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Computational modeling of cardiac fatty acid uptake and utilization

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Abstract

Fatty acids (FA) are important oxidizable substrates for the heart. These FA have to cross a distinct number of constraints before they are stored and/or oxidized in cardiac muscle cells (myocytes). The transport route of FA from the blood into the myocyte is not completely elucidated yet. Especially the mechanisms underlying transport of FA across biological membranes is a subject of continuous debate. Therefore, the main goal of this chapter was to elucidate the mechanism of transport of FA across the membrane of the myocyte, i.e., the sarcolemma.

This chapter showed that insight into the physiological background of the transport of FA could be enlarged when using a computer model that analyses the physiological phenomena involved. To investigate the transport pathway of FA in more detail, three models were used: Model I described the transport of FA across the sarcolemma of a resting cardiomyocyte in order to address the main goal. Model II was an extension of Model I including the glycocalyx (i.e. the carbohydrate-protein coat, surrounding the cell) to study the influence of the glycocalyx on the flux of FA, and Model III simulated the remaining part of the transport pathway of FA: the endothelial barrier. The trans-sarcolemmal flux of FA in a quiescent cardiomyocyte that resulted from Model I (∼ 14.7 nmol min\(^{-1}\) (g ww\(^{-1}\) cardiac tissue) corresponded well to experimental data (∼ 13.5 nmol min\(^{-1}\) (g ww\(^{-1}\))). It strongly indicated the involvement of an enhancer protein, a putative protein that improves the absorption of FA in a membrane, in the transport of FA across the sarcolemma. Model II revealed a substantial decrease in transport of FA to ∼ 10.5 nmol min\(^{-1}\) (g ww\(^{-1}\)) due to the presence of a glycocalyx. Based on the outcome of Model II, future experiments performed on cardiomyocytes should take the influence of the glycocalyx into account.

Finally, Model III showed that, given our current understanding of the endothelium as captured in the model, the endothelial barrier appears to lack a sufficient diffusion driving force and area for diffusion to account for the experimentally assessed rate of fatty acid uptake of ∼ 100 nmol min\(^{-1}\) (g ww\(^{-1}\)) in the intact, beating heart. The latter finding implies that further experimentation should focus on the endothelium as less understood barrier for cardiac fatty acid uptake and utilization.
Section I  Introduction

This chapter shows how computer models might be helpful tools to improve our understanding of important physiological processes, such as the uptake of fatty acids (FA) in heart and skeletal muscle. FA are important substrates for the heart, because they are the heart’s main source of energy under normal conditions (9). The transport route of FA from the blood into the heart muscle cells (myocytes) is one with many constraints (58), which have been subject of investigation in the last decades (Fig. 1). Controversy exists about the mechanisms underlying FA crossing biological membranes: is a special protein required to enhance the transport of FA across a membrane or is the rate of transmembrane diffusion of FA high enough to fulfil the heart's energy requirements? A computer model that simulates the uptake of FA differentiates between the different opinions about this subject. In this chapter, a spatial-temporal computer model will be introduced. It is composed of a set of mathematical equations (differential equations), which describes the dynamic behaviour of substrate concentrations at a given position in a biological compartment. Simulation results may confirm or reject the prevailing theory. Moreover, these computer models can be used as in silico tools to generate new hypotheses and optimise experimental design.

1.1 Benefits and drawbacks of modeling

A computer model has the advantage that it takes relatively little time to get results (32). In general, physiological experiments can consume a lot of time due to preparation and conduction. As a consequence, computer modeling is in general cheaper. Besides a computer and a mathematical package, other expensive experimental materials are not needed to perform computer simulations. Simulations can also be repeated with different parameter values very often, while the number of experiments that can be performed in real life is limited. A fourth advantage of modeling is the integration of heterologous information. In most cases, a physiological model contains data of different sources. Therefore, a computer model provides a quantitatively consistent description and reveals if the physiological theory or data of this system is ill defined. A fifth advantage of a computer model is that the number of laboratory animals that have to be included into the study can be decreased, because the results of the model lead to better experimental design. Unfortunately, the in silico approach has one major drawback: a model is a simplified representation of the reality. Every theory and related model contains a number of assumptions that are supposed to have little influence on the overall results. Sometimes, the implications of these assumptions are underestimated, which could substantially influence the end results, leading to erroneous conclusions.
Section II  Physiological background

To function properly the heart requires energy, which is obtained from oxidizable substrates such as glucose, fatty acids (FA), lactate, pyruvate, etc. Under normal conditions, about 70% of the energy is derived from mitochondrial oxidation of FA (9). The transport route of FA into the myocyte starts in the blood plasma of the capillaries (Fig. 1). In plasma, more than 99.9% of the unesterified FA are bound to the protein albumin (55). Another source of fatty acyl chains are triacylglycerol-containing lipoprotein particles, from which the enzyme lipoprotein lipase releases FA. The next step is transport of FA across the luminal endothelial membrane into the cytoplasm of the endothelial cells. After that, FA diffuse to the other side of the endothelial membrane: the abluminal membrane. The protein fatty acid-binding protein (FABP) could also be involved in the transport of FA across the endothelial cytoplasm. The abluminal membrane is the barrier that FA have to overcome before entering the interstitium. In this compartment, albumin is also present, which binds to the FA. Again, diffusion processes of FA are the main transport mechanism to reach the third membrane of the transport route: the sarcolemma. FA are moved across this membrane to enter the sarcoplasm of the myocyte. FABP in the cytoplasm of the myocyte binds to FA and facilitates transport before FA are converted to acyl-CoA. The acyl chain of acyl-CoA can be stored, e.g. in triacylglycerol pools, or are oxidized inside the mitochondria. Under normal physiological conditions, oxidation prevails over storage. Acyl-CoA is converted stepwise into acetyl-CoA and thereafter oxidized to CO₂ and H₂O to release chemical-bound energy in the form of ATP.

II.1  Blood-borne fatty acids

FA are present in the blood plasma of the capillaries in two possible chemical forms (71):

- Unesterified FA. These are present as acids and salts, and mainly bound to albumin. The concentration range of these FA lies between 0.2 and 1.0 mM under normal physiological conditions (71). The long non-polar tail makes FA less soluble in an aqueous environment. Albumin is also present in blood plasma with a concentration of ~ 0.6 mM (71). Albumin has a high affinity for FA and can bind a number of FA per albumin molecule. Although 8 and 12 binding sites per albumin molecules have been reported (4, 63), up to 3 binding sites for FA per albumin molecule are physiologically relevant. The affinity constants for the first 3 binding sites of FA to human serum albumin (HSA), which represent the ratio FA bound to HSA and unbound FA (uFA), are 1.45 x 10⁸ M⁻¹, 1.30 x 10⁸ M⁻¹ and 0.71 x 10⁸ M⁻¹ for the 1st (complex of albumin with 1 fatty acid: AF₁), 2nd (2 FA bound to albumin: AF₂) and 3rd binding site (3 FA bound to albumin: AF₃), respectively (55). Moreover, it was shown (55) that due to the strong binding of FA to albumin, only small amounts of uFA are present, ranging from 1.4 to 10.5 nM uFA (55, 71), i.e. about 0.001% of the total unesterified FA present.
**Esterified FA, like mono-, di- and triacylglycerols, phospholipids and cholesteryl esters. Very low-density lipoproteins (VLDL's) and chylomicrons, produced in the parenchymal liver cells and in the epithelial cells of the small intestine, respectively, are droplets of triacylglycerols, surrounded by a hydrophilic layer of phospholipids, cholesterol and apoproteins. Lipoprotein lipase (LPL), present in the glycocalyx of the cellular membrane, catalyses the release of FA from the chylomicrons and VLDL's by breaching the phospholipid coating and facilitating the hydrolysis of the triacylglycerol core. Although VLDL's and chylomicrons contain sufficient amounts of FA to theoretically fully cover the energy needs of the heart, its contribution under normal situations will not exceed 20 - 25% at maximum of the total amount of FA utilized (77).**

The corollary is that the normal heart relies heavily on blood-borne unesterified FA as energy source. The utilization of these substrates is therefore the subject of the present chapter.

**II.2 Transport routes of unesterified fatty acids: qualitative and quantitative considerations**

Blood-borne FA, either free or bound to albumin, have to cross a distinct number of barriers (Fig. 1), like membranes and aqueous compartments, before they reach the mitochondria inside the myocytes (Fig. 2). Under physiological conditions, FA are taken up at a constant rate. Between 40 - 60% of the FA present in the capillaries (38, 51, 79, 80) are absorbed by the endothelium during a capillary transit time for blood plasma, which lasts about \(~0.8\) sec (71). The amount of FA (in moles) that is transported across a certain cross-section is represented by the so-called flux of FA. An average concentration of FA under normal conditions is \(~0.5\) mM at an average plasma flow in the capillaries of \(3 \times 10^3 \text{ I min}^{-1}\) (g ww)\(^{-1}\) (76). This leads to a flux of about 200 nmol min\(^{-1}\) (g ww)\(^{-1}\) into the capillaries. Since about 40 - 60% of this flux is transported across the endothelium of an intact, beating heart, the flux of FA into the myocyte becomes on the order of 80 - 120 nmol min\(^{-1}\) (g ww)\(^{-1}\) under normal conditions, i.e. \(~100\) nmol min\(^{-1}\) (g ww)\(^{-1}\) on average.

**II.2.1 The vascular compartment**

The capillaries, with an average diameter of 8 \(\mu\text{m}\) (48), are filled with blood plasma, containing oxidizable substrates and binding proteins like FA and albumin, respectively. In general, the diffusion flux, \(J\), of a substance with concentration \(C\) is calculated as follows (Eq. 1):

\[
J = -DS \frac{\partial C}{\partial x} \tag{1}
\]

where \(D\) is the diffusion coefficient, \(S\) is the surface area of the crossing substrate and \(\frac{\partial C}{\partial x}\) is the first position-dependent derivative of substance concentration \(C\) (see also paragraph

\(^{11}\) (g ww)\(^{-1}\) stands for gram wet weight cardiac tissue, unless indicated otherwise
III.1.2), what could be interpreted as the difference in concentration $C$ ($\partial C$) across a given distance ($\partial x$). Before passing the endothelial barrier, FA diffuse from the lumen of the capillary to the luminal membrane of the endothelial cells, lining the capillary wall. In the capillary lumen, about 5 nM of uFA and 0.5 mM of the total concentration of FA in the blood plasma are present under normal conditions (55, 76). FA bound to albumin (AF$_1$, AF$_2$ and AF$_3$, when a maximum of 3 binding sites is used) diffuse at the same speed as free albumin (6). The diffusional flux of uFA at maximum (assuming an infinite sink for uFA at the luminal side of the endothelium) is obtained by putting a difference in concentration of 5 nM across a distance of 0.4 $\mu$m (length of endothelial glycocalyx on the luminal side, 75), $D = 3.0 \times 10^{-6}$ cm$^2$ s$^{-1}$ and $S = 500$ cm$^2$ (g ww)$^{-1}$ (see Table I) in Eq. 1, which results in a flux $J_{uFA}$ of $\sim 11$ nmol min$^{-1}$ (g ww)$^{-1}$. This flux of uFA alone is about 1/10 of the required flux ($\sim 100$ nmol min$^{-1}$ (g ww)$^{-1}$, see subsection II.2). The following calculations show the important influence of albumin-bound FA on the total flux: when FA bound to albumin could diffuse freely from the begining of the endothelial glycocalyx towards the endothelial cell membrane, again assumed to be an infinite sink for albumin-bound FA in this situation, this would theoretically increase the maximum flux of FA $J_{FA}$ to $\sim 2 \times 10^5$ nmol min$^{-1}$ (g ww)$^{-1}$ (Eq. 1 and diffusion coefficient of albumin in plasma used from Table I, calculation not shown). From a physiological point of view, this high contribution of albumin-bound FA to the total flux of FA can't be achieved, because the luminal side of the endothelial membrane isn't an infinite sink for albumin-bound FA. Furthermore, the presence of a glycocalyx most likely delays the diffusion rate of FA bound to albumin considerably (75). The glycocalyx is a part of the extracellular matrix (ECM) that surrounds every eukaryotic cell. This glycocalyx is a negatively charged, carbohydrate-rich coating of glycoproteins and glycolipids, which prevents the close approach of cells and macromolecules and forms a stagnant layer (6, 64). It takes albumin, a spherical, slightly negatively charged protein with a radius of $\sim 3.5$ nm (40), about 40 minutes to penetrate the glycocalyx with a thickness of 0.4 - 0.5 $\mu$m (75). This is too slow to account for a relevant contribution of albumin-bound FA to the total flux of FA to the luminal side of the endothelial cells, since the capillary transit time lasts only 0.8 seconds. In other words, about 0.8 seconds instead of 40 minutes are available to move sufficient FA (flux of $\sim 100$ nmol min$^{-1}$ (g ww)$^{-1}$) from the beginning of the glycocalyx to the surface of the endothelial membrane. Therefore, if the outcome of the in vitro experiments are correct (75), one should conclude that albumin-bound FA diffuse too slowly through a glycocalyx to account for a physiological reasonable flux of FA.

II.2.2 Endothelium

The capillary wall consists of a single layer of endothelial cells, which make up the endothelium, the second compartment that FA have to cross. Four mechanisms of crossing the endothelial cells lining the microvascular compartment have been proposed (7, 72)
1. Transcytosis: FA, bound to albumin, are transported across the endothelium by means of vesicles (25, 49, 61). It was shown that the FA-albumin complex binds to uncoated proteins on the endothelial membrane. These parts of the membrane form vesicles, which transport FA-albumin complexes through the endothelium to its abluminal side. Over there, the complex is released into the interstitial compartment. The time for these vesicles to cross the endothelium is 3 – 5 minutes (61) and by using Eq. 2 (75), one can deduce the diffusion coefficient $D$ for these vesicles:

$$D = \frac{d^2}{4t}$$

where $d$ is the diffusion distance, which a substrate has to cross within time $t$. Given a time of 4 min to cross an endothelial cell with a length $\sim 0.5 \mu m$ (Table I), this results in a $D \sim 2.6 \times 10^{-12} \text{cm}^2 \text{s}^{-1}$, which is about 6 orders in magnitude lower than other diffusion coefficients in Table I. This corresponds with a maximum flux of $\sim 0.8 \text{nmol FA min}^{-1} \text{(g ww)}^{-1}$ (Eq. 1). Therefore transcytosis is quantitatively of no importance for transport of FA.

2. Clefts: Intercellular junctions, called clefts, separate the individual endothelial cells. The cleft size is $\sim 6 – 7 \text{nm}$ (48), about the same as the diameter of an albumin molecule (40), and constitutes a total area of $\sim 5 \text{cm}^2 \text{(g ww)}^{-1}$ (48). Through these clefts, albumin-bound FA could diffuse towards the interstitium. However, the presence of a glycocalyx tethers albumin movement (7), reducing the flux of FA of albumin-bound FA to almost zero. A flux solely established by uFA is also negligible, as was calculated for uFA across the glycocalyx in paragraph II.2.1. Transport of FA through the clefts is therefore not sufficient to explain a physiologically relevant flux.

3. Diffusion through the endothelial cell: uFA are absorbed by the phospholipid bilayer of the endothelial membrane (luminal side). Subsequently, these FA are transported by two possible mechanisms to the abluminal side of the endothelium:

a. Peri-endothelial transfer (60): FA remain inside the membrane and diffuse via the membrane to the abluminal part of the membrane. This process will only occur if FA are present in huge amounts in the endothelial membrane ($\sim 7$ orders higher) compared to the phospholipid concentration in the membrane, as was shown by calculation (7). This is obviously not the case in physiological relevant situations. Therefore, peri-endothelial transfer of FA is inconsequential.

b. Transmembrane diffusion (22, 23, 28): (This process is described in more detail in paragraph II.3.1.) FA are absorbed in the membrane and move subsequently to the inner side of the membrane, where they are released in the cytoplasm. After the release from the membrane, FA diffuse through the endothelial cytoplasm to the abluminal membrane. There, the same process of absorption, movement across the membrane and release takes place to transport FA from the endothelial cytoplasm into the interstitial compartment.
The transport through the endothelial cytoplasm could be enhanced by fatty acid binding protein (FABP, (52, 53)), which binds tightly with FA ($K_a^{FABP} \sim 1 \times 10^8 \text{M}^{-1}$, (56, 57)). However, the concentrations of FABP in endothelial cells might be too small, about 0.5 $\mu$M (39), to contribute to cytoplasmic transport of FA (73).

c. Protein-mediated transport: It cannot be excluded that special proteins are present that enhance the transport of FA across the endothelial membranes (1, 8, 12, 19, 66, 67). Experimental data showed saturation effects that could be ascribed to the involvement of proteins (76). Also the use of agents that inhibit protein-mediated transport of FA strengthens this opinion (11, 37, 41, 44, 45). A few candidates for these proteins have been proposed: fatty acid translocase/CD36 (FAT/CD36, (1, 24)), plasma membrane fatty acid-binding protein (FABP pm, (66)), fatty acid transport protein (FATP, (59)) and albumin-binding protein (ABP, (54, 70)). The transport mechanisms of FA using these proteins remain unclear, but possible mechanisms will be discussed below (subsection 11.3).

Summarizing, from the possible FA pathways across the myocardial endothelium, only diffusion of FA through the endothelial membrane, either by transmembrane diffusion or protein-mediated, seems to be physiologically relevant.

### 11.2.3 Interstitial compartment

The interstitium is the compartment that divides the endothelial cells and myocytes. The interstitial width is about 0.5 $\mu$m (71). It consists of an aqueous solution, the interstitial fluid, containing among others uFA and albumin-bound FA (AF₁, AF₂ and AF₃). The albumin concentration in the interstitium is about half the concentration in blood plasma (26). Most of the interstitial fluid is "trapped" in a matrix of glycoproteins and proteoglycans, being constituents of the glycocalyx of both endothelial cells and myocytes. The crossing of the interstitial compartment is most likely due to simple diffusion (7) of both uFA and albumin-bound FA. But diffusion of AF₁, AF₂ and AF₃ may be substantially hampered, since macromolecules, such as albumin, diffuse slowly through the glycocalyx (75).

