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Transport of Free Fatty Acids from Plasma to the Endothelium of Cardiac Muscle: A Theoretical Study

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Abstract Fatty acids are transported in a multistep process from the plasma to the mitochondria, where they are oxidized in order to meet energy requirements of the myocardium. Some of those steps, mainly the crossing of the involved cells' membranes are far from being understood. Here, by means of mathematical modeling we address the problem of the fatty acid transport from the microvascular compartment to the endothelium. Values of parameters that are incorporated in the model are deduced from relevant experimental work. Concentration profiles are established as solutions of diffusion–reaction equations both numerically and using an analytical asymptotic approximation. The analytical solution accurately determines the fatty acid flux for any set of parameter values in contrast to off-the-shelf numerical solvers that fail under quite a few circumstances due to the stiffness of the differential equation system. Sensitivity analysis indicates that in spite of few uncertain parameter values, most of our conclusions are expected to be valid throughout the physiological range of operation. We find that in order to have an adequate fatty acid uptake rate it is essential for the luminal endothelial membrane to have very fast fatty acid transporters and/or specific sites that interact with the albumin-fatty acids complex.

Keywords Asymptotic solutions · Facilitated diffusion · Free fatty acids uptake · Mathematical modeling · Membrane transporters

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Introduction

Quite a few thorough investigations, both experimental and theoretical, have been pursued in order to decipher the mechanisms that underlie the fatty acid transport in the myocardium (Bassingthwaighte et al. 1989a, b; Vyska et al. 1991; van der Vusse et al. 1992; Goresky et al. 1994; van der Vusse et al. 2000; Bonen et al. 2007). Within the plasma, the endothelium, and the interstitium, fatty acid transport is substantiated as a passive facilitated diffusion process where the fatty acids are bound to proteins like albumin or cytoplasmic fatty acid binding protein, FABP in order to reduce the concentration of free fatty acids to levels that do not damage cell membranes while keeping it high enough as to satisfy the energy requirements. However, the transport mechanisms across the various membranes (the luminal and abluminal membranes and the sarcolemma) are still obscure; specifically, it is unclear whether transportation at the membranes is basically simple, passive one or is it facilitated by receptors for proteins that are scattered on the membrane faces and/or a combination of transporters that facilitate translocation across the membranes. Such facilitation might be needed as a pretty high fatty acids uptake rate, about 50–100 nmol(g min)$^{-1}$ is essential for the normal functioning of the human heart (van der Vusse et al. 2000).

The dispute has been going on for decades—years ago some experimental evidences (Ghitescu et al. 1986; Simionescu et al. 1987; Weisiger 1993; Trigatti and Gerber 1995) indicated that cells' walls are probably covered with albumin receptors. Hutter et al. (1984) detected the presence of such receptors in rats' myocardial tissue and other measurements (Goresky et al. 1994) indicated that receptors must facilitate the fatty acid uptake into the endothelial membrane but the subject was inconclusive as others

(Potter et al. 1989; Reed and Burrington 1989) found no evidence for such receptors in hepatic tissue. While recent works (Bonen et al. 2007; Hamilton 2007; Glatz et al. 2010; Hagberg et al. 2013; Iso et al. 2013) have substantiated the action of fatty acid transporters across the membrane, the action of receptors for albumin was not validated (or ruled out).

The difficulties involved with the experimental investigations of transport across membranes turn them to be prone to many inaccuracies—hence the importance of a theoretical modeling. Moreover, a theoretical analysis might help to determine a measurement protocol that is best fitted to detect the presence of transporters and receptors taking into account the sensitivity with respect to unknown or uncontrolled parameters. Here we establish a model, based on diffusion–reaction equations that accounts for the specific composition of the long-chain fatty acids and that assumes two fatty acid binding sites per any albumin molecule. The fatty acid crossing of the luminal membrane is either a simple, passive permeation or is enhanced by the action of transporters. The choice of the model's parameter values is backed by measurements and is tested for its effect on the model reliability by performing ad-hoc sensitivity analysis. Adjacent to the membrane there is a boundary layer where the gradual, moderate decrease of the unbound fatty acids, typical to most of the stagnant slab, is replaced by a steep decline of the concentration. This boundary layer turns off-the-shelf numerical solvers to be quite inefficient and/or inaccurate. However, using singular perturbation approach enabled us to formulate analytical expressions for the fatty acid concentrations. This method of solution yields accurate estimations for the flux of the fatty acids and might be adapted to other scenarios (with different putative permeation mechanisms via the membrane) as well.

We aimed to determine, based on our simulation, whether a presence of receptors is essential for the observed fatty acid uptake rate in vivo. Indeed we conclude that unless there are transporters that cross the membrane with an exceptionally high rate, sites of interaction with the fatty acid–albumin complex are essential for adequate functioning of the myocardium.

Mathematical Modeling

Past theoretical models (Barta et al. 2000; Musters et al. 2006) referred to the fatty acids as either pure palmitic or oleic acid; however, Saifer and Goldman (1961) found by gas–liquid chromatography that oleic acid, palmitic acid, linoleic acid, and arachidonic acid each comprises 33, 25, 20, and 5 %, respectively of the serum while the other 39 different acids are present in lower percentages. The association constants for the binding of those four acids with albumin, given by Table 1 in Richeri et al. (1993), yield the following weighted dissociation constants for the first five binding sites: 8.1, 9.0, 16.4, 34.4, 67.5 nM. Henceforth we refer just to the first two binding sites and ignore the others.

