Ontogeny of fetal hepatic and placental growth and metabolism in sheep

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Ontogeny of fetal hepatic and placental growth and in vitro oxygen consumption (Vo2) was investigated in fetal lambs at 75, 100, and 136 days postconception. Fetal hepatic relative weight and placental absolute and relative weights declined during this period. Oxygen consumption per gram dry weight of fetal liver and maternal placenta declined between mid and late gestation while fetal placental Vo2 was unchanged. Estimated Vo2 of the whole placentas did not change while the estimated total hepatic Vo2 increased more than threefold between 75 and 136 days. Total hepatic Vo2 was highly correlated with total placental Vo2 at 136 days (r = 0.84). The results suggest that the placenta reaches its maximum growth and metabolic capacity before 100 days and possibly at or before midgestation. Changes in hepatic weight-specific total Vo2, in addition to the declining relative size of the fetal liver, must contribute to the progressive decline in metabolic rate of the whole fetus during the second half of pregnancy. Correlations between placental and fetal liver weights and metabolic rates suggest the possibility of placental regulation of fetal hepatic growth and metabolism.

**THE PLACENTA** and fetal liver are vital organs for nutrient supply and metabolism in the growing fetus. The anatomic arrangement of these organs ensures intimate relations between their functions, similar to those between liver and gut in postnatal life. However, the gestational patterns of growth of the fetal liver and placenta vary considerably. In sheep, placental weight remains static or declines through the latter half of gestation (3, 5, 22), whereas the fetal liver continues to grow albeit at a slower rate than the rest of the fetus (6, 12). In vivo measurement of uteroplacental oxygen consumption (Vo2) during mid and late pregnancy suggests that placental metabolic rate does not change appreciably, despite a major increase in functional capacity of the placenta over this period (7, 8). Also, we have previously postulated that the gestational decline in relative weight of the liver may account for much of the decline in weight-specific metabolic rate of the whole fetus during the latter half of pregnancy, implying little change in weight-specific metabolic rate in the fetal liver (6). However, gestational changes in the metabolic rates of these organs, interrelations between them, and their consequences for energy expenditure in the whole conceptus have not been systematically studied.

Therefore, in the present study we have measured placental and fetal Vo2 in vitro in ovine tissues obtained at different stages of gestation and have related these metabolic rates to rates of growth of these organs between mid and late pregnancy.

**METHODS**

*Animals and feeding.* Twelve twin-pregnant and six single-pregnant Dorset and Finn-Dorset ewes of known mating date were scanned daily with a real time ultrasonic device (Johnson & Johnson Technicare 210 DX, New Brunswick, NJ) from day 45 postcoitus (PC) to verify litter size and gestational age. From 50 days PC the ewes were individually fed once daily a total mixed ration containing 2.5 Mcal metabolizable energy (ME) and 150 g crude protein (CP) per kilogram dry matter to fulfill estimated nutrient requirements depending on body weight, stage of pregnancy, and litter size according to National Research Council recommendations (20).

Six twin pregnant ewes were housed in individual floor pens from 50 days PC until slaughtered at 100 days PC. Another group of six twin pregnant ewes was maintained under similar conditions from 50 days until slaughtered at 136 days PC. This group underwent surgery on day 100 PC when one placentome was excised for the in vitro Vo2 studies, as described previously (23).

This procedure had no apparent detrimental effects on subsequent fetal and placental growth or gross morphology. The six single-pregnant ewes were similarly housed from 50 days PC until slaughter at 136 days PC.

**Dissection and tissue sampling procedures.** At slaughter ewes were stunned with a captive-bolt pistol and exsanguinated using procedures approved by the Cornell University Institutional Animal Care and Use Committee. The pregnant uterus was rapidly removed from the abdominal cavity, weighed, and dissected to separate each fetus and placenta. Placental weight was considered to be the aggregate weight of all placentomes for each fetus, trimmed of endometrium and fetal membranes. Fetuses were towel dried and weighed, and fetal livers were removed and weighed.

