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Ontogenetic Changes in the Rates of Protein Synthesis and Leucine Oxidation during Fetal Life

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ABSTRACT. Studies of fetal leucine metabolism and protein synthetic rate, using L-(1-^{14})leucine as tracer, were carried out in 12 pregnant ewes at midgestation and compared with similar studies in late gestation. The disposal rate of fetal plasma leucine ranged between 3.07 and 9.06 μmol/min and was correlated ($r = 0.89$) to fetal dry weight. The fluxes to CO_2 excretion and to protein synthesis were 18.6 ± 2.6 and 37.2 ± 2.6% of disposal rate, respectively. The flux of leucine molecules into the placenta was relatively large and correlated to the placental/fetal dry weight ratio ($r = 0.84$). The mean fractional protein synthetic rate was 0.216 ± 0.01 day^{-1}. Comparison with late gestation data showed that fractional protein synthetic rate is inversely correlated ($r = -0.87$) to gestational age and that fetal protein synthetic rate (PR_S, g/day) is related to fetal dry weight (DW, g) by the allometric equation:

$$\log PR_S = -0.503 + 0.754 \log DW$$

The 0.754 exponent is similar to the exponent relating fetal oxygen consumption to dry weight (0.729). This indicates that protein synthesis and energy metabolism per g dry weight decrease during fetal growth at approximately the same rate so that the protein synthesis/oxygen consumption ratio tends to remain constant. (*Pediatr Res* 22:688–692, 1987)

Abbreviations

a, umbilical arterial blood
d, days
DR, fetal disposal rate of leucine
DW, fetal dry weight
f, umbilical flow
GA, gestational age
K_F, fractional rate constant defining the time course of free leucine plasma specific activity
K_G, fractional growth rate of fetal dry weight
KIC, ketoisocaproic acid
K_S, fractional fetal protein synthetic rate
Leu_{PR}, amount of leucine in fetal proteins
Leu_{R_S}, flux of fetal plasma leucine into fetal protein synthesis
Lys_{R_S}, flux of fetal plasma lysine into fetal protein synthesis
PR, fetal proteins
PR_S, fetal protein synthesis rate
r, tracer leucine infusion rate
r_{CO_2}, excretion rate of $^{14}CO_2$ into placenta from umbilical circulation
R_{ox}, leucine decarboxylation rate
$R_{p,f}$, flux of fetal leucine into placenta
SA, specific activity of arterial free plasma leucine at steady state
SA_t, specific activity of arterial free plasma leucine at time t
SA_{PR}, specific activity of leucine in fetal proteins
v, umbilical venous blood
V_{O_2}, fetal oxygen uptake
IV, intravenous
PCS, phase combining system
GA, gestational age

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Studies of amino acid metabolism in fetal sheep have been limited to late gestation and do not provide an adequate foundation for ontogenetic studies (1–3). The importance of extending knowledge of amino acid metabolism from late to mid gestation is suggested by striking differences in fetal body composition, growth rate and oxygen requirements and in the placental/fetal mass ratio between these two stages of development (4, 5).

The present study applies tracer methodology to the investigation of fetal leucine metabolism at midgestation (73–88 days), a time when the fetus is only 5–10% of its birth weight. We focused attention on the essential amino acid leucine for two reasons: 1) it has dual importance as a constituent of body proteins and as a substrate of oxidative metabolism, and 2) the new information is directly comparable with results recently obtained by us in late gestation using similar techniques and data analysis (6). The study aims at providing the first description of the allometric relationships which pertain during fetal life for body size, metabolic rate, and whole-body protein synthesis.

METHODS

Biologic preparation. Twelve pregnant, Columbia-Rambouillet crossbred ewes with gestations of 73–88 days were studied. On the day of surgery the sheep were sedated with IV pentobarbital and given spinal anesthesia (6 mg pontocaine, 10% glucose). Streptomycin (1 g) was administered intramuscularly. Polyvinyl catheters were placed in the uterine vein draining the pregnant uterine horn, the maternal femoral artery, and the amniotic cavity. Catheters for sampling the fetal umbilical circulation were

