Perinatal changes in myocardial supply and flux of fatty acids, carbohydrates, and ketone bodies in lambs

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FATTY ACIDS are the major energy source for the myocardium in the adult (25), whereas lactate has been suggested to be the major energy source for the myocardium in the fetus (10). The differences in substrate use between the fetal and adult myocardium are speculated to be caused by differences in substrate supply. In the fetus the substrates supplied via the placenta are primarily the carbohydrates glucose and lactate (15). After birth, the suckling neonate is fed milk, which is composed primarily of fatty acids, so that fatty acids contribute >50% to energy intake (15). Because the myocardium is generally thought to be a scavenger, it is expected that the myocardium switches, parallel to the changes in substrate supply, from the use of carbohydrates before birth to the use of fatty acids after birth. However, it is questionable whether the myocardium of the newborn can use fatty acids as an energy source. In studies in isolated hearts from newborn pigs (2, 34) and rabbits (22) the oxidation rates of fatty acids were limited in the first days after birth. If the myocardium of the newborn cannot use fatty acids yet, carbohydrates must remain an important energy source. However, lactate supply by the placenta ceases after birth, leaving glucose as the primary energy source. Alternatively, ketone bodies could become an important energy source for the myocardium after birth (27). The concentration of ketone bodies rises shortly after birth in several species (15). It was suggested from studies in adult animals that ketone bodies are preferred to fatty acids and carbohydrates as substrates for the myocardium (7, 12).

Until now, only limited information on myocardial metabolism in vivo before or after birth was available. Fisher et al. (10) studied myocardial carbohydrate metabolism in fetal and young lambs, but they did not measure fatty acids or ketone bodies. Several investigators studied myocardial substrate uptake in isolated heart preparations from newborn pigs and rabbits (2, 22, 34). However, in those studies different sets of substrates in a wide range of concentrations were supplied. Moreover, different flow settings were used. These differences in experimental setup have led to a wide variation in myocardial substrate supply, which is the product of arterial concentration and coronary blood flow, and thus may have influenced myocardial substrate uptake.

Knowledge of myocardial metabolism around birth can lead to a better understanding of developmental physiology and can also be important, for instance, to understand how certain disorders in substrate metabolism, such as β-oxidation disorders, can lead to cardiomyopathy (18). Therefore, we studied myocardial delivery and flux of fatty acids, carbohydrates, and ketone bodies in vivo in fetal and newborn lambs. We intended to study the lambs in utero and, after delivery by caesarean section, to study them again after birth, thereby limiting the interanimal variation. For that purpose we chronically instrumented nine fetal lambs in utero. We successfully delivered only four fetal lambs; therefore, we also instrumented three newborn lambs within the first 2 days after birth. To further explore the developmental changes in myocardial substrate delivery and flux, we compared the data of the fetal and newborn lambs with those of a group of juvenile lambs that had been used as a control group in a previous study in our lab (17).

METHODS

We performed nine studies in nine fetal lambs and nine studies in seven newborn lambs of mixed western breed. Six of the studies in newborn lambs were performed in four lambs that were instrumented and studied in utero; the others were performed in three lambs instrumented after birth. If more than one study was performed in an animal, there was at least 1 day in between the two studies. We compared the data
from the fetal and newborn lambs with those from 12 7-wk-old (juvenile) lambs that participated in other studies in our lab (17). Surgical and experimental procedures were approved by the animal research committee of the University of Groningen.

