Myocardial Lactate Release During Ischemia in Swine
Relation to Regional Blood Flow

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To determine the relation between regional myocardial blood flow, contractile function, and myocardial lactate release during mild-to-moderate regional myocardial ischemia, nine open-chest swine were instrumented for measurement of regional myocardial blood flow (microsphere method), contractile function (sonomicrometry), and hemodynamics. L-[1-14C]Lactate or L-[U-13C]lactate was infused intravenously using a primed continuous infusion technique to quantify regional myocardial lactate release. D-[U-13C]glucose or D-[6-14C]glucose was simultaneously infused to determine the contribution of exogenous glucose to lactate release. Graded coronary ischemia (two to three levels) was created in the left anterior descending coronary arterial distribution by mechanically constricting the artery in five animals or by decreasing flow through a cannulated left anterior descending artery in four animals. In all nine animals, subendocardial blood flow was 0.99±0.21 (ml/min)/g during control and 0.34±0.14 (ml/min)/g during the most severe grade of underperfusion (p<0.001) in the left anterior descending coronary arterial distribution. Regional myocardial lactate release was 0.15±0.09 and 1.19±0.75 μmol/ml, respectively (p<0.003). A highly significant inverse correlation was observed between subendocardial blood flow and myocardial lactate release during the graded reductions in blood flow (r=-0.71, p<0.001). Results from sonomicrometry showed a significant reduction in contractile ventricular function in the anterior wall during the graded reductions in blood flow. The regional arterial-venous glucose difference increased significantly with underperfusion in the left anterior descending coronary arterial distribution, from 0.14±0.15 to 0.56±0.37 μmol/ml (p<0.003). The contribution of exogenous glucose to lactate release also increased significantly; 0.04±0.03 μmol/ml of the lactate came from exogenous glucose during control compared with 0.64±0.59 μmol/ml during the most severe underperfusion (p<0.02). A significant positive correlation exists between lactate release and lactate from exogenous glucose during graded underperfusion (r=0.96, p<0.001). In summary, these data demonstrate a close inverse relation between regional myocardial lactate release and regional subendocardial blood flow during graded ischemia. (Circulation 1990;81:1948–1958)

Myocardial lactate production has been used as a marker of ischemia in patients and in experimental animal studies.1 Lactate represents the end product of anaerobic or nonoxidative glycolysis. However, lactate is also an important substrate for the myocardium; lactate is converted to pyruvate, which undergoes oxidative decarboxylation to acetyl coenzyme A and subsequently enters the citric acid cycle. Using isotopically labeled lactate, we2,3 have shown that the myocardium releases or produces lactate when there is net chemical extraction in normal subjects and in patients with ischemic heart disease. This finding implies that both metabolic pathways, that is, nonox-

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idative glycolysis and lactate oxidation, are active simultaneously within the myocardium.

During atrial pacing in patients with significant coronary artery disease, there is a significant increase in lactate release in the absence of clinical ischemia; in subjects without coronary lesions, lactate release does not change with atrial pacing. These findings imply that an increase in isotopically measured myocardial lactate release is a marker of ischemia and that ischemia-induced biochemical alterations occur before the appearance of clinical symptoms of ischemic heart disease. Myocardial lactate release has also been shown to increase significantly during global hypoxia in a canine preparation.5

No published study, however, has investigated the relation between isotopically measured lactate release and contractile ventricular dysfunction during myocardial underperfusion. Thus, the purpose of this study was to determine the relation between lactate release, regional myocardial perfusion, and contractile function in an open-chest swine model of acute graded ischemia. Radiolabeled microspheres were used to quantify regional myocardial blood flow. Regional contractile function was assessed by sonomicrometry. Dual carbon-labeled isotopes (L-[1-14C]lactate and D-[U-13C]glucose, or L-[U-13C]lactate and D-[6-14C]glucose) were used to quantify myocardial lactate release and the contribution of exogenous glucose to lactate release.

Methods

Animal Preparation

The animals in this experimental protocol were handled in accordance with the guidelines for animal use provided by the American Physiological Society, and approval of this protocol was granted by the animal subjects committee of the University of California, San Diego. To ensure that the relation between lactate release and blood flow was not dependent on the method used to reduce perfusion, two different protocols (groups A and B) were used to alter blood flow in the left anterior descending coronary artery.