Tissue samples from the placenta and liver of one fetus per ewe were used for in vitro metabolic measurements. A single placentome and liver were washed in saline to remove blood and placed in complete media (M 199, Sigma Chemical, St. Louis, MO) saturated and continuously bubbled with 95% O2-5% CO2 at 4°C. During dissection one placentome was pinned to a dissecting dish placed on ice, covered with M 199 and continuously bubbled with 95% O2-5% CO2. Predominantly maternal tissue was excised from the very outer portion of the placentome, an area poor in trophoblastic interdigitation as judged by histological examination of many other placentomes. Predominantly fetal tissue was excised from the center of the fetal portion of the placentome near the chorionic surface. Tissue sections (25-50 mg) were blotted, weighed, and kept in individual 5-ml test tubes filled with M 199 saturated with 95% O2, 5% CO2 at 4°C until placed in the oxygen electrode chamber. The remaining placentomes were homogenized in a Waring blender and the homogenate was stored at -20°C.

Sections of fetal liver (25-50 mg) were dissected from the right lobe under the same conditions. Because of the small size of livers of 50-day fetuses, dry matter determinations were done on livers obtained from six other fetuses at the same gestational age, and the average of these values was applied to the Vo2 measurements.

**Analytical procedures.** Oxygen uptake of fetal and maternal placenta and fetal liver sections was measured with a Clark polarographic electrode in a biological oxygen monitoring system (YSI 5300, Yellow Springs Instruments, Yellow Springs, OH). Sections of tissue (25-50 mg) were placed in a chamber containing 4 ml of M 199 saturated with air and equilibrated to 37°C and pH 7.4. Oxygen consumption was monitored for 8-12
min then ouabain, a specific inhibitor of Na\(^+\)-K\(^+\)-adenosinetriphosphatase (ATPase; 15), was added to the chamber to a final concentration of 10\(^{-4}\) M, and \(\text{VO}_2\) was monitored for an additional 8–12 min. This dose was shown to produce a maximal response in preliminary experiments (B. W. McBride and I. Vatnick, unpublished observations). The difference between \(\text{VO}_2\) rates before and after addition of ouabain is defined as ouabain-sensitive respiration and is considered to represent the energy cost of Na\(^+\)-K\(^+\)-ATPase activity (16). Tissue sections respired linearly over a period of 2 h, and there was parallelism with doubling of section weights.

Placental and hepatic dry weights were determined by placing subsamples of wet tissues in a vacuum drying oven at 60°C for 48 h. Tissue DNA concentration was measured by a modification of the method of Munro and Fleck (19).

**Statistics.** Least squares regression analysis was used to determine relations between independent variables. Analysis of variance (ANOVA) with Scheffé’s modification was used for multiple comparisons of mean fetal and organ weights and hepatic respiration among all groups. A split plot ANOVA was used to analyze the in vitro placental respiration data.

**RESULTS**

Except where noted, the following results refer only to the 12 twin-pregnant ewes studied at 75, 100, or 136 days PC.

**Placental and fetal hepatic growth.** Fetal weight increased more than 15-fold from 232 ± 7 (SE) g at 75 days PC to 3,583 ± 228 g at 136 days PC. Placental wet weight declined 300 g from mid to late gestation, while dry weight did not change during this time. Total placental DNA content also remained constant between 75 and 136 days PC (Table 1). Placental weight was 2.75 times that of fetal weight at 75 days PC but was only 9% of fetal weight at 136 days PC (Table 1).

Fetal hepatic weight increased approximately sixfold between 75 and 136 days PC. Fetal liver was 6.4% of total fetal weight at 75 days PC but only 2.4% at 136 days PC (Table 1).

**Placental oxygen consumption.** Dry weight-specific \(\text{VO}_2\), and ouabain sensitive and ouabain insensitive \(\text{VO}_2\) of fetal placenta remained unchanged between mid and late pregnancy (Table 2).

Maternal total placental \(\text{VO}_2\) declined from 53 µL·min\(^{-1}\)·g dry tissue\(^{-1}\) at 75 days PC to 44 at 100 days PC and further declined to 31 µL·min\(^{-1}\)·g\(^{-1}\) at 136 days PC (P < 0.05, Table 2). Similarly, maternal ouabain-insensitive placental \(\text{VO}_2\) declined from 41 µL·min\(^{-1}\)·g dry tissue\(^{-1}\) at 75 days PC to 34 at 100 days PC and further to 26 µL·min\(^{-1}\)·g\(^{-1}\) at 136 days PC (P < 0.05). Ouabain-sensitive \(\text{VO}_2\) remained unchanged during this time (Table 2).

**Hepatic oxygen consumption.** Hepatic weight-specific \(\text{VO}_2\) tended to be higher (P < 0.1) at 50 days than at 75 days and declined sharply (P < 0.05) by 136 days PC (Fig. 1). Ouabain-sensitive \(\text{VO}_2\) remained unchanged between 50 and 75 days and tended to be lower (P < 0.1) at 136 days than at 75 days. Ouabain-insensitive \(\text{VO}_2\) also remained unchanged between 50 and 75 days but declined at 136 days PC (P < 0.05, Fig. 1).

