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Umbilical uptakes and transplacental concentration ratios of amino acids in severe fetal growth restriction

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BACKGROUND: This study examines the relationship between placental amino acid (AA) transport and fetal AA demand in an ovine fetal growth restriction (FGR) model in which placental underdevelopment induces fetal hypoxemia and hypoglycemia.

METHODS: Umbilical uptakes of AA, oxygen, glucose, and lactate were measured near term in eight experimental ewes (FGR group) and in eight controls (C group).

RESULTS: The FGR group demonstrated significantly reduced umbilical uptakes of oxygen, glucose, lactate, and 11 AAs per kg fetus. The combined uptake of glucose, lactate, and AAs, expressed as nutrient/oxygen quotients, was reduced almost to 1.00 (FGR: 1.05 vs. C: 1.32, $P \leq 0.02$). In contrast to a decrease in umbilical glucose concentration, all but one of the AAs that were transported from placenta to fetus demonstrated normal or elevated fetal concentrations, and five of the essential AAs were transported against a significantly higher feto/maternal (F/M) concentration ratio. This ratio peaked at the lowest fetal oxygen levels.

CONCLUSION: We conclude that, in the hypoxic FGR fetus, the reduction in AA uptake is not due to a disproportionally small placental AA transport capacity. It is the consequence of decreased fetal oxidative metabolism and growth rate, which together reduce fetal AA demand.

In the second half of a normal pregnancy, a progressively larger fraction of the placental barrier develops structural changes that make it more permeable to the transplacental diffusion of oxygen (1,2). In fetal growth restriction (FGR), both placental growth and the process of differentiation that increases oxygen diffusibility across the placenta are inhibited (3). As a consequence, the FGR placenta generates an abnormally large PO_2 difference between uterine and umbilical venous blood (4,5). In FGR, the transplacental glucose concentration difference is also greater than normal (6,7) because placental glucose transport capacity is disproportionally reduced with respect to the placental and fetal glucose utilization rates (7).

Tracer studies of placental amino acid (AA) transport have demonstrated that in FGR, there is a significant reduction in the transplacental flux/fetal turnover ratio of essential AAs (8–11). However, attempts to define the fetal plasma AA concentrations in FGR have produced inconclusive results. Although early studies showed significantly lower concentrations (9,12,13), subsequent human and animal studies could not confirm these original findings (10,11). Hence, the extent to which the reduction in transplacental flux represents a dysfunction of placental AA transport mechanisms and adaptation of these mechanisms to a reduction in the fetal demand for AAs remains unclear. We postulate that the variability in the degree of fetal hypoxia, which is associated with FGR, is one of the factors that causes variabilities in fetal AA concentration. More specifically, we postulate that fetal AA utilization depends on the availability of oxygen and that in severely hypoxic FGR fetuses, the reduction in fetal demand for AAs may become greater than the reduction in placental AA transport capacity.

This article presents the relation of umbilical AA uptake to AA concentration and level of oxygenation in a sheep model of severe FGR, in which uptakes and concentrations of oxygen, glucose, and lactate were measured simultaneously. Given the magnitude and complexity of the study, a detailed analysis of the results concerning placental oxygen transport has been presented separately (5).

RESULTS

In this article, the presentation of the O_2 data is limited to what is relevant to a discussion of AA transport and metabolism. The FGR group produced significantly smaller placentae. Placental and fetal weights were significantly correlated in the FGR group but not in the control group (**Figure 1**). The mean umbilical AA uptakes of the two groups are presented in **Table 1**. The uptakes are presented as uptakes per kg fetus and as AA/oxygen molar uptake ratios. In agreement with previous studies (14), there was a positive umbilical uptake of all the essentials and of eight nonessentials, a negative uptake of serine and glutamate, and virtually no uptake of aspartate, citrulline, or taurine. With the exception of lysine, the uptake per kg fetus of each essential was significantly reduced in the FGR group, and there was a significant reduction in the uptake of each of three nonessentials: arginine, tyrosine, and glutamine. The placental uptake of fetal glutamate was significantly reduced.

Mean arterial and uterine venous AA concentrations are presented in **Table 2**. In the FGR group, the mean maternal

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concentration of each AA was reduced. For 14 of these AAs, both the arterial and venous reductions in concentration were significant. Maternal plasma glucose was not significantly reduced (3.85 ± 0.12 vs. 3.87 ± 0.06 mmol/l). Mean umbilical venous and arterial AA concentrations are presented in **Table 3**. By contrast to the maternal data, only isoleucine and glutamate showed a significant decrease in arterial concentration, and six AAs: phenylalanine, lysine, glycine, alanine, asparagine, and taurine showed a significant increase. For 12 of the 16 AAs with a positive umbilical uptake, the arterial umbilical/maternal concentration ratio was significantly higher in the FGR group (**Table 4**). These data must be considered in conjunction with data on the uptakes of O_2, glucose, and lactate because the uptake per kg fetus of these metabolites was also reduced (**Table 5**). The reduction in fetal lactate uptake was associated with a significant increase in fetal lactate concentration (6.86 ± 1.74 vs. 1.93 ± 0.11 mmol/l, $P = 0.01$).

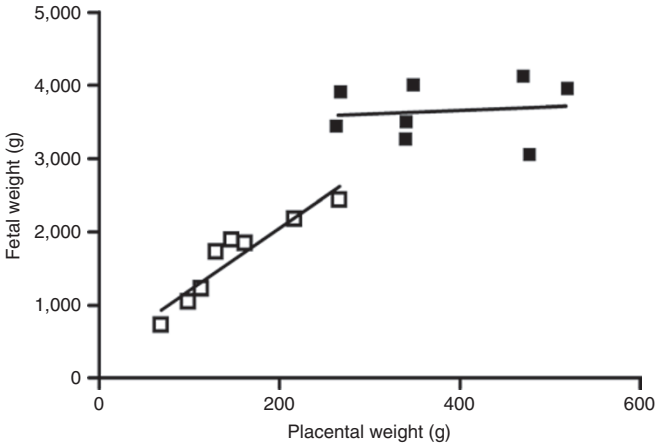

Figure 1. In the fetal growth restriction (FGR) group, placental and fetal weight are correlated ($R^2 = 0.88$, $P = 0.0005$), whereas they are not for control fetuses. ($R^2 = 0.01$, $P = 0.77$). Control, filled squares; FGR, open squares.

