Perinatal Changes in Myocardial Metabolism in Lambs

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Background—Lactate accounts for a third of myocardial oxygen consumption before and in the first 2 weeks after birth. It is unknown how the remainder of myocardial oxygen is consumed. Glucose is thought to be important before birth, whereas long-chain fatty acids (LC-FA) are the prime substrate for the adult. However, the ability of the myocardium of the newborn to use LC-FA has been doubted.

Methods and Results—We measured the myocardial metabolism of glucose and LC-FA with [U-13C]glucose and [1-13C]palmitate in chronically instrumented fetal and newborn lambs. In fetal lambs, myocardial oxidation of glucose was high and that of LC-FA was low. Glucose and LC-FA accounted for 48±6% and 2±6% of myocardial oxygen consumption, respectively. In newborn lambs, oxidation of glucose decreased, whereas oxidation of LC-FA increased. Glucose and LC-FA accounted for 12±3% and 83±19% of myocardial oxygen consumption. To test whether near-term fetal lambs could use LC-FA, we increased the supply of LC-FA with a fat infusion. In fetal lambs during fat infusion, the oxidation of LC-FA increased 15-fold. Although the oxidation of LC-FA was still lower than in newborn lambs, the contribution to myocardial oxygen consumption (70±13%) was the same as in newborn lambs.

Conclusions—These data show that glucose and lactate account for the majority of myocardial oxygen consumption in fetal lambs, whereas in newborn lambs, LC-FA and lactate account for the majority of myocardial oxygen consumption. Moreover, we showed that the fetal myocardium can use LC-FA as an energy substrate. (Circulation. 2000;102:926-931.)

Key Words: metabolism ■ glucose ■ fatty acids ■ blood flow ■ isotopes

Long-chain fatty acids (LC-FA) are the prime energy substrate for the myocardium in the adult.1 In the fetus, glucose and lactate are thought to be the major energy sources for the myocardium.2 In the newborn, the importance of glucose and lactate was thought to decrease1 due to a switch in substrate supply at birth. At birth, the placental supply of glucose and lactate ceases, and the newborn is fed milk, which largely consists of fatty acids.4 LC-FA were thought to become an important energy source after birth.3 However, many doubt that the myocardium of the newborn can use fatty acids as an energy source.5 This doubt was raised by studies in isolated heart preparations, which showed a limited capacity of the myocardium of newborn pigs to oxidize fatty acids.6,7 These observations were recently extended by studies in homogenated tissue from rabbit hearts.8 It was suggested that glycolysis may be the major energy source for the myocardium of the newborn.9 However, these studies in isolated heart preparations used different sets of substrates in a wide range of concentrations. The differences in experimental setup have led to a wide variation in myocardial substrate supply. This variation may have influenced myocardial substrate uptake in these studies, because the myocardium in the adult is generally considered an omnivore.10 Moreover, in these studies, a fixed insulin concentration was added to the perfusate; however the insulin concentration reportedly changes in the first weeks after birth.4

Until now, the myocardial oxidation of glucose and LC-FA has not been measured in vivo. Previous studies in lambs2,3,11 have used the myocardial oxygen extraction ratio, which is calculated with the arteriovenous concentration difference of a substrate, the amount of oxygen necessary to oxidize that substrate, and the arteriovenous concentration difference of oxygen across the myocardium. It is assumed, first, that the arteriovenous concentration difference of oxygen across the myocardium. It is assumed, first, that the arteriovenous concentration difference of oxygen across the myocardium reflects the actual uptake and, second, that all of the substrate apparently taken up is immediately oxidized. The first assumption may be true for glucose because the heart cannot produce glucose (it lacks the enzyme glucose-6-phosphatase).12 However, the assumption that all of the glucose taken up is immediately oxidized is probably not
valid. In studies in human adults\textsuperscript{13} and in isolated rat hearts,\textsuperscript{14} it was shown that only part of the glucose taken up was immediately oxidized; the rest was probably converted into lactate and glycogen. The only way to establish the actual contribution of a substrate to myocardial oxygen consumption is to measure its oxidation.

In a previous study, in which we measured myocardial lactate metabolism with the use of \textsuperscript{13}C-lactate, we found that lactate accounted for 36\% of myocardial oxygen consumption in fetal lambs and for 28\% of myocardial oxygen consumption in newborn lambs.\textsuperscript{15} It is unknown how the remainder of myocardial oxygen is used. On the basis of a previous study in which we calculated the myocardial oxygen extraction ratio,\textsuperscript{11} we speculated that in fetal and newborn lambs, glucose contributes to a substantial amount of myocardial oxygen consumption.

