Significance of myoglobin as an oxygen store and oxygen transporter in the intermittently perfused human heart: a model study

Volker Endeward, Gerolf Gros*, and Klaus D. Jürgens

Zentrum Physiologie, Vegetative Physiologie-4220, Medizinische Hochschule Hannover, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

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Aims
The mechanisms by which the left ventricular wall escapes anoxia during the systolic phase of low blood perfusion are investigated, especially the role of myoglobin (Mb), which can (i) store oxygen and (ii) facilitate intracellular oxygen transport. The quantitative role of these two Mb functions is studied in the maximally working human heart.

Methods and results
Because discrimination between Mb functions has not been achieved experimentally, we use a Krogh cylinder model here. At a heart rate of 200 beats/min and a 1:1 ratio of diastole/systole, the systole lasts for 150 ms. The basic model assumption is that, with mobile Mb, the oxygen stored in the end-diastolic left ventricle wall exactly meets the demand during the 150 ms of systolic cessation of blood flow. The coronary blood flow necessary to achieve this agrees with literature data. By considering Mb immobile or setting its concentration to zero, respectively, we find that, depending on Mb concentration, Mb-facilitated O2 transport maintains O2 supply to the left ventricle wall during 22–34 of the 150 ms, while Mb storage function accounts for a further 12–17 ms. When Mb is completely absent, anoxia begins to develop after 116–99 ms.

Conclusion
While Mb plays no significant role during diastole, it supplies O2 to the left ventricular wall for \( \leq 50 \) ms of the 150 ms systole, whereas capillary haemoglobin is responsible for approximately 80 ms. Slight increases in haemoglobin concentration, blood flow, or capillary density can compensate the absence of Mb, a finding which agrees well with the observations using Mb knockout mice.

Keywords
Myoglobin diffusion coefficient • Oxygen transport • Facilitated oxygen diffusion • Krogh cylinder • Haemoglobin

1. Introduction
Heart and skeletal muscle myoglobin (Mb) can serve as an oxygen store during times of restricted blood perfusion or apnoea phases. It can also enhance intracellular oxygen transport and, therefore, increase tissue oxygen conductance by loading oxygen at the capillary and diffusing to the mitochondria where it releases the oxygen, a mechanism called facilitated oxygen diffusion. It is still a matter of discussion as to what extent these functions contribute to the oxygen supply of red muscle tissues.1–6 Studies with Mb knockout mice proved that Mb is not essential for living. Nevertheless, compensatory adaptations of oxygen supply mechanisms have been demonstrated in their hearts, indicating that Mb does play a certain role during development7 and in later life.8

Numerous experimental attempts have been made to quantify this role, but they exhibit various limitations. Reagents blocking oxygen binding to Mb inhibit both storage function and facilitated diffusion and thus do not allow a quantitative distinction to be made between the two. More importantly, many blocking reagents are toxic to metabolism, causing defects in energy supply to tissue.2 Cryospectrophotometric measurements of intracellular Mb oxygen saturation (Mb-SO2) gradients turned out to be unreliable because the spatial resolution of the experimental setup had been greatly overestimated.9 Measurements of tissue Mb-SO2 by nuclear magnetic resonance (NMR) techniques have the disadvantage that they cannot measure local values but average over large tissue volumes, from which no reliable oxygen partial pressures (PO2s) can be calculated because of the non-linear shape of the Mb-oxygen binding curve.2
Significance of myoglobin in the beating heart

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earlier studies we have determined by using three different exper-

Krogh cylinder model to skeletal and heart muscle tissue. 2 The

cal, metabolic, and biochemical data, we have previously applied the

of model calculations. Based on experimentally obtained morphologi-

port and storage function of Mb.

role of Mb but omit the important function of capillary haemoglobin

as an oxygen store during restricted perfusion and—as all of the

above experimental approaches—cannot distinguish between trans-

port and storage function of Mb.

