Competition for nutrients in pregnant adolescents: consequences for maternal, conceptus and offspring endocrine systems.

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Abstract

The competition for nutrients that arises when pregnancy coincides with continuing or incomplete growth in young adolescent girls increases the risk of preterm delivery and low birthweight with negative after-effects for mother and child extending beyond the perinatal period. Sheep paradigms involving nutritional management of weight and adiposity in young biologically immature adolescents have allowed the consequences of differential maternal growth status to be explored. Although nutrient reserves at conception play a modest role, it is the dietary manipulation of the maternal growth trajectory thereafter which has the most negative impact on pregnancy outcome. Overnourishing adolescents to promote rapid maternal growth is particularly detrimental as placental growth, uteroplacental blood-flows and fetal nutrient delivery are perturbed leading to a high incidence of fetal growth-restriction and premature delivery of low birthweight lambs, whereas in undernourished adolescents further maternal growth is prevented and depletion of the maternal body results in a small reduction in birthweight independent of placental size. Maternal and placental endocrine systems are differentially altered in both paradigms with downstream effects on fetal endocrine systems, organ development and body composition. Approaches to reverse these effects have been explored, predominantly targeting placental growth or function. After birth, growth-restricted offspring born to overnourished adolescents and fed to appetite have an altered metabolic phenotype which persists into adulthood whereas offspring of undernourished adolescents are largely unaffected. This body of work using ovine paradigms has public health implications for nutritional advice offered to young adolescents before and during pregnancy, and their offspring thereafter.

Introduction

Adolescent fertility rate as measured by births per 1000 females aged 15-19years has steadily declined in virtually all countries across the globe from 1960 to present day (Worldbank,2016).

Nevertheless, pregnancy during adolescence still accounts for an estimated 11% of all births worldwide with more than 95% of these occurring in developing countries (WHO,2018). The antecedents of early childbearing are diverse and in the developed world include social disadvantage, low educational attainment, dysfunctional family structures, ethnicity and an intergenerational history of early childbirth, as well as a propensity for risky, aggressive and delinquent behaviours (Meade *et al.*2008; Gaudie *et al.*2010). In contrast, in developing countries, early marriage and childbearing are often the cultural norm particularly in communities where access to education for girls is limited and the supporting health infrastructure is weak (*Das et al.*2017). Irrespective of geographical location there is commonality in the hazards associated with early childbearing, and systematic reviews, multi-country surveys and population-based studies consistently report a higher risk of premature delivery, low birthweight, and neonatal morbidity and mortality in adolescent pregnancies (Salihu *et al.*2006; Gibbs *et al.*2012; de Azevedo *et al.*2012; Malabarey *et al.*2012; Kozuki *et al.*2013; Ganchimeg *et al.*2014). Relative to adult women, pregnancy at any age during adolescence is associated with a greater possibility of experiencing anaemia, eclampsia, puerperal endometritis and systemic infections while heightened risk of preterm delivery, low birthweight and neonatal mortality is most pronounced in very young girls (≤15years of age and/or within 2years of first menses) who are biologically immature (Ganchimeg *et al.*2014; Weng *et al.*2014; Torvie *et al.*2015; Neal *et al.*2018). A short cervix, small uterine volume and immature pelvis leading to cephalopelvic disproportion are more common in younger mothers and may predispose them to early delivery and complications such as obstetric fistula and other maternal near-miss events (Moerman,1982; Stevens-Simon *et al.*2000; Gadelha Da Costa *et al.*2004; Tebeu *et al.*2012; Ganchimeg *et al.*2014; Oliveira *et al.*2014). Maternal mortality is thankfully rare, but in low resource settings the adjusted risk of mortality is four-times higher for very young mothers compared with both older adolescents and adult women (Conde-Agudelo *et al.*2005).

Maternal-fetal competition for nutrients

Greater prevalence of adverse perinatal outcomes in very young adolescent mothers may additionally reflect that a significant proportion are either still-growing or have the potential to grow at the time of conception setting up a maternal-fetal competition for nutrients. Although the peak in growth velocity characteristic of adolescence is reached before menarche, girls carry on growing thereafter and on average gain a further 7cm in height before linear growth ceases (Roche & Davila,1972). In the USA-based Camden Adolescent Pregnancy and Nutrition Project, approximately 50% of young adolescents (≤16years) continued to grow as indicated by increases in knee-height over a 6-month period from mid-pregnancy to 4-6weeks post-partum. This drive to maternal tissue growth as reflected by higher gestational weight-gains, increased fat stores and greater post-partum weight-retention was associated with a three-fold higher risk of small-for-gestational-age birth and lower average birthweights than in both non-growing adolescents of equivalent age and mature women (Scholl *et al.*1994,1997). A similar alteration in nutritional priorities leading to smaller babies has been observed in young (<15years) 'still-growing' Peruvian girls: in this instance adolescent growth was defined as continuing or complete based on their height at delivery relative to parental height (Frisancho *et al.*1985). When pregnant adolescents are undernourished it is likely that both mother and fetus are compromised and in partial support there is evidence from rural Bangladesh that pregnant adolescents (average age 16.3years) cease linear growth and deplete their fat stores compared with non-pregnant adolescents of equivalent gynaecological age (Rah *et al.*2008). Perinatal outcomes were not reported but the effects on maternal growth were most pronounced in the girls who became pregnant at an earlier age. In contrast in a UK-based study involving older adolescents (average age 17.8years), continuing maternal growth measured as a change in knee-height of >2mm between 13 and 29 weeks gestation did not limit fetal growth relative to non-growing adolescents but in this instance both macronutrient and micronutrients intakes generally exceeded recommended levels (Jones *et*

97 *al.*2010). Together these studies suggest that the competition for nutrients that arises between
98 the maternal body and gravid uterus when pregnancy coincides with adolescence is specific to
99 very young girls and is likely to be moderated by maternal nutritional status and dietary-intake.

100 **Consequences for mother and child beyond the perinatal period**

101 The negative impact of adolescent pregnancy for mother and infant extends beyond the perinatal
102 period. For adolescent mothers themselves longer-term health outcomes include poorer mental
103 health scores and a two-fold higher rate of suicide (Webb *et al.*2011; Aitken *et al*.2016), more
104 premature deaths due to cervical and lung cancer (Otterblad-Olausson *et al.*2004; Webb *et*
105 *al.*2011), and a three-fold higher risk of diabetes-related mortality (Vandenheede *et al.*2012). A
106 young age at first birth is also associated with a higher prevalence of hypertension and
107 osteoporosis in postmenopausal women (Cho *et al.*2012; Park *et al.*2016), and with poor physical
108 performance and greater risk-scores for cardiovascular disease in later life (Pirkle *et al.*2014;
109 Rosendaal *et al.*2017). In contrast women with an early first birth have a reduced risk of breast
110 cancer and the earlier the pregnancy the lower the risk (Kelsey & Bernstein,1996). While some of
111 these relationships likely reflect the complex psychosocial needs and life choices of women who
112 experience early childbirth, others could have a physiological basis originating close to the initial
113 pregnancy. For example, women who give birth during adolescence in the developed world are
114 prone to greater weight-retention and adiposity than adolescents who did not experience
115 pregnancy and older pregnant women, and this may influence their metabolic health in later life
116 (Gunderson *et al.*2009; Thame *et al.*2010).

