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Research Article

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Umbilical Uptake of Amino Acids in the Unstressed Fetal Lamb

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Finally, the net uptake of nitrogen, carbon, and calories by the growing ovine fetus in the form of amino acids, glucose, and lactate is compared to estimated requirements as determined in previous studies.

INTRODUCTION

The observation that several amino acids, both essential and nonessential, can cross the placenta rapidly and against a concentration gradient (1–6) has led to the suggestion that the placenta may transport to the fetus all of the amino acids which are needed for protein synthesis (1). A related assumption is that in the fetus there is virtually no formation and degradation of amino acids (7). However, recent experimental evidence does not agree with these views. The rate of excretion of urea nitrogen in the fetal lamb is relatively high with respect to the nitrogen stored in the growing tissues (8). Furthermore, glutamate injected into the circulation of anesthetized pregnant rhesus monkeys is not readily transferred to the fetus (9). These observations point to the possibility that the relationship between placental transfer and fetal utilization of amino acids is more complex and offers more opportunity for physiologic regulation than has been implied.

The present study was designed to measure the concentration of 22 amino acids and the oxygen contents in the umbilical artery and umbilical vein of a chronic, unstressed fetal lamb preparation to: (a) determine whether a net fetal uptake of amino acid via the umbilical circulation is detectable; (b) document the relative contribution of individual amino acids and their subgroups to the placental supply of nitrogen and carbon to the fetus; and (c) compare the uptake of amino acids with known requirements of the fetus for growth and metabolism.

METHODS

Surgery. 13 Western breed ewes with gestational ages of 118 to 146 days (mean of 130 days) were studied (six singleton and seven twin pregnancies). The length of gestation in the sheep is 147 days. Catheters were placed in a fetal pedal artery (and advanced into the abdominal aorta), the umbilical vein, and the maternal femoral artery. Preparation for surgery, anesthesia, and the technique for arterial catheterizations were as previously described (10). A surgical approach new to this laboratory was used for catheterization of the common umbilical vein.[1]

At the conclusion of surgery, 500 mg of ampicillin was injected intra-amniotically and the abdominal incision was

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[1] In preparation.

closed. All catheters were exteriorized through a sub-cutaneous tunnel to a cloth pouch fixed on the ewe's flank. All animals were standing and eating within 6 h of the operation and were confined in specially constructed carts for the duration of the experiment. The catheters were irrigated daily with heparinized saline (30 U/ml).

Experimental design. After surgery the animals were maintained on food (dehydrated alfalfa pellets) and water ad libitum. The ewes were weighed weekly and showed normal weight gain (approximately 0.2 kg/day) over the study period. Blood sampling was begun on the 4th to 8th post-operative day, allowing a minimum 96-h period for recovery from operative stress. Samples for amino acid analysis (2.2 ml blood drawn into a dry 3-ml plastic syringe containing EDTA) were taken simultaneously from the umbilical artery (a),[2] umbilical vein (v), and maternal femoral artery (A) at approximately 10 a.m. daily, until either delivery of the fetus or catheter failure. 73 sets (a, v, A) of samples were obtained from the 13 animals (60 sets from singletons, 13 sets from twins). Oxygen content was determined simultaneously in a and v for 48 of the 73 sets collected, to relate the umbilical uptake of amino acids to fetal oxygen consumption.

After completion of the study each ewe was sacrificed and an autopsy was performed. Fetal weight, vertebral column, curved crown-rump, and straight crown-rump length measurements were made to verify the estimated gestational age by breeding dates (11). The umbilical vein and placenta were examined in each case for evidence of clot or tissue damage; no abnormalities were detected.

Amino acid analysis. 2 ml of whole blood was pipetted immediately after withdrawal from the animal into a 12-ml polyvinyl test tube containing 3.0 ml of distilled water. The mixture was shaken gently, frozen in acetone/dry ice, and rapidly thawed under warm water to induce complete lysis of all cellular components. The sample was then deproteinized by adding 1.0 ml of 10% sulfosalicylic acid containing the internal standard norleucine (600 μM) and centrifuged at 500 g for 8 min. The supernate was stored at −75°C until analysis.