Nine domestic swine (30–40 kg) were studied under isoflurane anesthesia (1–1.5%) after sedation with ketamine HCl (1 g i.m.) and initial anesthesia with thiamylal sodium (10 mg/kg i.v.). Ventilation was carefully controlled to keep blood gas and pH measurements within the physiological range; blood gas measurements were taken every 30 minutes after they had stabilized (model ABL2, Radiometer, Copenhagen, Denmark). The animal's body temperature was maintained with a circulating hot water pad and was kept above 37°C. Tygon (Fisher Scientific, Santa Clara, California) catheters were positioned in the thoracic aorta (through the carotid artery) for blood withdrawal and in the external jugular and saphenous veins for fluid replacement (Multisol-R 3X, Ceva Laboratories, Inc., Overland Park, Kansas). Through a left lateral thoracotomy, the pericardium was opened, and a high-fidelity micromanometer was placed within the left ventricle through the apex. To measure regional systolic segmental shortening, sonomicrometers were implanted in the lateral ventricular midwall (within the perfusion bed of the left circumflex coronary artery) and anterior midwall (within the perfusion bed of the distal left anterior descending coronary artery) perpendicular to the long axis of the heart so that they were parallel to the midwall fibers (group A, n=5).6

In group B (n=4), contractile function in the anterior wall was assessed by measurement of systolic wall thickening.7 Electrodes were attached to the left atrium for pacing. The left anterior descending coronary artery was dissected free approximately halfway from the left ventricular base to the apex. In group A animals, a rigid mechanical constrictor equipped with a micrometer was placed around the vessel to produce graded coronary stenosis. In group B animals, the left anterior descending coronary artery was cannulated with a polyethylene cannula equipped with a distal sidearm 5 mm from the cannula tip to measure pressure. Blood from the arterial catheter was circulated through an extracorporeal circuit consisting of a roller occlusive pump (Masterflex model, Cole-Parmer, Chicago, Illinois) and an in-line electromagnetic flow probe. Total coronary inflow was controlled by the inflow pump and was measured directly with an electromagnetic flow microcomputer (model RC 2000, Micron Medical, Los Angeles, California).

The large epicardial vein parallel to the left anterior descending coronary artery was dissected and cannulated adjacent to the position of the mechanical constrictor or cannula in the artery and then drained at atmospheric pressure; blood was returned to the animal through the jugular catheter with a second roller pump. Before the coronary artery or vein was cannulated, the animals were injected with 10,000 units of sodium heparin, and additional heparin (10,000 units) was administered every hour.

Regional Myocardial Blood Flow

Regional myocardial blood flow was measured with radiolabeled microspheres (15 μm diameter, E.I. Du Pont de Nemours & Co. Inc., Boston, Massachusetts) with standard techniques.8 Briefly, microspheres (approximately 6×10^8) were injected into the left atrium during arterial blood withdrawal at a constant rate.9 The ratio of the radioactivity of the arterial blood sample to the withdrawal flow rate was then used to calculate regional blood flow ([ml/min]/g). A matrix inversion technique was used to correct for overlapping spectral peaks from the radionuclides used in a given study.10 Formalin-fixed myocardial tissue was cut into transmural thirds, weighed, and placed in glass counting vials to determine the radioactivity with a gamma spectrometer (model 5901, Packard Instruments, Downers Grove, Illinois). The reported blood flow data correspond to the surrounding locations of the ultrasonic dimension.
Regional Myocardial Contractile Function

Regional myocardial contractile function was assessed by measurement of regional dimension (segment or wall thickness) changes occurring during systole and was expressed as a percent of end-diastolic dimension. End diastole was defined as the onset of the peak positive first derivative of left ventricular pressure (+LV dP/dt), and end systole was defined as the maximal shortening occurring within 20 msec of peak negative LV dP/dt.

Isotopically Labeled Lactate and Glucose

To quantify myocardial substrate use, dual carbon-labeled isotopes were infused. Four animals received D-[U-13C]glucose and L-[1-14C]lactate, and five received D-[6-14C]glucose and L-[U-13C]lactate. The stable isotopes, D-[U-13C]glucose and L-[U-13C]lactate (>99% enrichment), were obtained from Merck Sharp & Dohme, Montreal, Canada. L-[1-14C]lactate (specific activity, 55 mCi/mmol) and D-[6-14C]glucose (specific activity, 56 mCi/mmol) were obtained from New England Nuclear, Boston, Massachusetts. These were sterilized by microfiltration (0.22-μm bacteriologic filter, Millipore Corp., Bedford, Massachusetts) and diluted in 0.9% saline solution. After priming doses of 200 mg D-[U-13C]glucose and 20 μCi L-[1-14C]lactate, a continuous intravenous infusion of D-[U-13C]glucose at 125 mg/hr and L-[1-14C]lactate at 25 μCi/hr was begun. The doses for D-[6-14C]glucose were 32 μCi and 20 μCi/hr, respectively, and for L-[U-13C]lactate, 75 mg and 90 mg/hr, respectively.

