The effect of overnourishing singleton-bearing adult ewes on nutrient partitioning to the gravid uterus

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Overnourishing the singleton-bearing adolescent sheep throughout pregnancy promotes maternal tissue synthesis at the expense of the nutrient requirements of the gravid uterus. Consequently, the growth of the placenta is impaired and results in the premature delivery of low-birth-weight lambs relative to moderately fed adolescents of equivalent age. To establish if this phenomenon is unique to the growing animal, singleton pregnancies to a single sire were established by embryo transfer into primiparous adult ewes who had attained the normal mature body size for their genotype. Thereafter ewes were offered a maintenance or a high level of a complete diet throughout gestation. High maternal intakes resulted in elevated maternal insulin, no significant change in growth hormone or glucose, and attenuated progesterone and NEFA concentrations. Live weight gain during the first 93 d of gestation was 48 and 244 g/d, and adiposity score at term was 2.4 and 3.7 in the maintenance and high groups, respectively (P<0.001). In spite of achieving levels of adiposity similar to overnourished adolescents, placental (477 (SEM 30) v. 518 (SEM 41) g) and fetal (5190 (SEM 320) v. 5420 (SEM 250) g) weights were equivalent in maintenance and high groups. Gestation length was shorter (P<0.01) and colostrum yield at parturition lower (P<0.005) in high v. maintenance dams. Thus, adult sheep appear to be relatively insensitive to the oversupply of nutrients during pregnancy and have the ability to meet the nutrient requirements for normal conceptus growth in spite of their increased adiposity.

Abbreviation: GH, growth hormone.

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attained the normal mature body size for their genotype. The experimental design, sire, genotype, initial adiposity and diet were identical to those used in previous adolescent studies to facilitate direct comparisons of pregnancy outcome in the contrasting age cohorts.

The partitioning of oxygen, glucose and amino acids between the dam and her gravid uterus may be orchestrated by nutritionally sensitive endocrine hormones of maternal, placental and fetal origin (Bell & Bauman, 1997) and the circulating concentrations of many of these maternal and placental hormones have been examined in detail throughout pregnancy in the adolescent paradigm (Wallace et al. 1997a,b, 2001, 2004b; Thomas et al. 2001). Briefly, overnutrition during adolescent pregnancy is associated with elevated maternal insulin, insulin-like growth factor-1, leptin, triiodothyronine and thyroxine concentrations while the levels of progesterone, placental lactogen, pregnancy-specific protein B and growth hormone (GH) are attenuated. Similarly, in the present study, maternal blood samples were collected to examine peripheral hormone concentrations and metabolites in relation to pregnancy outcome.

Materials and methods

Animals and experimental design

All procedures were performed in accordance with the UK Animals (Scientific Procedures) Act of 1986 and approved by the Rowett Research Institute’s Ethical Review Committee.

Embryos from superovulated adult ewes (Border Leicester × Scottish Blackface), inseminated by a single sire, were recovered on day 4 after oestrus and transferred synchronously in singleton into the uterus of primiparous adult ewes (Dorset Horn × Mule) exactly as described previously by Wallace et al. (1997a). Donor ewes (n 9) were 4–5 years of age, multiparous and had a body condition score of 2·4 (SEM 0·04) at insemination. Embryo transfer was carried out during the mid-breeding season and the animals were housed in individual pens under natural lighting conditions at the Rowett Research Institute (57°N, 2°W). At the time of embryo transfer, the recipient ewes were approximately 19 months old, with a mean live weight of 56 (SEM 0·5) kg and a body condition score of 2·4 (SEM 0·02). Immediately following embryo transfer, recipient ewes were evenly allocated to one of two nutritional treatments on the basis of live weight, body condition score and ovulation rate at the time of transfer. Where possible, care was also taken to randomise for embryo source. Recipients were individually offered either a high or maintenance level of a complete diet (eighteen ewes per group). The latter control ration was calculated to supply the estimated metabolisable energy requirements for maintenance throughout gestation using the Feedbyte Ruminant Ration Formulation Program (Version 2.3; Scottish Agricultural College, Aberdeen, UK) and as such was predicted to optimise placental and fetal growth in this genotype. The complete diet supplied 10·2 MJ metabolisable energy and 137 g crude protein per kg as fed and was offered in two equal feeds at 08.00 and 16.00 hours daily. The diet contained 30% (w/w) coarsely milled hay, 50% barley, 10% molasses, 9% fishmeal, 0·3% salt, 0·5% calcium and 0·2% of a vitamin–mineral supplement and had an average DM content of 86%. Animals offered maintenance rations received their entire ration immediately while those offered high intakes had the level of feed increased gradually over a 2–3 week period until the level of daily feed refusal was approximately 15% of the total offered (equivalent to ad libitum intakes). The level of feed offered was reviewed three times weekly and adjusted for individual ewes on the basis of level of feed refused (recorded daily) and changes in live weight (recorded weekly). From day 95 of gestation until parturition, the feed intake of the maintenance fed group was adjusted weekly to meet the increasing nutrient demands of the developing fetus during the final third of pregnancy by maintaining individual ewe body condition score during this period. Body condition score was measured at about fortnightly intervals throughout the study. Body condition or adiposity score was measured on a five-point scale (1 = emaciated, 5 = obese), as described previously (Russel et al. 1969).

