Endotoxemia decreases matching of regional blood flow and O2 delivery to O2 uptake in the porcine left ventricle

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1Department of Intensive Care; 2Laboratory of Physiology, Institute for Cardiovascular Research; 3Centre for Integrative Bioinformatics; 4Department of Chemistry; and 5Medical Genomics Section, Department of Clinical Genetics, Vrije Universiteit and Vrije Universiteit Medical Center, Amsterdam; and 6Netherlands Consortium for Systems Biology, Amsterdam, The Netherlands

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Alders DJ, Groeneveld AB, Binsl TW, de Kanter FJ, van Beek JH. Endotoxemia decreases matching of regional blood flow and O2 delivery to O2 uptake in the porcine left ventricle. Am J Physiol Heart Circ Physiol 300: H1459–H1466, 2011. First published February 4, 2011; doi:10.1152/ajpheart.00287.2010.—Heterogeneity of regional coronary blood flow is caused in part by heterogeneity in O2 demand in the normal heart. We investigated whether myocardial O2 supply/demand mismatching is associated with the myocardial depression of sepsis. Regional blood flow (microspheres) and O2 uptake ([13C] acetate infusion and analysis of resultant NMR spectra) were measured in about nine contiguous tissue samples from the left ventricle (LV) in each heart. Endotoxemic pigs (n = 9) showed hypotension at unchanged cardiac output with a fall in LV stroke work and first derivative of LV pressure relative to controls (n = 4). Global coronary blood flow and O2 delivery were maintained. Lactate accumulated in arterial blood, but net lactate extraction across the coronary bed was unchanged during endotoxia. When LV O2 uptake based on blood gas versus NMR data were compared, the correlation was 0.73 (P = 0.007). While stable over time in controls, regional blood flows were strongly redistributed during endotoxin shock, with overall flow heterogeneity unchanged. A stronger redistribution of blood flow with endotoxin was associated with a larger fall in LV function parameters. Moreover, the correlation of regional O2 delivery to uptake fell from r = 0.73 (P < 0.001) in control to r = 0.18 (P = 0.25, P = 0.009 vs. control) in endotoxic hearts. The results suggest a redistribution of LV regional coronary blood flow during endotoxin shock in pigs, with regional O2 delivery mismatched to O2 demand. Mismatching may underlie, at least in part, the myocardial depression of sepsis.

myocardial depression of sepsis; heterogeneous coronary blood flow; regional oxygen uptake; coronary vasoregulation; isotope labeling; tricarboxylic acid cycle

REVERSIBLE SYSTOLIC myocardial dysfunction is a characteristic feature of human septic shock, a common syndrome in critically ill patients with a 50% or higher mortality rate (12, 22). The cause of this myocardial depression remains unknown and is probably complex (12, 22). Factors that may be involved include blood flow, metabolism, and structural alterations. Circulating or regional inflammatory mediators may induce nitric oxide (NO) synthase, leading to negative inotropy. Coronary dysregulated leads to blood flow redistribution, as shown in animal and human studies (4, 7–9, 14, 15, 20, 22–25).

Coronary blood flow, measured with microspheres, is highly heterogeneous and varies by a factor to up to 7 from one area to the other, even in the normal heart (13–15, 18). This heterogeneity can be largely attributed to heterogeneous distribution of O2 demand, i.e., O2 uptake in the normal resting heart at unobstructed coronary blood flow. This implies metabolic autoregulation not only globally for the whole heart but also at the regional level (2, 6, 13). Regional matching between O2 delivery and demand was demonstrated by our group by a good correlation between regional coronary (microsphere) blood flows and O2 uptake, measured in frozen tissue samples after [13C] acetate infusion and subsequent nuclear magnetic resonance (NMR) analyses (2, 6). We have also previously shown that in canine endotoxin shock, coronary blood flow heterogeneity is largely unchanged but flow distribution is completely altered (14). This was associated with a reduced net lactate extraction by the myocardium, suggesting a mismatching of regional O2 delivery to demand. Coronary blood flow redistribution was associated with impaired systolic cardiac function. However, the hypothesized mismatching of regional O2 delivery to demand and focal myocardial overperfusion at the cost of underperfusion elsewhere in the myocardium remained unproven in the absence of measurements of regional O2 uptake (14).