### 11.2.4 Sarcolemma

The final barrier, which FA have to cross before entering the myocardial cytoplasmic compartment, is the sarcolemma. The mechanism of transport of FA across this cell membrane might be different from the transport of FA across the endothelial membranes.

### 11.2.5 Metabolism of fatty acids in the myocyte

The sarcoplasm may be considered as a sink for FA. The concentration of FA in the sarcoplasm is very low compared with the extracellular concentrations (71), creating a
concentration gradient from the vascular compartment to the sarcoplasm. In the sarcoplasm, FA bind to FABP and this complex diffuses to intracellular sites of metabolic conversion. FA can be stored in the myocardial cytoplasm as triacylglycerols or metabolised in the mitochondria or peroxisomes (Fig. 2). Only the pathway of FA metabolism inside the mitochondria (Fig. 3) will be described. First, FA react with coenzyme A (CoA), catalysed by the enzyme acyl CoA synthetase (ACS) to form reactive acyl-CoA esters, which subsequently reacts with carnitine to yield acylcarnitine. This reaction is catalysed by carnitine acyltransferase, located in the outer mitochondrial membrane (CAT I). Acylcarnitine is transported across the inner mitochondrial membrane into the mitochondrial matrix by means of a carnitine-dependent shuttle protein, i.e. carnitine-acyl carnitine translocase (CAcT). Inside the mitochondrial matrix, carnitine acyltransferase (CAT II) located at the inner side of the inner mitochondrial membrane converts acylcarnitine back to acyl-CoA and carnitine. The next step is the β-oxidation of acyl-CoA, in which acyl-CoA is stepwise degraded to acetyl-CoA. Acetyl-CoA is degraded in the citric acid cycle and finally electron transfer chain activity leads to the formation of ATP. Although the mitochondrial conversion of FA into acyl-CoA is important for the driving force of fatty acid uptake (33, 34, 35), in this chapter the sarcoplasm is simplified as a single sink compartment.

II.3 Fatty acid transport across membranes

Different mechanisms have been proposed to explain FA crossing a biological membrane. The main controversy is about the necessity of a protein: is transmembrane diffusion of FA across the myocardial or endothelial membrane high enough to explain a physiological relevant flux of ~ 100 nmol min⁻¹ (g ww)⁻¹, or is a membrane-associated protein needed to enhance this transport process?

A biological membrane, like the myocardial or endothelial membrane, is composed of two leaflets: an outer (the layer of the membrane facing the exterior of the cell) and an inner leaflet (the layer of the membrane bordering the cell interior). In a standard membrane, about 50% of the mass of the membrane consists of phospholipids, which are composed of a polar head and 2 hydrophobic tails (2). The hydrophobic tails of the 2 leaflets point to each other, resulting in a phospholipid bilayer, composed of different phospholipids, e.g. phosphatidylcholine and sphingomyelin. The outer and inner leaflet differ a lot in phospholipid composition (phospholipid asymmetry), but the concentration of phospholipids on both sides of the membrane is globally equal (2), which will be used in our computer model. Proteins constitute the remainder of the mass in the membrane, although the number of proteins in the membrane is much lower than the amount of phospholipid molecules, i.e., about 1 protein per 100 phospholipids (2).

II.3.1 Transmembrane diffusion of fatty acids

The first mechanism proposed for FA transport across a membrane is transmembrane diffusion (Fig. 4). It’s divided in three different stages (22, 23, 28, 81):
1. **Absorption:** First, FA are absorbed from an aqueous phase, e.g. the blood plasma or interstitial fluid, into the phospholipid part of the membrane.

2. **Flipping:** FA, present in one of the leaflets, flips to the other leaflet.

3. **Desorption:** Finally, FA move from the outer leaflet to the aqueous phase.

The 3 different stages can be reversed, because the membrane is assumed to have same amounts of phospholipids in both leaflets. Moreover, the flux direction is only determined by the gradient of FA across the membrane: from the highest concentration to the lowest.

### II.3.2 Facilitated transport of fatty acids by a carrier protein

It has been hypothesized that transmembrane diffusion of FA across biological membranes is limited by the flipping rate of FA (1, 31). A so-called carrier protein could enhance the rate of movement of FA from one leaflet of the membrane to the other. This protein binds to uFA in the aqueous compartment, transports the uFA to the other side of the membrane and finally releases it over there. The principle is the same as in paragraph II.3.1, because three phases can be identified: absorption, flipping (solely uFA or complete carrier, depending on which mechanism is proposed) and desorption. However, transport of FA by means of a carrier protein doesn't require the phospholipids inside the membrane as transport medium, unlike transmembrane diffusion of FA (see Fig. 5).

### II.3.3 Receptor

FA are tightly bound to albumin (55). Release of these albumin-FA complexes could be facilitated by a so-called receptor (Fig. 6). This putative protein, attached to a cell membrane, is supposed to strongly bind to complexes of FA and albumin, creating a change in albumin conformation and thereby enhancing the release of FA. This leads locally to a higher concentration of FA in the stagnant water layer near the membrane and, hence, an increase in transport of FA across the membrane, due to the ensuing steeper diffusion gradient.

### II.3.4 Enhancer: improved uptake of fatty acids in the membrane

An enhancer could also increase the transmembrane transport of FA (Fig. 7). This putative protein increases the rate of absorption of uFA from the aqueous phase directly into the phospholipid layer of the membrane. The remaining pathway of membrane transport of FA (flipping and desorption) is the same as was already described in paragraph II.3.1 about transmembrane diffusion of FA. The overall effect of the enhancer is an increase in FA transport across the membrane.

### II.4 Differences between the sarcolemmal and endothelial barrier

Above, the global structure of FA crossing the vascular compartment, endothelium, interstitial compartment and sarcolemma has been dismissed. Two main barriers can be identified:
• The endothelial barrier. It consists of the vascular compartment, i.e. the endothelial glycocalyx on the luminal side, the endothelium and that part of the interstitial compartment that is composed of the endothelial glycocalyx on the abluminal side.

• The sarcolemmal barrier. This barrier consists of the myocyte-associated glycocalyx, the sarcolemma and the sarcoplasm.

Two differences exist between these two barriers, which might have important physiological implications. The first one is the area available for transmembrane transport. The area of the endothelial barrier is about 500 cm$^2$ (g ww)$^{-1}$ (7, 48), while the sarcolemmal barrier covers ~ 2000 cm$^2$ (g ww)$^{-1}$ (7). The second difference is the width of both barriers. The sarcolemmal barrier, composed of the glycocalyx of the myocyte and sarcolemma (cytoplasm of myocyte is a sink), is ~ 0.3 μm and the endothelial barrier, including the vascular compartment, endothelium and the abluminal side of the endothelium, has a length of ~ 1.2 μm. These quantitative differences might have a significant impact of flux rates of FA, as will be discussed in detail in paragraph V.3.2.
Section III  Modeling approach

III.1 Describing physiological conditions in mathematical equations

The mathematical model, required for uptake processes of FA, is a dynamic system for which the variables, concentrations of biological substrates in this case, are time-dependent (14). These dependent variables are described by differential equations, which are mathematical representations of the changes in a variable as function of time. Examples of biological processes that induce a change in concentration in time are chemical reactions, diffusion processes, and transport mechanisms by means of transport-facilitating proteins. A short guide to formulate mathematical equations of these processes is given in this section.

III.1.1 Ordinary differential equations

Differential equations that contain one or more derivatives of a dependent variable with respect to a single independent variable, time in this chapter, are called ordinary differential equations (ODE's). Each ODE describes the dynamic behaviour of one variable by means of a mathematical function of variables, parameters and inputs. A state equation is a special differential equation that can't be deduced from other differential equations. A model consisting of n state variables must be defined by a set of n equations, ODE's in this case. A set of n equations is described in mathematical terms as follows (Eq. 3):

\[ \dot{q} = f(t, q, u) \]  

(3)

where \( q \) is a n-element column, containing all the variables of the system, \( \dot{q} \) is vector whose elements are the time-derivatives of the corresponding elements in \( q \), \( u \) is the input vector of the system, and \( f(t, q, u) \) is a mathematical function that depends on time \( t \), the variables \( (q) \) and inputs \( (u) \). The parameters are assumed to have constant values in this equation and will be discussed in subsection III.2. An example to clarify ODE's: many biochemical reactions occur in the body and the reaction equation of a very simple one is shown here:

\[ C_1 + C_2 \xrightleftarrows{k_1}{k_{-1}} C_3 \]

It shows the reaction of two substrates with concentrations \( C_1 \) and \( C_2 \) that form one product with concentration \( C_3 \). The three different concentrations, the variables, can be rewritten as differential equations (Eq. 4 - 6):
\[
\dot{C}_1 = -k_1 C_1 C_2 + k_{-1} C_3 \\
\dot{C}_2 = -k_1 C_1 C_2 + k_{-1} C_3 \\
\dot{C}_3 = k_1 C_1 C_2 - k_{-1} C_3 
\]

(4)  
(5)  
(6)

where \( C_1 \), \( C_2 \) and \( C_3 \) are the two substrate and product concentrations, respectively, \( k_1 \) is the rate parameter of \( C_1 \) reacting with \( C_2 \) to form \( C_3 \), and \( k_{-1} \) is the rate parameter of the reverse reaction back to \( C_1 \) and \( C_2 \). \( \dot{C}_1 \), \( \dot{C}_2 \) and \( \dot{C}_3 \) on the left-hand side (LHS) are the time derivatives of substrate and product concentrations \( C_1 \), \( C_2 \) and \( C_3 \), respectively. Accordingly, Eq. 4 is defined as the change of \( C_1 \) in time and depends on two reaction rates:

a. The reaction of \( C_1 \) with \( C_2 \) to \( C_3 \) (the first part of Eq. 4: \( k_1 C_1 C_2 \)), and
b. The reverse reaction of \( C_3 \) back to \( C_1 \) and \( C_2 \) (the second part of Eq. 4: \( k_{-1} C_3 \))

A minus sign of the first part in Eq. 4 represents a decrease in \( C_1 \) as function of time, when \( C_1 \) reacts with \( C_2 \). The second part is preceded by a plus, because an increase in the conversion from \( C_3 \) to \( C_1 \) (and \( C_2 \)) leads to an increase in \( C_1 \). In the same way, the plus and minus signs are placed in Eq. 5 - 6 and notice the similarity between Eq. 4 - 5 and the opposite signs in Eq. 6. This is necessary, because the law of mass conservation must be obeyed: there is no mass loss or gain in the complete system. Summarizing, \( \dot{C}_1 = \dot{C}_2 = -\dot{C}_3 \) leads to the conclusion that three equations have to be solved, including only one state equation, to come to a solution for all three differential equations. Solutions are \( C_1(t) \), \( C_2(t) \) and \( C_3(t) \), which are concentrations as function of time (state trajectories). They are determined by certain initial concentrations and the inputs of the system (Eq. 3). The three concentrations converge after a certain time span to a final solution (meaning that the system is stable): the so-called steady state value. In steady state, there is no change in concentration (\( \dot{C} = 0 \)), provided that the input is constant.

III.1.1.1 Analytical vs. numerical solution

Possible solutions of differential equations, like ODE's, can be obtained analytically or by a numerical approximation. An analytical solution of a differential equation is an exact representation of the system in the form of a mathematical equation. However, this is only possible in a limited number of cases. Solutions of differential equations can also be approached by numerical methods, which are often used in more complicated situations, as is the case with most physiological processes. The analytical solution is very cumbersome to acquire or even impossible. Therefore the equations are solved numerically with mathematical computer programs, e.g., MATLAB (ode45) and Mathematica (DSolve).
III.1.2 Partial differential equations

Besides ODE’s that only depend on changes in time, there are differential equations that depend on changes in time and other independent variables. These are called partial differential equations (PDE’s) and this chapter will only discuss PDE’s that depend on changes in time and in distance. An example of a PDE is the diffusion of a substance with concentration \( C_1 \) across a given length \( L \) (Fig. 8). The diffusion in one dimension is described with the diffusion equation (68) by the following equation (Eq. 7):

\[
\dot{C}_1 = D \frac{\partial^2 C_1}{\partial x^2}
\]

(7)

where \( C_1 \) is the substrate concentration, \( D \) is the diffusion coefficient, and \( x \) is the location. \( \dot{C}_1 \) is the time derivative of concentration, \( \frac{\partial^2 C_1}{\partial x^2} \) represents the second position-dependent derivative of \( C_1 \). The solution of Eq. 7 is \( C_1(t, x) \), a concentration that not only varies with time, but also with the distance. Again, it is possible to obtain an analytical solution, but in complex biological situations a numerical approach is used to come to a solution. For example, the substrate with concentration \( C_1 \) diffuses only in the \( x \)-direction as shown in Fig. 8. The diffusion space, the rectangular box, is divided in a number of equally spaced smaller rectangulars: the segments (Fig. 9). Each segment represents a certain average concentration \( C_1 \), a value at a point, in a certain range with a length \( \Delta x \), which could differ per segment. This method of solving a PDE by creating a volume around one point is called the Finite Volume Method (FVM). The FVM is a way to rewrite the PDE as a coupled set of ODE’s, based on a spatial grid (Fig. 9), which has been deduced for the diffusion equation (Eq. 7). First, one has to know that the flux \( J \) per unit area \( S \) is defined in diffusion processes as (Eq. 8, also shown in Eq. 1, now slightly adapted):

\[
J = -DS \frac{\partial C_1}{\partial x}
\]

(8)

where \( \frac{\partial C_1}{\partial x} \) is the first position-dependent derivative of substrate concentration \( C_1 \), the other parameters have been described previously (Eq. 1). For segment 5, the concentration \( C_1^5 \) depends on the influx of substance from \( C_1^4 \) to \( C_1^5 \), \( J_{4 \rightarrow 5} \), and on the efflux from \( C_1^5 \) to \( C_1^6 \), \( J_{5 \rightarrow 6} \) (Fig. 9). The fluxes \( J_{4 \rightarrow 5} \) and \( J_{5 \rightarrow 6} \) are determined by discretising Eq. 8 (dividing in a finite number of segments) and using Fig. 9 (Eq. 9 - 10):

\[
J_{4 \rightarrow 5} = DS \frac{C_1^4 - C_1^5}{\Delta x_A}
\]

(9)

\[
J_{5 \rightarrow 6} = DS \frac{C_1^5 - C_1^6}{\Delta x_B}
\]

(10)
where \( \Delta x_A \) is the distance from the segment of interest, segment 5 in this example, to the neighbouring segment on the left, i.e., segment 4, \( \Delta x_B \) is the distance from the segment of interest to the right segment, i.e., segment 6 (Fig. 9).

The net flux, the amount of substrate per unit time moving into minus the amount of substrate per unit time moving out of the segment, divided by the total volume of segment 5 \( (V^5) \) in this case results in \( \dot{C}_i^5 \) (Eq. 11):

\[
\frac{J_{4 \rightarrow 5} - J_{5 \rightarrow 6}}{V^5_{\text{seg}}} = -\frac{DS}{V^5_{\text{seg}}} \left( \frac{C^5_1 - C^5_2}{\Delta x_A} - \frac{C^5_2 - C^5_1}{\Delta x_B} \right) = \frac{D}{\Delta x} \left( \frac{C^5_1 - C^5_2}{\Delta x_A} - \frac{C^5_2 - C^5_1}{\Delta x_B} \right) = \dot{C}_i^5
\]  

Eq. 11 is rewritten in a more general form as Eq. 12, which is a discretised representation of Eq. 7 for the segments \( i = 2 \) to \( N - 1 \), with \( N \) is the total number of segments:

\[
\dot{C}_i^1 = \frac{D}{\Delta x} \left( \frac{C_{i-1}^1 - C_i^1}{\Delta x_A} - \frac{C_i^1 - C_{i+1}^1}{\Delta x_B} \right) \quad \text{for} \quad i = 1
\]

with \( \dot{C}_i \) is an index that indicates the segment number of the system (it varies between 1 and 9 in Fig. 9). However, at \( i = 1 \) and \( i = 9 \), one can’t use Eq. 12, because the concentration in segment 0 and 10 doesn’t exist, respectively. Still, it’s possible to solve this numerical problem by reflection of diffusion (Eq. 13 - 14):

\[
\dot{C}_i^1 = \frac{J_{2 \rightarrow 1} - J_{1 \rightarrow 2}}{V^1_{\text{seg}}} = \frac{D}{\Delta x} \left( \frac{C_2^1 - C_1^1}{\Delta x_A} \right) \quad \text{for} \quad i = 1
\]

\[
\dot{C}_i^N = \frac{J_{N-1 \rightarrow N} - J_{N \rightarrow N-1}}{V^N_{\text{seg}}} = \frac{D}{\Delta x} \left( \frac{C_{N-1}^N - C_{N-1}^N}{\Delta x_A} \right) \quad \text{for} \quad i = N
\]

where \( N \) is the total number of segments, which the system consists of. Fig. 9 shows an example of 9 differential equations \( (N = 9) \) that needs to be solved to know the complete profile of \( C_1 \). But the more segments one uses to approximate Eq. 7, the better it approximates the analytical solution. Needless to say is that a higher number of segments leads to more equations, resulting in a longer calculation time.