The Governing Equations

The fatty acids that reach the endothelium are derived from the plasma where almost all of them are bound to albumin (e.g., Barta et al. 2000; Van der Vusse et al. 2000). Therefore computing their supply rate to the endothelium necessitates establishing fatty acid and protein profiles within the stagnant plasma layer near the capillary wall. The 1D region of solution extends from $x = 0$ (the centerline of the capillary in the in vivo heart or the interface with the plasma in perfusion in vitro experiments) to $x = x_1$ (the luminal endothelial membrane) and contains 4 constituents: Unbound fatty acids, F, albumin, A, and their two complexes, $AF1$ and $AF2$ with given volumetric concentrations: $\overline{C_F}, \overline{C_A}$, $\overline{C_{AF1}}$, and $\overline{C_{AF2}}$, respectively.

Within this region, albumin, and long-chain fatty acids react as follows (by our two binding sites assumption):

$$A + F \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} AF_1,\ AF_1 + F \underset{k_{-1}}{\overset{k_{+2}}{\rightleftharpoons}} AF_2. \tag{1}$$

In addition to the above reactions, there is a diffusion of all constituents and possibly, a metabolic consumption. Thus the time-dependent concentrations satisfy:

$$\frac{\partial \overline{C_A}}{\partial t} = D_A \frac{\partial^2 \overline{C_A}}{\partial x^2} - k_{+1}\overline{C_A C_F} + k_{-1}\overline{C_{AF1}} \tag{2}$$

$$\frac{\partial \overline{C_{AF2}}}{\partial t} = D_{AF} \frac{\partial^2 \overline{C_{AF2}}}{\partial x^2} + k_{+2}\overline{C_{AF1} C_F} - k_{-1}\overline{C_{AF2}} \tag{3}$$

$$\frac{\partial \overline{C_{AF1}}}{\partial t} = D_{AF} \frac{\partial^2 \overline{C_{AF1}}}{\partial x^2} + (k_{+1}\overline{C_A} - k_{+2}\overline{C_{AF1}})\overline{C_F} - k_{-1}(\overline{C_{AF1}} - \overline{C_{AF2}}) \tag{4}$$

$$\frac{\partial \overline{C_F}}{\partial t} = D_F \frac{\partial^2 \overline{C_F}}{\partial x^2} - (k_{+1}\overline{C_A} + k_{+2}\overline{C_{AF1}})\overline{C_F} + k_{-1}(\overline{C_{AF1}} + \overline{C_{AF2}}) - G_1\overline{C_F}, \tag{5}$$

where D_A, D_F, and D_{AF} are the diffusion coefficients of A, F and their two complexes respectively; k_{+1}, k_{-1}, k_{+2}, k_{-2} are the association and dissociation rate constants defined in Eq. 1 and G_1 is a metabolic consumption rate constant.

At $x = 0$ all concentrations equal their plasma values: C_{A0}, C_{F0}, C_{AF10}, C_{AF20} determined based on the data given in Table 1.

The membrane at $x = x_1$, is impermeable with respect to proteins therefore we write:

$$\frac{\partial \overline{C_A}(x_1,t)}{\partial x} = \frac{\partial \overline{C_{AF1}}(x_1,t)}{\partial x} = \frac{\partial \overline{C_{AF2}}(x_1,t)}{\partial x} = 0. \quad (6)$$

The flux of the fatty acids is given by Fick's law where P stands for the permeability constant of the membrane with respect to fatty acids and C_{Fendo} is the concentration of the free fatty acids at the outer face of the endothelial membrane:

$$D_F \frac{\partial \overline{C_F}(x_1,t)}{\partial x} = -P\left(\overline{C_F}(x_1,t) - C_{\mathrm{Fendo}}\right). \quad (7)$$

Alternatively, one may account for the activity of fatty acid transporters, T with total concentration C_{T} within the membrane. C_{T} is composed of concentrations C_{Ti} and C_{To} at the inner and outer faces of the membrane. Each of those is a sum of the concentrations of the unbound transporters and C_{FTi} (at the inner face) or C_{FTo} (at the outer face), the concentrations of the complex of the transporters and the fatty acids. Let the transporters cross the membrane with P_{T} as permeability constant then, Eq. 7 is replaced by:

$$D_F \frac{\partial \overline{C_F}(x_1,t)}{\partial x} = -P\left(\overline{C_F}(x_1,t) - C_{FTi} - C_{\mathrm{Fendo}} + C_{FTo}\right) - P_T(C_{FTi} - C_{FTo}). \quad (8)$$

Assuming that transporters and fatty acids are in equilibrium state, one may determine C_{FTo} by solving a quadratic equation that incorporates the given values of C_{To} and C_{Fendo}. Another quadratic equation for the inner side of the membrane enables us to express C_{FTi} as a function of $\overline{C_F}(x_1,t)$.

As for the needed initial conditions for Eqs. 2–5; we assume that all concentrations at $t = 0$ are zero throughout the region of solution except for at $x = 0$ where they are equal to their plasma values. Those are unrealistic initial conditions but as the transient period is short and does not affect the steady state situation (and, as there are no "realistic initial conditions" for the steady, working heart), the choice of these conditions is meaningless as long as one ensures compatibility with the boundary conditions.

Calibration of the Model (Parameter Values)

Here we extend on the choice of parameter values that are given in Table 1.

