Heterogeneity of local myocardial flow and oxidative metabolism

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Schwanke, Uwe, Andreas Deussen, Gerd Heusch, and Jochen D. Schipke. Heterogeneity of local myocardial flow and oxidative metabolism. Am J Physiol Heart Circ Physiol 279: H1029–H1035, 2000.—In mammalian hearts, local myocardial flow (LMF) varies between 20 and 200% of the mean. It is not clear whether oxidative metabolism has a similar degree of heterogeneity. Therefore, we investigated the relation between LMF and local oxidative metabolism in isolated rabbit hearts. Buffer oxygenation with 18O2 resulted in labeled myocardial oxidation water (H218O). In four hearts, myocardial oxygen consumption (MV02) was calculated from the H218O production and compared with that calculated according to Fick. In eight additional hearts, LMF was measured using microspheres. Coronary venous H218O kinetics and local H218O residues were determined and analyzed by mathematical modeling. MV02 recovery from H218O was >93% compared with that according to Fick. LMF ranged from 1.91 to 11.24 ml·min−1·g−1, and local H218O residue ranged from 0.41 to 1.04 μmol/g. Both variables correlated (r = 0.62, n = 64, P < 0.001). Measurements in nine hearts were fitted by modeling using capillary permeability-surface area products (PS) from 2 to 10 ml·min−1·g−1. With flow-proportional PS, a 3.33-fold difference in LMF was associated with a 6.45-fold difference in local MV02. Both LMF and local oxidative metabolism are spatially heterogeneous, and they correlate to one another.

The spatial distribution of local blood flow within mammalian myocardium is heterogeneous, with variations between 20% (low-flow areas) and 200% (high-flow areas) of the mean value (13, 22). Such perfusion patterns can remain stable for at least 15 min in isolated hearts (26).

Myocardial areas in which blood flow is <50% of mean flow cannot compensate for the low flow by increasing their oxygen extraction (13). On the other hand, mathematical modeling suggests that the oxygen concentration in capillaries in which flow is only 20% of the mean flow drops to zero after one-half the capillary length, assuming that cellular oxygen clearance is uniformly distributed (12). One would expect that such conditions would subject low-flow areas to hypoxia and, if prolonged, to necrosis. However, low-flow areas exhibit none of the typical signs of ischemia, such as increased lactate or cytosolic adenosine (33) or increased heat shock protein (HSP)-70 (27) concentrations. Hence, myocardial oxygenation in low-flow areas does not appear to be critical.

Previous studies have already studied the relation between local metabolism and local myocardial flow (LMF). In fact, extraction of iodophenylpentadecanoic acid correlated well with LMF (8, 18), and the local uptake of [3H]deoxyglucose in canine myocardium can only be explained quantitatively if it is assumed that the rate of phosphorylation of deoxyglucose is flow proportional (11).

On average, fatty acids and glucose each contribute ~30% to total myocardial oxidative metabolism under resting conditions (21). Their exact contributions, however, depend on the supply of substrate and the inotropic state, which makes it difficult to estimate local aerobic metabolism on the basis of local fatty acid or glucose uptake.

The most direct measure of local aerobic metabolism is the local rate of production of oxidation water. Because the rate of production is difficult to measure directly, the local residue of oxidation water may serve as a reasonable index of the local production rate. To test whether LMF and local tissue residue of oxidation water are correlated, we used a method of 18O labeling recently developed in our laboratory (31, 32). Because the local 18O-labeled water tissue residue depends on both intracellular production and simultaneous wash-out from the tissue, a mathematical model analysis (12) was used to account for these effects and to calculate the local rate of oxygen metabolism.

METHODS

Experiments on 12 isolated hearts from adult New Zealand White rabbits (age 4–8 mo, body mass 2,800–3,800 g) were performed in accordance with the animal welfare reg-

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ulations of the German federal authorities, which adhere to the guiding principles of the American Physiological Society. The hearts were connected to a temperature-controlled (38°C), modified Langendorff apparatus and were perfused with Krebs-Henseleit (KH) buffer. The KH composition was (in mM) 90 NaCl, 30 NaHCO3, 4 KCl, 1 Na2HPO4, 0.5 MgSO4, 2.5 CaCl2, 2.2 pyruvate, and 11.1 glucose. A servo-controlled roller pump (WM-505; Watson Marlow, Falmouth, UK) was used to adjust coronary perfusion pressure to 80 mmHg.

