Computational evidence for protein-mediated fatty acid transport across the sarcolemma

Mark W. J. M. MUSTERS*, James B. BASSINGTHWAIGHTE†, Natal A. W. VAN RIEL‡ and Ger J. VAN DER VUSSE§¹

*Department of Electrical Engineering, Eindhoven University of Technology, 5612 AZ Eindhoven, The Netherlands, †Department of Bioengineering, University of Washington, Seattle, WA 98195-7962, U.S.A., ‡Department of Biomedical Engineering, Eindhoven University of Technology, 5612 AZ Eindhoven, The Netherlands, and §Department of Physiology, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, 6200 MD Maastricht, The Netherlands

Long-chain fatty acids (FAs) are important substrates used by the heart to fulfil its energy requirements. Prior to mitochondrial oxidation, blood-borne FAs must pass through the cell membrane of the cardiac myocyte (sarcolemma). The mechanism underlying the sarcolemmal transport of FAs is incompletely understood. The aim of the present study was to estimate the trans-sarcolemmal FA uptake rate using a comprehensive computer model, in which the most relevant mechanisms proposed for cardiac FA uptake were incorporated. Our *in silico* findings show that diffusion of FA, present in its unbound form (uFA) in close proximity to the outer leaflet of the sarcolemma and serving as sole FA source, is insufficient to account for the physiological FA uptake rate. The inclusion of a hypothetical membrane-associated FA-TFPC (FA-transport-facilitating protein complex) in the model calculations substantially increased the FA uptake rate across the sarcolemma.

The model requires that the biological properties of the FA-TFPC allow for increasing the rate of absorption of FA into the outer leaflet and the ‘flip-flop’ rate of FA from the outer to the inner leaflet of the sarcolemma. Experimental studies have identified various sarcolemma-associated proteins promoting cardiac FA uptake. It remains to be established whether these proteins possess the properties predicted by our model. Our findings also indicate that albumin receptors located on the outer leaflet of the sarcolemma facilitate the transfer of FA across the membrane to a significant extent. The outcomes of the computer simulations were verified with physiologically relevant FA uptake rates as assessed in the intact, beating heart in experimental studies.

Key words: extracellular matrix, fatty acid, membrane protein, modelling, plasma albumin.

INTRODUCTION

To meet the energy requirements for proper electro-mechanical functioning, the myocardium relies heavily on the supply of blood-borne oxidizable substrates. Under normal physiological circumstances up to 70 % of cardiac metabolic energy is generated by oxidation of FAs (long-chain fatty acids) [1,2]. Because free uFAs (unbound FAs) are highly hydrophobic and almost insoluble in water, they comprise normally less than 0.01 % of all FAs in blood plasma [3]. Consequently, FAs are supplied to the heart either complexed to albumin or as triacylglycerols stored in the core of circulating lipoproteins. Prior to mitochondrial oxidation, FAs traverse a number of barriers to reach the cytoplasm of the cardiac muscle cell. These barriers include the endothelial cells lining the microvascular space, the interstitial fluid compartment containing the extracellular matrix, and the cardiac muscle cell membrane, i.e. the sarcolemma [2]. Originally, FAs were thought to cross the sarcolemma by unmediated diffusion [4–7]. Evidence has been provided, however, that membrane-associated proteins are likely to be involved in facilitating the transfer of FAs through the cardiomyocyte membrane. For instance, the rate of cellular FA uptake was significantly increased when cells in culture were transfected with the cDNAs of specific candidate proteins. Furthermore, FA transport across the plasma membrane exhibits both saturable and non-saturable uptake components [8–13]. Other observations suggested that direct interaction of the albumin–FA complex with the sarcolemma could also play a role in trans-

sarcolemmal FA transport [14–16]. Despite sophisticated experimental studies aiming to identify the key players in cardiac FA uptake, the precise mechanisms underlying trans-sarcolemmal transport of these substrates are still incompletely understood.

In recent years several attempts were made to obtain detailed information on FA uptake with the use of artificial membrane systems [17–19], isolated cardiac muscle cells [10,20] and computer models [21]. The experimental findings, however, did not either quantify to what extent protein-mediated FA transport is involved in FA transfer across the sarcolemma, or determine how membrane-associated proteins promote the transmembrane transfer of FAs. Experimental observations in hearts (*in situ* and isolated) and intact heart preparations of a variety of animal species, including human, indicate that the rate of FA utilization for cardiac energy conversion and, hence, sarcolemmal FA uptake rate, is in the order of 65 to 85 nmol/g ww (wet weight) of myocardial tissue per minute at normal cardiac workload and physiologically relevant albumin and FA concentrations [22,23]. Rating the *in vitro* findings of FA transport rates to determine their true physiological significance, is hampered by the fact that in most studies the physiologically relevant trans-sarcolemmal FA uptake rate values were not taken into account. Experimental methods for measuring parameter values are mostly conferred to specific processes in simplified physiological models. These various experimental conditions differ significantly from normal physiological conditions and, consequently, prevent an intuitive approach for an integrative interpretation. Therefore, computer models were

Abbreviations used: CD36/FAT, CD36/fatty acid translocase; FA, long-chain fatty acid; FABP_{PM}, plasmalemmal fatty acid-binding protein; FA-TFPC, a hypothetical FA-transport-facilitating protein complex; FATP, fatty acid transport protein; ISF, interstitial fluid; ODE, ordinary differential equation; uFA, unbound FA; ww, wet weight.

¹ To whom correspondence should be addressed (email vandervusse@fys.unimaas.nl).

considered that enable a detailed understanding of trans-sarcolemmal FA transport by combining the various experiments into one physiological concept.

The main objective of the present computational study was, therefore, to estimate trans-sarcolemmal FA uptake rates by including a variety of potential FA transport mechanisms and comparing the outcome of the calculations with physiologically relevant transport rates.

We recently calculated that the maximal rate of FA transfer across the sarcolemma achievable by unmediated diffusion of uFAs at their ambient interstitial concentrations does not exceed 15 nmol/(g min) [24]. This rate is obviously too low to explain the normal FA influx of 65 to 85 nmol/(g min) observed in the intact heart. This discrepancy prompted us to extend our computational model to investigate the presence and properties of additional mechanisms of trans-sarcolemmal FA transport. To this end, we included in our model analysis the putative processes involved either in altering the 'flip-flop' rate (i.e. the transfer of FA from the outer leaflet to the inner leaflet of the sarcolemma), or in enhancing the absorption of extracellular FAs into the outer leaflet. The potential role of a so-called albumin receptor, which was hypothesized to promote the dissociation of FA from FA-albumin-receptor complexes [14,15] was also explored. Since these transport processes are in series with the diffusion of the FA-albumin complex from the endothelium to the sarcolemma through the interstitial compartment, the hindrance to diffusion by the extracellular matrix was also included in our computational analysis.

The present model findings indicate that the extracellular concentrations of uFAs are not high enough to explain the physiological FA uptake rates across the sarcolemma by unmediated diffusion. We found that an albumin receptor could enhance trans-sarcolemmal FA transport to some extent, but still appears incapable of generating high enough FA transport rates on its own. These findings indicate that additional mechanisms should be involved enhancing the absorption and leaflet-to-leaflet 'flip-flop' rates, as measured in artificial phospholipid bilayers. This increase in absorption and flip-flop rate is most likely caused by a membrane-associated FA-TFPC (FA-transport-facilitating protein complex). The true nature of this hypothetical complex remains to be elucidated. At present, likely candidates to fulfil this role are FATP (fatty acid transport protein), FABP_{PM} (plasmalemmal fatty acid-binding protein), and/or CD36/FAT (CD36/fatty acid translocase) [11]. The concerted action of an albumin receptor and the hypothetical FA-TFPC was found to be required to raise FA absorption and translocation rates to physiologically relevant levels.

MATHEMATICAL MODELLING

Physiological background

The focus of the present paper lies in understanding the mechanism of FA transport across the myocardial sarcolemma. This barrier is the biological membrane that separates the interior of the cardiomyocyte, i.e. the myocardial cytoplasm and organelles, from the ISF (interstitial fluid) compartment outside the cardiac muscle cell. To meet the myocardial energy requirements, FAs must traverse the sarcolemma in sufficient amounts. These FAs are subsequently either incorporated in phospholipids and stored in triacylglycerols, or transported across the mitochondrial membrane via the carnitine shuttle and oxidized inside the mitochondrial matrix [2].