117 For the offspring of adolescent mothers, Demographic Health Surveys reveal evidence of low
118 height or stunting in infancy in 9 of 18 developing countries studied (Africa, Asia and Latin
119 America; Yu *et al.*2016) and similarly pooled data from five birth cohorts (Brazil, Guatemala, India,
120 The Philippines and South Africa, n~13,000; Fall *et al.*2015) reveals a 46% higher risk of stunting at

2years of age and a greater likelihood of offspring failing to complete their secondary education. The latter study also found a relationship between young maternal age and elevated offspring fasting glucose concentrations in early adulthood (n~10,000) independent of any difference in body composition or blood pressure. In high income countries an association between young maternal age (particularly ≤15years) and greater offspring developmental vulnerability aged 5 as assessed by school teachers has been reported (n=99,950; Falster *et al.*2018) and in a smaller study (n=2643), offspring born to adolescent mothers had a lower IQ score and a higher risk of low IQ at 21years of age (Khatun *et al.*2017). Furthermore, a nation-wide evaluation in Denmark (n= 1,793,681) has linked early childbearing (12-19years) with a greater risk of criminality, substance abuse and attempted suicide in offspring aged between 15 and 40years (Mok *et al.*2016). Depending on the context, each of the aforementioned studies adjusts the data to some degree for potential confounders, e.g. maternal smoking, child feeding practice, educational attainment, but residual confounding is likely to be an issue as it is unlikely that all the socioeconomic, environmental and biological/genetic influences that impact a young mother and her offspring from the peri-conception period forwards are sufficiently well documented. Animal models are of value in that specific known confounders can be controlled or removed, allowing researchers to better study the exposure of interest. This was the rationale behind the development of a sheep paradigm to explore the competition for nutrients when pregnancy coincides with continuing or incomplete growth of the young adolescent mother.

Adolescent sheep paradigms: approach

Sheep producers generally avoid breeding young females during adolescence because relative to adults they have an inferior reproductive performance characterised by a variable onset of puberty and short first breeding season, low ovulation rate, failure to be mated, fertilization failure and high embryo loss (Beck *et al.*1996; Kenyon *et al.*2014; Edwards *et al.*2016). The approach developed at the Rowett Institute bypasses many of these issues by using assisted

conception procedures to synchronise breeding and establish singleton pregnancies in peripubertal adolescent ewes of equivalent age (~7.5months), and standardized live-weight and adiposity at conception. Adult ewes of known reproductive history act as embryo donors, thus avoiding the inherently low viability of embryos arising from adolescents themselves (Quirke & Hanrahan,1977; McMillan & McDonald,1985; Morton,2008). Donor ewes in optimal body condition for breeding are superovulated using exogenous hormones, and semen from a single sire is deposited directly into the uterus to ensure fertilisation. Within each study high quality embryos harvested from any given embryo donor are then distributed evenly across the adolescent recipient study groups: this approach minimises the impact of the main peri-conceptual factors known to influence feto-placental growth and maximises the genetic homogeneity of the resulting fetuses (Wallace *et al.*1996).

Nutritional treatments normally begin immediately after embryo transfer and involve presenting the young still-growing adolescent recipient with different quantities of a nutritionally complete diet to manipulate gestational weight-gain and thus her growth and body composition. In the *overnourished adolescent* model, this involves offering a high dietary-intake throughout gestation (*ad libitum* intake, ~2 x maintenance requirements) to promote rapid maternal growth and is designed to mirror pregnancy in very young but relatively well nourished adolescent girls who continue to grow substantially while pregnant. By comparison in the *undernourished model*, the adolescent dams are prevented from growing further while pregnant (low-intake, ~0.7 x maintenance), mimicking the situation in very young and poorly nourished adolescents who prematurely cease linear growth during pregnancy. For both models the control group involves a moderate dietary-intake calculated to maintain maternal adiposity at a constant level throughout gestation (maintenance). This facilitates a small degree of maternal growth ensuring that the nutrient requirements for optimum conceptus growth are met: this is achieved by modest step-wise increases in maternal dietary-intake during the final-third of pregnancy.

Pregnancy outcome in rapidly growing adolescent sheep

The nutritional manipulation of gestational weight-gain in young adolescent dams to prioritise their own growth leads to adverse outcomes, consistent across multiple studies (Table 1). These include an increased incidence of late miscarriage or stillbirth, reduced placental growth, and a shorter gestation length prior to spontaneous delivery of lambs that are on average 30% lighter than those born to optimally-fed controls (Wallace *et al.*2004a). The extent of fetal compromise within individual studies is variable and closely relates to the degree of placental growth-restriction observed. Live-born fetuses are categorised as markedly growth-restricted if birthweight is more than two standard deviations below the mean sex-specific birthweight of fetuses in the control group. A summary analysis of contemporary trials revealed that almost half of overnourished pregnancies were in this category, and average placental and fetal weight was reduced relative to controls by ~ 45% (Wallace,2016). The remaining pregnancies were much less perturbed but average placental and fetal weights were still statistically lower than in controls (Figure 1a,b). Although male conceptuses were larger than females, both sexes were similarly perturbed by maternal nutrition. Within the overnourished groups maternal anthropometry reveals a greater weight-gain and increase in adiposity during the first-third of gestation in pregnancies which result in marked feto-placental growth-restriction versus those which are less perturbed (Figure 1d). This competition for nutrients between mother and fetus is independent of dietary protein content (Wallace *et al.*2006b) and exclusive to the young adolescent in that it does not occur in identically treated primiparous adult ewes of the same genotype (Wallace *et al.*2005a).

One of the most predictable features of pregnancy outcome in overnourished adolescents is a reduction in gestation length with viable lambs being born as early as day135 (term=145days in controls). Within overnourished adolescents, gestation length is unrelated to maternal growth or weight-gain at any stage of pregnancy and the difference in gestation length for growth-restricted

versus less perturbed fetuses of both sexes is less than one day (Figure 1c). This implies that it is primarily high dietary-intakes that underlie premature delivery and a rapid labour relative to controls. Lambs tolerate premature delivery badly irrespective of size, but it is the smallest individuals that face the most significant challenges in the neonatal period. Colostrum yield at parturition reflects placental mass (Wallace *et al.*1996, 2001), and is attenuated in overnourished dams (Wallace,2016). The lambs are generally vigorous at birth, and display normal teat seeking behaviour, but the mothering abilities of the adolescent dams are often poor, and the extremely low colostrum availability delays the formation of an appropriate ewe-lamb bond, and severely limits the supply of essential immunoglobulins and nutrients, making the lambs vulnerable to hypothermia and infection (Wallace *et al.*2006b). In the first study with this model, neonatal mortality rate was 62% (Wallace *et al.*1996), but subsequently a pre-emptive neonatal care protocol involving intensive monitoring, supplementary feeding and prophylactic antibiotic usage, irrespective of size at birth, ensured that most lambs survived.

Pregnancy outcome in undernourished adolescent sheep

Relative to the *overnourished model*, the perinatal outcomes associated with undernourishing pregnant adolescents are much less severe: placental size and gestation length are comparable with the control group and no fetal or neonatal deaths have been observed (Table 1). Preventing maternal growth by holding body weight at peri-conception levels progressively depletes maternal fat reserves and directly limits nutrient availability in the maternal and fetal circulations. This leads to a slowing of fetal soft tissue growth and by late gestation, and following spontaneous delivery at term, the fetus is 10-17% smaller than controls (Luther *et al.*2007a, b; Wallace *et al.*2012). Thus, while undernourishing adolescent mothers has a modest effect on fetal growth, it is the *overnourished model* which most closely mimics the human with respect to adverse perinatal outcomes.