Equilibration of KH buffer was performed with 95% O2-5% CO2. In a second perfusion system, 500 ml of KH buffer were equilibrated with 95% N2-5% CO2 for the removal of residual oxygen. This buffer was then equilibrated with recirculating 18O2. The experimental setup permitted switching between the perfusion media during the experiment.

A latex balloon (HSE no. 12-14; Hugo Sachs Elektronik, March-Hugstetten, Germany) was inserted into the left ventricle via the mitral valve. The balloon was connected to an artificial systemic circuit that was separated from the perfusion circuit and contained two valves to mimic the aortic and the mitral valves, a windkessel, and an ultrasonic flow probe for assessing cardiac output. Left ventricular pressure (TC-500; Millar Instruments, Houston, TX), aortic pressure (Statham P23; Gould Nicool, Erlensee, Germany), and cardiac output (T-206; Transonic Systems, Ithaca, NY) were measured in the systemic circuit. Coronary arterial pressure (Statham P23; Gould Nicolet), coronary flow (T-206; Transonic Systems), and coronary arterial and venous oxygen partial pressures (MT1-AC15; L. Eschweiler, Kiel, Germany) were measured in the perfusion circuit.

To validate our 18O-labeling technique, global myocardial oxygen consumption [MV(\(\dot{O}_2\)) (ml min\(^{-1}\) g\(^{-1}\))] was calculated according to Fick’s principle as

\[
MV(\dot{O}_2) = \alpha_{O_2} \cdot \left(\frac{P_{A,O_2} - P_{V,O_2}}{760}\right) \cdot CF
\]

with \(\alpha_{O_2} = 0.024\) ml O2 ml\(^{-1}\) mmHg\(^{-1}\), where \(P_{A,O_2}\) and \(P_{V,O_2}\) are coronary arterial and venous oxygen partial pressures (mmHg), respectively, and CF is normalized coronary flow (ml min\(^{-1}\) g\(^{-1}\)). This value was compared with the sum of the total H218O tissue residues plus the coronary venous H218O discharge in four hearts, collected over a period of 240 s.

In eight additional hearts, red microspheres were used to measure LMF. After sonication (Sonorex RK156; Bandelin Electronic, Berlin, Germany) and vigorous shaking (Vibrofix VF1; Janke & Kunkel, Staufen, Germany), the spheres were injected into the perfusion line immediately above the aortic root.

**Experimental protocol.** After stabilization, baseline hemodynamics were assessed over 15 min. Four hearts were then perfused with 18O-equilibrated solution for 240 s. During 18O perfusion, coronary venous effluent was collected at 0, 10, 20, 30, 40, 50, 60, 120, 180, and 240 s.

In eight hearts, 120,000 red microspheres were injected after measurement of baseline hemodynamics to assess LMF concomitantly with local oxidative metabolism. The KH buffer equilibrated with 95% O2-5% CO2 was replaced by the 18O2-equilibrated buffer for 240 s, and in five of these eight experiments, samples were also taken from the coronary venous effluent at 0, 10, 20, 30, 40, 50, 60, 120, 180, and 240 s. These five hearts and the four hearts mentioned above were used for mathematical model analysis of global oxidative metabolism (n = 9 hearts). At the end of the protocol, all hearts were immersed in liquid nitrogen.