**Correlations.** Weight-specific fetal hepatic \(\text{VO}_2\) was not significantly correlated with placental weight at 75 or 136 days. There was also no relation between fetal liver and placental weights or between total fetal hepatic \(\text{VO}_2\) and placental weight at 75 days. Fetal hepatic weight was highly correlated with placental weight (r = 0.87; Fig. 2A) and total fetal hepatic \(\text{VO}_2\) with total placental \(\text{VO}_2\) (r = 0.84; Fig. 2B) at 136 days. These correlations include individual data from a group of single-pregnant ewes not included in the ontogenic studies.

**DISCUSSION**

Despite many studies involving measurement of placental size in late pregnancy there is a lack of precise information on the gestational pattern of macroscopic and cellular growth of this organ in sheep and other species. In particular, the timing and metabolic basis for the abrupt cessation of hyperplastic ovine placenta growth are poorly defined. It is widely accepted that the formation of placentomes from cotyledonary and caruncular tissue is virtually complete by 40 days PC (2, 5) and that the placenta reaches its maximum weight sometime before term.

**Table 1. Fetal whole body, hepatic and placental weights**

<table>
<thead>
<tr>
<th></th>
<th>75 Days</th>
<th>136 Days</th>
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<tr>
<td>Fetal</td>
<td></td>
<td></td>
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<tr>
<td>Wet wt, g</td>
<td>232.2±6.9</td>
<td>3,563.0±228.2</td>
</tr>
<tr>
<td>Hepatic</td>
<td></td>
<td></td>
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<tr>
<td>Wet wt, g</td>
<td>14.8±0.5</td>
<td>88.0±7.3</td>
</tr>
<tr>
<td>Dry wt, g</td>
<td>2.9±0.1</td>
<td>17.2±1.4</td>
</tr>
<tr>
<td>% Fetal wt (wet)</td>
<td>6.4±0.2</td>
<td>2.4±0.1</td>
</tr>
<tr>
<td>Placental</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wet wt, g</td>
<td>635.0±39.1</td>
<td>332.5±41.2</td>
</tr>
<tr>
<td>Dry wt*, g</td>
<td>57.4±3.1</td>
<td>55.7±7.3</td>
</tr>
<tr>
<td>% Fetal wt (wet), g</td>
<td>274.6±16.9</td>
<td>8.9±0.8</td>
</tr>
<tr>
<td>Total DNA content*, g</td>
<td>1.2±0.08</td>
<td>1.3±0.30</td>
</tr>
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</table>

Values are means ± SE; n = 12 fetuses. * Not significantly different, P > 0.1.

**Table 2. In vitro oxygen consumption of fetal and maternal placenta**

<table>
<thead>
<tr>
<th>Gestational Age, days</th>
<th>75 Days</th>
<th>136 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>(\text{VO}_2), µL·min(^{-1})·g dry tissue(^{-1})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>36.8</td>
<td>52.8</td>
</tr>
<tr>
<td>Ouabain insensitive</td>
<td>25.2</td>
<td>41.2</td>
</tr>
<tr>
<td>Ouabain sensitive</td>
<td>11.2</td>
<td>12.7</td>
</tr>
</tbody>
</table>

Values are means with a pooled standard error (PSE) shown for each variable, n = 6. G: gestational age; F: fetal placenta tissue; and M, maternal placenta tissue. S, source of tissue (fetal or maternal). * Interaction between G and S.
Fig. 1. Effect of gestational age on fetal hepatic oxygen consumption. Histograms represent means for ouabain-insensitive (open) and ouabain-sensitive (closed) oxygen consumption. Vertical bars are SE; n = 6 fetuses. Values with superscripts a, b are different at P < 0.05.

Fig. 2. Relations between fetal liver weight and placental weight (A, y = 19.1 + 0.270x, r = 0.87, P < 0.01) and fetal hepatic oxygen uptake and placental oxygen uptake (B, y = 276 + 0.291x, r = 0.84, P < 0.01) in single-pregnant (●) and twin-pregnant (△) ewes at 136 days.

before 90 days, then declines in weight thereafter (3, 5). Recently communicated data from our laboratory (14) show that the sheep placenta reaches its maximum weight as early as 75 days PC and that then nuclear proliferation and, by inference, hyperplastic growth has ceased because there was no increase in total DNA content between 75 and 100 days. Those findings are entirely consistent with the present results, which show unchanged placental DNA content between 75 and 136 days.