Before analysis, the supernate was thawed and recentrifuged. Three samples were analyzed with the JEOL-6AH analyzer (JEOL Analytic Instruments, Cranford, N. J.) during a single 36-h run by means of an automated program. Good resolution of all amino acids studied (See Fig. 1) was obtained utilizing a two-column ion exchange chromatography system with JEOL LCR-2 resin (JEOL Analytic Instruments), three sodium and three lithium buffers, and column temperatures of 36° and 55°C (13, 14). The ninhydrin solution was made every 10–14 days and standards (100 μM) of the amino acids studied (Pierce Chemical Co., Rockford, Ill.) were analyzed in triplicate at the beginning and end of each batch of ninhydrin. The coefficient of variation of optical densities within sets of three standards analyzed in a 36-h run was 2% or less for all amino acids except glutamine (Table I).

Each amino acid was identified on the basis of elution time and the ratio of optical densities at 440 and 570 nm (15). The concentrations of the amino acids were determined on the 570-nm tracing with a single channel JEOL integrator 15SK (JEOL Analytic Instruments). In the analysis of blood samples, the internal standard norleucine was used to correct for possible dilution and recovery errors.

Further refinements of the methodology were introduced to measure glutamine accurately and to avoid the inter-

TABLE I

Reproducibility of Amino Acid Analysis Observed in Standard and Blood Samples. Each Number is the Coefficient of Variation Estimated from Repeated Three-Sample Analyses

Amino acid	Standards	Samples
Ornithine	0.9	0.8
Lysine	1.9	1.3
1-methyl Histidine	1.3	0.9
Histidine	0.9	1.3
3-methyl Histidine	1.5	1.5
Carnosine	2.2	6.3
Arginine	0.9	1.5
Taurine	2.3	1.9
Aspartate	0.8	1.2
Threonine	0.8	1.8
Serine	1.3	1.0
Asparagine	1.7	6.2
Glutamate	1.6	2.0
Glycine	1.3	2.6
Alanine	0.9	1.4
Citrulline	2.2	4.7
α-Aminobutyric acid	1.2	2.5
Valine	1.5	1.9
Isoleucine	1.5	1.7
Leucine	1.0	2.4
Tyrosine	1.0	2.7
Phenylalanine	1.4	1.5
Glutamine	—	—

ference of glutathione with the analysis of some amino acids.

Glutamine. Glutamine presents a particular problem in blood amino acid analysis, which accounts for the relative paucity of data on this amino acid in the literature. In the present buffer system, glutamine is cleanly separated from asparagine and glutamate. However, glutamine gradually cyclizes spontaneously to form pyrrolidone carboxylic acid at temperatures above 35°C. In addition, its concentration decreases slowly with time when stored at temperatures greater than −68°C (16). For these reasons, the samples and standards were stored at −75°C, and the column temperature for glutamine analysis was maintained at 36°C. In spite of these precautions, glutamine levels decreased over the 36-h analysis time for the three consecutive samples. This degradation of glutamine was not associated with an increase of glutamate concentration, as demonstrated by the high reproducibility of glutamate analysis (Table I). The rate of decay was fairly consistent in both sample and standard runs (20.8% between samples one and two, and 20.4% between samples two and three). Therefore, separate standard values could be calculated for glutamine for each of the three sample loops used during a single sequential analysis.

Glutathione. Both GSH and GSSG are present in high concentrations in sheep blood (17). GSH is eluted in our system as a sharp peak superimposed upon aspartate; GSSG is eluted as a broad peak primarily in the region of glycine but also overlapping with alanine and occasionally with citrulline and α-aminobutyric acid. Because of the interference with precise measurements of alanine and glycine, 13 sets of samples (a, v) were treated with 20 μl of performic acid to completely oxidize the glutathione (18). After such treatment, the glutathione is eluted at the extreme front
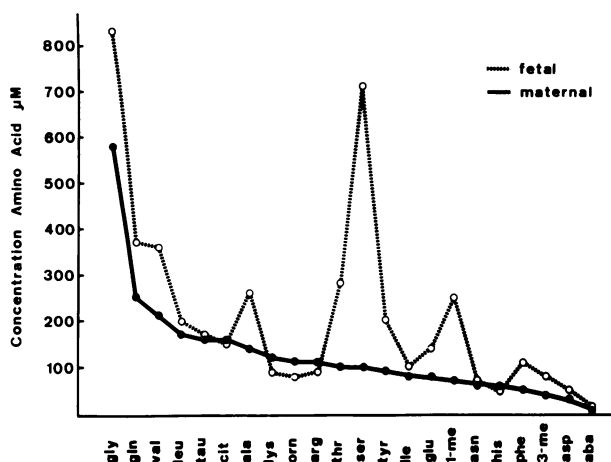
FIGURE 1 The aminograms of the mean maternal and umbilical arterial concentrations for all samples analyzed are shown. The concentrations of the basic amino acids (lysine, ornithine, arginine, and histidine) in fetal blood approximate maternal levels, while the neutral amino acids in general are present at higher concentrations in the fetus. For abbreviated designation of amino acids see (12).