C_{F_T}, C_{A_T}: The Total Concentrations of Free Fatty Acids and Albumin Within the Plasma

Vascular albumin concentration is about 0.6 mM (van der Vusse et al. 2000; Zhou et al. 2002; van der Vusse 2009). Total fatty acids concentration, C_{F_T} varies between different species e.g., 0.21 mM in rats (Zhou et al. 2002), 0.62 mM in rabbits (Gilbert et al. 1993), and was reported to vary from 0.25 to 3 mM in humans (Hamilton and Kamp 1999). It depends on age, being higher in children than in adults (Ijzerman et al. 2009), on nutritional status and exercise, and is chronically higher in individuals with obesity and/or diabetes. Normal circulating fatty acid concentration is lower than 0.6 mM (Lopaschuk et al. 2010). The ratio between C_{F_T} and C_{A_T} was found to vary from 0.5 to 0.8 (Potter et al. 1989). We infer a value of $C_{F_T} = 0.4$ mM or 2/3 of C_{A_T}. Assuming that all constituents are in equilibrium and implementing the above-mentioned two

Table 1 Literature-based and computed parameter values for the present simulation

Parameter	Value	References
Typical radius of a capillary (x_1)	0.0005 cm	
Total albumin concentration in plasma (C_{A_T})	0.6 mM	van der Vusse 2009
Total fatty acid concentration in plasma (C_{F_T})	0.4 mM	Potter et al. 1989
Consumption rate constant (G_1)	0 s^{-1}	
Equilibrium dissociation constants for albumin and fatty acids—the first two sites (k_{d})	8.1, 9 nM	Richeri et al. 1993
Equilibrium dissociation constant for transporters and fatty acids (k_{dt})	80 nM	
Rate constant for dissociation of fatty acids from albumin (k_{-1})	5.5 s^{-1}	van der Vusse 2009
Diffusion coefficient for albumin (D_A)	6 * 10^{-7}cm s^{-1}	Raj and Flygare 1974
Diffusion coefficient for free fatty acids (D_F)	3.3 * 10^{-6}cm s^{-1}	Smits 1976
Diffusion coefficient for *AF*1 & *AF*2 (D_{AF})	6 * 10^{-7}cm s^{-1}	Raj and Flygare 1974
Membrane permeability constants to fatty acids and fatty acid transporters (P and P_{T})	(0.1–15) cm s^{-1}	
Total transporter concentration (C_{T})	(0–0.1) mM	
Free fatty acid concentration at the endothelial face of the luminal membrane (C_{Fendo})	0.4 C_{F0}	

equilibrium dissociation constants, our choice of concentrations results in unbound fatty acid concentration in the plasma, $\overline{C_F}(0)$ of 8.4 nM i.e., just a tiny fraction of the fatty acids is unbound as claimed in previous studies (Saifer and Goldman 1961; Potter et al. 1989) and in agreement with the concentrations of 10 and 7.5 ± 2.5 nM measured by Goodman (1958) and Richeri and Kleinfeld (1995), respectively. This agreement between the measured and computed unbound fatty acid concentration is another justification for our choice to account for just two binding sites.

G_1: Metabolic Consumption Rate Constant

Since the consumption (uptake into micelles) is secondary compared to other processes and sensitivity with respect to this constant is very low (Barta et al. 2000), we use $G_1 = 0$.

k_{-1}: Rate Constant for Dissociation of Fatty Acids from Albumin

A wide range of rate constants can be found in the literature. The different measured values cannot be attributed to the type of albumin used (bovine or human) as those two were shown not to markedly differ (Sorrentino et al. 1988; van der Vusse 2009) but rather to the techniques of measurements (Weisiger 1993). While early measurements indicated on low rate constants (Burczynski and Cai 1994), more sophisticated, later measurements have established considerably higher values (Demant et al. 2002; van der Vusse 2009) that led us to use 5.5 s^{-1} as the value of the dissociation rate constant.

P: The permeability Constant of the Membrane with Respect to the Fatty Acids

Measuring the membrane permeability for fatty acids with a chain length above 12 carbons is difficult due to the low water solubility of those acids (Kamp and Hamilton 2006). Indeed, we could not find an established measured value for P as the plasma contains fatty acids with 16 (for Palmitic acid) to 22 (for Docosahexaenoic acid) carbons. Kamp and Hamilton (2006) cited P values of 0.1–0.22 cm s^{-1} for the permeability constant of lecithin bilayer membranes with respect to short-chain fatty acids and a monotonic increase of P values where the carbon chains are getting longer. Most measured values of P refer to transport in the liver. However, extrapolating from hepatic endothelium is not justified, as van der Vusse (2009) noted, due to fundamental differences between hepatic and cardiac endothelium. This uncertainty led us to incorporate a wide range of P values in our simulations in accord with Hamilton (2007) that cites a range of $0.1 < P < 1$ cm s^{-1}.

P_T and C_T: The Permeability Constant of the Membrane with Respect to the Transporters and Their Total Concentration

Few proteins, mainly CD36, FATP, and $FABP_{PM}$ have been suggested as potential endothelial membrane transporters (Bonen et al. 2007; Glatz et al. 2010; Carley and Kleinfeld 2011; Hagberg et al. 2013; Goldberg and Bornfeldt 2013) but others e.g., Hamilton and Kamp (1999), claim that their presence within the membrane does not necessarily mean that they are acting as transporters. Moreover, it is not agreed how they mediate the transport (do they cooperate or do they compete?) and what are their typical parameter values (concentrations, affinities to the fatty acids, mode and rates of transport, etc.). We chose to apply here the same Fick's law for both mediated and non-mediated transport. In case it is proved that the transporters act by flip-flop mechanism, the rate of flip-flop can be translated to "apparent permeability". Lacking the relevant data, we use a wide range of apparent permeability P_T values and putative concentrations C_T and few distributions of the transporters between the two faces of the membrane (either symmetric distribution where $C_{Ti} = C_{To}$ or asymmetric one).

k_{dt}: Equilibrium Dissociation Constant for Transporters and Fatty Acids

By our assumption, the transporters react just with the unbound fatty acids. Averaging over several values of reported dissociation constants (Stremmel 1987; Hui and Bernlohr 1997; Carley and Kleinfeld 2011) yields a value of 80 nM. A high degree of uncertainty characterizes this value as it is unclear exactly which proteins serve as transporters and as there is a high variability between the literature-based values of this constant. We apply the same k_{dt} value for reactions at both faces of the membrane.

