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Research Article

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Simultaneous Measurements of Lactate Turnover Rate and Umbilical Lactate Uptake in the Fetal Lamb

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ABSTRACT Lactic acid represents a major exogenous nutrient for the developing fetal lamb in utero. Our study was undertaken (*a*) to quantitate the net consumption of lactate by the fetus, (*b*) to quantitate the net lactate production and metabolism by the placenta, and (*c*) to compare the net fetal lactate consumption with fetal lactate use, measured simultaneously with radioactive tracers. 14 pregnant sheep were prepared with catheters in the maternal femoral artery and uterine vein and in the fetal aorta and umbilical vein. By simultaneous application of the Fick principle to the uterine and umbilical circulations, placental glucose consumption and placental lactate production were rapid, averaging 39.8±5.1 and 11.8±0.7 mg·min^{-1}. Net lactate umbilical uptake averaged 1.95±0.16 mg^{-1}·kg^{-1}·min^{-1}. During infusion of L-[^{14}C(U)]lactate, fetal lactate turnover was much more rapid, averaging 6.5±0.8 mg·kg^{-1}·min^{-1}, and lactate utilization within the anatomic fetus was 5.9±0.7 mg·kg^{-1}·min^{-1}. During infusion of tracer glucose, endogenous fetal lactate production from glucose and nonglucose substrates averaged 3.0 and 1.5 mg·kg^{-1}·min^{-1}, respectively. The present studies have quantitated under well oxygenated, steady-state conditions, the rapid placental metabolism and production of lactate, the net fetal consumption of lactate, and the rapid endogenous fetal lactate production from glucose and nonglucose substrates.

INTRODUCTION

Lactate, glucose, and amino acids are the major exogenous nutrients consumed by the fetal lamb, providing calories, carbon, and nitrogen for fetal growth and oxidative metabolism (1, 2). That amino acids and glucose are major exogenous nutrients for the fetus is not surprising, given the net accretion of protein during fetal growth and the apparent requirement for glucose by fetal organs, most notably brain (3). However, the physiologic significance of a large exogenous supply of lactate has received comparatively little attention (4, 5).

Earlier reports in several species had noted that the concentration of lactate in fetal blood is normally higher than in the mother, and concluded that fetal metabolism may be anaerobic (6–8). More recent studies in the chronically catheterized fetal lamb and calf (4, 5, 9), while confirming a higher fetal than maternal lactate concentration, have noted that the lactate concentration is higher in the fetal umbilical vein than in the fetal artery. Furthermore, the lactate concentration was noted to be higher in the uterine vein than in the maternal artery. Such observations imply net production of lactate by the uteroplacenta, with net consumption of this lactate both in the fetus and in the mother.

Our study was designed to quantitate separately the metabolism of lactate by the fetus and by the uteroplacenta, under in vivo steady-state conditions. Simultaneous measurements of lactate concentrations and uterine and umbilical blood flows were used to calculate net substrate fluxes into the umbilical and uterine circulations. Fetal lactate use was measured by tracer infusion into the fetus, allowing simultaneous estimation of endogenous fetal lactate production.

GLOSSARY

a	Fetal artery (as subscript)
A	Maternal artery (as subscript)
C	Concentration (as superscript) in mg·ml^{-1}
D	dpm·ml^{-1} (as superscript)
G	Glucose
GOQ	Glucose oxygen quotient
K_1	Constant defining distribution of production of fetal lactate from glucose and nonglucose substrates during infusion of tracer glucose (see text)
L	Lactate
LOQ	Lactate oxygen quotient

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LPR	Total lactate production rate within the fetus
LPR^G	Lactate production rate from glucose within the fetus
LPR^G_{min}	Minimum lactate production rate within the fetus necessary to account for specific activity relationships during tracer glucose infusion (see text)
LPR^{NG}	Lactate production rate from nonglucose substrates within the fetus
LTR	Lactate turnover rate
LUU	Lactate umbilical uptake
O	Oxygen
$\dot{Q}_f$	Umbilical blood flow ($ml \cdot min^{-1}$)
$\dot{Q}_F$	Uterine blood flow ($ml \cdot min^{-1}$)
S	Specific activity in $dpm \cdot mg^{-1}$ (as superscript)
v	Fetal umbilical vein (as subscript)
V	Maternal uterine vein (as subscript)

METHODS

Surgical preparation and general protocol. 14 Western-breed ewes were studied at a gestational age of 125–135 d. Surgery was carried out under pentobarbital sedation (5 $mg \cdot kg^{-1}$) and pontocaine spinal anesthesia (6 mg in 10% glucose). Polyvinyl catheters were placed in the fetal umbilical vein, fetal pedal artery, fetal pedal vein, maternal femoral artery, and maternal uterine vein as described (10, 11). The fetal pedal arterial catheter was advanced into the descending aorta to sample the blood supplying the umbilical artery. The catheter in the fetal umbilical vein was positioned before the portal sinus to sample the blood flowing through the common umbilical vein. Fetal pedal venous catheters were placed for infusions. These catheters were advanced to the level of the femoral vein. Animals were allowed to recover from surgery for at least 6 d, and free access to water and food (oats, alfalfa pellets, and hay) was permitted except as noted. Food intake of each animal was qualitatively monitored by weighing the feed and estimating the consumption of hay.

The study protocol compared measurements of fluxes of lactate, glucose, and oxygen with simultaneous measurements of lactate metabolism made with ^3H and ^{14}C tracers. A primed, constant infusion technique was used for both antipyrine and tracer infusions. A priming dose of antipyrine and of tracer was given through a fetal pedal vein, followed by constant infusion of antipyrine and tracer by pump, into the pedal vein. After a 60-min equilibration period whole blood samples were taken simultaneously from the fetal artery, umbilical vein, maternal artery, and maternal uterine vein at times of 60, 90, 120, and 150 min after the beginning of the infusions. Blood samples were analyzed for concentrations of glucose, lactate, oxygen, and antipyrine, and for specific activities of glucose, lactate, and CO_2.

Tracer infusions. 8 of the 14 fetuses were infused simultaneously with D-[Y-^{14}C]glucose and D-[X-^3H]glucose, obtained from Amersham Corp., Arlington Heights, IL. Tracer glucose (11) was labeled at carbon "X" and "Y", as detailed in Table III. Infusions used a primed, steady-state infusion technique (11). During continuous infusion of tracer glucose, steady-state conditions prevailed for specific activities of both glucose and lactate between 60 and 150 min of infusion.

The remaining six fetuses were infused with L-[^{14}C(U)]lactate, obtained from New England Nuclear (Boston, MA). Five of these six fetuses were infused simultaneously with D-[X-^3H]glucose. To determine the priming dose and rate of continuous infusion of L-[^{14}C(U)]lactate, a single pilot experiment was performed, in which a bolus of L-[^{14}C(U)]lactate was injected acutely. The volume of distribution of 600 $ml \cdot kg^{-1}$ fetal weight and the turnover rate of 6.5 $mg \cdot kg^{-1} \cdot min^{-1}$ were in approximate agreement with other reports (16). Using these values, the priming dose of tracer and the constant tracer infusion rate were chosen to obtain steady-state conditions rapidly during the infusion periods. In a typical experiment, the lactate specific activities in each vessel at each time point were within 5% of the mean specific activity for that vessel over the study period. Additionally, the relationships between the specific activities of lactate in the vessels sampled remained constant over the infusion periods.

Analytical methods. Whole blood was collected in iced syringes and immediately deproteinized for glucose analysis using a glucose oxidase method (12). Whole blood was collected in syringes frozen in ice, immediately deproteinized in perchloric acid, and assayed for lactate concentration using a fluorometric method (13). Whole blood was collected in iced, NaF-coated capillary tubes, and analyzed immediately for O_2 content using a Lex-O_2-Con (Lexington Instruments Corp., Waltham, MA). CO_2 was measured with a van Slyke apparatus. Antipyrine was measured in whole blood with a Technicon Auto Analyzer (Technicon Instruments Corp., Tarrytown, NY) (14).