An established mechanism for FA transport across lipid membranes is transmembrane diffusion [25]. The driving force for dif-

Figure 1 Transmembrane diffusion of FAs across the sarcolemma with uFAs as source

Distinct steps required for overall FA transport from the interstitium (ISF) to intracellular storage or oxidation in the cardiomyocyte are (I) FAs dissociate from albumin (Alb)-FA complexes, $[AF]_{isf}$, in the ISF, (II) absorption of uFAs into the outer leaflet of the membrane, (III) translocation of uFAs from outer to inner leaflet ('flip-flop'), (IV) desorption of uFAs into the myocardial cytoplasm (cyt) and (V) intracellular FA storage or oxidation.

fusion is the concentration gradient of free uFAs across the membrane, which is aided by the low cytoplasmic uFA concentration [2], so low that under normal circumstances there is a virtually unidirectional driving force for influx from the ISF into the cardiomyocyte.

It should be realized, however, that more than 99.9% of the FAs in the ISF are bound to albumin [3], which possesses at least three high affinity binding sites for FAs and acts as a buffer for uFAs [26,27]. A variety of physiological studies have pointed towards either albumin-bound FAs [14,15,28] or uFAs, after their release from the albumin-FA complex [18,29], as FA source for transmembrane diffusion. We included both sources of FAs for trans-sarcolemmal transport in the present analysis.

Finally, since experimental evidence is accumulating that membrane-associated proteins are involved in cardiomyocyte FA uptake [30,31], we investigated the effects of a sarcolemma-associated albumin receptor and a hypothetical FA-TFPC on the kinetics of trans-sarcolemmal FA transport rates.

uFAs as a source for transmembrane diffusion

Transmembrane diffusion with uFAs in the ISF as source is based on several consecutive steps (Figure 1). First, both uFAs and albumin-FA complexes diffuse through the ISF towards the sarcolemma. Since most of the FAs in the interstitial compartment are bound to albumin and the sarcolemma is virtually impermeable for albumin, FAs should dissociate from albumin prior to cellular uptake (I in Figure 1). Thereafter, FAs from the uFA pool in the stagnant water layer cross the membrane in three consecutive steps, namely absorption, translocation via 'flip-flop' and desorption (II, III and IV in Figure 1 respectively) into, through and from the lipid bilayer of the sarcolemma respectively. FA transport will be concluded by storage of FAs in the cytoplasmic esterified lipid pool, incorporation in membrane phospholipids or mitochondrial oxidation. In this situation the albumin-FA complex solely acts as buffer to replenish uFAs that disappear from the stagnant water layer.

In the model shown in Figure 1 several assumptions were made, namely: (1) diffusion was the main driving force of both uFAs and albumin-bound FAs to migrate from the central region of the interstitium to the sarcolemma; (2) only the first three binding sites for FAs on albumin were considered, because these sites

were most important under normal physiological conditions (the difference in uFA concentration between three and six binding sites was less than 10%) [3]; (3) albumin and its FA complexes possess the same diffusion coefficient.

General state equations

Together with the physiological properties of the system as outlined above, diffusion in the ISF is characterized by the following coupled set of PDEs (partial differential equations, Eqns 1–5), where all of the concentrations are functions of position x , which is defined as the radial distance from the middle of the ISF, and of time:

$$\begin{aligned} \partial[\text{uFA}]_{\text{isf}}/\partial t = & D_{\text{uFA}} \partial^2[\text{uFA}]_{\text{isf}}/\partial x^2 + k_{-1}([\text{AF}_1]_{\text{isf}} + [\text{AF}_2]_{\text{isf}} \\ & + [\text{AF}_3]_{\text{isf}}) - (k_{+1}[\text{Alb}]_{\text{isf}} + k_{+2}[\text{AF}_1]_{\text{isf}} \\ & + k_{+3}[\text{AF}_2]_{\text{isf}})[\text{uFA}]_{\text{isf}}, \end{aligned} \quad (1)$$

$$\begin{aligned} \partial[\text{Alb}]_{\text{isf}}/\partial t = & D_{\text{Alb}} \partial^2[\text{Alb}]_{\text{isf}}/\partial x^2 + k_{-1}[\text{AF}_1]_{\text{isf}} \\ & - k_{+1}[\text{Alb}]_{\text{isf}}[\text{uFA}]_{\text{isf}}, \end{aligned} \quad (2)$$

$$\begin{aligned} \partial[\text{AF}_1]_{\text{isf}}/\partial t = & D_{\text{Alb}} \partial^2[\text{AF}_1]_{\text{isf}}/\partial x^2 + k_{+1}[\text{Alb}]_{\text{isf}}[\text{uFA}]_{\text{isf}} \\ & + k_{-1}[\text{AF}_2]_{\text{isf}} - (k_{-1} + k_{+2}[\text{uFA}]_{\text{isf}})[\text{AF}_1]_{\text{isf}}, \end{aligned} \quad (3)$$

$$\begin{aligned} \partial[\text{AF}_2]_{\text{isf}}/\partial t = & D_{\text{Alb}} \partial^2[\text{AF}_2]_{\text{isf}}/\partial x^2 + k_{+2}[\text{AF}_1]_{\text{isf}}[\text{uFA}]_{\text{isf}} \\ & + k_{-1}[\text{AF}_3]_{\text{isf}} - (k_{-1} + k_{+3}[\text{uFA}]_{\text{isf}})[\text{AF}_2]_{\text{isf}}, \end{aligned} \quad (4)$$

$$\begin{aligned} \partial[\text{AF}_3]_{\text{isf}}/\partial t = & D_{\text{Alb}} \partial^2[\text{AF}_3]_{\text{isf}}/\partial x^2 \\ & + k_{+3}[\text{AF}_2]_{\text{isf}}[\text{uFA}]_{\text{isf}} - k_{-1}[\text{AF}_3]_{\text{isf}}, \end{aligned} \quad (5)$$

with $[\text{uFA}]_{\text{isf}}$ = uFA source concentration in ISF (in M); $[\text{Alb}]_{\text{isf}}$ = unbound albumin concentration in ISF (in M); $[\text{AF}_1]_{\text{isf}}$, $[\text{AF}_2]_{\text{isf}}$, and $[\text{AF}_3]_{\text{isf}}$ = albumin complex concentrations in ISF, which are bound to one, two and three FAs, respectively (in M); x = spatial variable (in cm); D_{uFA} = diffusion coefficient of uFA (in $\text{cm}^2 \cdot \text{s}^{-1}$); D_{Alb} = diffusion coefficient of albumin (in $\text{cm}^2 \cdot \text{s}^{-1}$); k_{+1} , k_{+2} , k_{+3} = binding rate constants of uFA with albumin, AF_1 and AF_2 , respectively (in $\text{M}^{-1} \cdot \text{s}^{-1}$); and k_{-1} = release rate constant of FA from the albumin–FA complex (in s^{-1}).

FA transport across the sarcolemma, located at position $x = x_{\text{ECM}}$, is described by the following set of ODEs (ordinary differential equations) [18]:

$$\begin{aligned} d[\text{uFA}]_{\text{o}}/dt = & k_{\text{on}}[\text{uFA}]_{\text{isf}}(x_{\text{ECM}}, t) - k_{\text{off}}[\text{uFA}]_{\text{o}} \\ & - k_{\text{flip}}([\text{uFA}]_{\text{o}} - [\text{uFA}]_{\text{i}}), \end{aligned} \quad (6)$$

$$d[\text{uFA}]_{\text{i}}/dt = k_{\text{on}}[\text{uFA}]_{\text{cyt}} - k_{\text{off}}[\text{uFA}]_{\text{i}} + k_{\text{flip}}([\text{uFA}]_{\text{o}} - [\text{uFA}]_{\text{i}}), \quad (7)$$

where x_{ECM} indicates the spatial location of a concentration directly adjacent to the sarcolemma; $[\text{uFA}]_{\text{o}}$ = uFA concentration in outer leaflet (in M); $[\text{uFA}]_{\text{i}}$ = uFA concentration in inner leaflet (in M); $[\text{uFA}]_{\text{cyt}}$ = uFA sink concentration in the cardiac cytoplasm and assumed to be negligible [2]; k_{on} = absorption rate constant of uFAs from an aqueous environment into an artificial phospholipid membrane (in s^{-1}); k_{off} = desorption rate constant of uFAs from an artificial phospholipid membrane into an aqueous environment (in s^{-1}); and k_{flip} = translocation ('flip-flop') rate constant of uFAs from one leaflet to the other (in s^{-1}). The partition coefficient for uFAs in the lipid–water phase $\lambda_{\text{m/w}} (= k_{\text{on}}/k_{\text{off}})$ is frequently reported in the literature to derive k_{on} . This coefficient is dependent on various factors, such as pH of the solution, pK_{a} and chain length of the FA, and temperature [32]. Since no differences in rate parameter values for k_{off} , k_{on} and k_{flip} between the outer and

inner leaflets of the sarcolemma were reported in the literature, we assumed them to be identical for both sides of the membrane.