In the current study, we therefore measured regional O2 delivery and uptake in the porcine heart in tissue samples with a spatial volume resolution of about 0.5 ml, before and after infusion of Escherichia coli endotoxin and induction of a septic shocklike state. These measurements were compared with controls. Regional blood flow (proportional to O2 delivery) was measured using radioactive microspheres, and regional O2 uptake was calculated using an NMR-based method using an incorporation of 13C-labeled carbon atoms via the tricarboxylic acid (TCA) cycle. The hypothesis tested in this study was that sepsis-induced myocardial depression is associated with regional mismatching of O2 delivery to demand across these small tissue samples.

MATERIALS AND METHODS

The study was approved by the Advisory Board for the Use of Experimental Animals of the Vrije Universiteit.

Experimental preparation. Thirteen male castrated pigs were divided into two groups [control, 35 kg (SD 7), n = 4; and endotoxin group, 35 kg (SD 2), n = 9]. After premedication by ketamine (15 mg/kg) and midazolam (1 mg/kg im), anesthesia was maintained by a continuous infusion of sufentanil (4 μg·kg−1·h−1), midazolam (0.5 mg·kg−1·h−1), and pancuronium (0.2 mg·kg−1·h−1). Lidocaine (initial dose 50 mg, followed by 9 mg·kg−1·h−1) was given to prevent cardiac arrhythmias. Pancuronium was given for muscle relaxation during mechanical ventilation. We checked for the absence of corneal
reflexes and of a reaction to a pain stimulus in the nasal cartilage before the administration of pancuronium to warrant adequate anesthesia. We closely monitored heart rate and blood pressure, which sensitively respond to pain, and found no sudden increases throughout the experiment. The procedure was in accordance with the American Physiological Society’s “Guiding Principles in the Care and Use of Animals,” which states that muscle relaxants may be used in conjunction with drugs known to produce adequate anesthesia.

The lungs were ventilated after tracheal intubation, with a mixture of 60% O2-40% room air. A relatively high O2 fraction was used to avoid arterial hypoxemia as a confounding factor during endotoxemia.

Five centimeters H2O of positive end-expiratory pressure was applied. Catheters were inserted for hemodynamic measurements and blood sampling as previously described (2). The maximum of the first derivative of left ventricular pressure (dP/dtmax) indicated cardiac contractile action. The thorax was opened via a midsternal incision, and the pericardium was opened. The left hemizygous vein was tied off to prevent an admixture of noncoronary venous blood during venous blood sampling from a coronary sinus catheter. About three million radioactive microspheres were injected into the left atrium to measure coronary blood flows. This ensured that there were more than 400 microspheres in each tissue sample. The left anterior descending coronary artery (LAD) was dissected free over a distance of about 2 cm and catheterized with a small catheter (24 gauge) for infusion of 13C-labeled acetate and measurement of mean coronary perfusion pressure. The electrocardiogram and the following hemodynamic parameters were recorded every 30 min: systolic, diastolic, and mean arterial pressure; systolic and diastolic left ventricular pressure and its dP/dt; mean pulmonary artery and pulmonary artery occlusion pressure (PAOP) after proper wedging; and mean coronary perfusion pressure. Pressures were measured with the fluid-filled catheters, zeroed to atmospheric pressure at the midchest level. Pigs were in the supine position. Cardiac output was measured by the bolus thermodilution technique with 5 ml of ice-cold glucose, 5% solution injected in the central venous port of the pulmonary artery catheter (Cardiac Output Computer 9520A, Edwards; triplicate measurements averaged). Left ventricular stroke work was calculated from stroke volume and the dP/dt.

Protocol. At the start (t = 0), systemic arterial and coronary venous blood samples were taken and baseline hemodynamics were measured. Cardiac output was measured at random points in the respiratory cycle. The first batch of microspheres (144Ce or 109Ru, in random order) was injected into the left atrium in 20 s. The reference method was used (2) with reference blood drawn from the femoral artery at 10.8 ml/min. The suspension containing the microspheres had been vigorously vortexed for 5 min and was continuously stirred during injection with a custom-built mixing device. A note in a sealed envelope indicated whether endotoxin (Escherichia coli type O127: B8, 10–15 mg/kg) or saline (in the control group) was infused intravenously over 30 min to randomize the treatment over the animals. Saline was given at a rate of 10–15 ml·kg−1·h−1. Thirty minutes after stopping endotoxin or saline infusion (i.e., at t = 60 min), an infusion of unlabeled sodium acetate (2.25 ml/min, 40 mmol/l in 0.9% sodium chloride) was started into the LAD for 30 min to achieve a metabolic steady state, after which the second batch of microspheres was injected, hemodynamics were measured, and arterial and coronary venous blood samples were taken (t = 90 min). Unlabeled acetate was switched to 2,13C-labeled acetate (at unchanged concentration) for exactly 4 min, after which another switch was made to [1,2-13C]acetate for 1.5 min. The first phase with unlabeled acetate infusion establishes a metabolic steady state. The two phases of labeled acetate infusion are optimal for metabolic flux quantitation and assessing regional aerobic metabolic flux from the isotope incorporation. This method is termed the Labeling with Isotope for a Pre-Steady-State Snapshots (LIPSSS) protocol and was extensively described and previously validated (6). Immediately after the last acetate infusion, a part (about 2 × 2 × 1 cm) of the ventral left ventricular free wall was quickly freeze clamped in situ between an aluminum clamp precooled to the temperature of liquid nitrogen, cut out of the heart, and stored in liquid nitrogen. The part cut out of the left ventricle is entirely perfused by the LAD (17). Samples were stored at −80°C until further analysis. Both kidneys were harvested for the determination of microsphere distribution.