The aim of this study was to determine the contribution of glucose and LC-FA to myocardial oxygen consumption in vivo before and in the first weeks after birth. Therefore, we measured the myocardial metabolism of glucose and LC-FA with the use of [U-\textsuperscript{13}C]glucose and [1-\textsuperscript{13}C]palmitate as tracers in chronically instrumented fetal and newborn lambs. To test whether the myocardium of near-term fetal lambs can use LC-FA as an energy substrate, we increased the supply of LC-FA in a group of fetal lambs with a fat infusion and subsequently measured LC-FA metabolism with [1-\textsuperscript{13}C]palmitate.

**Methods**

We studied 16 fetal lambs at 127 to 134 days of gestation and 13 newborn lambs with ages ranging from 3 to 15 days after birth. If \textgreater{}1 study was performed in an animal, the studies were performed at least 1 day apart. The fetal and newborn lambs were instrumented as described previously.\textsuperscript{11} We inserted polyvinyl catheters into the ascending aorta, superior caval vein, coronary sinus, left atrium, and the amniotic cavity (for zero pressure reference). We studied the lambs at least 2 days after surgery. Surgical and experimental procedures were approved by the animal research committee of the University of Groningen.

**Experimental Protocol**

On the day of the study, the ewe was placed in a cage in the study room with free access to food and water. Newborn lambs were weighed and placed in a sling. We gave the animals 60 to 90 minutes to get accustomed to the study room. To prevent interference with fatty acid metabolism, we removed heparin from the catheters \textgreater{}1 hour before blood samples were taken. Heart rate and blood pressures were recorded every 5 minutes throughout the experiment. We infused [U-\textsuperscript{13}C]glucose into the caval vein at a priming dose of 0.199 mmol/kg in 10 minutes and at a continuous rate of 1.99 mmol·kg\textsuperscript{-1}·min\textsuperscript{-1} thereafter.\textsuperscript{16} The dose needed for the fetal lambs was calculated assuming a body weight of 3 kg. To infuse palmitate, it must be bound to albumin. For that reason, an albumin-\textsuperscript{13}C-palmitate-complex was prepared,\textsuperscript{17} which was dissolved in 0.9\% NaCl. This solution was passed through a 0.20-\textmu m bacteriologic filter (Schleicher/Schuell) before it was infused.

We infused [1-\textsuperscript{13}C]palmitate into the caval vein at a continuous dose rate of 0.081 mg·kg\textsuperscript{-1}·min\textsuperscript{-1}. Studies in dogs had shown that no priming dose was needed.\textsuperscript{18} In previous experiments, it was shown that after 30 minutes of infusion, a steady state of arterial [1-\textsuperscript{13}C]palmitate was reached. After 30 and 45 minutes of infusion, we withdrew blood samples simultaneously from the ascending aorta and coronary sinus for the determination of \textsuperscript{13}C-enrichment of the substrate, CO\textsubscript{2} concentration, \textsuperscript{13}C-enrichment of CO\textsubscript{2}, oxygen saturation, hemoglobin concentration, hematocrit, pH, pCO\textsubscript{2}, pO\textsubscript{2}, and HCO\textsubscript{3} concentration and concentrations of glucose, lactate, and free fatty acids. Immediately after the collection of the last samples, we injected radionuclide-labeled microspheres into the left atrium.

To test whether near-term fetal lambs could use LC-FA as an energy substrate for the myocardium, we increased the arterial concentration of LC-FA in 9 fetal lambs by infusing a fat emulsion, Lipofundin 20\% (B. Braun Melsungen AG). Lipofundin was infused into the fetal jugular vein at a constant rate of 4.86 mL/h after a bolus injection of 4 mL. In pilot studies, we found that after 30 minutes, a steady state of arterial free fatty acid concentration was reached; this concentration was similar to that seen in newborn lambs. After 30 minutes of fat infusion, [1-\textsuperscript{13}C]palmitate was infused into the fetal caval vein, as was done in the fetal lambs without fat infusion. After 45 minutes of [1-\textsuperscript{13}C]palmitate infusion (that is, after 75 minutes of fat infusion), blood samples were withdrawn simultaneously from the ascending aorta and coronary sinus. A second and, in 5 lambs, a third set of blood samples were withdrawn after 100 and 115 minutes of fat infusion. Immediately after collection of the last blood sample, radionuclide-labeled microspheres were injected into the left atrium.

