Fatty Acid Uptake in Normal Human Myocardium

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Fatty acid binding protein has been found in rat aortic endothelial cell membrane. It has been identified to be a 40-kDa protein that corresponds to a 40-kDa fatty acid binding protein with high affinity for a variety of long chain fatty acids isolated from rat heart myocytes. It is proposed that this endothelial membrane fatty acid binding protein might mediate the myocardial uptake of fatty acids. For evaluation of this hypothesis in vivo, influx kinetics of tracer-labeled fatty acids was examined in 15 normal subjects by scintigraphic techniques. Variation of the plasma fatty acid concentration and plasma perfusion rate has been achieved by modulation of nutrition state and exercise conditions. The clinical results suggest that the myocardial fatty acid influx rate is saturable by increasing fatty acid plasma concentration as well as by increasing plasma flow. For analysis of these data, functional relations describing fatty acid transport from plasma into myocardial tissue in the presence and absence of an “unstirred layer” were developed. The fitting of these relations to experimental data indicate that the free fatty acid influx into myocardial tissue reveals the criteria of a reaction on a capillary surface in the vicinity of flowing plasma but not of a reaction in extravascular space or in an unstirred layer and that the fatty acid influx into normal myocardium is a saturable process that is characterized by the quantity corresponding to the Michaelis-Menten constant, $K_m$, and the maximal velocity, $V_{max}$, $0.24\pm0.024 \mu mol/g$ and $0.37\pm0.013 \mu mol/(g \cdot min)$, respectively. These data are compatible with a nondifusional uptake process mediated by the initial interaction of fatty acids with the 40-kDa membrane fatty acid binding protein of cardiac endothelial cells. (Circulation Research 1991;69:857–870)

Plasmonic nonesterified fatty acids (FAs) are transported to the heart via blood as albumin-bound FAs. Monomeric FAs are efficiently taken up by the myocardium, revealing a single-pass extraction rate of 40–60%. Although FAs are the major energy source of the myocardium, little is known about their transport into the myocardial tissue.

Previously, we described the method for free fatty acid (FFA) influx rate determination in vivo, discussed the clinical significance of FFA extraction fraction for the diagnostic purposes, and presented a first approximation analysis of the data, which indicates that the FFA extraction rate might provide some information about the biochemical processes involved in FFA transport into myocardial tissue. For this analysis, we postulated that in the endothelial cell membrane an FFA carrier has to be expected, and we assumed that the reaction of FFA with a putative carrier follows a modified Michaelis-Menten relation in which the supplied amount of FFA per gram and minute and not FFA plasma concentration is the determinant parameter. An axiogradient of FFA concentration along the capillary exchange unit could not be considered since no adequate theoretical model describing these conditions was available.

It was the aim of the present study 1) to evaluate whether an FFA carrier, which was found to be present in sarcolemma, can also be isolated from the endothelial cell membrane, 2) to provide a theoretical basis for the description of FFA uptake into myocardial tissue in which both the axiogradient of FFA concentration in the capillary and the surface

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interaction of FFA with the putative carrier are considered, and 3) to analyze how the experimental data obtained with our method apply to membrane transport at the level of the endothelial cell compared with cardiomyocyte transport.

In our present study, we evaluated the data obtained in the same patient group as described in our previous study.5

**Materials and Methods**

**Demonstration of a Membrane Fatty Acid Binding Protein in Enriched Rat Aortic Endothelial Cell Membrane Fractions**

**Membrane preparation.** The aorta of male Wistar rats (250 g body wt, Zentralinstitut für Versuchstiere, Hannover, FRG) was removed, and the lumens were rinsed with 20 ml cold saline. The lumen was opened by a longitudinal section, and the endothelium was scraped off gently with a glass slide. The scraping was suspended in 10 ml of 1 mM NaHCO₃ (pH 7.4) and homogenized in a Waring blender at full speed for 2.5 minutes. The homogenate was then filtered through cotton gauze to remove clumped material. Fractions enriched in endothelial plasma membranes were prepared by an established procedure for liver sinusoidal plasma membranes using differential centrifugation.9 The final membrane pellets were washed three times in 1 mM NaHCO₃ (pH 7.4) and centrifuged at 15,000g for 15 minutes. Protein determinations and detection of purity and contamination with other cell organelles were carried out as described previously.9,10

**Immunoblot analysis.** A fraction of endothelial plasma membrane proteins was solubilized with 1% (vol/vol) Triton X-100.10 After centrifugation at 100,000g for 60 minutes, the detergent was removed over a Bio-Beads SM-2 column, and the solubilized protein mixture was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (5–20% gradient gels; run for 12 hours at 10 mA/gel). Thereafter, the proteins were transferred to nitrocellulose (BA 85, Schleicher & Schuell GmbH, Dassel, FRG) for 4 hours at 250 mA and 8–10°C. Nitrocellulose was then washed with 0.1% Tween 20 in phosphate buffered saline (PBS) (blocking buffer). For antibody blots, nitrocellulose was thereafter incubated with the monospecific antibody to the rat liver membrane FA binding protein produced in rabbits as previously described10 in a 1:500 dilution with blocking buffer. After consecutive washing with PBS, the detergent NP-40 (Sigma Chemie GmbH, Diesenhofen, FRG) in a concentration of 1% in PBS, and PBS two times for 10 minutes, a peroxidase-coupled swine anti-rabbit immunoglobulin (Dako T260, 1:200 dilution with 0.1% Tween 20 in PBS) was added as second antibody. Nitrocellulose was washed again and stained with a solution containing 120 mg chloronaphthol, 40 ml methanol, and 120 μl of 30% H₂O₂ in a total volume of 200 ml PBS.

**Clinical Studies**

**Study protocol.** Fifteen individuals without risk factors for coronary heart disease and with normal coronary arteries (as demonstrated by coronary angiography), normal wall motion, and no abnormal findings in echocardiography or in electrocardiography were examined for social medical indications, or they were found to have, for example, esophagitis or chest pain from thoracic nerves, nerve roots, or the spinal cord. Each subject was examined by a physician, and informed consent was obtained for the radionuclide study. The study has been conducted according to a protocol approved by the Committee on Ethics of Human Investigation of the Heart Center North-Rhine Westphalia.