Tissue preparation and coronary (microsphere) blood flows. Cardiac tissue was freeze dried (Modulyo freeze-dryer; Edwards) for at least 48 h. The tissue was then divided in subendocardial, midmyocardial, and subepicardial layers. Each of these layers was divided into 3 to 4 contiguous samples of about 100 mg dry wt, representing about 0.5 ml of tissue. After being weighed, the samples were homogenized in 4 ml of ice-cold perchloric acid (0.6 mol/l) and centrifuged (10 min at 4,000 g). The sediment was used to count radioactive microspheres (1280 Compugamma, LKB-Wallac, Turku, Finland). Blood flow to the tissue sample was determined according to the following formula: Fb = (Ib/Ia) × Fa, where Ib is regional blood flow (in ml·min−1·g dry wt−1), Ia is regional radioactivity in the tissue sample, Ib is arterial reference radioactivity, and Fa is arterial reference flow. Blood flow in the left and right kidney differed by <5% in all animals, indicating an adequate mixing of the microspheres in the arterial blood.

NMR measurement and quantitation of aerobic metabolism (O2 uptake) with a computational model. The incorporation of 13C isotopes from the infused acetate into metabolites was measured with NMR spectroscopy. Computational analysis of the NMR peak intensities then allows to quantitate metabolic fluxes.

After neutralization to pH 7.0, the supernatant obtained from the tissue sample was centrifuged and freeze dried for 48 h. Thereafter, it was dissolved in 0.5 ml tridistilled water plus D2O, centrifuged again, and transferred to a 5-mm NMR tube. High-resolution 13C-NMR spectra were obtained at 100.62 MHz with a Bruker Avance400 spectrometer at 27°C with a WALTZ-16 broad-band H-decoupling pulse sequence, 13C-pulse angle of 45°, repetition time of 7.3 s, 32k data points, sweep width of 100 parts/million with 1,470 scans accumulated as described earlier (2). Up to nine glutamate NMR multiplet peak intensities (in μmol/g dry wt) were quantified (25) by comparison to a reference spectrum of a 50 mmol/l glutamate solution.

The NMR multiplet intensities were analyzed using a computational model of 13C incorporation into the TCA cycle as recently described in detail (6). In brief, the model describes the time course of isotope incorporation after infusion of 13C-labeled acetate, via the acetyl-CoA pool into intermediate pools of the TCA cycle. A single triplet was used in the modeling of the TCA cycle for NMR data analysis, the flux is assumed constant throughout the TCA cycle (6, and references cited therein). At the level of α-ketoglutarate, there is a fast exchange of 13C label with the glutamate pool, which is the largest metabolite pool reached by the label and provides the most intense peaks in the NMR spectrum. From the isotope composition of glutamate calculated using the model, the NMR multiplet intensities are predicted. The metabolic fluxes in the tissue sample are estimated with the correspondence between the 13C intensities predicted from the metabolic model and the NMR measurements. The optimization of the flux parameters is done with a nonlinear least square procedure. In this way the flux through the TCA cycle (Jcyc) and the fractional flux of acetate (fraction I – Pcyc) and other carbon sources (Pcyc) into acetyl-CoA are quantitated. When the estimated error for Jcyc was higher than 0.4 μmol·min−1·g dry wt−1, the sample was rejected for further analysis (see Ref. 6 for rationale and details on this quality control procedure).