Surgical procedure. The fetal and newborn lambs were instrumented as described previously (10, 11). The nino fetal lambs were instrumented at 125–126 days of gestation (term is 145 days). Briefly, the fetal lamb was exposed via a maternal midline laparotomy. Via the brachial artery and vein, we inserted polyvinyl catheters (ID 0.3 mm, OD 0.5 mm) into the ascending aorta and superior caval vein. Via a left thoracotomy we inserted catheters into the coronary sinus and left atrium. We also inserted catheters into the carotid artery and the jugular vein. A catheter was placed in the amniotic cavity for zero-pressure reference. All vascular catheters were filled with heparin sodium solution (500 IU/ml) and sealed. Antibiotics (gentamicin 80 mg, 1 cm) were administered at 80°C pending determination of the substrate concentration. After the last experiment the ewes and lambs were killed with an overdose of intravenous pentobarbital sodium. The fetal lambs were weighed, and this weight was used as body weight throughout the experiments. The heart and cerebral hemispheres were removed and weighed. The left ventricular wall was separated from the rest of the heart and was weighed. Radioactivity in the removed tissues was determined in a Packard 5550 gamma counter (Packard Instrument, Meriden, CT). Organ blood flows were calculated with the aid of a special computer program. Blood flows are expressed in milliliters per minute per 100 g of wet weight. Adequate mixing of microspheres was achieved by ascertaining that blood flow per 100 g of tissue of the two cerebral hemispheres did not differ by >10% (19). Oxygen delivery to organs was calculated as the product of arterial oxygen concentration and blood flow to that organ.

The concentrations of glucose, lactate, pyruvate, β-hydroxybutyrate, and acetoacetate were determined in duplicate in whole blood by enzymatic methods as described previously (17). For that purpose, the blood collected for substrate concentrations was transferred immediately to a tube containing a dash of NaF to stop glycolysis, mixed, and kept on ice. The concentrations of free fatty acids, total glycerol, and free glycerol were determined in duplicate in plasma as described previously (17). For these assays blood was mixed with NaF and centrifuged, and the plasma was removed and stored at −80°C pending determination of the substrate concentration. The intra-assay coefficients of variation for the assays for free glycerol, total glycerol, and free fatty acids were 0.66, 0.55, and 0.52%, respectively (6–15).

Because coronary sinus blood of lambs consists predominantly of venous blood from the left ventricle, we calculated oxygen consumption of the left ventricular free wall as the product of aorta-coronary sinus (A-CS) oxygen concentration difference and blood flow to the left ventricular free wall, obtained with the radionuclide-labeled microspheres. Left ventricular oxygen delivery was calculated as the product of arterial oxygen concentration and blood flow to the left ventricular free wall. Because A-CS difference of the substrates reflects the balance between substrate uptake and substrate release, we calculated substrate flux and not uptake. Myocardial substrate flux is calculated as the product of A-CS difference and blood flow to the left ventricular free wall. The relative contribution of a substrate to myocardial oxygen consumption, which is known as the oxygen extraction ratio, was calculated as the ratio of the A-CS difference of that substrate and the A-CS difference of O2 multiplied by a factor k, where k is the number of moles of oxygen necessary for combustion of that substrate. The value of k for glucose is 6, for lactate 3, for pyruvate 2.5 (data not shown), for β-hydroxybutyrate 4.5, for acetoacetate 4, for free fatty acids 25 (it is assumed that palmitate accounts for most of the free fatty acids), and for triglycerides 75. If the A-CS difference of a particular substrate was negative (indicating release of a substrate), the oxygen extraction ratio was assumed to be zero. The data for β-hydroxybutyrate and acetoacetate are pooled, and these values are presented together as values for ketone bodies.

Statistical analysis. Data are presented as means ± SE. Differences among the three groups (fetal, newborn, and juvenile lambs) were tested by ANOVA. Post hoc Newman-Keuls test was used to detect the differences. A P value < 0.05 was considered significant.
RESULTS

On the day of the study heart rate, blood pressure, oxygen saturation, and pH (Table 1) were normal in all lambs. The differences in baseline variables were related to the age of the lambs. Thus heart rates were lower in the fetal lambs than in the newborn lambs but higher than in the juvenile lambs. Arterial pressure, oxygen saturation, oxygen concentration, and pH were lower in the fetal lambs than in the newborn and juvenile lambs (Table 1). Blood flow to the left ventricle was the same in fetal and in newborn lambs but higher than in the juvenile lambs. Left ventricular oxygen delivery was highest in the newborn lambs. A-CS difference was significantly increased 10-fold in the newborn and juvenile lambs. Myocardial oxygen extraction and oxygen consumption were not significantly different among the three groups (Table 1).