The decline in relative weight of the liver during fetal life has been thoroughly characterized (6, 12). In this study relative weights are expressed on a wet weight basis, since fetal dry weight was not measured. Like all fetal organs and tissues, the liver steadily dehydrates during the latter half of gestation, but the pattern of change in hydration differs from that of the whole body (6). Therefore, relative weight of fetal liver expressed on a dry mass basis would be somewhat different from present values for relative wet weight.

The vascular anatomy of the placenta precludes direct in vivo measurement of placental Vo2 in conscious animals. Although in vivo measurements of uteroplacental Vo2 can be made (18), these measurements include metabolism of nonplacental uterine tissues. More specific measurements have been made on anesthetized ewes but values for placental Vo2 were much lower than those for uteroplacental Vo2 obtained in conscious animals (10, 18). In vitro measurements of highly active organs such as the placenta or liver inevitably underestimate metabolic rates in vivo (16) possibly because of inadequate oxygen delivery. The present data may at best provide an approximation of the relative developmental changes that occur in vivo. Calculations based on in vivo measurements of uteroplacental (8) and hepatic (9) Vo2 in late gestation indicate in vitro estimates of Vo2 for these organs at ~15 and 20% of in vivo measurements for the whole placenta and liver, respectively (see APPENDIX). The value for total placental Vo2 obtained in this study is similar to that obtained in a study of placental Vo2 in situ in anesthetized ewes (10). Weight-specific Vo2 of fetal placenta remained unchanged during the second half of gestation, whereas Vo2 of maternal tissues declined. These results contrast with those briefly reported by Reynolds and Redmer (21) who, using similar in vitro methodology, found an increase in weight-specific Vo2 of placental tissues over the same period. However, these authors expressed their results on a wet weight basis, disregarding the marked increases in placental dry matter content between mid and late pregnancy. Placental dry matter content is ~9% at 75 days PC and 17% at 136 days PC, which could account for the apparent gestational increase in total wet weight-specific Vo2 reported by these authors. Weight-specific Vo2 of human placenta in vitro declined 50% from early to late gestation (24).

Ouabain sensitive placental respiration remained constant through gestation and accounted for 20–30% of total Vo2 in fetal placenta and 18–24% in maternal placenta. Ouabain-sensitive respiration is considered to be an estimate of the metabolic costs of the Na+-K+-ATPase pump and therefore a measure of the energy costs for ion pumping (16). The decline in maternal weight-specific total Vo2 was apparently accounted for entirely by ouabain-insensitive Vo2, i.e., by energy costs other than ion pumping. This interpretation assumes that ouabain at 10−4 M does not cause nonspecific depression of other enzymes (15, 16).

Estimated Vo2 of the whole placenta (ml/min) remained constant between 75 and 136 days PC despite a tremendous increase in fetal demands for oxygen and nutrients. The high rate of placental metabolism in late pregnancy is presumably explained by the energy costs of meeting these demands. At midgestation the fetal nutrient requirements are much smaller and probably cannot account for the high metabolic rate of the placenta. The surprisingly high rate of placental Vo2 at this time may be more a consequence of the rapid growth of the placenta than of its functional energy costs. Although present in vitro values are quantitatively much lower than in vivo
estimates of placental $\text{V}_0$, it is notable that the latter also do not indicate a major change between mid and late gestation (7, 8).

In fetal sheep, weight-specific metabolic rate declines between mid and late gestation. The rate of this decline parallels that of the decline in the relative growth of the metabolically active organs, the liver in particular (6). This led to our suggestion that changes in relative organ size may have more influence on whole body metabolic rate than do changes in weight-specific cellular metabolism. Such a notion is also consistent with the proposal that the gestational decline in relative growth of the liver is due to a decrease in functional demand (12). However, present data suggest that changes in weight-specific hepatic $\text{V}_0$, in addition to declining relative size of the fetal liver, must contribute to the progressive decline in metabolic rate of the whole fetus during the second half of pregnancy. Measurements in vivo indicate that liver metabolic rate of the whole fetus during the second half of pregnancy = 1.89 ml/min = 1.89/12.8 = 14.8 g = 0.2 ml/min (estimated from Tables 1 and 3).