Two other laboratories have tried to replicate the effects of differentially feeding adolescent sheep, with variable success. Both groups have used natural mating following a synchronised oestrus, but the timing and extent of nutritional manipulation and thereby maternal growth rate differed. Swanson and colleagues (2008) report a shorter gestation length and a small overall reduction in birthweight (9%) when adolescents carrying singletons or twins were overnourished beginning on day50 of gestation, whereas when rations were restricted, gestation length was unperturbed and birthweight reduced by 13% relative to controls. In contrast, Peel and colleagues (2012) exposed singleton bearing adolescents to *ad libitum* intakes throughout pregnancy in two identical trials, and, in the trial where maternal weight and adiposity diverged earlier and to a greater extent, gestation length was reduced by 5days independent of an effect on lamb birthweight. Placental size was either unaffected (Swanson *et al.*2008) or not reported (Peel *et al.*2012). It is accepted that the use of assisted conception procedures may influence how the early embryo responds to differences in maternal nutritional status or diet in the Rowett model, but it is important to emphasise that maternal nutrition is the only variable that is manipulated with all other aspects controlled. Moreover, unlike human IVF/embryo transfer, the animals used have no known reproductive defects at baseline in that they respond appropriately to oestrus synchronisation and ovulation induction and conceive in high numbers following embryo transfer. Thus, it is the ability to achieve rapid maternal growth rates in early pregnancy that is most likely the root cause of placental growth-restriction and the adverse perinatal events described. Uterine immaturity is proposed as a primary driver of the placental dysfunction underlying preeclampsia, fetal growth-restriction and preterm delivery in young human adolescents (Brosens et al.2017) and the suggestion that preconditioning of the immature uterus by exposure to regular ovulatory menstrual cycles is required to prepare for appropriate trophoblast invasion has considerable merit bearing in mind that the studies reported here involve peripubertal animals.

Maternal weight and adiposity at conception and pregnancy outcome

In above-mentioned studies the emphasis was to manipulate gestational weight-gain and growth status immediately after embryo transfer and accordingly the adolescent recipients were deliberately of equivalent weight and adiposity level at conception. It is well established that pre-pregnancy underweight is associated with an increased risk of preterm delivery and low birthweight in adult women relative to those with normal body mass index (BMI, Han *et al.*2011). Similarly, although human adolescents enter pregnancy from a range of nutritional backgrounds, the majority in the developing world are likely to be underweight with low nutrient reserves at conception, and in very young girls this may interact with their gestational growth status to influence pregnancy outcome. This scenario has been modelled in two studies using different approaches: both involved selecting two groups of adolescent sheep of the same age but with a marked difference in weight and adiposity prior to embryo transfer. In the first study adolescents were subsequently overnourished, undernourished or fed a control-intake to drive maternal growth and gestational weight-gain in contrasting directions as described above (Wallace *et al.*2010), whereas in the second study the nutritional status of the embryo donor(s) was additionally manipulated (obese versus control), all these recipients were then overnourished throughout gestation and a contemporaneous control group was included as a reference point for optimal fetal growth (Wallace *et al.*2017). Together the design of these studies uniquely allows segregation of pre-, peri and post-conception nutritional exposures to evaluate their separate and/or interdependent influences. Donor ewe obesity did not influence conception rate or the feto-placental growth trajectory of high quality embryos following transfer into adolescent recipients, and irrespective of donor ewe nutrition and gestational intake, adolescents that were relatively light and thin at conception gave birth to lambs that were smaller than those born to adolescents who were relatively heavy and fat at conception (555g lighter in study 1 and 665g in study 2, $P<0.05$). This reduction in birthweight was mirrored by corresponding reductions in placental size but was independent of gestation length. The differences in estimated body fat at

the point of embryo transfer were relatively small but irrespective the thin adolescent dams had high lipid levels indicative of active catabolism, and low circulating nutrient and metabolic hormone concentrations which are likely to have directly impacted the early uterine environment contributing to compromised conceptus development. Accordingly, the incidence of marked fetal growth-restriction was two-fold higher in recipients that were thin versus fat at conception when all were subsequently overnourished (Wallace *et al.*2017). This clearly indicates that maternal nutritional status at the time of conception is an important consideration in predicting pregnancy outcome in still-growing adolescents, but comparison with the control groups in both studies unequivocally demonstrates that it is a high nutritional-intake to drive continued maternal growth and adiposity after conception which is most closely linked to the high incidence of fetal growth-restriction in young sheep (Wallace *et al.*2010, 2017). Similarly, a recent study of human adolescents (n=600) reported that gestational weight gains above the recommended levels for individual pre-pregnancy BMI categories, were associated with an increased risk of low birthweight (Sàmano *et al.*2018), but whether the girls (mean age 16years) were still growing was not assessed. This increased risk of low birthweight is unique to adolescent pregnancies as gestational weight gain above recommendations in adult women reduces the risk of SGA-birth (Goldstein *et al.*2017).

Consequences for maternal and placental endocrine systems

Overnourished model: The consequence of different dietary intakes for maternal hormone and nutrient status in adolescent sheep has been extensively described. Relative to controls, and consistent with the oversupply of nutrients, the overnourished dams have elevated peripheral insulin, insulin-like growth factor 1 (IGF-1), urea and glucose concentrations from ~ day 25-28 of gestation facilitating an early and sustained stimulus to maternal tissue growth at a time when the nutrient requirements of the fetus are negligible (Wallace *et al.*1997b; 1999; 2010). Accordingly, maternal weight-gains are rapid, and body-weight specific peri-renal fat depots and peripheral

leptin concentrations are enhanced by day50 of gestation (Thomas *et al.*2001; Redmer *et al.*2009), with the proportion of fat in the maternal carcass *per se* increasing between mid and late gestation (Wallace *et al.*2004a). In adult sheep, maternal insulin concentrations normally decline during the final third of gestation to promote increased fatty acid mobilization from adipose stores and to reduce glucose utilization by non-uterine tissues (McNeill *et al.*1997). However high insulin concentrations are maintained throughout in adolescent mothers and metabolic challenges at ~day97 of gestation reveal enhanced insulin insensitivity, namely greater glucose area-under-the-curve post insulin challenge, and higher glucose-stimulated insulin post glucose challenge. The high circulating glucose should in theory favour fetal growth but the reduction in placental size in these animals is a major constraint to fetal nutrient supply (see below). Indeed, reduced placental size and the attendant decrease in placental hormone secretion may compromise several of the maternal adaptations that normally underlie successful pregnancy and lactation (Napso *et al.*2018), thereby contributing to the poor perinatal outcomes characteristic of these rapidly growing adolescents. Notably, the overnourished adolescent dams are characterised by attenuated placental lactogen (also known as chorionic somatomammotropin hormone, CSH) – relative to controls, the detection of the hormone in the maternal circulation is delayed, and concentrations are reduced throughout the second two-thirds of pregnancy (Lea *et al.*2007). The generation of CSH-deficient sheep pregnancies by lentiviral-mediated RNA interference *in vivo* has recently provided convincing evidence of a causative role for this hormone in conceptus growth. Major reductions in placental and fetal weights were evident in late gestation corresponding to the decrease in CSH mRNA and protein (Baker *et al.*2016), and an equivalent study terminated at day50 revealed that the growth of the fetus was perturbed from early in pregnancy, possibly due to deficits in the paracrine actions of the hormone within the placenta (Jeckel *et al.*2018). In addition to low CSH concentrations, overnourished dams are typified by low placental reproductive steroid concentrations. This may reflect increased metabolic (hepatic) clearance due

to the high dietary-intakes or reduced placental steroid capacity, and there is evidence to support both scenarios (Lea *et al.*2007; Redmer *et al.*2012). Irrespective of the cause, progesterone and oestradiol-17ß levels are reduced relative to controls early in pregnancy, and remain attenuated thereafter (Wallace *et al.*1997a, 2003b, 2008a). Together low concentrations of these three lactogenic hormones, as well as reduced growth hormone concentrations (Wallace *et al.*1997b) are likely to underlie the impaired early lactation robustly observed in overnourished adolescents. Moreover, the attenuated concentrations of reproductive steroids, and an early decline in progesterone particularly (Taylor,1987), most likely triggers premature and rapid delivery in these pregnancies, while the deficit in circulating oestradiol is likely to be the origin of the poor mothering abilities (Dywer,2014) regularly observed in the adolescent dams that give birth to the most growth-perturbed lambs.