**Measurements**

We recorded heart rate and blood pressures and determined oxygen saturation, hemoglobin concentration, hematocrit, pH, pCO\textsubscript{2}, pO\textsubscript{2}, and HCO\textsubscript{3} concentrations as previously described.\textsuperscript{13} We measured blood flow to the myocardium (expressed in mL·min\textsuperscript{-1}·100 g wet weight\textsuperscript{-1}) with radionuclide-labeled microspheres (15 \mu m in diameter), as previously described.\textsuperscript{11} The blood collected for the determination of substrate concentrations was transferred immediately to a tube containing 25 mg NaF to stop glycolysis; it was then mixed and kept in ice. The concentrations of glucose, lactate, and free fatty acids were determined in duplicate by enzymatic methods,\textsuperscript{19} as previously described.\textsuperscript{11}

The \textsuperscript{13}C-enrichment of the glucose derivative was determined by gas chromatography mass spectrometry.\textsuperscript{20} Selective ion monitoring was performed at m/z 408 and 414, which correspond to m+0 and m+1, respectively. To obtain a calibration graph, we prepared standards containing 0\%, 2\%, 4\%, and 6\% [U-\textsuperscript{13}C]glucose by dilution with natural glucose. The coefficient of variation was 3.1\% (n=5).

To measure the enrichment of [1-\textsuperscript{13}C]palmitate in blood, we extracted free fatty acids from plasma with a chloroform/heptane/methanol mixture (49:49:2). The free fatty acids were made into derivatives with diazomethane. The derivative was dried under nitrogen and dissolved in hexane. The concentration of palmitate was determined with a gas chromatograph (HP 6890, Hewlett Packard) using heptadecanoic acid (C\textsubscript{17}) as an internal standard. The isotope enrichment of palmitate was determined with a Hewlett-Packard 5890 gas chromatograph interfaced to a VG Trio-2 quadrupole mass spectrometer (Fisons Instruments). The mass spectrometer was used in the electrical impact mode. Single ion monitoring was performed at m/z 270 (m+0) and 271 (m+1). To obtain a calibration graph, we prepared standards containing 0.0\%, 2.5\%, 5.0\%, and 7.5\% [1-\textsuperscript{13}C]palmitate by dilution with natural palmitate. The coefficient of variation was 3.7\% (n=13).

The blood samples used to determine CO\textsubscript{2} were withdrawn in heparinized Vacutainer tubes (Becton Dickenson) and stored at \textminus20°C until further analysis. The total CO\textsubscript{2} concentration and the isotope ratio of CO\textsubscript{2} were determined, and the molar fraction of CO\textsubscript{2} (F\textsubscript{CO\textsubscript{2}}) was calculated as previously described.\textsuperscript{13,16} The molar fraction was used to calculate the concentration of CO\textsubscript{2} as follows.

\[
[\text{CO}_2] = F_{CO_2} \times [CO_2]
\]

where [CO\textsubscript{2}] is the total CO\textsubscript{2} concentration.

**Calculations**

Blood O\textsubscript{2} concentration was calculated as the product of oxygen saturation, hemoglobin concentration, and a hemoglobin binding capacity of 1.36 mLO\textsubscript{2}/g Hb. Ventricular (LV) oxygen supply was calculated as the product of arterial oxygen concentration and blood flow to the LV free wall. Because the coronary sinus blood of lambs...
TABLE 1. Hemodynamic and Oxygen-Related Variables in Fetal and Newborn Lambs

<table>
<thead>
<tr>
<th></th>
<th>Fetal</th>
<th>Newborn</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>3.3±0.0</td>
<td>6.0±0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>163±1</td>
<td>214±1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aortic pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Systolic</td>
<td>65±0</td>
<td>95±0</td>
<td>&lt;0.001</td>
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<tr>
<td>Diastolic</td>
<td>38±0</td>
<td>55±0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean</td>
<td>50±0</td>
<td>73±0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean left atrial pressure, mm Hg</td>
<td>4±0</td>
<td>3±0</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin concentration, g/L</td>
<td>108±1</td>
<td>90±1</td>
<td>&lt;0.01</td>
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<tr>
<td>Aortic oxygen saturation, %</td>
<td>51±1</td>
<td>94±0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aortic pH</td>
<td>7.32±0.00</td>
<td>7.36±0.01</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LV blood flow, mL·min⁻¹·100 g⁻¹</td>
<td>192±7</td>
<td>282.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LV oxygen supply, μmol·min⁻¹·100 g⁻¹</td>
<td>611±18</td>
<td>1364±28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ao-CS oxygen, μmol/L</td>
<td>2030±36</td>
<td>3562±43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LV oxygen consumption, μmol·min⁻¹·100 g⁻¹</td>
<td>371±12</td>
<td>946±20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose concentration, mmol/L</td>
<td>0.91±0.01</td>
<td>5.52±0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LC-FA concentration, mmol/L</td>
<td>0.01±0.00</td>
<td>0.35±0.01</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean±SE. Ao-CS indicates aorto-coronary sinus concentration difference.

