BASIC SCIENCE: OBSTETRICS

Fetoplacental biometry and umbilical artery Doppler velocimetry in the overnourished adolescent model of fetal growth restriction

David J. Carr, MBBS, MRCOG; Raymond P. Aitken; John S. Milne; Anna L. David, MBChB, PhD, MRCOG; Jacqueline M. Wallace, BSc, PhD, DSc

OBJECTIVE: The purpose of this study was to evaluate ultrasonographically fetal growth trajectories, placental biometry, and umbilical artery (UA) Doppler indices in growth-restricted pregnancies of overnourished adolescent ewes and normally developing pregnancies of control-fed ewes.

STUDY DESIGN: Singleton pregnancies were established using embryo transfer in 42 adolescent ewes that were overnourished (n = 27) or control-fed (n = 15) and were scanned at weekly intervals from 83-126 days’ gestation and necropsied at 131 days’ gestation (term = 145 days).

RESULTS: Ultrasonographic placental measurements were reduced and UA Doppler indices were increased from 83 days’ gestation; measurements of fetal abdominal circumference and femur length, renal volume and tibia length, and biparietal diameter were reduced from 98, 105, and 112 days’ gestation, respectively, in overnourished vs control-intake pregnancies.

CONCLUSION: Overnourishment of adolescent sheep dams produced late-onset asymmetric fetal growth restriction that was commensurate with brain sparing. Ultrasonographic placental biometry was already reduced and UA Doppler indices increased by mid gestation in overnourished pregnancies, preceding reduced fetal growth velocity and indicating an early nutritionally mediated insult on placental development.

Key words: adolescent sheep, fetal growth restriction, placental biometry, ultrasound, umbilical artery Doppler indices


Fetal growth restriction (FGR) is defined as the failure of an individual fetus to achieve its genetically predetermined growth potential. FGR complicates 8% of pregnancies in developed countries1 and is a leading cause of perinatal death.2 It is also associated with significant morbidity both in neonatal3 and in later life.4 Most cases of FGR (60%) are due to uteroplacental insufficiency,5 for which there is currently no available treatment. Ongoing research in animal models of FGR is aimed at developing future therapies for translation into clinical practice.6,7 The pregnant sheep has been used widely to study fetal and placental physiology.8 There are several different methods by which FGR can be induced in ewes, which include premating carunclectomy (surgical removal of the future placental attachment sites), placental embolization (injection of microspheres into the uterine or umbilical arteries), single umbilical artery (UA) ligation, maternal hyperthermia, and nutritional manipulation.9

Our research group has demonstrated repeatedly that overnourishing adolescent sheep dams by providing a high dietary intake throughout pregnancy produces major placental and fetal growth restriction relative to the normally developing pregnancies of adolescent ewes that are fed a moderate (control) dietary intake.10 This paradoxical result of overnourishment is unique to the young still-growing adolescent. High dietary intakes promote a sustained anabolic drive that prioritizes maternal tissue deposition at the expense of the emerging nutrient requirements of the gravid uterus. However, irrespective of maternal age, this well-characterized ovine model has much wider applicability because it replicates many of the key features of human FGR secondary to uteroplacental insufficiency without the need for any surgical interference at a vascular or placental level. It is therefore a useful paradigm in which to evaluate novel therapeutic interventions aimed at improving outcome in FGR. Although there is an early insult on placental and vascular development,11–15 the resultant FGR is relatively late in its onset, equivalent to the third trimester of human pregnancy. Indeed the results of earlier studies that were terminated at different stages of gestation in...
attachment of fetal trophoblast at specialized sites (caruncles) to form functional units called placentomes. These may be up to 120 in number and are spread throughout both uterine horns. We previously validated a novel marker of ovine placental size, the “placentome index” but have not yet reported changes in this parameter as a function of gestational age.

In clinical practice, UA Doppler waveform analysis is recommended as the primary surveillance tool for fetuses that are recognized antenatally to be growth-restricted or small for gestational age. Measurement of UA Doppler indices represents a noninvasive method for the evaluation of umbilical blood flow and the impedance to blood flow in the UA without the need to measure absolute blood flow velocities. UA Doppler indices correlate well with directly measured blood flow and vascular resistance in normal sheep pregnancy and after maternal phenylephrine infusion, progressive external UA constriction with ligatures, and acute umbilicoplacental embolization. Terminal embolization studies in sheep illustrate a progressive reduction in end-diastolic blood flow that is reflected by increasing UA Doppler indices, followed by absent and finally reversed end-diastolic flow shortly before fetal death. This mirrors the sequence of events observed in human FGR.