C_{Fendo}: Concentration of the Free Fatty Acids at the Outer Face of the Endothelial Membrane

This unknown concentration has to be higher than 2.1 nM—its value at the interstitium (Musters et al. 2006) which is "down the road" from the endothelium. On the other hand, more than 50 % of the free fatty acids at the source are extracted within the region of solution (Barta et al. 2000). We use here 0.4 of the plasma concentration value or about 3.4 nM as a reference value.

Method of Solution

Numerical Solution

The high gradient of $\overline{C_F}$ near the membrane turns our boundary value problem to be a stiff one. This stiffness induces spatial fluctuations of the numerical solution (Gibbs phenomenon) and computation of reliable numerical solutions necessitates a very fine numerical grid. We tested two different methods of solution using off-the-shelf libraries;

1. Application of the Toms731 solver of JSim 2 library (other partial differential solvers of this library fail unless P is extremely low). This solver works unless the permeability constant with respect to the transporters, P_T is much different from that with respect to the fatty acids, P or where the distribution of the transporters is highly asymmetric.
2. Using NDSolve routine (which implements the method of lines) of Mathematica 10 library. This solver successfully copes just with trivial cases (e.g., P is very low) but yields highly inaccurate solutions otherwise so it is inferior compared to the JSIM.

Define the transient period as the time that elapses till the changes of the concentrations with time, starting from their initial values as specified above, become insignificant and a steady state is achieved. Then, the duration of this transient period was found to be shorter than 2 min for the range of relevant parameter values.

Analytical Solution

In light of the short transient time found in the present numerical solution and in past simulations (Barta et al. 2000) we aim at formulating an analytical solution for the steady state situation. Thus, the left-hand sides of Eqs. 2–5 are set to zero. Then, we normalize all the independent variables by dividing them by their values at $x = 0$ (e.g., $C_A = \overline{C_A}/C_{A0}$, $C_F = \overline{C_F}\ /C_{F0}$, etc.) and get the following equations for the normalized, steady state concentrations:

$$D_A \frac{d^2 C_A}{dx^2} = k_{+1} C_{F0}(C_A C_F - C_{AF1}) \tag{9}$$

$$D_{AF} \frac{d^2 C_{AF2}}{dx^2} = k_{-1}(-C_{AF1} C_F + C_{AF2}) \tag{10}$$

$$D_{AF} \frac{d^2 C_{AF1}}{dx^2} = \left(-k_{-1} C_A + k'_{-1} C_{AF1}\right) C_F + \left(k_{-1} C_{AF1} - k'_{-1} C_{AF2}\right) \tag{11}$$

$$D_F \frac{d^2 C_F}{dx^2} = \left(k'_{+1} C_A + k'_{+2} C_{AF1}\right) C_F - \left(k'_{+1} C_{AF1} + k'_{+2} C_{AF2}\right), \tag{12}$$

where

$k'_{-1} = k_{+2} C_{F0}$, $k'_{+1} = k_{+1} C_{A0}$, $k'_{+2} = k_{+2} C_{AF10}$.

Note that in Eq. 12 we omitted the term that represents the metabolic consumption as this term was found to hardly affect the solution (Barta et al. 2000).

While the right-hand sides of Eqs. 9–12 are composed of products of constants and normalized concentrations that are all O(1), the left-hand sides are products of very low diffusion constants (see Table 1) and second derivatives of concentrations. Thus, wherever there are moderate changes of concentrations and/or the concentrations change in an almost linear fashion, the left-hand sides might be approximated by zero and the differential equations turn to be simple, algebraic ones. Only at the boundary layer near the membrane the left-hand side of Eq. 12 cannot be nullified.

This situation calls for implementation of singular perturbation approach (Holmes 1995) namely—define an outer solution that is valid throughout the region except for the thin layer near the membrane and an inner solution that is valid exactly at this boundary layer. Then match those two in order to have a continuous solution that satisfies all boundary conditions. Here, we sketch the guidelines for the solution while more reasoning is given in the "Appendix" section.

Outer Solution

We determine the concentrations of all constituents as functions of the variable y, $y = x/x_1$ as follows: Based on previous solutions of more primitive models (Barta et al. 2000) and on the numerical solution, we expect the concentrations of the proteins to have a moderate, almost linear slope within the whole region. We try as a first approximation to determine a polynomial solution for the concentrations by satisfying: (a) the boundary conditions at $y = 0$ (i.e., all variables are equal to 1 there), (b) Eqs. 9–12 after nullifying their left-hand sides and, in addition, (c) the boundary conditions for the proteins at $y = 1$. Then, one gets:

$$C_A(y) = 1 + \varepsilon(y - y^2/2) + \mathrm{O}(\varepsilon^2) \tag{13}$$

$$C_{AF2}(y) = 1 - \varepsilon(y - y^2/2) + \mathrm{O}(\varepsilon^2) \tag{14}$$

$$C_{AF1}(y) = 1 + \mathrm{O}(\varepsilon^2) \tag{15}$$

$$C_F^o(y) = 1 - \varepsilon(y - y^2/2) + \mathrm{O}(\varepsilon^2), \tag{16}$$

where the constant ε satisfies: $|\varepsilon| < 1$ and should be determined by matching as described below. The expressions given in Eqs. 13–16 satisfy up to $\mathrm{O}(\varepsilon^2)$ conditions a, b, c specified above.