of the chromatogram (before taurine), leaving unaffected the peaks of alanine and glycine.

Sample analysis. In 33 sets of samples, either a or v was run in duplicate during a single 36-h run. On alternate runs, samples were loaded into the three sample loops of the analyzer in either of the following sequences: a_1, v_2, a_3 or v_1, a_2, v_3. In this manner reproducibility of analysis for blood samples could be documented in the same way as was done for amino acid standards. A coefficient of variation of less than 3% was found for all amino acids with the exception of glutamine, citrulline, asparagine and carnosine, in agreement with the results obtained from analysis of standards (Table I).

The remaining forty sets of samples were analyzed in the following manner. A single set of samples (a, v, A) from a given animal on the same day was loaded into the analyzer
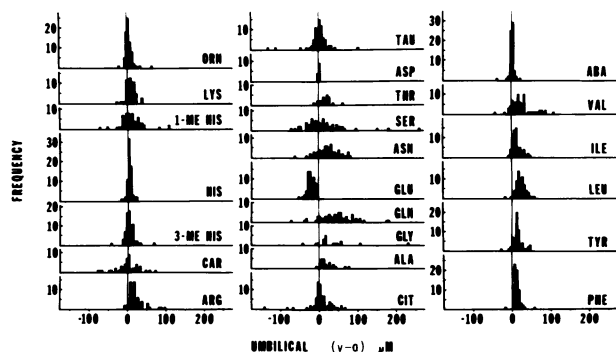

FIGURE 2 The frequency of umbilical venous-arterial concentration differences within 5 μM blocks is depicted for each amino acid for all samples analyzed. It is apparent that some amino acids demonstrate a fairly consistent positive (e.g., phenylalanine), negative (glutamate), or zero (e.g., aspartate) (v-a) difference, whereas others (e.g., glutamine) show a relatively wide scatter of values.

TABLE II
The Concentration of Amino Acids in v and a, and the Mean (v-a)

Amino acid	No.	Umbilical vein (v)	Umbilical artery (a)	(v-a)
		$\mu mol/liter \pm SEM$		
Acidic				
Aspartate	13	51±5.6	51±5.3	0±0.8
Glutamate	68	119±4.7	139±5.1	−20±1.2*
Taurine	73	160±6.3	160±6.1	0±3.3
Neutral				
Glycine	13	858±98.7	830±96.5	28±10.0‡
Alanine	24	287±14.7	264±14.3	23±4.0*
Threonine	23	302±12.9	284±12.1	18±3.4*
Serine	73	738±23.0	721±21.0	17±6.1
Valine	71	383±14.2	357±12.8	26±3.3*
Leucine	71	224±7.1	202±6.8	22±1.8*
Isoleucine	63	115±6.3	101±5.6	14±1.6*
Tyrosine	60	214±5.1	201±5.0	13±1.7*
Phenylalanine	73	130±3.4	118±3.0	12±1.1*
Asparagine	66	88±2.9	74±2.8	14±2.8*
Glutamine	71	414±12.5	365±12.7	49±5.0*
α-Aminobutyric acid	67	26±3.2	25±3.2	1±1.3
Basic				
Lysine	69	101±2.6	92±2.6	9±1.4*
Histidine	72	49±1.7	46±1.7	3±0.8*
Arginine	72	113±8.2	93±7.4	20±2.4*
1-methyl Histidine	72	255±11.9	245±11.3	10±3.3‡
3-methyl Histidine	71	84±5.9	80±5.3	4±1.6‡
Ornithine	72	87±3.3	83±3.1	4±1.2‡
Citrulline	72	151±6.0	146±5.9	5±3.5
Carnosine	53	110±6.8	110±7.6	0±4.4