Specific activities of glucose were measured as described (11). Lactate specific activity was determined on plasma, using the following modification of the method of Rognstead and Katz (17). A Dowex 1 × 8 (chloride) column (3 × 70 mm) was washed sequentially with 4 vol 1 N NaOH and 6 vol 1 N NaF, converting it to fluoride form. Blood was collected in iced syringes coated with EDTA and NaF, centrifuged, and immediately separated into plasma and cellular fractions. A volume of 200 μl plasma was then applied directly to the column without deproteinization. A wash of 2 vol 1 N NaF and 6 vol distilled demineralized water removed >99.9% of the protein and neutral compounds, including glucose and alanine. The column retained >98% of the lactate, which was then removed with 4 vol sequential washes of 0.3 N acetic acid and 2 N acetic acid. Approximately 85% of the lactate was recovered in the 2 N acetic acid fraction. Two-dimensional paper chromatography (18) was used in initial studies to confirm that >98% of the radioactivity in the 2 N acetic acid fraction was lactate. An aliquot of the 2 N acetic acid fraction was used for liquid scintillation counting and a second aliquot of this fraction was analyzed for lactate concentration. The ratio of disintegrations per minute and lactate concentration in this fraction yielded the specific activity of lactate. A plasma sample at each time point from each vessel was applied to at least three columns; thus, the mean steady-state lactate specific activity in each vessel was based on at least 12 determinations. Intraanimal variability was examined using replicate sample two-way analysis of variance. Although the differences in lactate concentration and in specific activity between the umbilical venous and fetal arterial samples were of small magnitude, these differences were significant to at least a level of $P < 0.05$. Intrareplicate variability typically contributed <5% to the total observed variance in these assays. The revised method combined the advantages of ease of processing large numbers of samples simultaneously, high specificity, and high precision.

Calculation of isotope present as lactate in whole blood ($dpm\text{-}lactate \cdot ml^{-1}$) was calculated as the product of lactate specific activity and the simultaneously measured whole blood lactate concentration in each vessel. Because lactate is generally considered to be freely diffusible across eryth-

rocyte membranes, the plasma specific activity was considered a reasonable estimate of whole blood specific activity for purposes of estimation of umbilical loss of dpm-lactate. The measured whole blood lactate concentrations were not significantly different from the whole blood lactate concentrations estimated from the measured lactate concentrations from plasma samples, supporting the assumption of free diffusion between erythrocyte and plasma lactate.

Symbolism. A standardized system of symbolism was adopted for description of the experimental findings and calculations. Conventions designed for kinetic analysis (19) were modified to include additional information required for analysis of these continuous infusion experiments. The modifications permit identification of the chemical compound analyzed and identification of the location sampled. It should be noted that this location is anatomic and need not represent a compartment in the kinetic sense.

According to this nomenclature, the capital letters L, O, and G represent lactate, oxygen, and glucose, respectively. The superscripts C, D, and S represent respectively "concentration" in $mg \cdot ml^{-1}$, "dpm" in $dpm \cdot ml^{-1}$, and specific activity in $dpm \cdot mg^{-1}$. The subscript refers to the vessel sampled, with a, v, A, and V referring to the fetal artery, umbilical vein, maternal artery, and maternal uterine vein, respectively, e.g., L_v^D = dpm-lactate $\cdot ml^{-1}$ in the umbilical vein. The symbols $\dot{Q}_F$ and $\dot{Q}_f$ refer to uterine and umbilical blood flow, respectively. Additional special symbols are listed in the Glossary.

Calculations. Uterine and umbilical blood flows were calculated from the antipyrine data according to the method of Meschia et al. (14). The Glucose Oxygen Quotient (GOQ) and Lactate Oxygen Quotient (LOQ) were calculated from the whole blood concentrations of glucose, lactate, and oxygen according to the formulae given in Table I. The GOQ and LOQ represent the fractions of the fetal oxygen consumption that could be accounted for if glucose or lactate were completely oxidized to carbon dioxide (1, 4, 15). Net substrate flux leaving the uterine circulation was calculated as the product of the maternal arteriovenous whole blood concentration difference times the uterine blood flow, and net substrate flux entering the fetus was calculated as the product of the umbilical venous-fetal arterial whole blood concentration difference times the umbilical blood flow (Table I). The net placental production or consumption of each substrate was taken as the difference between the uterine and umbilical substrate fluxes.

Tracer infused into the fetus may leave via the umbilical circulation (11), which represents a nonmetabolic irreversible loss of tracer. The amount of tracer leaving the fetus via the umbilical circulation was calculated as the product of the umbilical blood flow times the measured concentration difference of dpm-lactate, Eq. 11. The fetal Lactate Utilization Rate (LUR) corrects for this extrafetal nonmetabolic loss of lactate and represents the amount of lactate irreversibly consumed within the anatomic fetus in order to maintain the lactate specific activity measured in the umbilical artery. Eq. 13 calculates the LUR, which is derived in the accompanying Appendix. The use of the L_a^S specifies that both turnover rate and LUR represent the values as estimated by an observer in the umbilical arterial plasma (Appendix). Lactate Turnover Rate (LTR) was calculated according to Eq. 14 in Table I for steady-state infusion of isotope.

Two additional calculations were made from data in experiments where tracer glucose was infused and are described in the Appendix. The minimum lactate production rate from glucose (LPR^G_{min}) was calculated according to Eq. 18 and represents the minimum amount of lactate that must be produced from glucose in order to account for the observed concentrations of tracer and tracee lactate in the umbilical circulation. The actual production rate of lactate from glucose (LPR^G) and nonglucose substrates (LPR^{NG}) was calculated from Eqs. 17 and 20 in experiments in which the LUR was estimated as detailed in the Appendix.

Statistics. Determination of statistical significance used the Student's *t* test unless otherwise noted. Paired analysis was used where appropriate. In these calculations for each animal, the mean value of the replicate samples of a given determination (e.g., glucose concentration, lactate specific activity) in each vessel were entered as a single data point. Results are expressed as mean±SEM unless otherwise noted. Regression analyses used a standard least squares method.

RESULTS

Net uterine substrate fluxes. Maternal arterial and uterine venous catheters were maintained chronically in 11 pregnant ewes in late gestation. Concentrations of lactate, glucose, and oxygen were measured in whole blood samples from these catheters, and the net flux of these substrates exiting the uterine circulation was calculated by the Fick principle (Table II).

The 11 pregnant ewes were well oxygenated, with arterial oxygen concentrations averaging 5.88±0.15 mM. Maternal arterial lactate concentrations averaged 0.650±0.031 mM. In each of these pregnant ewes, arterial lactate concentration was less than the maternal venous lactate concentration (Fig. 1, $P < 0.01$) and net maternal lactate uptake averaged 4.97±0.67 $mg \cdot min^{-1}$. There was no significant relationship between maternal arterial lactate concentration and net maternal lactate uptake.

Net glucose exit from the maternal circulation averaged 54.3±6.3 $mg \cdot min^{-1}$ in the seven well fed animals. The remaining four had substantially lower glucose concentrations (Table II), resulting in a lower net uterine glucose uptake. Net uterine oxygen consumption was not significantly different in the two subgroups.

Net fetal substrate fluxes. Fetal arterial and umbilical venous catheters were maintained chronically in 14 fetuses. Concentrations of lactate, glucose, and oxygen were measured in blood samples from these vessels, and the net flux of substrates entering the fetus was calculated by the Fick principle (Table II).