placed by visualizing the vessels coursing inside the uterus along the lesser curvature of the pregnant horn through a small incision in the greater curvature. Using microsurgical techniques and operating telescopes, small cotyledonary branches of the umbilical vein and artery were cannulated with the tips placed 2–3 cm into the major vessels. In twin pregnancies a fetal infusion catheter was placed into the other main umbilical vein or into the fetal femoral vein. In singleton pregnancies a fetal infusion catheter was placed in an umbilical venous branch in the nonpregnancy horn. These techniques for catheterization of the mid gestation fetal lamb have been previously described in detail (5). All catheters were tunneled subcutaneously to a plastic pouch fixed to the ewe's flank and flushed daily with heparinized saline (100 U heparin·ml^{-1} for maternal catheters and 33 U heparin·ml^{-1} for fetal catheters). Ampicillin (250 mg) was administered into the amniotic cavity at surgery and on days 2 and 4 postoperatively.

Experimental procedure. After surgery the animals were housed in individual crates and allowed *ad libitum* access to alfalfa pellets and water. The studies were conducted 3–9 days postoperatively when the ewes were consuming adequate quantities of food (800–1600 g·day^{-1}) and water. A constant infusion of ^3H_2O (1.0 μCi/min) and L-[1-^{14}C]leucine (0.12 μCi/min) dissolved in saline was administered to the fetus through the venous infusion catheter. Fetal arterial samples were withdrawn serially beginning at 15 min until 240 min after starting the infusion and used for measurement of leucine specific activity. After isotopic plateau was reached, blood samples were simultaneously taken from the umbilical vein and artery and the uterine vein and maternal artery at 150, 180, 210, and 240 min. The samples were analyzed for oxygen content, amino acid concentrations, ^3H_2O, $^{14}CO_2$ and hematocrit. Blood samples for separate analysis of lactate plasma L-[1-^{14}C]leucine and tracee leucine were withdrawn at the same time from the fetal arterial catheter alone. The volume of fetal blood withdrawn (2.3 ml) was replaced immediately after each sampling with a transfusion of fetal blood.

At the end of the study the ewe and fetus were killed with rapid IV injection of T-61 euthanasia solution. The total infusion time was noted and the infusate saved for analysis of ^3H_2O and L-[1-^{14}C]leucine radioactivity. The ^{14}C infusate radioactivity infusion rate and time were used to calculate the total amount of L-[1-^{14}C]leucine administered to the fetus.

At autopsy the pregnant uterus, placental cotyledons, and study fetus were separated and weighed. The placenta and fetus were homogenized separately and frozen at −70° C for later analysis.

Analytical methods. Fetal and maternal plasma ^3H_2O activities were counted in a Packard Tri-Carb 460 C liquid scintillation counter after solubilization in Protosol and mixture with 15 ml of PCS (Amersham) liquid scintillation cocktail. Plasma ^3H_2O concentrations were converted to whole blood concentrations using the hematocrit data, as previously described (7).

Fetal and maternal blood samples for oxygen content were drawn into heparinized glass capillary tubes. Blood samples for lactate analysis were drawn into syringes encased in ice. Hemoglobin concentration and oxygen saturation were measured immediately with an automatic, direct reading photometer (Radiometer OSM-2), and blood oxygen content calculated. Blood for lactate analysis was deproteinized with perchloric acid. The filtrate was stored at −70° C and subsequently analyzed by means of a lactate dehydrogenase method (5).

Fetal arterial blood samples for amino acid analysis were immediately centrifuged at 5°C for 15 min, and the plasma frozen at −70° C. Plasma was deproteinized by adding 15% sulfosalicylic acid, the supernate collected, and the leucine concentration determined in an aliquot of the supernatant with a JEOL-200A amino acid analyzer using a norleucine internal standard. Another aliquot of the supernatant was used to determine the L-[1-^{14}C]leucine concentration with a JEOL-6 AH amino acid analyzer. The JEOL-6 AH analyzer was adapted with a short LCR-2 resin column fixed at a temperature of 41° C as described previously (6). Sodium citrate buffer (ph 4.25) was used for elution and fractions collected at 10-min intervals. We have shown that ^{14}C-leucine is effectively separated from other ^{14}C-labeled compounds with these column conditions (6). The sample was mixed with PCS scintillation fluid and the radioactivity measured in a Packard Tri-Carb 460 C liquid scintillation counter with internal quench correction.

Fetal and placental tissue homogenates were acid hydrolyzed as previously described (8). After hydrolysis the samples were dried and redissolved in buffer (pH 2.2) for measurements of leucine and tracer leucine content (6).