* Significantly different from 0 at $P < 0.001$ by the paired Student's t test.
‡ Significantly different from 0 at $P < 0.005$ by the paired Student's t test.

in random order, and the three-sample 36-h automated analysis performed. Randomization was done as a precaution to ensure that no systematic error inherent within the methodology would be introduced into the results.

Oxygen content. The collection and measurement of the oxygen content of a and v were performed as described previously (19) using a Lex-O₂-Con (Lexington Instruments Corp., Waltham, Mass.).

RESULTS

Arterial concentrations of amino acids. The concentrations of amino acids in maternal and fetal arterial blood are compared in Fig. 1. It is apparent that some amino acids, notably serine, have a higher concentration in fetal blood; whereas others, such as the basic amino acids lysine, arginine, and histidine, are approximately at the same concentration in the two blood streams. The total molar concentration of the amino acids measured in this study was 1.7 times greater in fetal blood (4.79 vs. 2.81 mM), in agreement with earlier observations about the α-amino

nitrogen content of maternal and fetal blood (4) or plasma (1, 20).

Umbilical venous-arterial differences for amino acids. The histograms of all the venous-arterial differences are presented in Fig. 2. Table II lists the mean umbilical concentration differences (v–a) in terms of micromoles of amino acid. It can be seen that for 16 of the 22 amino acids the mean (v-a) was significantly different from zero at the $P < 0.005$ level. When the mean amino acid (v-a) differences are ordered according to magnitude and sign (Fig. 3), a distinction between neutral, basic, and acidic amino acids becomes apparent. There is a net flux from the placenta into the umbilical circulation of neutral and basic amino acids, the bulk of which consists of neutral amino acids (87%) and arginine (7%). As to the acidic amino acids, there is no significant flux of aspartate and taurine in either direction, and a clearly discernable flux of glutamate from the umbilical circulation into the placenta.

The fetal uptake of glutamine via the umbilical circulation is significantly greater ($P < 0.05$, unpaired t test) than that of all the other amino acids, with the exception of glycine for which an inadequate number of observations is available. Among the basic amino acids, the uptake of arginine is highest ($P < 0.05$, unpaired t test between arginine and each of the other basic amino acids).

Estimate of umbilical blood flow and uptake of nitrogen and carbon by the fetus. In the present study the mean net umbilical venous-arterial difference of amino acid nitrogen was 6.29 $\mu g/ml$, and that of amino acid carbon was 16.3 $\mu g/ml$. In 48 sets of samples the umbilical venous-arterial difference of oxygen content was measured and found to be 1.62 ± 0.06 mM. These data were used to estimate amino
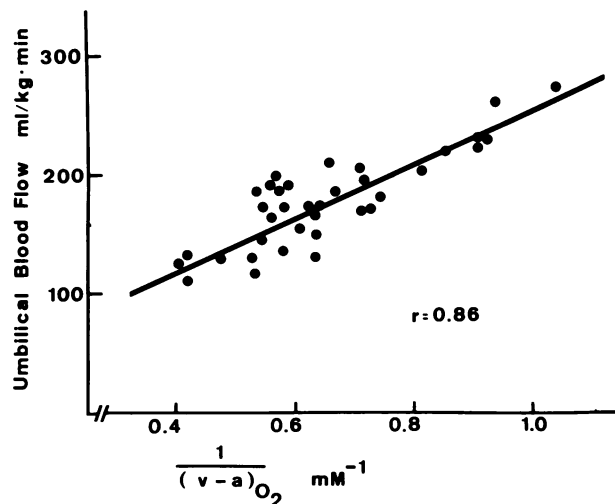

FIGURE 4 The inverse relationship of umbilical blood flow to the umbilical venous-arterial concentration difference of oxygen content is depicted for singleton pregnancies (21). The equation describing this relationship is:

$$f = 30.5 + \frac{222}{(v - a)_{O_2}}.$$

acid nitrogen and carbon uptake by the fetus as follows.