The absolute umbilical uptakes (μmol/min) of O_2, glucose, leucine, and lysine and the natural logs of the umbilical/uterine venous ratios of PO_2, glucose, leucine, and lysine concentration are plotted against placental weight in **Figure 2**. Two separate regression lines were calculated for the control and the FGR uptake data. The FGR lines in the O_2 and glucose uptake graphs have significantly smaller intercepts ($P < 0.05$ and $P < 0.0001$, respectively) than the control lines, and no significantly different slopes were observed. The FGR lines in the leucine

Table 1. Umbilical uptake of amino acids (μmol/min/(kg_{fetus})) and as amino acid/oxygen uptake molar ratio × 10^3

	Control (μmol/min/(kg_{fetus}))	FGR (μmol/min/(kg_{fetus}))	*P* value	Control (molar ratio × 10^3)	FGR (molar ratio × 10^3)	*P* value
Essential						
Val	4.66 ± 0.62	2.32 ± 0.20	0.003	13.81 ± 1.81	8.94 ± 0.88	0.03
Leu	4.26 ± 0.37	2.86 ± 0.13	0.003	12.49 ± 0.60	11.0 ± 0.66	NS
Ile	2.76 ± 0.30	1.59 ± 0.12	0.003	8.06 ± 0.53	6.14 ± 0.59	0.03
Thr	2.28 ± 0.35	1.27 ± 0.19	0.022	6.64 ± 0.82	4.79 ± 0.71	NS
Phe	1.51 ± 0.13	1.01 ± 0.10	0.008	4.49 ± 0.36	3.81 ± 0.34	NS
Met	1.07 ± 0.21	0.56 ± 0.07	0.036	3.09 ± 0.48	2.14 ± 0.27	NS
Lys	2.04 ± 0.22	1.75 ± 0.24	NS	6.00 ± 0.50	6.69 ± 0.87	NS
His	0.89 ± 0.15	0.54 ± 0.09	0.038	2.57 ± 0.25	2.03 ± 0.30	NS
Nonessential						
Ser	−0.77 ± 0.53	−0.38 ± 0.22	NS	−2.15 ± 1.53	−1.50 ± 0.86	NS
Gly	2.89 ± 0.37	1.99 ± 0.26	NS	8.57 ± 1.03	7.64 ± 1.09	NS
Ala	2.65 ± 0.41	1.87 ± 0.23	NS	7.84 ± 1.23	7.25 ± 1.04	NS
Pro	2.31 ± 0.35	1.98 ± 0.39	NS	6.71 ± 0.90	7.76 ± 1.78	NS
Arg	2.16 ± 0.26	1.23 ± 0.16	0.009	6.39 ± 0.75	4.71 ± 0.62	NS
Orn	0.10 ± 0.14	0.06 ± 0.12	NS	0.36 ± 0.39	0.25 ± 0.45	NS
Tyr	1.43 ± 0.18	0.85 ± 0.10	0.014	4.42 ± 0.59	3.24 ± 0.38	NS
Gln	6.37 ± 0.71	3.50 ± 0.68	0.011	18.72 ± 1.69	13.37 ± 2.62	NS
Glu	−5.22 ± 0.72	−1.42 ± 0.17	0.001	−15.44 ± 1.93	−5.44 ± 0.67	0.001
Asn	1.24 ± 0.16	0.77 ± 0.33	NS	3.65 ± 0.42	2.80 ± 1.34	NS
Asp	0.02 ± 0.09	−0.04 ± 0.04	NS	−0.02 ± 0.27	−0.18 ± 0.16	NS
Cit	0.23 ± 0.19	0.00 ± 0.23	NS	0.69 ± 0.57	−0.02 ± 0.87	NS
Tau	0.08 ± 0.12	0.18 ± 0.19	NS			

Data are means ± SEM for eight control and eight FGR ewes. NS, not significant ($P > 0.05$).

Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartate; Cit, citrulline; FGR, fetal growth restriction; Gln, glutamine; Glu, glutamate; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanine; Pro, proline; Ser, serine; Tau, taurine; Thr, threonine; Tyr, tyrosine; Val, valine.

Table 2. Maternal arterial and uterine venous concentrations of plasma amino acids (μmol/l) in the control and FGR groups

	Control maternal artery	FGR maternal artery	*P* value	Control uterine vein	FGR uterine vein	*P* value
Essential						
Val	325.79 ± 18.05	257.97 ± 35.21	NS	303.67 ± 18.55	245.99 ± 33.41	NS
Leu	230.14 ± 11.39	163.57 ± 16.69	0.005	212.26 ± 12.86	154.01 ± 15.80	0.02
Ile	169.37 ± 5.88	121.06 ± 12.54	0.004	155.99 ± 6.65	114.52 ± 11.83	0.02
Thr	222.31 ± 12.50	145.91 ± 20.96	0.007	209.63 ± 11.92	139.81 ± 19.92	0.02
Phe	71.35 ± 3.70	59.95 ± 4.57	0.03	69.05 ± 3.72	55.74 ± 4.32	0.04
Met	36.75 ± 1.30	23.31 ± 1.91	0.001	34.63 ± 1.47	22.01 ± 1.58	0.001
Lys	155.84 ± 8.99	109.61 ± 11.17	0.006	150.88 ± 10.79	105.81 ± 10.88	0.01
His	55.10 ± 3.04	47.21 ± 3.52	NS	52.85 ± 3.01	45.16 ± 3.26	NS
Nonessential						
Ser	78.96 ± 2.65	62.50 ± 6.19	0.03	70.73 ± 2.57	57.70 ± 5.30	NS
Gly	415.58 ± 19.76	375.94 ± 14.61	NS	415.30 ± 17.44	381.57 ± 15.43	NS
Ala	135.86 ± 8.38	105.87 ± 8.81	0.03	130.30 ± 8.06	102.28 ±7.97	0.04
Pro	136.01 ± 11.34	70.15 ± 5.56	0.001	130.60 ± 9.65	69.48 ± 4.99	0.001
Arg	160.90 ± 18.42	126.53 ± 18.69	NS	152.63 ± 17.91	124.61 ± 18.39	NS
Orn	141.83 ± 11.55	103.21 ± 12.19	0.04	131.20 ± 11.17	99.93 ± 11.93	0.05
Tyr	86.15 ± 4.29	63.66 ± 7.12	0.02	83.59 ± 4.01	62.38 ± 6.85	0.01
Gln	256.70 ± 10.51	237.00 ± 12.05	NS	244.26 ± 8.42	224.90 ± 11.82	NS
Glu	78.03 ± 4.05	59.36 ± 5.10	0.01	78.73 ± 4.27	59.41 ± 5.36	0.03
Asn	50.51 ± 3.51	39.63 ± 3.66	NS	48.97 ± 2.85	32.30 ± 3.00	0.002
Asp	8.02 ± 0.49	6.13 ± 0.50	0.02	9.04 ± 0.66	6.99 ± 0.45	0.03
Cit	262.94 ± 34.27	192.67 ± 12.18	NS	249.89 ± 32.13	186.64 ± 12.03	0.03
Tau	73.31 ± 3.92	45.36 ± 6.72	0.003	73.65 ± 3.78	44.64 ± 5.84	0.001

Data are means ± SEM for eight control and eight FGR ewes. *P* determined by unpaired Student's *t*-test. NS, not significant ($P > 0.05$).

Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartate; Cit, citrulline; FGR, fetal growth restriction; Gln, glutamine; Glu, glutamate; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanine; Pro, proline; Ser, serine; Tau, taurine; Thr, threonine; Tyr, tyrosine; Val, valine.

and lysine uptake graphs have significantly smaller slopes than the control lines ($P < 0.02$ for leucine and $P < 0.002$ for lysine). Polynomial analysis was used to construct the curves relating the natural log of the transplacental concentration ratio to placental mass. These curves have a descriptive purpose and are not derived from a theoretical model. The small number of observations did not allow us to establish whether the control and FGR ratios fit two separate curves. The four upper panels of **Figure 2** show that as placental weight decreased, it took progressively larger transplacental PO_2 and glucose concentration gradients to draw progressively smaller amounts of oxygen and glucose into the umbilical circulation. In association with a decrease in placental weight from about 500 to 100 g, umbilical venous PO_2 decreased from a maximum of 70% of uterine venous PO_2 (ln 0.7 = −0.36) to a minimum of 25% (ln 0.25 = −1.39). The umbilical venous glucose decreased from a maximum of 40% to a minimum of 12% of the uterine venous glucose concentration. The four lower panels of **Figure 2** show that in the FGR group, the decline in placental weight and in the umbilical uptakes of leucine and lysine was associated with an increase in the feto/maternal concentration ratio of these two AAs. This is in contrast to the decline in the PO_2 and glucose feto/maternal ratios.

The decrease in umbilical venous PO_2 was associated with a decrease in umbilical blood flow (5), and led to extremely low values of umbilical arterial O_2 content for five of the eight FGR fetuses (O_2 content < 1 mmol/l). The natural log of the umbilical/maternal arterial concentration ratio for each of the essential AAs is plotted against umbilical arterial O_2 content in **Figure 3**. Arterial concentration ratios are used in these graphs because they are plotted in relation to umbilical arterial O_2 content. Polynomial analysis was used to construct the curves in each panel. This figure demonstrates that for all these essential AAs, the feto/maternal concentration ratio tends to attain its highest value at the lowest O_2 concentration.

Under normal physiologic conditions, the umbilical uptake of nutrients has the two main functions of providing material for the accretion of new tissue and providing metabolic fuels to sustain fetal oxidative metabolism. Because in severe FGR the fetal growth rate approaches zero, virtually all of the umbilical uptake of nutrients should be reduced to the single function of sustaining fetal O_2 consumption. To explore the validity of this basic concept, the umbilical uptake of each of the substrates that were measured in this study was transformed into its umbilical nutrient/O_2 quotient. The results of this computation are presented in **Table 6** and in the cumulative

Table 3. Umbilical venous and arterial plasma amino acid concentration (μmol/l) in the control and FGR group

	Control umbilical vein	FGR umbilical vein	*P* value	Control fetal artery	FGR fetal artery	*P* value
Essential						
Val	516.08 ± 29.57	457.07 ± 39.76	NS	481.94 ± 27.03	429.64 ± 39.75	NS
Leu	205.73 ± 11.30	222.77 ± 20.59	NS	174.49 ± 9.36	189.62 ± 19.92	NS
Ile	137.96 ± 8.12	110.94 ± 10.68	NS	117.47 ± 6.43	92.49 ± 9.61	0.05
Thr	330.97 ± 26.68	336.87 ± 25.26	NS	314.01 ± 27.91	322.53 ± 24.84	NS
Phe	116.43 ± 11.07	171.56 ± 14.30	0.009	105.21 ± 10.56	159.93 ± 14.72	0.01
Met	85.57 ± 4.39	74.41 ± 7.20	NS	77.80 ± 4.62	68.03 ± 6.65	NS
Lys	63.07 ± 8.19	133.83 ± 26.48	0.023	48.12 ± 7.34	113.98 ± 26.47	0.04
His	54.00 ± 3.33	70.68 ± 6.84	0.046	47.73 ± 3.38	65.75 ± 8.38	NS
Nonessential						
Ser	689.29 ± 46.91	629.23 ± 91.65	NS	694.52 ± 47.35	633.51 ± 90.80	NS
Gly	370.55 ± 17.98	522.37 ± 61.21	0.032	349.51 ± 17.27	499.32 ± 60.04	0.031
Ala	296.85 ± 31.26	489.72 ± 83.88	0.049	277.58 ± 29.28	467.67 ± 82.65	0.048
Pro	173.33 ± 13.31	386.63 ± 117.77	NS	156.74 ± 11.44	363.35 ± 114.49	NS
Arg	76.61 ± 14.66	49.03 ± 7.96	NS	60.58 ± 13.48	34.56 ± 6.48	NS
Orn	68.18 ± 8.17	68.04 ± 7.64	NS	67.18 ± 7.97	67.36 ± 7.33	NS
Tyr	139.98 ± 10.64	164.10 ± 17.15	NS	128.78 ± 9.79	154.43 ± 17.37	NS
Gln	401.30 ± 22.84	474.20 ± 36.12	NS	354.98 ± 19.56	432.63 ± 38.06	NS
Glu	10.78 ± 2.29	12.89 ± 2.34	NS	48.42 ± 6.37	29.59 ± 3.73	0.023
Asn	48.23 ± 4.10	89.24 ± 14.57	0.017	39.09 ± 3.59	80.70 ± 16.56	0.028
Asp	19.50 ± 2.08	16.48 ± 1.68	NS	19.49 ± 2.02	16.94 ± 1.48	NS
Cit	162.51 ± 17.79	136.47 ± 12.29	NS	160.75 ± 17.00	136.83 ± 12.11	NS
Tau	77.85 ± 8.37	334.66 ± 66.59	0.002	77.07 ± 7.98	333.10 ± 65.37	0.002

Data are means ± SEM for eight control and eight FGR ewes. *P* determined by unpaired Student's *t*-test. NS, not significant ($P > 0.05$).

Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartate; Cit, citrulline; FGR, fetal growth restriction; Gln, glutamine; Glu, glutamate; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanine; Pro, proline; Ser, serine; Tau, taurine; Thr, threonine; Tyr, tyrosine; Val, valine.

graph of **Figure 4**. As expected, in the control group there was a large surplus in the umbilical uptake of oxidizable substrates (**Table 6**), providing the excess carbon and nitrogen required for growth.