Each of the 14 fetuses was well oxygenated, with umbilical venous oxygen concentrations averaging 5.23±0.16 mM. Net fetal oxygen consumption was 0.299±0.020 $mmol \cdot kg^{-1}$. Fetal arterial and venous lactate concentrations were each higher than the maternal levels ($P < 0.001$, paired) and umbilical venous lactate concentration was consistently higher than the fetal arterial concentration ($P < 0.001$, paired; Fig. 1).

Umbilical venous glucose concentration was in the normal fetal range for the 10 fetuses of normoglycemic ewes. Net glucose uptake via the umbilical circulation

TABLE I

Equations Used in Calculation of Substrate Flux and Turnover

Calculations involving substrate fluxes

1. Net fetal lactate uptake = $\dot{Q}_f(L_v^C - L_a^C)$
2. Net uterine lactate uptake = $\dot{Q}_F(L_V^C - L_A^C)$
3. Net placental lactate production = (Eq. 1) + (Eq. 2)
4. Net fetal glucose uptake = $\dot{Q}_f(G_v^C - G_a^C)$
5. Net uterine glucose loss = $\dot{Q}_F(G_A^C - G_V^C)$
6. Net placental glucose consumption = (Eq. 5) − (Eq. 4)
7. Glucose-oxygen quotient = $\dfrac{6 \times (G_v^C - G_a^C)}{(O_v{}^C - O_a{}^C)}$ (concentrations in mM)
8. Lactate-oxygen quotient = $\dfrac{3 \times (L_v^C - L_a^C)}{(O_v{}^C - O_a{}^C)}$ (concentrations in mM)

Calculations during tracer lactate infusion

9. Rate of dpm-lactate entering fetus via umbilical vein = $\dot{Q}_f \times L_v^D$
10. Rate of dpm-lactate leaving fetus via umbilical artery = $\dot{Q}_f \times L_a^D$
11. Net rate of extrafetal loss of dpm-lactate = (Eq. 10) − (Eq. 9)
12. Net irreversible loss of dpm-lactate within the Fetus = (rate of infusion Tracer Lactate) − Eq. 11
13. Fetal lactate utilization rate = (Eq. 12) ÷ (L_a^S)
14. Fetal lactate turnover rate = (rate of infusion tracer lactate) ÷ (L_a^S)

Calculations during tracer glucose infusion

15. Lactate specific activity = $\dfrac{\text{total rate of entry of dpm-lactate}}{\text{total rate of entry of lactate}}$
16. Total rate of entry of dpm-lactate $(\text{dpm} \cdot \text{min}^{-1}) = (LPR^G)(G_a^S) + (Q_f)(L_{v\text{-}a}^D)$
17. Total rate of entry of lactate $(\text{mg} \cdot \text{min}^{-1}) = (\dot{Q}_f)(L_{v\text{-}a}^C) + LPR^G + LPR^{NG}$
18. $LPR_{min}^G = \dfrac{(L_a^S)(\dot{Q}_f)(L_{v\text{-}a}^C) - (\dot{Q}_f)(L_{v\text{-}a}^D)}{(G_a^S) - (L_a{}^S)}$
19. $K_1 = \dfrac{(L_a^S)}{(G_a^S) - (L_a^S)}$
20. $LPR^G = LPR_{min}^G + (K_1)(LPR^{NG})$

for these fetuses averaged 4.31±0.31 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, in agreement with other reports (1, 10, 11). There have been no previous measurements in the lamb of fetal lactate umbilical uptake, but investigators have assessed fetal lactate metabolism in relation to oxygen consumption as the LOQ (Eq. 8). This quotient represents the fraction of fetal oxygen consumption that would be required to completely oxidize the fetal lactate uptake to carbon dioxide and water. Our measured LOQ of 0.212±0.016 is in agreement with these reports (4, 5). We now report that the net fetal lactate uptake averaged 1.95±0.16 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in these fetuses and that umbilical lactate uptake correlated well with LOQ (Fig. 2) ($r = 0.95$, $P < 0.01$).

Considering the fetuses of hypoglycemic ewes with arterial glucose concentrations < 2 mM, oxygen consumption was not significantly lower than the normoglycemic animals, while both fetal umbilical venous glucose concentration and net fetal uptake of glucose and lactate were significantly reduced (Table II).

Uteroplacental metabolism. Uteroplacental metabolism was calculated as the difference between the

TABLE II

Partition of Lactate, Glucose, and Oxygen in Pregnant Sheep with Normal and Low Maternal Arterial Glucose Concentration

Group	Units	Normal $(glucose)_A$	Low $(glucose)_A$	P
I Uterine		$n = 7$	$n = 4$	
A $[Glucose]_A$	mM	2.60±0.16	1.31±0.13	
B Net glucose exit	$mg \cdot min^{-1}$	54.34±6.34	28.06±3.17	<0.01
C $[Lactate]_A$	mM	0.66±0.05	0.64±0.03	NS
D Net lactate entry	$mg \cdot min^{-1}$	5.15±0.59	3.42±0.51	<0.05
E $[Oxygen]_A$	mM	5.77±0.22	6.05±0.27	NS
F Net oxygen exit	$mmol \cdot min^{-1}$	2.20±0.25	2.40±0.14	NS
II Fetal		$n = 10$	$n = 4$	
A $[Glucose]_v$	mM	1.25±0.06	0.61±0.02	<0.001
B Net glucose entry	$mg \cdot kg^{-1} \cdot min^{-1}$	4.31±0.31	1.15±0.48	<0.001
C $[Lactate]_v$	mM	1.69±0.15	1.24±0.12	<0.02
D Net lactate entry	$mg \cdot kg^{-1} \cdot min^{-1}$	1.95±0.16	1.01±0.07	<0.001
E $[Oxygen]_v$	mM	5.14±0.12	5.41±0.36	NS
F Net oxygen entry	$mmol \cdot kg^{-1} \cdot min^{-1}$	0.315±0.018	0.262±0.025	NS
G Lactate/oxygen quotient		0.212±0.016	0.131±0.012	<0.01
III Placental		$n = 7$	$n = 4$	
A Net glucose consumption	$mg \cdot min^{-1}$	39.820±5.08	23.223±1.43	<0.01
B Net lactate production	$mg \cdot min^{-1}$	11.727±0.69	7.721±0.65	<0.01
C Net oxygen	$mmol \cdot min^{-1}$	0.939±0.16	1.293±0.12	NS

amount of substrate exiting the uterine circulation and the amount of substrate entering the umbilical circulation (15). For the 11 animals in which catheters were maintained chronically in both the uterine and umbilical circulations, oxygen consumption by the uteroplacenta was extremely rapid, averaging 0.98±0.13 $mmol \cdot min^{-1}$, or ~45% of the oxygen leaving the maternal circulation.

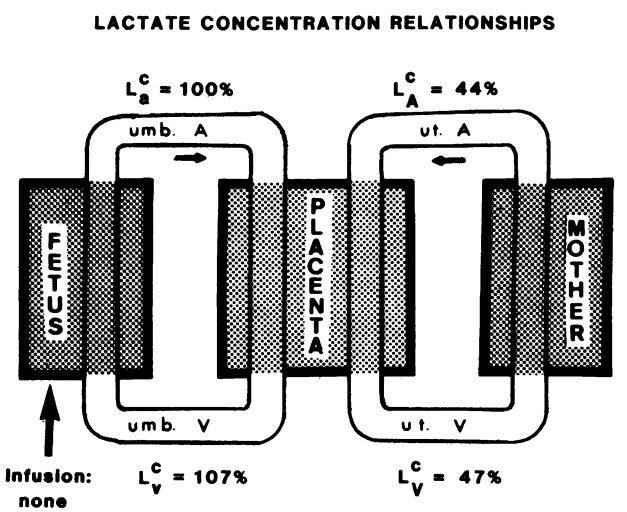

FIGURE 1 Relative lactate concentration relationships in the umbilical and uterine circulations of chronically catheterized sheep. Concentrations are expressed as the percentage of the concentration in the fetal artery, which is set at 100%.