Boundary and initial conditions

A constant supply of both uFAs and FA–albumin complexes is the source of this model at $x = 0$. For a physiologically relevant setting ($[\text{FA}]_{\text{tot}} = 0.09 \text{ mM}$, $[\text{Alb}]_{\text{tot}} = 0.3 \text{ mM}$; see Table 1), the steady-state concentrations of the five substrates ($[\text{uFA}]$, $[\text{Alb}]$, $[\text{AF}_1]$, $[\text{AF}_2]$ and $[\text{AF}_3]$) were calculated as was done previously [21,24] and are listed in Table 1. At $x = x_{\text{ECM}}$, 'no flux' boundary conditions were defined [21]; the myocardial cytoplasm was considered to be a sink for uFAs; the sarcolemma was impermeable for albumin and its complexes:

$$D_{\text{uFA}} \partial[\text{uFA}]/\partial x|_{x=x_{\text{ECM}}} = 0, \quad (8)$$

$$D_{\text{Alb}} \partial[\text{Alb}]/\partial x|_{x=x_{\text{ECM}}} = 0, \quad (9)$$

$$D_{\text{Alb}} \partial[\text{AF}_i]/\partial x|_{x=x_{\text{ECM}}} = 0 \text{ for } i = 1, 2, 3. \quad (10)$$

In addition, the dynamics of $[\text{uFA}](x_{\text{ECM}}, t)$ were described by the following ODE:

$$\begin{aligned} d[\text{uFA}]_{\text{isf}}(x_{\text{ECM}}, t)/dt = & k_{-1}\{[\text{AF}_1]_{\text{isf}}(x_{\text{ECM}}, t) \\ & + [\text{AF}_2]_{\text{isf}}(x_{\text{ECM}}, t) + [\text{AF}_3]_{\text{isf}}(x_{\text{ECM}}, t)\} \\ & + k_{\text{off}}[\text{uFA}]_{\text{o}} - \{k_{\text{on}} + k_{+1}[\text{Alb}]_{\text{isf}}(x_{\text{ECM}}, t) \\ & + k_{+2}[\text{AF}_1]_{\text{isf}}(x_{\text{ECM}}, t) + k_{+3}[\text{AF}_2]_{\text{isf}}(x_{\text{ECM}}, t)\} \\ & \times [\text{uFA}]_{\text{isf}}(x_{\text{ECM}}, t) \end{aligned} \quad (11)$$

The initial conditions (at $t = 0$) for all substrates were set to zero.

Involvement of an albumin receptor in trans-sarcolemmal FA transport

Albumin-bound FAs comprise a large source of FAs available for cardiac myocyte FA utilization. The release of FAs from albumin could be facilitated after binding of the FA–albumin complex to a membrane-associated protein, which acts as a docking place or 'receptor' for the albumin complexes [33] (Figure 2). Facilitated release of FAs from the FA–albumin complex implies a decline in affinity of FAs for albumin when FA–albumin is bound to its receptor, i.e. $k_{\text{rel}} > k_{-1}$. Albumin-bound FAs are released either into the ISF near the membrane (albumin–FA complexes as 'indirect' FA source, Figure 2A), or directly into the outer leaflet of the sarcolemma (albumin–FA complexes as 'direct' FA source, Figure 2B) [14,16]. The remaining steps of trans-sarcolemmal FA transport are identical to the corresponding steps of the model described for unmediated transmembrane diffusion. In the present model, direct interaction of albumin–FA complexes with the membrane without an albumin receptor was not included, since studies with artificial membranes failed to provide evidence for such first order and rate-limiting FA desorption from albumin [32].

The basics of the direct and indirect albumin receptor theories were described by Eqns 1–7, with boundary conditions, described by Eqns 8–11. Additional modifications were necessary for implementing these two theories as listed below.

Albumin–FA complexes as 'indirect' FA source

The first putative mechanism of the albumin receptor is the facilitated release of FAs from albumin–FA complexes (see above) which leads to a local elevation in uFA concentration in close proximity to the sarcolemma at $x = x_{\text{ECM}}$. Additional ODEs are required for taking into account the kinetics of albumin–FA

Table 1 Parameter values of the models

Parameter	Specification	Value	Description	Reference
$[FA]_{tot}(x,t)$	ISF:	0.09 mM	Total FA concentration (bound and unbound)	[22]
	Cytoplasm:	~ 0 mM	$[uFA]_{isf}(x,t) + \sum_{i=1}^3 i[AF_i]_{isf}(x,t)$	[2]
$[Alb]_{tot}(x,t)$	ISF:	0.3 mM	Total albumin concentration (bound and unbound)	[22]
			$[Alb]_{isf}(x,t) + \sum_{i=1}^3 [AF_i]_{isf}(x,t)$	[2]
$[uFA]_{isf}(0,t)$		2.07 nM	Source concentration of unbound FAs, available for transmembrane diffusion	[3]
$[Alb]_{isf}(0,t)$		0.23 mM	Source concentration of unbound albumin	[3]
$[AF_1]_{isf}(0,t)$		55.9 μ M	Source concentration of AF_1	[3]
$[AF_2]_{isf}(0,t)$		13.7 μ M	Source concentration of AF_2	[3]
$[AF_3]_{isf}(0,t)$		2.27 μ M	Source concentration of AF_3	[3]
$[R]_{tot}$		0.16 mM	Total available albumin receptor	Calculated
x_{ECM}		0.2 μ m	Extracellular matrix thickness in the interstitium	[42]
x_{mem}		5.0 nm	Membrane thickness	[45]
A_{heart}		2000 cm ² /g ww	Diffusion area of cardiomyocytes	[42]
D_{uFA}		3.0×10^{-6} cm ² · s ⁻¹	Diffusion coefficient of uFA in water	[46]
D_{Alb}	Unrestricted:	5.2×10^{-7} cm ² · s ⁻¹	Diffusion coefficient of albumin in water	[46]
	Restricted:	~ 0 cm ² · s ⁻¹		[34]
K_{d1}	uFA with Alb:	8.47 nM	Equilibrium dissociation constant of uFA with albumin(–FA complex)	[3]
K_{d2}	uFA with AF_1 :	8.47 nM		
K_{d3}	uFA with AF_2 :	12.5 nM		
k_{-1}	For oleate:	3.3 s^{-1}	Dissociation rate constant of FA from albumin–FA complex	[47]
$k_{+1} = k_{-1}/K_{d1}$		$3.89 \times 10^8 \text{ M}^{-1} \cdot \text{s}^{-1}$	Association rate constant of FA with albumin(–FA complex)	Derived
$k_{+2} = k_{-1}/K_{d2}$		$3.89 \times 10^8 \text{ M}^{-1} \cdot \text{s}^{-1}$		
$k_{+3} = k_{-1}/K_{d3}$		$2.64 \times 10^8 \text{ M}^{-1} \cdot \text{s}^{-1}$		
k_d^{rec}		1 μ M	Equilibrium dissociation constant of albumin with receptor	[14]
k_{dis}	Range:	0–100 s ⁻¹	Dissociation rate constant of albumin(–FA complex) from receptor	[14]
$k_{bind} = k_{-1}/k_d^{rec}$		10^7 – $10^8 \text{ M}^{-1} \cdot \text{s}^{-1}$	Association rate constant of albumin(–FA complex) with receptor	Derived
k_{rel}	Range:	0–100 s ⁻¹	Rate constant for release of FA from albumin–FA–receptor complex into ISF or outer leaflet	Estimated: assumed to have the same value as k_{dis}
$\lambda_{m/w}$	Artificial membrane:	4×10^5	Partition coefficient of unbound FAs in lipid/water phase	[18]
	Adipocyte membrane:	1.4×10^6		[36]
k_{off}		3 s^{-1}	Desorption rate constant of unbound FAs from membrane	[37]
$k_{on} = \lambda_{m/w} k_{off}$	For $\lambda_{m/w} = 4 \times 10^5$:	$1.2 \times 10^6 \text{ s}^{-1}$	Absorption rate constant of unbound FAs in membrane	Derived
	For $\lambda_{m/w} = 1.4 \times 10^6$:	$4.2 \times 10^6 \text{ s}^{-1}$		
k_{flip}	Range:	0.5 s^{-1}	Translocation rate constant of uFA from outer to inner membrane leaflet	[37]
α		$3.0 \times 10^4 \text{ dm}^3/\text{g}$	Constant to convert from mol/(dm ³ s) to nmol/(g min)	–
β		6.0×10^{10}	Factor for converting mol/s to nmol/min	–

complexes with the receptor:

$$\begin{aligned} d[R]/dt = & k_{dis}([AR] + [AF_1R] + [AF_2R] + [AF_3R]) \\ & - k_{bind}([Alb]_{isf}(x_{ECM}, t) + [AF_1]_{isf}(x_{ECM}, t) \\ & + [AF_2]_{isf}(x_{ECM}, t) + [AF_3]_{isf}(x_{ECM}, t))[R], \end{aligned} \quad (12)$$