The arterial concentration of free fatty acids was extremely low in the fetal lambs (Table 2) but was increased 10-fold in the newborn and juvenile lambs. Despite the increase in arterial concentration, A-CS difference did not change in the newborn lambs. In the juvenile lambs, A-CS difference was significantly increased compared with the newborn lambs. Only in this group was A-CS difference significantly different from zero (Fig. 1). Myocardial flux of free fatty acids was almost zero in the fetal and newborn lambs (Fig. 2A) but was increased in the juvenile lambs, although these changes did not reach statistical significance.

Arterial concentration of triglycerides was also very low in the fetal lambs, was higher in the newborn lambs, and was even negative in the newborn lambs (Fig. 2B). Flux of triglycerides was very low in both fetal and juvenile lambs and even negative in the newborn lambs (Fig. 2B).

Arterial concentration of glucose was highest in the newborn lambs and lowest in the fetal lambs (Table 2). A-CS difference did not change in the newborn lambs compared with the fetal lambs but appeared to be lower in the juvenile lambs. The same was true for the flux of glucose (Fig. 2C), although these changes did not reach statistical significance.

Arterial concentration of lactate was the same in the newborn as in the fetal lambs but was lower in the juvenile lambs (Table 2). A-CS difference was the same in the newborn as in the fetal lambs and was almost zero in the juvenile lambs. There was no detectable lactate flux in the juvenile lambs, as opposed to the fetal and newborn lambs (Fig. 2D).

Arterial concentrations of the ketone bodies increased with age and were highest in the juvenile lambs (Table 2). A-CS difference and myocardial flux

Table 2. Aortic concentration and myocardial A-CS difference of all substrates in chronically instrumented fetal, newborn, and juvenile lambs

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Fetal</th>
<th>Newborn</th>
<th>Juvenile</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Fatty acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-CS concn</td>
<td>0.02±0.00</td>
<td>0.36±0.07†</td>
<td>0.35±0.06†</td>
</tr>
<tr>
<td>A-CS</td>
<td>0.00±0.00</td>
<td>0.01±0.01</td>
<td>0.06±0.02*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-CS concn</td>
<td>0.03±0.01</td>
<td>0.46±0.13†</td>
<td>0.23±0.05*</td>
</tr>
<tr>
<td>A-CS</td>
<td>0.01±0.01</td>
<td>−0.03±0.03</td>
<td>0.01±0.01</td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-CS concn</td>
<td>1.07±0.09</td>
<td>5.62±0.32†</td>
<td>3.52±0.12†</td>
</tr>
<tr>
<td>A-CS</td>
<td>0.12±0.02</td>
<td>0.15±0.06</td>
<td>0.07±0.05</td>
</tr>
<tr>
<td>Lactate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-CS concn</td>
<td>1.30±0.19</td>
<td>1.44±0.12</td>
<td>0.82±0.08†</td>
</tr>
<tr>
<td>A-CS</td>
<td>0.39±0.04</td>
<td>0.26±0.06</td>
<td>−0.01±0.03†</td>
</tr>
<tr>
<td>Ketone bodies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-CS concn</td>
<td>0.09±0.02</td>
<td>0.17±0.05</td>
<td>0.49±0.06†</td>
</tr>
<tr>
<td>A-CS</td>
<td>0.03±0.01</td>
<td>0.07±0.03</td>
<td>0.17±0.02†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of studies. Concentrations are in mmol/l. *P < 0.05 vs. newborn lambs; † P < 0.05 vs. fetal lambs.
also gradually increased with age (Fig. 2E). The extraction coefficient of the ketone bodies was the same in the three age groups (30 ± 9, 28 ± 11, and 37 ± 3% in fetal, newborn, and juvenile lambs, respectively).

To estimate the possible contribution of all substrates to myocardial oxygen consumption, we calculated the oxygen extraction ratio assuming that all of a substrate taken up was completely oxidized. Figure 3 shows that lactate accounts for most of the myocardial oxygen consumption in the fetal lambs, whereas glucose is second best. In the newborn lambs glucose and lactate are still the major oxygen consumers, but in the juvenile lambs the free fatty acids and triglycerides have taken over.