The sire, genotype and diet used were identical to those used in all our previously published adolescent studies (for reviews, see Wallace et al. 2004a).

Pregnancy rate was initially estimated by non-return rates to twice daily exposure to a vasectomised ram between days 13 and 20 of the oestrous cycle. Pregnancy was subsequently confirmed by transabdominal ultrasound on about day 45 of gestation, when sixteen high- and fifteen maintenance-fed ewes had viability of their fetuses.

Pregnancy outcome

Pregnancy outcome was determined after spontaneous delivery at term. Lambs were dried and weighed after delivery and the sex recorded. Ewe colostrum yield was measured before lamb suckling and within 30 min of parturition. After intravenous injection of oxytocin (Oxytocin-S®, 6 IU per ewe; Intervet Ltd, Cambridge, UK) the ewes were milked by hand until all the colostrum had been removed from the udder. The colostrum was weighed and fed to the lamb by bottle at a rate of approximately 50 ml/kg live weight. In cases where the dam had insufficient colostrum, frozen ewe colostrum was substituted. After the placenta (fetal cotyledons and membranes) was delivered, it was weighed and the fetal cotyledons dissected, counted and weighed.

Blood sampling and biochemical analysis

Weekly blood samples were collected from a subset of twenty ewes from day 14 of gestation onwards by jugular venepuncture approximately 3 h after the morning feed. These samples were analysed for insulin, GH, progesterone, glucose and NEFA. Insulin, GH and progesterone concentrations were measured in duplicate by radioimmunoassays described previously (Ronayne & Hynes, 1990; MacRae et al. 1991; Wallace et al. 1997a). The limit of detection was 4 μU insulin/ml, 1 ng GH/ml and 0·5 ng progesterone/ml. The intra- and inter-assay CV were 4·4 and 8·6% for insulin, 5·6 and 9·9% for GH and 6·5 and 8·7% for progesterone, respectively. Plasma glucose and NEFA concentrations were determined as originally described by Peterson & Young (1968) and Matsubara et al. (1983).

Data analysis

The physical outcome data were analysed by Student’s t test. For the endocrine and metabolite data, the individual mean concentrations for three discrete periods spanning the first, second and third ‘trimesters’ of pregnancy were calculated. The resulting data were then analysed in Genstat 7th edition (VSN International...
The effect of dietary intake and stage of pregnancy on maternal endocrine and metabolite status throughout gestation was unexpected and resulted in a significant difference in the number of male fetuses per group.

Results

**Maternal dietary intake, weight and adiposity score**

Conception rate following embryo transfer was high (86 %) and unaffected by maternal nutrition. Maternal dietary intakes and changes in live weight and adiposity throughout gestation are presented in Table 1. Mean maternal dietary intakes were elevated in ewes fed ad libitum (high) compared with control (maintenance) ewes throughout gestation ($P<0.001$). On average in the high intake ewes this represented a 1.75-, 2.20- and 1.65-fold increase relative to the maintenance group during the first, second and third periods of gestation, respectively. From an equivalent starting point and as a result of these marked differences in dietary intake, maternal live weight and body condition scores were significantly elevated in the high vs. maintenance dams by day 28 and day 30 of gestation, respectively. Maternal body condition or adiposity score was successfully maintained at the initial level throughout gestation in the control dams, while the high intake dams became progressively obese. Live weight gain during the first 93 d of pregnancy was 48 and 244 g/d for maintenance and high intake groups, respectively ($P<0.001$). At approximately 24 h postpartum, maternal live weight was 63 (SEM 1·1) and 82 (SEM 1·8) kg and body condition score was 2·4 (SEM 0·04) and 3·7 (SEM 0·1) units for maintenance and high intake groups, respectively.