**Water extraction and processing.** This procedure has been described in detail previously (31, 32). The four hearts used for global MV(\(\dot{O}_2\)) assessment were lyophilized in toto. The extracted tissue water and coronary venous effluent were processed as mentioned below. For local flow/H218O analysis, the eight additional hearts were sliced into basal, median, and apical portions. The right ventricular free wall, septum, and left ventricular free wall were excised from these tissue slices. The basal, median, and apical portions of the left ventricular free wall were further cut into subendocardial and subepicardial layers. A total of eight tissue samples (100 ± 27 mg) was taken from each heart. The water was extracted from the tissue samples during lyophilization, and 7.5-μl aliquots were converted quantitatively to CO2 using the guanidine hydrochloride technique (15). The oxygen isotope ratio (18O/16O) within the CO2 samples was determined by mass spectrometry (type 251; Finnigan MAT, Bremen, Germany). The oxygen isotope ratios were converted from the standard mean ocean water (SMOW) values [% SMOW] (2, 10) to SI units and expressed as local H218O residue of cardiac tissue water per gram wet mass (μmol/g). LMF was measured by red microspheres as described previously (24) and is expressed in milliliters per minute per gram.

**Model analysis.** The overall configuration of the model (Fig. 1) is a simplification of that described in detail previously (12). In its present form, the model consists of a non-exchanging tube segment, arterial and venous vessels, and a tissue exchange unit arranged in series. The volume of the tube region representing the volume of the perfusion cannula was measured and kept constant for all simulations (0.30 ml/g). The volumes of the arterial (Vart) and venous (Vven) vessels representing nonexchanging arteries and veins were set to 0.03 and 0.07 ml/g, respectively, by using data from the...
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literature (4). The tissue exchange unit comprised an intracapillary and an extracapillary region arranged in a concentric fashion (4, 25). The volume of the intracapillary region \( V_c \) was assumed to be 0.07 ml/g, and that of the extracapillary region \( V_e \), which is a composite of endothelial, interstitial, and parenchymal cell regions, was set to 0.63 ml/g (17). Hence, the total tissue water space \( V_{tiss} \) amounted to 0.80 ml/g. Because no major differences between local volumes or ultrastructure of regions with high and low flow had been observed previously (17, 33), their \( V_{tiss} \) values were assumed to be identical. Production of \(^{18}\)O-labeled water was described by an extracapillary production term \( P_{e,i} \) in \( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1} \). In the present version of the model, \( P_e \) is the product of the extracapillary concentration of atomic oxygen \((C_e)\) and the extracapillary clearance rate \((C_{e}^{\text{c}})\); \( P_e = C_e^{\text{c}} \cdot G_{e} \).

The exchange of water between the capillary and the extracapillary regions was described by a permeability-surface area product \( (P_{S_e} \text{ in } \text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}) \), which is equivalent to an interregional clearance. The value of \( P_e \) was specified by setting the \( P_{S_e} \) for oxygen to a constant value of 20 \( \text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1} \) and adjusting the extracapillary clearance rate to give the desired \( P_e \) value. The flow \( (F_e \text{ in } \text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}) \) was set to the value determined by the local microsphere measurement. Because the experimentally determined arterial oxygen concentration and LMF are used, the model takes regional differences in oxygen appearance time into account. The concentration of \(^{18}\)O-labeled water in the capillary region can be summarized (4, 12) as

\[
V_c \cdot \partial C_c / \partial t = -F_e \cdot L \cdot \partial C_c / \partial x - [P_{S_e} \cdot (C_c - C_e)]
\]

and the concentration of \(^{18}\)O-water in the extracapillary region is given by

\[
V_e \cdot \partial C_e / \partial t = P_e - P_{S_e} \cdot (C_c - C_e)
\]

where \( \partial C_c / \partial x \) is the concentration change of \(^{18}\)O-labeled water along the length of the capillary, \( \partial C_e / \partial t \) is the concentration change as a function of time, and the subscripts c and e denote the capillary and extracapillary regions, respectively. \( L \) is an arbitrary capillary length, which is canceled by integration along the length of the capillary (6).