For assessment of FA influx rate, a dual isotope technique with ²⁰¹TI as perfusion tracer and 15-(p-¹²³I-iodophenyl)-pentadecanoic acid (IPPA) as FA tracer was used. The use of conventional single-photon-emitting tracers with gamma scintigraphy and double-tracer technique provides the possibility for examination of the FA transport in humans under physiological conditions (no invasive intervention, no pharmaceutical treatment). To overcome the known limitations of single-photon scintigraphy, we applied to all our data a background correction based on a modified algorithm of Goris et al,11 which provides a linear background interpolation between the values at the edges of the irregular area surrounding the target. Moreover, we restricted the analysis of the data to evaluation of invariant parameters such as slopes or ratios.5 By this procedure, effects such as the depth-dependent resolution of planar scintigraphy or the contamination of the raw data due to the activity in overlying and underlying tissue can be practically eliminated. The ²⁰¹TI data were corrected for the flow dependency of ²⁰¹TI uptake at high flows.5

The use of IPPA as FA tracer seems to be justifiable throughout, since the phenylpentadecanoic acid is an FA analogue used by Knoop12 for the proof of β-oxidation; therefore, it can be expected to follow the same metabolic pathways as physiological FAs. Moreover, the IPPA uptake was shown to be comparable with that of [¹⁴C]palmitate, and the data presented in Figure 7 also demonstrate that the extraction of [¹⁴C]palmitate is comparable with that of IPPA. These data suggest that IPPA behaves the same as physiological FAs, so that it can be considered to be a suitable indicator for studying the physiological mechanisms involved in FFA transport.

The measurements were carried out at exercise and at rest conditions. Parallel to the external IPPA detection, the determinations of FFAs and albumin concentration in serum were carried out as well. For these determinations, the blood samples were taken immediately before indicator application.

In exercise studies, upright bicycle ergometry was performed by stepwise increases of the workload (25 W/step) and was terminated in the case of exhaustion. The duration of one workload step was 1 minute. At
maximal exercise 1 mCi $^{201}$Tl and 2 mCi IPPA were injected simultaneously followed by a 10-ml saline flush via an intravenous cannula inserted before the start of the study. Exercise was continued for 60 seconds. Simultaneous with the indicator application, a dual-isotope registration with a single-crystal camera (DATAMO, Picker, Cleveland, Ohio) was started as described previously. The data were collected in anterior, left anterior oblique (LAO) 30°, LAO 45°, and LAO 75° projections; the collection period was 6 min/image.

This is a "single-pass" method. As demonstrated previously, in normal subjects both indicators ($^{201}$Tl and IPPA) are extracted by myocardium in a short period after their application. As demonstrated by myocardial $^{201}$Tl residue time–activity curve this short extraction period is followed by a very slow elimination phase; in the myocardial IPPA residue time–activity curve this short extraction period was found to be followed by a plateau phase† and a two-phase elimination period. In the plateau phase, no significant changes of $^{201}$Tl or IPPA tissue concentration were externally detected even when the plasma concentration of these indicators was significantly decreased; that is, by external detection, these indicators can be considered to behave as indicators that are trapped in tissue. Because of this particular property, the $^{201}$Tl and IPPA represent indicators that reflect the myocardial perfusion and FFA uptake under the metabolic conditions existing during the short period after indicator application (instant picture) and that provide this information for a longer time (frozen state), even when the myocardial perfusion and metabolism are already changing (a property necessary for a time-consuming myocardial scintigraphy).

For the examinations at rest 4 hours later, four images with the same camera (anterior, LAO 30°, LAO 45°, and LAO 75°) were registered to determine the residual activity (collection period was 6 minutes for each image). Subsequently, blood samples for determination of albumin, FFAs, and hematocrit were collected. After administration of 1 mCi $^{201}$Tl and 2 mCi IPPA, a registration of data was performed in the same projections as described above. The correction for residual activity was carried out by subtraction of corresponding scintigrams detected before and after the second indicator application. As described previously, the ratio of background-corrected IPPA and $^{201}$Tl images was used for the determination of the unidirectional (influx) IPPA extraction rate in myocardial tissue (EF*). The FA influx rate ($V_i$) was determined as follows:

$$V_i = F_i \cdot \bar{c}_f \cdot f_p$$  \hspace{1cm} (1)

where $f_p$ is the plasma flow rate and $\bar{c}_f$ is the FA plasma concentration. The changes of $f_p$ during the exercise were estimated on the basis of $^{201}$Tl uptake as described previously.

**Determination of the FA concentration in plasma.**
For the determination of FA concentration in plasma, an enzymatic colorimetric method (NEFA C kit, Wako Chemicals, Neuss, FRG) was used.

**Determination of albumin concentration in plasma.**
For the determination of albumin concentration in plasma, the nephelometric method for quantitative determination of human serum proteins was applied (N-Reagensenz kit, Behringwerke AG, Marburg, FRG).

**Statistical Analyses**
Model functions (see Equations 8 and 10 in "Theoretical Model" below) were fitted to the data by the method of nonlinear least squares. The quality of fit was judged by evaluation of the mean residual sum of squares as well as by analysis of the residuals (deviations of the data from the fitted function) with regard to a systematic error of the fit. For these analyses, the nonlinear least-squares regression procedure NLIN and the linear least-squares procedure REG of the statistical software package SAS Version 5 were used. The statistical quality of a parameter estimate $\hat{p}$ is given by the asymptotic standard deviation $\text{ASD}(\hat{p})$ in the form $\hat{p} \pm \text{ASD}(\hat{p})$. In analogy to models used in physiology for chemical mediation processes, a simplified stochastic model on the molecular level based on renewal process and queuing theory is formulated and analyzed, giving a theoretical argument for the empirically derived results.

The results of measurements are given as mean ± SD.

**Theoretical Model**
This section provides the theoretical basis for the analysis of FA transport from plasma into myocardial tissue in the presence and absence of an unstirred layer.

**Transport of FFA from plasma into endothelial cell membrane in absence of an unstirred water layer in capillary.** The theoretical description of local influx rate is in this case based on the assumption that, by transport of FA into myocardial tissue, unbound FA...
in moving plasma interacts with its reactive partner (putative carrier) fixed in the plasma membrane of the capillary endothelial cells and that the flow velocity is so high that the brownian molecular movement in the flow direction can be neglected. It is postulated that for the reaction of two molecules a collision between the reaction partners is necessary. In analogy to similar models used in biochemistry, it is assumed that the time interval during which the FA is binding to carrier is not negligible. During this time, the carrier cannot react with another FA molecule, so that the carrier molecule alternates between occupied and free states. In the free state, the carrier unit is waiting for a reaction partner. Therefore, the time of a transport cycle is the sum of waiting time (time between the end of one reaction and the beginning of the next one) and actual transport time. The mean duration of a transport cycle is the sum of mean waiting time \(m_w\) and the mean transport time \(m_T\). Yet, the mean number of transports performed by one carrier per unit of time is \(1/(m_T+m_w)\) (expressed as 1/min).