Experiments by James et al. (21) in our laboratory have shown that the umbilical venous-arterial difference of oxygen (v-a)$_{O_2}$ is inversely related to umbilical blood flow (f) according to the equation (Fig. 4):

$$f = 30.5 = \frac{222}{(v - a)_{O_2}} \quad (r = 0.86), \qquad (1)$$

where f is expressed as milliliters per kilogram per minute, and (v-a)$_{O_2}$ as millimolars. On the basis of Eq. 1, the mean umbilical blood flow in the present study was 168 ml/kg · min. Therefore, the mean fetal uptake of amino acid nitrogen was estimated as: 6.29 $\mu g/ml$ × 168 ml/kg · min × 1440 min/day = 1.52 g N/kg · day. Similarly, the fetal uptake of amino acid carbon was estimated to be 3.94 g of carbon/kg · day.

DISCUSSION

The results of the present investigation show that there is no net transfer from placenta to fetus of the two acidic amino acids, glutamate and aspartate, which are ordinary constituents of body proteins. This observation implies that glutamate and aspartate are formed in the fetus. The glutamate may originate from deamination of glutamine or from multiple sources. Furthermore, the amount of glutamate originating in the fetus appears to exceed the requirements of fetal protein synthesis, as demonstrated by the fact that the fetus ex-
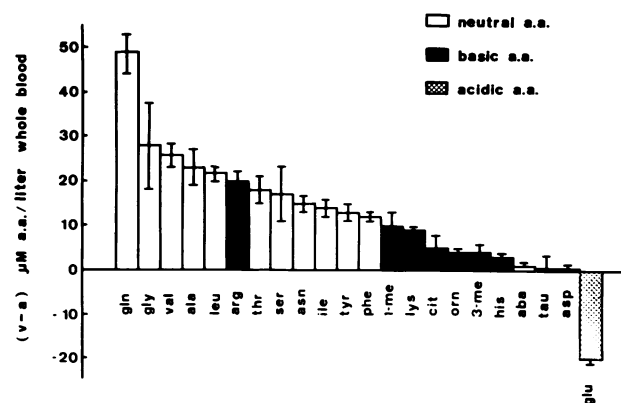

FIGURE 3 The mean umbilical venous arterial concentration differences±SEM for each amino acid are depicted in order of decreasing value. The acidic amino acids (taurine, aspartate, glutamate) show either no net flux or a negative (v-a) difference, whereas the neutral amino acids demonstrate a large positive uptake by the fetus from the placenta.
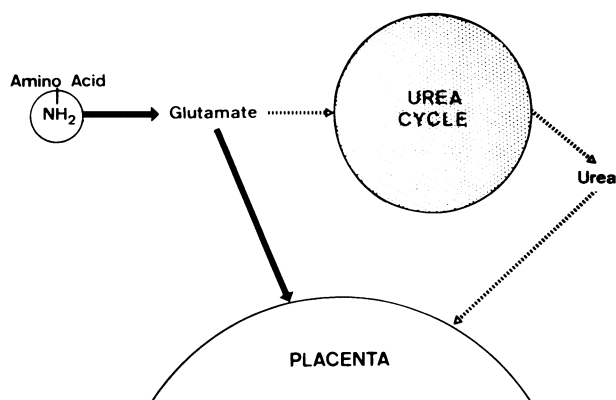
FIGURE 5 The figure depicts the two modalities of fetal nitrogen excretion that have been identified. As the immature fetus may not possess an efficient urea cycle (22), glutamate may serve as a primary carrier of excess nitrogen from fetus to placenta in early pregnancy.

cretes glutamate from the umbilical circulation into the placenta. On the basis of a comparison between mean glutamate and oxygen umbilical venous-arterial differences, it can be estimated that in late pregnancy the fetus delivers glutamate to the placenta at the rate of ~5 mmol/kg·day. Previous studies (8) have shown that fetal lambs of the same age produce ~26 mmol of urea nitrogen/kg·day. It is interesting to note that according to some evidence (22) the urea cycle is less active in immature fetuses. Thus it is possible that the transfer of glutamate from fetus to placenta represents a major pathway of nitrogen excretion in early intrauterine life (Fig. 5). This hypothesis, and the fate of glutamic acid taken up by the placenta, deserve further study.