Glucose consumption by the uteroplacenta was also rapid. For the seven normoglycemic animals, glucose consumption averaged 39.8±5.1 $mg \cdot min^{-1}$, or 78±2.9% of the glucose leaving the mother. The uteroplacenta also produced large amounts of lactate, of which 4.97±0.67 $mg \cdot min^{-1}$ were discharged into the maternal circulation, and 6.34±0.70 $mg \cdot min^{-1}$ into the fetal umbilical circulation. In the group characterized by low maternal arterial glucose concentration, consumption of glucose and production of lactate by the uteroplacenta were reduced ~35%.

Fetal L-[$^{14}C(U)$]lactate infusions. A major purpose of the study was to determine the fetal lactate utilization rate in the chronically catheterized sheep fetus and to compare it with simultaneously measured net lactate uptake via the umbilical circulation. Previous investigations have used bolus tracer injection (16) or have not corrected for nonfetal isotope loss (40). In the case of glucose turnover, the primed constant infusion technique corrected for nonfetal loss, has yielded substantially lower estimates of fetal glucose use than have

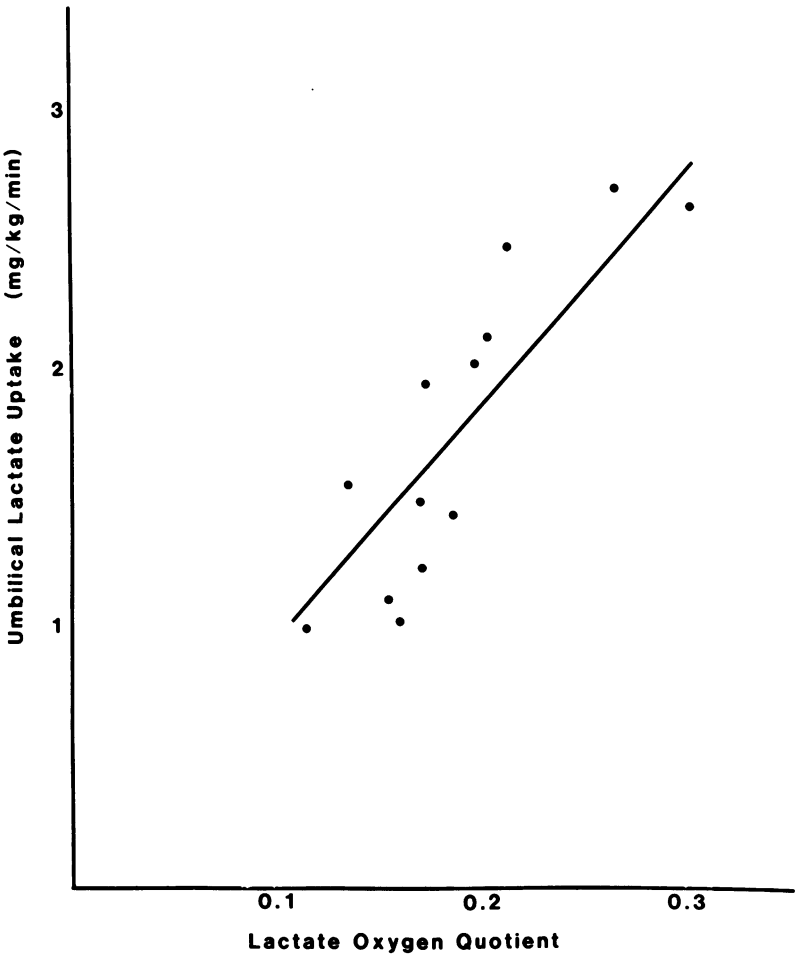

FIGURE 2 Correlation of simultaneously measured umbilical lactate uptake and umbilical lactate oxygen quotient in chronically catheterized fetal lambs.

other uncorrected injection techniques (11). Therefore, it seemed possible that a similar discrepancy might exist for lactate turnover as well.

Six fetuses were studied using the primed constant infusion technique. Steady-state specific activity of lactate was maintained over the sampling period. In all animals studied, lactate specific activity was lower in the umbilical vein than in the fetal artery ($P < 0.005$, paired t test), the difference amounting to an average of 9.02±1.3% of the specific activity in the umbilical artery. Similarly the dpm-lactate · ml^{-1} was consistently lower in the umbilical vein than the fetal artery during lactate tracer infusions ($P < 0.05$, paired t test). The dpm-lactate · ml^{-1} in the umbilical vein averaged 97.2±0.7% of that in the fetal artery. This decreased dpm-lactate · ml^{-1} in the vein compared with the artery reflects loss of dpm-lactate to the placenta. This extrafetal loss of tracer can be calculated according to the Fick principle (Eq. 11), and in the six fetuses infused with L-[^{14}C(U)]lactate, amounted to 8.0±1.0% of the rate of tracer infusion into the fetus. This loss of dpm-lactate, combined with the higher umbilical venous than fetal arterial lactate concentration (Fig. 1) results in a larger umbilical venous-fetal arterial difference for lactate specific activity than for dpm-lactate (9.0% vs. 2.8%).

The net fetal lactate utilization rate was determined as the steady-state arterial specific activity of lactate divided into the difference between the total L-[^{14}C(U)]lactate infusion rate and the nonfetal irreversible loss (Eq. 13). For the six animals, lactate utilization was 5.89±0.68 mg · kg^{-1} · min^{-1}. (Table III). Within the relatively narrow range observed in fetus oxygen concentration there was no apparent relationship between lactate utilization and fetal oxygen concentration. However, there was a significant correlation be-

TABLE III

Comparison of Net Lactate Umbilical Uptake with Simultaneously Measured Lactate Turnover Rate and Fetal Lactate Production Rate

	L-[^{14}C(U)]lactate fetal turnover rate	Lactate umbilical uptake	Fetal lactate production	D-[X-^{3}H]glucose LPR^{G}_{min}	(X)	D-[Y-^{14}C]glucose LPR^{G}_{min}	(Y)
	$mg \cdot kg^{-1} \cdot min^{-1}$	$mg \cdot kg^{-1} \cdot min^{-1}$	$mg \cdot kg^{-1} \cdot min^{-1}$	$mg \cdot kg^{-1} \cdot min^{-1}$		$mg \cdot kg^{-1} \cdot min^{-1}$	
W77-076	—	2.46	—	2.108	(6)	1.645	(1)
W77-080	—	2.69	—	2.872	(6)	1.916	(1)
W78-006	8.34	1.47	6.87	3.15	(6)	—	
W78-010	—	2.01	—	0.85	(6)	0.70	(1)
W78-018	—	2.61	—	0.63	(1)	0.56	(6)
W78-021	4.57	2.12	2.45	0.48	(6)	—	
W78-023	—	1.01	—	0.71	(6)	0.47	(6)
W78-030	5.32	1.23	4.09	0.48	(3)	—	
W78-038	—	1.13	—	1.34	(6)	1.40	(6)
W78-042	5.67	1.14	4.53	—		—	
W78-052	—	1.08	—	1.31	(6)	0.41	(6)
W78-063	7.42	1.57	5.85	1.93	(6)	—	
W78-064	—	1.94	—	—		—	
W78-070	4.03	0.85	3.18	—		—	
Mean	5.89	1.68	4.50	1.59		1.01	
SEM	±0.68	±0.16	±0.67	0.42		0.24	

tween fetal lactate utilization and fetal glucose uptake ($r = 0.89$, $P < 0.01$, Fig. 3) and between fetal lactate utilization and umbilical venous glucose concentration ($r = 0.84$, $P < 0.025$).