**DISCUSSION**

In this study in chronically instrumented fetal, newborn, and juvenile lambs we show that despite an increase in supply of fatty acids to the myocardium there is no net flux of fatty acids across the myocardium in the first week after birth. Instead, carbohydrates remain the major energy source for the myocardium in the first week after birth as they are in utero, because the flux of the carbohydrates glucose and lactate is the same in the newborn lambs as in the fetal lambs. In the juvenile lambs carbohydrates are eventually being replaced by fatty acids and ketone bodies. In that group there is a decrease in both supply and flux of carbohydrates and an increase in flux of fatty acids and ketone bodies. Thus, after birth, the myocardium does not immediately switch from the use of carbohydrates in utero to the use of fatty acids.

Fatty acids are generally believed to be the preferred substrate for the adult myocardium (25). That the myocardium of the fetal lamb does not use fatty acids can be explained by the lack of supply, because the arterial concentration of fatty acids is extremely low in
the fetal lambs (Table 2). However, it is unclear why there is no uptake of fatty acids by the myocardium of the newborn lamb although they are supplied in abundance. There are two possible explanations.

The first explanation is that the supply of other substrates attenuates the uptake of fatty acids. The high carbohydrate supply in the first week after birth can interfere with fatty acid oxidation. An inverse relation between carbohydrate supply and fatty acid oxidation has been described in myocytes (4, 28), isolated hearts (12, 32), and hearts of anesthetized dogs (9). In our study we did not find a relation between lactate supply and fatty acid flux. We did find an inverse relation between glucose delivery and fatty acid uptake. This was described by factors other than glucose delivery. That we did not find clues for substrate interaction in our study can be caused by either differences in substrate supply or differences in age. In the aforementioned studies (4, 9, 12, 28, 32) the concentration of lactate was higher and that of fatty acid lower than in our study. In the study by Drake et al. (9) lactate concentrations ranged from 0.8 to 10.6 mmol/l. Forsery et al. (12) added 5 mmol/l of glucose or lactate to the perfusate, in which the added lactate concentration was 0.1 mmol/l. In contrast, the lactate concentration in our study ranged from 0.5 to 2.6 mmol/l and the free fatty acid concentration ranged from 0 to 0.9 mmol/l. Thus, at physiological substrate concentrations, we do not have an indication that the high carbohydrate supply interferes with oxidation of fatty acids. Lopachuk et al. (23) suggested that immediately after birth high levels of malonyl-CoA suppress fatty acid uptake via inhibition of carnitine palmitoyltransferase (CPT)-I, an enzyme necessary to transport fatty acids into the mitochondrion, where they are oxidized. The authors also suggest that this inhibition is abolished during the neonatal period by a decrease in malonyl-CoA levels caused by a decrease in insulin levels. However, the isoform of CPT-I present in the heart (CPT-Ib) is extremely sensitive to malonyl-CoA inhibition compared with the isoform present in the liver (24). Because of this sensitivity to malonyl-CoA inhibition, CPT-Ib activity is expected to be completely blocked at the intracellular concentrations of malonyl-CoA measured in vitro (1, 26). Therefore, it has recently been speculated that fatty acid oxidation is not regulated by malonyl-CoA but rather by other intracellular intermediaries such as acetyl-CoA (1, 26).