In contradiction to the commonly made assumption (7) that the interconversion and catabolism of amino acids proceed at a slow rate in fetal life, it would appear that the nitrogen utilized by the fetus in the formation of urea, glutamate, and aspartate is a large fraction of its total nitrogen uptake, as shown by the calculations summarized in Table III. An important consequence of this result is that the umbilical uptake of some amino acids must exceed by a considerable margin their incorporation into fetal body proteins. Unfortunately, a comparison between umbilical uptake and accumulation of each amino acid in the fetal carcass is not feasible at present for two main reasons. The first is that the umbilical uptake of some amino acids could not be measured precisely due to large variability of the umbilical venous-arterial differences and (or) unresolved analytical problems. The second reason is that the results of carcass analysis, reported in the literature, do not include important amino acids such as glutamine and asparagine. Therefore, we have limited the comparison to a few amino acids. Accumula-

tion rates in the fetal lamb have been estimated from the known rate of nitrogen accumulation (23) and data on the amino acid composition per gram of nitrogen of fetal calves (24). The use of the latter seems justified, in view of the fact that results of carcass analysis in different species show good agreement. For example, the lysine content in the carcass of the rat (25) and a 40-wk-old cattle fetus (24) are 3.1 and 3.0 mmol/g of nitrogen, respectively. An unexplained exception is histidine, whose reported concentration in the fetal calf (24) is half that of the rat carcass (25). The results of the comparison are shown in Fig. 6. It can be seen that mean umbilical uptake and carcass accumulation are of the same order of magnitude in the case of lysine. In contrast, valine, leucine, isoleucine, arginine, phenylalanine, and tyrosine show mean uptakes which are three to five times greater than the estimated incorporation into fetal body proteins. It seems unlikely that discrepancies of this magnitude could be due entirely to inaccurate measurements and assumptions. Thus, a comparison of carcass analysis with umbilical uptake of amino acids lends additional support to the concept we proposed on the basis of fetal urea production rates, namely that some amino acids delivered to the fetus by the placenta are used extensively in transaminations and oxidative degradation. Conversely, it would be difficult to reconcile the experimental evidence with the idea that the role of the fetus in amino acid metabolism is limited to assembling amino acids into pro-

TABLE III
The Relationship of Fetal Aspartate, Glutamate, and Urea Production to Known Fetal Nitrogen Requirements

Conditions	Production	Reference
	g/kg·day	
(a) Nitrogen accumulated in the form of growth	0.65	22
(b) Accumulation of aspartate and glutamate nitrogen in fetal growth	0.078	23
(c) Fetal excretion of glutamate nitrogen	0.070	Present data
(d) Fetal excretion of urea nitrogen	0.364	8
(e) Production of aspartate, glutamate, and urea nitrogen (b + c + d)	0.512	
(f) Known fetal nitrogen requirements (a + c + d)	1.084	
(g) Aspartate, glutamate, and urea nitrogen production as fraction of known fetal nitrogen requirements (e/f)	0.47	

teins. Among the amino acids used by the fetus, glutamine is one of the most important as evidenced by its relatively large umbilical uptake. Glutamine is a major substrate for transamination, nucleotide synthesis, and gluconeogenesis (26–28).