In each of the six animals, fetal lactate utilization exceeded the simultaneously measured umbilical lactate uptake. This difference represents endogenous fetal lactate production, and averaged 4.45±0.67 mg·kg^{-1}·min^{-1}. Fetal lactate production also correlated significantly with umbilical glucose uptake ($r = 0.85$, $P < 0.025$).

Fetal glucose specific activity averaged 3.3±1.1% of the fetal lactate specific activity during infusion of L-[^{14}C(U)]lactate and was higher than maternal glucose specific activity. We conclude therefore that a small but detectable quantity of labeled glucose was produced from lactate within the fetus.

Samples were obtained from both uterine venous and maternal arterial catheters during fetal infusion of L-[^{14}C(U)]lactate in five animals. In each of these animals, both lactate specific activity and dpm-lactate·ml^{-1} were higher in the uterine venous sample compared to the maternal arterial sample. Net flux of labeled lactate into the uterine circulation averaged 1.6±4% of the fetal L-[^{14}C(U)]lactate infusion rate, significantly less than the rate of labeled lactate exiting the umbilical circulation of 8±1% cited earlier ($P < 0.01$, paired). This difference demonstrates that metabolism of L-[^{14}C(U)]lactate occurred within the placenta.

Fetal [^{14}C] and [^{3}H]glucose infusions. In eight studies lactate specific activity was measured during primed constant infusion of D-[Y-^{14}C]glucose into the fetal lamb, and in 13 studies lactate specific activity was measured during fetal infusion of D-[X-^{3}H]glucose. In both sets of studies lactate was rapidly labeled from glucose. Fetal lactate specific activity averaged 68±4% and 55±4% of glucose specific activity during infusions of D-[Y-^{14}C]glucose and D-[X-^{3}H]glucose, respectively.

In all experiments in which [^{14}C] and [^{3}H]glucose were infused into the fetus the specific activity of lactate in the umbilical vein was lower than in the umbilical artery ($P < 0.01$, paired). Two physiologically important conclusions can be inferred from this observation. The first is that the higher fetal arterial lactate specific activity can be maintained only if the fetus synthesizes lactate from the labeled tracer, glucose. The second conclusion is that the placenta infuses into the fetus lactate derived from unlabeled precursors. These conclusions follow from the assumption that unlabeled tracee lactate and labeled tracer lactate are metabolized identically. In the absence of isotope effects, removal of lactate from a well-mixed pool consumes tracer and tracee lactate proportionally, so that consistently maintained differences in specific activity at different sampling sites must reflect new entry of tracer or tracee at some anatomic site between the sampling sites.

Qualitatively, then, the observed concentrations of labeled and unlabeled lactate in the umbilical circu-

lation during fetal tracer glucose infusion require that the fetus produce lactate endogenously. Mathematically, further description of this fetal lactate production can be made, as described in the Appendix. When tracer glucose is infused the term LPR^{G}_{min} may be calculated by Eq. 18. This term represents the minimum production of lactate from glucose which must occur within the fetus in order to account for measured concentration differences. Secondly, a constant "k_1" may be derived, which partitions any additional endogenous fetal lactate production between glucose and nonglucose substrates. Values of LPR^{G}_{min} were calculated in experiments with fetal glucose tracer infusion (Table III).

The minimal amount of lactate the fetus must produce from glucose (LPR^{G}_{min}) averaged 1.59±0.42 mg·kg^{-1}·min^{-1} in D-[X-^{3}H]glucose infusions and 1.01±0.23 mg·kg^{-1}·min^{-1} in infusions of D-[Y-^{14}C]glucose. The values calculated during the simultaneous infusions of [^{14}C] and [^{3}H]glucose correlated well with each other ($r = 0.95$, $P < 0.001$), and were each significantly different from zero ($P < 0.05$) but not significantly different from each other ($P > 0.2$).

When it is possible to estimate independently the fetal lactate utilization rate during fetal infusion of labeled glucose, then it is possible to derive the actual endogenous fetal lactate production from glucose (LPR^{G}) and nonglucose (LPR^{NG}) substrates as described in Eq. 17 and in the Appendix. Using the simultaneous infusion of L-[^{14}C(U)]lactate to estimate the LUR, the lactate production rate from nonglucose sources, LPR^{NG}, in experiments with fetal D-[X-^{3}H]glucose infusion was 1.4±0.5 mg·kg^{-1}·min^{-1}. An additional estimate of LPR^{NG} can be obtained from the seven animals in whom D-[Y-^{14}C]glucose was infused into the fetus. In these animals an additional assumption is required; namely that the lactate turnover rate can be estimated from the relationship between umbilical glucose uptake and fetal lactate turnover rate shown in Fig. 4. The value calculated for these animals (1.6±0.1 mg·kg^{-1}·min^{-1}) is in good agreement with the previous estimate. From Eq. 17, an estimate of LPR^{G} appears in the range of 3 mg·kg^{-1}·min^{-1}.

DISCUSSION

Lactate metabolism must be interpreted very differently, depending on the physiologic and pathologic conditions pertaining at the time metabolic measurements are made (20). Under normal conditions, lactate may be an important intermediary in interorgan metabolism. Clinically, however, lactate metabolism is more frequently considered in the context of abnormal conditions producing lactic acidosis. In the perinatal literature, many observers have reported measurements of lactic acid concentrations in the maternal circulation as well as in cord blood samples at delivery. While the reports are occasionally in conflict, several general conclusions seem reasonable. First, lactate concentrations in maternal blood rise during labor (8, 21, 22) and at delivery achieve levels higher than prelabor values (8, 21–31). Second, fetal values may also rise during normal labor (8, 21, 33) and, at delivery, are usually reported to be higher than simultaneously obtained maternal values (8, 22–27, 29–35). Third, fetal lactate concentrations in both umbilical vessels increase further with elective cesarean section, moderate asphyxia, and severe or fatal asphyxia, demonstrating an enlarging fetal-maternal gradient of lactate concentration (23, 25, 26, 32, 35–37). Finally, while umbilical arterial lactate concentrations clearly exceed umbilical venous values during the high levels of lactate typical of asphyxia or distress, this arteriovenous difference narrows and may reverse at the lower fetal lactate concentrations more typical of intrauterine life in the human, sheep or calf (4–5, 8–9, 21–26, 31–38). In combination, these properties demonstrate fetal or cord lactate concentrations to be a sensitive indicator of fetal distress, and support the use of a low scalp pH, elevated lactate level or persistent neonatal lactate acidemia or base deficit as clinically useful measurements of intrapartum distress. Conversely, however, these same properties make it unlikely that lactate measurements from samples obtained at delivery can accurately describe the metabolic profile of the human fetus before labor (37). Although some authors have concluded that the human fetus uses anaerobic metabolism (6–8), others have interpreted their data to indicate that lactate is not normally the end-product of fetal glucose metabolism (39), and that human fetal metabolism is normally not anaerobic (23–28, 35–38).

This study was undertaken to quantitate the metabolism of lactate within the sheep fetus under chronic steady-state conditions. Previous studies in the fetal sheep have noted higher umbilical venous than fetal arterial lactate concentrations (4, 5), demonstrating net fetal lactate consumption. These studies have also demonstrated higher uterine venous than maternal arterial lactate concentrations, implicating net placental production of lactate. Our study has extended these findings by quantitating the magnitude of the placental lactate production. The rates of lactate infusion into the uterine and umbilical circulation were measured simultaneously by application of the Fick principle to each circulation. Total placental lactate production measured ~12 mg·min^{-1}, and supplied the fetus with ~2 mg·kg^{-1}·min^{-1} of lactate. Thus, lactate is quantitatively second only to glucose as an exogenous fetal nutrient (11), with infusion rates of individual amino

acids much below the rates for glucose or lactate (2). The LOQ has been used as a measure of fetal lactate uptake, and the present value of 0.21 agrees with previous reports (4, 5). The fetal lactate uptake measured in this study correlates well with the LOQ (Fig. 2), confirming the utility of the latter measurement.