In human pregnancies, failure of maternal plasma volume expansion and altered iron homeostasis are implicated in several adverse outcomes (Vricella,2017) and although a causative link has not been established, low placental progesterone and oestradiol concentrations have been associated with the reduced maternal plasma volume expansion observed in normotensive women with idiopathic fetal growth-restriction (Salas *et al.*1993, 2006). Similarly, deficits in these placental steroids may impact the renin-angiotensin-aldosterone system (Scaife & Mohaupt,2017) and hence salt and water retention in our animal model of fetal-growth restriction. Although this has not been directly examined, cross-sectional studies reveal that rapid growth during the first-half of pregnancy is linked to increased utilisation and hence early depletion of maternal liver iron stores, and a failure of normal blood volume expansion between mid and late pregnancy (Luther et al. 2010). The accompanying increase in haematocrit, haemoglobin and plasma protein concentrations is associated with an increase in blood viscosity by late gestation (Wallace et al. 2017) and this in turn may influence uteroplacental blood flow and fetal nutrient supply.

Plasma tri-iodothyronine and thyroxine concentrations mirror maternal nutrient-intake across a range of diet levels during pregnancy and around parturition (Lemley *et al.*2014) but do not significantly diverge in overnourished compared with control adolescents until the final-third of gestation (Wallace *et al.*1997b). This suggests that these hormones are unlikely to play a causative role in placental growth restriction although two independent human studies suggest that elevated maternal thyroid hormones negatively impact fetal growth (Leon *et al.*2015), and associate with the risk of miscarriage and fetal distress (Yang *et al.*2018). Higher plasma thyroid levels in overnourished dams may however influence colostrum IgG supply to the newborn as others have shown that supplementing a basal sheep diet with excess iodine for 4 weeks prior to parturition reduces serum IgG concentration in the neonate (McGovern *et al.*2016). Maternal plasma prolactin similarly reflects dietary-intake level (Lemley *et al.*2014) predominately in the final-third of pregnancy when circulating concentrations are elevated in overnourished dams and negatively correlated with fetal weight (Matsuzaki *et al.*2006). The significance of high maternal prolactin concentrations, if any, is unknown but aligns with similar observations in different models of fetal growth-restriction including hyperthermic sheep (Bell *et al.*1987; Regnault *et al.*1999) and protein-restricted rats (Fernandez-Twinn *et al.*2003). A degree of physiological stress is arguably common to these models and could theoretically impinge on the hypothalamic-pituitary axis promoting prolactin secretion, but intriguingly maternal cortisol concentrations were either equivalent or reduced relative to controls in all these animal models (Bell *et al.*1987; Wallace *et al.*2000, 2005; Fernandez-Twinn *et al.*2003).

Undernourished model: The maternal endocrine profiles and nutrient status of undernourished adolescent dams have been less rigorously studied but relative to optimally-fed controls are typified by lower peripheral insulin, IGF-1 and leptin concentrations, and similar cortisol levels (Luther *et al.*2007a, Wallace *et al.*2010). In contrast, decreased metabolic (hepatic) clearance rates in undernourished dams are thought to underlie the elevated concentrations of placental

reproductive steroids observed during the final third of pregnancy independent of any change in placental size (Luther *et al.*2007b). By late pregnancy maternal glucose, urea and specific amino-acid concentrations are reduced, and greater NEFA concentrations reflect dwindling internal adipose and carcass fat stores. Although maternal liver iron stores are independent of dietary treatment, a low haematocrit and haemoglobin is consistent with mild anaemia by this stage and collectively low availability of nutrients in the maternal circulation is the principal cause of the modest reduction in fetal growth observed. This is in stark contrast to overnourished pregnancies where regardless of an oversupply of nutrients in the maternal circulation, fetal growth is directly constrained by impaired placental development and function.

Consequences for placental development and uteroplacental blood flows: *overnourished model*

The competition for nutrients in the overnourished and rapidly growing adolescent dams influences placental development from early in gestation. At day50, cellular proliferation rates within both placental compartments are reduced (Rensick *et al.*2008), and capillary vessel size within the fetal cotyledon is compromised (Redmer *et al.*2009), while delayed and reduced appearance of placental lactogen and pregnancy specific protein-B concentrations in the maternal circulation suggests impaired trophoblast cell migration (Wallace *et al.*1997a; Lea *et al.*2007). By mid-pregnancy, angiogenic growth factor ligand and receptor mRNA expression, as well as markers of proliferation and apoptosis within the placenta are perturbed (Redmer *et al.*2005; Lea *et al.*2005). These adaptations precede the change in placental mass which does not significantly diverge from controls until 0.72 gestation (Wallace,2011), but nonetheless they are commensurate with an altered developmental and haemodynamic trajectory. Accordingly, uterine blood flow was attenuated by ~40%, and umbilical artery Doppler indices were greater in overnourished pregnancies at mid- gestation, and predictive of reduced fetal growth later in pregnancy (Wallace *et al.*2008b; Carr *et al.*2012). The latter is in line with observations at ~32weeks gestation in growing versus non-growing human adolescents (Scholl *et al.*1997). By ~

day133 of the ovine pregnancies, placental mass was ~ 45% lower than in controls and proportionate reductions in uterine and umbilical blood flows, uteroplacental glucose and oxygen consumption and lactate production, as well as placental glucose transport, were observed (Wallace *et al.*2002, 2003a).

Potential for manipulating placental function in overnourished adolescents

As restricted placental development is central to the pathology of fetal growth-restriction in this model, a few approaches have attempted to manipulate the placental growth trajectory to enhance fetal growth. The first of these simply involved switching dietary-intakes during specific windows of gestation and showed that normal placental development and fetal growth could be achieved by reducing maternal intakes from a high to a control level at day50 of gestation, whereas an abrupt increase in dietary-intake at this stage reduced placental and fetal growth to the same degree as in continuously overnourished dams (Wallace *et al.*1999). However, fetal growth could not be rescued by radically reducing dietary-intake and switching the mothers from a highly anabolic to a catabolic state during the final-third of pregnancy, despite changes in placental angiogenic growth factor and receptor gene expression consistent with blood vessel re-modelling (Redmer *et al.*2012). Together these studies highlight that the placenta is most sensitive to nutrition during its main proliferative growth phase. While fetal growth cannot be recovered once placental mass is reduced beyond its functional capacity, there is evidence of differential placental vascular adaptations in the late gestation placenta of continuously overnourished adolescent's dependent on the degree of fetal growth-restriction observed (Carr *et al.*2016a). The second approach to manipulating the placental growth trajectory of overnourished dams has involved supplementing maternal hormones. Progesterone supplementation of overnourished dams from day5 to 55 of gestation restored circulating hormone levels to control levels and increased lamb birthweight by 30% but this change was independent of a corresponding change in placental size at delivery, suggesting a direct effect of progesterone on the embryonic inner cell

mass (Wallace *et al.*2003b). Similarly, oestrogen replacement from day50-90 of gestation failed to impact feto-placental growth or placental vascularity as assessed in late gestation (Yunusova *et al.*2011). In contrast, when adolescent mothers received exogenous growth hormone (GH) during the main placental growth phase (day35-80), their accretion of adipose tissue was reduced, and in GH-treated overnourished dams this alteration in nutrient partitioning priorities favored uteroplacental and fetal growth at study end (day81, Wallace *et al.*2004b). In a second study, GH-treatment of overnourished dams targeted either the placental growth phase (day35 to 65) or later in pregnancy when placental growth is complete, and the nutrient demands of the fetus are high (day95 to 125). Both exposures had a profound influence on maternal metabolism resulting in insulin resistance, reduced lipogenesis and three-times higher circulating glucose concentrations. However, in this study, GH-treatment during early pregnancy did not have a sustained effect on placental or fetal size, whereas treatment during late pregnancy increased fetal weight by ~25% and had a large impact on fetal adiposity as measured at day130 of pregnancy, independent of placental size (Wallace *et al.*2006a). On balance GH supplementation has dramatic effects on maternal endocrinology and the partitioning of nutrients within the maternal body but the effects on the fetus reflect an abnormally high transplacental glucose supply rather than a modified placental growth trajectory. Furthermore, while increased fetal lipid deposition and the associated increase in hepatic glycogen stores could be an important energy source and protect the newborn from hypothermia in the neonatal period, it is likely to be maladaptive in the longer term. More worryingly, a similar GH-treatment regime in late pregnancy in a different model of fetal growth-restriction involving placental embolization was associated with hydanencephalic brain lesions in 3 of 5 fetuses (De Boo *et al.*2008). Recently an alternative approach directly targeting the uteroplacental circulation of compromised adolescent pregnancies has been evaluated. This was based on the premise that local uterine artery adenovirus (Ad.)-mediated overexpression of vascular endothelial growth factor (VEGF) in overnourished dams would increase uterine blood