### III.1.3 Combination of ODE’s and PDE’s

Based on the law of mass balance, the concentrations of the physiological important molecules can be described in terms of differential equations (e.g., Eq. 4 - 6 and Eq. 12 - 14), which are composed of different components, each representing a single process, which involves a change in concentration. For example, the right hand side (RHS) of Eq. 4 - 6 only consist of 2 components. For Eq. 4 this is the binding component, \(-k_1C_1C_2\), and the release component, \(+k_1C_3\). Both are lumped in one single rate component that contains the contribution of both components to the concentration change, dependent on the three substrate concentrations: \(-v_{\text{reaction}}(C_1,C_2,C_3)\). The same rate component is valid for Eq. 5 \((-v_{\text{reaction}}(C_1,C_2,C_3))\) and also for Eq. 6 \((v_{\text{reaction}}(C_1,C_2,C_3))\), except for the absence of a
minus sign, due to mass balance. Likewise, PDE’s can be described in terms of rate components. As mentioned earlier, diffusion processes are characterized by partial differential equations, which can be rewritten in terms of ODE’s for the different segments. These equations are represented in the discretised form as $\frac{D}{\Delta x} \left( \frac{C_{i-1} - C_i}{\Delta x_A} - \frac{C_i - C_{i+1}}{\Delta x_B} \right)$ (see also Eq. 12 - 14) and can also be lumped in one rate component for all $i$: $v_{\text{diffusion}}(C_i)$. Similarly, diffusion of another substrate with concentration $C_2$, in discretised form written as $\frac{D}{\Delta x} \left( \frac{C_{i-1}^{2} - C_i^{2}}{\Delta x_A} - \frac{C_i^{2} - C_{i+1}^{2}}{\Delta x_B} \right)$, is included in rate component $v_{\text{diffusion}}(C_2)$. Combinations of multiple rate components are also possible: a simple reaction of a substance with concentration $C_1$ and diffusion of this concentration at the same time, so a combination of Eq. 4 - 6 and Eq. 12 - 14, is rewritten in rate components as Eq. 15 - 17:

$$\begin{align*}
\dot{C}_1 &= -v_{\text{reaction}}(C_1, C_2, C_3) + v_{\text{diffusion}}(C_1) \\
\dot{C}_2 &= -v_{\text{reaction}}(C_1, C_2, C_3) + v_{\text{diffusion}}(C_2) \\
\dot{C}_3 &= v_{\text{reaction}}(C_1, C_2, C_3) + v_{\text{diffusion}}(C_3)
\end{align*}$$

(15) (16) (17)

These differential equations are state equations, which can't be rewritten in terms of each other, and show very clearly which components influence the dynamic behaviour of $C_1$, $C_2$ and $C_3$: the state variables. The rate components have to be determined: which processes are involved in changing the concentration of a given substrate in terms of rate components? If these are determined for the substrates and products, the following question has to be answered: what are the underlying equations of the required rate components? The final answer to this question is a system of one or more differential equations, which need to be solved. These equations are the structure of the model.

### III.2 Obtaining parameter values

State equations are composed of state variables, parameters and inputs, as mentioned in paragraph III.1.1. The parameters in the state equations provide a quantitative description of the different parts of a state equation. Examples of parameters are $k_1$, $k_{-1}$ (both from Eq. 4 - 6) and $D$ (Eq. 6), which values can be obtained from experimental data. Unfortunately, not all values of the parameters of interest are experimentally determined or are suitable for model use directly. It depends on what data is available and under which conditions the parameters are obtained, e.g., *in vivo* (measured in an intact cell or organism), or *in vitro* (measured in an isolated cell or cell-free homogenate, (2)). This subsection shows some important aspects of parameters that need attention, because a model greatly depends on the values of the parameters used. In the following paragraphs (III.2.1 – III.2.2), examples of parameters will be given, which are used in the mathematical model. All these parameter values are listed in Table 1.
III.2.1 Calculating parameter values: lipid content of a biological membrane

In some cases, parameter values are needed that can’t be determined directly from experiments, but need some post processing to get the value needed. An example is the determination of the concentration of lipids in a biological membrane, which is required to obtain a rate parameter value for the transport model of FA.

About $5 \times 10^6$ phospholipid molecules are located in a $1 \mu m \times 1 \mu m$ area of a phospholipid bilayer (2). The thickness of an average phospholipid bilayer is about 5 nm. Therefore, the volume of a phospholipid bilayer will become $(1 \times 10^{-5} \text{ dm}) \times (1 \times 10^{-5} \text{ dm}) \times (5 \times 10^{-6}) \text{ dm} = 5 \times 10^{-18} \text{ dm}^3$. The quantity of phospholipid molecules in this membrane is $5 \times 10^6$ phospholipid molecules, which is equal to $8.3 \times 10^{-16} \text{ mol}$, making use of the number of Avogadro. The average molecular mass of membrane phospholipids is 600 Da, so $(8.3 \times 10^{-16} \text{ mol}) \times (600 \text{ g mol}^{-1}) = 5 \times 10^{-15} \text{ g phospholipid}$ is present in $5 \times 10^{-18} \text{ dm}^3$. Dividing this mass by the volume leads to $1 \text{ kg dm}^{-3}$. This is calculated for a membrane that only contains phospholipids without proteins. However, only 50 wt % of the biological membrane consists of phospholipids (2). That leads to a phospholipid content in the biological membrane of $0.5 \text{ kg dm}^{-3}$. Converted back to molar: $(0.5 \times 10^3) / (600) = 0.8 \text{ M}$. A double check has been performed to confirm this result by using a typical canine erythrocyte membrane with an area of $195 \mu m^2$ and a thickness of 7.5 nm, which contains 0.7 pg phospholipids and 0.8 pg membrane proteins (3). The phospholipid concentration derived from these data (calculation not shown here) is in full agreement with the outcome of the previous calculation. Likewise, one derives a protein concentration in an average biological membrane of $\sim 8 \text{ mM}$, because the molar ratio proteins:phospholipids in the membrane is $\sim 1:100$ (2).

III.2.1.1 Adsorption layer

In the computer model of transport of FA, described in section IV, the concentration of phospholipids is needed to determine the value for the rate parameter $k_{on}$ of absorption (Eq. 18, (31)):

$$k_{on} = K_p [L] k_{off}$$

(18)

where $K_p$ is the so-called partition coefficient of unbound FA (uFA) in the phospholipid/water phase, $[L]$ is the concentration of phospholipids present in the membrane with adsorption layers and $k_{off}$ is the rate parameter of desorption. To determine $[L]$, one has to know, besides the phospholipid concentration in the membrane, that uFA are only absorbed by the membrane, if these are present in the close vicinity of the membrane. This region is called the adsorption 2) layer with a width of 1 fatty acid molecule ($\sim 2.5 \text{ nm} = \text{size of membrane leaflet}$) and is present on both sides of the membrane. The total volume of the solution of interest consists of both adsorption layers and the volume of the membrane. With this knowledge and a phospholipid concentration in the membrane of 0.8 M, one derives a value for $[L]$ of 0.4 M and a membrane-associated protein concentration of 0.4 mM (Table 1).

2) The adsorption layer has been defined as a region against the membrane, from which FA are absorbed into the membrane.
III.2.2 Controversy about parameter values

Experiments to obtain parameter values have been performed by different research groups. In a number of cases, this has lead to differences in values for the same parameters, which eventually resulted in opposing theories. To solve this problem, one has to double check every parameter value used to make sure that the parameter value is unique. If not, experimental conditions of the different experiments have to be scrutinously compared in order to find any crucial differences. Below (subparagraphs III.2.2.1 – III.2.2.3), three examples will illustrate this issue:

III.2.2.1 Fatty acids crossing a membrane

As mentioned above, controversy exists about the mechanism underlying transport of FA across membranes to explain a physiological normal flux of about $100 \text{ nmol min}^{-1} \text{ (gr wwr)}$, as measured in intact hearts. Experiments were performed on model membranes of small, large and giant unilamellar vesicles, which are abbreviated to SUV's (with a diameter $d \sim 25 \text{ nm}$), LUV's ($d \sim 100 \text{ nm}$) and GUV's ($d \sim 200 \text{ nm}$), respectively (23, 31). In these experiments, different values were found for the rate parameter $k_{\text{flip}}$, which is a measure that describes the amount of FA crossing the membrane per unit time and ranges from as low as $0.5 \text{ s}^{-1}$ (GUV's, 31) to values higher than $70 \text{ s}^{-1}$ (SUV's, 30), depending on the experimental methods used. Unfortunately, it is unclear which experiment approaches the in vivo physiological situation as close as possible. Moreover, the presence of both protein-mediated and transmembrane diffusion of FA is supported by a substantial number of experiments. In addition, FA are present in the ionised or un-ionised form in the membrane; ionised FA “flip” very slowly across a phospholipid bilayer, in contrast to un-ionised FA (20, 28, 29). Which form of FA (ionised or un-ionised) is predominantly present, depends on the $pK_a$ (acid ionisation constant) and pH (measure for the degree in acidity of a solution) of the environment. Its relation is described by the Henderson-Hasselbalch equation (Eq. 19):

$$\text{pH} - pK_a = \log\left(\frac{[X^-]}{[HX]}\right)$$

where $[X^-]$ and $[HX]$ represent the ionised and un-ionised concentration of substance $X$, respectively. Eq. 19 shows that if $\text{pH} > pK_a$, the ionised form predominates, which is the case when FA are present in an aqueous environment: the $pK_a$ is $\sim 4.5$ and the pH is 7.4 (23). Therefore FA are mainly present as ionised monomeric molecules in aqueous compartments. The $pK_a$ is approximately 7.6 in a phospholipid environment ($\text{pH} = 7.4$, (23)), indicating a higher quantity of un-ionised FA in the membrane. Consequently in this chapter, FA in the phospholipid membrane are assumed to be present in the un-ionised form.

It’s of great importance to incorporate the opposing views about transmembrane transport of FA in our computer model and compare the in silico findings with experimental data (see section V).
111.2.2 Diffusion of FABP in the cytosol

Parameters have been obtained by using different methods. Results could however be biased by the conditions chosen in the experimental set-up. An illustrative example is the determination of the diffusion coefficient of cytoplasmic FABP present. Experiments were performed with FABP bound to labelled 12-(N-methyl)-N-[((7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-octadecanoic acid (NBD-stearic acid) in order to determine the diffusion coefficient of FABP in liver cells of male rats (5). The value obtained was about $0.8 \times 10^{-9} \text{cm}^2 \text{s}^{-1}$, which is 2000 times lower than assessed in another experiment (69): $1.7 \times 10^{-6} \text{cm}^2$ (this value is corrected to a temperature of 37 degrees Celsius; see subparagraph 111.2.2.3). Another remarkable result of the experiments performed with NBD-stearic acid (5) was the differences in diffusion coefficients between male ($0.8 \times 10^{-9} \text{cm}^2 \text{s}^{-1}$) and female rats ($4.8 \times 10^{-9} \text{cm}^2 \text{s}^{-1}$).

Compared with proteins of the same size as FABP with a molecular weight of 15 kDa (62), the values for FABP in the NBD-stearic acid experiments (5) are surprisingly low. These low values can be ascribed to the NBD-stearic acid used as fluorescent marker. A drawback of this marker is that it doesn’t solely bind to FABP: it was shown that NBD-stearic acid also interacts with cell membranes (46, 47), which leads to a decrease in mobility of this compound. This can be interpreted as a decrease in diffusion coefficient and is probably the cause of the low values found (5). The higher values, obtained in female rats, can be explained by the higher amount of FABP present in female cells, so more NBD-stearic acid binds to FABP, which results in a higher diffusion coefficient compared to experiments performed on male rats (47).

111.2.2.3 Translation of experimental data to physiological relevant conditions

Most experiments performed to obtain parameter values are executed at room temperature, cellular homogenates, etc. These values must be extrapolated to physiological conditions, e.g. body temperature and quantity per gram tissue. An example is converting the experimental results performed on SUV’s (23) to physiological conditions. The value of rate parameter $k_{\text{on}}$ was determined as $3.6 \times 10^6 \text{s}^{-1}$ per molar phospholipid in the membrane with adsorption layers (= [L]). The experiments were performed at a phospholipid concentration of $0.2 \times 10^{-3} \text{M}$ (23), which lead to a $k_{\text{on}}$ of 720 s$^{-1}$. But this doesn’t apply on a real physiological situation with a value for [L] of 0.4 M: this will lead to a value for $k_{\text{on}}$ of $1.4 \times 10^6 \text{s}^{-1}$. Compared to the $k_{\text{on}}$ (720 s$^{-1}$) in the SUV experiment, this is considerably higher.

One has to bear in mind that even in experiments performed on biological cells, e.g. on isolated myocytes (45), a number of assumptions are made that could effect the results. For example, experiments to determine the uptake of FA in isolated myocytes were performed on quiescent cells (45), implicating that these cells didn’t contract. This leads to a lower energy requirement and therefore a reduced uptake of FA. Moreover, the isolated myocytes were
obtained by destroying the glycocalyx, which might have a profound effect the mobility of albumin (75).

### III.2.3 Monte Carlo method

Physiological experiments generate results with a certain probability distribution of the parameters, which can be incorporated in the model. This is implemented by simulating the same model several times (about 500 cycles), but with changed values for the parameters. During each cycle, all the parameter are chosen according to their own probability distribution (e.g., with the command `normrnd` in MATLAB), independent of each other. The results of the model of each cycle is stored and subsequently the simulation is repeated with other values for the parameters, chosen according to their probability distribution and finally the results are stored again. This process is repeated as often as possible and leads to a collection of saved results that has a certain mean and standard deviation, which can be analysed and compared with experimental data. The name for this method of varying parameters according to their probability distribution is called the *Monte Carlo* method (17) and its advantage is to create a better estimate of the model uncertainty (74).

### III.3 The flux test and unit check

Till now, only methods have been discussed to create a model, but not to test whether it's correctly implemented. Possible methods are to perform a *flux test* or check the units of the system, i.e., the *unit check*.

#### III.3.1 Flux test

The processes in a stable system reach a steady state value when a constant input is applied (see paragraph III.1.1). This can only be achieved if all the derivatives in time of the differential equations become zero (e.g., $\dot{C}_1 = \dot{C}_2 = \dot{C}_3 = 0$ in Eq. 4 - 6, or $\dot{C}_i = 0$ for $i = 1$ to $N$ in Eq. 12 - 14). Therefore, the different parts on the RHS of the state equations have to counterbalance each other in steady state. A special case is a system in which diffusion processes are involved. Though the sum of the rate components in these equations has to be equal to zero in steady state, the flux is *not* zero. Paragraph III.1.2 showed how the influx (Eq. 9) and efflux (Eq. 10) have been related to the discretised state equation (Eq. 12). This state equation has to be equal to zero in steady state (see paragraph III.1.1), which results in identical values for both influx and efflux. The flux test checks whether the influx and efflux satisfies these requirements. Therefore, a mathematical model of a discretised diffusion process is only technically correct, if the fluxes across the different segments are equal.

#### III.3.2 Unit check

Differential equations consist of algebraic combinations of parameters, variables and inputs, each having their own units. Therefore, all differential equations must be consistent in units.
An example is to check the units of Eq. 4: \( \dot{C}_1 = -k_1 C_1 C_2 + k_{-1} C_3 \). The state equation consists of a change in concentration per unit time, \( \dot{C}_1 \), on the left-hand side (LHS), e.g. the units are \( \text{M} \text{ s}^{-1} \) (molar per second). The RHS is composed of two subcomponents, \(-k_1 C_1 C_2\) and \(k_{-1} C_3\), which also have to be in the units molar per second, just like the LHS. Both subcomponents contain the variables \( C_1, C_2 \) and \( C_3 \), concentrations with unit \( \text{M} \) (molar). The units for the parameters \( k_1 \) and \( k_{-1} \) are determined to be (using Eq. 4): \( \text{M}^{-1} \text{ s}^{-1} \) and \( \text{s}^{-1} \), respectively, which is in agreement with the literature (31).
Section IV  Computer model of cardiac fatty acid uptake

With the knowledge described in the previous sections of this chapter, one can theoretically create a model of the FA uptake processes across the vascular compartment, the endothelial cells, the interstitial compartment, the sarcolemma and finally into and through the sarcoplasm of the myocyte. The source of the complete system is located in the vascular compartment, at the beginning of the luminal side of the endothelial glycocalyx. Leaving esterified FA present in the core of chylomicrons and VLDL's out of consideration, the FA in this compartment are mainly present as complexed to albumin, only a minor portion is in the unbound form present in the aqueous solution (55). The sarcoplasm is considered to be a sink, i.e. $[\text{FA}] \approx 0$ M.

This section will split the transport pathway of FA from blood plasma to myocardial sarcoplasm in three parts, starting with a model of FA crossing the sarcolemma. The goal of this model is to elucidate which mechanism is involved in the transport of FA across the sarcolemma: is it transmembrane diffusion or protein-mediated? The second model is an extension of the first model by incorporating a glycocalyx to show the effect of a glycocalyx on the flux of FA. The third model describes the transport of FA from the vascular compartment to the abluminal glycocalyx of the endothelial compartment. This model is to complete the total uptake process of FA from blood to sarcoplasm and to see how large the flux is across this barrier. When the last two models are linked together, they form the complete transport pathway of FA.

IV.1  Model I:  The myocardial sarcolemma

The precise transport mechanism of FA across the sarcolemma is not elucidated yet. Three possible transport mechanisms will be described and implemented in the computer model, presented in subsection V.1.

IV.1.1  Transmembrane diffusion

The first mechanism of FA to overcome the sarcolemma as a constraint is transmembrane diffusion (13,15,21,28) by means of "flip-flop" through the phospholipid bilayer (36). The three steps are absorption, flipping and desorption, which are described mathematically. The model of transport of FA across the sarcolemma consists of four compartments:

1. The interstitial compartment in close vicinity of the outer leaflet of the sarcolemma. In this model, the interstitial compartment is only a constant supplier of FA (only the unbound form will be considered) and no diffusion or reaction processes are involved yet. Because the supply of FA is constant (no change in FA per unit time), the ODE describing the change of FA in this compartment is set to zero.
2. The outer leaflet is the phospholipid layer of the sarcolemma, which borders on the interstitial compartment. The FA, present in this layer, are located in between the phospholipid molecules.