An alternative approach to evaluate the role of tissue Mb is the use

of model calculations. Based on experimentally obtained morphologi-

cal, metabolic, and biochemical data, we have previously applied the

Krogh cylinder model to skeletal and heart muscle tissue. The

Krogh cylinder is a simple model of O2 supply to the tissue originally

introduced by August Krogh,11 which considers the tissue to be com-

posed of cylinders, with each cylinder being supplied with O2 from a

capillary running along its centre. A crucial parameter in these calcu-

lations is the intracellular diffusion coefficient of Mb (Dmb), which in

earlier studies we have determined by using three different exper-

imental approaches and with all of them found it to be approximately

2 × 10^-7 cm^2/s in heart and skeletal muscle at 37°C.12–15 Previous

model studies of continuously perfused skeletal muscle led to the

conclusion that the role of Mb-facilitated oxygen diffusion is minor,

even at maximal oxygen consumption, VO2 of the tissue.2,12 Only at

the periphery of the venous end of the Krogh cylinder, an appreciable

oxygen desaturation of Mb is seen, while in the bulk of the cylinder

there is no significant radial Mb-SO2 gradient and thus no significant

facilitated oxygen diffusion occurs.

Deviant from the model assumptions used in these previous

studies, coronary perfusion is subject to marked fluctuation. In the

left ventricle myocardial blood flow, Q, is drastically reduced during

systolic contraction, and especially in the subendocardial region, Q

falls to very low values or even zero because blood vessels are

occluded by the intraventricular systolic pressure.16

Therefore, in this study we investigate how long the amount of

oxygen stored in the muscle tissue at the end of diastolic perfusion

can prevent the occurrence of anoxic tissue regions during the follow-

ing systolic cessation of Q. Because this will be most critical at very

high myocardial VO2, we use model parameters representing

maximal cardiac work. A probably unique advantage of this model

is that the role of oxygen storage and of Mb-facilitated oxygen diffu-

sion can be quantified separately. In addition, we can estimate how

large the changes of other tissue parameters like capillary density

(CD), haematocrit (hct), or Q must be to compensate the lack of

Mb-facilitated O2 diffusion and/or Mb-O2 storage. The necessary

changes of these parameters can then be compared with those

reported for Mb knockout mice. The study sheds light on the mech-

anisms that serve the left ventricular wall to survive the systolic inter-

val without anoxia.

2. Methods

For maximally working human heart muscle, we calculated the local distri-

bution of Mb-SO2, PO2, as well as the fractional contribution of

Mb-facilitated oxygen diffusion to total oxygen diffusion (IO2fac).

We used the modified Krogh cylinder model developed by Groebe17

that consists of three concentric cylinders—the inner one representing

the interior of the capillary, the middle one extending from the surface

of the red blood cell to the sarcolemma (the ‘carrier-free’ space), and

the outer one representing the Mb-containing muscle tissue. Only radial

oxygen diffusion is considered. The model takes into account a discrete

distribution of red cells within the central capillary. For further details

of the calculations see Supplementary material online.

As model parameters we used normal values at 37°C for oxygen

half-saturation pressure of blood (P50Hb = 27 mmHg), Hill’s n of the blood-

oxygen binding curve (nH = 2.8), hct (=45%), and intraerythrocytic

haemoglobin concentration (MCHC = 5 mmol/L). Arterial PO2 was

assumed to be 94 mmHg, leading to an arterial haemoglobin-SO2 of 97%.

A capillary radius of 2 μm was used. The Krogh cylinder radius (Rc)

was set to 10 μm, corresponding to a human left ventricle CD of

2680 mm^-2, which is in the range of data found in the literature.18,19 As

a fraction of the capillary volume filled with red cells (fractional length

of red blood cell column within the capillary, lmb) a value of 0.625 was inserted.

The width of the carrier-free region17 was set to 1.5 μm. The other values

used are: oxygen half-saturation pressure of Mb (P50mb = 2.8 mmHg),20

cytosplasmic Mb concentration (Cmb = 190 μmol/L),21 diffusion coefficient

of Mb (Dmb = 2 × 10^-7 cm^2/s),15 Krogh’s O2 diffusion constant in muscle

tissue (Ko2m = 1.3 × 10^-9 mmol/cm/min/mmHg),22 Krogh’s O2 diffusion

constant in water (Ko2w = 2.6 × 10^-9 mmol/cm/min/mmHg),23 and O2

solubility in muscle tissue (αO2 = 1.5 × 10^-6 mmol/mL/mmHg, the mean

of two data sets).24,25

VO2 of human left ventricle at rest26 amounts to 85–120 mL/min/kg.