Calculations. Umbilical flow (f) was measured by the steady-state transplacental diffusion method using tritiated water as the test molecule (9).

Leucine fluxes. The DR (μmol/min) was calculated using the r (DPM/min), and the SA (DPM/μmol):

$$DR = r/SA \quad (1)$$

As shown in a previous study (6), this disposal rate has three major components: the R_{ox} (μmol/min), the Leu_{R_s} (μmol/min), and the $R_{p.f}$ (μmol/min).

The R_{ox} rate was calculated using the excretion rate of $^{14}CO_2$ into the r_{CO_2} (μmol/min) and SA:

$$R_{ox} = r_{CO_2}/SA \quad (2)$$

The r_{CO_2} rate was measured by application of the Fick principle to the umbilical excretion of $^{14}CO_2$ (6):

$$r_{CO_2} = f\,(^{14}CO_{2_a} - {}^{14}CO_{2_v}) \quad (3)$$

where $^{14}CO_{2_a}$ and $^{14}CO_{2_v}$ represent the concentration of $^{14}CO_2$ (DPM/ml) in umbilical arterial and venous blood, respectively. Previous experiments of ^{14}C-bicarbonate infusion into the fetus have demonstrated that in the steady state the rate of CO_2 excretion via the umbilical circulation is equal to the rate of $^{14}CO_2$ production within the fetus (7). Therefore, r_{CO_2} represents the rate of tracer leucine decarboxylation within the fetus. This rate may also include decarboxylation of KIC molecules reentering fetal blood after the placental deamination of fetal leucine (6).

The rate at which fetal plasma leucine enters fetal protein synthesis ($^{Leu}R_s$) was calculated from the equation:

$$^{Leu}R_s = K_s \times (Leu)_{PR}/1440 \quad (4)$$

where K_s is the fractional synthetic rate per day, Leu_{PR} is the amount of leucine in fetal proteins (μmol), and 1440 is the number of min/day. The fractional rate K_s was estimated by means of a two step procedure. A first estimate of K_s was made using the formula:

$$K_s = -t^{-1} \ln [1 - (SA_{PR}/SA)] \quad (5)$$

where t is the total infusion time in days and SA_{PR} is the specific activity of leucine in fetal proteins at the end of infusion. Equation 5 accounts for the backflow of tracer leucine into fetal plasma due to fetal protein degradation (2) and is preferable to the equation that neglects tracer backflow:

$$K_s = t^{-1} (SA_{PR}/SA) \quad (6)$$

However, in the 4-h infusion the specific activity of leucine in fetal proteins increased to approximately 3.5% of the plasma leucine specific activity so that the use of equation 6 would have underestimated K_s by only 2%. Both equations 5 and 6 assume that SA was constant during the infusion. This assumption is not exactly correct because in these experiments the specific activity of plasma leucine attained steady state approximately 30 minutes after the start of the infusion. Therefore, a more refined calculation was made using Swick's equation (10):

$$(SA_{PR}/SA) = 1 - [(K_1 e^{-K_s t}) - (K_s e^{-K_1 t})]/(K_1 - K_s) \quad (7)$$

where K_F is the rate constant with which plasma specific activity attained equilibrium. The K_F constant was estimated by plotting $\ln(SA - SA_t)/SA$ versus t for all observations prior to t = 30. The estimated value was 110 day^{-1}. The K_s constant was then calculated by an iterative procedure using the value calculated by means of equation 5 as the initial estimate. Since K_F is much greater than K_s, this calculation does not require precise knowledge of K_F (10). The K_s thus estimated was approximately 5% higher than the K_s estimated with equation 5.

The flux of leucine into the placenta from the fetus ($R_{p,f}$) was estimated as the difference between DR and the (R_{ox} + $^{Leu}R_s$) sum.