In the FGR group, the sum of all the umbilical O_2 quotients was ~1.0. This indicates that, on average, the fetal uptake of carbon and nitrogen was just sufficient to meet oxidative requirements. Of interest is the role that each substrate played in this radically altered physiological state. The glucose/O_2 quotient was not significantly different in the control and FGR groups. There was, however, a disproportional decrease of the umbilical lactate/O_2 quotient. Despite the decrease in fetal O_2 consumption, the umbilical uptake of glucose and lactate was insufficient to sustain fetal oxidative metabolism. In this article, the lactate/O_2 quotient calculation was based on plasma lactate measurements and assumed zero lactate uptake by the red cells ($\Phi = 1$ in equation 3). The alternative calculation ($\Phi = 0$) yields lactate/O_2 quotients equal to 0.21 ± 0.02 and 0.02 ± 0.08 for the control and FGR groups, respectively.

The umbilical uptake of AAs filled the gap in the availability of oxidizable metabolites. **Figure 4** shows that within the limits of experimental error, the AAs with the largest uptake in the normal condition were also the ones that contributed most to the metabolic balance of the FGR fetus. The mean placental uptake of fetal glutamate in the FGR group was reduced to about 27% of that of the control group and was significantly correlated to the disproportionate decrease in the fetal liver/fetal weight ratio that characterizes FGR (**Figure 5**). The uterine nutrient/O_2 quotient computation (**Table 7**) agrees with the umbilical computation. It shows a surplus in the uptake of oxidizable substrates in the control group and no surplus in the FGR group. From a methodological point of view, this agreement is an important validation of the experimental results because the calculation of the uterine and umbilical O_2 quotients depends on two sets of independent measurements. Note that the role of each substrate in balancing supply and demand is somewhat different across the uterine and the umbilical circulation. Both circulations show the quantitatively important negative uptake of one substrate that balances the positive uptake of all the other substrates. However, in the uterine circulation, lactate, fulfills this role and in the umbilical circulation, glutamate does. The assumption that the maternal red cells did not pick up lactate ($\Phi = 1$) may have underestimated the uterine lactate excretion. The alternative assumption ($\Phi = 0$) would have increased the uterine lactate excretion, expressed as lactate/O_2 quotient by 0.04 for both the control and FGR groups.

Table 4. Comparison of control vs. FGR of the umbilical arterial (α)/maternal arterial (A) concentration ratios for the 16 amino acids with a positive umbilical uptake and the 5 that did not display a positive umbilical uptake

	Control α/A	FGR α/A	*P* value
Val	1.49 ± 0.08	1.75 ± 0.14	NS
Leu	0.77 ± 0.05	1.20 ± 0.14	0.006
Ile	0.70 ± 0.04	0.80 ± 0.10	NS
Thr	1.41 ± 0.11	2.38 ± 0.24	0.002
Phe	1.46 ± 0.09	2.88 ± 0.30	0.001
Met	2.14 ± 0.15	3.08 ± 0.39	NS
Lys	0.30 ± 0.03	1.13 ± 0.31	0.001
His	0.85 ± 0.04	1.43 ± 0.18	0.004
Gly	0.85 ± 0.04	1.37 ± 0.20	0.031
Ala	2.07 ± 0.20	4.79 ± 1.00	0.009
Pro	1.18 ± 0.07	5.29 ± 1.62	0.001
Arg	0.37 ± 0.06	0.28 ± 0.04	NS
Orn	0.47 ± 0.03	0.69 ± 0.08	0.025
Tyr	1.49 ± 0.08	2.63 ± 0.41	0.009
Gln	1.38 ± 0.04	1.85 ± 0.17	0.018
Asn	0.77 ± 0.06	2.03 ± 0.37	0.001
Ser	8.83 ± 0.62	10.85 ± 1.96	NS
Glu	0.64 ± 0.09	0.50 ± 0.06	NS
Asp	2.48 ± 0.29	2.89 ± 0.30	NS
Cit	0.63 ± 0.04	0.71 ± 0.05	NS
Tau	1.07 ± 0.11	8.86 ± 2.24	0.001

Data are means ± SEM for eight control and eight FGR ewes. *P* determined by unpaired Student's *t*-test. NS, not significant ($P > 0.05$).

Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartate; Cit, citrulline; FGR, fetal growth restriction; Gln, glutamine; Glu, glutamate; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanine; Pro, proline; Ser, serine; Tau, taurine; Thr, threonine; Tyr, tyrosine; Val, valine.

Table 5. Umbilical uptakes of oxygen, glucose, lactate, and total amino acids in control and FGR fetuses (μmol/min/kg_{fetus})

	Control	FGR	*P* value
Oxygen	340.0 ± 23.3	261.6 ± 6.9	0.006
Glucose	34.5 ± 2.9	25.5 ± 1.3	0.010
Lactate	16.4 ± 2.2	−1.2 ± 4.2	0.002
Amino acids	33.0 ± 4.0	22.5 ± 1.3	0.02

Data are means ± SEM for eight control and eight FGR ewes. *P* determined by unpaired Student's *t*-test. NS, not significant ($P > 0.05$).

FGR, fetal growth restriction.

DISCUSSION

Human FGR is associated with abnormal development of the placenta, with a decreased proportion of terminal villi relative to stem and intermediate villi (3) and with asymmetric growth of the fetus. FGR is a major cause of fetal and neonatal morbidity and mortality and is a major risk factor for cardiovascular disease, type 2 diabetes mellitus, and obesity in adulthood (15). The ovine FGR that is the subject of this investigation is also characterized by asymmetrical fetal growth. It is characterized by a reduction in placental weight, which is associated with a disproportionate reduction in placental permeability to the transplacental diffusion of oxygen and glucose. (**Figure 2**). A significant enlargement of the transplacental PO_2 and glucose concentration differences has also been observed in human FGR (4,6).

The primary focus of this study was to investigate the relative importance of placental transport vs. fetal utilization in determining the umbilical uptake of AAs in the hypoxic growth-restricted fetus. One way to evaluate these factors is through use of the AA fetal/maternal (F/M) concentration ratio. For any given essential AA, for which uterine and umbilical uptakes are virtually equal, the F/M ratio is an expression of the balance between two factors: (i) the rate at which the placenta clears the AA from the maternal circulation (placental transport clearance) and (ii) the rate at which fetal metabolism clears the AA from the fetal circulation (fetal metabolic clearance). The uterine clearance (factor 1) is simply the uterine uptake of the AA (Q_{ut}) divided by its maternal arterial concentration (A). Thus, Q_{ut}/A = uterine clearance. The fetal clearance of the AA is the umbilical uptake (Q_{ft}) divided by its fetal arterial concentration (a). Thus, Q_{ft}/a = fetal clearance of the AA.