The TCA cycle flux is stoichiometrically coupled to O2 uptake by the mitochondria. The relation O2 uptake [(2 + Pcyc) × Jcyc] is used to calculate regional O2 uptake from the TCA cycle flux (see Ref. 6). For a detailed description of the NMR method and flux parameter quantification, see Refs. 6, 12, and 13.
Arterial PO2 fell during endotoxemia, although it still averaged the same experimental conditions without endotoxin (Table 1). Lactate content in arterial and coronary venous blood samples (in mmol/l) was measured using an L(+)-lactate kit (Sigma Diagnostics). Global myocardial O2 uptake was measured according to standard methods: blood gases were measured with a blood gas analyzer (Radiometer, Brønshøj, Denmark), whereas hemoglobin (Hb) and O2 saturation (SO2, in %) were measured with a hemoximeter (OSM3, Radiometer). Blood O2 content (in μmol/ml) was computed as follows: (Hb × 0.621 × SO2) + (0.0031 × PO2), with Hb content in mg/dl, SO2 in %, PO2 in mmHg. Mean myocardial O2 delivery was computed as the product of arterial O2 content and mean myocardial microsphere blood flow in the tissue samples of each heart. Regional myocardial O2 delivery was calculated for the individual samples. Mean myocardial O2 uptake in the whole left ventricle (blood gas based) was calculated as the product of mean coronary microsphere blood flow and the difference in O2 content between arterial and coronary venous blood on the one hand. Mean myocardial O2 uptake (NMR) was also determined by averaging the O2 uptakes assessed by the LIPSSS method in individual samples (regional O2 uptakes, NMR based). We also calculated mean and regional O2 extractions by dividing O2 uptake by O2 delivery. Three samples in which regional O2 extraction (higher O2 uptake than delivery) was erroneously >1, based on the LIPSSS method, were excluded from the reported mean values but are shown in Fig. 3.

Statistical analysis. Because of the relatively low number of experiments, the data were summarized as median and interquartile range, even though they were normally distributed (Kolmogorov-Smirnov test, $P > 0.05$). The results were compared between groups with the nonparametric Mann-Whitney U-test and within the endotoxin group with the paired Wilcoxon test in case of between-group differences. We normalized tissue data for means per pig and expressed heterogeneity as the coefficient of variation (CV = SD/mean) for pooled normalized data, which again were normally distributed (Kolmogorov-Smirnov test, $P > 0.05$) as previously reported (14, 18). Groups were compared with respect to their heterogeneities with Levene’s test, which compares equality of variances. Where applicable, partial correlation coefficients were calculated for repeated measurements in the same hearts: the partial correlation coefficient is a measure of the strength of association between a dependent variable and one independent variable when the effect of all other independent variables is removed. Partial correlation therefore provides an appropriate method to adjust the correlation coefficients for repeated measurements in the same subjects by removing the effect of interindividual variation between animals. Correlation coefficients were compared between groups after $z$ transformation. The relation of blood flow between time points was determined per pig heart using the Pearson linear correlation coefficient. Generalized estimating equations were used to determine group differences in regional data, adjusted for repeated measurements in the same hearts.

RESULTS

Global hemodynamics. After infusion of endotoxin, the pigs developed hypotension, whereas cardiac output remained unchanged (see Table 1). There was a fall in left ventricular stroke work and dP/dt at unchanged diastolic left ventricular and PAOP. In contrast, the control group was stable under the same experimental conditions without endotoxin (Table 1). Arterial PO2 fell during endotoxemia, although it still averaged 99 mmHg, but hemoglobin levels did not change. Arterial blood pH in endotoxin-treated animals decreased from 7.51 ± 0.36 to 7.36 ± 0.11 ($P = 0.002$), whereas in the control group pH did not significantly change (7.53 ± 0.03 to 7.52 ± 0.04). The lower pH may contribute to global vasodilatation, although severe acidosis did not occur. Despite coronary arterial hypotension, coronary blood flow and O2 delivery were maintained during endotoxemia (Tables 1 and 2). Lactate accumulated in arterial blood but lactate extraction across the coronary bed was not significantly changed during endotoxemia (Table 1).

Myocardial O2 uptake from blood and NMR data. The correlation coefficient between mean O2 uptake and extraction per heart based on blood gas and NMR data at $t = 90$ min was 0.73 and 0.76 ($P = 0.007$), respectively, taking control and endotoxin groups together (Fig. 1). The NMR method only measures O2 uptake linked to the TCA cycle. The blood gas-based measurement of O2 uptake also measures other O2-consuming reactions. Therefore, the O2 uptake calculated from NMR data is expected to be systematically lower than that by blood gas-based measurements (Fig. 1).

Despite unchanged O2 extraction across the coronary vascular bed, the mean blood gas-based O2 uptake in myocardium tended to decrease in endotoxin versus control pigs, although this effect was not significant (Table 1). However, the NMR-based O2 uptake was significantly decreased by about 40% (Table 2). Please note that blood gas-based O2 extraction was measured for the heart as a whole (Table 1), whereas the NMR method has a much finer spatial resolution (Table 2).