If \(n_u\) is the number of carriers per unit of the length of capillary (1/cm) and \(dz\) is the length of the capillary segment (cm), then the total number of transports in unit of time in this segment is \(n_u \cdot dz/ (m_T+m_w)\) (expressed as 1/min).

If, because of the plug flow in myocardial capillaries in the vicinity of the capillary wall, the unstirred layer cannot develop, then the flow velocity at the wall would not be zero. In this case, by increasing the supply of FA the mean waiting time can be made arbitrarily small, so that the process can be saturated. Each FA molecule that is a potential candidate for collision with a carrier has to pass the cross section \((Q)\) of the capillary at the position of the carrier unit. The mean number of FA molecules passing \(Q\) per unit of time is the product of the average FA plasma density \((\bar{k}_f, the average number of fatty acid molecules per \(cm^3\)), the average plasma velocity \((\bar{v})\), and the area of the capillary cross section \((Q)\); that is, \(\bar{k}_f \cdot \bar{v} \cdot Q = \bar{k}_c \cdot f_c\), where \(f_c\) is capillary flow rate. As demonstrated in Appendix 1, the mean number of FA collisions with one carrier unit is proportional to the supplied amount of FA to a capillary cross section \(Q\) \((\bar{k}_c \cdot f_c)\), and the mean waiting time is proportional to the reciprocal of the supplied amount of FA; that is, \(m_w = K/(\bar{k}_c \cdot f_c)\), where \(K\) is the proportionality constant. (The comparison of Equations 2 and A5 indicates that the constant \(K\) is the reciprocal of probability \(R\).) Therefore, the number of transports per unit of time in a capillary segment \(dz\) (expressed as 1/min) is

\[
\frac{n_u \cdot dz}{m_W + m_T} = \frac{n_u \cdot dz}{m_T} \cdot f_c \cdot \bar{k}_f
\]

This relation indicates that the local influx of FA from plasma into the endothelial capillary wall is determined, in the case of absence of an unstirred layer, by the product of local FA plasma density and capillary plasma flow.

To describe the FA influx in the whole capillary where an axiogradient of FA concentration develops because of high consumption of FA, it has to be considered that, according to mass conservation law, the loss of FA in plasma in a short segment of capillary, \(d(f_c \cdot \bar{k}_f)\), is equal to the negative value of the FA influx into myocardial tissue in this segment; that is,

\[
d [f_c \cdot \bar{k}_f(z)] = \frac{n_u \cdot dz}{m_T} \cdot f_c \cdot \bar{k}_f (z)
\]

By dividing this relation by Avogadro's number, \(N_{AV}\) \((N_{AV}=6.03 \cdot 10^{27} \text{ molecules}/\mu \text{mol})\), the average FA loss in a capillary segment \(dz\) is obtained in units usually used in biochemistry:

\[
d [f_c \cdot \bar{c}_f(z)] = \frac{n_u \cdot dz}{m_T N_{AV}} \cdot f_c \cdot \bar{c}_f (z)
\]

where \(\bar{c}_f = \bar{k}_f/N_{AV}\) is the plasma FA concentration (\(\mu \text{mol}/\text{ml}\)).

By integrating this local FA losses in the whole capillary, the FA loss in one capillary is obtained:

\[
\int_0^l d [f_c \cdot \bar{c}_f(z)] = \frac{n_u \cdot n_c}{m_T N_{AV}} \cdot \int_0^l f_c \cdot \bar{c}_f (z) dz
\]

where \(l\) is the length of capillary.

If it is assumed that the extraction behavior of all capillaries is the same, then the FA losses in 1 g tissue (in \(\mu \text{mol}/[\text{min} \cdot \text{g}]\)) are obtained by multiplication of this relation with the average number of capillaries per gram of myocardial tissue \(n_c\):

\[
\int_0^l d [f \cdot \bar{c}_f(z)] = \frac{n_u \cdot n_c}{m_T N_{AV}} \cdot \int_0^l f \cdot \bar{c}_f (z) \cdot \bar{c}_f (z) \cdot \tau
\]

where \(f=n_c \cdot f_c\) is the plasma flow per gram of tissue and \(\tau\) is the unit of time. Since \(1/m_T\) is the maximal number of transports that can be performed by one carrier unit and \(n_u \cdot l \cdot n_c\) is the total number of carriers in gram of tissue, \(n_u \cdot l \cdot n_c/(m_T \cdot N_{AV})\) is the...
maximal achievable transport velocity per gram of tissue \( V_{\text{max}} \). The term \( K \cdot n \cdot \tau / (m \cdot N_{AV}) \) in Equation 5 is a constant that equals the amount of FA being supplied to 1 g in a unit of time, at which exactly the half maximal velocity is achieved. We labeled it as \( K_{\text{mv}} \). Consequently, Equation 5 can be rewritten as follows:

\[
\int_0^t \mathrm{d} [f \cdot \bar{c}(z)] = - \int_0^t \frac{V_{\text{max}} \cdot f \cdot \bar{c}(z) \cdot \tau}{K_m + f \cdot \bar{c}(z) / \tau} \cdot \frac{\mathrm{d}z}{t} \tag{6}
\]

The solution of this equation is as follows (see Appendix 1):

\[
f(\bar{c}_a - \bar{c}_v) = (K_m / \tau) \cdot \ln (\bar{c}_v / \bar{c}_a) + V_{\text{max}} \tag{7}
\]

where \( \bar{c}(z) = \bar{c}(0) = \bar{c}_a \) and \( \bar{c}(z) = \bar{c}(l) = \bar{c}_v \) are the arterial and the venous FA plasma concentration, respectively, and \( f \cdot (\bar{c}_a - \bar{c}_v) \) is the FA influx rate per gram of tissue.

If it is considered that \( \bar{v}_i / (f \cdot \bar{c}_a) = (\bar{c}_a - \bar{c}_v) / \bar{c}_a \), then the following implicit relation for FA influx in a gram of tissue is obtained:

\[
\bar{v}_i = (K_m / \tau) \cdot \ln [1 - \bar{v}_i / (f \cdot \bar{c}_a)] + V_{\text{max}} \tag{8}
\]

This relation was fitted to our data.