This value can increase approximately five- to six-fold during exercise,

as can be estimated from the increase of the heart rate–blood pressure

product (RPP). For our calculation, therefore, a value of 600 mL/min/kg

was chosen for the maximally working left ventricle.

The myocardial Q was allowed to be adjusted to the value required to fulfil the criterion

that the amount of oxygen stored in the tissue at the end of the diastole

is sufficient to last for 150 ms of systolic interruption of blood flow (time to beginning of anoxia, t),

such that no anoxic region occurs in the Krogh cylinder at any time. The time 150 ms is equivalent to the duration of a

systole in a human heart beating at 200 beats/min, assuming a relation

of diastole to systole of 1:1.27 It turned out that under this premise the

appropriate value for the mass-specific diastolic blood flow (Q) amounts to 4400–4700 mL/min/kg.

This is a very reasonable physiological range for maximally perfused human heart muscle: in humans mean

resting myocardial Q18,25 amounts to 800–1000 mL/min/kg, leading to 4000–5000 mL/min/kg under a five-fold increase during maximal

work.30 We conclude that heavily working human heart muscle is well

represented by our model with regard to all relevant functional

parameters.

3. Results

3.1 Spatial distribution of PO2, Mb-SO2, and contribution of facilitated diffusion

For the condition of continuous diastolic perfusion during maximal

work, we calculate an end-capillary PO2 of 16.9 mmHg, a mean tissue

PO2 of 24.5 mmHg, and a mean Mb-SO2 of 85% (Table 1). As

depicted in Figure 1A, in a large part of the volume of the Krogh cylin-

der Mb is almost completely saturated with oxygen because the PO2

by far exceeds the P50mb of 2.8 mmHg. Only at the venous end of the


cylinder a marked drop is seen, and only here appreciable Mb-O2 gra-

dients occur that lead to Mb-facilitated oxygen diffusion. The latter’s

mean contribution to total oxygen transport in the cylinder amounts to only 1.3%, but even at the venous end, where the capillary

PO2 has dropped to 16.9 mmHg, this contribution does not exceed

5.1%.

 Interruption of Q in the left ventricle wall during systole leads to a

continuous decrease of PO2 in the whole cylinder. As chosen in this

model, it takes 150 ms, the duration of the systole, before the PO2

Downloaded from https://academic.oup.com/cardiovascres/article-abstract/87/1/22/333052 by guest on 10 September 2018
Table 1 Oxygen supply parameters during continuous diastolic perfusion of the left ventricle

<table>
<thead>
<tr>
<th>End-capillary PO₂ (mmHg)</th>
<th>Mean muscle tissue PO₂ (mmHg)</th>
<th>Mean myoglobin saturation (%)</th>
<th>Mean contribution of facilitated O₂ diffusion fO₂fac (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.9</td>
<td>24.5</td>
<td>85.0</td>
<td>1.3</td>
</tr>
</tbody>
</table>

V = 600 mL/(min kg); Q = 4395 mL/(min kg); R = 10 μm; PaO₂ = 94 mmHg

Figure 1 The spatial distribution of the oxygen partial pressure PO₂ (upper panel), myoglobin oxygen saturation (Mb-SO₂, middle panel), and fraction of myoglobin-facilitated oxygen diffusion fO₂fac (lower panel) within the Krogh cylinder is shown. The capillary length is normalized and given in percentages. (A) Distribution during continuous diastolic blood flow; (B) distribution after systolic cessation of blood flow, just before anoxic regions start to develop.
reaches 0 at the outer surface of the venous end of the cylinder. The end-capillary PO₂ prevailing at the end of systole is calculated in such a way that a PO₂ of 0 mmHg at the periphery of the venous end of the cylinder is reached at the moment when 150 ms have elapsed.

As shown in Table 2 and illustrated in Figure 1B, even at the end of the systolic interruption of blood flow, the mean Mb-SO₂ of the muscle tissue still amounts to 77.5%, the mean PO₂ to 15.7 mmHg. Under these conditions, the mean overall contribution of Mb-facilitated O₂ diffusion amounts to 3.5%. At the venous end, the capillary PO₂ has dropped to 10.5 mmHg, and here the facilitated O₂ diffusion reaches a maximal contribution at the outer circumference of the cylinder of 26.3% of total O₂ diffusion.