Inner Solution

Near the membrane ($y = 1$) there is a boundary layer where C_F changes in a steep manner. It is expressed as a function of a stretched variable, z, $z = (x - x_1)/\delta$, δ is of the order of magnitude of the boundary layer thickness. Assuming that protein concentrations might be approximated by their values at the membrane face, Eq. 12 converts to:

$$\frac{d^2C_F^i}{dz^2} = \frac{\delta^2 k'_{+1}}{D_F}\left\{\left(C_A(y=1) + \frac{k'_{+2}}{k'_{+1}}C_{AF1}(y=1)\right)C_F^i(z) - \left(C_{AF1}(y=1) + \frac{k'_{+2}}{k'_{+1}}C_{AF2}(y=1)\right)\right\}. \quad (17)$$

We seek an inner solution with the following functional form,

$$C_F^i(z) = \varphi_0(z) - \varepsilon\varphi_1(z)/2 + \mathrm{O}(\varepsilon^2), \quad (18)$$

$\varphi_0(z) \to 1$, $\varphi_1(z) \to 1$, where $z \to -\infty$ so that the inner and outer solutions coincide at the left side of the boundary layer or:$C_F^i(-\infty) = C_F^o(1)$.

Define δ as $\sqrt{D_F/k'_{+1}}$ then from Eqs. (12–15,17,18) one gets,

$$\frac{d^2(\varphi_0 - \varepsilon\varphi_1/2)}{dz^2} = \left(1 + \frac{k'_{+2}}{k'_{+1}} + \frac{\varepsilon}{2}\right)(\varphi_0 - \varepsilon\varphi_1/2) - (1 + \frac{k'_{+2}}{k'_{+1}} - \frac{\varepsilon}{2}\frac{k'_{+2}}{k'_{+1}}). \quad (19)$$

Equating the free terms at both sides of the equation above results with:

$$\frac{d^2\varphi_0}{dz^2} = \left(1 + \frac{k'_{+2}}{k'_{+1}}\right)(\varphi_0 - 1), \quad (20)$$

where there are no transporters, the boundary condition at the membrane, $z = 0$ yields:

$$\frac{d\varphi_0}{dz} = -\frac{P}{\sqrt{D_F k'_{+1}}}(\varphi_0 - C_{Fendo}). \quad (21)$$

The only solution of Eqs. 20, 21 that is finite where $z \to -\infty$ or : to the left of the boundary layer, is:

$$\varphi_0 = 1 - \frac{P(1 - C_{\mathrm{Fendo}})e^{z\sqrt{1+k'_{+2}/k'_{+1}}}}{P + \sqrt{D_F(k'_{+2} + k'_{+1})}}. \quad (22)$$

In a similar fashion, equating the coefficients of ε in both sides of Eq. 19 results with:

$$\frac{d^2\varphi_1}{dz^2} = \left(1 + \frac{k'_{+2}}{k'_{+1}}\right)(\varphi_1 - 1) - (\varphi_0 - 1), \quad (23)$$

where at $z = 0$,

$$\frac{d\varphi_1}{dz} = -\frac{P}{\sqrt{D_F k'_{+1}}}\varphi_1. \quad (24)$$

Therefore,

$$\varphi_1(z) = 1 - \frac{Pe^{z\sqrt{1+k'_{+2}/k'_{+1}}}}{P + \sqrt{D_F(k'_{+2} + k'_{+1})}}\left\{1 + \frac{(1 - C_{\mathrm{Fendo}})}{2(1 + k'_{+2}/k'_{+1})} \times \left(\frac{\sqrt{D_F(k'_{+2} + k'_{+1})}}{P + \sqrt{D_F(k'_{+2} + k'_{+1})}} - z\sqrt{1 + k'_{+2}/k'_{+1}}\right)\right\} \quad (25)$$

Substitution of Eqs. 22, 25 in Eq. 18 yields the inner solution $C_F^i(z)$.

Transporters' presence changes the boundary conditions, Eqs. 21, 24, and leads to a different, more cumbersome expression for the inner solution but the general functional dependence of φ_0, φ_1 on z remains unchanged.

Matching

Conservation of mass implies that the flux of the fatty acids at the entrance ($y = 0$) equals the flux at the membrane ($y = 1$ or $z = 0$), namely:

$$(D_F C_{F0} + D_{AF} C_{AF20})\varepsilon/x_1 = P(C_{F0}C_F^i(z = 0) - C_{\mathrm{Fendo}}). \quad (26)$$

The above linear equation uniquely determines ε and this concludes the solution.

Results

In order to gage the inaccuracy involved with the asymptotic solution we compared numerical and analytical solutions. It was found that for the physiological range of parameter values, as far as the flux and the fatty acid concentrations at the endothelial membrane are concerned, our analytical approach yields exceptionally accurate results. The profiles of the concentrations computed by the two approaches differ within the region of solution but as those profiles have no clinical meaning and as they cannot be experimentally validated, this difference shouldn't bother us.