Regional coronary blood flow, O2 delivery and uptake, and their relations. There were 12 samples for each of the three layers (subepicardial, midmyocardial, and subendocardial) in control hearts (total 36 samples from 4 hearts) and 28, 29, and 30 samples (total 87 samples from 9 hearts), respectively, in endotoxemic hearts. Sample weights are given in Table 2. Parameters quantified for 11 samples from control hearts and 45 samples from endotoxin-treated hearts showed high asymptotic SEs and did not pass our quality control criterion developed and described in Binsl et al. (6). Therefore, these samples were excluded from further analysis. The changes in blood flow during endotoxemia were not noticeably different between the three layers. In control and endotoxemic pig hearts, regional blood flows at $t = 0$ min had CVs ($\pm$SD/mean) of 27.6 and 29.5% and at $t = 90$ min of 19.8 and 25.8%, respectively (not significantly different). This high CV indicates substantial true spatial heterogeneity of blood flow, with a relatively small contribution by measurement error (16). Regional blood flows were highly correlated in time at partial $r = 0.89$ ($P < 0.001$) in the control group, but the correlation of blood flows before and during endotoxemia was much lower: $r = 0.37$ ($P < 0.001$, $P < 0.001$ vs. control; Fig. 2). Please note that partial correlation coefficients correct for individual behavior. The decrease in correlation was therefore for a large part caused by increased scatter around the line of unity in the hearts during endotoxemia. In addition, one heart tended to show decreased flow in all tissue samples, whereas one other heart showed increased flow. After normalization for mean flow per heart, regional blood flows correlated in time with partial $r = 0.73$ ($P < 0.001$) in control and showed low correlation, $r = 0.09$ ($P = 0.39$, $P < 0.001$ vs. controls), in endotoxin hearts.
toxemia. Regions with low or average O2 uptake often showed relatively often in controls but were found rarely during endo-

toxemia. Importantly, the correlation between regional O2 uptake

and delivery was much less in endotoxemia than in controls (Fig. 3). After normalization for the mean flow per individ-

ual heart, regional O2 uptake related to regional O2 delivery at partial r = 0.51 (P = 0.011) in controls and −0.19 (P = 0.24,

P = 0.008 vs. controls) in endotoxin hearts. Regions with relatively high O2 uptake (>20 μmol·g−1·min−1) were found relatively often in controls but were found rarely during endotoxemia. Regions with low or average O2 uptake often showed higher blood flow during endotoxemia than in controls.

Regional O2 delivery at t = 0 correlated with regional O2 uptake at t = 90 at partial r = 0.44 (P = 0.005) in the endotoxin group and 0.70 (P < 0.001) in the control group (not significantly different from each other).

Relation to left ventricular stroke work and dP/dt. A fall in correlation coefficient for regional blood flows between t = 0 and t = 90 min, calculated per heart, was associated with a fall in left ventricular stroke work (r = 0.54, P = 0.058) and dP/dt (r = 0.60, P = 0.029) (Fig. 4). This suggests that greater redistribution of coronary blood flows during endotoxemia is

Table 1. Global and myocardial hemodynamics

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<td>Systemic</td>
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<tr>
<td>HR, beats/min</td>
<td>99 (15)</td>
<td>102 (30)</td>
<td>97 (29)</td>
<td>111 (35)</td>
<td>0.94</td>
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<tr>
<td>CO, l/min</td>
<td>2.75 (0.56)</td>
<td>2.45 (0.92)</td>
<td>2.84 (0.70)</td>
<td>2.41 (1.12)</td>
<td>0.94</td>
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<td>PAOP, mmHg</td>
<td>8 (3)</td>
<td>9 (4)</td>
<td>9 (2)</td>
<td>9 (9)</td>
<td>0.93</td>
</tr>
<tr>
<td>MPAP, mmHg</td>
<td>13 (3)</td>
<td>14 (2)</td>
<td>12 (3)</td>
<td>14 (2)</td>
<td>0.57</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>143 (19)</td>
<td>143 (30)</td>
<td>132 (48)</td>
<td>97 (25)</td>
<td>0.60</td>
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<tr>
<td>DBP, mmHg</td>
<td>106 (19)</td>
<td>113 (29)</td>
<td>97 (33)</td>
<td>57 (19)</td>
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<td>MAP, mmHg</td>
<td>118 (19)</td>
<td>124 (28)</td>
<td>109 (37)</td>
<td>70 (27)</td>
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<td>LVSP, mmHg</td>
<td>41 (13)</td>
<td>43 (19)</td>
<td>36 (18)</td>
<td>14 (9)</td>
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<td>LVDP, mmHg</td>
<td>6 (2)</td>
<td>6 (1)</td>
<td>9 (9)</td>
<td>4 (7)</td>
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<td>dP/dt, mmHg/s</td>
<td>3,001 (374)</td>
<td>2,900 (455)</td>
<td>2,315 (1,707)</td>
<td>2,099 (1,321)</td>
<td>0.50</td>
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Values are medians (interquartile ranges), also for mean values per heart (e.g., median of the mean blood flow). HR, heart rate; CO, cardiac output; PAOP, pulmonary arterial occlusion pressure; MPAP, mean pulmonary artery pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; LVSP, left ventricular systolic pressure; LVDP, left ventricular diastolic pressure; dP/dt, first derivative of left ventricular pressure; PAOP, partial O2 pressure; SO2, O2 saturation. O2 delivery and uptake were calculated based on blood gas values. For change vs. baseline: *P = 0.012, **P = 0.008, ***P = 0.015, ****P = 0.006, and *****P = 0.012.