flow as demonstrated in normally developing adult pregnancies (Mehta *et al.*2011). In two separate studies Ad.VEGF administration in mid-pregnancy (day89) increased fetal growth velocity as measured by ultrasound 3 and 4 weeks after injection. At 0.9 gestation there were fewer markedly growth-restricted fetuses (Carr *et al.*2014) and at term average birthweight was increased by 20% (Carr *et al.*2016b). Importantly there were no adverse responses to the gene therapy and lambs continued to thrive and exhibit appropriate growth and body composition for their size in the early postnatal period.

Consequences for placental development and uteroplacental blood flows: *undernourished model*

Final placental size is unperturbed in undernourished adolescents but the relatively low nutrient availability in these mothers does significantly impact on vascular development within the placenta. Thus a 20% reduction in capillary area density within the maternal caruncle was observed at both mid and late gestation (Luther *et al.*2007b), and closely mirrored an average reduction in uterine blood flow of 22% measured between these two stages using perivascular flow probes (Wallace,2016). These haemodynamic differences between undernourished and control groups are likely to contribute to low fetal nutrient supply but the increasingly hypoglycaemic conditions in undernourished dams are still considered the major limitation to fetal growth in these pregnancies. In support the reduction in capillary area density persisted when underfed mothers were switched to control-intakes between mid and late gestation, but despite this fetal weight was partially restored in direct response to re-alimentation by study end (Luther *et al.*2007b).

Consequences for fetal and offspring endocrine systems: *overnourished model*

Serial ultrasonography of multiple indices of prenatal size reveals reduced growth velocity from around day100 of gestation forwards in fetuses of overnourished dams (Carr *et al.*2012).

Regression analysis using natural logarithms of data obtained from fetuses necropsied at day130 of gestation demonstrates that individual organ weights, including those of the main endocrine organs (namely pituitary, thyroid, adrenal glands, gonads and pancreas) were predicted by the weight of the fetus (P=0.001 or less), rather than the dam's nutritional treatment. The allometric plots of the fetal brain compared with the liver, and the lungs compared with the kidneys, are illustrated and serve to emphasis the extent of brain sparing in fetuses of overnourished dams (Figure 2a, b). The latter fetuses were further categorised as markedly growth-restricted when their bodyweight was more than two standard deviations below the mean sex-specific weight of fetuses in the control group. This approach revealed that, in addition to the brain, the relative pituitary, kidney, and adrenal gland weights were higher in growth-restricted compared with non-perturbed and control groups (Figure 2 c-f).

Ovine paradigms permit access to the placental and fetal circulations to examine fetal endocrine and nutrient status, nutrient uptakes and fetal metabolism. Accordingly, by late gestation and relative to normally growing controls, the growth-restricted fetuses of overnourished dams are hypoglycaemic, mildly hypoxic, have low insulin and IGF-1 concentrations, and high lactate levels. Absolute umbilical (fetal) uptakes of glucose, oxygen and amino acids are reduced but are normal when expressed on a fetal weight-specific basis (Wallace *et al.*2002, 2003b). Similarly, when the fetal sensitivity to insulin and glucose was examined during fetal hyperinsulinaemic-euglycaemic and hyperglycaemic-euinsulinaemic clamps, normal body weight-specific responses to short-term experimental increases in insulin and/or glucose were observed (Wallace *et al.*2007). These maintained mechanisms of insulin action and glucose uptake/utilisation capacity allow the fetus to protect essential metabolic functions while growth velocity slows. Surprisingly, these growth-restricted fetuses have a comparatively fat phenotype prenatally in that bodyweight-specific perirenal fat mass and carcass fat content is modestly increased compared to controls by late gestation (Matsuzaki *et al.*2006), and plasma cholesterol and LDL concentrations are higher at

493 birth (Wallace *et al.*2012). Greater adiposity in these otherwise growth-restricted fetuses may be
494 caused by exposure to higher maternal and thus fetal glucose early in gestation in overnourished
495 dams (Redmer *et al.*2009) influencing adipocyte development before placental size and hence
496 fetal glucose supply is impaired. Definitive causation in the overnourished model is lacking, but in
497 the undernourished model key genes involved in adipocyte proliferation and function are
498 expressed in fetal perirenal fat tissue at mid-gestation when they are down-regulated by maternal
499 undernutrition and the associated low glucose supply leading to reduced fetal adiposity by late
500 gestation (Wallace *et al.*2015). Glucose is the main fetal fuel and it is notable also that
501 anorexigenic neuropeptide expression in the fetal hypothalamus is sensitive to fetal
502 hyperglycaemia at mid-gestation with effects persisting throughout fetal life (Adam *et al.*2008,
503 2011). Once released from the nutritional constraint imposed by the *in-utero* environment,
504 growth-restricted lambs born to overnourished adolescents display rapid fractional growth rates
505 relative to their size at birth. Growth is particularly rapid during the suckling period and the extent
506 of growth compensation at the point of weaning (11weeks of age) depends on the degree of
507 prenatal growth impairment. Thus, when average birthweight is reduced by a modest 22% relative
508 to controls, complete catch-up in terms of weight is observed by weaning (Wallace *et al.*2010,
509 2012), but when birthweight is ~40% lower, the lambs remain lighter at weaning, and continue to
510 have modestly reduced weight and stature at study end in mid-adulthood despite being fed to
511 appetite (109weeks, Wallace *et al.*2018). In the latter study, serial dual-energy-X-ray
512 absorptiometry (DEXA) revealed that prenatally growth-restricted lambs had lower bone mineral
513 density than controls throughout the life-course (11, 41, 64 and 107 weeks). The small fat
514 phenotype observed in growth-restricted fetuses in late gestation was also apparent in both sexes
515 at weaning but not at the adolescent or early adult stages when lean tissue growth was the
516 dominant nutrient partitioning priority. The metabolic phenotype of these lambs has also been
517 serially documented: during the suckling period and into adolescent life, fasting insulin

concentrations and insulin secretion after glucose challenge are greater in growth-restricted offspring, in line with their higher fractional growth rates and increased bodyfat percentage at weaning. This enhanced insulin sensitivity does not persist but the prenatally growth-restricted offspring of overnourished dams are consistently characterised across studies by higher fasting glucose concentrations and/or greater glucose area-under-the-curve after glucose challenge, indicative of glucose intolerance (Wallace *et al.*2012, 2014a, 2018). During the suckling period in offspring of overnourished dams, relatively higher fasting glucose concentrations in prenatally growth-restricted versus non-perturbed lambs, reflect an inverse relationship between birthweight and hepatic mRNA expression and activity of a key gluconeogenic enzyme, Glucose 6-phosphatase (Figure 3). In the life-course study, altered glucose metabolism was evident at all ages and by the time mature body size had been reached in mid-adulthood the animals had experienced a prolonged period of glucose intolerance and the associated alteration in tissue glucose uptake and hence an obese phenotype was once again evident, particularly in females that reach peak bone mass and adult size before their male counterparts (Wallace *et al.*2018). Further there is evidence that the metabolic forerunner of this adverse phenotype, namely glucose intolerance at ~12months of age, can be ameliorated by restricting nutrient-intake between adolescent and young adult life, an effect specific to females (Wallace *et al.*2012). Thus, when considering any potential target of developmental programming it is important to differentiate between the sexes when possible. For example, differences in adipose tissue development are evident in fetal life with normally growing females having a greater carcass fat percentage at mid-pregnancy, and a higher bodyweight-specific perirenal fat mass and leptin gene expression in late pregnancy than males (Figure 4). This early dissimilarity in adiposity is likely to reflect differences in sex steroid status and is maintained postnatally: independent of birthweight at 11weeks of age, females are characterised by greater visceral fat mass, leptin gene expression, adipocyte size and carcass fat while males exhibit faster growth rates in line with reduced hepatic