We have previously shown that, at rest, 20 minutes is required to achieve equilibration of arterial and coronary venous specific activities and the myocardial CO2 pool when lactate is labeled with a tracer and that 25–30 minutes are required for a glucose tracer. Thus, to ensure equilibration, the first control blood samples were obtained at least 30 minutes after the priming bolus and the start of the continuous isotope infusion.

Arterial and coronary venous blood samples were drawn simultaneously. Samples were obtained for chemical concentrations of glucose and lactate, isotopic analyses (14C and 13C) of glucose and lactate, and 13CO2 content. Immediately after the samples were drawn as paired sets, blood flow measurements were performed. A final set of samples was taken after the blood flow measurement to verify steady-state conditions throughout the protocol.

Chemical Analyses

Blood samples for analysis of lactate, glucose, specific activities, and 13C enrichments were mixed immediately with a measured volume of cold 7% perchloric acid and centrifuged. The protein-free supernatant was removed and stored at −4°C C for future analysis. The coefficient of variation and the methodologies used in our laboratory for analyses of chemical substrates, determination of specific activities, measurement of 14CO2, and analysis of [U-13C]lactate have been published previously. The lactate concentration was determined by an enzymatic spectrophotometric method. Glucose was measured by the hexokinase–glucose-6-phosphate dehydrogenase–coupled enzymatic method. Free fatty acids were measured by a modified gas chromatography (GC) method of Ko and Royer. For determination of specific activities, glucose, lactate, and pyruvate were separated by ion-exchange chromatography as previously described. Portions of the eluates were assayed by the described enzymatic methods; other portions were mixed with Aquasol (New England Nuclear), and then 14C was measured in a scintillation counter. The specific activity was calculated from these results as disintegrations per minute per micromole (dpm/μmol).

[U-13C]lactate content was measured by GC–mass spectrometry (MS) after conversion to a trimethylsilyl ether derivative of methyl lactate. [U-13C]glucose content was determined with a modification of the method developed by Bier et al for 6,6-dideuteroglucose. Glucose was isolated from the protein-free supernatant by ion-exchange chromatography, lyophilized, and converted to a butane–boronic acid acetate derivative. This derivative was analyzed by GC-MS as described by Bier et al by measuring the masses at 297 (M-C4H9) and 296, corresponding to unlabeled glucose, and at 303, corresponding to [U-13C]glucose. The enrichments were compared with a standard curve prepared by diluting 99%-enriched D-[U-13C]glucose with unlabeled glucose. 14CO2 was collected directly from blood by a diffusion method and was measured by scintillation counting. All isotopic and chemical analyses were performed in duplicate.

Calculations

The chemical extraction (μmol/ml) for a given substrate was calculated from the arterial and coronary venous substrate chemical concentrations as [A]−[CV], where [A] is the arterial concentration, and [CV] is the regional epicardial coronary venous concentration. The isotopic lactate extraction ratio (percent) for [1-13C]lactate was calculated from the specific activities (dpm/μmol) of lactate and from [A] and [CV] as

\[
\left[\frac{[A] \times \text{specific activity of lactate in artery} - [CV] \times \text{specific activity of lactate in vein}}{[A] \times \text{specific activity of lactate in artery}}\right] \times 100
\]

For [U-13C]lactate, the isotopic extraction ratio was calculated as

\[
\left[\frac{[A] \times \% \text{ }^{13}C_3 \text{ in artery} - [CV] \times \% \text{ }^{13}C_3 \text{ in vein}}{[A] \times \% \text{ }^{13}C_3 \text{ in artery}}\right] \times 100
\]

where
% $^{13}$C$_3$=[$^{13}$C$_3$]lactate/chemical lactate×100

The myocardial isotopic lactate extraction (μmol/ml) was determined from either the [1-$^{14}$C]lactate or [U-$^{13}$C]lactate extraction ratio as


Myocardial lactate release (μmol/ml) represents the difference between the isotopic lactate and the chemical lactate extraction and was calculated as

\[
\text{Isotopic lactate extraction} = (A) \times \text{isotopic lactate extraction ratio (\%)/100}
\]

The oxidation of lactate labeled with $^{14}$C (μmol/ml) was calculated from the myocardial production of $^{13}$CO$_2$ and the arterial specific activity of lactate as

\[
\frac{(14CO_2 \text{ dpm/ml in CV}) - (14CO_2 \text{ dpm/ml in A})}{\text{arterial specific activity of lactate}}
\]