Table 2. Regional blood flow and myocardial O2 balance

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<td>Dry weight, g</td>
<td>5.9 (0.9)</td>
<td>5.2 (1.0)</td>
<td>5.6 (1.0)</td>
<td>5.2 (1.0)</td>
<td>0.15</td>
<td>0.71</td>
</tr>
<tr>
<td>Mean coronary perfusion pressure, mmHg</td>
<td>115 (30)</td>
<td>128 (47)</td>
<td>112 (37)</td>
<td>78 (22)</td>
<td>0.57</td>
<td>0.004</td>
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<td>Mean coronary blood flow (whole heart), ml·min−1·g dry wt−1</td>
<td>5.2 (3.3)</td>
<td>6.0 (2.9)</td>
<td>5.6 (3.1)</td>
<td>6.4 (5.1)</td>
<td>0.41</td>
<td>0.94</td>
</tr>
<tr>
<td>Mean O2 delivery (blood gas), μmol·min−1·g dry wt−1</td>
<td>31.1 (19.6)</td>
<td>35.3 (21.4)</td>
<td>29.6 (28.4)</td>
<td>33.3 (17.8)</td>
<td>0.60</td>
<td>0.88</td>
</tr>
<tr>
<td>Mean O2 uptake (blood gas), μmol·min−1·g dry wt−1</td>
<td>20.0 (8.9)</td>
<td>23.9 (12.0)</td>
<td>22.3 (6.2)</td>
<td>19.4 (6.7)</td>
<td>0.71</td>
<td>0.33</td>
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Lactate, mmol/l

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<td></td>
<td>0.9 (1.0)</td>
<td>1.0 (1.2)</td>
<td>0.5 (1.3)</td>
<td>2.9 (3.7)</td>
<td>0.94</td>
<td>0.01</td>
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Lactate extraction fraction, dimensionless

| 0.31 (0.35) | 0.08 (1.4) | 0.23 (1.0) | 0.18 (0.25) | 0.60 | 0.82 |

Values are medians (interquartile ranges); n, number of observations. Myocardial O2 delivery and uptake were calculated based on NMR data. GEE, generalized estimating equations.
DISCUSSION

Our results suggest a mismatching of regional left ventricular \textsuperscript{O}2 delivery to demand, associated with diminished global cardiac contractile function, in porcine endotoxin shock. The porcine model hemodynamically resembles human septic shock with hypotension and maintained cardiac output, indicative of peripheral vasodilation (8, 9, 12, 22). Moreover, the fall in left ventricular stroke work and \textit{dP/dt\textsubscript{max}} suggests myocardial contractile depression and a fall in contractility rather than a fall in preload because left ventricular diastolic pressure and PAOP were unchanged. This interpretation might have to be changed if left ventricular end-diastolic volume would have fallen. However, septic myocardial depression is often associated with a rise rather than a fall in cardiac volumes (12, 22). The fall in coronary perfusion pressure along with maintained global coronary blood flow suggests global coronary vasodilation, as found in humans (8, 9). As previously reported by Austin et al. (3), the coronary reserve in the pig heart is profoundly heterogeneous. Myocardial blood flow as found in the basal state increases much more in one myocardial region than the other when the coronary vessels are maximally vasodilated. The redistribution of blood flow in endotoxin shock thus suggests that vasodilation was more profound in some regions than in others.