It is evident that lactate does not diffuse in net from fetus to mother along the observed fetal-maternal lactate concentration gradient. Instead, the placenta produces lactate that is distributed asymmetrically, with the greater portion transferred to the umbilical circulation with its higher lactate concentration. The processes governing these transfers require further investigation.

Glucose appears the most likely precursor of the uteroplacental lactate production. In agreement with this interpretation, during infusion of tracer glucose to the fetus, we observed higher dpm-lactate $\cdot$ ml^{-1} in the uterine vein than maternal artery and in the umbilical vein than the fetal artery indicating that some uteroplacental lactate production originated from glucose. It should be noted that the production of lactate does not necessarily imply anaerobic metabolism by the uteroplacenta. Glycolysis occurred despite normal oxygen concentrations in the vessels perfusing the uteroplacenta. Placental oxygen consumption of 0.939 mmol $\cdot$ min^{-1} demonstrates a rapid rate of oxidative metabolism occurring concurrently with the lactate production.

The metabolism of lactate was further quantified by the infusion of tracer into the fetus. By measurement of both tracer lactate (dpm-lactate $\cdot$ ml^{-1}) and tracee lactate (mg $\cdot$ ml^{-1}) in both fetal artery and umbilical vein, several important features were noted. During tracer infusion into the fetus, the tracer lactate concentration in the fetus is experimentally maintained higher than in the mother. Although the metabolism of tracer and tracee lactate are presumed identical, the transfer of tracer and tracee lactate between the fetal, uteroplacental, and maternal compartments may be very different due to the very different concentration relationships observed for tracer and tracee (Fig. 1). Thus, at the 30:1 tracer concentration gradient from fetus to mother maintained by the fetal tracer lactate infusion, there was a net flux of tracer lactate from the fetus to the placenta. In contrast, the concentration gradient for tracee lactate was ~2:1, during which net tracee entered the fetus. Thus, the net flux of tracer did not reflect in magnitude or direction the net flux of tracee. A second finding was that net tracer lactate entering the placenta from the umbilical circulation exceeded the net tracer lactate exit from the placenta to the uterine circulation, indicating placental consumption of tracer. This consumption did not reflect the simultaneously measured net production of tracee lactate. Therefore, in interpretation of fetal tracer studies, tracer and tracee must be considered separately in calculating net tracer and tracee transfer between anatomical compartments, and in calculating net tracer and tracee consumption or production within anatomical compartments. A similar situation exists in tracer glucose infusions into the fetus (11).

During fetal infusion of tracer lactate, the fetal lactate turnover rate was 6.5±0.8 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}, in agreement with other reports (16, 40). Correcting for net extrafetal irreversible loss of tracer (Eq. 12), this study estimated the fetal lactate utilization rate as 5.9 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}. This value is 12% lower than that estimated by Warnes et al. (16) and 25% lower than Prior (40), both of whom did not correct for extrafetal nonmetabolic loss of tracer from the umbilical circulation.

The interpretation of the LTR has been the subject of considerable discussion. Lactate is rapidly equilibrated with other three-carbon intermediaries, including pyruvate and alanine. In experiments in which lactate is injected as a bolus, a substantial part of the injected label is distributed in pools in reversible equilibrium with the plasma lactate pool, and the apparent turnover rate therefore includes both reversible and irreversible loss of lactate label. Under steady-state conditions, it may be expected that tracer returns from these rapidly reversible pools at a rate approximately equal to the rate of tracer exit from lactate into these pools. For this reason, such reversible losses effectively cancel, and the turnover rate measures only the net of irreversible rates of entry or exit to the plasma lactate pool (47). We therefore make the assumption that the measured net rate of irreversible loss of lactate tracer infused into the fetus represents fetal irreversible metabolism (40), which at steady state permits calculation of the total rate of irreversible lactate consumption by the fetus. Under steady-state conditions, the net consumption of lactate by the fetus is equal to the total entry of lactate into the fetus from all endogenous and exogenous (umbilical) sources.

Our study is the first to compare simultaneous measurements of umbilical lactate uptake, representing the exogenous fetal supply of lactate, with fetal lactate utilization, representing net fetal consumption. The difference between the umbilical uptake (1.5 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}) and the lactate utilization (6 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}) represents the fetal production rate of lactate (Fig. 4), equal to ~4.5 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}.

The substrates from which lactate is produced within the fetus are not entirely known. To address this question, we have used a second tracer technique, the examination of labeled and unlabeled lactate metabolism during infusion of tracer glucose. Qualita-

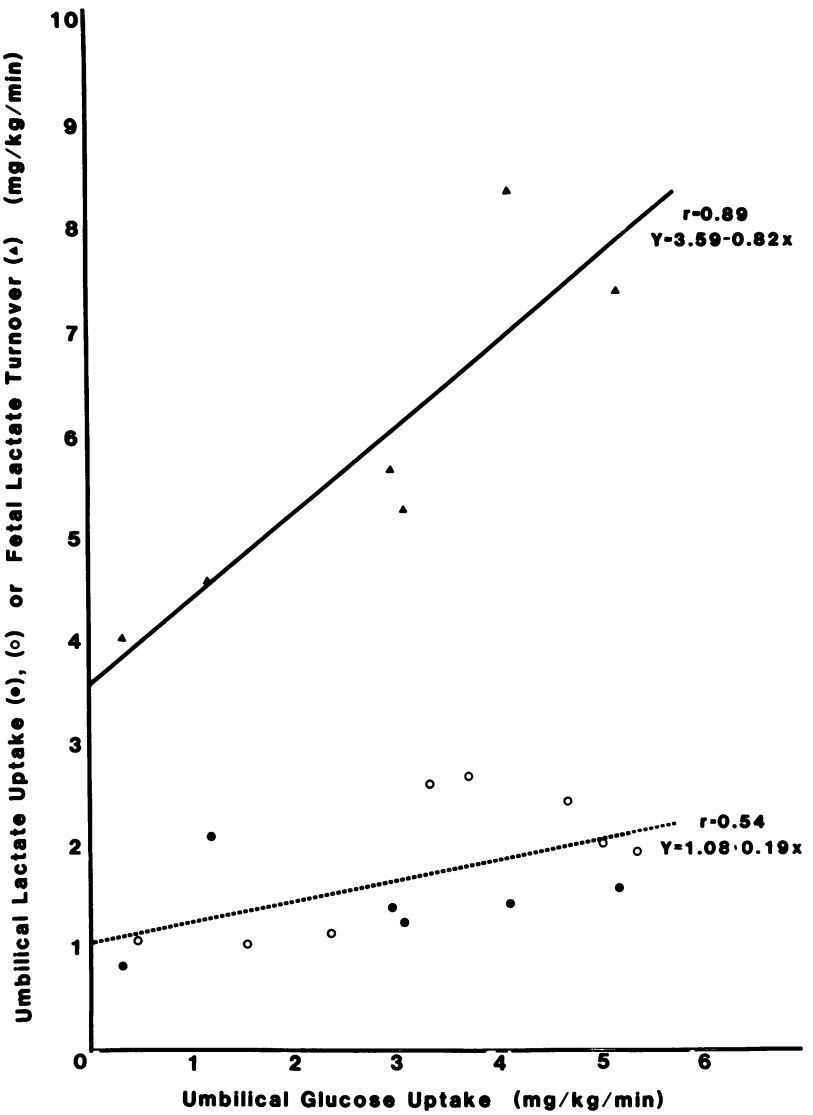

FIGURE 3 Fetal lactate turnover (——) and lactate umbilical uptake (-----) as a function of umbilical glucose uptake. Animals denoted by solid symbols (▲, ●) were infused with ^{14}C-(U)-lactate.