Because other substrates are labeled secondarily when tracers are infused, the (CV−A) $^{14}$CO$_2$ was corrected for the possible oxidation of the secondarily labeled substrates as previously published.2,3

The use of dual carbon-labeled isotopes of glucose and lactate and measurement of the enrichment of the stable isotopes and the specific activities of glucose and lactate in the artery and coronary vein allows quantification of the conversion of exogenous glucose to lactate being released by the myocardium. The equations for experiments with D-[6-$^{14}$C]glucose and L-[U-$^{13}$C]lactate have been published.3 In the experiments with D-[U-$^{13}$C]glucose and L-[1-$^{13}$C]lactate, the contribution of exogenous glucose to released lactate was calculated from the observed and theoretical $^{13}$C$_3$ contents of lactate per milliliter of blood in the coronary vein and the $^{13}$C$_3$ enrichment of arterial glucose as

\[
\frac{(\text{Observed} - \text{theoretical}) \text{ lactate} \times \text{content in coronary vein}}{\text{13C$_3$ enrichment of arterial glucose}}
\]

Note that, despite the fact that two molecules of lactate are produced for each molecule of glucose, a factor of 2 is not included because each molecule of lactate is completely labeled, as universally labeled glucose was infused.

The actual (or observed) $^{13}$C$_3$ content of lactate per milliliter of blood in the coronary vein was calculated as

\[
(CV) \times (\% \text{ $^{13}$C$_3$ lactate enrichment in coronary vein})
\]

The theoretical $^{13}$C$_3$ content of lactate per milliliter of blood was determined as

\[
\frac{(A) - [1-^{14}$C$lactate \text{ uptake in } \mu\text{mol/ml}] \times (\% \text{ $^{13}$C$_3$ enrichment of arterial lactate})}{100}
\]

Protocol

Thirty minutes after the start of isotope infusion, control measurements were made. Duplicate, simultaneous samples were taken from the arterial and coronary venous catheters approximately five minutes apart and were followed by measurement of blood flow with the microsphere method. An additional set of blood samples was taken after the blood flow measurement to ensure a steady state. Changes in regional myocardial contractile function or LVP more than 5% occurring during an observation period were indicative of failure to maintain a steady state, and such observations were not included in analysis. After the control sampling, myocardial underperfusion in the left anterior descending coronary arterial distribution was then created by tightening the mechanical constrictor (group A) or by decreasing the inflow in the cannulated vessel (group B). The contractile function in the anterior wall was monitored closely during induction of each level of myocardial ischemia; the aim of the study was to reduce contractile function by 10% or more from control or from the previous ischemic level. Once a stenosis or flow was set, no adjustments were made for 15 minutes preceding data collection. Duplicate blood samples were taken during the stenosis or reduced-flow period, and then microspheres were injected to measure myocardial blood flow. Immediately after the myocardial blood flow measurement, a final set of blood samples was again taken during the hypoperfusion period. The constrictor was then adjusted to produce slightly more stenosis (group A), or inflow was further reduced (group B), and the above routine was repeated. Because of the rigid criteria for acceptance of data, only two or three levels of underperfusion were successfully completed in each animal.

After the last level of underperfusion, blood flow was restored (release of mechanical occluder in group A or return of pump-controlled blood flow to control level in group B). Blood samples were obtained, microspheres were injected, and contractile function data were recorded as described above.

Control Studies

Sham-operated, time-matched (total time, three hours) control studies were performed in five additional animals to determine the stability of hemodynamics, regional myocardial function, blood flow, and lactate metabolism for the duration of the study. Measurements were made in a manner similar to that in the experimental group.

Data Acquisition and Analysis

Hemodynamic data, including LVP, +LV dp/dt, and regional myocardial contractile function, were recorded on a Brush forced-ink recorder with 1/2-in. magnetic tape (Hewlett-Packard, Palo Alto, California) for subsequent A-D conversion and beat averaging by computer (model LSI-11, Digital Equipment Corp., Nashua, Massachusetts). Ten sequential beats during each steady-state condition were averaged at the time of microsphere injections. The reported chemical and isotopic data represent the mean of the three samples taken through the steady-state
period (two samples 5 minutes apart before microsphere injection and the third sample after microsphere injection).

Since different numbers of acceptable graded stenoses or reduced flows were achieved in the animals, analysis of variance was not used for statistical comparison. However, for each experimental study, the various levels of ischemia were compared with the control value by paired t test.20 The stability of the control studies was verified by one-way analysis of variance.

