 15/6 hybridoma supernatant) (heart 1, lower left panel) and, in a second heart, with control perfusion buffer (heart 2, upper right panel) and buffer-containing non-specific antibody (myeloma NS-1 supernatant) (heart 2, lower right panel). From Goresky et al.¹⁰ with permission.