IGF-1 DNA methylation, higher IGF-1 mRNA expression and greater plasma IGF-1 concentrations (Wallace *et al.*2014a, b; Carr *et al.*2015). The early divergence in growth and body composition is also reflected in the brain centres involved in energy balance with females having higher hypothalamic expression of anorexigenic genes and lower expression of orexigenic genes than males (Adam *et al.*2013). Furthermore, serial DEXA measurements suggest that sex differences in adiposity (females>males) and bone mineral density (males>females) are life-long (Wallace *et al.*2018).

Theoretically, altered hypothalamic-pituitary-adrenal (HPA) axis function may underlie and contribute to the adverse metabolic phenotype of growth-restricted offspring. The greater bodyweight specific adrenal gland mass (Figure 2 f) and premature activation of the fetal HPA axis leading to early delivery (Figure 1 c) in the most severely growth-restricted pregnancies are arguably commensurate with enhanced *in utero* stress although no evidence of altered cortisol concentrations in mother or fetus was found, albeit measured at a single timepoint in late gestation (Wallace *et al.*2000). Similarly, when stress tests involving corticotropin-releasing hormone plus arginine vasopressin challenge were performed in growth-restricted versus normally-grown females at three ages up to 24months (40% birthweight differential), or in growth-restricted or normal offspring of both sexes at 6months of age (20% birthweight differential), baseline and stimulated adrenocorticotrophin and cortisol concentrations were independent of maternal nutrition and birthweight category (Wallace *et al.*2011). On this basis nutritionally-programmed alterations in the development and function of the HPA axis are unlikely to be central to the phenotype of the prenatally growth-restricted offspring described above.
In contrast, there is robust evidence that the developing reproductive axis is impacted by maternal nutrition, particularly in female fetuses. The resting reserve of primordial follicles that determines lifetime supply of potentially fertilisable eggs is established before birth (McNatty *et al.*1995). Accordingly, in the adolescent paradigm, large reductions in primordial follicle number (up to 80%

less) were measured at both mid and late gestation in fetuses from overnourished dams and reflected the attenuated placental weight, and hence fetal nutrient supply, measured at both stages (Da Silva *et al.*2002, 2003). Pituitary gonadotrophin mRNA was unaffected at mid-pregnancy but by late gestation LHβ mRNA was higher in the most growth-restricted fetuses. This indicates reduced oestrogen feedback from the placenta and/or fetal ovary directly regulating fetal pituitary function, or alternatively a temporal delay in the maturation of the pituitary gland. Irrespective, these prenatally growth-restricted females reach puberty at an equivalent weight and age as normally-grown controls, and the normality and duration of ovarian cyclicity during the first breeding season is also similar (Da Silva *et al.*2001). Nevertheless, the severely diminished ovarian reserve is likely to impact ovulation rate and hence fertility as the animal ages. Although males continuously produce new spermatozoa after puberty, the number of Sertoli cells, the primary determinant of sperm production and testes size in adulthood, is determined by proliferation during the fetal, neonatal and peripubertal periods (Sharpe *et al.*2003). Nonetheless, the number of Sertoli cells, seminiferous cords and pituitary gonadotroph mRNA expression was not impacted by maternal nutrition when assessed at mid-gestation in our model (Da Silva *et al.*2003). However, in contrast to females, male lambs with a 47% suppression in birthweight had slower absolute growth rates, delayed age at puberty, attenuated testosterone concentrations and a smaller testicular volume per unit live-weight between 28 and 35weeks of age (Da Silva *et al.*2001). As Sertoli cells set the upper limit for sperm production and continue to proliferate until puberty it is likely that impaired fetal growth velocity and a delay in reaching an appropriate pubertal weight will impact initial sperm quality and quantity, but this has not been expressly tested.

Consequences for fetal and offspring endocrine systems: *undernourished model*

The phenotype of fetuses and offspring from undernourished adolescent dams has been less intensively studied. Relative to controls, the modestly growth-restricted fetuses of undernourished dams do exhibit relative brain sparing but all other organ weights are unperturbed, and while

plasma glucose levels at late gestation necropsy are consistent with hypoglycaemia there is only a tendency towards lower peripheral insulin concentrations, and IGF-1 levels are not perturbed (Luther *et al.*2007a). Liver glycogen stores are depleted, and body composition analysis indicates a thin phenotype with reduced adiposity but preserved skeletal growth (Luther *et al.*2007a, Wallace *et al.*2015). Unlike the overnourished model, the fetal phenotype with respect to glycaemia, bodyweight and carcass fat percentage can be partially rescued by switching undernourished dams to a control-intake between mid and late pregnancy. The impact of this switch is also seen within the fetal hypothalamus in that the increased expression of three orexigenic neuropeptides in response to nutrient deficit can largely be normalised after improving nutrition (Adam *et al.*2015). After delivery there was no evidence of compensatory growth in the offspring of continuously undernourished dams and likewise at 6months of age glucose metabolism and adrenal responses to stress tests in both sexes were equivalent to the same sex control groups (Wallace *et al.*2010, 2011, 2012).

Conclusion

These ovine paradigms unequivocally demonstrate that the maternal-fetal competition for nutrients that arises during pregnancy in young adolescents is sensitive to maternal nutrition. While nutrient reserves at conception play a role, it is the dietary manipulation of maternal growth and body composition thereafter which has the most negative influence on pregnancy outcome: rapid maternal growth constrains placental development and function and is more detrimental than preventing maternal growth. Maternal and placental endocrine systems and associated nutrient partitioning priorities are differentially altered in both paradigms with downstream consequences for fetal nutrient supply, organ system development, body composition and metabolism, some of which are life-long. From a public health perspective, strategies to prevent pregnancy occurring during adolescence should remain the priority but in cultures where early marriage soon after menarche is the norm, girls with a low BMI should be counselled to gain

weight and achieve a normal BMI before conception. Subsequently dietary-intakes should be sufficient to maintain maternal nutrient reserves throughout pregnancy and thereby meet fetal fuel requirements in the final trimester: documenting changes in skinfold thickness as well as measuring weight gain may be a simple and effective approach and requires evaluation. Where pregnancies are unplanned, and food readily available, biologically immature mothers should be made aware of the potential consequences of excessive gestational weight gain with respect to placental development, and additional early monitoring of placental size and uteroplacental blood flows may help identify those at risk of perinatal complications. For the offspring of young adolescent mothers, individuals with the lowest birthweight are likely to be vulnerable to impaired fertility and adverse metabolic health and should be cognisant of this when making their own life-choices.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Figure Legends

Figure 1. Placental weight (a), lamb birthweight (b), gestation length (c) and early pregnancy maternal weight gain (d) in adolescent sheep carrying a single male or female fetus and offered a control nutrient intake or overnourished (ON) throughout gestation. Overnourished pregnancies were categorised as fetal growth-restricted (FGR) if lamb birthweight was less than two standard deviations below the sex-specific birthweight of the optimally nourished control group, thus <4108g for males and <3798g for females. Numbers per group shown in (a) and summarized from eight studies (Wallace, 2016). The significance of main effects is shown and there were no significant nutrition/growth category x sex interactions. Post hoc Tukey comparisons are shown and where letters differ, $P<0.01$.