The most meaningful outcome of this model is the fatty acid uptake rate which is computed by multiplying the flux at the membrane face, given by Eqs. 7 or 8, by the total surface area of the capillaries in the myocardium. This surface area was found to equal 500 $\mathrm{cm}^2\ \mathrm{g}^{-1}$ where the weight refers to canine left ventricular wet myocardial tissue (Bassingthwaighte et al. 1974). The difference between the radii of canine and human capillaries (the latter is about 1.5 times the former) counteracts the difference between the densities of the capillaries (being about 2/3 at human heart), inducing quite a similar diffusional surface area for these two species. Substituting the concentrations

Table 2 Normalized concentrations and uptake rates (in nmol(g min)$^{-1}$) computed by a model without transporter activity. Parameter values are given in Table 1

P	C_F (x_1)	C_{AF2} (x_1)	Total uptake rate
0.1 (cm s^{-1})	0.94	0.999	14
0.5 (cm s^{-1})	0.79	0.997	49
2.5 (cm s^{-1})	0.56	0.993	101
5 (cm s^{-1})	0.49	0.992	117
15 (cm s^{-1})	0.435	0.991	130

computed by our model in this expression yields the estimated uptake rate, see Table 2.

Our solutions indicate that we cannot meet the physiological need of 100 nmol(g min)$^{-1}$ fatty acid uptake rate (van der Vusse et al. 2000; Musters et al. 2006) unless P is higher than the values reported in the literature.

Replacing the boundary condition in order to account for transporters' activity and changing Eq. 26 accordingly yields results for a membrane covered with transporters. By our assumption the transporters react just with the free fatty acids which have an extremely low concentration. Therefore, unless the transporters have an extremely low concentration as well, almost all the fatty acids are associated with the transporters and unmediated fatty acid flux becomes negligible compared to the transporters assisted one. Thus the total flux is determined to a leading order by the value of P_T, see Table 3. Moreover, for a symmetric distribution of transporters ($C_{Ti} = C_{To}$) and where $P_T \gg P$ the mediated flux equals the one derived in the simple (no transporters) scenario where P_T replaces P.

Sensitivity Analysis

A sensitivity analysis is essential due to the high degree of uncertainty in determining some of the parameter values.

Sensitivity to P

Figure 1 demonstrates the dependence of the fatty acid uptake rate on the permeability constant. Evidently, where P is low, its change induces a major effect on the flux and an accurate determination of it is crucial. In contrast, where P is high enough there is a saturation process and raising

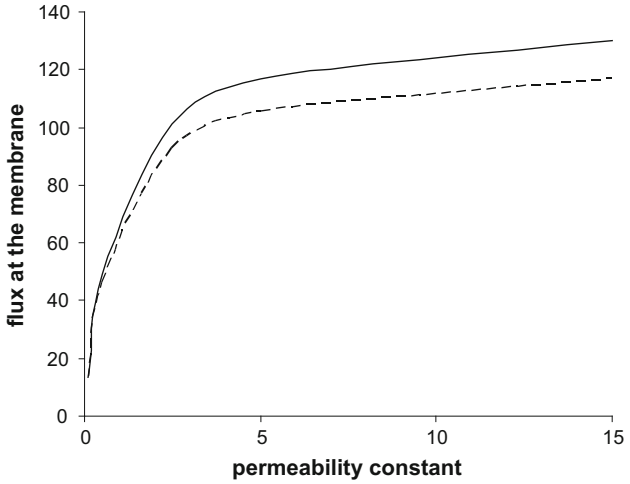

Fig. 1 Fatty acids flux given in nmol(g min^{-1}) as a function of P (in cm s^{-1}). *Solid line* $x_1 = 0.0005$ cm, *dashed line* $x_1 = 0.005$ cm

P will have a negligible effect. The same holds with respect to P_T.

Sensitivity to x_1

This parameter represents the diffusional distance i.e., a radius of a capillary in the in vivo heart or the thickness of the stagnant layer attached to the endothelium in perfusion experiments (usually, it is about 0.005 cm e.g., Goresky et al. 1994). Due to variation in the reported values of capillary radii and in order to check the relevance of perfusion experiments we run the simulation for a wide range of x_1 values and concluded that while it has a significant impact on the protein concentrations (a higher variation of the protein concentrations where the slab is thicker) it has a low impact on the uptake rate. In Fig. 1 we show that multiplying x_1 by 10 has a minor effect on the fatty acid uptake rate for physiological P values.

Sensitivity to C_{Fendo}

Figure 2 demonstrates the relatively high sensitivity of the uptake rate with respect to this parameter. As expected, where C_{Fendo} is lowered the flux increases, as the membrane tends to act like a sink e.g., lowering $C_{Fendo}/\overline{C_{F0}}$ from 0.5 to 0.3 enhances the flux by 40 %.