One consistent observation in the overnourished adolescent paradigm, like many other sheep models of FGR, is a variable response to nutritional manipulation that results in a range of placental growth trajectories and hence a variable degree of fetal growth constraint. Historically, 52% of fetuses are markedly growth-restricted at birth, defined as having a birthweight that falls >2 SDs below the mean birthweight of the normally grown fetuses of contemporaneous control-intake ewes. In these genuinely FGR pregnancies, average placental and fetal weights are reduced by approximately 48%. By contrast, the remaining 48% of fetuses are far less perturbed as a group, with 23% and 10.5% reductions in placental and fetal weight, respectively. Although these still represent statistically significant differences relative to controls, this is biologically far less important; hence, these much less compromised fetuses may be termed “non-FGR.” A means of distinguishing between fetuses that are destined to become FGR or non-FGR in late gestation would be useful because it would allow putative therapeutic interventions to be evaluated in the most compromised pregnancies.

The aims of this study were (1) to measure fetal growth velocity in overnourished pregnancies and to determine the temporal relationship of attenuated growth in the final one-third of gestation (relative to normally growing controls) by ultrasound measurement of a variety of biometric parameters, (2) to examine for differences in the UA Doppler waveform between nutritional treatments, and (3) to assess the ability of ultrasound to identify those fetuses that are destined to be markedly growth-restricted in late gestation (by retrospective secondary analysis) and to determine how their growth trajectories differ from their ultimately less compromised non-FGR counterparts.

### Materials and Methods

**Experimental animals and study design**

All animal procedures were approved and regulated by the UK Home Office under the Animals (Scientific Procedures) Act 1986 and by local ethics committee review. Animals were housed in individual pens under natural lighting conditions at the Rowett Institute of Nutrition and Health (57°N, 2°W). Animals were bred on site and drawn from the institute’s flocks. To generate singleton pregnancies and maximize genetic homogeneity, embryos were harvested from superovulated adult donor ewes (Scottish Blackface × Border Leicester) 4 days after laparoscopic intrauterine insemination with semen from a single sire (Dorset Horn) and synchronously transferred into the uterus of 51 recipient adolescent ewe lambs (Dorset Horn × Mule) as previously described. Immediately after embryo transfer, ewes were allocated to 1 of 2 nutritional treatments: (1) a control intake that was designed to meet, but not exceed, the en-
ergy requirements of pregnancy and thereby promote normal fetal growth or (2) a high intake that was designed to overnourish the mother and promote her own body growth and adiposity at the expense of the pregnancy, resulting in relative placental and fetal growth restriction. Overnourished and control-intake ewes were offered different amounts of the same complete diet, which provided 12 mJ metabolizable energy and 140 g crude protein per kilogram and contained (per kilogram) 422.5 g rolled barley, 300 g coarsely chopped hay, 167.5 g soybean meal, 100 g molasses, 3.5 g salt, 2.5 g limestone, 2.5 g dicalcium phosphate, and 1.5 g vitamin and minerals (Norvite; Insch, Aberdeenshire, UK). Overnourished ewes consumed approximately 2.25 times the control-intake group ration. The diet composition and regimen used to generate this model of FGR are described in further detail elsewhere. Brief ultrasound examination at 45 days’ gestation confirmed viable pregnancies in 15 of 17 control and 27 of 34 overnourished ewes, giving pregnancy rates of 88.2% and 79.4%, respectively.

Serial ultrasound examination
At 83 ± 0.1 days’ gestation each ewe underwent a detailed baseline ultrasound examination, which was then repeated at approximately weekly intervals between 98 ± 0.1 and 126 ± 0.3 days’ gestation. All scans were carried out by a single operator (D.J.C.) accredited in advanced obstetric ultrasound using a GE Logiq 400 CL machine with a 5.0 MHz curvilinear probe (GE Healthcare, Little Chalfont, Bucks, UK). With the sheep awake and standing upright, the following fetal biometric parameters were measured as previously described; abdominal circumference (AC), renal volume (RV), biparietal diameter (BPD), tibial length (TL), femur length (FL) and placentome index, which was calculated as the sum of the individual cross-sectional areas of 10 representative placentomes. In addition, the deepest vertical pool of amniotic fluid was quantified, and UA Doppler waveform analysis was performed. For the latter, a free loop of cord was identified as close as possible to the fetal abdo-

Pregnancy outcome
At 131 ± 0.3 days’ gestation all ewes were killed humanely with an overdose of intravenous pentobarbitual sodium (Euthatal; Merial Animal Health Ltd, Harlow, UK). Their fetuses were delivered by hysterotomy and promptly killed by the same method before being dried and weighed. The abdominal girth was measured at the level of the umbilicus, and the biparietal head diameter measured with a pair of calipers. Full dissection was carried out, and the weights of all major internal organs were recorded. In addition, the femur and tibia were cleared of all overlying connective tissue so that the shaft of each bone could be measured with calipers. Finally, whole placentomes comprising both the fetal (cotyledonary) and maternal (caruncular) tissues were dissected, and their number and total weight were recorded.