$$d[AR]/dt = k_{bind}[Alb]_{isf}(x_{ECM}, t)[R] + k_{rel}[AF_1R] - k_{dis}[AR], \quad (13)$$

$$\begin{aligned} d[AF_1R]/dt = & k_{bind}[AF_1]_{isf}(x_{ECM}, t)[R] + k_{rel}([AF_2R] \\ & - [AF_1R]) - k_{dis}[AF_1R], \end{aligned} \quad (14)$$

$$\begin{aligned} d[AF_2R]/dt = & k_{bind}[AF_2]_{isf}(x_{ECM}, t)[R] + k_{rel}([AF_3R] \\ & - [AF_2R]) - k_{dis}[AF_2R], \end{aligned} \quad (15)$$

$$d[AF_3R]/dt = k_{bind}[AF_3]_{isf}(x_{ECM}, t)[R] - (k_{rel} + k_{dis})[AF_3R], \quad (16)$$

where $[R]$ = unbound albumin receptor concentration (in M); $[AR]$, $[AF_1R]$, $[AF_2R]$ and $[AF_3R]$ = complexes of albumin receptor with albumin, AF_1 , AF_2 and AF_3 , respectively (in M); k_{dis} = dissociation rate constant of albumin and FA–albumin complexes from the albumin receptor (in s⁻¹); k_{bind} = association rate constant

of albumin and FA–albumin complexes with the albumin receptor (in M⁻¹ · s⁻¹); and k_{rel} = release rate constant of FA from the FA–albumin–receptor complexes into the ISF ('indirect' FA source) (in s⁻¹).

Boundary conditions

The boundary condition in Eqn 11 was replaced by Eqn 17. Furthermore, additional boundary conditions were required.

$$\begin{aligned} d[uFA]_{isf}(x_{ECM}, t)/dt = & k_{-1}([AF_1]_{isf}(x_{ECM}, t) + [AF_2]_{isf}(x_{ECM}, t) \\ & + [AF_3]_{isf}(x_{ECM}, t)) + k_{off}[uFA]_o \\ & + k_{rel}([AF_1R] + [AF_2R] + [AF_3R]) \\ & - \{k_{on} + k_{+1}[Alb]_{isf}(x_{ECM}, t) \\ & + k_{+2}[AF_1]_{isf}(x_{ECM}, t) + k_{+3}[AF_2]_{isf} \\ & \times (x_{ECM}, t)\}[uFA]_{isf}(x_{ECM}, t), \end{aligned} \quad (17)$$

$$\begin{aligned} d[Alb]_{isf}(x_{ECM}, t)/dt = & k_{-1}[AF_1]_{isf}(x_{ECM}, t) + k_{dis}[AR] - \{k_{bind}[R] \\ & + k_{+1}[uFA]_{isf}(x_{ECM}, t)\}[Alb]_{isf}(x_{ECM}, t), \end{aligned} \quad (18)$$

Figure 2 Transmembrane diffusion of FAs across the sarcolemma, assisted by an albumin receptor

Two different mechanisms are proposed. **(A)** The albumin receptor enhances the dissociation of FAs from the FA–albumin–receptor complex into the ISF ('indirect' FA source). The overall transfer process consists of at least six consecutive steps, i.e. (I) binding of an albumin–FA complex to the albumin receptor, (II) enhanced dissociation of FAs from this FA–albumin–receptor complex, and (III)–(VI) are similar to (II)–(V) of transmembrane diffusion (Figure 1). **(B)** The albumin receptor enhances FA dissociation from the FA–albumin–receptor complex directly into the outer leaflet of the sarcolemma ('direct' FA source). This process corresponds to Figure 2A; only steps (II) and (III) are replaced by direct absorption of FA from the FA–albumin–receptor complex into the outer leaflet. In addition, uFAs are also able to traverse the sarcolemma as indicated Figure 1, but not displayed in this figure.

$$\begin{aligned} d[AF_1]_{\text{isf}}(x_{\text{ECM}}, t)/dt = & k_{+1}[Alb]_{\text{isf}}(x_{\text{ECM}}, t)[uFA]_{\text{isf}}(x_{\text{ECM}}, t) \\ & + k_{-1}[AF_2]_{\text{isf}}(x_{\text{ECM}}, t) + k_{\text{dis}}[AF_1R] \\ & - \{k_{\text{bind}}[R] + k_{-1} + k_{+2}[uFA]_{\text{isf}} \\ & \times (x_{\text{ECM}}, t)\}[AF_1]_{\text{isf}}(x_{\text{ECM}}, t), \end{aligned} \quad (19)$$

$$\begin{aligned} d[AF_2]_{\text{isf}}(x_{\text{ECM}}, t)/dt = & k_{+2}[AF_1]_{\text{isf}}(x_{\text{ECM}}, t)[uFA]_{\text{isf}}(x_{\text{ECM}}, t) \\ & + k_{-1}[AF_3]_{\text{isf}}(x_{\text{ECM}}, t) + k_{\text{dis}}[AF_2R] \\ & - \{k_{\text{bind}}[R] + k_{-1} + k_{+3}[uFA]_{\text{isf}} \\ & \times (x_{\text{ECM}}, t)\}[AF_2]_{\text{isf}}(x_{\text{ECM}}, t), \end{aligned} \quad (20)$$

$$\begin{aligned} d[AF_3]_{\text{isf}}(x_{\text{ECM}}, t)/dt = & k_{+3}[AF_2]_{\text{isf}}(x_{\text{ECM}}, t)[uFA]_{\text{isf}}(x_{\text{ECM}}, t) \\ & + k_{\text{dis}}[AF_3R] - (k_{\text{bind}}[R] + k_{-1}) \\ & \times [AF_3]_{\text{isf}}(x_{\text{ECM}}, t), \end{aligned} \quad (21)$$

Albumin–FA complexes as 'direct' FA source

A second hypothetical mechanism of the albumin receptor is the facilitated release of FAs from the albumin–FA complexes directly into the outer leaflet of the sarcolemma. The equations of this mechanism are identical to Eqns 1–5, 7, 12–16 with boundary conditions Eqns 8–11, 17–21. Eqn 6 is now replaced by

$$\begin{aligned} d[uFA]_o/dt = & k_{\text{on}}[uFA]_{\text{isf}}(x_{\text{ECM}}, t) + k_{\text{rel}}([AF_1R] + [AF_2R] \\ & + [AF_3R]) - k_{\text{off}}[uFA]_o - k_{\text{flip}}([uFA]_o - [uFA]_i), \end{aligned} \quad (22)$$

Extracellular matrix as a potential hindrance to albumin mobility

Experimental observations have shown that the glycocalyx could hamper albumin mobility [34]. It was felt that including albumin hindrance, in the interstitial compartment, by the extracellular matrix should make our models more realistic. Therefore, we incorporated the extracellular matrix as a diffusional barrier for albumin ($D_{\text{Alb}} = 0$) in the models with unmediated transmembrane diffusion (Eqns 1–7), mediated transmembrane diffusion (Eqns 1–7, but with larger $\lambda_{m/w}$, i.e. the involvement of specific membrane-associated proteins in trans-sarcolemmal FA transport, see below) and mediated transmembrane diffusion with an albumin receptor for 'direct' FA absorption (Eqns 1–5, 7, 12–16, 22). The impact of tethered albumin on the overall FA transport rate was tested *in silico* by analysing the maximum effect of albumin restriction on FA transport rates. It should be noted that complete blocking of albumin diffusion has not been observed under physiological circumstances.

Parameter values, simulation procedures and experimental validation

Most parameter values could be retrieved from the literature (Table 1), using oleate as main FA representative, since it comprises the largest percentage of FA species in the total plasma FA pool [35]. The total albumin receptor concentration, $[R]_{\text{tot}}$, was determined indirectly by calculation, which is explained in the Appendix in more detail.

For the transmembrane diffusion part of the model, parameter value ranges were taken from various sources, using measuring techniques like stopped-flow measurements with vesicles containing the pH-sensitive fluorophore pyranine or ADIFAB (acrylodan-labelled intestinal fatty acid binding protein) [6,18,36]. The most recent reinvestigation of this issue took both methods into account, which resulted in more reliable values: $k_{\text{off}} = 3 \text{ s}^{-1}$ and $k_{\text{flip}} = 0.5 \text{ s}^{-1}$ in artificial membranes, solely composed of phospholipids [37]. A considerable variation in membrane partition coefficient $\lambda_{m/w}$ has been reported: in artificial membranes (4×10^5) [32], in plasma membrane vesicles (1×10^6) [32], and in adipocyte membranes (1.4×10^6) [36]. The variation in values of $\lambda_{m/w}$ was almost 3-fold, and may readily be ascribed to differences between artificial and biological membranes. It should be emphasized that membrane-associated proteins such as FABP_{PM}, FATP and CD36/FAT [30,39,41] could enhance the absorption of uFAs into the outer leaflet of biological membranes, resulting in larger values of $\lambda_{m/w}$, whereas the artificial membranes used by Richieri et al. [32] were devoid of these proteins [37].