A decrease in fetal clearance tends to increase the F/M ratio, whereas a decrease in placental transport clearance (uterine clearance) has the opposite effect. In the FGR group of the current study, the F/M ratio of most of the AAs that are transported by the placenta into the umbilical circulation was significantly higher than in the control group (**Table 2**). This finding indicates that the decrease in fetal clearance was the dominant factor in limiting uptake.

Furthermore, for the two essential AAs leucine and lysine, the F/M ratio showed a tendency to increase in an inverse manner to placental weight and to umbilical uptake (**Figure 2**). Therefore, the data in **Figure 2**, showing an increasing F/M ratio with deceasing placental weight, lead to the conclusion that at the lowest placental weight, the fetal ability to metabolize leucine and lysine had decreased even more than the ability of the placenta to transport these AAs into the fetal circulation.

The evidence that the transplacental PO_2 gradient is inversely related to placental weight indicates fetal hypoxia as the condition that contributes to a decrease in the fetal plasma clearance of AAs and causes the smallest FGR placentas to transport AAs against higher F/M concentration ratios. Experiments in sheep of graded fetal hypoxemia have shown that an umbilical arterial content of about 2 mmol/l defines the threshold below which, in the fetal lamb, the oxygen consumption of the organs perfused via the descending aorta begins to decrease. This decline accelerates in inverse relation to O_2 content so that below the 1.5 mmol/l level oxygen uptake by the fetal hind limbs is drastically reduced (16). In the current study, six of the eight fetuses of the FGR group had an umbilical arterial O_2 content at or below 1.5 mmol/l. From 1.5 mmol/l downward, the F/M ratio of all the essential AAs tended to increase in inverse relation to O_2 content (**Figure 3**). This suggests that under significantly reduced oxygenation, an increased F/M ratio defines a decreased clearance of AAs from fetal circulation.

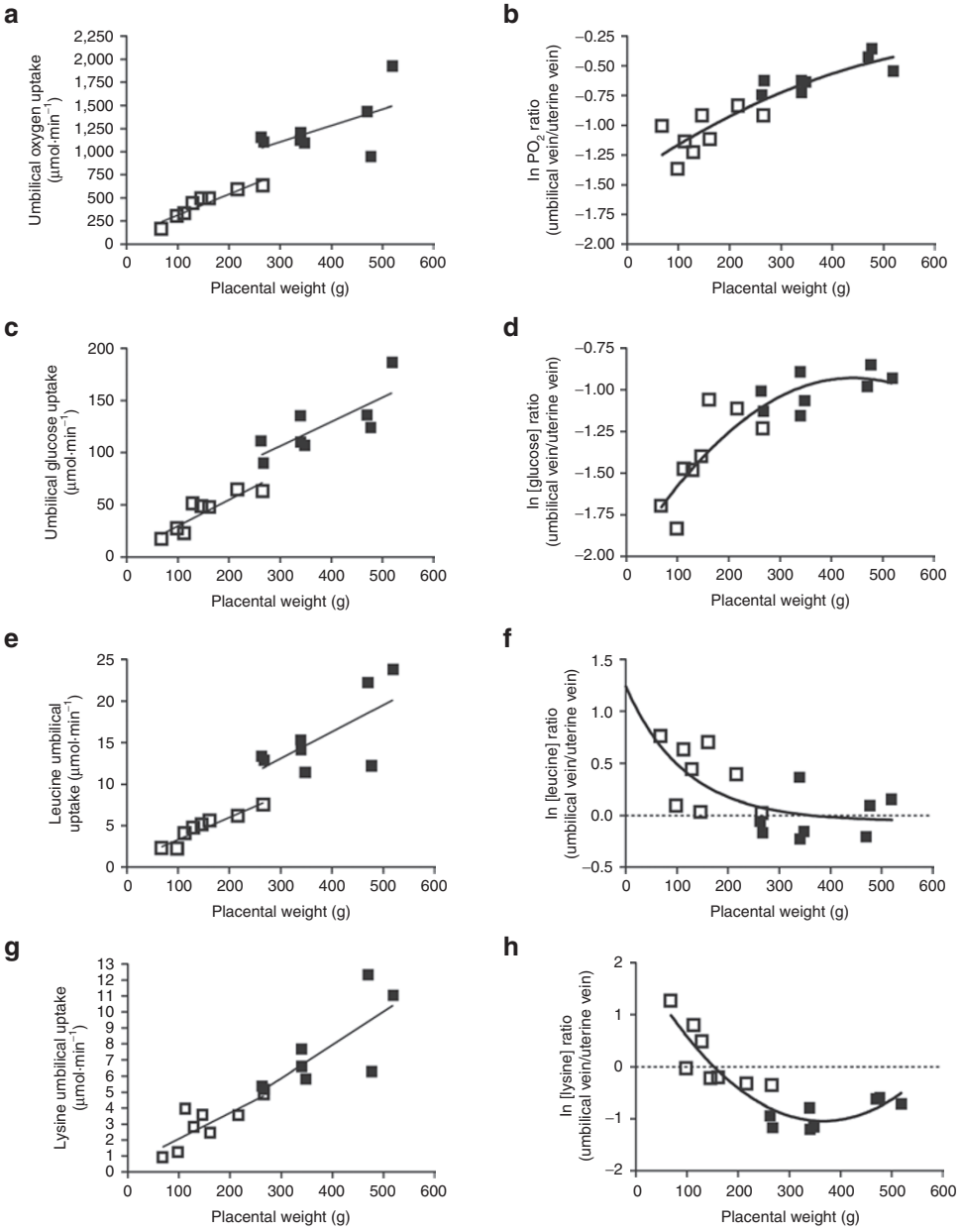

Figure 2. Umbilical uptakes of oxygen, glucose, leucine, and lysine, and the natural log (ln) of the umbilical/uterine venous concentration ratios of these metabolites are plotted against placenta weight. In the four left graphs, umbilical uptakes (μmol/min) of (**a**) O_2, (**c**) glucose, (**e**) leucine, and (**g**) lysine. In the four right graphs, the ln of the (**b**) PO_2, (**d**) glucose, (**f**) leucine, and (**h**) lysine ratios. Polynomial analysis was used to draw the curves in these graphs. Two separate regression lines were calculated for the FGR and control uptakes. Control, filled squares; FGR, open squares. The horizontal dotted line in graphs **f** and **h** defines a fetal/maternal concentration ratio of one. FGR, fetal growth restriction.