tively, the observation that the lactate specific activity during tracer glucose infusion is higher in the fetal artery than vein requires the synthesis of labeled lactate from tracer glucose within the fetus. Quantitatively, we have derived two estimators of the magnitude of the conversion of glucose to lactate. The first, LPR^{G}_{min} estimates the minimal amount of glucose that must be converted to lactate in order to account for the observed glucose and lactate specific activities. This minimum conversion was 1–1.5 $mg \cdot kg^{-1} \cdot min^{-1}$, requiring therefore that the total fetal lactate consumption must exceed the net exogenous umbilical lactate entry. The second estimator, LPR^{G}, is the actual rate of lactate conversion from glucose, and additionally requires independent estimation of the total lactate utilization rate within the fetus. Using two methods of estimation of fetal lactate utilization, we have estimated that the fetal production rate of lactate from glucose was ~3 $mg \cdot kg^{-1} \cdot min^{-1}$, or ~50% of the fetal lactate utilization rate. These analyses demonstrate that glucose is not the sole precursor of lactate within the fetus.

Considering possible metabolic fates of lactate within the fetus, the maintenance of a stable fetal lactate con-

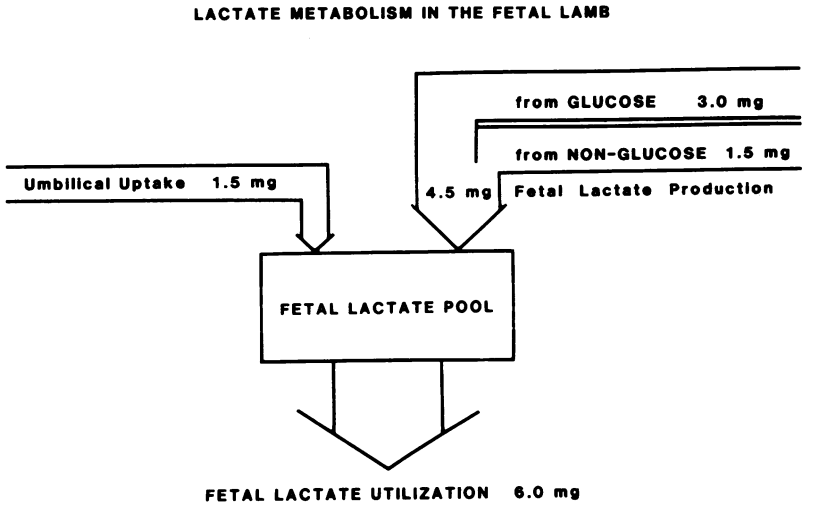

FIGURE 4 Summary of lactate metabolism in the chronically catheterized fetal lamb. Input to the fetal lactate pool is the sum of umbilical lactate uptake (1.5 mg · kg^{-1} · min^{-1}) and fetal lactate production from glucose (4.5 mg · kg^{-1} · min^{-1}) and nonglucose substrates (1.5 mg · kg^{-1} · min^{-1}).

centration requires that the fetus be a net consumer of lactate in order to dispose of the continuous infusion of lactate from the placenta into the umbilical circulation. The net consumption of lactate requires conversion to pyruvate and metabolism subsequently via the pathways available to pyruvate. In the case of the fetus, restriction of the reversal of glycolysis at the level of PEPCK would divert the lactate into the citric acid cycle. The measured fetal lactate utilization rate of 6 mg · kg^{-1} · min^{-1} is equivalent to an irreversible loss of ~3.5 g carbon · kg^{-1} · d^{-1} from the lactate pool (6 × 0.4 × 1,440). Net carbon accretion in the sheep fetus is ~3.2 g C · kg^{-1} · d^{-1} during late gestation (2), so that some oxidation of lactate appears necessary. Because reversal of glycolysis may be restricted by low fetal PEPCK, and because it is unlikely that lactate is the major source of carbon for accretion in the fetus, the rapid fetal lactate utilization implies aerobic metabolism of lactate by the fetus.

The implications of lactic acid as a major fetal nutrient are only beginning to be understood. Like glucose, lactic acid shares the ($C \cdot H_2O$) chemical formulation of carbohydrates, and the oxidation of lactic acid is similar with regard to caloric value (3.62 vs. 3.75 kcal/g) and ATP production (36 vs. 38 ATP/6 carbons). Unlike glucose, lactate is poorly transferable from the fetal circulation, and during fetal tracer lactate infusion in the sheep, we observed only an 8% loss of tracer with a 30:1 fetal-maternal dpm-lactate · ml^{-1} concentration gradient. Thus, for the fetal sheep, carbon and energy provided as lactate from placental metabolism remains relatively trapped within the fetus. For the primate (39) and guinea pig (41) transfer into and from the fetus may be more rapid, although experimental differences preclude direct comparison of rates as well as whether simple or facilitated diffusion governs the transfer (41).

Although glucose is generally considered obligatory for fetal brain metabolism, there is no known obligatory requirement for lactate by any organ. Only fetal heart (42) and portal drained viscera (43) have been shown to consume lactate in the chronically catheterized fetal lamb. Calculated from the published values of lactate consumption, these organs could consume about 0.15 and 0.20 mg · kg^{-1}, substantially below either umbilical lactate uptake or fetal lactate utilization demonstrated in the present studies. Liver and kidney appear to be the largest consumers of lactate in the adult (20), although net lactate consumption by these organs has not been reported in the fetal sheep.

This study confirms that lactate is an important exogenous nutrient for the fetal sheep, and defines metabolic relationships between lactate, glucose, and nonglucose fetal nutrients. A similar role for lactate in fetal metabolism may be present in other species. Fetal lactate uptake has been measured in the chronically catheterized fetal calf (9), and appears to be of even larger magnitude than for the sheep. Acute studies in several species have demonstrated umbilical venous-fetal arterial lactate concentration relationships compatible with net fetal lactate consumption (34). The placental production of lactate observed in the sheep may also be a general finding, as rapid placental production of lactate under aerobic conditions has been

demonstrated in vitro in many species, including man (44–46).

APPENDIX

Measurement of lactate turnover in the fetus

Lactate turnover in a simple system. Consider a system that is sampled at a fixed location (X) and infused with tracer lactate at a constant net rate (rate of infusion of tracer lactate = R_IL^*). During the initiation of the constant tracer infusion, the concentration of tracer lactate (e.g., dpm-lactate/ml) sampled at "x" ($=L_x^D$) may vary. Steady state is defined for the tracer lactate as a time period during which the concentration of tracer lactate dpm sampled at "x" (L_x^D) cannot be shown to change further with time, within given confidence limits. At steady state, the rate of irreversible loss of lactate tracer (rate of consumption of lactate tracer = R_CL^*) from the location sampled at "x" is equal to the lactate tracer entry rate R_IL^*. If the concentration of unlabeled tracee lactate is also measured at "x" ($=L_x^C$), then one may define a steady state for unlabeled tracee lactate as a time period during which the concentration of lactate sampled at "x" cannot be shown to change with time within known confidence limits. During steady-state conditions for tracee lactate, the net rate of entry of unlabeled tracee lactate (R_IL) sampled at "x" is equal to the rate of irreversible loss of tracee lactate from "x" (R_CL). If one assumes that tracer R_CL and tracee lactate are metabolized identically, then the relationship between tracer concentration (L_x^D) and the net tracer infusion rate (R_IL^*) is the same as the relationship between the tracee concentration (L_x^C) and the net tracee entry rate (R_IL), and the following relationship can be shown to be true (47):

$$\frac{R_IL}{L_x^C} = \frac{R_IL^*}{L_x^D} \qquad \text{(A1)}.$$

By rearrangement:

$$R_IL = LTR_x = \frac{R_IL^*}{(L_x^D/L_x^C)} = \frac{R_IL^*}{(L_x^S)} \qquad \text{(A2)},$$

where the ratio of L_x^D to L_x^C is defined as L_x^S, the lactate specific activity measured at "x". Note that when the L_x^C and the L_x^D are at steady state, then the L_x^S must also be at steady state. At steady state, the R_IL and R_CL are equal and define the lactate turnover rate (LTR_x). Thus, the lactate turnover rate represents the net amount of lactate at the specific activity measured at "x" that would need to enter and exit the system to account for the known rate of irreversible loss of tracer.