The parameter values k_{dis} and k_{rel} were varied within ranges that were reported in the literature [14]. The other parameter values were obtained from various sources (Table 1).

The trans-sarcolemmal FA uptake rate J_{FA} (in nmol/g min) was used as a measure and determined from the steady-state solution of Eqns 6–7 (for the albumin receptor with 'direct' FA absorption, Eqns 7 and 22 were used):

$$J_{\text{FA}} = \alpha k_{\text{flip}}([uFA]_o - [uFA]_i) \quad (23)$$

With

$$\alpha = \beta A_{\text{heart}} x_{\text{mem}}/2, \quad (24)$$

where α = constant for converting mol/(dm³ s) to nmol/(g min); β = factor for converting mol/s to nmol/min; A_{heart} = diffusion area of myocardial sarcolemma per g ww (in dm²/g); x_{mem} = membrane thickness (in dm).

The partial derivatives in Eqns 1–5 were replaced with central, second-order, finite differences, as was done previously [21,24]. A spatial discretisation of 50 segments was applied, which gave an accurate approximation of the partial derivatives in Eqns 1–5 when tested against using more segments [24]. The resulting set of first-order equations were solved with ode15s, a ‘stiff’ differential equation solver in MATLAB 6.5 (The MathWorks, Inc.).

The computer models were validated by comparing the predicted FA transport rates with experimentally found FA uptake rates in the intact heart. A trans-sarcolemmal FA uptake rate of 65 to 85 nmol/(g min) was taken as a physiologically relevant range under normal conditions [11,23].

RESULTS AND DISCUSSION

Unmediated transmembrane diffusion of uFAs is physiologically insufficient

FA uptake rates by means of transmembrane diffusion with uFAs in the ISF as source using $\lambda_{m/w} = 4 \times 10^5$, as measured in artificial membranes [32], were calculated with Eqns 23–24 and are displayed in Figure 3. In Figure 3(A), the range of values for ‘flip-flop’ rate parameter k_{flip} (0 to 80 s⁻¹) and the desorption rate parameter k_{off} (0 to 15 s⁻¹) was chosen within the range published in earlier studies [17,18,29]. It can be appreciated from Figure 3(A) that physiologically relevant values for FA uptake rates, i.e. 65 nmol/(g min), could only be obtained at values for k_{flip} and k_{off} around 10 s⁻¹ for each parameter. However, a recent reinvestigation of k_{flip} - and k_{off} -values in phospholipid membranes by Cupp et al. [37] has shown that the original values [17,29] were incorrect, because the experimental setup required very high uFA concentrations that might perturb the membrane structure and consequently result in deviant FA transport rates. Therefore, the experimental conditions were adapted to more appropriate physiological conditions and the recently published parameter values of k_{flip} and k_{off} were in fact much lower ($k_{\text{flip}} = 0.5$ s⁻¹ and $k_{\text{off}} = 3$ s⁻¹) [37]. Applying the value found by Cupp et al. [37], the estimated FA uptake rate J_{FA} was found to be on the order of 9.0 nmol/(g min) (Figure 3B, white cross). Therefore, compared to physiologically relevant rate values of 65 to 85 nmol/(g min), the simulated FA transport rate through a membrane, consisting solely of phospholipids, with uFAs as source [37] and a partition coefficient $\lambda_{m/w}$ of 4×10^5 [35] was found to be totally insufficient to fulfil myocardial FA requirements.

Membrane-associated proteins putatively involved in trans-sarcolemmal FA transport

Kamp et al. [36] reported in a study on adipocyte membranes a more than three-times larger value for $\lambda_{m/w}$ than was found for artificial membranes, i.e. 1.4×10^6 compared with 4×10^5 [32]. The substantial increase in parameter value can most likely be ascribed to differences between artificial and biological membranes, such as phospholipid composition, cholesterol content and/or the presence of membrane-associated proteins. Simulations using the larger $\lambda_{m/w}$ value, reflecting a larger k_{on} if k_{off} is assumed constant, and the recently determined parameter values of k_{flip} and k_{off} [37] produced an estimated J_{FA} of approx. 29 nmol/(g min) (Figure 4, white cross).

Again, this value was not large enough to explain a physiological rate of 65 to 85 nmol/(g min). Physiologically relevant FA uptake rates were achieved only with k_{flip} - and k_{off} -values being much higher than measured in plain phospholipid bilayers, i.e., $k_{\text{flip}} = 0.5$ s⁻¹ and $k_{\text{off}} = 3$ s⁻¹ [37]. As hypothesized by Abumrad et al. [30], the potential involvement of membrane-

Figure 3 FA transport rates in nmol/(g min) during transmembrane diffusion of uFAs for $\lambda_{m/w} = 4 \times 10^5$

This value of $\lambda_{m/w}$ was obtained by [32] with artificial membranes (see Table 1). (A) The grey-scale of the contour plot indicates the magnitude of the trans-sarcolemmal FA transport rate; the numbers on the contour lines represent the corresponding rate values in nmol/(g min). The parameter values for translocation of FAs from outer to inner leaflet, k_{flip} , and desorption of FAs from the membrane, k_{off} , were varied over a large range to explore their effect on FA transport rates. (B) Magnification of (A) with recently determined parameter values for $k_{\text{off}} = 3$ s⁻¹ and $k_{\text{flip}} = 0.5$ s⁻¹ [37] (white cross) indicates a FA transport rate of approx. 9 nmol/(g min).

associated proteins could increase both k_{on} and k_{flip} . In fact, a physiologically relevant FA uptake rate of approx. 65 nmol/(g min) was observed in our simulations for $k_{\text{on}} = 4.2 \times 10^6$ (corresponding to $k_{\text{off}} = 3$ s⁻¹, $\lambda_{m/w} = 1.4 \times 10^6$) and a 10 times larger k_{flip} value (5 s⁻¹) than measured by Cupp and colleagues in plain phospholipid membranes [37] (Figure 5, solid line). To the best of our knowledge alterations in k_{off} due to membrane-associated proteins have not been reported in literature and were therefore not explored in the present study.

It should be emphasized that FA uptake rate values higher than 85 nmol/(g min) could only be obtained at very high k_{flip} values, i.e. $k_{\text{flip}} \gg 20$ s⁻¹ (Figure 5A). Such high k_{flip} values are, however, from a biophysical point of view unlikely. Moreover, the FA uptake rate rapidly approaches a limit value for larger k_{flip} values. This notion strongly suggests the presence of additional factors, contributing to the transport rate of FA across the sarcolemma, when the cardiac need for FAs is in the higher range, for instance, during increased

Figure 4 FA transport rates in nmol/(g min) during transmembrane diffusion of uFAs with $\lambda_{m/w} = 1.4 \times 10^6$, in other words for a larger k_{on}

This value for $\lambda_{m/w}$ was obtained in adipocyte membranes [36] and assumed to hold for the myocardial sarcolemma as well. The grey-scale of the contour plot indicates the trans-sarcolemmal FA transport rate; the numbers on the contour lines represent the corresponding rate values in nmol/(g min). Again, the values for translocation of FAs from outer to inner leaflet, k_{flip} , and desorption of FAs from the membrane, k_{off} , were varied over the range as reported by [37]. These parameter values ($k_{off} = 3 \text{ s}^{-1}$ and $k_{flip} = 0.5 \text{ s}^{-1}$, see Table 1), are also indicated (white cross), corresponding to a FA transport rate of approx. 29 nmol/(g min).

workload. Therefore, the potential role of a sarcolemmal albumin receptor in transmembrane FA diffusion was investigated.

Albumin receptor contributes to an additional increase in trans-sarcolemmal FA transport

In general, the role of an albumin receptor is supposed to increase the supply of uFAs to the outer leaflet of the phospholipid bilayers and, hence, to create a steeper FA gradient across the sarcolemma. We investigated two different potential mechanisms of the albumin receptor. In the first mechanism we considered the facilitated release of FAs from the FA–albumin–receptor complexes into the stagnant water layer of the ISF near the sarcolemma giving rise to a local [uFA] increase in close proximity to the membrane (uFA as an ‘indirect’ source, Figure 2A), i.e. the traditional line of thought. The second mechanism relates to facilitated release of FAs from the FA–albumin–receptor complex followed by a direct transfer of uFAs from albumin into the outer leaflet of the sarcolemma (uFAs as a ‘direct’ source, Figure 2B). Both cases were simulated with $k_{on} = 4.2 \times 10^6$ and k_{flip} values up to 20 s^{-1} (Figure 5A for uFA as ‘indirect’ source and Figure 5B for uFA as ‘direct’ source).