The second explanation is that the enzymes necessary to transport or to oxidize fatty acids are immature. Age-related differences in enzyme expression and activity have been described in tissues from several species (15). Three steps are necessary before fatty acids can be oxidized in the mitochondrion. They must be transported into the cell, activated to their CoA ester in the cytoplasm, and transported into the mitochondrion. Several enzymes are necessary for these steps. Fatty acids can cross membranes both by simple diffusion or by a carrier-mediated process (20, 33). Three possible transporters for fatty acids have been isolated so far (29–31), but little is known about the perinatal expression and activity of these transporters. However, in previous studies, increased amounts of intracellular fatty acyl-CoA have been found despite reduced fatty acid oxidation rates (4, 35). Therefore, transport into the cell and activation in the cytoplasm of fatty acids were not considered to be rate limiting. Because fatty acids are oxidized in the mitochondria, they must be transferred across the mitochondrial membranes. This occurs via a transport system that consists of CPT-I located on the outer mitochondrial membrane, carnitine acylcarnitine translocase (CAC) located between the mitochondrial membranes, and CPT-II located on the inner membrane. There is no information available on either expression or activity of the CAC. CPT-I activity increased in the first week after birth in isolated mitochondria from rat hearts (6) but not in rabbit hearts (23). In the rat hearts there was a switch in CPT-I expression after birth, from the liver-specific form (CPT-Ia) to the muscle-specific form (CPT-Ib) (6). An age-dependent enzyme expression of CPT-II has been described in rat (39) and mouse (13) hearts. CPT-II activity also increases immediately after birth in isolated mitochondria from rat hearts (6). The capacity to oxidize fatty acids also increases with age in isolated mitochondria from rats and pigs (16, 38).

Thus developmental changes in the capacity to transport or to oxidize fatty acids in the mitochondrion can possibly explain the lack of myocardial flux of fatty acids in the newborns. More studies on enzyme expression and activity in the developing heart are necessary to elucidate the mechanisms involved in the development of myocardial fatty acid metabolism. Knowledge of myocardial metabolism in the newborn as well as in the fetus can be useful to explain how disorders in long-chain fatty acid metabolism lead to cardiomyopathy (18). If uptake into the mitochondrion rather than oxidation is the limiting factor, it is expected that medium-chain fatty acids can be used shortly after birth, because they can pass the mitochondrial membrane without the CPT system. However, the regular diet contains little medium-chain fatty acids. Both in humans (3) and in our lambs, most of the fatty acids supplied are long-chain fatty acids (using a gas chromatograph analysis we found no significant amounts of fatty acids shorter than 16 carbon atoms in the blood of our lambs). Thus these medium-chain fatty acids would have to be added separately to the diet. Therefore, studies are necessary to evaluate the effect of addition of medium-chain fatty acids to the diet on myocardial metabolism and heart function in children with long-chain fatty acid oxidation disorders.

This study provides indirect evidence that not all of the substrates taken up are immediately and completely oxidized. This is demonstrated by the fact that the sum of the oxygen extraction ratios is well above 1 in the fetal and newborn lambs (Fig. 3). From studies in
the adult human myocardium it is known that only 20% of the glucose taken up by the myocardium is immediately and completely oxidized (36) and 13% is converted into lactate. Moreover, of the free fatty acids only 85% of the uptake was immediately oxidized (37). Therefore, it is possible that the contribution of glucose and fatty acids to oxygen consumption is overestimated in all groups. Irrespective of the method of calculation used, lactate accounts for the majority of myocardial oxygen consumption in the fetal lamb. Unexpectedly, lactate continues to do so in the first week after birth. The arterial lactate concentration was also the same in the newborn lambs as in the fetal lambs (Table 2), despite the fact that lactate production from the placenta had stopped. In other studies in newborn lambs (11) and infants (5, 8), lactate concentrations were found that were similar to those of our newborn lambs. We therefore assume that the lactate concentrations in our newborn lambs are physiological. The relatively high lactate concentration in the normal newborn is unexplained. We speculate that it might be caused by lower removal by the liver due to lower gluconeogenic capacity in the newborn.

Glucose appears to be of lesser importance for myocardial ATP production than lactate both before and after birth. The myocardial flux we measured in our fetal lambs was the same as reported previously by Fisher et al. (10), but the glucose flux in their newborn lambs (18 ± 9 µmol·min⁻¹·100 g⁻¹, mean ± SE; Ref. 11) was even lower than in ours, despite a similar supply of glucose. These differences can be explained by the difference in age range between the two studies. They studied newborn lambs ranging from 4 to 25 days, whereas we studied newborn lambs from 1 to 4 days. The relatively small contribution of glucose to energy production in these studies is in contrast with a previous study from Lopaschuk et al. (22) in isolated rabbit hearts. They found that immediately after birth 44% of ATP production was derived from glycolysis and 18% from glucose oxidation; thus >60% of ATP production was derived from glucose. However, in that study 11 mmol/l of glucose were added to the perfusate. This concentration is much higher than the physiological concentration of glucose obtained in our study. These differences in substrate concentration can account for the differences in substrate use between the studies. Care should be taken when comparing data obtained from studies in isolated, perfused hearts with those from in vivo experiments because of the differences in experimental setup, such as substrate supply, and hormonal and neuroendocrine influences.