3. The inner leaflet is the layer of the sarcolemma that faces the sarcoplasm.

4. The sarcoplasm of the myocyte is the sink compartment for FA. Because FA bind directly to FABP and are subsequently converted to acyl-CoA, the concentration of uFA is almost negligible (71). Therefore, the assumption is made that the ODE for this compartment is also equal to zero.

In short, only two ODE's are necessary to describe the transport of FA across a membrane (Fig. 11). If one assumes that the source and sink concentrations, the concentration of FA in the interstitial compartment and sarcoplasm, respectively, remain constant. The change in concentration of the remaining two compartments is described by the following two state equations (Eq. 20 - 21):

**Outer leaflet:**

\[
\frac{dC_{FA}}{dt}_{outer} = k_{on}C_{FA}^{source} - k_{off}C_{FA}^{outer} + k_{flip}(C_{FA}^{inner} - C_{FA}^{outer})
\]  

(20)

**Inner leaflet:**

\[
\frac{dC_{FA}}{dt}_{inner} = k_{on}C_{FA}^{sink} - k_{off}C_{FA}^{inner} + k_{flip}(C_{FA}^{outer} - C_{FA}^{inner})
\]  

(21)

where \(\dot{C}_{FA}^{outer}\) and \(\dot{C}_{FA}^{inner}\) represent the change of FA per unit time in the outer and inner leaflet, respectively. The state variables are \(C_{FA}^{source}\), \(C_{FA}^{outer}\), \(C_{FA}^{inner}\) and \(C_{FA}^{sink}\), which are the concentration of uFA in the interstitial compartment, outer leaflet, inner leaflet and sarcoplasm, respectively. The parameters \(k_{on}\), \(k_{off}\) and \(k_{flip}\) represent the rate parameters of absorption, desorption and "flip-flop" of FA in a phospholipid membrane, respectively. The two state equations (Eq. 20 - 21) consist only of the three processes involved in transmembrane diffusion of FA. In Eq. 20, the absorption step is described by \(k_{on}C_{FA}^{source}\), which represents the uptake of FA from the interstitial compartment into the outer leaflet of the myocardial sarcolemma. Besides absorption, also desorption from the outer leaflet into the interstitial compartment takes place: \(-k_{off}C_{FA}^{outer}\) (in Eq. 20). The remaining part of Eq. 20, \(k_{flip}(C_{FA}^{inner} - C_{FA}^{outer})\), is the "flip-flop" step, with the "flip" defined as the application of FA from outer to inner leaflet, \(k_{flip}C_{FA}^{outer}\), and the "flop" as the reverse movement (from inner to outer leaflet), \(k_{flip}C_{FA}^{inner}\), assuming that "flipping" and "flopping" of FA occur at an equal rate \((k_{flip} = k_{flop})\). Similarly, one can identify the three processes (absorption, desorption and "flip-flop") in Eq. 21: absorption of FA from the sarcoplasm into the inner leaflet is \(k_{on}C_{FA}^{sink}\), desorption of FA is the reverse reaction (from inner leaflet to sarcoplasm): \(-k_{off}C_{FA}^{inner}\) and "flip-flop" is represented as \(k_{flip}(C_{FA}^{outer} - C_{FA}^{inner})\), with \(k_{flip}C_{FA}^{outer}\) as the "flop" (from inner to outer leaflet) and \(-k_{flip}C_{FA}^{inner}\) as the "flip" (from outer to inner leaflet).
Values for the parameters involved in the absorption, flipping and desorption of FA were derived by conducting experiments on SUV's, LUV's (23, 30) and GUV's (31). The values found are listed in Table 1, which were put in the computer model. Table 1 clearly shows that the parameter values differ substantially among the various experiments: the ones performed on SUV's and LUV's (23, 30) resulted in higher parameter values compared to experiments with GUV's (31). Two possible explanations are the involvement of a fluorescent marker and the kind of vesicles used. In the GUV experiments (31) an anthroyloxy group as marker, bound to FA, was utilized to obtain a value for $k_{\text{flip}}$. However, this marker could alter the characteristics of FA bound to this marker, probably leading to artefacts in the measurements (30). The other explanation is the differences in vesicles used: SUV's and LUV's are black membranes (artificial membranes, only consisting of phospholipids) with a diameter of $\sim 25$ and $\sim 100$ nm, respectively. This is much smaller than the diameter of normal biological cells ($\sim 500$ nm on average), which have membranes that also contain other components, e.g. cholesterol. For that reason, GUV's (diameter $\sim 200$ nm) with cholesterol (31) match biological cells better than SUV's and LUV's.

It is important that in order to solve the equations, initial conditions are defined. These are values for the state variables ($C_{\text{FA}}^{\text{source}}$, $C_{\text{FA}}^{\text{inner}}$, $C_{\text{FA}}^{\text{outer}}$ and $C_{\text{FA}}^{\text{sink}}$ in this case) at $t = 0$. The initial concentrations for the source and sink remain constant and are shown in Table 1. Now the total model is defined, it has to be solved by an ODE solver. The results obtained are shown in section V.

**IV.1.2 Carrier protein**

A second theory about FA crossing a membrane involves a so-called carrier protein, which transports the FA through the membrane (Fig. 5). Its transport mechanism shows great similarity with the transport of FA by transmembrane diffusion ("flip-flop") due to a 3-step transport process. The difference is that with a carrier protein the transport medium is not the phospholipid bilayer, but the carrier protein itself. The three steps to be taken are explained in the section about the physiological background of transport of FA (section II), from which one could deduce four state variables: the concentration of available sites on the carrier protein at the outer and inner side of the membrane ($C_{\text{CP}}^{\text{outer}}$ and $C_{\text{CP}}^{\text{inner}}$, respectively), and the concentration of FA bound to carrier protein sites at the outer and inner membrane ($C_{\text{CPFA}}^{\text{outer}}$ and $C_{\text{CPFA}}^{\text{inner}}$, respectively). Rewritten in state equations, transport of FA by a carrier protein is mathematically expressed as (Eq. 22 - 25), irrespective of the exact molecular mechanism of transport:

\[
\begin{align*}
C_{\text{outer}}^{\text{CP}} &= -k_{\text{bind}CPFA} C_{\text{FA}}^{\text{source}} C_{\text{CP}}^{\text{outer}} + k_{\text{release}CPFA} C_{\text{CPFA}}^{\text{outer}} + k_{\text{flip}CPFA} (C_{\text{inner}}^{\text{CP}} - C_{\text{CP}}^{\text{outer}}) \quad (22) \\
C_{\text{outer}}^{\text{CPFA}} &= k_{\text{bind}CPFA} C_{\text{FA}}^{\text{source}} C_{\text{CP}}^{\text{outer}} - k_{\text{release}CPFA} C_{\text{CPFA}}^{\text{outer}} + k_{\text{flip}CPFA} (C_{\text{inner}}^{\text{CPFA}} - C_{\text{CP}}^{\text{outer}}) \quad (23) \\
C_{\text{inner}}^{\text{CP}} &= -k_{\text{bind}CPFA} C_{\text{CP}}^{\text{inner}} + k_{\text{release}CPFA} C_{\text{CPFA}}^{\text{inner}} + k_{\text{flip}CPFA} (C_{\text{outer}}^{\text{CP}} - C_{\text{CP}}^{\text{inner}}) \quad (24)
\end{align*}
\]
\[
\dot{C}_{CP,FA} = k_{bind}^{CP,FA} C_{CP} - k_{release}^{CP,FA} C_{CP,FA} + k_{flip}^{CP,FA} (C_{CP} - C_{CP,FA})
\] (25)

where \(k_{bind}^{CP,FA}\), \(k_{release}^{CP,FA}\) and \(k_{flip}^{CP,FA}\) represent the rate parameters of binding and release of FA to and the "flip-flop" of the carrier protein, respectively. Moreover, it is assumed that the rate parameters \(k_{bind}^{CP}\) and \(k_{release}^{CP}\) are identical on both sides of the membrane and that the "flip"-rate equals the "flop"-rate. The state variables and source and sink concentrations have already been explained in section III. Unfortunately, no experimental data is available about the carrier protein, because its existence is not proven yet. Therefore, values for rate parameters and the initial concentration of carrier protein in the membrane have been based on estimations within physiological range (Table 1). The initial source and sink concentrations of FA are the same as used in the transmembrane diffusion of FA, so only concentrations of uFA are used.

IV.1.3 Enhancer

The 3rd transport mechanism is almost similar to transmembrane diffusion, but facilitated by an enhancer protein. The state equations of an enhancer are identical to the state equations for transmembrane diffusion of FA (Eq. 20 - 21). The main difference is the value for the uptake parameter \((k_{on})\) on the outer side of the membrane, because the enhancer elevates this value, which in turn increases the flux of FA across the membrane. The state equation of FA in the inner leaflet is shown in Eq. 21, the other state equation for FA in the outer leaflet is virtually identical to Eq. 20 (Eq. 26):

\[
\dot{C}_{FA} = k_{on}^{enhancer} C_{source} - k_{off}^{outer} C_{FA} + k_{flip} (C_{FA} - C_{FA}^{outer})
\] (26)

where \(k_{on}^{enhancer}\) is the rate parameter for absorption of FA, which is augmented due to the presence of the enhancer.

A remarkable difference between biological and black membranes in the uptake of FA is the difference in partition coefficient \((K_p)\): the \(K_p\) was shown to be \(\sim 6.0 \times 10^5\) M\(^{-1}\) [L] in black (57), but \(\sim 1.8 \times 10^6\) M\(^{-1}\) [L] in biological membranes (adipocytes, (27)) for palmitate. The presence of a protein that increases the value of \(K_p\) could be responsible for the 3 times higher value in biological membranes than in black membranes. Therefore, a \(K_p\) of 6.0 \(\times 10^5\) M\(^{-1}\) [L] will be used for all models to come, in which no protein is present and results in a low value for \(k_{on}\) (as was shown in Eq. 18: \(k_{on} = k_{off} K_p [L]\)). The enhancer is modelled with the high value for \(K_p\) (1.8 \(\times 10^6\) M\(^{-1}\) [L]), which results in an increased value for \(k_{on}\). The other variables and parameters have been described in paragraph IV.1.1, just like the initial concentrations.
IV.2 Model II: Extension of sarcolemma with a glyocalyx

IV.2.1 Diffusion of albumin and its complexes

Model I, with transport of FA across the sarcolemma by means of transmembrane diffusion, has been extended with a glyocalyx. Diffusion processes and binding reactions of uFA, albumin and albumin-bound FA have been incorporated in Model II.

Eq. 20 - 21 describe the state equations for the transport across the sarcolemma, but the following equations (Eq. 27 - 31) are added to describe the influence of a glyocalyx on the transport of FA:

For the segments \( i = 2 \) to \( N - 2 \)

\[
\frac{\mathrm{d} C_{i}^{\text{uFA}}}{\mathrm{d} x} = \frac{D_{\text{uFA}}}{(\Delta x)^2} (C_{i+1}^{\text{uFA}} + C_{i-1}^{\text{uFA}} - 2C_{i}^{\text{uFA}}) - k_{1} C_{i}^{\text{ Alb}} C_{i}^{\text{xuFA}} - k_{2} C_{i}^{\text{AF1}} C_{i}^{\text{uFA}} - \ldots
\]

\[
- k_{3} C_{i}^{\text{AF2}} C_{i}^{\text{uFA}} + k_{-1} C_{i+1}^{\text{AF1}} + k_{-2} C_{i+1}^{\text{AF2}} + k_{-3} C_{i}^{\text{AF3}}
\]

(27)

\[
\frac{\mathrm{d} C_{i}^{\text{ Alb}}}{\mathrm{d} x} = \frac{D_{\text{ Alb}}}{(\Delta x)^2} (C_{i+1}^{\text{ Alb}} + C_{i-1}^{\text{ Alb}} - 2C_{i}^{\text{ Alb}}) - k_{1} C_{i}^{\text{ Alb}} C_{i}^{\text{uFA}} + k_{-1} C_{i}^{\text{AF1}}
\]

(28)

\[
\frac{\mathrm{d} C_{i}^{\text{AF1}}}{\mathrm{d} x} = \frac{D_{\text{AF1}}}{(\Delta x)^2} (C_{i}^{\text{AF1}} + C_{i+1}^{\text{AF1}} - 2C_{i}^{\text{AF1}}) + k_{2} C_{i}^{\text{AF1}} C_{i}^{\text{uFA}} - k_{3} C_{i}^{\text{AF2}} C_{i}^{\text{uFA}} - k_{-1} C_{i}^{\text{AF1}} + k_{-2} C_{i}^{\text{AF2}}
\]

(29)

\[
\frac{\mathrm{d} C_{i}^{\text{AF2}}}{\mathrm{d} x} = \frac{D_{\text{AF2}}}{(\Delta x)^2} (C_{i}^{\text{AF2}} + C_{i+1}^{\text{AF2}} - 2C_{i}^{\text{AF2}}) + k_{2} C_{i}^{\text{AF1}} C_{i}^{\text{uFA}} - k_{3} C_{i}^{\text{AF2}} C_{i}^{\text{uFA}} - k_{-2} C_{i}^{\text{AF2}} + k_{-3} C_{i}^{\text{AF3}}
\]

(30)

\[
\frac{\mathrm{d} C_{i}^{\text{AF3}}}{\mathrm{d} x} = \frac{D_{\text{AF3}}}{(\Delta x)^2} (C_{i}^{\text{AF3}} + C_{i+1}^{\text{AF3}} - 2C_{i}^{\text{AF3}}) + k_{3} C_{i}^{\text{AF2}} C_{i}^{\text{uFA}} - k_{-3} C_{i}^{\text{AF3}}
\]

(31)

where \( i \) is a index, ranging between 1 and \( N \), which represents the segment number of the interstitial compartment (divided in \( N \) segments). The state variables are concentrations of uFA, albumin, AF1, AF2 and AF3 at segment \( i \): \( C_{i}^{\text{uFA}}, C_{i}^{\text{ Alb}}, C_{i}^{\text{AF1}}, C_{i}^{\text{AF2}} \) and \( C_{i}^{\text{AF3}} \), respectively. The description of the remaining variables is listed in Table 1. The state equations for segment \( i = 1 \) are the same as Eq. 27 - 31, with the difference that the diffusion part on the RHS \( \frac{D}{(\Delta x)^2} (C_{i+1}^{\text{uFA}} + C_{i-1}^{\text{uFA}} - 2C_{i}^{\text{uFA}}) \) is replaced by Eq. 32:

\[
\frac{D}{\Delta x} \left( \frac{C_{\text{source}} - C_{1}^{\text{uFA}} - C_{1}^{\text{uFA}}}{2} \right) \equiv \frac{D}{(\Delta x)^2} (2C_{\text{source}}^{\text{source}} - 3C_{1}^{\text{uFA}} + C_{2}^{\text{uFA}})
\]

(32)

using the theory outlined in paragraph III.1.2. For the state equations at segment \( i = N - 1 \), the diffusion part on the RHS of Eq. 27 - 31 is replaced by Eq. 33 – 34:

\[
\frac{D}{\Delta x} \left( \frac{C_{N-2}^{\text{xuFA}} - C_{N-1}^{\text{xuFA}}}{2} \right) \equiv \frac{D}{(\Delta x)^2} (2C_{\text{source}}^{\text{source}} - 3C_{1}^{\text{uFA}} + C_{2}^{\text{uFA}})
\]

(33)

and

\[
\frac{D}{\Delta x^{\text{ads}}} \left( \frac{C_{N-1}^{\text{xuFA}} - C_{N}^{\text{xuFA}}}{2} \right) \equiv \frac{2D}{\Delta x^{\text{ads}}} (C_{N-1}^{\text{uFA}} - C_{N}^{\text{uFA}})
\]

(34)

respectively. The final adaptation of the set of state equations for segment \( i = N \) is the absorption of uFA in the membrane. If one assumes that FA are absorbed in the membrane
within an adsorption layer of length 2.5 nm (~ length of a fatty acid molecule), which is half the membrane size, the state equation for uFA becomes (Eq. 35):

\[
\dot{C}_{uFA}^N = \frac{2D_{uFA}}{\Delta x}\left(N_{uFA}^N - C_{uFA}^N\right) - k_1 C_{Alb}^N C_{uFA}^N - k_2 C_{AF1}^N C_{uFA}^N - k_3 C_{AF2}^N C_{uFA}^N - k_4 C_{AF3}^N C_{uFA}^N - k_{on} C_{AF1}^N C_{uFA}^N
\]

\[
\dot{C}_{AF1}^N = \frac{2D_{AF1}}{\Delta x}\left(N_{AF1}^N - C_{AF1}^N\right) + k_1 C_{Alb}^N C_{uFA}^N + k_2 C_{AF2}^N C_{uFA}^N + k_3 C_{AF3}^N C_{uFA}^N - k_{off} C_{AF1}^N C_{uFA}^N
\]

\[
\dot{C}_{AF2}^N = \frac{2D_{AF2}}{\Delta x}\left(N_{AF2}^N - C_{AF2}^N\right) + k_1 C_{Alb}^N C_{uFA}^N + k_2 C_{AF3}^N C_{uFA}^N - k_{off} C_{AF2}^N C_{uFA}^N
\]

\[
\dot{C}_{AF3}^N = \frac{2D_{AF3}}{\Delta x}\left(N_{AF3}^N - C_{AF3}^N\right) + k_1 C_{Alb}^N C_{uFA}^N + k_2 C_{AF1}^N C_{uFA}^N - k_{off} C_{AF3}^N C_{uFA}^N
\]

\[
\dot{C}_{uFA}^N = \frac{2D_{uFA}}{\Delta x}\left(N_{uFA}^N - C_{uFA}^N\right) - k_1 C_{Alb}^N C_{uFA}^N - k_2 C_{AF1}^N C_{uFA}^N - k_3 C_{AF2}^N C_{uFA}^N - k_4 C_{AF3}^N C_{uFA}^N - k_{on} C_{AF1}^N C_{uFA}^N
\]

IV.2.2 Receptor

The supply of FA to the membrane could be enhanced by an albumin-FA complex receptor. This receptor is only useful when albumin and albumin-bound FA can diffuse freely, like in Model II A through the glycocalyx. If the assumption is made that the receptor is only present in the adsorption layer of the interstitial compartment (i = N), the state equations remain the same as in Model II A, except for segment i = N (Eq. 36 - 40):

\[
\dot{C}_{uFA}^N = \frac{2D_{uFA}}{\Delta x}\left(N_{uFA}^N - C_{uFA}^N\right) - k_1 C_{Alb}^N C_{uFA}^N - k_2 C_{AF1}^N C_{uFA}^N - k_3 C_{AF2}^N C_{uFA}^N - k_4 C_{AF3}^N C_{uFA}^N
\]

\[
\dot{C}_{AF1}^N = \frac{2D_{AF1}}{\Delta x}\left(N_{AF1}^N - C_{AF1}^N\right) + k_1 C_{Alb}^N C_{uFA}^N + k_2 C_{AF2}^N C_{uFA}^N + k_3 C_{AF3}^N C_{uFA}^N - k_{off} C_{AF1}^N C_{uFA}^N
\]

\[
\dot{C}_{AF2}^N = \frac{2D_{AF2}}{\Delta x}\left(N_{AF2}^N - C_{AF2}^N\right) + k_1 C_{Alb}^N C_{uFA}^N + k_2 C_{AF3}^N C_{uFA}^N - k_{off} C_{AF2}^N C_{uFA}^N
\]

\[
\dot{C}_{AF3}^N = \frac{2D_{AF3}}{\Delta x}\left(N_{AF3}^N - C_{AF3}^N\right) + k_1 C_{Alb}^N C_{uFA}^N + k_2 C_{AF1}^N C_{uFA}^N - k_{off} C_{AF3}^N C_{uFA}^N
\]

where state variables \(C_{receptor}^N, C_{R^*AF1}, C_{R^*AF2}\) and \(C_{R^*AF3}\) represent the concentrations of the receptor, receptor-AF1-, receptor-AF2- and receptor-AF3 complexes, respectively. The state equations for the receptor and its complexes are shown in Appendix A.