Data analyses
Statistical analyses were performed using the Statistical Package for the Social Sciences software (version 19.0; SPSS Inc, Chicago, IL). Correlations were assessed by Pearson’s product moment test. After testing for normality with Q-Q plots and equality of variance by Levene’s test, comparisons were made between overnourished and control groups with the use of the unpaired Student t test. After determination of fetal weight at necropsy, the overnourished cohort was further subdivided into FGR and non-FGR groups on the basis of the –2 SD cutoff relative to the mean fetal weight in the control-intake group. These 2 groups were compared with the control-intake group with the use of 1-way analysis of variance. Post-hoc testing was carried out when appropriate using the test of least significant difference. All data are presented as mean ± SEM, unless otherwise specified.

RESULTS

Fetal growth curves
Figure 2 shows serial ultrasound measurements of the 5 different fetal biometric parameters (BPD, AC, RV, FL and TL) and the calculated BPD:AC ratios, by nutritional treatment. At baseline examination at 83 ± 0.1 days’ gestation, there were no significant differences in any of these measurements of fetal size. At 98 ± 0.1 days’ gestation ultrasound measurements of AC and FL were reduced in overnourished vs control groups (198 ± 2.6 mm vs 206 ± 3.6 mm [P = .035] and 30.2 ± 0.38 mm vs 31.7 ± 0.51 mm [P = .029], respectively). Measurements of RV and TL were additionally reduced in overnourished compared with control groups at 105 ± 0.1 days’ gestation (4.4 ± 0.18 cm² vs 5.2 ± 0.15 cm² [P = .01] and 50.1 ± 0.54 mm vs 52.3 ± 0.70 mm [P = .016], respectively), whereas differences in BPD between groups did not reach statistical significance until 112 ± 0.1 days’ gestation (48.1 ± 0.26 mm vs 49.4 ± 0.21 mm; P = .002). Once each biometric marker had deviated significantly for the first time, it remained significantly reduced at all subsequent time points examined (P ≤ .001 to .038), with the single exception of TL at 126 ± 0.3 days’ gestation, at which point the significant differences that had been observed at the previous 3 time points were no longer present. BPD:AC ratios were reduced significantly in control vs overnourished groups from 105 ± 0.1 days’ gestation, which reflects the early shift in AC and the relatively late shift in BPD in the latter group of pregnancies.

Placental biometry
Figure 3, A, shows serial measurements of placentome index by nutritional treatment. At baseline examination, the pla-
centome index was already reduced by 24% in overnourished vs control groups (4.0 ± 0.17 cm² vs 5.2 ± 0.25 cm²; P ≤ .001) and remained significantly lower throughout the study period. Measurements of placentome index progressively fell with advancing gestation in both groups, in a parallel fashion. Consequently, the lines of best fit were similar in slope and differed significantly only in their intercept (y = 15.3 + 0.17x + 0.0006 x² in the overnourished group and y = 12.2 + 0.14x + 0.0005 x² in the control group, respectively). Figure 3, B, shows a scatterplot of placentome index at final ultrasound examination (126 ± 0.3 days’ gestation) with placentome weight at necropsy (131 ± 0.3 days). Placentome index shortly before necropsy correlated positively with total placentome weight (r = 0.685; P ≤ .001; n = 27) and fetal weight (r = 0.572; P = .002; n = 27) in overnourished but not control groups (P ≥ .1). Placentome weight also correlated, albeit relatively weakly, with baseline placentome index measurements at 83 ± 0.1 days’ gestation again in the overnourished (r = 0.501; P = .018; n = 27) but not the control group.

**UA Doppler indices**

Figure 4 shows serial measurements of UA Doppler indices (comprising UA pulsatility index, resistance index and systolic-to-diastolic ratios) and deepest vertical pool of amniotic fluid from 83 ± 0.3 until 126 ± 0.3 days’ gestation. UA PI, resistance index and systolic-to-diastolic ratios were all increased signif-
cantly in overnourished relative to control groups at 83 ± 0.3 days’ gestation (1.46 ± 0.039 vs 1.30 ± 0.063 [P = .272], 0.80 ± 0.008 vs 0.73 ± 0.021 [P = .001], and 5.17 ± 0.295 vs 3.72 ± 0.263 [P = .002], respectively). All 3 indices decreased with advancing gestation, but highly significant differences remained between nutritional treatments at all subsequent time points (P ≤ .001 to .004). Measurements of deepest vertical pool also fell with increasing gestation, but there were no differences between the 2 groups at any stage.

**Late gestation necropsy**

Placental weight and fetal anthropometric data at the point of necropsy are shown in Table 1. Relative to the normally growing control-intake pregnancies, total placentome weight and fetal body weight in overnourished pregnancies were 31% and 20% lower, respectively. There were fewer placentomes in the overnourished group (P = .001); average placenta weight was also reduced compared with controls (P ≤ .001). Fetal weight was correlated significantly with total placenta weight in overnourished (r = 0.715; P ≤ .001; n = 27), but not control groups. All postmortem physical measurements were reduced in fetuses from overnourished relative to control pregnancies (P = .001 to .003). Renal, liver, and perirenal fat weights were also lower but were not significantly different when expressed relative to fetal body weight (ie, per kilogram fetus; data not shown). Conversely, there was only a tendency towards lower absolute fetal brain weight in the overnourished group (P = .06) and relative brain weight (in grams per kilogram body weight) was actually increased (P = .001) compared with the control group. Accordingly the brain-to-liver weight ratio was also higher in the overnourished group (P = .003).