Table 3 Uptake rates (in nmol(g min)$^{-1}$) computed by a model that accounts for transporters' activity. $C_{Ti} = C_{To}$, $P_T = 1.5$ cms^{-1}. Other parameter values are given in Table 1

C_T (mM)	0		0.0001		0.001		0.01		0.1	
P (cms^{-1})	0.1	0.5	0.1	0.5	0.1	0.5	0.1	0.5	0.1	0.5
Uptake rate	13.6	49.0	55.5	67.2	81.4	82.8	85.5	85.5	86.1	86.1

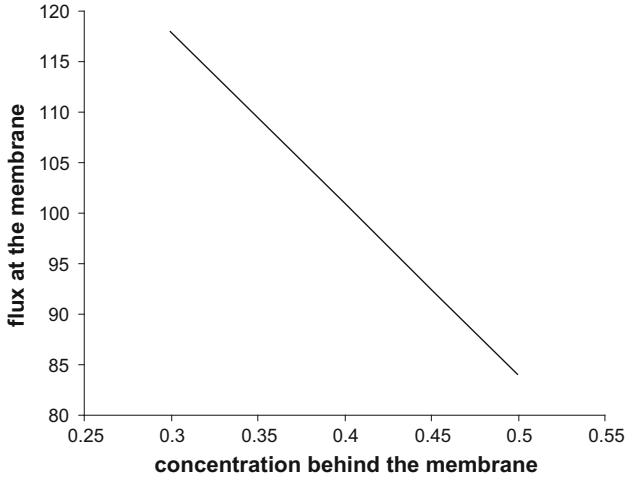

Fig. 2 Fatty acid flux given in nmol(g min)$^{-1}$as a function of $C_{\mathrm{Fendo}}/\overline{C_{F0}}$, $P = 2.5$ cm s^{-1}

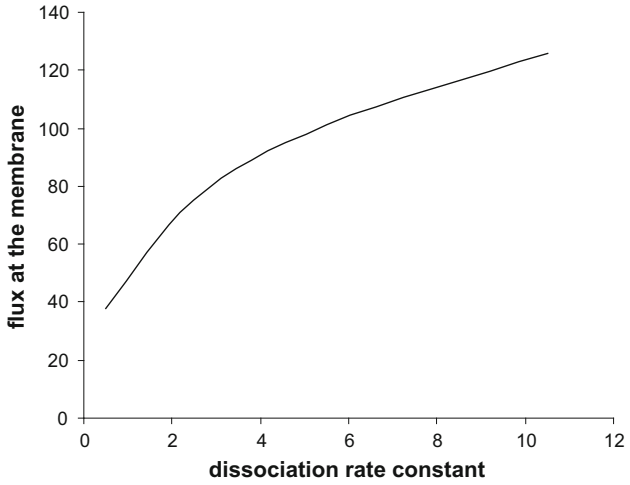

Fig. 3 Fatty acid flux given in nmol(g min)$^{-1}$ as a function of k_{-1} given in s^{-1}, $P = 2.5$ cm s^{-1}

Sensitivity to k_{-1}

As discussed above, while in the past this rate constant was estimated to be O(0.1 s^{-1}), it is now agreed that it is ten to hundred times higher. This parameter actually determines the distribution between bound and stripped fatty acids and therefore has a high impact on the flux especially in the lower range of parameter values as demonstrated in Fig. 3.

Sensitivity to Parameter Values k_{dt}, C_T of the Transporters

Due to the typical extremely low C_F values, most of the free fatty acids are bound at quite low C_T beyond which saturation occurs and the flux is practically insensitive to changes in either k_{dt} or transporter concentrations at any face of the membrane, see Fig. 4. The saturation point depends on the values of all involved parameters—a typical value is lower than 1 μM, see Table 3. The higher is the difference between P and P_T, the more pronounced is the effect of the transporters and so is the sensitivity to their parameter values.

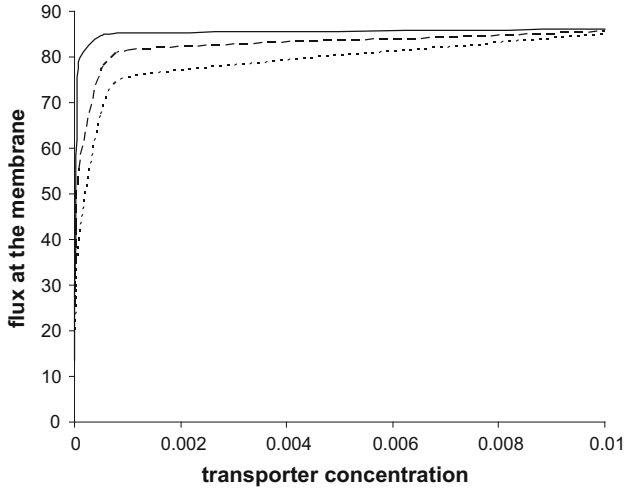

Fig. 4 Fatty acid flux given in nmol(g min)$^{-1}$ as a function of C_T given in mM, $P = 0.1$ cm s^{-1}, $P_T = 1.5$ cm s^{-1}. *Solid*, *dashed*, and *dotted lines* stand for $k_{dt} = 10$, 80, and 200 nM, respectively

Discussion

The mechanisms that govern fatty acid transport in the myocardium are far from being fully deciphered (Hutter et al. 1984; Bassingthwaighte et al. 1989b; van der Vusse et al. 2000; Carley and Kleinfeld 2011). This is mainly due to the difficulties involved with getting clear-cut experimental evidences for the activity of receptors for proteins and of fatty acid transporters at the membranes' faces. Thus, any theoretical simulation that elucidates their effect on the transport is helpful. Unfortunately, few theoretical simulations have been formulated (Barta et al. 2000; Musters et al. 2006) not only due to scarce physiological knowledge regarding the governing mechanisms that are involved and lack of substantiated parameter values, but because such models face difficulties in computing reliable numerical solutions and no attempt has been done hitherto to formulate an analytical solution.

The present simulation accounts for the specific composition of the fatty acids and for the two main sites of binding on the albumin face. All involved parameter values are either determined by rigorous reasoning based on measurements or are being analyzed in order to gage their effect on the end result. The diffusion–reaction system of equations which is stiff and presents numerical difficulties

is solved both numerically and analytically using singular perturbation approach. The same analytical approach might be applied for other simulations and is expected to serve as an efficient tool in solving futuristic models.