Global myocardial \textit{O}2 uptake and extraction, averaged from NMR data, correlated well to those obtained from blood analyses, indicating the validity of the NMR method as previously shown for baseline and a dobutamine stress condition (6). The blood gas-derived \textit{O}2 uptake was systematically higher than the NMR-derived \textit{O}2 uptake, as previously observed (2, 6). The reason for this is that the NMR-based \textit{O}2 uptake only includes \textit{O}2 uptake linked to the TCA cycle flux, whereas blood gas \textit{O}2 uptake additionally includes other \textit{O}2-consuming reactions. This explains why blood gas \textit{O}2 uptake tends to be higher.

Global myocardial \textit{O}2 uptake (NMR and blood gas based) was lower during endotoxemia: the NMR-based global \textit{O}2 uptake was significantly lower by about \textbf{40\%} (Table 2), but the smaller decrease in median value of blood gas-based \textit{O}2 uptake was not significant (Table 1). This suggests that \textit{O}2 uptake coupled to the TCA cycle is decreased, whereas other \textit{O}2-consuming reactions are maintained or perhaps even increased. It remains to be determined how the various components of \textit{O}2 metabolism affect blood flow regulation.

Global coronary blood flow was maintained despite a diminished workload by the left ventricle, whereas the fall in myocardial efficiency in endotoxin shock, suggested by a greater fall in cardiac work than in \textit{O}2 uptake (Table 1), agrees with
Earlier observations (14). Increased O2 costs for excitation-contraction coupling may help to explain this finding and the associated maintenance of global coronary blood flow levels due to metabolic autoregulation following relatively high maintained O2 uptake (1, 14, 21, 23).

During endotoxemia, a substantial portion of regions showed lower O2 extraction than in the control group, indicated by a lower O2 uptake to O2 delivery ratio (Fig. 3). This suggests regional overperfusion or a relatively low O2 uptake for a given O2 supply in many regions during endotoxemia (Table 2). However, note that O2 supply and uptake were measured at a volume resolution of 0.5 ml. It is therefore possible that O2 supply within this volume is inadequate in hypothetical cases where O2 diffusion cannot compensate for disturbed distribution of blood flow at small spatial scales. Conversely, during endotoxemia, there were relatively fewer regions with O2 uptake > 20 μmol-min⁻¹·g⁻¹ than in controls, suggesting that O2 uptake had fallen during endotoxemia in some regions.

Arterial lactate levels rose, characteristic for endotoxin or septic shock (7–9, 14, 26), indicating that lactate production systemically increased, although not in the heart. Lactate is a preferred substance for the heart, particularly in endotoxin shock (19, 26). However, net myocardial lactate extraction was not changed, as also previously observed (8, 9, 13, 25), suggesting that endotoxemia caused no increase in the overall myocardial lactate production as a consequence of ischemia. However, several myocardial tissue samples showed high O2 uptake-to-O2 delivery ratios, especially after endotoxin (Fig. 3). It cannot be excluded that there is an increased lactate extraction in some myocardial regions due to the increased arterial lactate concentration during endotoxemia. This increased lactate extraction might then compensate for the increased lactate production in other myocardial regions. It remains therefore to be elucidated whether some regions produce lactate due to focal ischemia. However, there was no evidence for global ischemia during endotoxemia, in agreement with previous studies (8, 9, 14–16, 25). However, some areas in the left ventricle may have been underperfused relative to demand (see Fig. 3), perhaps at the cost of underperfusion in other areas.

Indeed, on a regional basis, endotoxin shock was characterized by a redistribution of regional blood flows at only minimally increased overall heterogeneity as indicated by the CV of regional blood flows. In other words, the correlation is lost but the overall probability density function of flows is virtually unchanged. This was reported by us before in dogs (14) and agrees with a previous report on pigs (15). The high correlation between regional blood flows at subsequent time points in the control group conforms to the idea that spatial blood flow heterogeneity is far greater than temporal heterogeneity (18), demonstrating a stable but heterogeneous blood flow distribution in the heart. Here, the measurement error of about 5% of microsphere blood flows in the heart has to be taken into account (13, 14, 18). The correlation between regional O2 uptakes and regional blood flows in the control group (Fig. 3) suggests that spatial heterogeneity expressed by the variance of blood flow is at least in part (almost 50%) caused by regional differences in O2 demand (13, 14, 18), as previously described by us in the baseline state (2, 6). In contrast, the redistribution of regional microsphere blood flows in endotoxin shock was associated with clearly diminished matching of regional O2 delivery to demand (Fig. 3). In fact, regional blood flows before endotoxin correlated to regional O2 uptake after endotoxin, as in controls. The combination of the two observations suggests a largely unaltered distribution of O2 demand and a mismatch of regional O2 delivery caused by the redistribution