**Comparison of ultrasonographic and postmortem measurements**

Fetal AC measurements at 126 ± 0.1 days’ gestation correlated strongly with subsequent fetal weight (r = 0.802; P ≤ .001; n = 39; Figure 5, A) and umbilical girth (r = 0.665; P ≤ .001; n = 39) at necropsy, irrespective of nutritional treatment. By contrast, significant correlations between fetal weight and the other ultrasound indices of fetal size were observed only in the overnourished group (RV: r = 0.752 [P = .001]; FL: r = 0.731 [P ≤ .001]; TL: r = 0.669 [P ≤ .001]; BPD: r = 0.638 [P = .011]; n = 27 each). Similarly, significant correlations between ultrasonographic and postmortem physical measurements of TL and FL and between RV and renal weight were observed only for the overnourished pregnancies (TL: r = 0.547 [P = .003]; FL: r = 0.720 [P ≤ .001]; RV: r = 0.798 [P ≤ .001]; n = 27 each). Ultrasound measurements of BPD did not correlate significantly with postmortem BPD or fetal brain weight in either group. Estimated fetal weight values, which were calculated with our previously validated regression equation [Log estimated fetal weight = 2.115 + 0.003 AC + 0.12 RV − 0.005 RV²] ²² correlated strongly with actual fetal weight at necropsy, irrespective of nutritional treatment (r = 0.819; P ≤ .001; n = 39; Figure 5, B).

**Comparisons between FGR and non-FGR overnourished pregnancies**

Figure 6 shows fetal weight and total placentome weight at necropsy in control-intake and overnourished pregnancies after the latter group had been subcategorized into FGR (n = 17; 63%) and non-FGR (n = 10; 37%) groups based on a 2-SD cutoff relative to the mean fetal weight in the control group (<4222 g in the present study). Further comparisons across these 3 groups are detailed in Table 2. Pregnancies in the non-FGR category did not differ from control-intake pregnancies with respect to fetal weight (4824 ± 208 g vs 5084 ± 124 g; P = .248), absolute or relative fetal organ weights, or postmortem physical measurements. The single exception to this was the total placentome weight, which

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**FIGURE 4**

**UA Doppler indices in overnourished and control-intake ewes**

The graphs show serial ultrasonographic measurements of UA Doppler indices (comprising pulsatility index, resistance index, and systolic-to-diastolic ratio) and deepest vertical pool of amniotic fluid in 42 singleton-bearing adolescent ewes from 83 ± 0.3 to 126 ± 0.3 days’ gestation. Ewes were offered a control dietary intake (closed circles) or overnourished (open circles) to generate normal vs restricted fetal growth, respectively. The error bars indicate SEM. The asterisks denote statistically significant differences: *P ≤ .05; **P ≤ .01; ***P ≤ .001.

UA, umbilical artery.

**Carr. Ultrasound assessment of ovine FGR. Am J Obstet Gynecol 2012.**
was reduced by 22% in non-FGR overnourished relative to control-intake pregnancies (406 ± 26.9 g vs 521 ± 23.8 g; P = .005). Consequently, fetal-to-placental weight ratios were increased in non-FGR overnourished pregnancies relative to the controls (12.1 ± 0.53 vs 9.9 ± 0.42; P = .018) and, in fact, did not differ significantly from the FGR overnourished group (12.1 ± 0.53 vs 11.7 ± 0.62; P = .618). The FGR overnourished pregnancies exhibited a 28% reduction in fetal weight (3639 ± 117 g vs 5084 ± 124 g; P = .001) and a 37% reduction in total placentome weight (330 ± 23.8 g vs 521 ± 23.8 g; P = .0001) relative to the control group at 131 ± 0.3 days’ gestation. Ultrasound measurements from each time point in gestation were subsequently retrospectively compared across all 3 groups. There were no significant differences in fetal biometry between FGR and non-FGR groups between 83 ± 0.3 and 119 ± 0.1 days’ gestation, and there were no differences in placentome index or UA Doppler indices at any point during the study. However, at 126 ± 0.3 days’ gestation, measurements of the following ultrasound parameters were reduced in FGR vs non-FGR overnourished pregnancies: AC (259 ± 2.5 mm vs 270 ± 4.4 mm; P = .017); RV (7.7 ± 0.2 cm³ vs 8.8 ± 0.51 cm³; P = .031); FL (54.4 ± 0.69 mm vs 59.3 ± 1.26 mm; P = .022); and TL (72.8 ± 1.11 mm vs 76.4 ± 1.23 mm; P = .038). Measurements of BPD also tended to be lower (52.5 ± 0.43 mm vs 53.9 ± 0.76 mm; P = .056).