Results

Hemodynamics

An original recording from one study with coronary stenosis is illustrated in Figure 1. Heart rate was controlled by atrial pacing in all but two animals; therefore, changes in heart rate during underperfusion were eliminated. The mean heart rate was 114±9 beats/min. Two or three grades of underperfusion were achieved in each animal. With the first level of underperfusion, peak LVP was 93.2±6.9 mm Hg compared with a control value of 95.4±10.2 mm Hg (p=0.25); during the most severe underperfusion period, peak LVP was 86.8±6.5 mm Hg (p<0.05). LV end-diastolic pressure was 9.3±4.8 mm Hg during control, 12.1±6.3 mm Hg (p=0.06) during the first level of underperfusion, and 13.5±6.5 mm Hg (p<0.05) during the most severe underperfusion period. LV dP/dt was 1,326±172, 1,225±158 (p=0.05), and 1,103±144 mm Hg/sec (p<0.005), respectively, during these periods.

Regional Myocardial Blood Flow

During the control period, subendocardial blood flow in the anterior wall was 0.99±0.21 (ml/min)/g. With graded underperfusion (created by the mechanical stenosis or reduced pump flow through the cannula), the subendocardial flow varied from 0.87 to 0.11 (ml/min)/g. The mean subendocardial blood flow during the most severe underperfusion level was 0.34±0.14 (ml/min)/g (p<0.001). During that time, subepicardial blood flow did not change significantly from the control measurements (1.06±0.32 vs. 0.71±0.44 [ml/min]/g, p=0.10); thus, primarily subendocardial ischemia was present. No changes were observed in the subendocardial blood flow to the lateral wall of the left ventricle during underperfusion of the left anterior descending coronary artery (1.07±0.29 vs. 0.99±0.47 [ml/min]/g).

Regional Myocardial Contractile Function

Regional myocardial contractile function, expressed as a percent of control values, is plotted...
against subendocardial blood flow for the anterior wall in Figure 2. There was a direct relation between reductions in subendocardial blood flow and reductions in regional myocardial contractile function. As indicated in Figure 2, the reduction in systolic transmural wall thickening for a given flow reduction tended to be greater than the reduction in midwall shortening. During underperfusion of the anterior descending coronary artery, contractile function of the nonischemic lateral wall was not affected.

**Myocardial Lactate Release**

Activity in the nonoxidative (anaerobic) glycolytic pathway can result in myocardial lactate release (production). Lactate can also be used as a substrate for oxidative metabolism by the myocardium; lactate is converted to pyruvate, undergoes oxidative decarboxylation, and enters the citric acid cycle. The arterial-venous chemical lactate difference represents the net result of these two major metabolic pathways. Thus, because lactate is a major fuel for myocardial oxidative metabolism, changes in the nonoxidative glycolytic pathway can occur that are not reflected in net chemical lactate production.

We have previously shown\(^3\) that the nonoxidative pathway is active and that lactate is released by the myocardium in normal human subjects. Leunissen and Piatnek-Leunissen\(^21\) and we\(^22\) have also found that lactate is released by an anesthetized, open-chest, nonischemic canine preparation. In these experiments, \(\text{L-}[1-^{14}\text{C}]\text{lactate}\) was infused as a tracer, and an isotopic lactate extraction ratio was calculated. The isotopic extraction ratio was greater than the chemical extraction ratio, which indicates that the myocardium is releasing lactate.

All nine swine demonstrated myocardial lactate release during control nonischemic conditions in the present study; the mean value was 0.15±0.09 \(\mu\text{mol/ml}\). Four of these animals received \(\text{L-}[1-^{14}\text{C}]\text{lactate}\), and myocardial lactate oxidation was assessed by measuring myocardial production of \(^{14}\text{CO}_2\). In these four animals, the amount of lactate released during control conditions was 0.16±0.07 \(\mu\text{mol/ml}\); the lactate isotopic uptake was 0.59±0.32 \(\mu\text{mol/ml}\) compared with the chemical lactate difference of 0.44±0.32 \(\mu\text{mol/ml}\). The \(^{14}\text{CO}_2\) data demonstrated that 0.52±0.30 \(\mu\text{mol/ml}\) lactate was oxidized. Thus, 86±4\% of the isotopic lactate uptake was oxidized immediately. Similar calculations for chemical lactate extraction implied that 130±21\% of the lactate extracted was oxidized. Thus, the \(^{14}\text{CO}_2\) measurement, which is independent of the isotopic extraction ratio, indicates that the chemical lactate difference underestimates lactate use and that myocardial lactate oxidation is very closely correlated with isotopic lactate uptake. During the most severe underperfusion state, all four animals exhibited net myocardial lactate production. However, isotopic analysis showed that 0.42±0.17 \(\mu\text{mol/ml}\) lactate was extracted, and the \(^{14}\text{CO}_2\) data revealed that 92±25\% of the isotopic uptake was oxidized.