The total tissue residue \( (Q_{tiss}) \) is the sum of the residues in the various model regions

\[ Q_{tiss} = Q_{ven} + Q_{art} + Q_c + Q_e \]

with \( Q_{art} \) and \( Q_{ven} \) representing the residues of \(^{18}\)O-labeled water in the nonexchanging vessels, \( Q_c \) representing that of the capillary region, and \( Q_e \) representing that of the extracapillary region. The units for the tissue residues \( (\mu \text{mol} / \text{g}) \) can be converted into concentrations \( (\mu \text{mol} / \text{ml}) \) by dividing by the volume of the region \( (\text{ml} / \text{g}) \). To describe intracapillary concentration gradients along the vessel length realistically, the capillary region was represented by 10 segments of tissue exchange units in series. The flow and exchange processes of the capillary region are dealt with in the model by the Lagrangian sliding fluid element approach (3). The individual tissue regions were assumed to consist of parallel independent pathways, each differing from the others only with respect to the local flow. To account for possible effects of flow heterogeneity within tissue samples, 10 pathways of parallel capillary tissue exchange units were simulated (23). Thus each of the 10 parallel pathways contained a capillary region represented by 10 tissue exchange units arranged in series. The flow distribution chosen for the exchanging vessel segment was characterized by a coefficient of variation of 0.3 and a skew of 0.6.

Statistics. Data were processed on a personal computer using SYSTAT 5.0 software (SPSS, Chicago, IL) and are expressed as means \( \pm \) SE. Correlations between local oxidative metabolism and LMF, coronary venous \( H_2^{18}O \) kinetics and perfusion time, and global \( PS_e \) value and global myocardial flow were determined by linear least-squares regression analysis. A \( P \) value \( < 0.05 \) was considered to indicate statistical significance.

RESULTS

Global \( MV_{O_2} \) assessment with analysis of oxidation water. During stable baseline hemodynamics, the global \( MV_{O_2} \) in four hearts calculated on the basis of \( H_2^{18}O \) production was \( > 93\% \) of the global \( MV_{O_2} \) calculated by the standard Fick method (Table 1).

Correlation of local \( H_2^{18}O \) residue and LMF. Hemo-
dynamic variables and \( MV_{O_2} \) were as follows \((n = 8)\): heart rate, 190 \( \pm 15 \) beats/min; left ventricular pressure, 84 \( \pm 12 \) mmHg; aortic flow, 52 \( \pm 8 \) ml/min; coronary flow, 6.6 \( \pm 0.7 \) ml/min \( ^{18}O \cdot g^{-1} \); and \( MV_{O_2} \), 0.08 \( \pm 0.01 \) ml/min \( ^{18}O \cdot g^{-1} \).

In 64 tissue samples, LMF ranged between 1.91 (low-flow areas) and 11.24 ml/min \( ^{18}O \cdot g^{-1} \) (high-flow areas). Local \( H_2^{18}O \) residue varied between 0.41 (low-flow areas) and 1.04 \( \mu \text{mol} / \text{g} \) (high-flow areas).

Linear regression analysis between both variables gave the equation \( H_2^{18}O = 0.04 \text{LMF} + 0.44 \), with \( r = 0.62 \), \( n = 64 \), and \( P < 0.001 \) (Fig. 2).

Table 1. Global \( MV_{O_2} \) from oxidation water versus measurements according to Fick.