Rate of protein synthesis. To estimate rates of fetal protein synthesis from the incorporation of tracer amino acids in fetal proteins, it is necessary to estimate the protein content of the fetus. The grams of protein present in the fetus were estimated in an earlier study from our laboratory (2) as 6.25 times the nitrogen content of the fetal carcass. This calculation assumes an average protein nitrogen content of 16% and that virtually all of the fetal nitrogen is protein nitrogen. Subsequent data (8) showed that this calculation may overestimate the amino acid content in fetal proteins by as much as 10%. Therefore, in the present study we have calculated the g of protein per g of leucine in the following manner: the total protein amino acid/leucine ratio was determined at different gestational ages from an acid hydrolysis of homogenates of the fetal carcass. This ratio increases with GA (days) according to the regression equation:

$$(\text{g total amino acids})/(\text{g leucine}) = 10.7 + 0.0235\ GA \quad 8)$$

This increase is likely to represent changes in the relative amounts of different proteins present in the fetus since there is a marked increase in fetal hydroxyproline content with fetal age indicating an increase in collagen and/or elastin (8). The equation used to calculate protein synthesis rate from the tracer leucine data (PR_s, g/day) was:

$$PR_s = {}^{Leu}R_s \times 1440 \times 131.18 \times 10^{-6} (10.7 + 0.0235\ GA) \quad 9)$$

where 131.18 is the molecular weight of leucine. To compare protein synthesis rates estimated by the use of tracer leucine with rates previously estimated in our laboratory by the use of tracer lysine (2), the following equation was applied to the lysine data:

$$PR_s = {}^{Lys}R_s \times 1440 \times 146.19 \times 10^{-6} (11.8 + 0.0210\ GA) \quad 10)$$

where 146.19 is the molecular weight of lysine. The linear regression of the fetal protein amino acids/lysine (g/g) ratio *versus* gestational age is encompassed in the parentheses. All regression analyses were done by the least squares method.

RESULTS

The 12 fetuses ranged in gestational age from 73 to 88 days. Among fetuses, arterial oxygen saturation ranged between 56 and 75% (mean 68%), umbilical venous oxygen saturation between 86 and 94% (mean 91%), and arterial lactate between 0.7 and 1.5 mM (mean 1.1 mM). Individual fetal and placental weights, umbilical blood flows, and oxygen uptakes were as shown in Table 1. The data in all tables are presented in order of increasing fetal age.

Leucine fluxes. The DR ranged between 3.07 and 9.06 μmol/min (Table 2) and was correlated (r = 0.89) to DW (DR = 1.98 + 0.077 DW). In each fetus the disposal rate of leucine carbon on position one had three major components, namely: the flux into CO_2 excretion (R_{ox}, range 5.7 to 30.5% of DR), the flux into protein synthesis (R_s, range 22.9 to 52.4% of DR), and a third residual component representing primarily the flux of fetal leucine molecules into placental metabolism ($R_{p,f}$, range 21.0 to 64.5% of DR). The variability of the latter component could be accounted for primarily by individual variability in the relationships of placental and fetal masses. The placental/fetal dry weight ratio ranged between 2.86 and 0.77 (Table 3) and was significantly correlated (r = 0.84) with the $R_{p,f}$/DR ratio (Fig. 1).

There was a strong correlation (r = 0.94) between plasma leucine flux into fetal proteins (R_s) and fetal dry weight (R_s = −0.125 + 0.0562 DW). The R_s per g of fetal dry weight (R_s/DW, mean 0.0534 ± 0.0021 $\mu mol \cdot min^{-1} \cdot g^{-1}$) had a relatively small coefficient of variation (±13.9%) and was not significantly correlated (r = −0.02) with plasma leucine concentration. By contrast, the fetal plasma leucine decarboxylation rate per g of dry weight (R_{ox}/DW, mean 0.0275 ± 0.0043 $\mu mol \cdot min^{-1} \cdot g^{-1}$) had a large coefficient of variation (± 53.8%) and was significantly correlated ($r = 0.63$, $p < 0.05$) with plasma leucine concentration. Plasma leucine concentration ranged between 0.186 and 0.079 μmol/ml and had a mean value of 0.122 ± 0.008 μmol/ml.