Variability in the degree of fetal hypoxemia may be one of the reasons why a low concentration of fetal plasma AAs is not a standard feature of FGR. In a previous study of ovine FGR, fetal plasma leucine was significantly lower than in controls and consistently lower than maternal leucine (9). This contrasts with the data in the current study, which show no significant decrease in the mean FGR fetal leucine concentration and a reversal of the mean F/M leucine ratio from <1 in the control group to >1 in the FGR group. A major difference between the two studies is that, in the tracer leucine study, the mean FGR umbilical arterial O_2 content was much higher than that in the current study (2.4 vs. 1.2 mmol/l). This higher level of oxygenation was associated with higher umbilical uptakes of O_2, glucose, and leucine per kg fetus and with a positive rate of fetal protein accretion (9).

The tracer leucine study demonstrated that in FGR, both the flux of maternal leucine into the fetus and the back-flux of fetal leucine into the placenta were reduced in relation to placental and fetal weight and that the back-flux correlated with fetal plasma leucine concentration (9). This evidence supports the hypothesis that placental underdevelopment causes FGR by limiting the transport of maternal AAs into the fetal

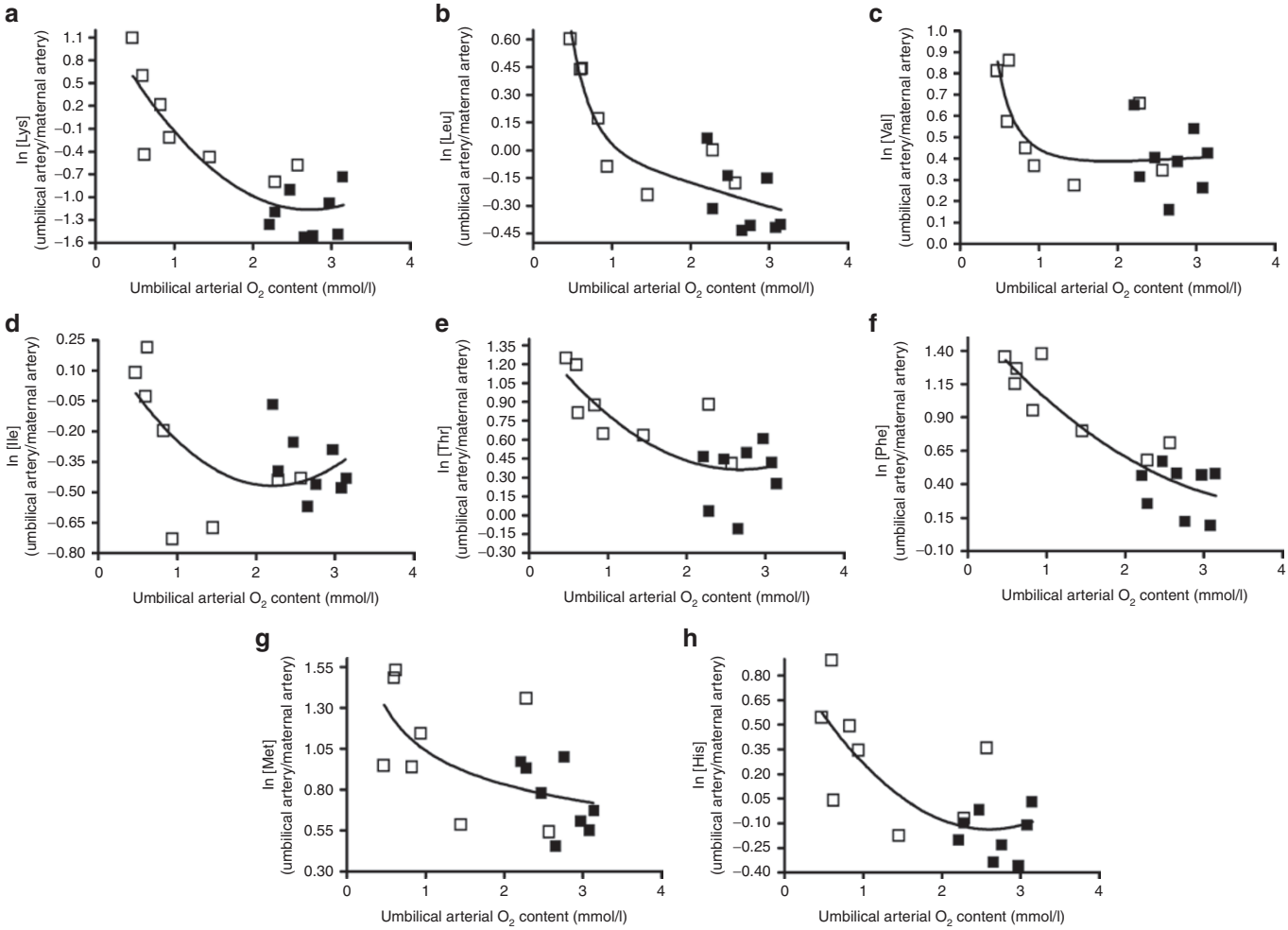

Figure 3. The natural log (ln) of the umbilical arterial/uterine arterial concentration ratio for essential amino acids is plotted against the umbilical arterial O_2 content (mmol/l). (**a**) Lys, (**b**) Leu, (**c**) Val, (**d**) Ile, (**e**) Thr, (**f**) Phe, (**g**) Met, (**h**) His. Polynomial analysis was used to construct the curves that are drawn through the data. Control, filled squares; fetal growth restriction, open squares. His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Thr, threonine; Val, valine.

Table 6. Umbilical uptakes of glucose, lactate, and total amino acids expressed as nutrient/O_2 quotients and their sum in control and FGR fetuses

	Control	FGR	*P* value
Glucose	0.61 ± 0.03	0.59 ± 0.03	NS
Lactate	0.14 ± 0.02	−0.02 ± 0.05	0.01
Amino acids	0.56 ± 0.05	0.48 ± 0.03	NS
Sum	1.32 ± 0.08	1.05 ± 0.06	0.017

Data are means ± SEM for eight control and eight FGR ewes. *P* determined by unpaired Student's *t*-test. NS, not significant ($P > 0.05$).

FGR, fetal growth restriction.

circulation. However, a subsequent study, which was focused on the investigation of hepatic metabolism in a normally grown fetus, produced the unexpected result that a 20-h infusion of a glucagon-somatostatin solution into the fetal circulation inhibits the placental transport of both neutral and basic AAs (17). This finding indicates that hormonal mechanisms, which regulate fetal metabolism, are also important in the regulation of placental AA transport. Thus, an alternative hypothesis is that in FGR, these mechanisms adapt fetal growth to placental underdevelopment via a downregulation of placental AA transport.