Our simulation is limited by four types of simplifications imbedded in it:

A. Simplifications that concern the configuration/geometry. We refer here just to the radial penetration of the fatty acids neglecting the axial direction (the direction of blood flow within the vessels). Extending the simulation to the 3D case will considerably increase the complexity of the equations but is not expected to have a significant effect on the results since it would not change the distance that is being diffused.
B. We account for just two sites of reaction between albumin and fatty acids. Evidently, this is far from being accurate (van der Vusse (2009) mentions 7 binding sites for fatty acids). Using two-sites assumption is a kind of a golden pass between the simplistic one-site assumption that results with non-physiologic high unbound fatty acid concentration and multiple binding sites assumption that results with a larger system of differential equations and incorporates more parameters that unavoidably decrease the reliability of the simulation.
C. The transport across the membrane is represented by one parameter, a permeability constant, P. The transporters' activity namely, their permeation rate represented by P_T and their equilibrium dissociation constant with the fatty acids represented by k_{dt}, is simplified as well. These simplifications should be removed once the detailed mechanisms that govern transport (flip-flop? fatty acids pump? or another mechanism), absorption, and desorption rates (reported to be different, Hamilton 2007) are deciphered and their parameters are calibrated. Till then, we think that the least speculative boundary condition is one that relates to the membrane as one compartment where flux is driven by a concentration gradient.
D. We ignore the contribution of the lipoprotein-associated triglycerides. Fatty acids from the two sources, albumin complex or triglycerides, cannot be distinguished at the membrane face (van der Vusse 2009; Hagberg et al. 2013). Van der Vusse et al. (2000) stated that the contribution of LPL to total fatty acid utilization in the isolated rat heart is 25 % at maximum. Accounting for the LPL-derived fatty acids might enhance present computed fluxes by up to 25 %.

Simulation of fatty acid transport within the human myocardium is hindered by the scarce measurements of the relevant parameters. We used educated guesses of those parameter values when they were not available, by inferring from measurements in other species and/or other organs. Evidently, the lack of substantiated, measured parameter values necessitates a detailed sensitivity analysis with respect to those unknown parameter values. Our model predicts insufficient fatty acid supply where there are no transporters and no receptors and where the permeability constant is lower than 2.5 cm s^{-1}. Where there are transporters, it is crucial to have an estimation of their density; the diminished sensitivity of the flux with respect to transporter parameter values (k_{dt}, C_T, distribution within the membrane) that our simulation predicts, see Table 3 and Fig. 4, means that the accurate determination of these parameters is ancillary unless transporter concentration is low. In any case, it is essential to know the rate with which the transporters cross the membrane. Other meaningful data are the concentration of the unbound fatty acids within the endothelial cell, C_{Fendo} and the rate constant for the dissociation of the fatty acids from the albumin, k_{-1}. These findings might guide the experimentalists in formulating a protocol of measurements aimed to decipher the transmembrane transport by pointing to the more affecting parameters.

In light of the extremely low concentration of the unbound fatty acids within the plasma and the high physiological uptake rate of free fatty acids, as long as the membrane cannot be crossed by the albumin complexes, there have to be mechanisms that enhance the dissociation of the fatty acids from the albumin at the membrane. These might be either receptors for albumin (that act independently or in collaboration with transporters) or transporters that attach not just to the unbound fatty acids but to the bound ones as well and expel the albumin before crossing the membrane. By our simulation, such receptors' activity can be avoided just in case the fatty acid transporters cross the membrane with a very high rate and/or are distributed in an asymmetric manner within the membrane (more transporters at the inner face of the luminal membrane than on the outer face).

We plan to extend our model and include putative receptors on the membrane in order to characterize the density and affinity of receptors that will induce physiological fluxes of fatty acids. Other required extensions are: A. Accounting for the adjacent endothelium by adding another compartment to the model (Barta et al. 2000)—it involves mathematical difficulties but will enable us to entail a more accurate C_{Fendo} value. B. Removing some of the simplifications regarding the mechanisms that govern adsorption, crossing of the membrane and desorption.

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Appendix

The four independent variables of the differential system of equations, Eqs. 9–12, have two typical profiles: C_F is characterized by a moderate linear slope followed by a steep decline within a boundary layer near the membrane while the proteins are characterized by a linear slope followed by a much thinner boundary layer where they hardly change. This difference between the profiles is due to the different values of the diffusion constant of the two "species" and (mainly) due to the different boundary conditions imposed at the membrane (the fatty acids are evacuated but the proteins are confined within the region). Accordingly, establishing a solution using a singular perturbation approach necessitates expressing the variables near the membrane as series with two scaled coordinates (for the two types of boundary layers) and a solution of four simultaneous equations for each term that appear in each series. These formalistic steps mean a most formidable task. Instead, we expressed the protein concentrations by uniformly valid functional forms, Eqs. 13–15, that satisfy all boundary conditions and the differential equations after nullifying their left-hand sides. Any series that includes a deviation of $\varepsilon(y - y^n/n)$, $n > 1$ from the plasma concentrations will do for this purpose. Choosing $n = 2$ involves constant and minimal 2nd derivatives (and errors). Outside the boundary layer the differential equations are satisfied up to $O(D_A\varepsilon/x_1^2)$. Inside the boundary layer the inaccuracy is a bit higher. This approach induces normalized concentrations that differ at the membrane by less than one percent from the numerically computated ones for our range of parameter values.

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