If the biological activity of the putative albumin receptor only increased the ‘indirect’ source for uFAs (Figure 5A), the FA uptake rate could readily be elevated to 65 nmol/(g min) for $k_{flip} = \text{approx. } 2 \text{ s}^{-1}$ when k_{dis} and k_{rel} were set at 100 s^{-1} . As expected, the stimulatory effect of the receptor was found to be dependent on the choice of parameters k_{dis} and k_{rel} , i.e. the dissociation rate constant of albumin–FA complex from the albumin receptor and the release rate of FAs from the FA–albumin–receptor complexes respectively. For the sake of simplicity, the values for k_{dis} and k_{rel} were assumed to be similar in the present computer calculations. In general, larger values of the release rate of the FAs from the FA–albumin–receptor complex resulted in greater FA uptake. ‘Direct’ absorption of FAs from the FA–albumin–receptor complex into the outer leaflet (Figure 2B) was found to be more effective and increased FA uptake to even larger trans-sarcolemmal rates, i.e.

Figure 5 Trans-sarcolemmal FA transport rates in nmol/(g min) when an albumin receptor was taken into account

Two different FA uptake mechanisms were simulated as function of k_{flip} . (A) ‘Indirect’ FA source: FAs are released from the FA–albumin–receptor complex into the stagnant water layer (see also Figure 2A). The values for k_{rel} and k_{dis} were varied within physiological range and are listed together with the other parameter values in Table 1. Assigning larger values for k_{rel} and k_{dis} resulted in increased FA uptake rates. For example, an approx. 30% increase (compared with no albumin receptor present) in FA uptake rate was observed if $k_{rel} = k_{dis} = 100 \text{ s}^{-1}$. This rise in FA uptake rates becomes quantitatively significant for larger k_{flip} -values. (B) ‘Direct’ FA source: FAs are released directly into the outer leaflet of the sarcolemma (see also Figure 2B). Simulations were conducted with the same parameters as used for the ‘indirect’ FA source. Once again, larger k_{rel} and k_{dis} enhanced FA transport, but this was more pronounced than with the ‘indirect’ method: an approx. 55% rise was found for $k_{rel} = k_{dis} = 100 \text{ s}^{-1}$.

approx. 85 nmol/(g min) for $k_{flip} = 2 \text{ s}^{-1}$ (Figure 5B), than in the ‘indirect’ option, because the release into the ISF and subsequent absorption of uFAs into the outer leaflet are bypassed in the ‘direct’ option. Since details about the mechanism of action of the albumin receptor in FA transport are lacking in the literature, at present no conclusion can be drawn as to whether the albumin receptor-mediated absorption of uFAs is ‘direct’ or ‘indirect’.

Our simulations indicate that, even if an albumin receptor is involved, physiologically relevant FA uptake rates could only be realised for k_{flip} values of the order of 2 s^{-1} and higher, irrespective of the mechanism(s) underlying the increased release of FAs from the FA–albumin–receptor complex (Figure 5). It should be emphasized that a k_{flip} of 2 s^{-1} is considerably higher than 0.5 s^{-1} , as found in artificial phospholipid membranes by Cupp et al.

Table 2 Effect of albumin mobility on FA uptake rates

	$(J_{FA})_{D=5.2}$ (nmol/(g min)) $D_{AF} = 5.2 \times 10^{-7} \text{ cm}^2 \cdot \text{s}^{-1}$	$(J_{FA})_{D=0}$ (nmol/(g min)) $D_{AF} = 0 \text{ cm}^2 \cdot \text{s}^{-1}$	$(J_{FA})_{D=0}/(J_{FA})_{D=5.2}$
Unmediated transmembrane diffusion*	8.9	8.0	0.9
Mediated transmembrane diffusion†	76.1	37.3	0.49
Mediated transmembrane diffusion with albumin receptor‡	102.3	35.1	0.34

The rate parameters from Table 1 were used, unless indicated otherwise. For transmembrane diffusion, the following rate parameters were applied:

* $k_{flip} = 0.5 \text{ s}^{-1}$, $\lambda_{m/w} = 4 \times 10^5$.

† $k_{flip} = 5 \text{ s}^{-1}$, $\lambda_{m/w} = 1.4 \times 10^6$.

‡ $k_{flip} = 5 \text{ s}^{-1}$, $\lambda_{m/w} = 1.4 \times 10^6$, $k_{rel} = k_{dis} = 100 \text{ s}^{-1}$, 'direct' FA absorption.

[37]. This notion strongly suggests that membrane-associated proteins, other than the putative albumin receptor, are involved in translocation of FAs across the sarcolemma. The biological activity of these proteins should be increasing the value of k_{flip} . The presence of a protein which facilitates FA absorption into the outer leaflet was already implicated by setting $\lambda_{m/w}$ at 1.4×10^6 instead of 4×10^5 . These conclusions underscore the notion that the albumin receptor may only fulfil an assisting role in trans-sarcolemmal FA transport.

Unimpeded mobility of albumin-FA complexes is required for high sarcolemmal transport rates of FAs

It should be stated that in the above calculations we assumed an unrestricted diffusion of the FA-albumin complexes through the ISF. This might not be the case if the extracellular matrix hinders the diffusion of albumin, analogous to the obstructing effect of the glycocalyx on albumin diffusion in the microvascular compartment [34].

Because the extracellular matrix might impede albumin mobility in the ISF, obstruction of albumin complex mobility was incorporated in the modelling by reducing its diffusion coefficient D_{AF} to zero. We performed additional simulations with $D_{AF} = 10^{-17} \text{ cm}^2 \cdot \text{s}^{-1}$ (values obtained from studies on the microvascular glycocalyx [34]), but the FA uptake rates differed marginally from the worst case scenario ($D_{AF} = 0 \text{ cm}^2 \cdot \text{s}^{-1}$). The effects of the latter on the FA uptake rates are depicted in Table 2. As can be seen, complete hindrance of albumin reduced the FA transport rate in the case of unmediated transmembrane diffusion only by 10%. These percentages become more evident for higher rates, i.e. approx. 51% and approx. 66% reduction in sarcolemmal FA transport if membrane-associated proteins were involved, without and with an albumin receptor respectively. These findings imply that the overall FA uptake rate is highly sensitive to diffusional hindrance of the albumin-FA complex, when sarcolemmal albumin receptors and membrane-associated proteins are assumed to be involved to reach physiologically relevant FA uptake rates. However, it is uncertain whether, under the physiological circumstances in the beating heart, the impact of the extracellular matrix on albumin-coupled FA transport is so large. The possibility cannot be excluded that the mobility of albumin is substantially increased by continuous stirring of solutes in the interstitial compartment as a consequence of the repeated contraction and relaxation cycle of the cardiac muscle cells.

The nature of membrane-associated transport proteins putatively involved in trans-sarcolemmal FA transport

The classical transmembrane transporter designed to channel substrates through membranes, such as the glucose transporter, was not considered in this paper as a potential facilitator of trans-sarcolemmal FA transport, since the structure of the various candi-

dates are not suitable for channeling uFAs through a pore in the membrane, as will be explained below. It is known that FAs traverse the lipid phase of artificial phospholipid membranes and, therefore, the classical type of transmembrane transporter is most probably not involved to facilitate FA flux across a biological membrane [14,16,38]. Moreover, with the use of a computational model we recently failed to find any support for the plausibility of a transport protein channelling FAs through the sarcolemma analogous to glucose transport [24]. In contrast, possible candidates that could assist FA transfer across biological membranes by alternative mechanisms are the membrane-associated proteins CD36/FAT [9,30], FATP [39] and FABP_{PM} [41]. Expression of the cDNAs of these candidates in various cell types resulted in increased, saturable cellular FA uptake. Native CD36/FAT was identified in adipocyte membranes as an 88 kDa protein with two transmembrane spanning regions, which reversibly binds to uFAs [40]. In comparison, FATP has multiple membrane-associated domains that are associated with the inner membrane leaflet and no specific binding sites for uFAs, but is closely linked to the synthesis of very long-chain fatty acyl-CoA [39]. A third candidate for which a role in FA uptake has been established, i.e. the 43 kDa protein FABP_{PM}, has shown to be identical to mitochondrial aspartate aminotransferase [41].