One of the main functions of placental AA transport is to supply the fetus with substrates of oxidative metabolism, even in severe FGR. This is a quantitatively important function, as demonstrated by the finding that in the FGR group of the current study, despite evidence of virtually zero growth (**Figure 4**), the umbilical uptake of AAs per kg fetus was reduced only to ~70% of normal (**Table 5**). The data in **Table 5** suggest that this relatively small decrease in AA uptake compensated for a large deficit in umbilical lactate uptake.

Fetal AA oxidation in FGR may be accomplished in part via the routing of AA carbon into fetal hepatic gluconeogenesis and the subsequent oxidation of the produced glucose by other fetal organs. An increase in fetal hepatic gluconeogenesis may be one of the reasons why the fetal plasma glutamate concentration and the placental uptake of fetal glutamate were significantly reduced in the FGR fetuses (**Tables 1** and **3**). However,

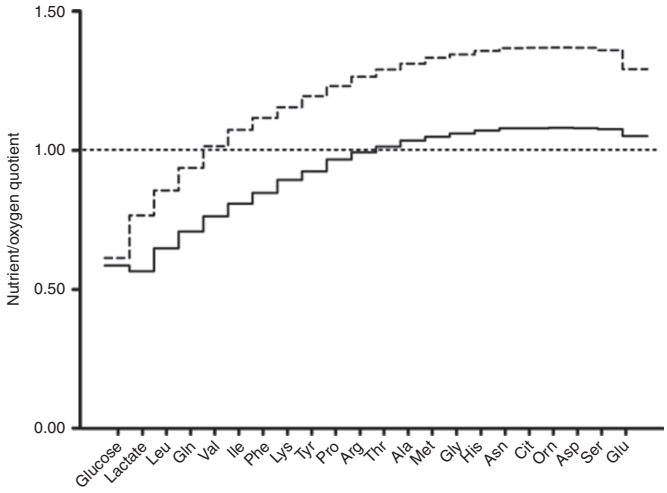

Figure 4. Individual umbilical nutrient/O_2 quotients were determined and are presented as a cumulative graph comparing control ($n = 8$) (dashed line) and FGR ($n = 8$) (solid line) umbilical nutrient/O_2 quotients for all the metabolites that were measured in this study. From left to right, the quotients are ordered according to their magnitude in the control group. The FGR quotients were significantly smaller for lactate, valine, isoleucine, and glutamate (**Tables 1** and **6**). The sum of the FGR quotients was significantly less than control (1.05 vs. 1.32, $P = 0.017$) and approached the 1.00 level (dotted line) that defines zero growth. Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartate; Cit, citrulline; FGR, fetal growth restriction; Gln, glutamine; Glu, glutamate; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine.

Table 7. Uterine uptakes of glucose, lactate, and total amino acids expressed as O_2 quotients and their sum in control and FGR fetuses

	Control	FGR	*P* value
Glucose	0.96 ± 0.09	0.74 ± 0.03	0.03
Lactate	−0.09 ± 0.01	−0.12 ± 0.02	NS
Amino acids	0.53 ± 0.09	0.32 ± 0.04	0.04
Sum	1.40 ± 0.15	0.94 ± 0.05	0.01

Data are means ± SEM for eight control and eight FGR ewes. *P* determined by unpaired Student's *t*-test. NS, not significant ($P > 0.05$).

FGR, fetal growth restriction.

the changes in fetal glutamate metabolism may have multiple causes, including a decrease in the hepatic/fetal body mass ratio (**Figure 5**) and fetal hepatic ischemia.

This study of AA uptake and utilization by growth-restricted fetal lamb helps in interpreting data related to human FGR. Clinical studies of FGR pregnancies have shown relationships between placental weight and fetal weight similar to those shown in **Figure 1** (18). A significant relationship of placental weight and fetal oxygenation in human FGR has also been demonstrated (8). Tracer studies of placental AA transport across the placenta of severely growth-restricted human fetuses demonstrated that the transplacental fluxes of leucine and phenylalanine were a smaller fraction of the fetal plasma turnover of these AAs than in a normal pregnancy (8,10). However, the umbilical venous concentration of these AAs was not below normal (8,10). This result was puzzling because it

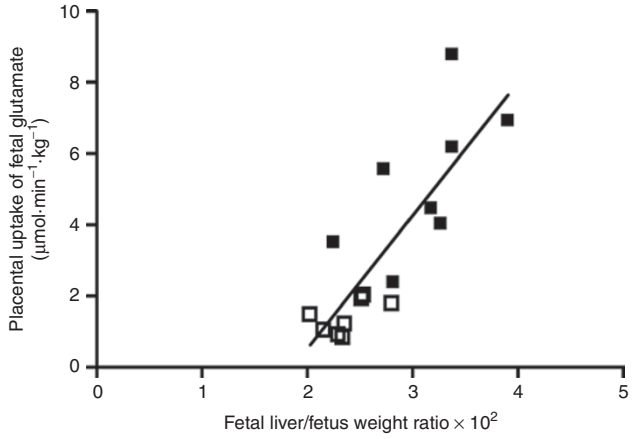

Figure 5. Placental glutamate uptake was significantly correlated ($R^2 = 0.67$, $P < 0.001$) to the fetal liver/body weight ratio. Control, filled squares; FGR, open squares. FGR, fetal growth restriction.

was contrary to the expectation that the availability of AAs for fetal protein synthesis would be at its lowest level in the most growth-restricted fetuses. Because these fetuses had a much lower mean umbilical venous O_2 saturation than the controls (44.7 vs. 72.5%) and had an abnormally high impedance to umbilical blood flow, it is likely that hypoxia had decreased the clearance of AAs from the fetal circulation, resulting in increased F/M ratios with decreased placental weights, which is consistent with the findings of this study.

In conclusion, this study indicates that in the near term growth-restricted fetus, impairment of placental O_2 transport may induce a state of severe hypoxia that in turn reduces the ability of the fetus to utilize metabolic substrates. This reduction in metabolic rate may be disproportionally greater than the reduction in placental AA transport capacity and may produce the finding of normal, or even greater than normal, fetal AA concentrations. It is important to note that severe fetal hypoxia is not a constant aspect of FGR. According to studies of human FGR, it may represent a late stage of this disease. Therefore, the current study does not contradict the hypothesis that an impairment of placental AA transport may play a crucial role in the development of FGR. Rather, it cautions against the assumption that FGR is nothing more than a case of nutrient deprivation.