Table 2 shows that preventing Mb diffusion by setting $D_{Mb} = 0$ reduces the time $t_a$ to the beginning of anoxia at the periphery of the venous end of the cylinder by 22 ms to 128 ms (diastolic Q unchanged). This reflects the contribution of Mb-facilitated O₂ diffusion. In this case, a larger PO₂ drop across $R_C$ is required because by the loss of facilitated diffusion, oxygen conductance of the cylinder is decreased. The calculated end-capillary PO₂ at the beginning of anoxia then amounts to 11.7 mmHg and the mean cylinder SO₂ is slightly increased to a value of 78.7%.

Removing Mb from the tissue by setting $C_{Mb} = 0$ further reduces $t_a$ by another 12 ms to 116 ms. This decrease in $t_a$ is because of the loss of the Mb oxygen store. End-capillary PO₂ and mean muscle PO₂ are not affected by this step because tissue O₂ conductance is identical whether Mb is immobile or whether it is completely absent.

We conclude that, in the absence of Mb-facilitated O₂ diffusion but in the presence of the Mb oxygen store, part of the heart muscle tissue will become anoxic during 15% of the systole. Removing in addition the Mb oxygen store reduces $t_a$ by a further 8% and then during 23% of the systole part of the tissue becomes anoxic. Intracapillary haemoglobin contributes significantly more, namely approximately 55%, and the dissolved O₂ of the entire tissue including blood contributes the remainder 22%.

### 3.1.1 Effect of reduced capillary volume during systole

It has been reported that during systole not only is Q greatly reduced, but in addition the volume of the capillary bed of the ventricular wall may be reduced by approximately 40% because of the elevated intramyocardial pressure. This implies that the important O₂ reservoir associated with intracapillary red cells may be smaller than that assumed in our standard model calculation as described in Table 2 and the uppermost part of Table 3. We have simulated this by using a normal hct of 45% for modelling the diastolic phase, but reducing the hct to 27% during the systolic phase. The result is given in the second section of Table 3, which shows that, in order to maintain $t_a$ at 150 ms, Q must rise by 10% from 4400 to 4860 mL/min/kg, a number still in the range of expected Q values (see Methods). The effects of eliminating first Mb diffusion and then Mb concentration are roughly equivalent to what we obtain for standard conditions (first section of Table 3), although the overall contribution of haemoglobin to $t_a$ decreases moderately from 55% to 50%. The relative contributions of Mb–O₂ diffusion and of Mb storage to $t_a$ are about 1:1, while being approximately 2:1 in the standard condition.

### 3.1.2 Effect of higher sarcoplasmic Mb concentration

In the third section of Table 3 we repeat the calculation of the first section for an Mb concentration of 300 μM. This value has been estimated in the dry tissue of a less representative part of cardiac tissue, human heart papillary muscle. Similar values have been obtained by an NMR technique and have been postulated from other data by considering that almost 40% of the intracellular space may be taken up by mitochondria and sarcoplasmic reticulum, thus increasing the effective sarcoplasmic Mb concentration. With $[Mb] = 300 \mu M$, the necessary Q is slightly reduced from 4400 to 4290 mL/min/kg, illustrating the economizing effect of Mb. In the absence of facilitated diffusion, the time to beginning of anoxia is reduced from 150 ms to 116 ms ($-34 \text{ ms}, -23\%$), and by additionally abolishing the storage function $t_a$ is further reduced to 99 ms ($-17 \text{ ms}, -11\%$). In comparison with the uppermost part of Table 3, the contributions of both processes to $t_a$ facilitated oxygen diffusion and oxygen storage, increase approximately linearly with increasing Mb concentration and again show a relation of approximately 2:1. The relative contribution of Hb falls only slightly, the greatest reduction being seen in that of dissolved O₂.

The lowest section of Table 3 shows the results for a reduced systolic capillary volume at $[Mb] = 300 \mu M$. One obtains rather similar effects on $t_a$ by abolishing facilitated O₂ diffusion ($t_a = 127 \text{ ms}$) and by removing Mb entirely ($t_a = 102 \text{ ms}$) as seen in the first section of the table. The value of Q required to model this condition, 4675 mL/min/kg is higher than that seen with unaltered systolic hcts (first and third sections of Table 3), but is somewhat lower than with $[Mb] = 190 \mu M$ and systolic reduction of hct (second section), which again illustrates the economizing effect of Mb on the heart.