IV.2.3 No diffusion of albumin and its complexes

Model II B takes the reduced mobility of albumin and its complexes into account by using exactly the same state equations as described in Model II A. This is realized by putting the diffusion coefficients for albumin, AF1, AF2 and AF3 to 1.7 x 10^{-13} cm^2 s^{-1} (using Eq. 2, with d = 0.4 \mu m and t = 40 min). The effect of no albumin diffusion on the total flux of FA is compared with Model II A and shown in the subsection V.2.
IV.3 Model III: From blood plasma to interstitium

In the previous sections, models have been described for transport of FA across the sarcolemma and glycocalyx. A model of the transport of FA from blood plasma to interstitium is composed likewise. One can identify the following five transport barriers for FA in this pathway: the glycocalyx attached to the luminal side of the endothelial membrane, the luminal membrane, the endothelial cytoplasm, the abluminal membrane and finally a glycocalyx on the abluminal side of the endothelium. The state equations are composed of the same processes as shown in Model I and II: diffusion of substrates (uFA, albumin, AF₁, AF₂, AF₃), binding reactions (binding of uFA to albumin and complexes) and transport of FA across membranes by a “flip-flop” mechanism. Calculations in section II have excluded transport of FA by means of transcytosis, peri-endothelial transfer or through the clefts. One process has been added: the binding of uFA to FABP in the endothelial cytoplasm. The total set of state equations for the transport of FA from blood plasma to the interstitial compartment (Fig. 13) can be found in Appendix A, just like all the other state equations described above.
Section V  Results and discussion

The equations of the 3 models are listed in Appendix A and are solved by the command ode15s (see also Appendix A) from the mathematical package MATLAB 6.5 (The MathWorks Inc.). In Model I and II, the Monte Carlo method has been incorporated in the model to encompass the probability distribution of the different parameters.

V.1 Results of Model I:  The myocardial sarcolemma

Model I is a simplification of the myocardial sarcolemma, consisting of a pure phospholipid bilayer. The source of FA in this model is the interstitial compartment, near the sarcolemma, where $[FA]_{\text{total}}$ was assumed to be 0.09 mM (the total concentration of FA, (45)) and $[\text{Alb}]_{\text{total}} = 0.3$ mM (total concentration of albumin, (45, 71)). The sink of this model is the sarcoplasm of the myocyte, i.e. $[FA]_{\text{sarcoplasm}} \approx 0$.

V.1.1 Experimental data

The fluxes determined in experiments performed on quiescent, isolated myocytes (45) will be used as comparison for the model results. The experiments showed also that sulfo-N-succinimidyl-oleate (SSO) influences the fluxes of FA. SSO inhibits membrane-associated proteins, putatively facilitating membrane transport of FA. The flux in SSO-treated myocytes (no proteins involved) is used as reference value in the normal situation (low $K_p$) for the models. SSO-treated myocytes showed a flux of FA of $\sim 11.4$ nmol min$^{-1}$ (g ww$^{-1}$ myocyte) (45), which corresponds to $\sim 6.7$ nmol min$^{-1}$ (g ww$^{-1}$ cardiac tissue, since 1 g ww cardiac tissue consists of $\sim 0.59$ g ww myocyte (Vinnakota, personal communication). The non-SSO treated myocytes showed a higher flux of FA: 13.5 nmol min$^{-1}$ (g ww$^{-1}$ heart (22.9 nmol min$^{-1}$ (g ww$^{-1}$ myocyte, (45)), which is taken as reference value for the models with a high $K_p$ (enhancer situation). The flux value found by (45) in the normal situation is $\sim 7$ times lower (13.5 nmol min$^{-1}$ (g ww$^{-1}$)) than under physiological conditions ($\sim 100$ nmol min$^{-1}$ (g ww$^{-1}$)). A variety of explanations for this difference can be put forward: the absence of insulin (42), no electrical stimulation of the myocytes (43), no physiologically relevant metabolic requirements, etc.

V.1.2 Transmembrane diffusion and enhancer

Models with parameters derived from SUV’s and LUV’s by (23) lead to relatively high fluxes of FA of 110 and 100 nmol min$^{-1}$ (g ww$^{-1}$). These values are higher than those obtained from experiments performed on isolated cells, i.e., 13.5 nmol min$^{-1}$ (g ww$^{-1}$) (45). The parameters from GUV experiments (31) show significantly lower results: 8.4 nmol min$^{-1}$ (g ww$^{-1}$). The experimental value for $k_{\text{flip}}$ is probably underestimated by (31) as was shown in paragraph IV.1.1. To account for this underestimation, the value for $k_{\text{flip}}$ of the GUV (31) is replaced by
the value for $k_{\text{flip}}$ of the SUV and LUV experiments. This resulted in a slightly higher flux value: 15.5 and 15.0 nmol min$^{-1}$ (g ww)$^{-1}$, respectively. The final sub-model is still based on the parameters of (31), but now the value of $k_{\text{flip}}$ has been obtained by extrapolation of data from (23) (see Fig. 15 and Table 1). Compared with the experimental data for both the normal situation and the presence of an enhancer (45), the model of (31) with the extrapolated $k_{\text{flip}}$ gives the best approximation of this situation. The flux in a normal situation is 5.3 nmol min$^{-1}$ (g ww)$^{-1}$, the enhancer leads to a flux of 14.7 nmol min$^{-1}$ (g ww)$^{-1}$. The latter differs only $\sim$ 8% from the experimental data.

V.1.3 Carrier protein

Like for the putative enhancer, the effect of a carrier protein depends on many factors, e.g. the concentration of the carrier protein and the affinity of the carrier for uFA. These have been varied in the model within physiological range to check its effect (shown in Table 1 and in Fig. 16). Unfortunately, no experimental evidence about carrier proteins that transport FA across the sarcolemma is available. Linear behaviour of the flux of FA is shown (Fig. 16), when the affinity constant of the carrier for uFA and the release of uFA from the carrier are raised from $1 \times 10^6 - 1 \times 10^8$ M$^{-1}$ and from $0.1 - 10$ s$^{-1}$, respectively. The concentration of carrier protein is assumed to be 1 mM. With a high affinity of $1 \times 10^8$ M and a fast release ($k_{\text{release}}^C = 10$ s$^{-1}$), the flux of FA is 12.0 nmol min$^{-1}$ (g ww)$^{-1}$, which is about the same flux as obtained with an enhancer (13.5 nmol min$^{-1}$ (g ww)$^{-1}$).

V.1.4 Transport across sarcolemma is most likely by enhanced transmembrane diffusion

In summary, if one considers the outcome of the computational SUV and LUV models (Fig. 14), based on parameters of (23), pure transmembrane diffusion would be more than sufficient to satisfy a flux of 13.5 nmol min$^{-1}$ (g ww)$^{-1}$, as was obtained from experiments on isolated, quiescent cells (45). On the other hand, if one compares the adapted results (for $k_{\text{flip}}$) for GUV (31) with the experimental results (45), these correspond much better with the experiments than the computational SUV and LUV models did. This implies that the adapted GUV model (31) gives the best representation of the experimental data of (45) and the data of this model will be used in Model II and III. The model of the sarcolemma with an enhancer showed a physiologically feasible concentration of FA in the sarcolemma ($\sim 6 \times 10^{-4}$ M, more than three orders lower in magnitude compared to the phospholipid concentration in the membrane).

The effect of a carrier protein is only relevant in a relatively high protein concentration of 1 mM, which corresponds with $\sim$ 25% of all proteins present in the membrane (total protein concentration in membrane with adsorption layers is $\sim$ 4 mM, see Table 1). Therefore, the involvement of a carrier with properties summarized in Table 1 is not very likely in the transport of FA across the sarcolemma.
V.2 Results of Model II: Extension of sarcolemma with a glycocalyx

Model II simulates the effects of diffusion of uFA and albumin-bound FA, which are AF$_1$, AF$_2$, and AF$_3$. Model I has been extended with a glycocalyx with a variable thickness of 0.2 - 0.5 $\mu$m. The influence of this glycocalyx on a mobile albumin situation (Model II A, diffusion of albumin) and the effect of immobile albumin (Model II B, no diffusion of albumin) has been tested. The effect of a receptor with varying properties (shown in Table 1) on the flux is described for a glycocalyx thickness set at 0.4 $\mu$m. The source and sink concentrations of this model are identical to Model I, except that the source is now located in the middle of the interstitial compartment instead of at the adsorption layer of the sarcolemma.

V.2.1 Model II A and Model II B

The fluxes of FA in steady state for Model II A (mobile albumin) and II B (immobile albumin) at different glycocalyx thickness are shown in Fig. 17. The fluxes of FA for the mobile albumin situation remain the same, varying between $\sim 13.3 - 14.5$ nmol min$^{-1}$ (g ww)$^{-1}$, irrespective of the glycocalyx thickness, and are in agreement with the flux obtained in Model I (Fig. 14). The influence of mobile albumin on the total flux (in steady-state) is shown in Fig. 18. When albumin diffuses freely, the albumin-bound FA contribute the most to the flux of FA in the interstitial fluid, but near the sarcolemma, primarily uFA take care of the flux. The summation of the four separate fluxes at a given point in the interstitial compartment gives a required constant flux in steady state. However, the presence of a glycocalyx restricts albumin mobility significantly (paired student's $t$-test, $P > 0.05$). The flux has been reduced to a minimum of $\sim 9.7$ nmol min$^{-1}$ (g ww)$^{-1}$. This is about 70% compared to a situation with diffusing albumin, if the glycocalyx width equals 0.3 $\mu$m or more (Fig. 17). Unfortunately, only in vitro experiments are performed to investigate the effect of the glycocalyx on the diffusion of macromolecules (75), which might represent an extreme vision (no mobile albumin) of the real physiological situation. To rate these findings on their true physiological significance experiments should be performed. Therefore, the only two extremes are presented in this chapter: either the glycocalyx is no barrier for albumin or albumin is immobilized (75).

V.2.2 Receptor

Model II A has been extended with a receptor protein. Fig. 19 shows the effect of a receptor on the normalized concentration profile of uFA in the interstitial compartment at different receptor concentrations. The high concentration of uFA near the sarcolemma is explained by the relatively slow diffusion of uFA, which originate from albumin-FA complexes. Since the release of FA from albumin is facilitated by the receptor, the concentration of uFA is further increased. Fig. 20 shows that the higher the concentration of receptor, the greater is the flux of FA across the membrane. For a receptor concentration of 0.01 M, which is 2.5 times the
total protein concentration in the membrane (~ 4 mM, calculated in section III), the calculated flux is ~ 140 nmol min\(^{-1}\) (g ww\(^{-1}\)), i.e. in the same order of magnitude as the required flux of ~ 100 nmol min\(^{-1}\) (g ww\(^{-1}\)). Diminishing the receptor concentration in the membrane to 0.001 M (~ 25 % of all membrane proteins), reduces the flux to ~ 25 nmol min\(^{-1}\) (g ww\(^{-1}\)). The required concentrations of receptor protein present in the membrane are physiologically too high to make it likely that a receptor contributes considerably to the total flux of FA. Moreover, in the extreme case of a strong reduction in albumin mobility due to the glycocalyx (75), the effect of a receptor protein is eliminated and leads to an even larger reduction in flux of FA.

V.2.3 Glycocalyx decreases the flux of fatty acids

Summarizing, the glycocalyx reduces the diffusion of albumin considerably (~ 6 orders in magnitude as shown in Table 1 and by (75)), which would make the contribution of albumin to the transport of FA almost negligible, implicating that only uFA can diffuse freely. As was shown in Fig. 17, the flux of FA in the immobile albumin situation (75) was ~ 70% of that in the situation with mobile albumin when AF\(_1\), AF\(_2\) and AF\(_3\) contribute substantially to the flux of FA (see also Fig. 18). If the experimental findings of Vink and Duling are correct (75), the contribution of albumin-bound FA diffusion to overall diffusion of FA to reach the sarcolemma is minor. The influence of a receptor could also be considerable, but only if one assumes receptor concentrations that are physiologically too high. The transport mechanism of a receptor is also based on mobile albumin and its complexes.

V.3 Results of Model III: From blood plasma to the interstitium

The final model represents the endothelial barrier, which is composed of the vascular compartment, endothelium and the first half of the interstitial compartment (Fig. 13). The source is located in the vascular compartment, at the beginning of the endothelial glycocalyx on the luminal side, and the sink in the interstitial fluid, at the end of the abluminal glycocalyx of the endothelium. The concentration of [FA]\(_{\text{total}}\) and [Alb]\(_{\text{total}}\) present in the source and sink are listed in Table 1, from which one could deduce the concentrations uFA, albumin and albumin-bound FA (55).

V.3.1 Transmembrane diffusion and enhancer

Similar to the sarcolemma, the transport of FA across the endothelial membranes has been simulated with transmembrane diffusion and the incorporation of an enhancer. The K\(_p\) values for the enhancer, FABP concentration in the endothelial cytoplasm and the diffusion coefficient of FABP have all been varied to test its effect on the overall flux. As can be seen in Table 2, the flux of FA is ~ 0.8 nmol min\(^{-1}\) (g ww\(^{-1}\)) under standard conditions. Moreover, the flux of FA across the endothelium is most sensitive to enhanced K\(_p\): a 30-fold increase in K\(_p\) leads to an almost 4-fold increase in flux of FA. The increase of FABP concentration or
V.3.2 Enhancer alone is not sufficient to transport enough fatty acids across the endothelial barrier

In summary, the physiologically required flux of FA found by (76) and also calculated in section II, \( \sim 100 \text{ nmol min}^{-1} \text{ (g ww)}^{-1} \), is much higher than obtained with endothelial transmembrane diffusion alone or with the aid of an enhancer (Fig. 14). FABP in the endothelial cytoplasm plays no role of importance in the transport of FA: elevation of diffusion coefficient and FABP concentration barely raised the flux of FA (Table 2).

Explanations for the low flux of FA across the endothelial barrier compared to the sarcolemmal barrier are:

- Differences in available area for diffusion. The area of the sarcolemma available for diffusion is 2000 cm² (g ww)⁻¹ (7), which is 4 times larger than the area of the endothelium: 500 cm² (g ww)⁻¹ (7). A small area of diffusion reduces the flux of FA considerably (Eq. 1).

- The gradient of uFA (\( \frac{\partial \text{C}_{\text{uFA}}}{\partial x} \)), the driving force for the diffusion flux, is bigger across the sarcolemmal barrier than across the endothelial barrier, which is shown by the following calculation: 1.9 nM (source: concentration uFA in interstitial compartment) - 0 nM (sink: concentration of uFA in sarcoplasm) = 1.9 nM over \( \sim 0.3 \mu\text{m} \) (see Table 1) results in a gradient of \( 6.3 \times 10^{-5} \text{ M cm}^{-1} \) for the sarcolemmal barrier versus 5.1 nM (source: concentration of uFA in vascular compartment) - 1.9 nM (sink: concentration of uFA in interstitial compartment) = 3.2 nM over \( \sim 1.2 \mu\text{m} \) leads to a gradient across the endothelial barrier of \( 2.7 \times 10^{-5} \text{ M cm}^{-1} \).

These two major differences between the sarcolemmal and endothelial barrier are the main cause for the low fluxes of uFA across the endothelial barrier. The combination of both effects in contribution shows a large reduction in flux across the endothelium compared to the sarcolemma. How a flux of FA of \( \sim 100 \text{ nmol min}^{-1} \text{ (g ww)}^{-1} \) across the endothelium could be achieved in the intact heart, remains a puzzling question and requires further experimentations.

V.4 Conclusion & future perspectives

The goal of this chapter was to develop a computer model that would help to elucidate the controversy of FA crossing the sarcolemma. The computer model showed clearly that transport of FA across the sarcolemma is most likely mediated by transmembrane diffusion with an enhancer. A simplified model of the sarcolemma (transmembrane diffusion of FA, facilitated by a membrane-associated enhancer) approached the experimental data of FA crossing the sarcolemma of an isolated cardiac muscle cell very well. The enhancer facilitated
the absorption of uFA in the membrane. Moreover, a carrier protein wasn't feasible to explain the experimental results, because it had to be present in too large quantities to arrive at the physiologically relevant flux.