Transport of FFA from extravascular space into myocardial cell or from plasma into endothelial cell membrane in presence of an unstirred water layer in capillary. In the case of the presence of an unstirred layer, it has to be expected that the dominant component for the FA–carrier reaction is not the translation of FA molecules but brownian molecular movement; thus, it is reasonable to assume that the local FA influx follows the standard Michaelis-Menten relation (see Appendix 2); that is,

\[
V_{\text{max}} \cdot \bar{c}(z) = \frac{V_{\text{max}} \cdot \bar{c}(z) / \tau}{K_m + \bar{c}(z) / \tau} \tag{9}
\]

Under these conditions the following relation describing the FA influx rate per gram of myocardial tissue is obtained:

\[
\bar{v}_i = f \cdot K_m \cdot \ln [1 - \bar{v}_i / (f \cdot \bar{c}_a)] + V_{\text{max}} \tag{10}
\]

This relation was also fitted to our data.

Results

In Vitro Studies

Demonstration of a membrane FA binding protein in enriched rat aortic endothelial cell membrane fractions. The initial step in any carrier-mediated uptake process represents binding of the ligand to specific membrane binding sites. In the overall uptake process of FAs by the heart, the first membrane that has to be passed is the cell membrane of the capillary endothelial cell. Therefore, we examined whether the membrane FA binding protein (MFABP), which was found to be present in sarcolemma, can also be identified in the endothelial cell membrane. In fact, a monospecific antibody to MFABP reacted with a single 40-kDa protein of the solubilized rat aortic endothelial cell membrane proteins (Figure 1). Comparison with MFABP from the liver, heart, and intestine, isolated by Stremmel and colleagues revealed no apparent physicochemical differences to the identified protein from rat endothelium. Moreover, staining was abolished after preabsorption of the antibody with purified MFABP isolated from rat liver. The results suggest that the identified endothelial protein shares antigenic determinants with the other MFABPs previously isolated by Stremmel and may possibly be identical.

Clinical Results

To analyze the FA uptake process, unidirectional FA influx rate into myocardial tissue was determined in 15 normal subjects both during exercise and at rest, providing a wide range of FA concentrations in plasma as well as various flow rates.

The data obtained in this study are summarized in Table 1. The albumin plasma concentrations ranged from 3,350 to 4,040 mg/dl. The average heart rate was 123 ± 47 beats/min during exercise and 80 ± 13 beats/min at rest.

The FFA influx rate dependence on FFA plasma concentration is demonstrated in Figure 2. In this figure, a large scatter of the data is apparent; the mean residual sum of squares for fitting with a function \( ax / (b + x) \) was 0.12 × 10⁻².
TABLE 1. Subject Data

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<td>0.83</td>
<td>ANT</td>
<td>0.191*</td>
</tr>
<tr>
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<td>0.45</td>
<td>ANT</td>
<td>0.115†</td>
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<tr>
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<td>0.66</td>
<td>0.50</td>
<td>ANT</td>
<td>0.177</td>
</tr>
</tbody>
</table>

$\bar{c}_p$, Free fatty acid plasma concentration; $f_p$, plasma flow rate; $\bar{v}_i$, fatty acid influx rate; ANT, anterior projection; L75, left anterior oblique 75° projection.

*Data evaluated for analysis of free fatty acid influx rate at "constant" free fatty acid plasma concentration. Average free fatty acid plasma concentration was $0.51 \pm 0.03$ µmol/ml.

†Data evaluated for analysis of free fatty acid influx rate at "constant" plasma flow. Average plasma flow was $0.44 \pm 0.03$ [ml/(g·min)].

‡Preprandial and postprandial study.

To analyze this scatter, we considered the data obtained in subjects with a "constant" myocardial plasma flow rate of $0.44 \pm 0.03$ ml/(g·min) (see Table 1) and data obtained in subjects with a constant FA plasma concentration of $0.51 \pm 0.03$ µmol/ml (see Figure 3 and Table 1). In the first group, the FFA plasma concentration ranged from 0.32 to 1.17 µmol/ml. This increase of the FFA plasma concentration led to an increase of FA influx rate from 0.096 to 0.24 µmol/(g·min). In the second group, the increase of plasma flow rate from 0.42 to 1.20 ml/(g·min) led to an increase of the FA influx rate from 0.12 to 0.23 µmol/
(g·min). In both groups, the scatter of data is remarkably lower; fitting a function ax/(b+x) to the data of the first and second group led to the mean residual sum of squares of 0.019×10^-2 and 0.018×10^-2 respectively.

These data, which indicate that in case of "constant flow" the scatter of data is remarkably lower and that at "constant FA plasma concentration" the FA influx is a function of flow, suggest that under in vivo conditions the FA plasma concentration is not the only determinant of FA influx rate.

The classical theory postulates that the flow dependence of FA influx is an attribute of axiogradient of FA concentration developing along the capillary exchange unit due to significant FA consumption. The magnitude of axiogradient is determined by the degree of a local influx. In the case that the carrier reacts with FFA dissolved in a stationary solution (cell culture, unstirred water layer in the capillary, or transport from extravascular space into myocardial cell), the local FA influx is usually assumed to follow a Michaelis-Menten relation (see Equation 9). Under these conditions, the FA influx rate in 1 g tissue is described by Equation 10.

This function was fitted to all data obtained in this study. The mean residual sum of squares and the parameter estimates for this case are given in Table 2. The deviations of the observed data from the theoretical estimate are shown in Figure 4 as a function of plasma flow. In this figure, a trend can be recognized pointing out that the functional relation shown in Equation 10 overestimates the influx rate at low plasma flow and underestimates it at high plasma flow. The significance of the trend was tested by fitting a straight line to all data by linear least squares. The trend is found to be significant at p<0.001.

This analysis suggests that the description of the local FA influx rate by the standard Michaelis-Menten relation is not sufficient to explain our data; that is, along with axiogradient-related effects of flow.

---

**TABLE 2. Statistical Analysis of Fatty Acid Influx Rate Data**

<table>
<thead>
<tr>
<th>Model</th>
<th>Mean residual sum of squares [μmol/(g·min)]</th>
<th>V_mmax [μmol/(g·min)]</th>
<th>K_m [μmol/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Michaelis-Menten</td>
<td>0.057×10^-2</td>
<td>0.40±0.033</td>
<td>0.43±0.085</td>
</tr>
<tr>
<td>Flow-dependent local FFA influx rate†</td>
<td>0.017×10^-2</td>
<td>0.37±0.013</td>
<td>0.24±0.024</td>
</tr>
</tbody>
</table>

FFA, free fatty acid. Model functions shown in Equations 10 and 8 were fitted to the data by nonlinear least-squares regression procedure NLIN and the linear least-squares procedure in the statistical software package SAS Version 5. The statistical quality of parameter estimates are given by the asymptotic standard deviation.

*See Equation 10. †See Equation 8.

---

**FIGURE 2.** Free fatty acid influx rate into normal myocardium plotted as a function of the free fatty acid plasma concentration.