**Statistical Analysis**

Data are presented as mean±SE. We applied Student’s t test and ANOVA with post hoc Newman-Keuls when necessary. P<0.05 was considered significant. For comparison, we used data obtained in a previous study in lambs.15 No differences existed in baseline values between the 2 studies.

**Results**

On the day of the study, all lambs had a normal heart rate, blood pressure, oxygen saturation, and pH (Table 1). No difference existed in the arterial enrichment of [U-13 C]glucose or [1-13 C]palmitate between the blood samples obtained at the different time points (ie, after 30, 45, 60, or 90 minutes of infusion of the label). Several differences existed in the baseline variables between fetal and newborn lambs (Table 1); these were similar to those described in previous studies.11,15 The arterial concentrations of glucose and LC-FA were higher in the newborn lambs (Table 1). Because LV blood flow was also higher in newborn lambs, there was a 9-fold and 30-fold perinatal increase in the LV supply of glucose and LC-FA, respectively.

Despite the increased supply of glucose in the newborn, LV uptake of glucose was the same in both newborn and fetal lambs, and LV oxidation of glucose was significantly lower in newborn than in fetal lambs (Figure 1). In contrast to glucose, LV uptake and oxidation of palmitate were higher in newborn than in fetal lambs (Figure 1). In fetal lambs, the oxidation of glucose and lactate contributed most to LV oxygen consumption, whereas in newborn lambs, the oxidation of LC-FA and lactate contributed most to LV oxygen consumption (Figure 2).

In fetal lambs, LV uptake and oxidation of palmitate were almost zero because there was no supply of fatty acids. Infusion of the fat emulsion into fetal lambs significantly increased the arterial concentration of LC-FA (Table 2) and...
the myocardial supply of LC-FA (2.8±1.1 to 72.5±9.6 μmol·min⁻¹·100 g⁻¹, P<0.05). The arterial concentration of LC-FA was the same in the fetal lambs during fat infusion as it was in the newborn lambs (Table 1). Fat infusion did not affect heart rate, mean aortic pressure, oxygen saturation, or oxygen and carbon dioxide tensions, nor did it affect the concentration of ketone bodies and glucose (Table 2). Fat infusion increased the concentration of lactate and decreased pH and HCO₃⁻ concentration. LV uptake of palmitate gradually increased (Figure 3), although only the difference between fetal and newborn lambs was statistically significant. LV oxidation of palmitate also increased, and it was significantly higher in the newborn lambs. However, the contribution of LC-FA to LV oxygen consumption was the same in fetal lambs during fat infusion as it was in newborn lambs (Figure 4, left). The oxidation of LC-FA increased with an increase in LV oxygen consumption (Figure 4, left). The relative contribution of the oxidation of LC-FA to LV oxygen consumption seemed to increase with LV oxygen consumption, especially in the group of fetal lambs during fat infusion (Figure 4, right).

**Discussion**

In this study, in which we measured myocardial oxidation of glucose and LC-FA in vivo before and after birth, we showed that the myocardium of fetal lambs uses mostly glucose, whereas that of newborn lambs uses mostly LC-FA. In fetal lambs, LC-FA were not used as a substrate because they were not supplied. When we supplied LC-FA to the fetal lambs, the uptake and oxidation of LC-FA increased. Although the LV uptake and oxidation of LC-FA were still lower in fetal lambs during fat infusion than in newborn lambs, the contribution of LC-FA to LV oxygen consumption was the same in fetal and newborn lambs.

The contribution of glucose oxidation to myocardial oxygen consumption in fetal lambs was relatively high, and it fit with the expectation that glucose and lactate account for the majority of myocardial oxygen consumption before birth. The contribution of glucose to myocardial oxygen consumption decreased to 12%, which is in contrast to the expectations raised from the previous study in which we measured only the myocardial flux of glucose. The decrease in the contribution of glucose to myocardial oxygen consumption is due to both a decrease in myocardial glucose oxidation and an increase in myocardial oxygen consumption. The increase in myocardial oxygen consumption in newborn lambs reflects an increase in myocardial energy demand, which is due to 2 phenomena. First, at birth, the low-resistance placental circulation is removed, which leads to an increase in systemic vascular resistance and, hence, an increase in afterload. Second, pulmonary vascular resistance decreases, and right ventricular output is directed to the pulmonary circulation. The left and right ventricle are now connected in series instead of in parallel. Because total body oxygen consumption also increases after birth, LV output increases. This increase is accomplished, despite the increase in afterload, by an increase in heart rate and stroke volume.