On the basis of the strong correlations between AC and fetal weight at necropsy (Figure 5), we tested the ability of a −2-SD ultrasound AC cutoff (roughly equivalent to the third percentile) to identify the genuinely FGR fetus. Mean ± SD measurements of AC at 126 ± 0.3 days’ gestation for the control group were 286 ± 8.0 mm, giving a cutoff of 270 mm. At this gestation, 15 of 17 fetuses in the FGR group had an AC measurement below this threshold; hence, the sensitivity of the AC to predict an FGR fetus was 88.2%. Notably 5 of the 10 non-FGR fetuses were also noted to have an AC that plotted >2 SD below the control mean at this stage, which gave a specificity of 50.0%.

**FIGURE 5** Relationship between ultrasound AC/estimated fetal weight and necropsy weight

Scatterplot shows A, AC by ultrasound and B, estimated fetal weight (calculated using the following equation: log estimated fetal weight = 2.115 + 0.003 AC + 0.12 renal volume − 0.005 renal volume²) at 126 ± 0.3 days’ gestation with fetal weight at necropsy at 131 ± 0.3 days’ gestation in ewes offered a control dietary intake (closed circles) or overnourished (open circles) to generate normal vs restricted fetal growth, respectively. Linear regression lines are shown. Parameters were significantly correlated within both groups and across groups (AC: r = 0.802; estimated fetal weight: r = 0.819; P ≤ .001).

AC, abdominal circumference.


**TABLE 1** Necropsy data at 131 days’ gestation in overnourished and control-intake ewes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control intake (n = 15)</th>
<th>Overnourished (n = 27)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal weight, g</td>
<td>5084 ± 124</td>
<td>4078 ± 153</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Biparietal head diameter, mm</td>
<td>71 ± 0.8</td>
<td>67 ± 0.7</td>
<td>.001</td>
</tr>
<tr>
<td>Length of femoral shaft, mm</td>
<td>90 ± 1.0</td>
<td>84 ± 1.0</td>
<td>.001</td>
</tr>
<tr>
<td>Length of tibial shaft, mm</td>
<td>106 ± 1.24</td>
<td>100 ± 1.15</td>
<td>.003</td>
</tr>
<tr>
<td>Umbilical girth, mm</td>
<td>363 ± 4.9</td>
<td>332 ± 5.0</td>
<td>.001</td>
</tr>
<tr>
<td>Brain weight, g</td>
<td>47.0 ± 0.95</td>
<td>44.7 ± 0.67</td>
<td>.004</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>161.3 ± 6.99</td>
<td>126.0 ± 6.10</td>
<td>.002</td>
</tr>
<tr>
<td>Renal weight, g</td>
<td>27.5 ± 0.77</td>
<td>22.7 ± 0.91</td>
<td>.002</td>
</tr>
<tr>
<td>Perirenal fat weight, g</td>
<td>21.1 ± 0.57</td>
<td>18.0 ± 0.76</td>
<td>.002</td>
</tr>
<tr>
<td>Brain-to-liver weight ratio</td>
<td>0.30 ± 0.012</td>
<td>0.37 ± 0.014</td>
<td>.003</td>
</tr>
<tr>
<td>Relative brain weight of fetus, g/kg</td>
<td>9.27 ± 0.239</td>
<td>11.22 ± 0.300</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Placentome, n</td>
<td>113 ± 5.2</td>
<td>98 ± 3.1</td>
<td>.017</td>
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<tr>
<td>Total placentome weight, g</td>
<td>521 ± 23.8</td>
<td>358 ± 19.1</td>
<td>&lt;.001</td>
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<tr>
<td>Average placentome weight, g</td>
<td>4.7 ± 0.20</td>
<td>3.7 ± 0.15</td>
<td>&lt;.001</td>
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<tr>
<td>Fetal-to-placental weight ratio</td>
<td>9.92 ± 0.422</td>
<td>11.84 ± 0.429</td>
<td>.01</td>
</tr>
</tbody>
</table>

All data are given as mean ± SEM. Groups were compared using the Student t-test, with the exception of the male-to-female sex ratio for which the Fisher exact test was used.

The bar charts show A, fetal weight and B, total placental weight at necropsy (131 ± 0.3 days’ gestation) in 42 singleton-bearing adolescent ewes that were offered a control dietary intake (closed bars; n = 15) or overnourished (n = 27). Overnourished pregnancies were further subcategorized into FGR (vertically striped bars; n = 17) and non-FGR (horizontally striped bars; n = 10) groups on the basis of a minus 2 SD cutoff in fetal weight relative to the control mean (<4222 g for the present study). The error bars indicate SEM. The asterisks denote statistically significant differences: *P ≤ .05; **P ≤ .01; ***P ≤ .001.