**FIGURE 3.** Free fatty acid influx rate into normal myocardium plotted as a function of flow rate. Data were obtained in subjects with an average free fatty acid (FA) plasma concentration of 0.51±0.03 μmol/ml and the plasma flow rate ranging from 0.42 to 1.20 ml/(g·min).

**FIGURE 4.** Deviations of the observed data from the theoretical estimate plotted as a function of plasma flow. The estimates were calculated under the assumption that the local fatty acid influx follows the standard Michaelis-Menten relation and that the dependence of fatty acid influx on the flow is a sole function of the axiogradient of fatty acid concentration along the capillary exchange unit.
on influx rate, direct interaction of flow with local FA transport mechanism might also be present.

As demonstrated in Appendix 1, the dependence of local FA influx rate on flow can be expected when no unstirred layer is present in capillary and when the FFAs dissolved in flowing medium react with a carrier immobilized in the endothelial cell wall. Under these conditions, the local FA influx rate is described by Equation 8.

Also, this function was fitted to all data obtained in this study. The mean residual sum of squares and the parameter estimates are given in Table 2. The essential improvement of agreement between predicted and observed values is indicated by a significantly lower mean residual sum of squares. The deviations of the observed data from the theoretical estimate are shown in Figure 5 as a function of plasma flow.

In Figure 6, the FA influx rates, observed in all subjects examined in this study, are plotted as a function of the amount of FA available per gram of tissue and unit of time.

The nonlinear least-squares fit of the relation (Equation 8) to the observed data revealed that the myocardial FA influx follows saturation kinetics with a $K_m$ and $V_{max}$ of $0.24\pm0.024 \mu mol/g$ and $0.37\pm0.013 \mu mol/(g \cdot min)$, respectively (Table 2).

Discussion

Plasmatic nonesterified FAs are transported to the heart via blood bound to albumin. However, the cells do not appear to internalize albumin at a significant rate.\textsuperscript{18} The conventional theory of the sequestration of protein bound ligands assumes that only unbound ligand participates in the uptake process. Since the unbound fraction of ligand is very small when compared with uptake of ligand, the conventional theory assumes that the bound fraction equilibrates virtually instantaneously with the unbound fraction.\textsuperscript{19-21} This assumption has been the cause of much confusion and uncertainty. Recent studies of Weisiger and Ma\textsuperscript{22} and Sorrentino et al\textsuperscript{20} showed, however, that 1) the rate of FA dissociation from the albumin molecule is sufficient to cover the hepatic uptake, 2) under physiological plasma albumin concentrations, the conventional model is applicable, and 3) at physiological plasma albumin concentrations, the rate of FA transport correlates also with the albumin bound concentration. Therefore, the FA dissociation from albumin is commonly not thought to be a rate-limiting step for FA transport into myocardial tissue.\textsuperscript{20-22}

The transfer of FA from plasma to cardiac cells does not involve solely a phase boundary (plasma–capillary wall) process; it also involves a sequence of membrane and aqueous phase translocation processes. These include passage of FFAs across the capillary wall and movement through the intercellular space before they are presented to the cardiomyocytes. The mechanism of this directed transport across various extracellular and intracellular compartments, which is assumed to be highly regulated to ensure sufficient energy supply for the working heart under various metabolic conditions, is still unclear.

For transport of FA across the nonfenestrated myocardial capillary endothelium, three hypotheses are discussed: penetration via clefts, lateral diffusion in the plasmalemma, and transendothelial transport. Since recent studies\textsuperscript{18} indicate that the first two possibilities are unlikely, it was concluded that the transendothelial transport begins with the translocation of FA from plasma into the luminal membrane of the endothelial cell.

Several years ago Spector\textsuperscript{23,24} proposed that the FFAs enter the plasma membrane of cells by a process of simple diffusion. During the last decade, however, evidence cumulated that the entry of FA into the cell is mediated by special carrier proteins. So, for exam-
ple, Stremmel\textsuperscript{8} was able to isolate a specific 40-kDa membrane FA transport protein from the membrane of myocardial cells and to show that it participates in the FA uptake. Yet, the prevalent view today is that the FA uptake in myocardial tissue is a carrier-mediated process.\textsuperscript{8,10,16,17,25–28}

Therefore, it was of potential interest to evaluate whether an FA carrier, which was found to be present in sarcolemma,\textsuperscript{8} can be also isolated from the endothelial cell membrane.

By Western blot analysis (Figure 1) of a protein fraction isolated from rat aortic endothelial cells, it has been shown that an MFABP corresponding to that isolated by Stremmel\textsuperscript{8} from sarcolemma is also a constituent of endothelial plasma membranes. This protein is distinct from the 12-kDa cytosolic FA binding protein\textsuperscript{29} and does not reveal apparent homology to other cellular or extracellular proteins. We believe that this membrane FA binding protein might also mediate the translocation of FA from plasma into the endothelial plasma membrane of the capillary.

To support this hypothesis, it was necessary to characterize the kinetic properties of the FA transport system under in vivo conditions. For this, we determined myocardial FA influx in 15 normal subjects at different metabolic states. By evaluating these data two questions were of particular interest: 1) Does in vivo fatty acid influx into myocardial tissue reveal saturation kinetics? 2) Do the data obtained in our study apply to membrane transport at the level of the endothelial cell or do they reflect the myocardial cell membrane transport?

At first we studied the dependence of FA influx rate on FA plasma concentration. As demonstrated in Figure 2, our data reveal, in fact, saturation kinetics. However, a large degree of scatter of the data was present. On the other hand, in measurements in which the FA influx rate was determined at conditions of constant flow but at different FA plasma concentrations, the scatter of the data was evidently lower. These results indicate that the FA plasma concentration cannot be considered as the only determinant of FA influx rate. Indeed, the data obtained under conditions of constant FA plasma concentration but at different flows (Figure 3 and Table 1) showed that the FA influx rate is also dependent on plasma flow. This dependence could be confirmed by Reske\textsuperscript{30} and Schöhn\textsuperscript{31} in animal experiments. These authors examined in eight open-chest mongrel dogs the relations existing between the regional myocardial blood flow (\textsuperscript{133}I microspheres) and regional uptake of IPPA (radioactivity in heart slices was determined in a well scintillation counter and corrected for radioactive decay, background, and cross contamination) under ischemic and normal conditions and during pacing-induced stimulation.

Initially, we tried to interpret the dependence of FA influx rate on flow by the axiogradient of FA plasma concentration along the capillary exchange unit. The axiogradient develops under the conditions of a significant consumption of FA. Under these conditions, the downstream concentration is lower than the upstream concentration; therefore, the FA influx is higher upstream than downstream. The increase of the flow leads to reduction of the axiogradient and thus to an increase of the overall influx rate.