### 3.2 Studies of compensatory mechanisms

Here we study how anoxic intervals during systole can be avoided by compensatory changes of morphological and functional parameters. An increase in CD reduces $R_C$, an increase of hct enhances the

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**Table 2 Oxygen supply parameters after systolic interruption of coronary perfusion**

<table>
<thead>
<tr>
<th></th>
<th>End-capillary PO₂ (mmHg)*</th>
<th>PO₂ on arterial side of capillary</th>
<th>Mean muscle tissue PO₂ (mmHg)*</th>
<th>Mean myoglobin saturation (%)*</th>
<th>Time to beginning of anoxia, $t_a$ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D_{Mb}$ = 0</td>
<td>10.5</td>
<td>47.1</td>
<td>15.7</td>
<td>77.5</td>
<td>150</td>
</tr>
<tr>
<td>$C_{Mb}$ = 190 μmol/L</td>
<td>11.7</td>
<td>49.8</td>
<td>16.5</td>
<td>78.7</td>
<td>128</td>
</tr>
<tr>
<td>$D_{Mb}$ = 0</td>
<td>11.7</td>
<td>49.8</td>
<td>16.5</td>
<td>—</td>
<td>116</td>
</tr>
<tr>
<td>$C_{Mb}$ = 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- $V_C = 600 \text{ mL/(min kg)}$; $Q = 0 \text{ mL/(min kg)}$; $R_C = 10 \mu m$.
- *Results refer to the beginning of anoxia at the end of the systolic time interval indicated in the last column.
Table 3 Times to anoxia, $t_a$, and left ventricular perfusion rates, $Q$, under various conditions of $C_{Mb}$, $D_{Mb}$, and systolic hct, together with compensatory changes of the parameters $Q$, hct, and CD necessary to restore times to anoxia to 150 ms

<table>
<thead>
<tr>
<th></th>
<th>$t_a$ (ms)</th>
<th>$Q$ necessary to bring $t_a$ to 150 ms (mL/min/kg)</th>
<th>hct necessary to bring $t_a$ to 150 ms</th>
<th>CD needed to bring $t_a$ to 150 ms (mm$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$C_{Mb}$, 190 $\mu$M, $D_{Mb}$, $2 \times 10^{-7}$ cm$^2$/s</td>
<td>150</td>
<td>4400</td>
<td>0.450</td>
</tr>
<tr>
<td></td>
<td>$C_{Mb}$, 190 $\mu$M, $D_{Mb}$ = 0</td>
<td>128</td>
<td>4530 (+3.0%)</td>
<td>0.461 (+2.4%)</td>
</tr>
<tr>
<td></td>
<td>$C_{Mb}$ = 0, $D_{Mb}$ = 0</td>
<td>116</td>
<td>4630 (+5.2%)</td>
<td>0.469 (+4.2%)</td>
</tr>
<tr>
<td>2</td>
<td>$C_{Mb}$, 190 $\mu$M, $D_{Mb}$, $2 \times 10^{-7}$ cm$^2$/s hct (diastole → systole) 0.45 → 0.27</td>
<td>150</td>
<td>4860</td>
<td>0.450</td>
</tr>
<tr>
<td></td>
<td>$C_{Mb}$, 190 $\mu$M, $D_{Mb}$ = 0 hct (diastole → systole) 0.45 → 0.27</td>
<td>135</td>
<td>5025 (+3.4%)</td>
<td>0.462 (+2.7%)</td>
</tr>
<tr>
<td></td>
<td>$C_{Mb}$ = 0, $D_{Mb}$ = 0 hct (diastole → systole) 0.45 → 0.27</td>
<td>118</td>
<td>5300 (+9.1%)</td>
<td>0.480 (+6.7%)</td>
</tr>
<tr>
<td>3</td>
<td>$C_{Mb}$, 300 $\mu$M, $D_{Mb}$, $2 \times 10^{-7}$ cm$^2$/s</td>
<td>150</td>
<td>4290</td>
<td>0.450</td>
</tr>
<tr>
<td></td>
<td>$C_{Mb}$, 300 $\mu$M, $D_{Mb}$ = 0</td>
<td>116</td>
<td>4475 (+4.3%)</td>
<td>0.467 (+3.8%)</td>
</tr>
<tr>
<td></td>
<td>$C_{Mb}$ = 0, $D_{Mb}$ = 0</td>
<td>99</td>
<td>4630 (+7.9%)</td>
<td>0.479 (+6.4%)</td>
</tr>
<tr>
<td>4</td>
<td>$C_{Mb}$, 300 $\mu$M, $D_{Mb}$, $2 \times 10^{-7}$ cm$^2$/s hct (diastole → systole) 0.45 → 0.27</td>
<td>150</td>
<td>4675</td>
<td>0.450</td>
</tr>
<tr>
<td></td>
<td>$C_{Mb}$, 300 $\mu$M, $D_{Mb}$ = 0 hct (diastole → systole) 0.45 → 0.27</td>
<td>127</td>
<td>4910 (+5.0%)</td>
<td>0.468 (+4.0%)</td>
</tr>
<tr>
<td></td>
<td>$C_{Mb}$ = 0, $D_{Mb}$ = 0 hct (diastole → systole) 0.45 → 0.27</td>
<td>102</td>
<td>5300 (+13%)</td>
<td>0.495 (+10%)</td>
</tr>
</tbody>
</table>