**Pregnancy outcome**

Pregnancy outcome data following spontaneous vaginal delivery at term are presented in Table 1. The mean duration of gestation was shorter ($P<0.01$) in high compared with maintenance intake ewes (Fig. 2). However, no measured placental parameter or lamb birth weight per se was influenced by maternal dietary intake during pregnancy. The ratio of fetal weight:placental weight was identical in both groups and fetal placental weight and lamb birth weight were positively correlated in both the maintenance and high intake groups ($r = 0.611$ and 0.657, $P<0.005$ and <0.01, respectively). There was no relationship detected between maternal live weight gain and either placental weight or lamb birth weight, either within nutritional treatments (data not shown) or when the treatments were combined ($r = 0.232$ and 0.079, respectively, $n = 29$). Gestation length was positively associated with lamb birth weight in the high ($r = 0.573$, $n = 16$, $P<0.005$) but not the maintenance ($r = 0.267$) group. Colostrum yield at parturition was higher ($P<0.05$) in maintenance compared with high intake ewes. Irrespective of treatment group, no correlation was found between gestation length, placental weight, lamb birth weight, maternal live weight gain, and colostrum yield.

**Maternal endocrine and metabolite status throughout gestation**

The effect of dietary intake and stage of pregnancy on maternal endocrine and metabolite status throughout gestation is summarised in Table 2. Maternal plasma insulin concentrations were not influenced by dietary intake during the first third of pregnancy and remained relatively static in the maintenance dams during the second and third periods of gestation. In contrast, insulin concentrations in the high intake dams increased during the second third and reached peak levels in the final third of gestation (diet × period of gestation interaction, $P=0.002$). GH concentrations were highly variable and did not change markedly throughout pregnancy in either dietary group. Although GH concentrations were higher in maintenance compared with high intake dams during all three periods of gestation, this difference did not achieve formal significance ($P=0.079$). Peripheral progesterone concentrations increased significantly throughout gestation ($P<0.001$) and were higher in maintenance compared with high intake dams during all three periods ($P=0.005$, Fig. 3). Maternal glucose concentrations were not affected overall by diet or stage of pregnancy. Lower maternal glucose concentrations during the first third of pregnancy in high intake dams were unexpected and resulted in a significant diet × period of gestation interaction ($P=0.01$). Plasma NEFA concentrations were higher in maintenance than in high intake dams throughout gestation but remained well within the acceptable range for ewes fed to meet maternal requirements (Russel
The increase in maternal NEFA as gestation progressed was most pronounced in high intake dams. No relationship was detected between maternal insulin, GH, glucose or NEFA concentrations during the first, second or third period of gestation and pregnancy outcome (gestation length, placental weight, lamb birth weight or colostrum yield). Irrespective of treatment group, maternal progesterone concentrations during the final third of pregnancy were positively associated with lamb birth weight ($r = 0.508$, $n = 20$, $P < 0.05$). A similar relationship was evident between late pregnancy peripheral progesterone concentrations and fetal placental mass ($r = 0.530$, $P < 0.05$). Maternal progesterone concentrations on day 140 of gestation (the final blood sample prior to parturition for the majority of ewes sampled) was positively associated with gestation length ($r = 0.671$, $P < 0.002$).

### Table 1. Gestation length and morphometric data relating to pregnancy outcome in primiparous adult ewes offered a maintenance or high dietary intake from day 4 of gestation (Mean values with standard error of the mean, or range)

<table>
<thead>
<tr>
<th>Maternal dietary intake</th>
<th>Maintenance</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SEM</td>
<td>Mean SEM</td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>Gestation length (d)</td>
<td>147.4 0.41</td>
<td>145.1 0.53</td>
</tr>
<tr>
<td>Range (g)</td>
<td>144–150</td>
<td>142–149</td>
</tr>
<tr>
<td>Lamb birth weight (g)</td>
<td>5190 320</td>
<td>5420 250</td>
</tr>
<tr>
<td>Range (g)</td>
<td>2250–7030</td>
<td>3380–6800</td>
</tr>
<tr>
<td>Number of male lambs</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Fetal placental weight (g)</td>
<td>477 30</td>
<td>518 41</td>
</tr>
<tr>
<td>Range (g)</td>
<td>181–428</td>
<td>230–499</td>
</tr>
<tr>
<td>Number of fetal cotyledons</td>
<td>82 2–8</td>
<td>85 4–7</td>
</tr>
<tr>
<td>Total fetal cotyledon weight (g)</td>
<td>156 12</td>
<td>169 21</td>
</tr>
<tr>
<td>Range (g)</td>
<td>79–260</td>
<td>76–380</td>
</tr>
<tr>
<td>Fetal weight:placental weight</td>
<td>11.2 0.66</td>
<td>11.1 0.55</td>
</tr>
<tr>
<td>Colostrum yield (g)</td>
<td>525 49</td>
<td>386 45</td>
</tr>
</tbody>
</table>