We based our calculation of the losses of FA along the capillary exchange unit (axiogradient of FA plasma concentration) on the assumption that the local FA influx rate follows the standard Michaelis-Menten relation \( \frac{V_{\text{max}}}{K_m + [S]} \).\textsuperscript{32} As described in Appendix 2, this assumption implies that in the vicinity of the capillary wall exists the unstirred layer or that our data reflect the FFA transport from the stationary interstitial fluid into the myocardial cell. By the use of this model, a function describing the FA influx rate into myocardium has been derived (Equation 10). This function was fitted to the observed data. In Figure 4, the deviations of the observed data from the data predicted on the basis of the functional relation shown in Equation 10 are presented as a function of plasma flow. It can be seen that at low plasma flow rates, FA influx is overestimated by this model, whereas at high flows it is underestimated. Therefore, we concluded that this model is not sufficient to explain the observed influx dependence on flow and that some kind of interaction between flow and local FA influx rate has to be expected.

Such an interaction would be expected in the case of reaction of FFA dissolved in flowing plasma with the carrier fixed in the endothelial cell wall in the absence of an "unstirred layer." In myocardial tissue, where, probably because of the plug flow in myocardial capillaries, an unstirred layer in the vicinity of capillary wall cannot develop, such conditions are possible throughout.

To analyze this situation, the classical model mentioned above had to be reshaped. We decided to describe the transport process, in this case, on the molecular level. For this, we first analyzed the possible interactions of the FA molecule with the carrier. We based our analysis on the collision theory, which postulates that the reaction of two molecules can only occur if it comes to a collision between the reaction partners. Based on this hypothesis, a function describing the number of collisions of the FA molecule leading to reaction with the carrier was developed (Equation 2). This relation suggests that the expected number of collisions and thus the local FA influx rate is determined by the amount of substrate being supplied to a gram of tissue in a unit of time (product of FA concentration and flow). This would mean that the carrier fixed in the endothelial cell membrane interprets an increase of flow velocity as an increase of FA plasma concentration. By the use of Equation 2, the relation describing the axiogradient and global FA influx rate into gram of myocardial tissue was developed (Equation 8). The function was fitted to the observed data. In Figure 5, where the deviations of the observed data from the predicted values are plotted as a function of plasma flow, it can be seen that, in
by Julien et al.36

contrast to the model based on the standard Michaelis-Menten description of local FA influx rate, this model appears to be sufficient to predict the observed data. These results, which indicate that the FFA influx into myocardial tissue reveals the criteria of a reaction on a capillary surface in the vicinity of flowing plasma but not of a reaction in extravascular space or in an unstimulated layer, suggest that in the main process of FFA influx into normal myocardial tissue the translocation across the endothelial cell represents a determinant of the overall uptake process.

To substantiate our results, we compared our data with results of physiological measurements of arterial coronary sinus estimates of fatty acid ([14C]palmitic acid) influx done by Most et al.14 in normal humans and by Opie and colleagues,33,34 Spitzer,35 and Julien et al.36 in experimental animals (see Figure 7 and Table 3). In Figure 7, it can be seen that our data, and thus the model, correlate well with physiological measurements of arterial coronary sinus estimates of FA influx performed both in humans and experimental animals.

The fitting of Equation 8 to experimental data revealed, moreover, that the FA influx into myocardium is characterized by the quantity corresponding to the Michaelis-Menten constant, $K_m$, and $V_{max}$ being $0.24 \pm 0.024 \, \mu mol/g$ and $0.37 \pm 0.013 \, \mu mol/(g \cdot min)$, respectively (see Table 2). For comparison, in Table 2 the results of a fit of the conventional model (Equation 10) to our data are also presented. Whereas the latter values correspond quite closely to the values described in our previous study,5 the value of $K_m$ obtained by the use of the present model (Equation 8) is approximately twofold different. These results indicate that conventional relations developed initially for biochemistry as well as the model used in our previous study,5 which does not consider axiogradients effects even if providing some valuable clinical information, might lead to a significant overestimation by evaluation of pathophysiological mechanisms involved in FFA transport.

The analyses presented in the present study thus indicate that data obtained by our method reflects endothelial cell membrane transport rather than myocardial cell transport, that the FA carrier might be present in endothelial cell membrane, and that the FA influx into human myocardium reveals saturation kinetics with $K_m$ and $V_{max}$ of $0.24 \pm 0.024 \, \mu mol/g$ and $0.37 \pm 0.013 \, \mu mol/(g \cdot min)$, respectively. Therefore, we proposed that the influx of FA into human myocardium might be facilitated by a carrier

![Figure 7. Plot comparing data obtained in this study (see Figure 6) with data presented in literature (see Table 3). The curve represents the values predicted by the functional relation shown in Equation 8 (see "Materials and Methods"). X, our data; A, data obtained by Most et al.; V, data obtained by Opie et al.33,34; W, data obtained by Spitzer.35; D, data obtained by Julien et al.36](image-url)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Hct (%)</th>
<th>HR (beats/min)</th>
<th>FFA $\bar{c}_i$ (\mu mol/ml)</th>
<th>$[14C]$Palmitate $f_p$ EF</th>
<th>$f_p \cdot \bar{c}_i$</th>
<th>$\bar{v}_i$</th>
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<td>0.38</td>
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<td>0.44*</td>
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<td>Greyhounds</td>
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<td>...</td>
<td>0.778</td>
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<td>Study 233,34 (n=7)</td>
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<td>0.15</td>
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<td>Mongrel dogs</td>
<td>Study 133 (n=7)</td>
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<td>162</td>
<td>0.688</td>
<td>0.44</td>
<td>0.65</td>
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<tr>
<td>Study 236 (n=16)</td>
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<td>167</td>
<td>0.598</td>
<td>0.62</td>
<td>0.62*</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Hct, hematocrit; HR, heart rate; FFA $\bar{c}_i$, free fatty acid plasma concentration; EF, extraction fraction; $f_p$, plasma flow rate $=f \cdot (1-Hct)$, where $f$ is myocardial blood flow, $\bar{v}_i$, fatty acid influx rate $=EF \cdot f_p \cdot \bar{c}_i$; PT, paroxysmal tachycardia; MI, mitral insufficiency.

*Estimate of flow at rest in normal human myocardium.37,38
†Estimate of flow in normal dog myocardium.35
fixed in the membrane of the capillary endothelial cells.