A decrease in glucose oxidation after birth was also found in studies of isolated hearts from rabbits. To compare our data with those measured in the isolated heart preparations, we converted their oxidation rates, which were expressed per gram of dry weight, to oxidation rates expressed per 100 g of wet weight, assuming a dry/wet ratio of 0.2. The oxidation of glucose in isolated hearts from rabbits decreased from...
energetic pathway for the myocardium of the newborn.9 hearts, it was speculated that glycolysis might be an important metabolic pathways are used. From the study in isolated rabbits. The decrease in glucose oxidation concomitant with an increase in myocardial energy demand indicates that other regions that depend on glycolysis rather than on aerobic metabolism.

Using the data from our previous study in lambs,15 we calculated the relative contribution of glycolysis to ATP production from carbohydrates. The release of lactate was 13.6±5.6 μmol · min⁻¹ · 100 g wet weight⁻¹ in fetal lambs and 37.0±10.5 μmol · min⁻¹ · 100 g wet weight⁻¹ in newborn lambs.15 The amount of lactate released is equivalent to the same amount of ATP produced, because the glycolysis of 1 mol glucose yields 2 mol lactate and 2 mol ATP. The glycolytic rates in the isolated heart preparations are much higher (1300 μmol · min⁻¹ · 100 g wet weight⁻¹ in hearts from 1-day-old rabbits and 250 μmol · min⁻¹ · 100 g wet weight⁻¹ in hearts from 7-day-old rabbits).9

Apart from the already mentioned differences in experimental setup, differences in oxygenation may add to the differences in glycolytic rates. Under physiological conditions, oxygen is carried to the capillaries by erythrocytes, thereby limiting the fall in capillary oxygen tension throughout the tissue perfused. In contrast, in isolated heart preparations, oxygen is supplied entirely by physically dissolved oxygen; hence, the diffusion gradient of oxygen will rapidly decrease toward the venous end of the capillaries. This may lead to an increase in regions that depend on glycolysis rather than on aerobic metabolism.

An unexpected finding was the ability of the myocardium of near-term fetal lambs to use LC-FA as an energy source. LC-FA are generally believed to be the prime substrate for the adult myocardium.1 However, the myocardium of the fetus and newborn was supposed to be limited in its capacity to use LC-FA as an energy source.6,7,9,24 This limitation was assumed to be localized at the level of the transport into the mitochondria.9 From a previous study in which we only measured the arteriovenous concentration differences across the myocardium, we concluded that there was no oxidation of LC-FA by the myocardium of the fetus or newborn.11 However, the arteriovenous concentration difference does not reflect actual oxidation, because the myocardium contains a rather high amount of intracellular triglycerides that can also be used for oxidation.25 In the present study, we showed that in fetal lambs under physiological conditions, LC-FA were not used as an energy substrate by the myocardium because they were not supplied. When LC-FA were supplied to fetal lambs by the infusion of the fat emulsion, an increase in the uptake and oxidation of palmitate occurred (Figure 3). In newborn lambs, LC-FA were the prime substrate for the myocardium (Figure 2).

These results show that LC-FA can be used by the myocardium of the fetus and newborn. From these results, no firm conclusions can be drawn as to whether the ability to oxidize LC-FA by the myocardium of the newborn is limited.5 However, several arguments suggest that no such limitation exists. The relative contribution of the oxidation of LC-FA to LV oxygen consumption was the same in fetal lambs during fat infusion as it was in newborn lambs (Figure 3). This was partly due to the lower LV oxygen consumption...
in fetal compared with newborn lambs. Moreover, the LV oxidation of LC-FA significantly increased with an increase in LV oxygen consumption (Figure 4, left), suggesting that when LV energy demand increases, LC-FA oxidation also increases. The contribution of the oxidation of LC-FA to LV oxygen consumption appeared to increase with oxygen consumption, especially in the fetal lambs during fat infusion (Figure 4, right). Moreover, we did not find a significant relation between age and LC-FA oxidation in our newborn lambs. Hence, these results are in favor of the conclusion that there is no limitation in the ability to use LC-FA.

In summary, in this study in chronically instrumented fetal and newborn lambs, we showed that in fetal lambs, glucose and lactate are the major energy source for the myocardium, whereas in newborn lambs, glucose is replaced by LC-FA. We also showed that in vivo, LC-FA do not contribute to myocardial energy production in fetal lambs because they are not supplied. However, when LC-FA are supplied, they can be used by the myocardium. No limitation seems to exist in the ability of the myocardium to use LC-FA.

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