**Subendocardial Blood Flow Versus Lactate Release**

The data demonstrate that with decreasing perfusion in the left anterior descending coronary artery, there was an increase in regional myocardial lactate release. Table 1 compares subendocardial blood flow in the anterior wall with biochemical analysis of the blood samples obtained simultaneously from the artery and anterior cardiac vein. Samples obtained when the subendocardial blood flow was between 0.41

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**TABLE 1. Comparison of Subendocardial Blood Flow With Biochemical Data**

<table>
<thead>
<tr>
<th>Subendocardial blood flow (ml/min)/g</th>
<th>n</th>
<th>Arterial lactate ((\mu\text{mol/ml}))</th>
<th>A-V chemical lactate ((\mu\text{mol/ml}))</th>
<th>Lactate release ((\mu\text{mol/ml}))</th>
<th>Lactate from glucose ((\mu\text{mol/ml}))</th>
<th>Arterial glucose ((\mu\text{mol/ml}))</th>
<th>A-V chemical glucose ((\mu\text{mol/ml}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;0.80</td>
<td>9</td>
<td>1.68±0.30</td>
<td>0.60±0.31</td>
<td>0.15±0.11</td>
<td>0.06±0.05</td>
<td>5.02±0.64</td>
<td>0.13±0.12</td>
</tr>
<tr>
<td>0.61–0.80</td>
<td>9</td>
<td>1.29±0.37</td>
<td>0.41±0.35</td>
<td>0.19±0.07</td>
<td>0.08±0.06</td>
<td>5.09±0.90</td>
<td>0.12±0.13</td>
</tr>
<tr>
<td>0.41–0.60</td>
<td>8</td>
<td>1.66±0.30</td>
<td>0.09±0.38</td>
<td>0.64±0.32</td>
<td>0.27±0.14</td>
<td>5.04±0.93</td>
<td>0.29±0.14</td>
</tr>
<tr>
<td>&lt;0.41</td>
<td>8</td>
<td>1.33±0.34</td>
<td>−0.75±0.87</td>
<td>1.28±0.73</td>
<td>0.73±0.59</td>
<td>5.06±1.02</td>
<td>0.66±0.32</td>
</tr>
</tbody>
</table>

Data represent mean±SD.
A–V, Arterial-venous difference.
and 0.60 (ml/min)/g showed that lactate release was four times the control value. With further reduction of blood flow to less than 0.41 (ml/min)/g, regional lactate release increased to eight times that of control. The individual data points for regional lactate release and anterior subendocardial blood flow are plotted in Figure 3; a highly significant inverse correlation is demonstrated (r = -0.71, p < 0.001).

Figure 4 shows control conditions and three levels of graded underperfusion in two different animals. The open triangles denote an animal in which a mechanical rigid occluder was used to create stenoses in a graded fashion and for which contractile function (Figure 4B) was measured as a percent of midwall segmental systolic shortening. In the animal denoted by the closed triangles, coronary flow was controlled through an extracorporeal circuit and a cannula inserted into the left anterior descending coronary artery, and contractile function was assessed by changes in wall thickening. These data show that, as perfusion to the subendocardium decreases, lactate release increases in a graded fashion and that the relation between flow and lactate release is similar for both methods of underperfusion (Figure 4A). Figure 4C demonstrates the relation between arterial-venous chemical lactate difference and flow. In the animal symbolized by closed triangles, there was a 0.76 μmol/ml increase in lactate release and a 55% reduction in wall thickening before net production of chemical lactate. This indicates that chemical lactate production may not be a sensitive index of myocar- dial underperfusion or ischemia.

Exogenous Glucose to Released Lactate

Using dual carbon-labeled isotopes enabled us to quantify not only myocardial lactate release but also the contribution of exogenous glucose to lactate release. As shown in Table 1, the amount of released lactate derived from exogenous glucose (column 6) increased with underperfusion of the subendocardium. During control, 35% of the released lactate came from exogenous glucose. As subendocardial blood flow was decreased, lactate release increased, and the contribution of exogenous glucose also increased. With subendocardial blood flow values of less than 0.41 (ml/min)/g, exogenous glucose was the source of 57% of the released lactate. These changes are also reflected in the arterial-venous glucose differences (Table 1, column 8).