Q, coronary perfusion of left ventricle; hct, blood haematocrit; CD, capillary density of left ventricle wall. Considered are cardiac Mb concentrations of 190 $\mu$M (sections 1 and 2) and of 300 $\mu$M (sections 3 and 4). Sections 2 and 4 of the table illustrate the situation, when during systole not only $Q$ is reduced to zero, but in addition 40% of the red cells are forced out of the capillary bed owing to compression of the left ventricle wall. This reduction of red cells present in the capillary bed is expressed in the model in terms of reduction of the hct by 40%.

Table 3 shows that relatively slight changes of these parameters are sufficient to avoid systolic anoxia. With $C_{Mb}$ = 190 $\mu$M, after the elimination of facilitated diffusion, $t_a$ is restored to 150 ms by a reduction of $R_C$ from 10 to 9.6 $\mu$m, equivalent to an 8.6% increase in CD. In the absence of both facilitated $O_2$ diffusion and Mb oxygen storage, reduction of $R_C$ to 9.3 $\mu$m or a 15.7% increase in CD is necessary for compensation. The same effects are achieved by increasing the hct by 2.4% and 4.2%, respectively, or the diastolic Q by 3 and 5.2%, respectively.

Relative compensatory changes in all the three parameters: Q (+13%), hct (+10%), and CD (+40%) among those seen in Table 3. It should be noted that this latter condition is well in the range of realistically possible physiological situations.

4. Discussion

4.1 Reliability of the Krogh model and physiological significance of myoglobin

The Krogh cylinder model leads to results that are in good agreement with data measured in a heavily working myocardium. The end-capillary blood $PO_2$ (11–17 mmHg) as well as the arterio-venous differences of blood oxygen content (74–76% of blood oxygen capacity) are in agreement with literature data on maximally working heart muscle. The calculated high mean tissue Mb-SO$_2$ of 78% (systole, left ventricle) to 85% (diastole) is in accordance with the results obtained by NMR measurements of heavily working rat heart (76%) by Jelicks and Wittenberg as well as dog hearts (80%) by Bache et al. As mentioned before, there are no reliable literature data on local or mean $PO_2$ values of working muscles, and it is not correct to calculate a mean $PO_2$ from a mean Mb-SO$_2$ with the Hill equation because of the pronounced nonlinear shape of the Mb-oxygen binding curve.

Previous Krogh cylinder calculations, which were based on continuous perfusion of a working muscle, yielded a very minor contribution of Mb-facilitated oxygen transport. This is confirmed by the
present results for the diastolic phase of coronary perfusion. On an average, only 1.3% of total oxygen transport within heart muscle tissue is caused by diffusion of Mb. A negligible role of Mb for oxygen transport has also been suggested by Lin et al.,3 who even with a three- to four-fold higher $D_{\text{Mb}}$ (calculated from them by NMR measurements) than measured by us,12–15 estimated no significant contribution of Mb-facilitated oxygen diffusion in rat heart muscle.