Addition of reversibly connected subsystems. In the above construction, the location "x" may be considered to sample a region "P" throughout which the concentration of tracer (L_x^D) and of tracee (L_x^C), are constant. L_x^{SA} must therefore also be constant throughout the region "P". Note that this region represents a mathematical construct, and need not correspond to an anatomic or physiologic entity. Consider now a second region "P_r", such that all entry of tracer and tracee must originate from region "P", and such that all exit of tracee and tracer must go to region "P". At steady state, there may then be unidirectional fluxes of tracer and tracee to and from "P_r". However, net fluxes of tracer and/or tracee from "P" to "P_r" would lead to alteration of L_x^D or L_x^C, and are therefore not consistent with a steady state of L_x^D or L_x^C. Therefore, the addition of rapidly reversible regions to the region "P" will not affect the net flux of tracer or tracee from region "P" at steady state. However, the presence of such regions would affect the unidirectional fluxes entering or leaving the region "P". The mathematical notion of a rapidly reversible region may reflect diverse physical phenomena, including equilibration across membranes, as well as rapidly reversible metabolic interconversions.

Application of turnover methodology to the fetus. The mother, fetus, and placenta represent interconnected, anatomically definable regions. To estimate the portion of irreversible lactate loss that occurs within the anatomic fetus, it is necessary to exclude that portion of the tracer which is irreversibly lost to the placenta via the umbilical circulation (11). The dpm-lactate leaving the fetus via the umbilical artery may be calculated as the product of the dpm-lactate $\cdot$ ml^{-1} measured in the umbilical artery times the umbilical blood flow (Eq. 10). Similarly, the dpm-lactate entering the fetus via the umbilical vein may be calculated as the product of the dpm-lactate $\cdot$ ml^{-1} in the umbilical vein times the umbilical blood flow (Eq. 9). The difference between Eqs. 9 and 10 represents the net rate of extrafetal loss of lactate during fetal lactate infusion (Eq. 11). The difference between the net rate of extrafetal tracer loss, and the total fetal rate of infusion represents the actual rate of irreversible loss of tracer within the anatomic fetus (Eq. 12). This value divided by the lactate specific activity in the umbilical artery (Eq. 13), defines an estimate of the actual net lactate utilization rate (LUR) occurring within the anatomic fetus. This figure represents the amount of tracee lactate (mg $\cdot$ min^{-1}) required to be irreversibly lost within the anatomic fetus in order to account for the known fetal irreversible loss of tracer (Eq. 12) at the measured fetal lactate specific activity (L_a^S).

The choice of L_a^S specifies that this is the apparent fetal irreversible loss of tracee from the viewpoint of an observer located in the umbilical artery. At steady state, the umbilical circulation acts as a source of tracee lactate and a sink of tracer lactate, so that the specific activities in the umbilical artery and umbilical vein are each constant with time but are maintained at different absolute levels. Both the LTR and the LUR in the fetus must therefore be specified in terms of the site of sampling. From a practical standpoint, the measured differences of L_a^S and L_v^S are very small, and the LUR and LTR measured at either the umbilical vein or umbilical artery will yield very similar results. We have arbitrarily chosen the L_a^S for computation of Eqs. 13 and 14.

Consideration of the error in determining steady state. The foregoing has assumed a system at a mathematically defined steady state, such that the concentrations of tracer and tracee remain constant at the sampling site, and that all reversibly-connected subsystems have fully equilibrated. Under these conditions, the LTR and LUR calculated by the above equations estimate the net irreversible loss of tracee from the total system, and from the anatomic fetus, as seen by an observer at the sampling site. In real experiments, it is unlikely either that the steady state can be proven with certainty, or that all reversibly connected regions have fully equilibrated within the relatively short time of lactate infusion.

If one considers a simplified system in which the actual L_a^C, LTR, and LUR are constant and in which the fetus is infused with an unprimed constant infusion of an ideal tracer L^* distributed in a volume V_d, one can define the potential error in estimation of LTR due to the possibility of nonachievement of steady state. If at experimentally chosen "steady state", a change in specific activity of "C" percent

per minute cannot be excluded statistically by the actual data, then the dpm/minute (R) that could contribute to such a rise may be expressed:

$$R = (C)(L_a^S)(L_a^C)(V_d)/100 \quad \text{(A3)}.$$

Thus, the corrected rate of tracer lactate infusion partitioned to irreversible pathways is given by:

$$R_IL\ corr = R_IL - R \quad \text{(A4)}.$$

Using values of C = 5%/75 min, V_d = 600 ml · kg^{-1} (16), fetal weight = 4 kg, a measured LUR of 6 mg · kg^{-1} · min^{-1}, and typical values of R_IL and L_a^S from the present studies, then the permitted confidence limit for estimation of LUR would be ~ ±1%. Restated, the presence of unequilibrated pools reversibly connected to the blood lactate pool would permit an error of as large as ±1%.

Observations during fetal infusion of tracer glucose

Conceptually, the specific activity of lactate at steady state may be considered as the ratio of the infusion rate of tracer lactate to the infusion rate of tracee lactate (Eq. 15). During infusion of tracer glucose into the fetus, this relationship permits calculation of the rate of lactate production from glucose and nonglucose substrates. The exogenous rate of tracer lactate infusion into the anatomic fetus equals the measured umbilical blood flow ($\dot{Q}_f$) times the measured umbilical venous-arterial difference in dpm-lactate (Eq. 11). Similarly, the rate of endogenous production of dpm-lactate from glucose during infusion of labeled glucose can be taken as the product of the measured glucose specific activity (GSA_a) times the rate of conversion of glucose to lactate occurring within the anatomic fetus (defined as LPR^G). The total rate of entry of dpm-lactate is the sum of the endogenous production and exogenous entry rates (Eq. 16). By similar reasoning, the total infusion rate of unlabeled lactate into the anatomic fetus may be considered as the sum of the lactate umbilical uptake (Eq. 1) plus the endogenous fetal lactate production from glucose (LPR^G) and nonglucose (LPR^{NG}) substrates (Eq. 17). The lactate specific activity at steady state is given as the ratio of Eq. 16 to Eq. 17, from which can be derived the relationships shown in Eqs. 18, 19, and 20. In these equations, the term LPR^G_{min} represents the minimum production of lactate from glucose occurring within the anatomic fetus that is required to account for the observed concentrations of labeled and unlabeled lactate in the umbilical vein and artery. The actual LPR^G may exceed this minimum value, and the proportion of this excess production of lactate derived from glucose and nonglucose sources is defined by the constant K_1. If the total rate of lactate utilization within the anatomic fetus (LUR) is also known, then the actual values of LPR^G and LPR^{NG} can be determined from Eqs. 17 and 20.

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