**FGR**; fetal growth restriction.

**Carr. Ultrasound assessment of ovine FGR. Am J Obstet Gynecol 2012.**

**FIGURE 6**

**Fetal and placental weights in FGR vs non–FGR overnourished and control-intake ewes**

**TABLE 2**

**Necropsy data in FGR/non–FGR overnourished vs control-intake ewes**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control intake (n = 15)</th>
<th>Non–FGR (n = 10)</th>
<th>FGR (n = 17)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal weight, g</td>
<td>5084 ± 124</td>
<td>4824 ± 208</td>
<td>3639 ± 117</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Biparietal head diameter, mm</td>
<td>71 ± 0.8</td>
<td>69 ± 1.3</td>
<td>65 ± 0.7</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Length of femoral shaft, mm</td>
<td>90 ± 1.0</td>
<td>88 ± 0.9</td>
<td>82 ± 1.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Length of tibial shaft, mm</td>
<td>106 ± 1.2</td>
<td>104 ± 1.5</td>
<td>98 ± 1.3</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Umbilical girth, mm</td>
<td>363 ± 4.9</td>
<td>353 ± 6.5</td>
<td>320 ± 5.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Brain weight, g</td>
<td>47.0 ± 0.95</td>
<td>46.2 ± 0.97</td>
<td>43.7 ± 0.85</td>
<td>.034</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>161.3 ± 6.99</td>
<td>143.8 ± 8.46</td>
<td>115.5 ± 7.33</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Renal weight, g</td>
<td>27.5 ± 0.77</td>
<td>26.2 ± 1.46</td>
<td>20.5 ± 0.83</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Perirenal fat weight, g/kg fetus</td>
<td>21.1 ± 0.57</td>
<td>21.3 ± 1.14</td>
<td>16.1 ± 0.65</td>
<td>&lt;.001</td>
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<tr>
<td>Brain-to-liver weight ratio</td>
<td>0.23 ± 0.003</td>
<td>0.23 ± 0.003</td>
<td>0.23 ± 0.003</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Relative brain weight, g/kg fetus</td>
<td>9.3 ± 0.24</td>
<td>9.7 ± 0.25</td>
<td>12.1 ± 0.27</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Placentome, n</td>
<td>113 ± 5.2</td>
<td>107 ± 3.7</td>
<td>93 ± 4.0</td>
<td>.005</td>
</tr>
<tr>
<td>Total placental weight, g</td>
<td>521 ± 23.8</td>
<td>406 ± 26.9</td>
<td>330 ± 23.8</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Average placental weight, g</td>
<td>4.7 ± 0.20</td>
<td>3.8 ± 0.22</td>
<td>3.6 ± 0.21</td>
<td>.002</td>
</tr>
<tr>
<td>Fetal-to-placental weight ratio</td>
<td>9.9 ± 0.42</td>
<td>12.1 ± 0.53</td>
<td>11.7 ± 0.62</td>
<td>.033</td>
</tr>
<tr>
<td>Male-to-female fetal sex ratio</td>
<td>9:6</td>
<td>4:6</td>
<td>7:10</td>
<td>.573</td>
</tr>
</tbody>
</table>

*Overnourished ewes were subcategorized into FGR and non–FGR groups on the basis of a minus 2 SD cutoff in fetal weight relative to the control mean (<4222 g for the present study). All data are given as mean ± SEM. Groups were compared using 1-way analysis of variance and post-hoc test of least significant difference, with the exception of the male-to-female sex ratio for which the Fisher exact test was used. Overall analysis of variance P values are shown. Mean values within a row with unlike subscripts (a, b, and c) were significantly different (P = .05 for individual comparisons). FGR, fetal growth restriction.