The picture drastically changes when systolic interruption of blood flow is considered. Despite a low Mb diffusivity, the Mb of human heart ventricle has a significant effect on $O_2$ supply of the muscle tissue during systolic cessation of blood flow in the left coronary artery. During approximately 10–20% of the systole (Table 3), anoxic regions will develop if the oxygen conductance is reduced by immobilization of Mb. In addition, the storage function of Mb contributes significantly to avoid anoxia. The release of oxygen from immobile Mb ensures an oxygen supply sufficient for another 8–17% of the systole (Table 3). Thus, the presence of Mb in heart muscle tissue renders possible a 21–34% longer period of systolic cessation of blood flow and, therefore, prevents a substantial loss of mechanical tension in the contracting myocardium. Most of the residual time of the 150 ms systolic interval remains free of anoxia because of the presence of oxyhaemoglobin in the capillaries. Table 3 shows that, independently of the details of the model parameters, Mb is responsible for $O_2$ supply of not more than 50 ms of the 150 ms systolic period.

It has been shown39 that the subendocardial Mb concentration in the human heart is 10% higher than in the subepicardial muscle tissue, i.e. it is higher in that tissue layer in which reduction of $Q$ and reduction of intracapillary red cell content presumably are most pronounced. As illustrated in the third section of Table 3, this should help avoiding anoxia in this critical zone. Inversely, as illustrated here by the calculations with $C_{\text{Mb}} = 0$, the beneficial effect of Mb on $O_2$ supply will be lost, when dramatic losses of Mb from the myocardium occur as observed in various conditions of heart failure.40–42 An event expected to further impair the condition of these hearts.

4.2 Role of the intracellular myoglobin diffusion coefficient

In a sensitivity analysis for the model parameters given in Supplementary material online, Table S4, it is apparent that, compared with other model parameters, the results are not extremely sensitive to changes in Mb properties like $P_{50\text{Mb}}, D_{\text{Mb}}$, and $C_{\text{Mb}}$. This agrees with the results of Tables 2 and 3 and is because of the fact that under all conditions studied, a release of oxygen from Mb takes place only in a small volume fraction of the Krogh cylinder, because in most of the cylinder Mb-SO$_2$ is high owing to the low $P_{50\text{Mb}}$. However, applying a substantially larger $D_{\text{Mb}}$, like the approximately four times higher value of $7.5 \times 10^{-7}$ cm$^2$/s (after conversion to 37°C using a $Q_{10}$ of 1.46) from the recent studies of Lin et al.,3,4 changes the picture because facilitated $O_2$ diffusion acquires greater importance. Therefore, $D_{\text{Mb}}$ is a very crucial parameter inspite of the data of Supplementary material online, Table S4, and deserves a special discussion in view of the controversy about its value. If the model is used to generate a time to beginning of anoxia of 150 ms for the higher Mb diffusivity of Lin et al.,3,4 (the necessary $Q$ then reduces to $4205 \text{ mL/kg/min}$), the immobilization of Mb results in a considerably greater anoxic interval lasting for 56 ms, i.e. 37% of the systole. Removing Mb completely, however, prolongs only the anoxic interval only by another 9 ms, or 6%, respectively. Under this condition, Mb-facilitated $O_2$ diffusion appears to be six times more important than Mb storage function, although the overall role of Mb during systole is only moderately increased and accounts for $O_2$ supply during 65 out of 150 ms. In other words, with the high $D_{\text{Mb}}$ of Lin et al.,3,4 it is mainly the role of Mb-facilitated $O_2$ diffusion that is increased during systole, while the overall contribution of Mb is enhanced only to a limited extent. During diastole, the role of Mb-facilitated $O_2$ diffusion remains minor because of the absence of significant MbO$_2$ gradients in the tissue.