Figure 2. Allometric plots of brain, liver (a), kidneys and lungs (b) of fetuses from adolescent dams offered a control nutrient intake (blue symbols) or overnourished (red symbols) and killed at day 130 of gestation. Bodyweight specific weight of fetal brain (c), pituitary (d), kidneys (e) and adrenals (f) in relation to maternal nutrition and growth-status. Overnourished (ON) pregnancies were categorised as fetal growth-restricted (FGR) if fetal weight was less than two standard

1005 deviations below the sex-specific weight of the optimally nourished control group, thus <3755g for
1006 males and <3638g for females. Numbers per group shown in (c) and summarized from four studies
1007 (Wallace et al. 2006a; Yunusova et al. 2011; Carr et al 2014 and unpublished). For c-f, post hoc
1008 Tukey comparisons are shown and where letters differ, P<0.01.
1009
1010 Figure 3. Mean fasting glucose concentrations at 7 weeks of age in male and female offspring of
1011 overnourished dams in relation to prenatal growth category (a). Pregnancies were categorised as
1012 fetal growth-restricted (FGR) or non FGR based on a two times standard deviation cut-off below
1013 the mean birthweight of normally grown controls from earlier studies. Relationship between
1014 birthweight and relative hepatic glucose-6-phosphatase gene expression (b) and between
1015 birthweight and hepatic glucose-6-phosphatase enzyme activity in the same animals at 11 weeks
1016 of age. Glucose data from Wallace et al. 2014a, other data JM Wallace, JS Milne, RP Aitken
1017 unpublished.
1018
1019 Figure 4. Carcass fat percentage at day 80 of gestation (a), relative perirenal fat mass (b) and
1020 relative leptin gene expression (c) at day 130 of gestation in normally growing male and female
1021 fetuses from optimally nourished control adolescents. Inset in (c) is haematoxylin and eosin-
1022 stained sections of perirenal fat from a representative male and female fetus showing differences
1023 in unilocular cell size (JM Wallace, JS Milne, RP Aitken, unpublished data). Hepatic IGF-1 DNA
1024 methylation percentage in male and female offspring of overnourished dams (d) and the
1025 relationship between relative hepatic IGF-1 mRNA gene expression and plasma IGF-1
1026 concentrations in the same animals at 11 weeks of age (e). Data from Carr et al. 2015.
1027

Table 1. Key characteristics of adolescent pregnancy and offspring outcomes in the overnourished and undernourished models expressed relative to optimally nourished (maintenance-fed) controls

	Overnourished Model	**Undernourished Model**
Maternal Dietary Intake	High, 2 x maintenance	Low, 0.7 x maintenance
Maternal Growth	Rapid	Prevented
Maternal Body Composition	Progressive fat deposition	Progressive fat depletion
Maternal Metabolic Hormones	High insulin, IGF-1 and leptin High T_3, T_4 and prolactin Low GH, normal cortisol	Low insulin, IGF-1 and leptin Normal cortisol
Maternal Nutrients	High glucose and urea Low NEFA	Low glucose, urea and individual amino acids. High NEFA
Placental Growth	Impaired, ~40% smaller at term	Normal
Placental Hormones	Low placental lactogen, P_4 and E_2	High P_4 and E_2
Uterine Blood Flow	Reduced by 40% at 0.6gestation	Mildly attenuated, 0.6 to 0.9gestation
Fetal Growth	Impaired, ~30% smaller at term	Impaired, ~10% smaller at term
Fetal Body Composition	Marked brain sparing and increased relative adiposity	Reduced adiposity, preserved skeletal growth
Fetal Hormones and Nutrients	Low insulin, IGF-1 and glucose	Low insulin and glucose, normal IGF-1
Incidence of marked FGR*	45%	14%
Gestation Length	~ 5 days shorter	Normal
Colostrum Yield	Inadequate in >50% of mothers	Inadequate in <15% of mothers
Neonatal Morbidity and Mortality	Increased but largely preventable	Normal
Offspring Growth	Rapid compensatory growth but reduced adult size	Normal
Offspring Metabolism	Persistent glucose intolerance	Normal
Offspring Body Composition	Persistently low bone mineral density Higher adiposity in adult life	Normal

* Categorised as marked fetal growth restriction (FGR) if birthweight is more than two standard deviations below the mean sex-specific birthweight of fetuses in the optimally nourished control group.