### Table 2. Mean maternal plasma insulin, growth hormone, progesterone, glucose and NEFA concentrations during the first, second and third period of pregnancy in primiparous adult ewes offered a maintenance (M) or high (H) dietary intake from day 4 of gestation (Mean values with standard error of the mean)

<table>
<thead>
<tr>
<th>Period of gestation</th>
<th>First</th>
<th>Second</th>
<th>Third</th>
<th>Dietary intake</th>
<th>Period of gestation</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (µU/ml)</td>
<td></td>
<td></td>
<td></td>
<td>M intake</td>
<td>29.9 3.28</td>
<td>23.2 2.26</td>
</tr>
<tr>
<td></td>
<td>H intake</td>
<td>30.6 2.37</td>
<td>37.0 3.22</td>
<td>52.5 4.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth hormone (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td>M intake</td>
<td>3.0 0.82</td>
<td>2.5 0.71</td>
</tr>
<tr>
<td></td>
<td>H intake</td>
<td>1.8 0.12</td>
<td>1.7 0.12</td>
<td>1.7 0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td>M intake</td>
<td>4.4 0.33</td>
<td>6.7 0.51</td>
</tr>
<tr>
<td></td>
<td>H intake</td>
<td>3.7 0.33</td>
<td>4.8 0.28</td>
<td>10.1 0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td>M intake</td>
<td>3.8 0.09</td>
<td>3.5 0.05</td>
</tr>
<tr>
<td></td>
<td>H intake</td>
<td>3.3 0.17</td>
<td>3.4 0.19</td>
<td>3.6 0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEFA (mmol/l)</td>
<td></td>
<td></td>
<td></td>
<td>M intake</td>
<td>0.14 0.008</td>
<td>0.13 0.011</td>
</tr>
<tr>
<td></td>
<td>H intake</td>
<td>0.09 0.004</td>
<td>0.10 0.006</td>
<td>0.14 0.011</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

The results of the present study demonstrate that overnourishing the singleton-bearing primiparous adult ewe throughout gestation markedly increases adiposity but does not alter nutrient partitioning to the gravid uterus. Consequently, fetal placental mass and lamb birth weight at term were identical to those of maintenance-fed ewes. This contrasts with the major alterations in fetoplacental growth consistently observed during the final third of gestation and at term in overnourished adolescents (Wallace et al. 2002a,b, 2004a) and implies that the growing animal is particularly sensitive to alterations in maternal dietary intake. The pubertal animals used in our adolescent studies are gynaecologically immature. Similarly, in human cohorts, the rates of spontaneous miscarriage and very preterm birth (logically immature. Similarly, in human cohorts, the rates of spontaneous miscarriage and very preterm birth (≤32 weeks) are highest in girls aged 13–15 years (Scottish Needs Assessment Programme, 1994; Olausson et al. 1999). Furthermore, babies born at term to young mothers (12–16 years) are lighter and smaller (P<0.0001) in all body dimensions than the offspring of both older adolescents (17–19 years) and adult women (20–29 years; Kirchengast & Hartmann, 2003), suggesting that gynaecological immaturity is a major factor predisposing human adolescents to poor pregnancy outcome. However, in the present highly controlled adult sheep study, placental and fetal weights in both groups (Table 1) were identical to those of maintenance-fed (control) adolescents (491 and 5136 g, respectively; averaged over nine individual studies as detailed in Wallace et al. 2004a). This critically suggests that the singleton fetus gestated in an adolescent uterus has the capacity to achieve the same growth trajectory and final birth weight as those gestated in primiparous adults providing that the dam is optimally nourished. In both adult and adolescent animals, this was achieved by increasing maternal dietary intake in a stepwise manner during the final third of gestation to maintain maternal adiposity and prevent the ewe becoming catabolic during the period of rapid fetal growth (for review, see Robinson, 1983). Clearly this observation has implications for the nutritional management of both young pregnant sheep and human adolescents.