Two important goals in the study of fetal amino acid metabolism are to describe fetal nitrogen balance and to estimate the contribution that amino acids make to the caloric needs of the fetus. As shown in Table III, the combined fetal nitrogen requirements for growth and urea production amount to approximately 1 g of nitrogen/kg · day. On the other hand, the average, net uptake of amino acid nitrogen measured in the present study was 1.5 g/kg·day. In addition, several amino acids (proline, tryptophan, cystine, cystathionine, and methionine) were not measured in the present study. The reasons for the discrepancy between estimated supply and demand are obscure. The discrepancy could be due to the cumulative effect of errors in estimating each component of the balance. Alternatively, urea and glutamate may not be the only forms of nitrogen excretion by the fetus. It is of interest that a similar calculation of a metabolic balance based on presently known sources of carbon rather than nitrogen gives less of a discrepancy. The estimated mean uptake of carbon in the form of amino acids was 3.9 g/kg · day in this study. Carbon is also taken up by the ovine fetus in the form of glucose (1.8 g/kg · day) and lactate (1.4 g/kg · day). The total estimated uptake of carbon in the form of amino acids, glucose, and lactate is therefore 7.1 g/kg · day. The estimated requirements of the ovine fetus for carbon are the sum of the carbon accumulated by growth (3.2 g/kg · day) (21) and the carbon excreted in the form of CO_2 (4.4 g/kg · day) and urea (0.2 g/kg · day). This gives a total requirement of 7.8 g/kg · day, which compares favorably with the estimated uptake. However, it is important to realize that such an estimate is rather crude, as other potential sources of carbon (e.g., the amino acids previously mentioned as well as short chain fatty acids) were not measured.

In the case of fetal caloric needs, data are also available for a rough comparison between caloric supply and demand. The caloric value of the net flux of glucose (21), lactate (29), and amino acids (present data) can be estimated as 16.8, 10.5, and 43.1 kcal/kg · day, respectively (30, 31). The flux of urea in the other direction represents a loss of 2.0 kcal/kg · day, and therefore the net supply of calories is 68.4 kcal/kg · day. To estimate fetal caloric requirements we have proceeded as follows. The mean caloric value of the sheep fetal carcass at 130 days gestational age is 0.895 kcal/g (23). Since at that age the fetus gains weight at the mean rate of 36 g/kg · day (23), the fetus stores 30.4 kcal/kg · day in the form
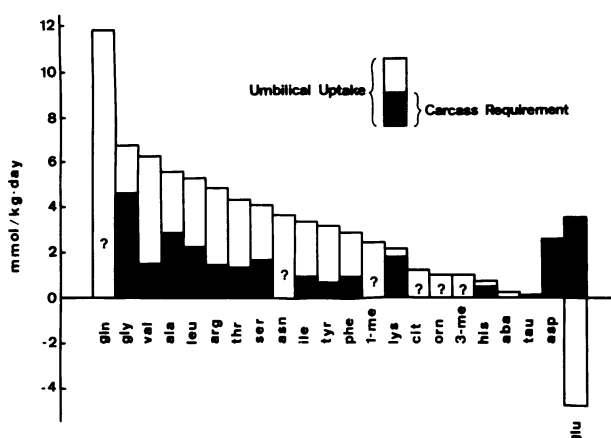

FIGURE 6   The estimated carcass requirements of the ovine fetus are compared to estimated mean umbilical uptakes for each amino acid. The requirements for some amino acids are not known (?). The estimated uptakes of glutamate and aspartate are designated by the open bars, and the carcass requirements are shown by the solid bars. For the remainder of the amino acids, the estimated carcass requirements (solid bars) are superimposed upon the estimated umbilical uptakes (open bars). It is evident that the uptakes of lysine and histidine closely approximate the requirements for protein synthesis, whereas other amino acids are taken up in considerable excess of estimated need.

of new tissue. This may be considered the anabolic caloric requirement. The caloric requirement associated with oxidative metabolism can be estimated from fetal oxygen consumption, which is approximately 8.6 liters/kg · day. On the assumption of a caloric equivalent for oxygen of 4.9 kcal/liter (32), the caloric requirement of fetal oxidative metabolism is approximately 42.1 kcal/kg · day. Therefore, the total caloric requirement of the growing fetus appears to be 72.5 kcal/kg · day, or 6% higher than the estimated calories delivered in the form of glucose, lactate and amino acids. It should be noted that some of the energy derived from oxidative metabolism is stored in the growing tissue. As a consequence, the sum of calories accumulated in the carcass and produced by oxidative metabolism overestimates the true caloric requirements. However, the magnitude of this overestimate appears to be negligible (33).

It can be surmised from the above calculations that glucose, lactate, and amino acids comprise the major sources of energy and substrate for the unstressed, growing fetal lamb. However, the discrepancy between estimated supply and requirement of nitrogen deserves further study.

ACKNOWLEDGMENTS

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