The model of the sarcolemma was extended with a glycocalyx, which hampered the diffusion of macromolecules such as albumin (75) and reduced the flux of FA with ~ 30% and has to be taken into account in future experiments. Till now, experiments of the transport of FA don't take the glycocalyx into consideration. The possible role of a receptor in the sarcolemma, which facilitates the release of FA from albumin, is less likely, since very high receptor protein concentrations were required and the mobility of AF₁, AF₂ and AF₃ was impaired due to the presence of the glycocalyx (75).

The 3rd model revealed a flux of FA across the endothelium, which was too low to account for a physiological relevant flux. Increase in FABP concentration and mobility of albumin in the endothelial glycocalyx could not raise the flux of FA to physiological relevant values: diffusion of uFA alone through the different compartments isn't sufficient either. Therefore, the endothelium barrier is most likely rate governing in overall transport of FA in the heart. The present calculations indicate that the flux of FA across the endothelium by diffusion is not high enough. The diffusion gradient across the endothelial barrier and the area covering this barrier are the most sensitive model parameters. Another transport mechanism has to compensate for the low flux of FA across the endothelium, but its nature remains an open question and has to be investigated in future research.

An extension of the models presented in this chapter is the incorporation of the oxidation and storage of FA in the myocyte. The models assumed a constant sink, regardless of the metabolic processes in the myocyte. Small disturbances, however, could exert profound effects on the uptake processes of FA, for instance under diabetic conditions. The oxidation of FA is closely related to the metabolism of carbohydrates, e.g. glucose, or contraction of the heart (calcium related). An important challenge is to link computer models of all these separate processes to obtain more detailed insight in the interrelationship of metabolic and mechanical processes in the heart.
Table 1  Model parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
<th>Value $^{1}$</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{a,1}$</td>
<td>HSA:</td>
<td>$1.45 \times 10^8$ M$^{-1}$</td>
<td>Affinity constant: uFA with BSA/HSA</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>BSA:</td>
<td>$1.22 \times 10^8$ M$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{a,2}$</td>
<td>HSA:</td>
<td>$1.30 \times 10^8$ M$^{-1}$</td>
<td>Affinity constant: uFA with $AF_1$</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>BSA:</td>
<td>$1.53 \times 10^8$ M$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{a,3}$</td>
<td>HSA:</td>
<td>$0.71 \times 10^8$ M$^{-1}$</td>
<td>Affinity constant: uFA with $AF_2$</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>BSA:</td>
<td>$0.75 \times 10^8$ M$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{-1}$, $k_{-2}$, $k_{-3}$</td>
<td></td>
<td>$6.9 \pm 0.6$ s$^{-1}$</td>
<td>Dissociation rate of $AF_1$, $AF_2$ and $AF_3$</td>
<td>16</td>
</tr>
<tr>
<td>$k_1$ $^{*1}$</td>
<td>HSA:</td>
<td>$1.00 \times 10^9$ M$^{-1}$ s$^{-1}$</td>
<td>Association rate: uFA with BSA/HSA</td>
<td>16, 55</td>
</tr>
<tr>
<td></td>
<td>BSA:</td>
<td>$8.42 \times 10^8$ M$^{-1}$ s$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_2$ $^{*1}$</td>
<td>HSA:</td>
<td>$8.97 \times 10^8$ M$^{-1}$ s$^{-1}$</td>
<td>Association rate: uFA with $AF_1$</td>
<td>16, 55</td>
</tr>
<tr>
<td></td>
<td>BSA:</td>
<td>$1.06 \times 10^9$ M$^{-1}$ s$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_3$ $^{*1}$</td>
<td>HSA:</td>
<td>$4.90 \times 10^8$ M$^{-1}$ s$^{-1}$</td>
<td>Association rate: uFA with $AF_2$</td>
<td>16, 55</td>
</tr>
<tr>
<td></td>
<td>BSA:</td>
<td>$5.18 \times 10^8$ M$^{-1}$ s$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_p$</td>
<td>Low (normal):</td>
<td>$6.0 \times 10^5$ per M [L]</td>
<td>Partition coefficient of uFA in lipid/water phase</td>
<td>27, 31</td>
</tr>
<tr>
<td></td>
<td>High (enhancer):</td>
<td>$1.8 \times 10^6$ per M [L]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{off}$</td>
<td></td>
<td>$0.8 \pm 0.1$ s$^{-1}$</td>
<td>Desorption rate of uFA from membrane</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$6.0$ s$^{-1}$</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>$k_{on}$ $^{*1}$</td>
<td>Low:</td>
<td>$4.8 \times 10^5$ s$^{-1}$</td>
<td>Absorption rate of uFA in membrane</td>
<td>31</td>
</tr>
</tbody>
</table>
High: 1.4 x 10^6 s\(^{-1}\)

LUV: 20 s\(^{-1}\)

SUV: 70 s\(^{-1}\)

**k\(_{\text{flip}}\)**

GUV: 0.5 ± 0.075 s\(^{-1}\) Flip rate of uFA from outer to inner leaflet of membrane

**K\(_{a}\)**

9.8 x 10^7 M\(^{-1}\) Affinity constant: uFA with FABP

**k\(_{\text{FABP}}\)**

4.5 s\(^{-1}\) Dissociation rate of FABP-bound FA

**k\(_{\text{FABP}}^{-1}\)**

4.4 x 10^8 M\(^{-1}\) s\(^{-1}\) Association rate FA with FABP

**D\(_{u\text{FA}}\)**

Water (37°C): 5.0 x 10^6 cm\(^2\) s\(^{-1}\) Diffusion coefficient of unbound FA in different media

Plasma: 3.0 x 10^6 cm\(^2\) s\(^{-1}\)

Cytoplasm: 1.9 x 10^6 cm\(^2\) s\(^{-1}\)

**D\(_{HSA\approx D_{AF1}=D_{AF2}}\approx D_{AF3}\)**

Water (37°C): 8.8 x 10^7 cm\(^2\) s\(^{-1}\) Diffusion coefficient of albumin, AF\(_1\), AF\(_2\), and AF\(_3\)

Plasma: 5.2 x 10^7 cm\(^2\) s\(^{-1}\)

**D\(_{FABP\approx D_{uFA+FABP}}\)**

Water (37°C): 1.7 x 10^8 cm\(^2\) s\(^{-1}\) Diffusion coefficient of FABP & FABP-bound FA

Cytoplasm: 1.7 x 10^7 cm\(^2\) s\(^{-1}\)

**S\(_{\text{cardio}}\)**

2000 cm\(^2\) (g ww\(^{-1}\)) Area of myocytes

**S\(_{\text{endo}}\)**

500 cm\(^2\) (g ww\(^{-1}\)) Area of endothelium

**x\(_{\text{mem}}\)**

5 nm Membrane width

**x\(_{\text{GLYC}}\)**

Endothelial luminal side: 0.4 μm Glycocalyx width
Endothelial abluminal side: 0.3 μm
Myocyte: 0.3 μm

\( x_{\text{ENDO}} \)
\( x_{\text{ISF}} \)

0.5 μm
0.5 - 1.0 μm

Endothelium width
Interstitial width

\([L]\)

0.4 M
Phospholipid concentration in membrane with adsorption layers

\([P]\)

0.4 mM
Membrane protein concentration in membrane with adsorption layers

\( K_{\text{CP}} \)

1 \( \times \) 10\(^6\) - 1 \( \times \) 10\(^8\) M\(^{-1}\)
Affinity constant of uFA for carrier protein
Physiological range

\( k_{\text{bind}} \) **)

1 \( \times \) 10\(^5\) - 1 \( \times \) 10\(^9\) M\(^{-1}\) s\(^{-1}\)
Association rate: uFA with carrier
Physiological range

\( k_{\text{release}} \)

0.1 - 10 s\(^{-1}\)
Dissociation rate: uFA from carrier-bound FA
Physiological range

\( k_{\text{flip}} \)

50 s\(^{-1}\)
Flip rate of carrier from outer to inner leaflet
Physiological range

\( K_{\text{A}} \)

4 \( \times \) 10\(^{10}\) M\(^{-1}\)
Affinity constant: albumin-bound FA for receptor
Adapted

\( k_{\text{1}} \)

4 \( \times \) 10\(^{16}\) M\(^{-1}\) s\(^{-1}\)
Association rate: albumin-bound FA with receptor
Adapted

\( k_{\text{-1}} \)

1 \( \times \) 10\(^6\) s\(^{-1}\)
Dissociation rate: albumin-bound FA with receptor
Adapted

\( k_{2} \)

50 s\(^{-1}\)
Release rate of FA from albumin-bound FA complexed to the receptor
Adapted

\([FA]_{\text{total}}\)

Blood plasma: 0.5 mM
Total concentration FA

Interstitial fluid: 0.09 mM
26, 71, 76
Sarcoplasm: ~ 0 mM

Blood plasma: 0.5 mM  Total albumin concentration 71, 76
Interstitial fluid: 0.3 mM

Endothelial cytoplasm: 0.6 μM  Total FABP concentration 39, 71

Sarcoplasm: 0.3 mM

Blood plasma: 5.1 nM  Concentration unbound FA 55
Interstitial: 1.9 nM

*) All values have a standard deviation of 10% of their mean value, unless indicated otherwise.

**) Related to other variables
Table 2  Resulting fatty acid flux for different values of key parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter value</th>
<th>Flux of fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_p$</td>
<td>$1.8 \times 10^6$ per M [L]</td>
<td>$1.6 \text{ nmol min}^{-1} (\text{g ww})^{-1}$</td>
</tr>
<tr>
<td></td>
<td>$1.8 \times 10^6$ per M [L]</td>
<td>$2.8 \text{ nmol min}^{-1} (\text{g ww})^{-1}$</td>
</tr>
<tr>
<td></td>
<td>$1.8 \times 10^7$ per M [L]</td>
<td>$3.1 \text{ nmol min}^{-1} (\text{g ww})^{-1}$</td>
</tr>
<tr>
<td>$[\text{FABP}]_{\text{total}}$</td>
<td>$0.6 \times 10^5$ M</td>
<td>$0.9 \text{ nmol min}^{-1} (\text{g ww})^{-1}$</td>
</tr>
<tr>
<td></td>
<td>$0.6 \times 10^4$ M</td>
<td>$0.9 \text{ nmol min}^{-1} (\text{g ww})^{-1}$</td>
</tr>
<tr>
<td>$D_{\text{FABP}}$</td>
<td>$1.7 \times 10^5$ cm$^2$ s$^{-1}$</td>
<td>$0.9 \text{ nmol min}^{-1} (\text{g ww})^{-1}$</td>
</tr>
<tr>
<td></td>
<td>$1.7 \times 10^5$ cm$^2$ s$^{-1}$</td>
<td>$0.9 \text{ nmol min}^{-1} (\text{g ww})^{-1}$</td>
</tr>
</tbody>
</table>

The flux in the standard situation with parameter values $K_p = 6 \times 10^5$ per M [L], $[\text{FABP}]_{\text{total}} = 0.6 \text{ \mu M}$ and $D_{\text{FABP}} = 1.7 \times 10^{-7}$ cm$^2$ s$^{-1}$ is $0.8 \text{ nmol min}^{-1} (\text{g ww})^{-1}$. 
Fig. 1
A simplified representation of the cardiac transport route of FA. FA are present in the capillary (vascular compartment), mostly complexed to albumin or in esterified form as triacylglycerols in very low-density lipoproteins (VLDL’s) and chylomicrons. FA are transported across the endothelial cytoplasm and interstitial compartment (interstitium), where FA bind to FABP and albumin, respectively. From the interstitium, FA move across the sarcolemma into the sarcoplasm of the myocyte. Subsequently, FA are either stored in the myocyte (triacylglycerols) or metabolised in the mitochondria, which results in energy production for the heart.
Fig. 2
Electron micrograph of the orientation of a capillary, which is surrounded by myocytes. The photograph shows 1) a myocyte, 2) mitochondria inside the myocyte, 3) the nucleus of an endothelial cell, 4) the intravascular compartment, 5) the interstitial compartment, 6) the nucleus of a myocyte and 7) a cleft between two adjacent endothelial cells. (Picture taken from (10))
A more detailed description of the fate of FA in the myocyte. In the sarcoplasm, FA are transported by FABP to intracellular sites of metabolic conversion followed by storage (triacylglycerol) or oxidation. Near the outer mitochondrial membrane, FA are converted to acyl-CoA, catalysed by acyl-CoA synthetase (ACS). Acyl-CoA moves across the outer mitochondrial membrane, where carnitine acyltransferase I (CAT I) converts acyl-CoA to acylcarnitine, which is transported through the inner mitochondrial membrane by action of carnitine/acyl carnitine translocase (CAcT). In the mitochondrial matrix, carnitine acyltransferase II (CAT II) catalyses the reaction of acylcarnitine back to acyl-CoA. This substrate is broken down successively by β-oxidation and the citric acid cycle activity.
Transmembrane diffusion of non-protein bound FA (uFA, indicated with u in the figure). uFA present in the aqueous compartment are 1) absorbed into the outer leaflet of the membrane, 2) they flip from the outer to the inner leaflet and 3) are released (desorbed) in the aqueous compartment on the other side of the membrane.

Carrier protein. A possible representation of a carrier protein is shown here: 1) binding of non-protein bound FA (uFA, u) to the carrier (bound FA are represented with b), 2) transport of uFA to the other side of the membrane (a confirmation change in carrier protein is shown here as the transport mechanism), and 3) uFA are released to the other side of the membrane.

Receptor. The receptor binds to complexes of FA (b) and albumin (Alb, Step I). The normally tight binding of albumin to FA is weakened as a result of binding of the albumin-FA complex and the receptor. This leads to an increased release of FA from the albumin-FA complex (Step II), which causes a raised concentration of uFA (u) in the stagnant water layer near the membrane. Eventually, this creates a steeper gradient across the membrane, which increases the flux of FA due to transmembrane diffusion.
Enhancer. The enhancer (Enh.) binds to non-protein bound FA (uFA, u), present in the aqueous solution, which increases the concentration of FA in the outer leaflet of the membrane. Subsequently, the FA flip from the outer to the inner leaflet and are released on the other side of the membrane. Therefore, the enhancer improves the absorption step of uFA in transmembrane diffusion: more uFA are absorbed from the aqueous compartment in the biological membrane, when an enhancer is present.

Fig. 8  
Diffusion of a substrate in the x-direction of a system with length L. At $t = 0$, a substrate is present in the middle only and at time $t_1$ and $t_2$ ($t_2 > t_1$), the substrate diffuses to both sides.

Fig. 9  
A system with substrate concentration $C_1$ is divided in 9 segments ($N = 9$, segment number is shown as superscript). The 5th segment has a length of $\Delta x$ and its distance to the middle of the left and right segment is $\Delta x_A$ and $\Delta x_B$, respectively. In this figure, $\Delta x_A = \Delta x_B$.
Fig. 10
A schematical representation of the total model. The sizes of the different parts of the models are also shown here.

Fig. 11
Model I consists of a sarcolemma only, composed of 2 leaflets (outer and inner). The source is the interstitial compartment, the sarcoplasm is the sink.

Fig. 12
Model II is an extension of Model I: a glycocalyx has been added. The segments have a thickness $\Delta x$, and the adsorption layer a thickness $\Delta x_{ads}$. The source is located in the middle of the interstitium, the sink remains the same: the sarcoplasm.

Fig. 13
This figure shows Model III: the endothelial barrier. It consists of 2 times an endothelial glycocalyx, an luminal and abluminal endothelial membrane and the endothelial cytoplasm. The source is the beginning of the luminal side of the endothelial glycocalyx, the sink is the middle of the interstitium (= source of Model II).
The results of Model I. This figure shows the flux of FA across the sarcolemma. These vary between \(-3\) nmol min\(^{-1}\) (g ww\(^{-1}\)) for GUV \(^3\), to \(-40\) nmol min\(^{-1}\) (g ww\(^{-1}\)) for SUV \(^2\), using a low partition coefficient. For a high partition coefficient, the flux ranges between \(-8\) nmol min\(^{-1}\) (g ww\(^{-1}\)) to \(-110\) nmol min\(^{-1}\) (g ww\(^{-1}\)). The Monte Carlo method was used (500 cycles) to obtain these results.

1) Experimental results, based on data of (45)
2) Values for \(k_{\text{flip}}\) and \(k_{\text{off}}\) from (30)
3) Values for \(k_{\text{flip}}\) and \(k_{\text{off}}\) from (31)
4) Values for \(k_{\text{flip}}\) and \(k_{\text{off}}\) from (30) and (31), respectively
5) Value for \(k_{\text{off}}\) from (31), value for \(k_{\text{flip}}\) obtained by extrapolation (see Fig. 15)
Fig. 15
Derivation of $k_{flip}$ for GUV by extrapolation. Extrapolating $t_{1/2}$ is used to derive the rate parameter $k_{flip}$ for GUV: $k_{flip} = \frac{\ln(2)}{t_{1/2}}$. The $t_{1/2}$-values for SUV and LUV are obtained from (30). A linear plot results in a $t_{1/2}$ for GUV to be 70 ms, which leads to $k_{flip} \approx 10$ s$^{-1}$.

Fig. 16
Influence of carrier protein on the flux. The 3 plots (logarithmic scale) show that a 10-fold increase in $k_{\text{release}}^{CP}$ leads to a 10-fold increase in flux. Likewise, a 10-fold increase in affinity constant of non-protein bound FA ($uFA$), raises the flux also 10-fold. These simulations were performed with a carrier protein concentration of 1 mM. The highest flux in this figure is $\approx 12$ nmol min$^{-1}$ (g ww)$^{-1}$ obtained with an affinity constant of $1.0 \times 10^8$ M and a $k_{\text{release}}^{CP}$ of 10 s$^{-1}$.