Figure 5 demonstrates the relation between lactate release and the contribution of exogenous glucose to the lactate released under experimental conditions. A highly significant positive correlation was observed between these two values during the graded under- perfusion protocol (r = 0.96, p < 0.001).

Reperfusion Data

In eight animals, blood samples were obtained, microspheres were injected, and contractile function was measured after reperfusion. As shown in Table 2, subendocardial blood flow returned to control values during the reperfusion period. In both groups A and B, lactate release measured during the reperfusion period was not significantly different from control values. With reperfusion, the contractile dysfunction that had occurred in the anterior wall during the underperfusion period improved in both groups (Table 2). These data indicate that the graded increase in lactate release observed during the experimental protocol was related to underperfusion of the left anterior descending coronary artery and not secondary to myocardial injury induced during preparation.

Control Studies

No changes in either regional myocardial blood flow or lactate release occurred during the 3-hour observation of the five sham-operated, time-matched control animals. Lactate release was 0.16 ± 0.04 μmol/ml at the onset vs. 0.20 ± 0.05 μmol/ml after 3 hours. Likewise, regional myocardial contractile function did not change over time in these control studies.
Discussion

These data demonstrate for the first time that there is a close relation between myocardial blood flow and changes in lactate release. With decreases in subendocardial flow, increases in lactate release were observed. This finding, together with the development of regional contractile dysfunction, suggests that significant ischemia was present. The use of dual carbon-labeled isotopes also permitted calculation of the amount of released lactate derived from exogenous glucose. Our findings show that during ischemia, glucose uptake by the myocardium is increased and that this glucose is metabolized to lactate.

It is commonly believed that, under normal conditions, the myocardium is entirely aerobic and does not produce lactate; this belief is based on the observation that the myocardium exhibits net chemical extraction of lactate. However, evidence exists that the normoxic myocardium produces lactate despite net chemical extraction. Probst et al. have found that cultured adult ventricular myocytes release lactate when incubated with room air or 100% O2. Leunissen and Piatnek-Leunissen showed that the myocardium released lactate, despite net chemical extraction of this substrate, in an anesthetized nonischemic canine preparation. Recently, we reported similar findings in a normoxic, nonischemic canine preparation and have also demonstrated that the normal human myocardium releases lactate at rest. These data indicate that the nonoxidative (i.e., anaerobic) glycolytic pathway is active even in the normal healthy myocardium.

Early investigators have shown that the nonoxidative glycolytic pathway is active during hypoxia and ischemia. More recent investigators have raised the question of the importance of the nonoxidative glycolytic pathway during normoxic, nonischemic conditions. Several studies have shown evidence that high-energy phosphates derived from nonoxidative glycolysis may be preferentially used for the sarcoplasmic reticulum and are necessary for myocardial relaxation. Another possible explanation for the lactate release observed during basal nonischemic conditions is heterogeneous blood flow and metabolism.
the intact, working myocardium may be transiently underperfused, which may account for lactate release during control conditions. Evidence also exists that indicates that the subendocardial layers may be hypoperfused in the working heart during peak systolic pressures. Myocardial biopsy studies in animals have shown a transmural gradient of enzymes and substrate stores between the endocardial and epicardial regions. The subendocardium contains more glycogen, has increased levels of glycolytic enzymes, and has increased phosphorylase activity. These findings suggest that glycolytic activity is higher in the subendocardial regions, and these regions may be the source of lactate release in the control state.

The present study does not reveal the mechanism for lactate release during normoxic, nonischemic conditions. However, it shows that graded decreases in coronary flow that result in contractile dysfunction (i.e., graded myocardial ischemia) are associated with graded increases in lactate release as measured by an isotopic method. Net chemical lactate production from the myocardium has long been regarded as a reliable marker of acute myocardial ischemia. This study demonstrates that increases in the activity of the nonoxidative glycolytic pathway can occur that do not result in net chemical lactate production as measured in the epicardial vein (Figure 4).