Fetal protein synthesis. Table 2 presents the fractional rates of plasma leucine flux into the protein leucine pool (K_s, day^{-1}) and the estimated rates of fetal protein synthesis (PR_s, g/day). The mean K_s value was 0.216 ± 0.010 day^{-1}. Comparison of the K_s data in Table 2 with previously measured K_s values of leucine and lysine in older fetuses (2, 6) showed a significant decline (r = −0.87) of the fractional synthetic rate with fetal age (Fig. 2). The rate of protein synthesis ranged between 2.14 and 11.44 g/day and was correlated (r = 0.94) with dry weight. For the purpose of estimating the allometric relationship between PR_s and DW, all available data were used in a plot of 1 g PR_s *versus*

Table 1. *Fetal age, fetal and placental wt, umbilical blood flow, and fetal oxygen uptake ($\dot{V}_{O_2}$)*

Animal	Fetal age (days)	Fetal wt		Placental wt		Umbilical blood flow (ml/min)	$\dot{V}_{O_2}$ (ml_{STP}/min)
		Wet	Dry	Wet	Dry		
1	73	160	15.4	411	44.1	62.7	1.46
2	75	180	21.3	400	51.6	66.1	2.37
3	75	198	21.2	438	52.6	167.0	2.28
4	75	215	23.1	424	44.1	65.6	1.65
5	78	323	35.9	586	61.5	133.8	3.87
6	79	291	36.0	514	57.7	131.0	3.55
7	81	308	35.9	424	56.4	81.0	2.98
8	87	414	47.3	285	36.3	133.7	4.48
9	87	427	46.8	398	50.1	105.9	3.63
10	87	446	53.4	440	58.7	140.9	4.80
11	87	577	74.7	578	66.1	212.9	6.11
12	88	487	55.2	352	44.7	155.4	4.95
$\bar{X}$	81	335	38.9	437	52.0	121.3	3.26
± SEM	1.7	39	5.0	25	2.5	13.4	0.46

Table 2. *Fetal plasma leucine concentration, DR, R_{ox}, and R_s, K_s, PR_s*

Animal	Plasma leucine (μmol/ml)	DR	R_{ox} (μmol/min)	R_s	K_s (day^{-1})	PR_s (g/day)
1	0.113	3.07	0.17	0.91	0.218	2.14
2	0.186	4.62	1.09	1.06	0.208	2.57
3	0.135	4.21	0.47	1.06	0.194	2.50
4	0.105	3.10	0.22	1.52	0.257	3.58
5	0.079	4.27	0.41	1.64	0.201	3.88
6	0.147	6.44	1.79	1.96	0.225	4.65
7	0.092	5.37	1.64	1.96	0.249	4.67
8	0.127	5.05	1.34	2.19	0.179	5.27
9	0.126	6.83	1.20	2.66	0.222	6.40
10	0.105	6.42	0.86	2.79	0.213	6.72
11	0.128	9.06	2.41	4.75	0.275	11.44
12	0.120	6.18	1.46	2.22	0.155	5.35
$\bar{X}$	0.122	5.39	1.09	1.89	0.216	4.93
± SEM	0.008	0.49	0.20	0.33	0.010	0.73

Table 3. *Leucine fluxes R_{ox}, R_s, and $R_{p,f}$ expressed as % of leucine disposal rate, and leucine fluxes R_{ox} and R_s expressed per g of fetal dry wt*

Animal	R_{ox} (%)	R_s (%)	$R_{p,f}$ (%)	R_{ox}/g (μmol·min^{-1}·g^{-1})	R_s/g
1	5.7	29.8	64.5	0.0113	0.0594
2	23.6	22.9	53.5	0.0512	0.0512
3	11.0	25.2	63.8	0.0219	0.0500
4	7.2	49.0	43.8	0.0097	0.0658
5	9.7	38.4	51.9	0.0115	0.0457
6	27.8	30.4	41.8	0.0497	0.0544
7	30.5	36.5	33.0	0.0457	0.0546
8	26.5	43.4	30.1	0.0283	0.0463
9	17.6	38.9	43.5	0.0256	0.0568
10	13.4	43.5	43.1	0.0161	0.0522
11	26.6	52.4	21.0	0.0323	0.0636
12	23.6	35.9	40.5	0.0264	0.0402
$\bar{X}$	18.6	37.2	44.2	0.0275	0.0534
± SEM	2.6	2.6	3.7	0.0043	0.0021

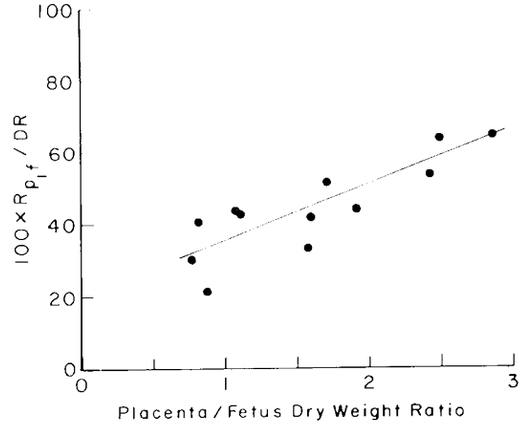

Fig. 1. The flux of leucine molecules into the placenta from fetal plasma, expressed as percent of fetal plasma leucine disposal rate is plotted against the placenta/fetus dry weight ratio. Regression line (r = 0.84) drawn according to equation: y = 20 + 15.4x.