Fig. 17
Effect of glycocalyx width on flux of FA. Immobile albumin, due to the glycocalyx, reduces the flux of FA to $\sim 70\%$ of the value obtained with mobile albumin. Monte Carlo method was used (10 cycles).

* Significantly different from value found with mobile albumin, $P < 0.05$, paired student's $t$-test.
Contribution of the different components to the total flux. The results of mobile albumin (not influenced by glycocalyx) are shown in this figure. As can be seen, the contributions of albumin-bound FA ($AF_1$, $AF_2$ and $AF_3$) is higher in the beginning of the glycocalyx, but decreases near the membrane on the right. The flux of uFA increases at that point. The black dotted line shows the total flux of all separate components, so the summation of uFA, $AF_1$, $AF_2$ and $AF_3$.

Fig. 19
The normalized concentration profile of non-protein bound FA (uFA) at different receptor concentrations of albumin-bound FA. This profile is located in the glycocalyx of the myocyte. The source is the middle of the interstitium, the sarcolemma is located at the right part of this figure. For this simulation only, the glycocalyx width of the interstitium is taken to be 0.4 μm, instead of the usual 0.3 μm. Assumption: albumin diffuses freely as in Model IIA.

Fig. 20
Effect of receptor protein concentration on the flux of FA. The flux of FA is high for a receptor concentration of 0.01 M: ~145 nmol min$^{-1}$ (g ww$^{-1}$), but is reduced at lower receptor concentrations. These results were obtained by performing a Monte Carlo simulation with 10 cycles.
Fig. 21

Normalized concentration profile of uFA (steady-state) across the vascular, endothelial and interstitial compartment. The concentration profiles are normalized (divided by the source concentration) and linear across the different compartments.
Appendix A

In general

Bovine serum albumin (BSA) has a lower affinity constant for FA, compared with HSA, indicating that less FA bind to BSA than to HSA. This creates significant higher concentrations (paired student’s t-test, \( P < 0.05 \)) of uFA for BSA than for HSA (55). Because BSA is used in most experimental set-ups, all simulations were also performed with BSA.

Model I (see Fig. 11)

Source:
The adsorption layer of the sarcolemma in the sarcoplasm of the myocyte.
\[ [\text{FA}]_{\text{total}} = 0.09 \text{ mM} \text{, } [\text{Alb}]_{\text{total}} = 0.3 \text{ mM} \text{ (see Table 1)} \]
This results in \([\text{uFA}] = C_{\text{FA}}^{\text{source}} \approx 1.9 \text{ nM} \text{ (for BSA)} \text{ and } \approx 1.8 \text{ nM (for HSA).} \]

Sink:
The adsorption layer of the sarcolemma in the interstitial compartment:
\[ [\text{uFA}] = C_{\text{FA}}^{\text{sink}} = 0 \text{ M (see Table 1)} \]

Equations:

Normal situation

Outer leaflet of sarcolemma:
\[
\frac{dC_{\text{FA}}}{dt} = k_{\text{on}} C_{\text{FA}}^{\text{source}} - k_{\text{off}} C_{\text{FA}}^{\text{outer}} + k_{\text{flip}} (C_{\text{FA}}^{\text{inner}} - C_{\text{FA}}^{\text{outer}})
\]

Inner leaflet of sarcolemma:
\[
\frac{dC_{\text{FA}}}{dt} = k_{\text{on}} C_{\text{FA}}^{\text{sink}} - k_{\text{off}} C_{\text{FA}}^{\text{inner}} + k_{\text{flip}} (C_{\text{FA}}^{\text{outer}} - C_{\text{FA}}^{\text{inner}})
\]

Enhancer

Identical to equations described above, except for higher \( k_{\text{on}} \) (see Table 1).

Carrier

Outer leaflet of sarcolemma:
\[
\frac{dC_{\text{CP}}}{dt} = -k_{\text{bind}} C_{\text{FA}}^{\text{source}} C_{\text{CP}} + k_{\text{release}} C_{\text{CP}}^{\text{outer}} + k_{\text{flip}} (C_{\text{CP}}^{\text{inner}} - C_{\text{CP}}^{\text{outer}}) + k_{\text{CP}}^{\text{flip}} (C_{\text{CP}}^{\text{source}} - C_{\text{CP}}^{\text{outer}})
\]

Inner leaflet of sarcolemma:
\[
\frac{dC_{\text{CP}}}{dt} = -k_{\text{bind}} C_{\text{FA}}^{\text{inner}} C_{\text{CP}} + k_{\text{release}} C_{\text{CP}}^{\text{inner}} + k_{\text{flip}} (C_{\text{CP}}^{\text{outer}} - C_{\text{CP}}^{\text{inner}}) + k_{\text{CP}}^{\text{flip}} (C_{\text{CP}}^{\text{source}} - C_{\text{CP}}^{\text{inner}})
\]
$\dot{C}_{\text{CP}^*\text{FA}} = k_{\text{bind}}^{\text{CP}} C_{\text{FA}} - k_{\text{release}}^{\text{CP}^*\text{FA}} C_{\text{CP}^*\text{FA}} + k_{\text{flip}}^{\text{CP}} (C_{\text{CP}^*\text{FA}} - C_{\text{CP}^*\text{FA}})$

**Model II (see Fig. 12)**

**Source:**
The middle of the interstitial compartment, the conditions are further the same as in Model I.

**Sink:**
See Model I.

**Equations:**

**Normal situation**

**Interstitial compartment (segment i = 1):**

\[ \dot{C}^{i}_{\text{UFA}} = \frac{D_{\text{UFA}}}{\langle \Delta x \rangle^2} (2C^{i}_{\text{source}} - 3C^{i}_{\text{UFA}} + C^{i+1}_{\text{UFA}}) - k_{-1} C^{i}_{\text{UFA}} C^{i}_{\text{UFA}} - k_{2} C^{i}_{\text{AFI}} C^{i}_{\text{UFA}} \ldots 
\]

\[ ... - k_{3} C^{i}_{\text{AF2}} C^{i}_{\text{UFA}} + k_{-1} C^{i}_{\text{AF1}} + k_{-2} C^{i}_{\text{AF2}} + k_{-3} C^{i}_{\text{AF3}} \]

\[ \dot{C}^{i}_{\text{Alb}} = \frac{D_{\text{Alb}}}{\langle \Delta x \rangle^2} (2C^{i}_{\text{source}} - 3C^{i}_{\text{Alb}} + C^{i}_{\text{Alb}}) - k_{1} C^{i}_{\text{Alb}} C^{i}_{\text{Alb}} + k_{-1} C^{i}_{\text{AF1}} \]

\[ \dot{C}^{i}_{\text{AF1}} = \frac{D_{\text{AF1}}}{\langle \Delta x \rangle^2} (2C^{i}_{\text{source}} - 3C^{i}_{\text{AF1}} + C^{i}_{\text{AF1}}) + k_{1} C^{i}_{\text{Alb}} C^{i}_{\text{UFA}} - k_{2} C^{i}_{\text{AF1}} C^{i}_{\text{AF1}} \ldots 
\]

\[ ... - k_{3} C^{i}_{\text{AF2}} C^{i}_{\text{UFA}} + k_{-1} C^{i}_{\text{AF1}} + k_{-2} C^{i}_{\text{AF2}} + k_{-3} C^{i}_{\text{AF3}} \]

\[ \dot{C}^{i}_{\text{AF2}} = \frac{D_{\text{AF2}}}{\langle \Delta x \rangle^2} (2C^{i}_{\text{source}} - 3C^{i}_{\text{AF2}} + C^{i}_{\text{AF2}}) + k_{2} C^{i}_{\text{AF1}} C^{i}_{\text{AF1}} \ldots 
\]

\[ ... - k_{3} C^{i}_{\text{AF2}} C^{i}_{\text{UFA}} + k_{-1} C^{i}_{\text{AF1}} + k_{-2} C^{i}_{\text{AF2}} + k_{-3} C^{i}_{\text{AF3}} \]

\[ \dot{C}^{i}_{\text{AF3}} = \frac{D_{\text{AF3}}}{\langle \Delta x \rangle^2} (2C^{i}_{\text{source}} - 3C^{i}_{\text{AF3}} + C^{i+1}_{\text{AF3}}) + k_{3} C^{i}_{\text{AF2}} C^{i}_{\text{AF1}} - k_{-3} C^{i}_{\text{AF3}} \]

**Interstitial compartment (segment i = 2 to N-2):**

\[ \dot{C}^{i}_{\text{UFA}} = \frac{D_{\text{UFA}}}{\langle \Delta x \rangle^2} (C^{i+1}_{\text{UFA}} + C^{i-1}_{\text{UFA}} - 2C^{i}_{\text{UFA}}) - k_{1} C^{i}_{\text{Alb}} C^{i}_{\text{UFA}} - k_{2} C^{i}_{\text{AF1}} C^{i}_{\text{UFA}} \ldots 
\]

\[ ... - k_{3} C^{i}_{\text{AF2}} C^{i}_{\text{UFA}} + k_{-1} C^{i}_{\text{AF1}} + k_{-2} C^{i}_{\text{AF2}} + k_{-3} C^{i}_{\text{AF3}} \]

\[ \dot{C}^{i}_{\text{Alb}} = \frac{D_{\text{Alb}}}{\langle \Delta x \rangle^2} (C^{i+1}_{\text{Alb}} + C^{i-1}_{\text{Alb}} - 2C^{i}_{\text{Alb}}) - k_{1} C^{i}_{\text{Alb}} C^{i}_{\text{UFA}} + k_{-1} C^{i}_{\text{AF1}} \]

\[ \dot{C}^{i}_{\text{AF1}} = \frac{D_{\text{AF1}}}{\langle \Delta x \rangle^2} (C^{i+1}_{\text{AF1}} + C^{i-1}_{\text{AF1}} - 2C^{i}_{\text{AF1}}) + k_{1} C^{i}_{\text{Alb}} C^{i}_{\text{UFA}} - k_{2} C^{i}_{\text{AF1}} C^{i}_{\text{AF1}} \ldots 
\]

\[ ... - k_{2} C^{i}_{\text{AF1}} C^{i}_{\text{UFA}} - k_{-1} C^{i}_{\text{AF1}} + k_{-2} C^{i}_{\text{AF2}} \]

\[ \dot{C}^{i}_{\text{AF2}} = \frac{D_{\text{AF2}}}{\langle \Delta x \rangle^2} (C^{i+1}_{\text{AF2}} + C^{i-1}_{\text{AF2}} - 2C^{i}_{\text{AF2}}) + k_{2} C^{i}_{\text{AF1}} C^{i}_{\text{AF1}} \ldots 
\]

\[ ... - k_{3} C^{i}_{\text{AF2}} C^{i}_{\text{UFA}} - k_{-2} C^{i}_{\text{AF2}} + k_{-3} C^{i}_{\text{AF3}} \]

\[ \dot{C}^{i}_{\text{AF3}} = \frac{D_{\text{AF3}}}{\langle \Delta x \rangle^2} (C^{i+1}_{\text{AF3}} + C^{i-1}_{\text{AF3}} - 2C^{i}_{\text{AF3}}) + k_{3} C^{i}_{\text{AF2}} C^{i}_{\text{AF1}} - k_{-3} C^{i}_{\text{AF3}} \]
Interstitial compartment (segment $i = N-1$):

$$\dot{C}_{N-1}^{uFA} = \frac{D_{uFA}}{\Delta x} \left( \frac{C_{N-2}^{uFA} - C_{N-1}^{uFA}}{\Delta x} - \frac{C_{N-1}^{uFA} - C_{N}^{uFA}}{\Delta x} \right) - k_1 C_{N-1}^{uFA} C_{N-1}^{uFA} - k_2 C_{AF1}^{uFA} C_{N-1}^{uFA} \ldots$$

$$\ldots - k_3 C_{AF2}^{N-1} C_{uFA}^{N-1} + k_{-1} C_{AF1}^{N-1} + k_{-2} C_{AF2}^{N-1} + k_{-3} C_{AF3}^{N-1}$$

$$\dot{C}_{N-1}^{Alb} = \frac{D_{Alb}}{\Delta x} \left( \frac{C_{N-2}^{Alb} - C_{N-1}^{Alb}}{\Delta x} - \frac{C_{N-1}^{Alb} - C_{N}^{Alb}}{\Delta x} \right) - k_1 C_{Alb}^{N-1} C_{N-1}^{Alb} - k_2 C_{AF1}^{Alb} + k_{-1} C_{N-1}^{Alb}$$

$$\dot{C}_{AF1}^{N-1} = \frac{D_{AF1}}{\Delta x} \left( \frac{C_{N-2}^{AF1} - C_{N-1}^{AF1}}{\Delta x} - \frac{C_{N-1}^{AF1} - C_{N}^{AF1}}{\Delta x} \right) + k_2 C_{AF1}^{N-1} C_{N-1}^{AF1} \ldots$$

$$\ldots - k_3 C_{AF2}^{N-1} C_{uFA}^{N-1} + k_{-2} C_{AF2}^{N-1} + k_{-3} C_{AF3}^{N-1}$$

$$\dot{C}_{AF2}^{N-1} = \frac{D_{AF2}}{\Delta x} \left( \frac{C_{N-2}^{AF2} - C_{N-1}^{AF2}}{\Delta x} - \frac{C_{N-1}^{AF2} - C_{N}^{AF2}}{\Delta x} \right) + k_2 C_{AF1}^{N-1} C_{N-1}^{AF1} \ldots$$

$$\ldots - k_3 C_{AF2}^{N-1} C_{uFA}^{N-1} + k_{-2} C_{AF2}^{N-1} + k_{-3} C_{AF3}^{N-1}$$

$$\dot{C}_{AF3}^{N-1} = \frac{D_{AF3}}{\Delta x} \left( \frac{C_{N-2}^{AF3} - C_{N-1}^{AF3}}{\Delta x} - \frac{C_{N-1}^{AF3} - C_{N}^{AF3}}{\Delta x} \right) + k_3 C_{AF2}^{N-1} C_{N-1}^{AF2} - k_{-3} C_{AF3}^{N-1}$$

Adsorption layer in interstitial compartment (segment $i = N$):

$$\dot{C}_{N}^{uFA} = \frac{2D_{uFA}}{\Delta x_{ads}} \left( \frac{C_{N-1}^{uFA} - C_{N}^{uFA}}{\Delta x} \right) - k_1 C_{N}^{uFA} C_{N}^{uFA} - k_2 C_{AF1}^{uFA} C_{N}^{uFA} \ldots$$

$$\ldots - k_3 C_{AF2}^{N} C_{uFA}^{N} + k_{-1} C_{AF1}^{N} + k_{-2} C_{AF2}^{N} + k_{-3} C_{AF3}^{N} - k_{on} C_{uFA}^{N} + k_{off} C_{FA}^{outer}$$

$$\dot{C}_{N}^{Alb} = \frac{2D_{Alb}}{\Delta x_{ads}} \left( \frac{C_{N-1}^{Alb} - C_{N}^{Alb}}{\Delta x} \right) - k_1 C_{N}^{Alb} C_{N}^{Alb} - k_{-1} C_{AF1}^{N}$$

$$\dot{C}_{AF1}^{N} = \frac{2D_{AF1}}{\Delta x_{ads}} \left( \frac{C_{N-1}^{AF1} - C_{N}^{AF1}}{\Delta x} \right) + k_1 C_{AF1}^{N} C_{N}^{AF1} \ldots$$

$$\ldots - k_2 C_{AF2}^{N} C_{uFA}^{N} - k_{-2} C_{AF1}^{N} + k_{-3} C_{AF2}^{N}$$

$$\dot{C}_{AF2}^{N} = \frac{2D_{AF2}}{\Delta x_{ads}} \left( \frac{C_{N-1}^{AF2} - C_{N}^{AF2}}{\Delta x} \right) + k_2 C_{AF1}^{N} C_{N}^{AF1} \ldots$$

$$\ldots - k_3 C_{AF2}^{N} C_{uFA}^{N} - k_{-2} C_{AF2}^{N} + k_{-3} C_{AF3}^{N}$$

$$\dot{C}_{AF3}^{N} = \frac{2D_{AF3}}{\Delta x_{ads}} \left( \frac{C_{N-1}^{AF3} - C_{N}^{AF3}}{\Delta x} \right) + k_3 C_{AF2}^{N} C_{N}^{AF2} - k_{-3} C_{AF3}^{N}$$

Outer leaflet of sarcolemma:

$$\dot{C}_{outer} = k_{on} C_{N}^{uFA} - k_{off} C_{FA}^{outer} + k_{flip} (C_{inner}^{outer} - C_{FA}^{outer})$$

Inner leaflet of sarcolemma:

$$\dot{C}_{FA}^{inner} = k_{on} C_{FA}^{sink} - k_{off} C_{FA}^{inner} + k_{flip} (C_{FA}^{outer} - C_{FA}^{inner})$$

For Model II B, no diffusion of albumin and its complexes is possible, so the values for $D_{Alb}, D_{AF1}, D_{AF2}$ and $D_{AF3}$ are zero in Model II B.
Receptor

Equations are identical to Model II, except for segment \( i = N \).