Recent investigations have suggested that these candidate proteins could play a crucial role in modulating myocardial FA uptake, although the exact mechanism by which these proteins facilitate transmembrane FA transport is still largely unknown [10]. One could envision that the absorption of FAs into the membrane (k_{on}) or the FA translocation rate from the outer to the inner leaflet (k_{flip}) is enhanced by decreasing the activation energy for movement of the polar carboxy group through the membrane, as previously hypothesized by Abumrad et al. [31]. Given the values chosen for parameters characterizing the process of trans-sarcolemmal FA transport in the present study, our computer model clearly shows that both FA absorption into the outer leaflet and translocation of FA to the inner leaflet of the sarcolemma have to be elevated simultaneously to increase the overall FA uptake rate from ISF to the cytoplasm of the cardiomyocyte to physiologically relevant levels. This notion corroborates the earlier proposed, but still hypothetical, properties of CD36/FAT, although it does not exclude the participation of other membrane-associated proteins in facilitating FA transfer through the sarcolemma. Therefore, in the present study we propose a putative FA-TFPC, which promotes the absorption of uFAs in the outer leaflet of the membrane and the subsequent facilitated translocation process through the phospholipid bilayer of the sarcolemma. Whether the FA-TFPC consists of a single protein or a combination of multiple proteins remains to be established. In this respect it is of interest also to reiterate the potential role of the albumin receptor in accomplishing physiologically relevant trans-sarcolemmal FA uptake rates if the real physiological values of

Figure 6 Schematic representation of the hypothetical process of FAs transport across the myocardial sarcolemma

According to the outcome of our simulations, the following trans-sarcolemmal FA uptake process is proposed: (I) an albumin–FA complex interacts with a sarcolemmal albumin receptor; this interaction facilitates the release of FA from the FA–albumin–receptor complex. FA is either released into the ISF (IIa), followed by facilitated absorption into the outer leaflet by the hypothetical FA–TFPC or directly transferred into the outer leaflet (IIb). The membrane-associated protein complex is assumed to enhance FA translocation ('flip-flop') from outer to inner leaflet of the sarcolemma (III). Thereafter FAs desorb (IV) from the inner leaflet of the sarcolemma into the cytoplasm and are stored or oxidized intracellularly (V).

k_{flip} for FA movement through the phospholipid bilayer are in the low range. The collected findings of the present study are summarized in the hypothetical model of a protein-mediated FA uptake process across the myocardial sarcolemma in Figure 6.

General conclusion

In the present paper, computer models of trans-sarcolemmal FA transport were developed to delineate the mechanisms underlying FA transfer across the sarcolemma. Various hypotheses regarding trans-sarcolemmal FA transport were simulated in a computer model to integrate recent findings obtained in a variety of experimental models, ranging from artificial vesicles to intact hearts, and to predict the kinetic properties of putative processes and proteins involved in transmembrane FA trafficking. Our simulations showed that unmediated transmembrane diffusion across the myocardial sarcolemma is quantitatively insufficient to reach

physiologically relevant FA uptake rates into cardiac muscle cells, under the assumption that the recently determined parameter values for k_{off} and k_{flip} [37] were correct and applicable to the contracting cardiomyocyte. A recent study in which $\lambda_{\text{m/w}}$ was determined for the plasmalemma of fat cells, indicated a substantially larger $\lambda_{\text{m/w}}$ in biological than in artificial membranes [36]. This finding could be considered as indirect evidence for an increased absorption of uFAs in biological membranes, possibly mediated by membrane-associated proteins, putatively involved in trans-plasmalemmal FA transport [31]. Although implementing the larger $\lambda_{\text{m/w}}$ in our computational model resulted in appreciably higher trans-sarcolemmal FA uptake rates, physiologically relevant transport rates of the order of 65 to 85 nmol/(g min) were still not reached; detailed analysis revealed that translocation of FAs from outer to inner leaflet, characterized by k_{flip} , represented a rate-limiting step. Interestingly, in earlier studies Abumrad et al. [31] have hypothesized that membrane-associated proteins might increase the rate of the translocation step itself. According to our simulations, only if both the absorption of uFAs from the ISF into the membrane (k_{on}) and the translocation of FAs from outer to inner leaflet of the sarcolemma (k_{flip}) become sufficiently large can FA uptake rates as observed in the intact heart be achieved by our model. Finally, the present model calculations also indicated that a facilitating role of the albumin receptor in FA uptake is highly feasible. The presence of an albumin receptor resulted in a further increase of the trans-sarcolemmal FA uptake rate of approx. 55 % by accelerating the release of FA from the FA–albumin–receptor complex. This increment could be of importance especially if high FA transport rates are required, for instance when cardiac workload is increased resulting in an enhanced need for oxidizable substrates.

It should be realized that experimental models are limited in their ability to measure, for instance, the real values of k_{flip} in the sarcolemma, because of difficulties in separating the trans-membrane movement of FAs from supply, absorption into, and desorption from the biological membrane in the intact sarcolemma. Therefore studies have been limited to preparations of artificial membranes (liposomes) or vesicles derived from biological membranes, the properties of which may be changed by the isolation procedure. It is felt that a mathematical model which can predict these values is of great help to elucidate the mechanisms underlying the transport of FAs across the sarcolemma and to quantify each step in the trans-sarcolemmal FA uptake process.

APPENDIX

Most parameter values could be obtained from the literature. Two parameters were calculated as shown in this Appendix. The average human cardiomyocyte has a diameter d of approx. 15 to 18 μm and a length l of 75 to 90 μm . A cylindrical geometry with $d = 16.5 \mu\text{m}$ and $l = 82.5 \mu\text{m}$ was assumed and, hence, a single cardiomyocyte surface area A_{cell} of $4.7 \times 10^3 \mu\text{m}^2$ could be deduced. The total sarcolemmal surface area A_{heart} is approx. 2000 cm^2/g [42]. In addition, 10^7 binding sites for albumin were reported to be present per adipocyte [14,43]. The average diameter of a spherical adipocyte is approx. 100 μm and assuming the same distribution of these binding sites on the cardiomyocyte surface results in approx. 1.5×10^6 binding sites for a single cardiomyocyte. Hence, the total number of albumin receptors per g ww might be 6.4×10^{13} , which corresponds to 1.1×10^{-10} mol. The receptors were assumed to bind with albumin molecules that are within direct contact of this receptor. An estimate of the diameter of albumin is approx. 3.5 nm [44] and combined with A_{heart} gives a volume of

$7.0 \times 10^{-7} \text{ dm}^3$. Eventually, the receptor concentration becomes $1.1 \times 10^{-10} / 7.0 \times 10^{-7} = 1.6 \times 10^{-4} \text{ M}$. We assumed therefore an albumin receptor concentration of $[R]_{\text{tot}} = 0.16 \text{ mM}$.

REFERENCES

- Bing, R. J., Siegel, A., Ungar, I. and Gilbert, M. (1954) Metabolism of the human heart. II. Studies on fat, ketone and amino acid metabolism. *Am. J. Med.* **16**, 504–515
- van der Vusse, G. J., Glatz, J. F., Stam, H. C. and Reneman, R. S. (1992) Fatty acid homeostasis in the normoxic and ischemic heart. *Physiol. Rev.* **72**, 881–940
- Richieri, G. V., Anel, A. and Kleinfeld, A. M. (1993) Interactions of long-chain fatty acids and albumin: determination of free fatty acid levels using the fluorescent probe ADIFAB. *Biochemistry* **32**, 7574–7580
- Broring, K., Haest, C. W. and Deuticke, B. (1989) Translocation of oleic acid across the erythrocyte membrane. Evidence for a fast process. *Biochim. Biophys. Acta* **986**, 321–331
- Cooper, R. B., Noy, N. and Zakim, D. (1989) Mechanism for binding of fatty acids to hepatocyte plasma membranes. *J. Lipid Res.* **30**, 1719–1726