Despite the tendency of hepatic ouabain-sensitive $\text{V}_0$ to decline from early to late pregnancy, the fractional energy cost of ion pumping increased in late pregnancy. The reciprocal decline in contribution of ouabain-insensitive $\text{V}_0$ to total weight-specific $\text{V}_0$ suggests that processes other than ion pumping account for most of the decline in hepatic metabolic activity (Table 1). The liver’s role in erythropoiesis diminishes substantially between mid and late pregnancy (12), but whether this is involved in the decrease in $\text{V}_0$ during this time is not clear. Certainly, the fractional rate of protein synthesis in the whole fetus decreases markedly between mid and late gestation (17); it is likely that a similar decline occurs in the fetal liver.

The high correlation between placental and fetal hepatic weights in late gestation appears to account for a similar degree of correlation between placental and hepatic metabolic rates. This suggests that fetal hepatic growth and metabolism may be closely related to the ability of the placenta to supply nutrients, as indicated by its size in late pregnancy, and may explain the special sensitivity of hepatic growth to placental insufficiency in growth-retarded fetuses (1). The anatomic basis for such a relation is strong because much of the umbilical venous blood leaving the placenta perfuses the liver before reaching the fetal systemic circulation (13). This provides a direct means of communication and coordination of metabolic activity of these two vital organs. Specific examples of interrelated metabolic processes include cycling of amino acids and their products or precursors between the placenta and fetal liver (e.g., glutamate-glutamine, serine-glycine), and hepatic conversion to urea of ammonia originating from placental amino acid catabolism (4, 11).

In conclusion, major findings of this study are first, that there is surprisingly little change in placental $\text{V}_0$ on a dry weight-specific basis between mid and late gestation despite major increases in functional activity and associated metabolic demands over this period. The source(s) of the relatively high metabolic rate of the midgestation placenta warrants further study. Second, we found that in contrast to that of the placenta, weight-specific $\text{V}_0$ of the fetal liver declines markedly from mid to late gestation. Our suggestion that the size, metabolic rate, and, presumably, functional properties of the fetal liver near term may be directly influenced by size and functional capacity of the placenta requires direct investigation. This should be possible with the availability of techniques for simultaneously measuring fetal liver metabolism (9) and functional characteristics of the placenta (8) in vivo in normal and placentally insufficient fetuses.

APPENDIX

Calculations and Assumptions: Liver

1) Fetal hepatic $\text{V}_0$ in vivo at late gestation is 4 ml/min$^{-1}$·kg$^{-1}$, accounting for 17% of total $\text{V}_0$ (9).
2) Fetal hepatic $\text{V}_0$ in vitro at midgestation is 0.75 ml/min$^{-1}$·kg$^{-1}$ (estimated from Tables 1 and 3), i.e., 19% of in vivo value.
3) Fetal hepatic $\text{V}_0$ in vitro at midegestation is 1.34 ml/min$^{-1}$·kg$^{-1}$ (estimated from Tables 1 and 3).
4) Fetal hepatic $\text{V}_0$ in vitro at midgestation (75 days PC) is assumed to be also 19% of in vivo value. Therefore estimated in vivo fetal hepatic $\text{V}_0$ = 0.2/0.19 = 1.1 ml/min.
5) Total fetal $\text{V}_0$ in vivo at 75 days is ~10.5 ml/min$^{-1}$·kg$^{-1}$ (6). Therefore estimated total $\text{V}_0$ = 10.5 × 0.232 = 2.44 ml/min.
6) Estimated contribution of hepatic to total $\text{V}_0$ in midgestation = 1.1/2.44 = 45%.

Calculations and Assumptions: Placenta

1) Estimates of uteroplacental $\text{V}_0$ in vivo at late gestation range between 12 and 22 ml/min (assume mean of 16 ml/min; Refs. 8 and 18). Placental $\text{V}_0$ is assumed to account for ~80% of uteroplacental oxygen consumption (18); therefore, placental $\text{V}_0$ = 0.8 × 16 = 12.8 ml/min.
2) Placental $\text{V}_0$ in vitro was measured in placental slices that included both fetal and maternal tissue. Assuming equal weights of these tissues, mean placental $\text{V}_0$ in vitro is 34 ml/min$^{-1}$·g dry tissue$^{-1}$ (Table 2). From this and placental dry weight (Table 1), calculated $\text{V}_0$ of the whole placenta in vitro is 1.89 ml/min = 1.89/12.8 = 15% of the estimated in vivo value.

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