The failure of sustained high maternal intakes to alter either initial conception rate or placental growth in the present study is perhaps surprising in view of the weight of published evidence. High maternal dietary intakes operating via nutritionally induced decreases in peripheral progesterone concentrations are associated with reduced pregnancy rates and embryo survival (Cumming et al. 1975; Parr et al. 1987). In contrast, the consensus of a review of studies examining the role of mid-pregnancy nutrition concludes that a high compared with a low plane of nutrition in mid-pregnancy is beneficial to ovine placental growth, particularly when the dams are in relatively poor body condition at mating (Kelly, 1992). There is a paucity of comparable animal data on the effect of excess nutrients on fetal growth in late pregnancy. However, a recent study reveals that while increasing global nutrient intake (1.55 × maintenance) in mature multiparous ewes between days 115 and 140 of gestation increases maternal and fetal glucose concentrations, it did not alter placental mass, fetal weight or fetal fat mass at autopsy (Muhlhansler et al. 2002). Similarly, a number of human studies carried out in developed countries have failed to establish a beneficial relationship between macronutrient intake and fetoplacental growth (Mathews et al. 1999; Sloan et al. 2001; Kramer, 2002).

Indeed, in adult human subjects a high pre-pregnancy BMI and/or excessive weight gain during pregnancy has variously been associated with a plethora of negative outcomes including fetal macrosomia, stillbirth and premature delivery (Cnattingius et al. 1998; Schieve et al. 2000; Stephansson et al. 2001; Castro & Avina, 2002). Similarly, in our overnourished adolescents, high maternal weight gains and the associated increase in adiposity are associated with higher spontaneous abortion rates and a reduction in gestation length (Wallace et al. 2001). The latter effect has primarily been attributed to accelerated maturation of the fetal hypothalamic–pituitary axis in the nutritionally stressed growth-restricted fetus. However, in the present study, the reduction in gestation length in overnourished dams is independent of growth restriction and gestation length per se is positively correlated with peripheral progesterone concentrations. Maternal dietary intakes and peripheral progesterone concentrations are inversely related (Williams & Cumming, 1982; Wallace et al. 1994) due to an increase in blood flow throughout the gut and the liver of high intake animals (Parr, 1992). Thus, similar nutritionally induced alterations in placental progesterone metabolism may have triggered premature parturition in the overnourished dams in the present study.

As high dietary intakes did not alter fetoplacental growth in the adult ewe, we carried out only a limited analysis of the molecules putatively involved in nutrient partitioning during pregnancy. Maternal insulin concentrations positively reflected maternal dietary intake and were broadly similar to those reported and discussed previously for adolescent ewes on high v. maintenance rations (Wallace et al. 1997a, 1999b). Insulin, acting via its receptor on the adipocyte, is generally accepted as the major stimulator of lipogenesis in the subcutaneous and omental fat of pregnant sheep (Vernon et al. 1981; Guinnet et al. 1991), and the marked increase in insulin secretion and adiposity in the high intake dams is commensurate with this role. However, in contrast to previous adolescent studies, this sustained anabolic stimulus to maternal fat deposition did not occur at the expense of placental growth. Furthermore, we found no evidence of insulin resistance in the overnourished adult dams in that maternal glucose concentrations were maintained at equivalent levels in both groups. The relatively higher NEFA concentrations in the maintenance group, and the gestational increase in NEFA levels in both groups, indicate increased turnover of lipid stores to maintain the maternal glucose pool as pregnancy progresses and fetal demand for glucose increases.
Comparison of the current endocrine profiles (insulin, GH and progesterone) for overnourished v. maintenance-fed adult dams indicates that the relative differences between groups is markedly less than those reported previously for adolescent dams (Wallace et al. 1997a,b, 1999b, 2004b). For example, in the current study, maternal GH concentrations were not significantly different between nutritional groups, while in identically treated adolescent dams GH concentrations are significantly perturbed throughout gestation. As these studies were carried out using the same radio-immunoassay procedures, this indicates that the mature endocrine system of adult ewes is less sensitive to changes in maternal nutrition while in the peripubertal adolescent dams both the somatotropic and sex steroid axes are actively maturing.

The decrease in colostrum yield at parturition in the overnourished dams was in broad agreement with previous observations in adolescent sheep (Wallace et al. 2001) but the relative decrease in yield was only 26% in the present adult study compared with 62% in adolescents overall (Wallace et al. 2001). The observed nutritionally induced alterations in circulating lactogenic hormone concentrations (progesterone, GH) are the most probable cause in the absence of an effect on placental mass per se in the present study. High pre-pregnancy BMI and obesity are similarly associated with a failure to initiate and sustain lactation in many human populations (Rasmussen et al. 2002; Hilson et al. 2004).

In conclusion, adult sheep appear to be relatively insensitive to the oversupply of nutrients during pregnancy and have the ability to meet the nutrient requirements for normal conceptus growth in spite of their increased adiposity. In contrast, the associated endocrine changes have the potential to negatively alter gestation length and initial colostrum production.

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