days’ gestation. Meanwhile, growth of the fetal BPD was relatively well preserved until 112 days’ gestation. This asymmetric growth pattern is indicative of fetal brain sparing, which is an adaptive response to reduced substrate supply in which growth of the brain is prioritized. This phenomenon is seen commonly in human FGR secondary to uteroplacental insufficiency. Our additional findings of increasing ultrasound BPD to AC ratios and a higher fetal brain-to-bodyweight ratio in the last one-third of gestation provide further evidence of this effect. It is unsurprising that the AC was the first parameter to be affected because it is the most sensitive indicator of underlying fetal size in sheep and human pregnancy and is potentially the best ultrasonographic indicator of current fetal metabolic status because it reflects both subcutaneous fat and liver glycogen stores. By comparison, changes in RV would not be expected to manifest until there had been a degree of nutritional deficiency severe enough to impact the growth and development of major internal organs. Ultrasound studies of longitudinal fetal growth velocity in another well-characterized sheep paradigm of FGR induced by maternal hyperthermic exposure also found that the AC was the first parameter to be reduced relative to normally growing control fetuses (from as early as 73 days’ gestation) and that the BPD was the last parameter to be affected. The findings of the present study support the observation that exposure of pregnant sheep to hyperthermic conditions produces a similarly asymmetric but relatively more severe and earlier onset form of FGR compared with overnourishment of the pregnant adolescent ewe.
With respect to the long bones, it has been suggested that TL may be a better marker of longitudinal fetal growth than FL because it demonstrates a greater acceleration with advancing gestation. However, in the present study, FL was impacted by prenatal nutrition earlier than TL and subsequently showed stronger correlations with both postmortem measurements and fetal weight in late pregnancy. Furthermore, TL was the only parameter in which significant differences did not remain at 126 days’ gestation. This is likely to reflect difficulties in measuring TL in late gestation rather than a genuine catch up in TL growth, because subsequent postmortem measurements of the tibial shaft differed significantly between treatment groups (P = .003). Towards the end of pregnancy, the tibia was technically more challenging to identify and measure accurately than the femur because of variable fetal leg position and difficulty visualizing the entire tibial shaft, which can measure up to 100 mm at term. It is also interesting that the BPD was the only parameter in the present study that failed to correlate significantly with postmortem biometry. Satisfactory views were harder to obtain in late pregnancy for BPD than for the other parameters as the fetal head was often deeply engaged in the maternal pelvis, which is likely to have affected the accuracy of its measurement. Furthermore, variable extrinsic compression of the fetal head, particularly in late gestation, is known to affect the accuracy of the BPD measurement in human fetuses; for this reason, it is not recommended for use as a marker of fetal growth in UK obstetric practice; the head circumference is preferred instead.

Placental biometry

Despite the lack of significant differences in fetal biometry at baseline ultrasound examination, we demonstrated that ultrasound indices of placental size were already attenuated by 83 days’ gestation. This observation was relatively unexpected, because previous studies in which pregnancies were interrupted at days 80-90 of gestation have not shown differences in total placental wet weight at this stage. Nevertheless, these studies have provided evidence of altered placental development and secretory function by mid pregnancy including a lower proliferation index, increased apoptosis, attenuated levels of circulating progesterone and ovine placental lactogen, and reduced messenger RNA expression of vascular endothelial growth factor, angiopoietins 1 and 2, nitric oxide synthase 3, and fms-related tyrosine kinase 1 (vascular endothelial growth factor receptor 1). Putative alterations in placental density (perhaps secondary to compensatory changes in placental vascular adaptability and/or differences in carbohydrate metabolism and water content) might explain the lack of a difference in weight per se, despite obvious differences in ultrasound appearance and indices of size in the present study. To date few studies have assessed ovine ultrasonographic placental biometry. Doize et al. evaluated transrectal measurements of placentome diameter as a method of pregnancy dating in both sheep and goats. Selecting between 2 and 5 average-sized placentomes, they demonstrated a rapid increase in diameter up until 70-90 days’ gestation. Their placentome measurements correlated with gestational age in does, but not ewes; the authors concluded that placental biometry is unsuitable for dating sheep pregnancies. Kaulfuss et al. also used transrectal ultrasound scanning to measure the maximum diameter of 10 representative placentomes in 4 different sheep breeds. They too observed that placentome dimensions reached a peak in mid gestation and declined thereafter. This is consistent with the observed trends in both experimental groups in the present study as well as with the findings of serial slaughter studies in normal sheep pregnancy, which indicate that total placentome weight peaks at approximately 80 days’ gestation. The positive correlation between ultrasonographic placentome index in both mid and late gestation and eventual total placentome weight suggests that early identification of compromised placentas with the use of ultrasound is feasible in the sheep. Moreover, the positive correlation between late-gestation placentome index and fetal weight reinforces the observation that it is placental size per se that provides the major constraint to fetal growth in overnourished adolescent ewes. Notably, these correlations were only significant in the overnourished group, which is likely to be explained by the relative lack of variability within the control-intake group, with respect to total placentome weight. The same applies to the observed correlations between fetal weight, postmortem measurements, and late-gestation ultrasound parameters, which were significant only among fetuses of overnourished adolescent ewes.

UA Doppler velocimetry

In the present study, UA Doppler indices already were increased significantly by 83 days’ gestation in overnourished, compared with control-intake, pregnancies and remained so through to late gestation. In normally developing sheep pregnancies, UA Doppler indices decline with advancing gestation, with the most rapid fall in values between 66 and 109 days’ gestation. Between 109 and 139 days’ gestation, studies in chronically instrumented normal sheep pregnancy have shown that UA pulsatility index, resistance index and systolic-to-diastolic ratios continue to fall progressively and that all 3 correlate positively with directly measured umbilical vascular resistance and negatively with umbilical and placental blood flow. In late gestation (approximately day 133), the growth-restricted pregnancies of overnourished adolescent ewes are characterized by a 37% reduction in umbilical blood flow, as measured directly using the Fick principle; therefore, the changes in UA Doppler indices demonstrated herein likely reflect this reduction in absolute blood flow and/or putative increased downstream vascular impedance to blood flow. Although umbilical blood flow has not been assessed directly by the same method in mid gestation, uterine blood flow measured using indwelling flow probes is reduced by 42% relative to controls by as early as 88 days’ gestation. Given that umbilical and uterine blood flows are generally closely matched, at least in late gestation, it is likely that a corresponding reduction in umbilical blood flow does