The present method provides the possibility of quantitative measurements of FA influx rate in normal myocardium. It is, however, limited in the case of quantitative measurements under pathological conditions, where the homogeneous behavior of myocardium cannot be expected and the overlapping of normal and pathological tissue might influence the results. Moreover, under pathological conditions in areas of low-activity accumulation, the underestimation that occurs by applying the background correction might represent a potential source of error in quantitation and the resultant plasma flow rates and IPPA transfer rates. Yet, in pathological cases, the planar photon tomography should be replaced by single-photon tomography.

**Appendix 1**

This section provides the mathematical basis for the descriptive theoretical analysis of FA influx presented in “Materials and Methods.”

By modeling the transport of FA molecules from blood into the myocardial tissue, it was considered that under physiological conditions the efflux of nonmetabolized FA from normal myocardial tissue can be neglected.\(^{14}\)

In total, eleven assumptions were made. We assumed 1) that if FA reacts with carrier, it will be transported; that one carrier can bind only one FA molecule; and that the time for which FA binds to a carrier is finite and positive; 2) that FAs are transported from blood into myocardial tissue by carrier molecules that are randomly distributed in the capillary wall; 3) that, in the vicinity of each carrier unit, an acquisition area exists, in which the FA molecule can react with the carrier; 4) that there are no interactions between the single carrier molecules; 5) that the transport behavior of the carrier unit is independent of time and of transport intensity; 6) that the flow velocity is so high that the molecular movement in flow direction and the time during which the fatty acid molecule is passing through the acquisition area of the carrier unit are negligible (i.e., that the passage time through the acquisition area can be considered to be zero); and 7) that the system is in a steady state.

Under these conditions, the transport process can be considered as consisting of two components: a waiting time (W) during which the carrier is waiting for the FA molecule “to come into its acquisition area” and 2) a transport time (T) during which the FA molecule is transported from blood into an endothelial cell (i.e., the total time for the transport of an FA molecule into an endothelial cell is given by \(X=W+T\)).

The schematic description of such a process for one molecule is presented in Figure A1. Let us consider a carrier unit at a fixed position on the capillary surface. The expected average number of FA molecules, which are transported in time interval \(t\) into the endothelial cell \(\langle N(t)\rangle\), can be determined from the joint distribution of the \(X_i=W_i+T_i\), where \(W_i\) is the waiting time of the carrier unit between the end of the \((i-1)\)th and the beginning of the \(i\)th transport process, \(T_i\) is the transport time needed for the \(i\)th transport, and \(N_i\) is the number of completed transport processes in an observation period \(t\). This distribution can be derived from the distributions of \(W_i\) and \(T_i\). We assume that \(T_i\)s are independent and identically distributed random variables (see assumptions 5 and 7).

To determine the distribution of \(W_i\), we consider a cross section \(Q\) of the capillary at the position of the carrier unit under consideration (assumption 6). This cross section must be passed by all FA molecules that are potential candidates for transport with this carrier unit.

Let \(k_i(a_1,a_2)\) be the concentration of FAs (average number of FA molecules per cm\(^2\) [1/cm\(^2\)]\(^3\)) and \(v(a_1,a_2)\) be the velocity (cm/min) in point \((a_1,a_2)\)Q. Then the density of FA molecules passing point \((a_1,a_2)\) in a unit of time is \(\dot{n}=k_i(v)\) (expressed as 1/min cm\(^2\)), and the average density of FA molecules passing \(Q\) in a unit of time (expressed as 1/min cm\(^2\)) is

\[
\dot{n}=\frac{1}{|Q|}\int_Q \int k_i(a_1,a_2)\cdot v(a_1,a_2)da_1da_2=\hat{k}_i\cdot \vec{v}
\]

(A1)

where \(\vec{v}=(1/|Q|)\int_Q v\cdot da_1da_2\) is the average flow velocity (cm/min), \(k_i(1/|Q|)\int_Q |k_i| \cdot v'\cdot da_1da_2\) is average FA concentration in \(Q\) (1/cm\(^3\)), \(|Q|\) is the area of cross section \(Q\) (cm\(^2\)), and \(v'=v/\vec{v}\) (1) is the velocity profile.

Let us, furthermore, assume 8) that the positions at which the fatty acid molecules pass the cross section \(Q\) are independent and identically distributed random variables (assumption 7) that are independent of the arrival times of molecules passing \(Q\).

When \(A\) is the coordinate-vector of the random point at which an FA molecule passes the cross section \(Q\), then the probability density function of \(A\) (expressed as 1/cm\(^3\)) is

\[\text{Figure A1. Schematic representation of the sequence of the events during the transport of fatty acids into myocardial tissue.} x_i\]
where \( k' = k_0/k_f \) is the concentration profile.

For each point \((a_1,a_2)\in Q\) a probability \( p(a_1,a_2) \) exists that the fatty acid molecule passing \( Q \) in \((a_1,a_2) \) is "ready for transport" (RFT), which means that the fatty acid will be transported if the carrier unit is waiting (assumptions 3 and 4). The probability \( p(a_1,a_2) \), which describes the reaction radius of a carrier unit, is influenced by the orientation of the FA molecule in the stream, by the configuration of the FA molecule, by the configuration of the active center of the carrier unit, and by the albumin concentration in plasma.

The probability \( R \) that a random fatty acid molecule passing \( Q \) is RFT is then

\[
R = \int_Q \int p(a_1,a_2)f_a(a_1,a_2)da_1da_2 \tag{A3}
\]

and the expected value of fatty acid molecules passing \( Q \) in a unit of time that is RFT (expressed as 1/min) is (under assumption 8):

\[
\mathbb{E}[N(t)]_{RFT} = R \cdot n_0 \cdot |Q| = R \cdot |Q| \cdot \bar{v} \cdot k_f \tag{A4}
\]

If it is further assumed 9) that the fatty acid flow through \( Q \) is a Poisson process, then (under assumption 8) the flow of RFTs through \( Q \) is a Poisson process, too.39

Since the expected value of RFTs passing \( Q \) in a unit of time is determined to be \( R \cdot n_0 \cdot |Q| \), the intensity parameters of this Poisson process is \( R \cdot n_0 \cdot |Q| \). Under these conditions, the differences (Z) between appearance times (\( \tau \)) of RFTs, \( Z_i = \tau_{i+1} - \tau_i \), are independent and identical exponentially distributed variables, with an expectation of \( 1/(R \cdot n_0 \cdot |Q|) \).

The waiting time \( W_i \) during which the carrier unit is waiting for the arrival of the next RFT is a part of a certain \( Z_i \) (assumption 6); it represents the forward recurrence time of a Poisson process40 and has, because of the "lack of memory" of exponential distribution, the same distribution as \( Z_i \). Thus, \( W_i \) is independent and identically exponentially distributed variables with parameter \( R \cdot n_0 \cdot |Q| \) and expectation \( n_0 = 1/(R \cdot n_0 \cdot |Q|) \) (expressed in minutes).