<table>
<thead>
<tr>
<th>Steps</th>
<th>Parameter</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Coronary flow, ( \text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1} )</td>
<td>4.75 ( \pm 0.40 )</td>
</tr>
<tr>
<td>2</td>
<td>Wet mass, g</td>
<td>10.80 ( \pm 0.99 )</td>
</tr>
<tr>
<td>3</td>
<td>Dry mass, g</td>
<td>2.33 ( \pm 0.45 )</td>
</tr>
<tr>
<td>4</td>
<td>Tissue water, ml</td>
<td>8.48 ( \pm 0.73 )</td>
</tr>
<tr>
<td>5</td>
<td>CVE (240 s), ml</td>
<td>234.25 ( \pm 44.37 )</td>
</tr>
<tr>
<td></td>
<td>( H_2^{18}O ) enrichment (240 s)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Tissue water, ( \mu \text{mol} / \text{ml} )</td>
<td>1.12 ( \pm 0.12 )</td>
</tr>
<tr>
<td>7</td>
<td>CVE, ( \mu \text{mol} / \text{ml} )</td>
<td>0.71 ( \pm 0.07 )</td>
</tr>
<tr>
<td></td>
<td>( H_2^{18}O ) production</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Tissue water, ( \mu \text{mol} / \text{min} )</td>
<td>2.36 ( \pm 0.44 )</td>
</tr>
<tr>
<td>9</td>
<td>CVE, ( \mu \text{mol} / \text{min} )</td>
<td>44.67 ( \pm 4.89 )</td>
</tr>
<tr>
<td></td>
<td>( ^{18}O ) consumption</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Tissue water, ( \mu \text{mol} / \text{min} )</td>
<td>1.18 ( \pm 0.22 )</td>
</tr>
<tr>
<td>11</td>
<td>CVE, ( \mu \text{mol} / \text{min} )</td>
<td>22.34 ( \pm 2.44 )</td>
</tr>
<tr>
<td>12</td>
<td>( MV_{O_2}, \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1} )</td>
<td>49 ( \pm 11 )</td>
</tr>
<tr>
<td>13</td>
<td>Fick ( MV_{O_2}, \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1} )</td>
<td>54 ( \pm 6 )</td>
</tr>
</tbody>
</table>

The coronary flow \((1)\) in four hearts was normalized to the wet mass \((2)\). The difference between wet and dry mass \((3)\) is equal to tissue water \((4)\). The coronary venous effluent (CVE; \(5)\) was collected over 240 s. The \( H_2^{18}O \) enrichment in the tissue water \((6)\) and the CVE \((7)\) was assessed via mass spectrometry from standard mean ocean water (SMOW) values. The \( H_2^{18}O \) production in the tissue \((8)\) and the CVE \((9)\) was equal to the \( H_2^{18}O \) enrichment in the tissue water \((6)\) and the CVE \((7)\) times their respective absolute amounts, i.e., (4) and (5) and divided by 4 min. The \( ^{18}O \) consumption in the tissue \((10)\) and the CVE \((11)\) was equal to the \( H_2^{18}O \) production divided by 2. The sum of \((10)\) and \((11)\) was then converted from \( \mu \text{mol} \) to \( \mu \text{l} \) and normalized for the wet mass \((12)\). This latter value largely agreed with the myocardial \( O_2 \) consumption \((MV_{O_2})\) according to Fick \((13)\) because the difference was only \(-7\%\). Values are means \( \pm \) SE; \( n = 4 \) experiments.
The coronary venous kinetics of $^{18}$O-labeled oxidation water were determined from five rabbit hearts. Ten seconds after the onset of $^{18}$O perfusion, $^{18}$O-labeled oxidation water appeared in the coronary venous effluent with an increase of the $H_2^{18}$O concentration ($C$) of $0.20 \pm 0.12$ µmol/ml. The increase became statistically significant after 40 s ($0.35 \pm 0.09$ µmol/ml). Over time, the slope of the curve flattened, indicating that $H_2^{18}$O synthesis and $H_2^{18}$O removal were approaching equilibrium. At 240 s, the coronary venous $H_2^{18}$O concentration was increased by $0.58 \pm 0.12$ µmol/ml. Fitting the results to a logarithmic function gave the following equation with a significant correlation: $C = 0.14 \ln$ (perfusion time) $- 0.16$, with $r = 0.96$, $n = 53$, and $P < 0.05$ (Fig. 3).

**Model analysis.** Experimental measurements of global myocardial flow and arterial $^{18}$O concentration from nine hearts were used as model input parameter values. The experimental switch to perfusion with $^{18}$O was simulated by an inflow step increase of the $^{18}$O$_2$ concentration from 0 to $1.19 \pm 0.20$ µmol/ml, which is equal to twice the concentration of molecular $^{18}$O$_2$ in water at a partial pressure of 600 mmHg and a temperature of 38°C. Thus 1 mol of atomic oxygen yielded 1 mol of oxidation water. The model output was constrained by using the experimental value of oxygen consumption according to Fick. The $PS_c$ for water was used as a free parameter. Satisfactory model solutions were obtained for individual data sets by choosing a $PS_c$ between 2 and 10 ml·min$^{-1}$·g$^{-1}$. $PS_c$ linearly correlated with the global myocardial flow (GMF) according to the equation $PS_c = 2.23GMF - 4.24$, with $r = 0.70$ and $P < 0.05$ (Fig. 4). The average $PS_c$ value was $5.11 \pm 2.56$ ml·min$^{-1}$·g$^{-1}$, and the median was $5$ ml·min$^{-1}$·g$^{-1}$. In each experiment, a single $PS_c$ value could be used for fitting the residue and outflow data. The agreement of experimental results and model solutions is summarized in Table 2.