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exist in mid gestation in overnourished adolescent ewes, especially considering the highly significant differences in UA Doppler indices at 83 days’ gestation in the present study. UA Doppler indices have been measured previously in 2 other established sheep models of FGR. Indices were found to be increased from 80-135 days’ gestation after maternal hyperthermia, an intervention that has been shown to reduce absolute umbilical blood flow in late pregnancy. By contrast, Doppler indices were not influenced by premating carunclectomy, which appears to restrict fetal growth through a reduction in total placentome number in the absence of specific effects on the fetoplacental circulation.

In the present study, we found that early identification of fetuses that are destined to be FGR by ultrasound scanning was not possible because they did not differ from the non-FGR group with respect to placental biometry or UA Doppler indices at any stage of pregnancy, and no differences in fetal biometry were detected between 83 and 119 days’ gestation. Rather, it was only when examined at 126 days’ gestation that significant differences in any fetal measurements became apparent. This is perhaps not surprising because by 121 days’ gestation the fetus has only achieved approximately 70% of its final weight (Figure 1), irrespective of nutritional treatment. Hence, although differences between control and overnourished pregnancies begin to emerge at 98 days’ gestation, more subtle differences within the overnourished group would intuitively be harder to detect. It appears that, despite an initial reduction in placental biometry and increased UA Doppler indices relative to control-intake pregnancies, non-FGR overnourished fetuses are subject subsequently to a lesser degree of placental constraint during the exponential period of fetal growth compared with their FGR counterparts. This is reinforced by the significant differences between the 2 groups in total placentome weight at 131 days’ gestation. Given their greater placental mass, it is likely that overall placental transport capacity is relatively preserved in the late gestation non-FGR overnourished pregnancy at a time when the supply of nutrients from the maternal circulation is plentiful because of overnutrition, which allows the fetus to continue to thrive. The placenta has a certain intrinsic reserve capacity, and we have consistently observed that placental growth needs to be restricted by >20% before it significantly impacts fetal growth (J.M.W., unpublished data, 2006), which is consistent with estimates derived from experiments involving midpregnancy placentome ablation. In spite of the lack of early differences between FGR and non-FGR overnourished pregnancies, fetal biometry nonetheless performed well in the identification of the most compromised pregnancies in late gestation; an AC of >2 SD below the control mean had a sensitivity of 88.2% to predict an FGR fetus. However, the relatively low specificity of 50% indicates that ultrasound did not perform as well in identifying non-FGR pregnancies and therefore is likely to be limited in its ability to reliably distinguish between FGR and non-FGR pregnancies.

**Relevance to human pregnancy**

The main strength of this FGR model lies in the fact that it is characterized by an early insult on uteroplacental development, which represents the most common pathology underlying the human condition. However, this particular model also highlights the potential risks of excessive gestational weight gain independent of the degree of adiposity at the time of conception, which is equivalent in the overnourished and control-intake dams. Although dietary excess during pregnancy and maternal obesity per se are both established risk factors for fetal macrosomia, gestational weight gain in excess of the limits recommended by the Institute of Medicine is also associated with other adverse pregnancy outcomes, which include the delivery of low birthweight infants, irrespective of body mass index at booking.

Our results indicate that the sequence of events observed in this unique paradigm mimic those that are observed in uteroplacental insufficiency in clinical practice, namely an asymmetric pattern of growth restriction and increased impedance to blood flow in the UA. However, given the differences in placentaion and the experimental approach to generate FGR, care should be taken when extrapolating the findings of the present study to human pregnancy.

Fetal ultrasonographic biometric markers were reduced from 98 days’ gestation onwards in overnourished adolescent sheep pregnancies relative to normally growing control-intake pregnancies. The fetal abdominal circumference and femur length were reduced first, shortly followed by the renal volume and tibia length. The biparietal head diameter was the last parameter to be affected, which indicates an asymmetric growth pattern commensurate with fetal brain sparing. Although ultrasound was unable to discriminate between fetuses who were destined to be FGR or non-FGR before 126 days’ gestation, at this stage fetal biometry performed well in the identification of those fetuses who weighed >2 SD below the control mean. At 83 days’ gestation, ultrasonographic placental biometry was already reduced, and UA Doppler indices were increased in overnourished vs control-intake pregnancies, before any demonstrable shift in fetal growth velocity. These findings suggest an early insult on placentonal development resulting in reduced umbilical blood flow or at least increased impedance to blood flow secondary to increased fetal cotyledonary resistance.

**REFERENCES**