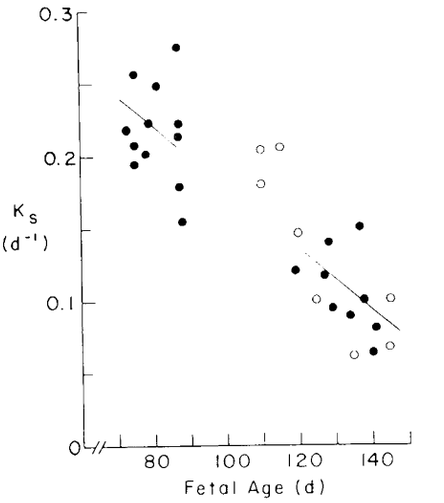

Fig. 2. The K_s is inversely related to fetal age. *Dots* indicate rates measured using tracer leucine and *open circles* indicate rates measured using tracer lysine. Regression line (r = −0.87) drawn according to equation: y = 0.39 − 0.021x.

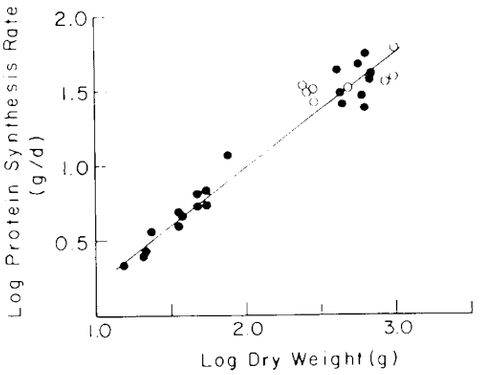

Fig. 3. Log-log plot of fetal protein synthesis rate versus fetal dry weight. *Symbols* as in Figure 2. Regression line (r = 0.97) drawn according to equation: y = −0.503 + 0.754x.

1 g DW (Fig. 3). The slope of the regression equation was less than one (0.754), indicating that over the latter half of intrauterine life the rate of protein synthesis increases less rapidly than fetal mass.

DISCUSSION

The present study, together with our previous work on the late gestation fetus (2, 6), demonstrates that the K_s decreases significantly with fetal age from approximately 23% per day at midgestation (74 day) to less than 10% per day at term (148 day). This decrease is concomitant with a decrease in the K_G. In this breed of sheep estimates of K_G at mid gestation vary between 13% per day (5) and 9% per day (6), whereas in late gestation K_G is approximately 4% per day (6).

Protein synthesis includes both protein accretion due to growth and protein turnover due to degradation and resynthesis of existing proteins. Therefore, a relatively high rate of fetal protein synthesis at mid gestation could not have been predicted solely from the higher rate of fetal growth because protein accretion can be increased either by increasing synthesis or by decreasing degradation. The higher value of K_s with respect to K_G shows that protein turnover is a quantitatively important component of fetal protein synthesis.

We have previously demonstrated that there is a decrease in the oxygen consumption rate per unit dry weight as the fetus grows to maturity (4). This study demonstrated that fetal oxygen consumption ($\dot{V}_{O_2}$, liter day^{-1}) is related to DW (g) by the allometric equation:

$$\dot{V}_{O_2} = 0.323\ DW^{0.729} \quad 11)$$

Since a decrease in protein synthetic rate represents a decline in energy requirements, it is interesting to compare the changes in fetal protein synthesis and oxygen consumption rate that occur during fetal growth. The present study (see Fig. 3) shows the allometric relation between rate of protein synthesis (PR_s, g day^{-1}) and dry weight to be:

$$PR_s = 0.314\ DW^{0.754} \quad 12)$$

The similarity of the two exponents (0.729 *versus* 0.754) indicates that protein synthesis and energy metabolism decelerate during growth at approximately the same rate so that the $PR_s/\dot{V}_{O_2}$ ratio tends to remain constant. According to equations 11 and 12, as the fetus grows from 25 to 650 g DW, the $PR_s/\dot{V}_{O_2}$ ratio only changes from 1.05 to 1.14 g/liter. Given that the synthesis of 1 g of protein requires the expenditure of approximately 0.8 kcal (11) and that the caloric equivalent of oxygen in the lamb fetus is approximately 4.9 kcal/liter of oxygen (12), we can estimate from the $PR_s/\dot{V}_{O_2}$ ratio that the energy cost of protein synthesis during the latter half of fetal life represents approximately 18% of fetal oxidative metabolism. This estimate is remarkably similar to the figure (17%) calculated by Garlick *et al.* (13) for the cost of protein synthesis in pigs, rats and humans.

It is important to note, however, that we base this comparison on data obtained by similar methods, namely, measurements of tracer amino acid flux from circulating plasma into tissue proteins. These methods tend to underestimate the rate of protein synthesis by the whole organism for two main reasons: 1) some amino acid molecules originating from protein degradation reenter protein synthesis without cycling through the general circulation, and 2) the synthetic rate of proteins with high turnover rates is underestimated by methods that measure protein specific activity after several hours of tracer infusion (10, 14). Although the entry rate of amino acids from the vascular compartment into body proteins does not represent total entry rate, measurements of plasma amino acid fluxes into proteins in postnatal life have demonstrated conclusively that the weight specific protein synthetic rate declines as body size increases and that this decline is proportional to the decline in weight specific oxygen consumption (14). It is apparent from our results that a similar relationship exists in prenatal life. To demonstrate this relationship we used fetal dry weight as the basis for comparison. During fetal growth there is a considerable decrease in percent fetal water content which makes fetal wet weight an unreliable basis for comparing metabolic requirements at different stages of fetal growth (4).

Our results demonstrate that there is a relatively rapid rate of leucine oxidation in the immature fetus. In the present study (mean fetal age 81 ± 1.7 day) the mean rate of fetal leucine oxidation per gram dry weight was significantly higher than in a group of older fetuses (6) with mean age 133 ± 3.3 day (0.0275 *versus* 0.0110 $\mu mol \cdot min^{-1} \cdot g^{-1}$, $p < 0.02$). The higher oxidation rate occurred despite a lower plasma leucine concentration in the younger fetuses (0.122 *versus* 0.167 μmol/ml, $p < 0.05$). This finding may simply represent another aspect of the high metabolic rate of the immature fetus. Another possibility, however, is that the placenta stimulates the catabolism of leucine by deaminating fetal leucine and by returning to the fetus KIC molecules which are then oxidized. Although the interaction of placental and fetal metabolism needs to be considered at all stages of fetal development, it is likely to be particularly important at midgestation when the mass of placental cotyledons exceeds fetal mass by a large margin (Table 3). Since leucine is an essential amino acid, its net flux is from placenta to fetus (15). Tracer leucine data have demonstrated, however, that this net flux is the algebraic sum of two large and opposite unidirectional fluxes that continuously exchange leucine molecules between placenta and fetus (6). When the placenta is large in relation to the fetus, the placental-fetal exchange represents the largest component of fetal leucine disposal rate (Fig. 1).

The placental contribution to disposal of fetal amino acids may explain in part why some estimates of fetal protein synthesis have been surprisingly high. For example, Schaefer and Krishnamurti (3) studied tyrosine metabolism in the late gestation fetal lamb and concluded that the fetus synthesizes proteins at the rate of 63 g/day/kg wet weight. This rate is approximately four to six times the value obtained by us using either tracer lysine (2) or tracer leucine (6). Inspection of their experimental data shows that after 8 h of fetal infusion with tracer tyrosine the protein bound/free plasma specific activity ratio in different organs ranged from 3.1 to 1.8%. Calculation of K_s values by means of these figures would give results similar to those obtained in our laboratory. However, the calculation of fetal whole body protein synthetic rate was based on a two-pool model of fetal-maternal tyrosine exchange that corrects for the small flux of fetal amino acid into the mother but does not correct for the potentially large flux of tyrosine into the placenta. Estimates of placental protein synthesis rate in sheep have yielded relatively high values (16).

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