After calibration of this model with regard to its $PS_c$ value, it was used to calculate the local metabolic rate of oxygen. With a flow-proportional $PS_c$ value (Fig. 4), local flow differences between 3 and 10 ml·min$^{-1}$·g$^{-1}$ corresponded to a 6.45-fold difference in the metabolic rate of oxygen.

**Table 2. Agreement between experiment and model**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment Mean</th>
<th>Experiment SE</th>
<th>Model Mean</th>
<th>Model SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_c$, ml·min$^{-1}$·g$^{-1}$</td>
<td>4.19</td>
<td>0.80</td>
<td>4.19$^a$</td>
<td>0.80</td>
</tr>
<tr>
<td>$MV_0\mu$, µmol·min$^{-1}$·g$^{-1}$</td>
<td>1.70</td>
<td>0.46</td>
<td>1.66$^a$</td>
<td>0.44</td>
</tr>
<tr>
<td>$C_{out}$, µmol/ml</td>
<td>0.71</td>
<td>0.18</td>
<td>0.78$^a$</td>
<td>0.10</td>
</tr>
<tr>
<td>$Q_{stat}$, µmol/g</td>
<td>0.93</td>
<td>0.12</td>
<td>0.88$^a$</td>
<td>0.10</td>
</tr>
<tr>
<td>$PS_c$, ml·min$^{-1}$·g$^{-1}$</td>
<td>nd</td>
<td>nd</td>
<td>5.11</td>
<td>2.56</td>
</tr>
</tbody>
</table>

Values for coronary flow ($F_c$) and the arterial (atomic) oxygen concentration ($C_{out}$, 1.7 µmol/ml) were set to the experimentally measured values. The intracellular clearance term was then adjusted to give an $MV_0\mu$ within 3% of the experimental measurement. Finally, the $PS_c$ for water was adjusted to fit measurements of the coronary venous outflow concentrations ($C_{out}$) and the tissue residue ($Q_{stat}$) of $^{18}$O-labeled water equally well ($n = 9$). $^a$Model input parameters constrained by the experimental data. $^\dagger$Model output parameters. nd, No experimental measurement, estimated by the model analysis.
DISCUSSION

Recent studies in anesthetized dogs indicate that local myocardial substrate metabolism is as heterogeneous as LMF (9, 11). However, substrate consumption depends strongly on experimental conditions and cannot be reliably extrapolated to oxidative metabolism.

Our technique of evaluating local myocardial oxidative metabolism by labeling oxidation water with $^{18}$O was intended to circumvent this problem (31, 32). Under physiological, normoxic conditions as used in this study, the myocardium incorporates about 98% of its oxygen uptake into oxidation water, whereas oxidant generation is negligible and amounts to only 1–2% (7, 34). Thus our technique is well suited for direct measurements of myocardial oxidative phosphorylation.