![Fig. 3](http://ajpheart.physiology.org/)
of blood flow during endotoxemia. The factors that may cause blood flow dysregulation throughout the left ventricle include regional production of vasodilating NO, vasconstricting endothelin, microvascular obstruction by accumulated leukocytes (11, 24), exhausted regional vasodilation during coronary hypotension, and altered extravascular compression following altered contraction and left ventricular pressure (3, 10). The regulatory state of the coronary vasculature was therefore quite different between endotoxemia and the basal state. The release of cytokines or NO might also be responsible for the fall in the ability to use generated ATP for contractile work (21, 25).

The catheter for infusion of labeled acetate was placed proximally in the LAD to reach the largest possible LAD-perfused area. We freeze clamped part of the LAD territory in the left ventricle extending toward the apex (~2 × 2 cm). It has been documented that the LAD perfuses the left ventricular wall plus most of the apex (17). Thus anatomical data support that the part of the left ventricle we sampled is perfused from the LAD. It is unlikely that collaterals reach the area where tissue samples were taken, because they are rare in the pig heart. Even in the unlikely case that the 13C-enriched acetate infused in the LAD is diluted, this would have no effect on the quantitation of the TCA cycle flux. The fraction of acetyl-CoA entering the TCA cycle that gets labeled is measured, not assumed (6). Variations in this fraction will not influence the quantitation of the TCA cycle flux. Indeed, an important feature of the 13C-NMR method is that, irrespective of the fractional contribution of labeled acetate to the TCA cycle flux, as soon as a sufficient 13C-signal is detected, TCA cycle flux can be estimated. Finally, it would be hard to explain that the hypothetical random variation explains the high correlation which was found between measured blood flow and O2 uptake.

The present study was not designed to separate the direct effect of endotoxemia on the coronary vasculature from the indirect effects of extravascular compression (3, 10), cardiac contraction, and left ventricular pressure, which may all have been altered. Even though regional blood flow redistribution correlated with the fall in left ventricular stroke work and \( \frac{dP}{dt_{\text{max}}} \), suggestive of a fall in cardiac function during endotoxemia, the functional consequences and mechanisms of regional coronary blood flow mismatching deserve further study. Future studies in which \( \frac{dP}{dt} \) is decreased in various ways may reveal how regional blood flow is affected by regional mechanical factors. There may be extravascular mechanical mechanisms depending on ventricular contraction that affect regional blood flow (3, 10), although heterogeneous O2 delivery correlates to heterogeneous O2 demand in the normal heart (Ref. 2, and this study) and regional vasodilation is not exhausted in any part of the heart at rest (3, 13). In a previous study using metabolic vasodilatation by infusion of glucose-insulin-potassium, workload increased (cf. 13 and references cited therein) but the CV of the blood flow distribution was unchanged and the increased regional blood flow at increased workload was strongly correlated with blood flow in the basal state, suggesting regional metabolic autoregulation during changing workload. In the present study, the \( t = 0 \) regional blood flow before the administration of endotoxin correlated with the \( t = 90 \) regional O2 uptake, even during endotoxemia, suggesting (partial) O2 supply to demand matching before endotoxemia and unchanged relative regional O2 demand distribution after endotoxin injection. Thus the latter may have occurred despite the fall in workload. Neither coronary anatomy nor the mechanical effects of contraction disturb the relation between local O2 uptake and local myocardial perfusion in the normal heart (this study, and Ref. 2) when vasomotor tone is present (3). However, it is possible that extravascular compression becomes a greater determinant of regional coronary blood flow in regions with a fall in vascular tone during endotoxemia. The limitations of our study further include the observation that we could fit only about 70% of cardiac tissue samples from controls successfully to the TCA cycle model and even an even lower fraction in endotoxin shock because of relatively low NMR peak intensities with some NMR peaks below detection limits. This may relate to the diminished activity of TCA cycle enzymes (20), although other studies suggest that sepsis does not impair the TCA cycle or ATP generation in the heart (16, 23, 25). The glutamate concentration was not decreased in contrast with other observations (16).

In conclusion, this study finds a redistribution of coronary blood flow in the left ventricle in endotoxin shock in pigs, associated with a fall in global cardiac contractile function. Mismatching of regional left ventricular O2 delivery to demand may thus underlie, at least in part, the myocardial depression of sepsis.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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