Adsorption layer in interstitial compartment (segment \( i = N \)):

\[
\begin{align*}
\dot{C}_{uFA}^N &= \frac{2D_{uFA}}{\Delta x_{ads}} \left( C_{uFA}^{N-1} - C_{uFA}^N \right) - k_1 C_{Alb}^N C_{uFA}^N - k_2 C_{AF1}^N C_{uFA}^N \\
&\quad - k_3 C_{AF2}^N C_{uFA}^N + k_{-1} C_{AF1}^N + k_{-2} C_{AF2}^N + k_{-3} C_{AF3}^N \\
&\quad + k_{\text{receptor}} (C_{R^*AF1} + C_{R^*AF2} + C_{R^*AF3}) - k_{on} C_{uFA}^N + k_{off} C_{FA}^N \\
\dot{C}_{Alb}^N &= \frac{2D_{Alb}}{\Delta x_{ads}} \left( C_{Alb}^{N-1} - C_{Alb}^N \right) - k_1 C_{Alb}^N C_{uFA}^N + k_{-1} C_{AF1}^N \\
&\quad + k_{\text{receptor}} C_{R^*AF1} - k_{\text{receptor}} C_{Alb} C_{receptor} + k_{\text{receptor}} C_{R^*Alb} \\
\dot{C}_{AF1}^N &= \frac{2D_{AF1}}{\Delta x_{ads}} \left( C_{AF1}^{N-1} - C_{AF1}^N \right) + k_1 C_{Alb}^N C_{uFA}^N - k_2 C_{AF1}^N C_{uFA}^N \\
&\quad - k_3 C_{AF1}^N + k_{-2} C_{AF2}^N + k_{\text{receptor}} C_{R^*AF2} - k_{\text{receptor}} C_{AF1} C_{receptor} + k_{-1} C_{R^*AF1} \\
\dot{C}_{AF2}^N &= \frac{2D_{AF2}}{\Delta x_{ads}} \left( C_{AF2}^{N-1} - C_{AF2}^N \right) + k_2 C_{AF1}^N C_{uFA}^N - k_3 C_{AF2}^N C_{uFA}^N \\
&\quad - k_{-3} C_{AF2}^N + k_{-2} C_{AF3}^N + k_{\text{receptor}} C_{R^*AF3} - k_{\text{receptor}} C_{AF2} C_{receptor} + k_{-1} C_{R^*AF2} \\
\dot{C}_{AF3}^N &= \frac{2D_{AF3}}{\Delta x_{ads}} \left( C_{AF3}^{N-1} - C_{AF3}^N \right) + k_3 C_{AF2}^N C_{uFA}^N - k_{-3} C_{AF3}^N \\
&\quad - k_{-3} C_{AF3}^N + k_{-2} C_{AF3}^N + k_{\text{receptor}} C_{AF3} C_{receptor} + k_{-1} C_{R^*AF3} \\
\dot{C}_{receptor} &= -k_{\text{receptor}} C_{Alb} C_{AF1} + k_{\text{receptor}} C_{Alb} + C_{AF1} + C_{AF2} + C_{AF3} \\
&\quad + k_{-1} C_{Alb} C_{receptor} + k_{\text{receptor}} C_{R^*AF1} \\
\dot{C}_{R^*Alb} &= k_{\text{receptor}} C_{Alb} C_{AF1} - k_{-1} C_{Alb} C_{AF1} + k_{\text{receptor}} C_{R^*AF1} \\
\dot{C}_{R^*AF1} &= k_{\text{receptor}} C_{AF1} C_{AF1} - k_{\text{receptor}} C_{AF1} + k_{\text{receptor}} (C_{R^*AF2} - C_{R^*AF1}) \\
\dot{C}_{R^*AF2} &= k_{\text{receptor}} C_{AF2} C_{AF2} - k_{\text{receptor}} C_{AF2} + k_{\text{receptor}} (C_{R^*AF3} - C_{R^*AF2}) \\
\dot{C}_{R^*AF3} &= k_{\text{receptor}} C_{AF3} C_{AF3} - k_{\text{receptor}} C_{AF3} - k_{\text{receptor}} C_{R^*AF3}
\end{align*}
\]

Model III (see Fig. 13)

Source:
The beginning of the endothelial glycocalyx on the luminal side

\([FA]_{\text{total}} = 0.5 \text{ mM}, [Alb]_{\text{total}} = 0.5 \text{ mM} \) (see Table 1)

Sink:
The middle of the interstitium. This is also the source for Model II.
Normal situation

Vascular compartment:

Identical to the equations for the interstitial compartment of Model II, only \( N_i \) has to be substituted for \( N \) in these equations and the term \( k_{\text{off}}^{\text{outer}} \) in the absorption layer for uFA have to be replaced with \( k_{\text{off}}^{\text{outer}}^{-1} \).

Luminal endothelial membrane:

\[
\dot{C}_{\text{FA}}^{\text{outer}}^{-1} = k_{\text{on}}^{\text{outer}} - k_{\text{off}}^{\text{outer}}^{-1} + k_{\text{flip}} (C_{\text{inner}}^{\text{outer}}^{-1} - C_{\text{FA}}^{\text{outer}}^{-1})
\]

\[
\dot{C}_{\text{FA}}^{\text{inner}}^{-1} = k_{\text{on}}^{\text{inner}} - k_{\text{off}}^{\text{inner}}^{-1} + k_{\text{flip}} (C_{\text{outer}}^{\text{inner}}^{-1} - C_{\text{FA}}^{\text{inner}}^{-1})
\]

Endothelial cytoplasm (i = 1):

\[
\dot{C}_{\text{uFA}}^{\text{cytoplasm}} = \frac{2D_{\text{cytoplasm}}^{\text{uFA}}}{\Delta x_{\text{ads}} \Delta x} (C_{\text{FA}}^{\text{uFA}} - C_{\text{uFA}}^{\text{cytoplasm}})...
\]

\[
- k_{\text{FABP}} C_{\text{FABP}}^{\text{1,endo}} C_{\text{uFA}}^{\text{cytoplasm}} + k_{\text{1}} C_{\text{FA}}^{\text{1,endo}} FABP - k_{\text{on}}^{\text{1,endo}} C_{\text{uFA}}^{\text{cytoplasm}} + k_{\text{off}}^{\text{1,endo}}
\]

\[
\dot{C}_{\text{FABP}}^{\text{cytoplasm}} = \frac{2D_{\text{cytoplasm}}^{\text{FABP}}}{\Delta x_{\text{ads}} \Delta x} (C_{\text{FA}}^{\text{FABP}} - C_{\text{FABP}}^{\text{cytoplasm}})...
\]

\[
- k_{\text{FABP}} C_{\text{FABP}}^{\text{1,endo}} C_{\text{uFA}}^{\text{cytoplasm}} + k_{\text{1}} C_{\text{FA}}^{\text{1,endo}} FABP - k_{\text{on}}^{\text{1,endo}} C_{\text{FABP}}^{\text{cytoplasm}} + k_{\text{FABP}}^{\text{cytoplasm}}
\]

\[
\dot{C}_{\text{FA}}^{\text{cytoplasm}} = \frac{2D_{\text{cytoplasm}}^{\text{uFA}}}{\Delta x_{\text{ads}} \Delta x} (C_{\text{FA}}^{\text{1,endo}} - C_{\text{FA}}^{\text{2,endo}})...
\]

\[
+ k_{\text{FABP}} C_{\text{FABP}}^{\text{1,endo}} C_{\text{uFA}}^{\text{cytoplasm}} - k_{\text{1}} C_{\text{FA}}^{\text{1,endo}} FABP
\]

Endothelial cytoplasm (i = 2):

\[
\dot{C}_{\text{uFA}}^{\text{cytoplasm}} = \frac{D_{\text{cytoplasm}}^{\text{uFA}}}{\Delta x (\Delta x)^2} (C_{\text{FA}}^{\text{2,endo}} - C_{\text{uFA}}^{\text{cytoplasm}} - C_{\text{FA}}^{\text{2,endo}} - C_{\text{uFA}}^{\text{cytoplasm}})...
\]

\[
- k_{\text{FABP}} C_{\text{FABP}}^{\text{2,endo}} C_{\text{uFA}}^{\text{cytoplasm}} + k_{\text{1}} C_{\text{FA}}^{\text{2,endo}} FABP - k_{\text{on}}^{\text{2,endo}} C_{\text{uFA}}^{\text{cytoplasm}} + k_{\text{off}}^{\text{2,endo}}
\]

\[
\dot{C}_{\text{FABP}}^{\text{cytoplasm}} = \frac{D_{\text{cytoplasm}}^{\text{FABP}}}{\Delta x (\Delta x)^2} (C_{\text{FA}}^{\text{2,endo}} FABP - C_{\text{2,endo}} FABP - C_{\text{FA}}^{\text{2,endo}} FABP)...
\]

\[
- k_{\text{FABP}} C_{\text{FABP}}^{\text{2,endo}} C_{\text{uFA}}^{\text{cytoplasm}} + k_{\text{1}} C_{\text{FA}}^{\text{2,endo}} FABP - k_{\text{on}}^{\text{2,endo}} C_{\text{FABP}}^{\text{cytoplasm}} + k_{\text{FABP}}^{\text{cytoplasm}}
\]

Endothelial cytoplasm (i = 3 to \( N_i \) - 2):

\[
\dot{C}_{\text{uFA}}^{\text{cytoplasm}} = \frac{D_{\text{cytoplasm}}^{\text{uFA}}}{(\Delta x)^2} (C_{\text{FA}}^{(i-1,\text{endo})} - C_{\text{uFA}}^{\text{cytoplasm}} - 2C_{\text{i,endo}})...
\]

\[
- k_{\text{FABP}} C_{\text{FABP}}^{i,\text{endo}} C_{\text{uFA}}^{\text{cytoplasm}} + k_{\text{1}} C_{\text{FA}}^{i,\text{endo}} FABP - k_{\text{on}}^{\text{i,endo}} C_{\text{uFA}}^{\text{cytoplasm}} + k_{\text{off}}^{\text{i,endo}}
\]
\[\dot{C}_{\text{AF}_1}^{1, \text{isf}} = \frac{2D_{\text{AF}_1}}{\Delta x_{\text{ads}}} \left( C_{\text{AF}_1}^{2, \text{isf}} - C_{\text{AF}_1}^{1, \text{isf}} \right) + k_1 C_{\text{Alb}}^{1, \text{isf}} C_{\text{uFA}}^{1, \text{isf}} \]

\[... - k_2 C_{\text{AF}_1}^{1, \text{isf}} C_{\text{uFA}}^{1, \text{isf}} - k_3 C_{\text{AF}_1}^{1, \text{isf}} + k_{-2} C_{\text{AF}_2}^{1, \text{isf}}\]

\[\dot{C}_{\text{AF}_2}^{1, \text{isf}} = \frac{2D_{\text{AF}_2}}{\Delta x_{\text{ads}}} \left( C_{\text{AF}_2}^{2, \text{isf}} - C_{\text{AF}_2}^{1, \text{isf}} \right) + k_2 C_{\text{AF}_1}^{1, \text{isf}} C_{\text{uFA}}^{1, \text{isf}} \]

\[... - k_3 C_{\text{AF}_2}^{1, \text{isf}} C_{\text{uFA}}^{1, \text{isf}} - k_{-2} C_{\text{AF}_2}^{1, \text{isf}} + k_{-3} C_{\text{AF}_3}^{1, \text{isf}}\]

\[\dot{C}_{\text{AF}_3}^{1, \text{isf}} = \frac{2D_{\text{AF}_3}}{\Delta x_{\text{ads}}} \left( C_{\text{AF}_3}^{2, \text{isf}} - C_{\text{AF}_3}^{1, \text{isf}} \right) + k_3 C_{\text{AF}_2}^{1, \text{isf}} C_{\text{uFA}}^{1, \text{isf}} - k_{-3} C_{\text{AF}_3}^{1, \text{isf}}\]

**Interstitial compartment (segment i = 2):**

\[\dot{C}_{\text{uFA}}^{2, \text{isf}} = \frac{D_{\text{uFA}}}{\Delta x} \left( C_{\text{AF}_1}^{1, \text{isf}} - C_{\text{AF}_2}^{1, \text{isf}} - C_{\text{AF}_2}^{2, \text{isf}} - C_{\text{AF}_3}^{2, \text{isf}} \right) - k_1 C_{\text{Alb}}^{2, \text{isf}} C_{\text{uFA}}^{2, \text{isf}} - k_2 C_{\text{AF}_1}^{2, \text{isf}} C_{\text{uFA}}^{2, \text{isf}} \]

\[... - k_3 C_{\text{AF}_2}^{2, \text{isf}} C_{\text{uFA}}^{1, \text{isf}} - k_{-2} C_{\text{AF}_2}^{2, \text{isf}} + k_{-3} C_{\text{AF}_3}^{2, \text{isf}}\]

\[\dot{C}_{\text{Alb}}^{2, \text{isf}} = \frac{D_{\text{Alb}}}{\Delta x} \left( C_{\text{AF}_1}^{2, \text{isf}} - C_{\text{AF}_2}^{2, \text{isf}} - C_{\text{AF}_2}^{3, \text{isf}} - C_{\text{AF}_3}^{3, \text{isf}} \right) - k_1 C_{\text{Alb}}^{2, \text{isf}} C_{\text{uFA}}^{2, \text{isf}} + k_{-1} C_{\text{AF}_1}^{2, \text{isf}} \]

\[\dot{C}_{\text{AF}_1}^{2, \text{isf}} = \frac{D_{\text{AF}_1}}{\Delta x} \left( C_{\text{AF}_1}^{1, \text{isf}} - C_{\text{AF}_2}^{1, \text{isf}} - C_{\text{AF}_2}^{2, \text{isf}} - C_{\text{AF}_3}^{2, \text{isf}} \right) + k_1 C_{\text{Alb}}^{2, \text{isf}} C_{\text{uFA}}^{2, \text{isf}} \]

\[... - k_{-2} C_{\text{AF}_2}^{2, \text{isf}} + k_{-3} C_{\text{AF}_3}^{2, \text{isf}}\]

\[\dot{C}_{\text{AF}_2}^{2, \text{isf}} = \frac{D_{\text{AF}_2}}{\Delta x} \left( C_{\text{AF}_2}^{1, \text{isf}} - C_{\text{AF}_2}^{2, \text{isf}} - C_{\text{AF}_3}^{2, \text{isf}} \right) + k_2 C_{\text{AF}_1}^{2, \text{isf}} C_{\text{uFA}}^{2, \text{isf}} \]

\[... - k_{-3} C_{\text{AF}_3}^{2, \text{isf}} \]

**Interstitial compartment (segment i = 3 to N, - 1):**

Same equations as in Model II for the interstitial compartment (segment i = 2 to N-2), except N has been substituted by N_3.

**Interstitial compartment (segment i = N_3):**

\[\dot{C}_{\text{uFA}}^{N_3} = \frac{D_{\text{uFA}}}{\Delta x^2} \left( 2C_{\text{sink}}^{N_3} - C_{\text{AF}_1}^{N_3} - C_{\text{AF}_2}^{N_3} - C_{\text{AF}_3}^{N_3} \right) - k_1 C_{\text{Alb}}^{N_3} C_{\text{uFA}}^{N_3} - k_2 C_{\text{AF}_1}^{N_3} C_{\text{uFA}}^{N_3} \]

\[... - k_{-3} C_{\text{AF}_3}^{N_3} C_{\text{uFA}}^{N_3} \]

\[\dot{C}_{\text{Alb}}^{N_3} = \frac{D_{\text{Alb}}}{\Delta x^2} \left( 2C_{\text{sink}}^{N_3} - C_{\text{AF}_1}^{N_3} - C_{\text{AF}_2}^{N_3} - C_{\text{AF}_3}^{N_3} \right) - k_1 C_{\text{Alb}}^{N_3} C_{\text{uFA}}^{N_3} + k_{-1} C_{\text{AF}_1}^{N_3} \]

\[\dot{C}_{\text{AF}_1}^{N_3} = \frac{D_{\text{AF}_1}}{\Delta x^2} \left( 2C_{\text{sink}}^{N_3} - C_{\text{AF}_1}^{N_3} - C_{\text{AF}_2}^{N_3} - C_{\text{AF}_3}^{N_3} \right) + k_1 C_{\text{Alb}}^{N_3} C_{\text{uFA}}^{N_3} - k_{-2} C_{\text{AF}_2}^{N_3} \]

\[... - k_{-3} C_{\text{AF}_3}^{N_3} \]

\[\dot{C}_{\text{AF}_2}^{N_3} = \frac{D_{\text{AF}_2}}{\Delta x^2} \left( 2C_{\text{sink}}^{N_3} - C_{\text{AF}_1}^{N_3} - C_{\text{AF}_2}^{N_3} - C_{\text{AF}_3}^{N_3} \right) \]

\[\dot{C}_{\text{AF}_3}^{N_3} = \frac{D_{\text{AF}_3}}{\Delta x^2} \left( C_{\text{sink}}^{N_3} - C_{\text{AF}_1}^{N_3} - C_{\text{AF}_2}^{N_3} - C_{\text{AF}_3}^{N_3} \right) \]

\[\dot{C}_{\text{AF}_1}^{N_3} = \frac{D_{\text{AF}_1}}{\Delta x^2} \left( 2C_{\text{sink}}^{N_3} - C_{\text{AF}_1}^{N_3} - C_{\text{AF}_2}^{N_3} - C_{\text{AF}_3}^{N_3} \right) \]
ode solver configuration

The steady state solution of Model I was obtained by putting the two time-derivatives, \( \dot{C}_{\text{outer}} \) and \( \dot{C}_{\text{inner}} \), to zero. The 2 unknowns, \( C_{\text{outer}} \) and \( C_{\text{inner}} \), could then be deduced from the two equations by substitution in MATLAB.

Model II and III are too complex to solve by substitution, so it’s solved by a ordinary differential equation solver. Both models are composed of ‘stiff’ differential equations. Definition from Mathworks: (50) "An ordinary differential equation problem is stiff if the solution being sought is varying slowly, but there are nearby solutions that vary rapidly, so the numerical method must take small steps to obtain satisfactory results." These ‘stiff’ differential equations are solved in MATLAB with the command ode15s, a ‘stiff’ solver. The time span ranges from 0 - 5 \times 10^6 \text{ seconds}, long enough to get a steady state solution for every compartment. The absolute tolerance, a measure to indicate the accuracy of the solver, is set to \( 1 \times 10^{-10} \), because else the solution converges to a wrong end solution. Albumin and FABP are equally distributed across the corresponding compartments and taken as initial concentrations.
References


48. R. S. MacLeod. Bioengineering Course About Systems Physiology: Microcirculation (Presentation).