The application of oxygen isotopes to the assessment of oxidative metabolism has a long history. As early as 1960, $^{18}$O-labeled water was used to determine the turnover of energy-rich phosphates (ATP, ADP) in isolated muscle fibers (16). We have replaced $^{18}$O-labeled water with $^{18}$O-oxygenated buffer and extended this technique to use in whole hearts. Similarly, $^{18}$O$_2$ was used in previous experiments on isolated, blood-perfused rabbit hearts (12), but only an extensive mathematical model analysis of tracer kinetics made it possible to distinguish between the produced oxidation water and nonmetabolized oxygen using this technique. In contrast, the present technique permits the analytic separation of the $^{18}$O-labeled water from the $^{18}$O-labeled oxygen. In another study in anesthetized dogs, $^{18}$O-labeled erythrocytes were injected into the coronary circulation, and the $^{18}$O tracer transient in the coronary venous outflow was analyzed (29). Because of sensitivity limitations, $^{18}$O-labeled water as reaction product could not be determined in the coronary venous effluent and, furthermore, the local tissue tracer residue was not analyzed. On the other hand, measurements of the tissue residue of $^{17}$O$_2$ using nuclear magnetic resonance, $^{17}$O$_2$ using positron-emission tomography, or $^{18}$O$_2$ using mass spectrometry require mathematical model analysis to correct the time-dependent changes in the tissue residue for the effects of water transport.

Capillary permeability-surface area product. The use of $^{18}$O-labeled water tissue residues in the present study provided physiologically significant data after the measurements were subjected to a rather simple mathematical model analysis. Assuming that one of the major model parameters influencing the results, namely, the $PS_c$, for water, was proportional to flow, the model estimated a 6.45-fold difference in local H$_2^{18}$O production rate for a 3.33-fold difference in LMF.

Because the extraction fraction of oxygen can be increased by only about 50% in the myocardium, changes in flow are expected to be of similar magnitude to changes in oxidative metabolism. Thus we expected a difference smaller than 6.45-fold for the H$_2^{18}$O production rate as predicted by the model. To obtain an estimate of the lower possible limit of the local H$_2^{18}$O production rate, a constant $PS_c$ was also calculated from our data; this value (5.5 ml·min$^{-1}$·g$^{-1}$) gave a 2.6-fold difference in local H$_2^{18}$O production rate.

In the present study, the $PS_c$ for water was estimated by modeling the kinetics of intracellularly produced water. Previous studies have used intracoronary bolus injections of $^3$H-labeled water and analyzed the kinetics of the resulting coronary effluent concentration (1, 30). The key difference between these approaches is that, with parenchymal production of labeled water, there is only net outward diffusion into the capillary region, whereas there is a time-dependent net inward and net outward diffusion of labeled water in classic indicator dilution experiments. In one such previous study, the capillary wall $PS_c$ was estimated to be 2.8 ml·min$^{-1}$·g$^{-1}$ (1), whereas in another, capillary and parenchymal cell membrane $PS_c$ was 18.0 and 3.8 ml·min$^{-1}$·g$^{-1}$, respectively (30). These estimates refer to blood flow ranges between 0.8 and 1.5 ml·min$^{-1}$·g$^{-1}$.

Our $PS_c$ values varied between 2 and 10 ml·min$^{-1}$·g$^{-1}$ (mean = 5.11 ± 2.56 ml·min$^{-1}$·g$^{-1}$) and were used to fit the kinetics of intracellularly produced water. To reduce the number of degrees of freedom in the model analysis, it was assumed that one lumped $PS_c$ value was sufficient to characterize the water-exchanging processes between parenchymal cells and the capillary region. Because parenchymal cell membranes and capillary walls represent two barriers in series, $PS_c$ may be calculated as $PS_{tot} = 1/[(1/PS_1) + (1/PS_2)]$, where $PS_{tot}$ represents the lumped $PS_c$ and $PS_1$ and $PS_2$ represent the individual permeability-surface area products. This simplification seems justified for the conditions of the present study, because the labeled water is produced in the extracapillary region, from which it slowly escapes into the capillary region. In agreement, the $PS_c$ value found in another study [3.8 ml·min$^{-1}$·g$^{-1}$ (30)] falls within the range of lumped $PS_c$ values calculated in the present study.

One important limitation of the present modeling is the uncertainty of the estimated local $PS_c$ values and, hence, local MVO$_2$. Previous studies conducted on the global heart have provided evidence for a significant direct relationship between flow rate and $PS_c$ (1, 19). Because both variables were correlated in the present experiments, a flow-proportional $PS_c$ was used for the analysis of local MVO$_2$. However, mean coronary flow varied between 3.10 and 5.25 ml·min$^{-1}$·g$^{-1}$ in the experiments that we used to calibrate the model with respect to the $PS_c$, i.e., mean coronary flow did not cover a wide range. In addition, only a single estimate of $PS_c$ was obtained for each heart, ranging from 2 to 10 ml·min$^{-1}$·g$^{-1}$.