It is also assumed 10) that \( T_i \), independent of \( W_i \) and of the random position \( A_i \) at which the fatty acid passes \( Q \) and that the expectation \( n_0 \) for the transport time exists.

Then the total transport times \( X_2,X_3,X_4, \ldots \) are a series of independent and identically distributed random variables. An exception is represented by the value \( X_1 \), which has a different distribution. Since, however, we consider a relatively long time interval \( t \), which consists of a large number of \( X_i \) values, the importance of \( X_1 \) is relatively low, and \( W_i \) and \( T_i \) can be considered as an alternating renewal process in equilibrium (assumption 7),40 and the expected value of FA molecules transported in a unit of time (expressed as 1/min) is given by the following40:

\[
\mathbb{E}[N_i] = \frac{1}{m_w + m_T} = \frac{1}{m_T + \bar{v} \cdot k_f} \tag{A5}
\]

where \( m_T \) is the expected value of the transport time (expressed in minutes).

To determine the properties of a carrier unit randomly distributed on the surface of a capillary, \( S \), we first consider the carrier unit fixed in \((x,y,z)\) (coordinates of the selected carrier unit on \( S \)), where the \( z \) axis is the capillary axis. For each carrier we select in \( Q \) local axes \( a_1 \) and \( a_2 \). So, the center of \( Q \) is \((0,0)\) and the position of the carrier in \( Q \) is \((0,0)\), with \( r \) being the radius of capillary.

We assume 11) that \( Q\), \( v(a_1,a_2) \), \( m_T \), and \( p(a_1,a_2) \) and \( k'(a_1,a_2) \) are independent of \( x,y,z \).

Then the probability \( R \) (1) is independent of \( x,y,z \):

\[
R = \int_Q \int p(a_1,a_2)k'(a_1,a_2)v'(a_1,a_2)da_1da_2 \tag{A6}
\]

and \( k_f \) (expressed as 1/cm) is independent of \( x,y \) and only function of \( z \):

\[
\bar{k}_f(z) = \int_Q k_f(a_1,a_2,z) \frac{v(a_1,a_2)}{\bar{v}} da_1da_2 \tag{A7}
\]

Let \( S(z_1,z_2) \) be the surface of a segment of capillary cylinder between \( z_1 \) and \( z_2 \); then the expected value of molecules transported into an endothelial cell in unit of time by a carrier randomly positioned in \( S(z_1,z_2) \) is as follows (expressed as 1/min):

\[
\mathbb{E}[N_i(z_1,z_2)] = \int_{S(z_1,z_2)} \int_{S(z_1,z_2)} \frac{1}{m_T} \cdot \bar{v} \cdot \bar{k}_f(z) dS(x,y,z) \tag{A8}
\]

If \( n_s \) is the number of carriers per unit length of capillary (1/cm), then the number of carrier units in \( S(z_1,z_2) \) is \( n_s \cdot (z_2 - z_1) \), and the number of transports performed in this segment is \( n_s \cdot (z_2 - z_1) \cdot \mathbb{E}[N_i(z_1,z_2)] \).
The amount of FA transported across the capillary wall in segment S(z1,z2) is equal to the losses of FA in plasma in this segment:

\[ f_c \bar{k}(z) - f_c \bar{k}(z) = -n_u \cdot (z_2-z_1) \cdot \bar{E}[N(z_1,z_2)] \]  
(A9)

where \( f_c \) is the capillary plasma flow.

The limit \( (z_2-z_1) \rightarrow 0 \) gives the following differential equation for \( \bar{k}(z) \):

\[ f_c \frac{d\bar{k}(z)}{dz} = -n_u \cdot \frac{1}{m_T} - \frac{cf}{\bar{k}(z)} \]  
(A10)

or expressed in biochemical terms:

\[ f_c \frac{d\bar{c}(z)}{dz} = -n_u \cdot \frac{l}{m_T \cdot N_{AV}} - \frac{cf \cdot \bar{c}(z)}{\tau} \]  
(A11)

where \( \bar{c} = k/N_{AV} \) is the plasma FA concentration, \( N_{AV} \) is Avogadro’s number, \( l \) is average capillary length, and \( \tau \) is unit of time.

Equation A11 leads to the implicit equation:

\[ \frac{\bar{c}(z) - \bar{c}(z_0)}{\bar{c}(z_0)} = \frac{\tau (m_T \cdot N_{AV})}{f_c \bar{c}(z_0)} \ln \frac{\bar{c}(z)}{\bar{c}(z_0)} + \frac{[n_u/(m_T \cdot N_{AV})] \cdot (z-z_0)}{f_c \bar{c}(z_0)} = 1 \]  
(A12)

After multiplying this equation with the number of capillaries per gram of tissue, the following is obtained for \( \bar{c}(z) = \bar{c}(z_0) = \bar{c}_c \):

\[ f_c \cdot (\bar{c}_a \cdot \bar{c}_v) = n_u / (m_T \cdot N_{AV}) \cdot \ln (\bar{c}_i / \bar{c}_a) + n_u \cdot l / (m_T \cdot N_{AV}) \]  
(A13)

where \( \bar{c}(z_0) = \bar{c}_a \) and \( \bar{c}_v \) are the arterial and venous FFA plasma concentrations, respectively, \( f = n_c \cdot f_c \) is the plasma flow per gram of tissue, and \( (z_0-z_0) = l \) is the average length of the capillary.

Appendix 2

In classical biochemical reactions (homogeneously mixed solutions), the collisions are caused by brownian molecular movement, and the mean number of collisions of one enzyme (carrier) molecule with the substrate molecule is proportional to both the substrate and enzyme concentration; that is, the mean number of collisions is proportional to the product of these concentrations. This relation is known as the mass action law. This principle can be applied to FA transport across the capillary wall in the case that an unstirred layer exists near the capillary wall or to FA transport from stationary interstitial fluid into the myocardial cell. In this case, the mean number of FA collisions with one carrier is proportional to the local FA concentration. Therefore, the mean time between two collisions is proportional to the reciprocal concentration of FA, and this will also be valid for the mean waiting time. So we get \( m_u = K/k_f \) and the expected value of molecules transported in capillary segment \( dz \) in unit of time is as follows (expressed as \( 1/min \)):

\[ \bar{E}[N(z)] \cdot n_u \cdot \frac{dz}{K + k_f} \]

This relation corresponds to the standard Michaelis-Menten relation.32

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