The $D_{\text{Mb}}$ obtained by NMR3,4 is close to the self-diffusion coefficient of $7.6 \times 10^{-7}$ cm$^2$/s (converted to 37°C using a $Q_{10}$ of 1.31) measured by Riveros-Moreno and Wittenberg41 in a 20 g% Mb solution, a protein concentration that is lower than the intracellular (soluble plus structural) protein concentration of approximately 24 g%.42 It does not appear conceivable that Mb diffuses as easily as it does in a 20 g% Mb solution in an intracellular compartment, which contains numerous structural diffusion obstacles and whose total protein concentration is even higher than 20 g%. The conclusion of Lin et al.,3,4 that cell architecture does not impose tortuosity on the translational diffusion path of Mb contradicts many other results on intracellular protein diffusion.12–15,42–44 It has been shown by us with three different methods and for a variety of differently sized proteins12–15 that diffusivity within muscle cells is one-half to one-tenth of the diffusivity found even in a 24 g% protein solution, a concentration equivalent to the total protein concentration of muscle. Obviously, $D_{\text{Mb}}$ derived by pulsed-field gradient NMR from root mean-square molecular displacement, suggested to range between maximally 2.5 and 3.5 μm, is markedly higher than the translational diffusion coefficient measured by tracking the diffusion path of intracellularly generated or injected met-Mb over distances between 10 μm15 and up to 1000 μm12–14,43 within the muscle cell. All measurements of $D_{\text{Mb}}$ over diffusion distances between 10 and 1000 μm yield almost identical values of $2 \times 10^{-7}$ cm$^2$/s, when considered at 37°C. The $D_{\text{Mb}}$ of Lin et al.,3,4 may therefore reflect a local or short range Mb diffusivity, determined mainly by the viscosity of the microenvironment. This view is confirmed by the observation of Lin et al.,4 that the effect of the intracellular environment as observed by NMR is identical on the translational diffusion coefficient and the rotational diffusion coefficient of Mb. The latter is expected to be determined more or less exclusively by the microenvironment and much less by structural obstacles. It has indeed been reported by various groups45–47 that rotational diffusion of macromolecules is much less reduced by high protein concentration or by an intracellular environment than is translational diffusion of the same molecules. This view of a short-range diffusion coefficient being observed by NMR would be compatible with the NMR measurements of Budhiraja et al.,48 who report a lower dependence on protein concentration of the self-diffusion coefficients of Mb, human haemoglobin, and earthworm haemoglobin than has been observed in classical diffusion measurements by Riveros-Moreno and Wittenberg41 and Gros.49 Taken all this together, it appears that macromolecular diffusivity as measured by NMR depends less on widely spaced obstacles and represents a short-range diffusivity, while the various classical diffusion techniques all yield long-range diffusivities as they need to be considered for physiological processes such as Mb-facilitated $O_2$ diffusion.12–15,41–43,49 The present calculations indicate that $O_2$ transport in heart tissue has to overcome a diffusion distance of
about 10 μm in the optimal situation in which all capillaries are perfused.

If indeed there is a significant increase in D02, upon reducing the observed diffusion distance from 10 μm to approximately 3 μm, this has interesting implications for the nature of the main diffusion obstacles for Mb within the muscle cell. It indicates that the decisive obstacles occur at distances of between 3 and 10μm, which would eliminate the myofilaments. Z disks and M lines are also unlikely, because Mb diffusion is equally reduced in radial and longitudinal direction.6,13 This leads to the conclusion that sarcoplasmic reticulum, t-tubules, and mitochondria represent the major obstacles for Mb diffusion in muscle cells, because their spacing is in that order of magnitude. Such an interpretation agrees with a recent theoretical study by Novak et al.,50 in which diffusion of macromolecules in a simulated intracellular space is studied and intracellular membranes are found to be more important diffusion obstacles than cytoskeletal filaments.

4.3 Comparison with Mb knockout animals

Much more than by properties of Mb, the time to beginning of anoxia is influenced by the properties of haemoglobin (mainly its concentration, additionally the position of the blood oxygen-binding curve), Q, oxygen diffusion distance, and Vo2 (Tables 2 and 3; Supplementary material online, Table S4). These parameters largely determine oxygen supply and, therefore, the PO2 gradient between capillary and tissue. Haemoglobin offers a greater advantage for O2 supply under ischaemia than Mb owing to two features: its monomeric amount per Krogh cylinder or unit volume of tissue is approximately twice as large as the amount of Mb (at least during diastole) and, secondly, its lower oxygen affinity improves the availability of oxygen far more than Mb does. Minor adaptations in the variables hct (+4.2 to +10%) or Q (+5.2 to +13%) can completely compensate for the absence of Mb. The same can also be achieved by a slight structural change in the tissue, an increase of the CD by 16–40%, causing a reduction in Mb. The same can also be achieved by a slight structural change in the tissue, an increase of the CD by 16–40%, causing a reduction in Mb. The same can also be achieved by a slight structural change in the tissue, an increase of the CD by 16–40%, causing a reduction in Mb.

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Supplementary material

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