METHODS

Animal Care and Surgery

Sixteen time-mated 2–3-y-old Columbia-Rambouillet ewes pregnant with a single fetus were studied as previously described (5). Animal care was in compliance with US National Institutes of Health guidelines within an American Association for Accreditation of Laboratory Animal Care–certified facility. The University of Colorado Health Sciences Center Animal Care and Use Committee approved the study. At ~38 d gestational age (dGA; term = 147 ± 3 dGA), the ewes were allocated to either the thermoneutral control (C) or hyperthermic (FGR) treatment, a treatment documented to induce placental insufficiency intrauterine growth restriction (5). Ewes in the FGR environment had their feed and water intakes recorded daily and were fed a diet of alfalfa hay pellets. Control animals were fed the daily FGR ewes' intake. Ewes were maintained in the FGR environment until 120 ± 1 dGA, after which they were moved to the control environment

room where feeding continued *ad libitum*. The feed intake never fell below 9.75 Mj/kg/d.

At 127 ± 1 dGA, ewes underwent surgery for placement of fetal and maternal catheters as previously described (5). Following surgery, sheep were placed in individual carts with free access to water and alfalfa hay pellets, and daily intakes were recorded. Ampicillin (500 mg) was injected daily into the amniotic cavity for the first 3 d after surgery. Animals were allowed to recover from surgery for at least 5 d before study.

Study and Analytic Methods

At ~134 dGA, under control room conditions (19 ± 2 °C) and at animal core body temperature of ~39.1 °C, four sets of 1 ml blood samples were drawn at 20-min intervals, each set consisting of samples drawn simultaneously from the maternal femoral artery, uterine vein, umbilical vein, and fetal femoral artery. The fetal femoral arterial and umbilical arterial blood had identical composition. A detailed description and schematic drawing of this preparation has been published (19). The samples were analyzed for maternal and fetal PO_2; O_2 saturation and O_2 capacity; glucose, lactate, and AA concentrations; and the blood flow indicator (ethanol) (5). PO_2 was measured with a PO_2 electrode (Radiometer America BMS 3 MK_2, Westlake, OH). Oxygen saturation and O_2 capacity were measured spectrophotometrically (OSM2, Radiometer). Plasma glucose and lactate were measured with a glucose-lactate analyzer (model 2700 Select and YSI Dual Standard, Yellow Springs, Yellow Springs, OH). Ethanol was measured enzymatically (Sigma Catalog no. Alcohol 332-UV, Sigma, St Louis, MO).

Plasma AA determination was as previously described (14). Briefly, on the day of analysis, samples were thawed quickly and deproteinized with 15% sulfosalicylic acid containing 0.3 µmol/l norleucine as an internal standard. The pH was then adjusted to 2.2 with 1.5 N LiOH. After centrifugation, the supernatant was analyzed with a Dionex (Dionex, Sunnyvale, CA) high-performance liquid chromatography AA analyzer. Concentrations were measured after reaction with ninhydrin at 570 nm, except for proline, which was measured at a wavelength of 440 nm. The same high-performance liquid chromatography column was used for all samples from an individual animal. Reproducibility within the same column had a mean value of ±2%.

Calculations

The umbilical uptake of O_2 (µmol/min) was calculated by means of the equation:

$$O_2 \text{ umbilical uptake} = f(\gamma O_2 - \alpha O_2) \quad (1)$$

where f is umbilical blood flow (ml/min) and γO_2 and αO_2 are the O_2 content (µmol/ml) in umbilical venous and umbilical arterial blood, respectively.

The umbilical uptakes of the metabolic substrates that were measured in plasma (glucose, lactate, and AAs) were calculated by means of the equation:

$$(\text{Umbilical uptake})x = f(\gamma - \alpha)x\,(1 - \phi x \text{Htf}) \quad (2)$$

where $(\gamma - a)x$ is the concentration difference per ml of plasma of substrate x across the umbilical circulation (µmol/ml), Htf is the fractional hematocrit of fetal blood, and Φx is a coefficient whose value depends on the contribution of fetal erythrocytes to uptake. The Φx coefficient was set to 0.24 for glucose (7) and to 1.0 for AAs (14). The value of Φx for lactate has not been determined. In this article, we set Φx for lactate to 1. If there is rapid lactate exchange between plasma and red cells, this calculation underestimates the true uptake.

The umbilical nutrient/O_2 quotients were calculated according to the equation:

$$(\text{Umbilical nutrient/O}_2 \text{ quotient})x = (n\text{O}_2)_x \frac{(\gamma - \alpha)x(1 - \phi_x \text{Htf})}{\gamma O_2 - \alpha O_2} \quad (3)$$

where $(n\text{O}_2)x$ is the number of O_2 molecules that are needed to oxidize one molecule of nutrient x to CO_2, water, and urea. The uterine nutrient/O_2 quotients were similarly calculated.

For each nutrient, we computed the whole-blood arterial–venous concentration difference across the uterine circulation and divided this difference by the uterine oxygen (arterial–venous concentration) difference. This molar ratio was then multiplied by the $n\text{O}_2$ coefficient.

In the conversion of plasma to whole-blood concentration, the Φ coefficient was set to 1.0 for AAs and lactate and to 0.87 for glucose. The glucose Φ coefficient is much higher for maternal than fetal blood because in adult sheep, most of the blood glucose is carried in the plasma.

A goal of this study was to compare the changes in the transplacental concentrations of metabolites as diverse as free oxygen, glucose, and AAs. In ovine FGR, there is an enlargement of the uterine venous–umbilical venous PO_2 difference (5) and of the maternal arterial–umbilical arterial plasma glucose concentration difference (7). Studies of AA transport across the FGR placenta have described changes in the umbilical venous/maternal arterial concentration ratios (10,12). In comparing transplacental PO_2, glucose, and AA concentrations in any given animal, it is necessary to compare samples that have been drawn from the same vessel, and it is preferable to avoid the difficulty of comparing differences with ratios. This is one of the reasons to use the natural logs of the umbilical/uterine venous PO_2, plasma glucose, and plasma AA ratios for a direct comparison of concentration changes (**Figure 2**). A second reason is that this log transformation focuses attention on the energetics of placental transport. The umbilical uptake of O_2 and glucose is energized by fetal metabolism. As a consequence, the natural logs of the transplacental feto/maternal PO_2 and glucose concentration ratios are negative. The umbilical uptake of AAs is energized both by placental metabolism (active transport) and fetal metabolism (fetal consumption). As a consequence, the natural log of the transplacental concentration ratio of AAs can vary from negative to positive, depending on the balance between placental active transport and fetal consumption.

Statistical Analysis

All data are presented as either mean ± SEM or as individual marks, where n is the number per control or FGR group. For parameters studies, a nonpaired t-test was used in the calculations for statistical differences between groups, dependent on normal distribution of data. $P < 0.05$ was taken as the significance level. In some graphs, visual inspection suggested a curvilinear relation between x and y. For each of these graphs, polynomial analysis was used to construct a descriptive curve. In all the other graphs, linear regression was used to describe the y vs. x relation and to establish the significance ($P < 0.05$) of slope and intercept.

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