- 6 Hamilton, J. A., Guo, W. and Kamp, F. (2002) Mechanism of cellular uptake of long-chain fatty acids: Do we need cellular proteins? *Mol. Cell. Biochem.* **239**, 17–23
- 7 Zakim, D. (1996) Fatty acids enter cells by simple diffusion. *Proc. Soc. Exp. Biol. Med.* **212**, 5–14
- 8 Storch, J., Lechene, C. and Kleinfeld, A. M. (1991) Direct determination of free fatty acid transport across the adipocyte plasma membrane using quantitative fluorescence microscopy. *J. Biol. Chem.* **266**, 13473–13476
- 9 Harmon, C. M. and Abumrad, N. A. (1993) Binding of sulfosuccinimidyl fatty acids to adipocyte membrane proteins: isolation and amino-terminal sequence of an 88-kD protein implicated in transport of long-chain fatty acids. *J. Membr. Biol.* **133**, 43–49
- 10 Luiken, J. J. F. P., Van Nieuwenhoven, F. A., America, G., van der Vusse, G. J. and Glatz, J. F. C. (1997) Uptake and metabolism of palmitate by isolated cardiac myocytes from adult rats: involvement of sarcolemmal proteins. *J. Lipid Res.* **38**, 745–758
- 11 van der Vusse, G. J., van Bilsen, M. and Glatz, J. F. C. (2000) Cardiac fatty acid uptake and transport in health and disease. *Cardiovasc. Res.* **45**, 279–293
- 12 Pelsers, M. M. A. L., Lutgerink, J. T., van Nieuwenhoven, F. A., Tandon, N. N., van der Vusse, G. J., Arends, J.-W., Hoogenboom, H. R. and Glatz, J. F. C. (1999) A sensitive immunoassay for rat fatty acid translocase (CD36) using phage antibodies selected on cell transfectants: abundant presence of fatty acid translocase/CD36 in cardiac and red skeletal muscle and up-regulation in diabetes. *Biochem. J.* **337**, 407–414
- 13 Brinkmann, J. F., Abumrad, N. A., Ibrahimi, A., van der Vusse, G. J. and Glatz, J. F. C. (2002) New insights into long-chain fatty acid uptake by heart muscle: a crucial role for fatty acid translocase/CD36. *Biochem. J.* **367**, 561–570
- 14 Ockner, R. K., Weisiger, R. A. and Gollan, J. L. (1983) Hepatic uptake of albumin-bound substances: albumin receptor concept. *Am. J. Physiol.* **245**, G13–G18
- 15 Hutter, J. F., Piper, H. M. and Spieckermann, P. G. (1984) Kinetic analysis of myocardial fatty acid oxidation suggesting an albumin receptor mediated uptake process. *J. Mol. Cell. Cardiol.* **16**, 219–226
- 16 Rose, H., Hennecke, T. and Kammermeier, H. (1990) Sarcolemmal fatty acid transfer in isolated cardiomyocytes governed by albumin/membrane-lipid partition. *J. Mol. Cell. Cardiol.* **22**, 883–892
- 17 Kamp, F., Westerhoff, H. V. and Hamilton, J. A. (1993) Movement of fatty acids, fatty acid analogues, and bile acids across phospholipid bilayers. *Biochemistry* **32**, 11074–11086
- 18 Kleinfeld, A. M., Chu, P. and Romero, C. (1997) Transport of long-chain native fatty acids across lipid bilayer membranes indicates that transbilayer flip-flop is rate limiting. *Biochemistry* **36**, 14146–14158
- 19 Hamilton, J. A. (1998) Fatty acid transport: difficult or easy? *J. Lipid Res.* **39**, 467–481
- 20 Luiken, J. J. F. P., Koonen, D. P. Y., Willems, J., Zorzano, A., Becker, C., Fischer, Y., Tandon, N. N., van der Vusse, G. J., Bonen, A. and Glatz, J. F. C. (2002) Insulin stimulates long-chain fatty acid utilization by rat cardiac myocytes through cellular redistribution of FAT/CD36. *Diabetes* **51**, 3113–3119
- 21 Barta, E., Sideman, S. and Bassingthwaighe, J. B. (2000) Facilitated diffusion and membrane permeation of fatty acid in albumin solutions. *Ann. Biomed. Eng.* **28**, 331–345
- 22 van der Vusse, G. J., Roemen, T. H., Prinzen, F. W., Coumans, W. A. and Reneman, R. S. (1982) Uptake and tissue content of fatty acids in dog myocardium under normoxic and ischemic conditions. *Circ. Res.* **50**, 538–546
- 23 Vyska, K., Meyer, W., Stremmel, W., Notohamiprodjo, G., Minami, K., Machulla, H. J., Gleichmann, U., Meyer, H. and Korfer, R. (1991) Fatty acid uptake in normal human myocardium. *Circ. Res.* **69**, 857–870
- 24 Musters, M. W. J. M., Bassingthwaighe, J. B., Panday, V., van Riel, N. A. W. and van der Vusse, G. J. (2004) Computational modeling of cardiac fatty acid uptake and utilization. In *Lipobiology* (van der Vusse, G. J., ed.), pp. 173–224, Elsevier, Amsterdam
- 25 Hamilton, J. A., Johnson, R. A., Corkey, B. and Kamp, F. (2001) Fatty acid transport: the diffusion mechanism in model and biological membranes. *J. Mol. Neurosci.* **16**, 99–108
- 26 Spector, A. A., Fletcher, J. E. and Ashbrook, J. D. (1971) Analysis of long-chain free fatty acid binding to bovine serum albumin by determination of stepwise equilibrium constants. *Biochemistry* **10**, 3229–3232
- 27 Ashbrook, J. D., Spector, A. A., Santos, E. C. and Fletcher, J. E. (1975) Long chain fatty acid binding to human plasma albumin. *J. Biol. Chem.* **250**, 2333–2338
- 28 Weisiger, R., Gollan, J. and Ockner, R. (1981) Receptor for albumin on the liver cell surface may mediate uptake of fatty acids and other albumin-bound substances. *Science (Washington, D.C.)* **211**, 1048–1051
- 29 Hamilton, J. A. and Kamp, F. (1999) How are free fatty acids transported in membranes? Is it by proteins or by free diffusion through the lipids? *Diabetes* **48**, 2255–2269
- 30 Abumrad, N., Harmon, C. and Ibrahimi, A. (1998) Membrane transport of long-chain fatty acids: evidence for a facilitated process. *J. Lipid Res.* **39**, 2309–2318
- 31 Abumrad, N., Coburn, C. and Ibrahimi, A. (1999) Membrane proteins implicated in long-chain fatty acid uptake by mammalian cells: CD36, FATP and FABPm. *Biochim. Biophys. Acta* **1441**, 4–13
- 32 Richieri, G. V., Ogata, R. T. and Kleinfeld, A. M. (1995) Thermodynamics of fatty acid binding to fatty acid-binding proteins and fatty acid partition between water and membranes measured using the fluorescent probe ADIFAB. *J. Biol. Chem.* **270**, 15076–15084
- 33 Popov, D., Hasu M., Ghinea N., Simionescu, N. and Simionescu, M. (1992) Cardiomyocytes express albumin binding proteins. *J. Mol. Cell. Cardiol.* **24**, 989–1002
- 34 Vink, H. and Duling, B. R. (2000) Capillary endothelial surface layer selectively reduces plasma solute distribution volume. *Am. J. Physiol. Heart Circ. Physiol.* **278**, H285–H289
- 35 Richieri, G. V. and Kleinfeld, A. M. (1995) Unbound free fatty acid levels in human serum. *J. Lipid Res.* **36**, 229–240
- 36 Kamp, F., Guo, W., Souto, R., Pilch, P. F., Corkey, B. E. and Hamilton, J. A. (2003) Rapid flip-flop of oleic acid across the plasma membrane of adipocytes. *J. Biol. Chem.* **278**, 7988–7995
- 37 Cupp, D., Kampf, J. P. and Kleinfeld, A. M. (2004) Fatty acid-albumin complexes and the determination of the transport of long chain free fatty acids across membranes. *Biochemistry* **43**, 4473–4481
- 38 Rose, H., Hennecke, T. and Kammermeier, H. (1989) Is fatty acid uptake in cardiomyocytes determined by physicochemical fatty acid partition between albumin and membranes? *Mol. Cell. Biochem.* **88**, 31–36
- 39 Schaffer, J. E. and Lodish, H. F. (1994) Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein. *Cell* **79**, 427–436
- 40 Storch, J. and Thumser, A. E. (2000) The fatty acid transport function of fatty acid-binding proteins. *Biochim. Biophys. Acta* **1486**, 28–44
- 41 Stremmel, W., Strohmeyer, G., Borchard, F., Kochwa, S. and Berk, P. D. (1985) Isolation and partial characterization of a fatty acid binding protein in rat liver plasma membranes. *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4–8
- 42 Bassingthwaighe, J. B., Noodleman, L., van der Vusse, G. J. and Glatz, J. F. C. (1989) Modeling of palmitate transport in the heart. *Mol. Cell. Biochem.* **88**, 51–58
- 43 Brandes, R., Ockner, R. K., Weisiger, R. A. and Lysenko, N. (1982) Specific and saturable binding of albumin to rat adipocytes: modulation by epinephrine and possible role in free fatty acid transfer. *Biochem. Biophys. Res. Commun.* **105**, 821–827
- 44 Luby-Phelps, K. (2000) Cytoarchitecture and physical properties of cytoplasm: volume, viscosity, diffusion, intracellular surface area. *Int. Rev. Cytol.* **192**, 189–221
- 45 Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. and Watson, J. D. (1994). *Membrane structure*. In *Molecular Biology of the Cell* (Robertson, M., Adams, R. and Goertzen, D., eds.), pp. 478–506, Garland Publishing, New York
- 46 Weisiger, R. A., Pond, S. and Bass, L. (1991) Hepatic uptake of protein-bound ligands: extended sinusoidal perfusion model. *Am. J. Physiol.* **261**, G872–G884
- 47 Demant, E. J., Richieri, G. V. and Kleinfeld, A. M. (2002) Stopped-flow kinetic analysis of long-chain fatty acid dissociation from bovine serum albumin. *Biochem. J.* **363**, 809–815

Received 27 May 2005/4 October 2005; accepted 6 October 2005

Published as BJ Immediate Publication 6 October 2005, doi:10.1042/BJ20050869