A possible limitation of this study is that we measure the A-CS difference of the substrates. This value represents the balance between uptake and release of a substrate and not uptake alone. Myocardial release of fatty acids has been described in the fasting adult (21, 37). The released free fatty acids can be derived from hydrolysis of triglycerides in adipocytes surrounding the heart, endothelial cells, or myocytes. However, in our group of newborn lambs the flux of fatty acids was −0. If this is the result of a balanced uptake and release, it is highly unlikely that fatty acids are being used to a significant extent. Only in the juvenile lambs is there a positive flux of free fatty acids (Fig. 1A), which can underestimate the real uptake. Release of glucose by the myocardium is rare. The myocardium cannot convert glucose 6-phosphate into glucose because it does not have the enzyme glucose 6-phosphate dehydrogenase. Theoretically, small amounts of glucose can be formed from glycogen by the enzyme α-glucosidase in lysosomes; however, it is unlikely that the quantities involved with this process interfere with our data. Release of lactate simultaneously with uptake was described by Gertz et al. (14) in human adult myocardium. In that study lactate release was −40% of uptake. If this also occurred in our study, we underestimate the contribution of lactate to the energy production. Further studies using labeled substrates are necessary to separate uptake and release of, especially, fatty acids and lactate and to determine how much of the substrate taken up is completely oxidized.

The role of ketone bodies in myocardial metabolism immediately after birth remains to be established. Ketone bodies are suggested to be an alternative energy source for many tissues (27). Several investigators have even thought that ketone bodies are the preferred substrate for the myocardium (7, 12). However, in those studies high doses of ketone bodies were supplemented, ranging from 1 to 5 mmol/l. In our study the physiological concentration of ketone bodies was much lower, ranging from 0.1 to 0.5 mmol/l. Both the arterial concentration and the A-CS difference of ketone bodies increase with age in our study. The increase in supply of ketone bodies can be an age-related difference or a species-specific phenomenon, the latter because ruminants produce more short-chain fatty acids in the stomach. It is noteworthy that the extraction coefficient for ketone bodies was the same in all three age groups, indicating that the uptake of ketone bodies is limited by the supply. This is further confirmed by the close correlation between the arterial concentration and the A-CS difference (Y = 5.34 + 0.34X; P < 0.001; r² = 0.791). Thus it appears that the role of ketone bodies in myocardial metabolism after birth is limited under physiological conditions but can become important when ketosis develops, as in fasting. Children who do not produce ketone bodies during fasting, as in fatty acid oxidation disorders, often present with acute cardiorespiratory failure (18). This acute failure could possibly be caused by an energy deficit, because neither glucose nor fatty acids or ketone bodies are present. Alternatively, intracellular accumulation of intermediates may disturb myocardial metabolism. More knowledge of the role of ketone bodies in myocardial metabolism is necessary to elucidate the mechanism responsible for acute cardiac failure during fasting in children with fatty acid oxidation disorders.

In conclusion, we show in this study that despite an increase in supply of fatty acids to the myocardium there is no net myocardial flux of fatty acids in the first week after birth. Instead, carbohydrates remain the major energy source for the myocardium in the first
week after birth, as they are in utero. Carbohydrates are eventually being replaced by fatty acids and ketone bodies. Thus it appears that the myocardium does not immediately switch from the use of carbohydrates in utero to the use of fatty acids after birth. Further studies are necessary to elucidate the mechanism involved in the development of myocardial fatty acid oxidation. This knowledge can lead to a better understanding of developmental physiology and can also be important, for instance, to understand how certain disorders in fatty acid oxidation can lead to cardiomyopathy.

This work was supported by the Netherlands Heart Foundation (94.149).

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Received 1 December 1997; accepted in final form 20 February 1998.

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