In addition to being the end product of the nonoxidative glycolytic pathway, lactate is also an important substrate for oxidative metabolism. The myocardial lactate extraction ratio is relatively high but is dependent on a number of factors, such as circulating levels of free fatty acids and lactate. Myocardial lactate extraction has been shown to be inversely related to the arterial free fatty acid level but directly correlated with arterial lactate level. In this study, heparin was administered to prevent thrombosis in the extracorporeal circuit and intravascular catheters. However, in addition to its anticoagulation effects, heparin stimulates lipoprotein lipase to hydrolyze triglycerides and thereby raises the levels of circulating free fatty acids. Blood samples for substrate analysis were not obtained before heparin administration in this study. In previous swine experiments, we have observed that this heparin dose (10,000 units) can raise circulating free fatty acid levels from a baseline value of 0.24±0.12 to 0.48±0.30 μmol/ml at 20 minutes after heparin administration. During the course of the present study, the mean arterial free fatty acid level was 0.27±0.08 μmol/ml; we believe that this postheparin level was relatively low because the first metabolic samples were obtained at least 1 hour after initial heparin administration. This, although lactate extraction may be slightly reduced compared with baseline (i.e., no heparin), it is probably not significantly decreased due to heparin administration.

The chemical arterial-venous lactate difference represents the net balance between myocardial lactate extraction and lactate generated by activity of the nonoxidative glycolytic pathway and then released. This report shows that ischemia results in significant increases in lactate release and suggests that an increase in lactate release may be a more sensitive index of ischemia than the chemically derived arterial-venous lactate difference. However, if all the factors that influence myocardial lactate extraction are held constant, then changes in lactate release are directly reflected in the chemical arterial-venous differences.

To ensure that increases in lactate release were not secondary to myocardial injury induced by the procedure, ischemia was created by two methods. Both methods (A and B) revealed a similar relation
between blood samples and lactate release (Figures 3 and 4). In addition, blood samples and measurements were obtained when coronary flow had returned to baseline after the underperfusion protocol (Table 2). This revealed that subendocardial flow, as measured by the microsphere method, had returned to control levels and that the biochemical data and contractile function had normalized. Thus, graded increases in lactate release and decreases in contractile function observed over the time course of the experiment were not secondary to a deteriorating preparation.

We hypothesize that the increase in lactate release observed in this study came from the subendocardial area. Quantification of blood flow by the microsphere method allows measurement of blood flow in relatively small (0.5–1.0 g) samples of myocardial tissue, thus providing a truly regional assessment of perfusion. In this study, the microsphere measurements showed a significant decrease in subendocardial flow (p<0.001) while the flow to the epicardial layers was not significantly altered. Thus, primarily subendocardial ischemia was created. This finding agrees with other previously published studies that have shown that, because intramyocardial pressure is greatest in the subendocardium, this area is the most susceptible to ischemia when flow in the epicardial vessel is limited.

The assessment of regional contractile function by measuring the midwall rather than the subendocardial segmental shortening or wall thickening was done to ensure correct orientation between the ultrasonic crystal alignment and the primary fiber orientation of the local myocardium. The fiber orientation of the subendocardial layer is difficult to estimate from external landmarks, whereas midwall fiber orientation is very predictable, which greatly facilitates correct placement of the gauges. However, since ischemia was more pronounced in the subendocardial area, the use of midwall (vs. subendocardial) segmental shortening may have underestimated the severity of contractile dysfunction in relation to the lactate released. This could also explain why decrements of blood flow were associated with greater changes in systolic wall thickening than segmental shortening (Figure 2).

Another limitation of the techniques is that the sampling site for the coronary venous sample was an epicardial vein adjacent to the site of coronary stenosis. Therefore, the sample was a mixture of blood not only from the ischemic subendocardium but also from the overlying, well-perfused epicardial region. In this study, relative changes in lactate release are compared; absolute amounts of released lactate are not equated with a certain degree of ischemia. Clearly, the closer the sampling site is to the area of ischemia, the higher the lactate concentration one measures. However, during very severe ischemia, our measurement of released lactate may underestimate the actual value. Apstein et al have demonstrated that during severe ischemia, metabolic by-products such as lactate are not released because of the very low flow. Similarly, Rovetto et al found that glucose use was decreased in a severely limited flow model. Thus, to avoid these problems, most levels of ischemia were in the mild-to-moderate range in this study.

In summary, this study shows for the first time that mild-to-moderate episodes of subendocardial ischemia associated with regional contractile dysfunction are accompanied by increases in lactate release that appear related to the intensity of ischemia. The relation between subendocardial blood flow and lactate release suggests that increases in lactate release are a graded phenomenon rather than a simple “on-off” mechanism, in which chemical lactate extraction is equated with normoxic perfusion and net chemical production indicates ischemia. This study suggests that increases in lactate release may be a sensitive marker of ischemia during stress in patients with coronary artery disease or during investigation of various pathophysiological states in experimental animal models.

References

**KEY WORDS** • subendocardial blood flow • lactate release • glucose uptake • segmental contractile function
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B D Guth, J A Wisneski, R A Neese, F C White, G Heusch, C D Mazer and E W Gertz

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