On the other hand, we are confident that the $PS_c$ values used in the present study were reliable, because in contrast to most previous analyses, they were constrained by independent measurements of coronary outflow concentrations and tissue residues. There is only a narrow range in which the estimated $PS_c$ parameter fits both measures equally well. It is reassuring that the analysis of classic indicator dilution curves after intracoronary bolus injection of tracer water (1,
30) yielded results similar to those of the present study.

The relatively high 6.45-fold difference of local \( H_2^{18}O \) can only be explained with difficulty. Using a constant \( P_{Sc} \) gives a value that is certainly too low, once a flow-proportional \( P_{Sc} \) is accepted (1, 19). An increase in the extraction fraction of oxygen would also increase local \( H_2^{18}O \). Thus we must attribute the uncertainty in estimating \( P_{Sc} \) values to an unknown rest.

\( H_2^{18}O \) tissue residues and LMF. The 2.5-fold difference in the measured local \( H_2^{18}O \) tissue residues represents a considerable underestimate of the true disparity of local oxidative metabolism between low- and high-flow areas. The relatively small difference of \( H_2^{18}O \) residues between low- and high-flow areas (see Fig. 2) is due to the constant drain of \( ^{18}O \)-labeled water with the coronary effluent, which is influenced by the following two factors: 1) the metabolic rate turns out to be higher in high-flow areas, which gives rise to a higher extravascular \( ^{18}O \)-labeled water concentration in these areas; and 2) because of the higher flow rate, washout of vascular \( ^{18}O \)-labeled water occurs more rapidly, thus lowering the concentration in the capillary region. These two factors result in a greater concentration difference between extracapillary and capillary regions in high-flow areas.

Errors in assessing LMF might also account for the flat slope of the relation between the \( H_2^{18}O \) tissue residues and LMF, but they are likely to be small, because tissue sample mass averaged 100 ± 27 mg, which is close to the optimal size (80 mg) for local flow determinations in rabbit hearts using microspheres (5). By estimating the precision (V) of flow measurements according to \( V = 1.96\sqrt{n/T} \) (14, 28), with \( n = 600-4,000 \) microspheres per sample of 100 ± 27 mg, the methodological error ranged between 3 and 9% and therefore seems not to obscure the observed large flow heterogeneity. Potential temporal flow fluctuations, on the other hand, are unlikely to occur during 240 s and, if anything, would tend to underestimate LMF. Thus the 2.5-fold difference of local \( H_2^{18}O \) residues as measured in this study must be regarded as a minimum difference associated with the observed 5.8-fold flow range.

In conclusion, we have quantitatively characterized the relationship between LMF and the production of oxidation water. Oxidative metabolism and hence \( \text{MV}_2\text{O}_2 \) differ between low- and high-flow areas, and our data thus confirm previous studies (35, 36) using other techniques such as microbiopsies and spectrophotometry. In context with recent results on the lower flow threshold that is associated with an increase in local cytosolic adenosine concentration in myocardial low-flow areas (27), the results of the present study indicate that low-flow samples do not represent hyperperfused areas. In turn, high-flow samples do not represent regions of luxury perfusion but, rather, regions in which aerobic metabolism is above average (27). Because no local histological differences have so far been demonstrated and, in particular, because the mitochondria are uniformly distributed within myocardial tissue, we propose that oxidative metabolism is controlled by cellular mechanisms as yet unknown. Thus the present study provides the first experimental evidence that, in structurally homogeneous myocardium (13, 33), both \( \text{MV}_2\text{O}_2 \) and flow are heterogeneous but correlated to each other. Whether low oxidative metabolism and low flow are secondary to low contractile function on the same spatial level, potentially representing physiological hibernation, remains unknown at present (20).

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REFERENCES