Figure 1

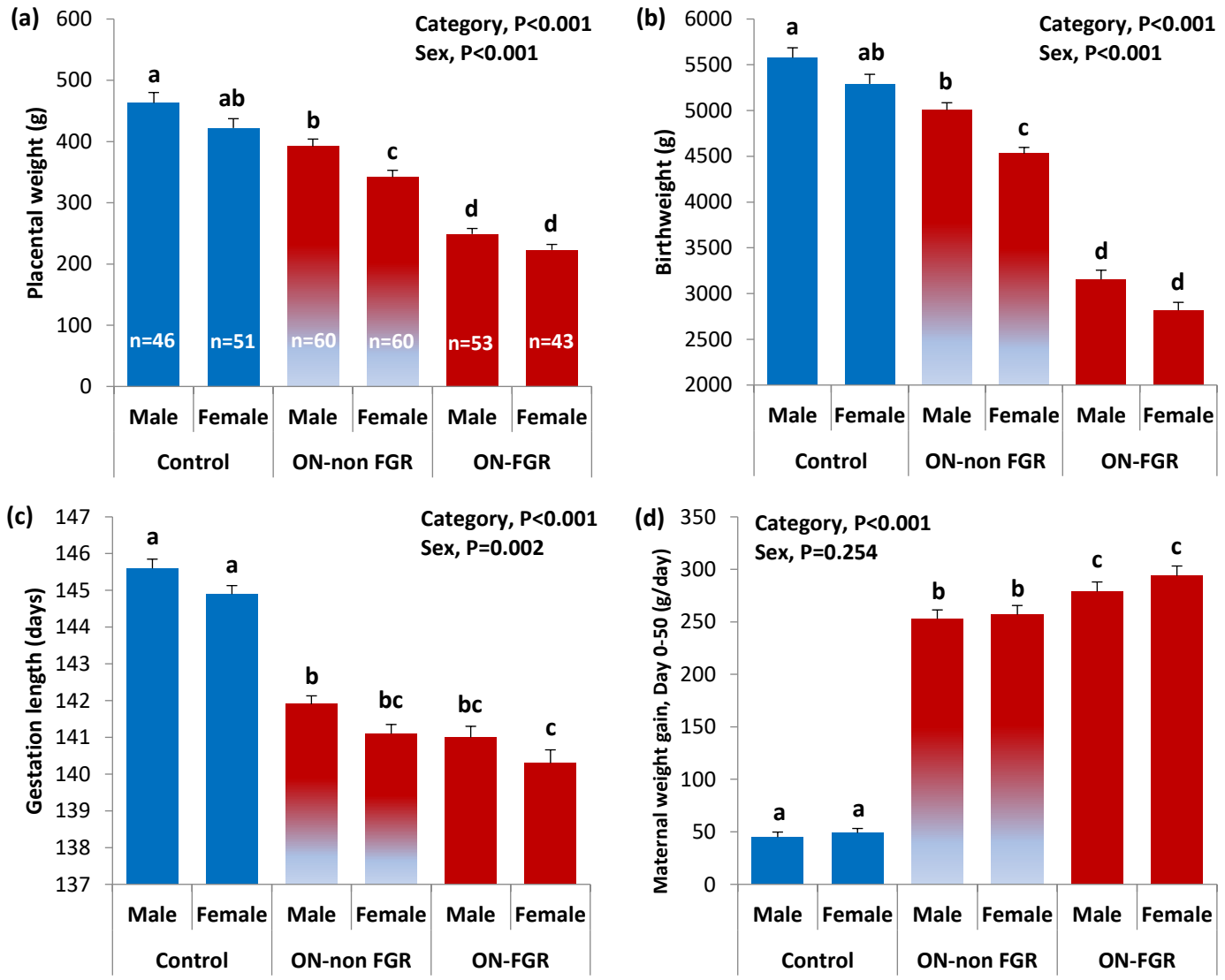
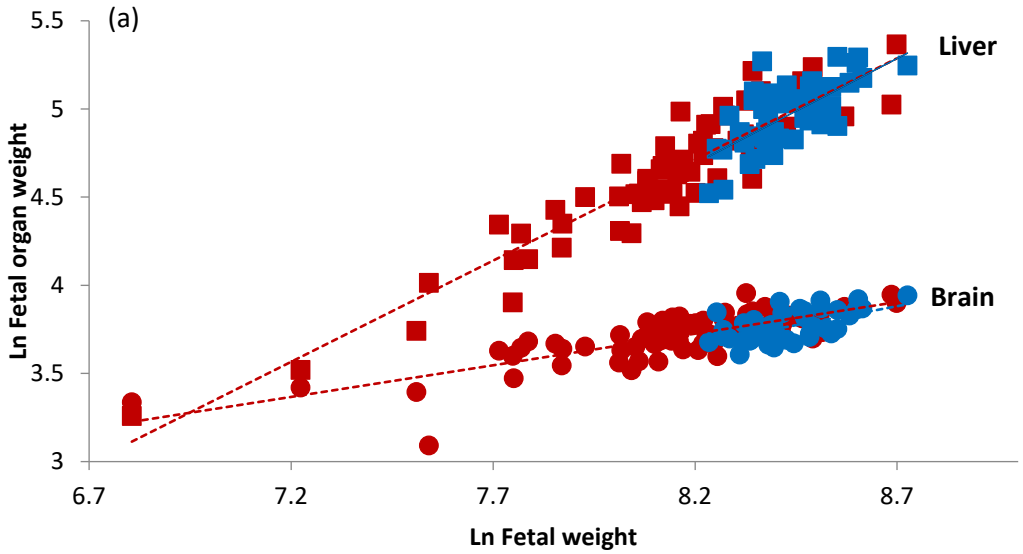
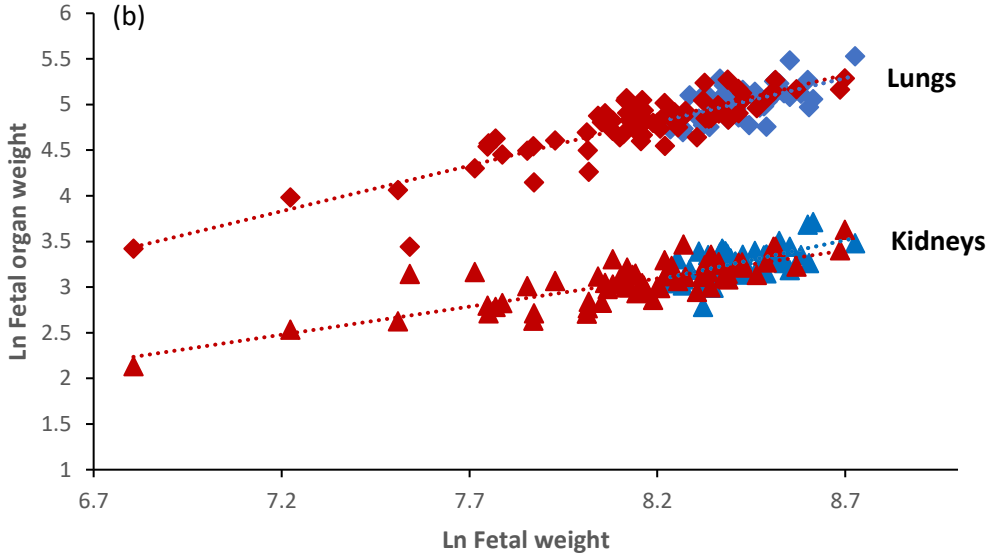
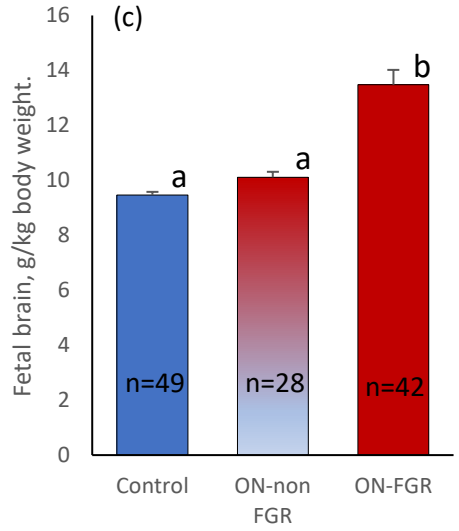
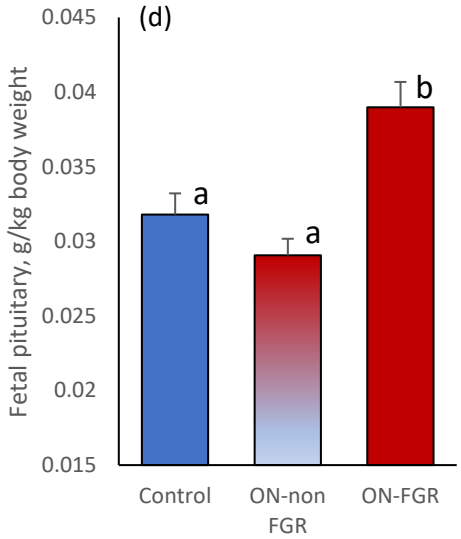

(a)
Placental weight (g)
Category, P<0.001
Sex, P<0.001
a
ab
b
c
d
d
n=46
n=51
n=60
n=60
n=53
n=43
Male
Female
Control
ON-non FGR
ON-FGR
(b)
Birthweight (g)
Category, P<0.001
Sex, P<0.001
(c)
Gestation length (days)
Category, P<0.001
Sex, P=0.002
(d)
Maternal weight gain, Day 0-50 (g/day)
Category, P<0.001
Sex, P=0.254

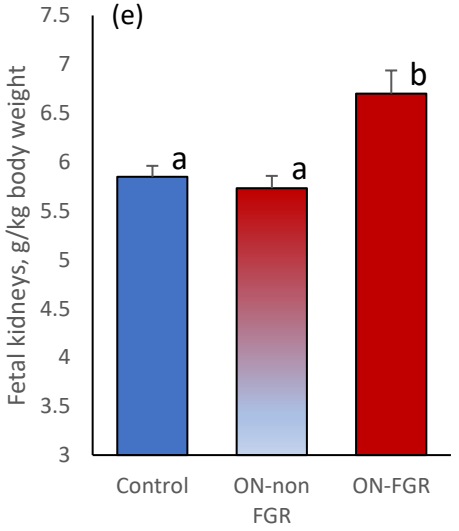
Figure 2


(a)
Liver
Brain
Ln Fetal organ weight
Ln Fetal weight
5.5
5
4.5
4
3.5
3
6.7
7.2
7.7
8.2
8.7


(b)
Lungs
Kidneys
Ln Fetal organ weight
Ln Fetal weight
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
6.7
7.2
7.7
8.2
8.7


(c)
Fetal brain, g/kg body weight.
n=49
n=28
n=42
a
a
b
Control
ON-non FGR
ON-FGR


(d)
Fetal pituitary, g/kg body weight
a
a
b
Control
ON-non FGR
ON-FGR


(e)
Fetal kidneys, g/kg body weight
a
a
b
Control
ON-non FGR
ON-FGR

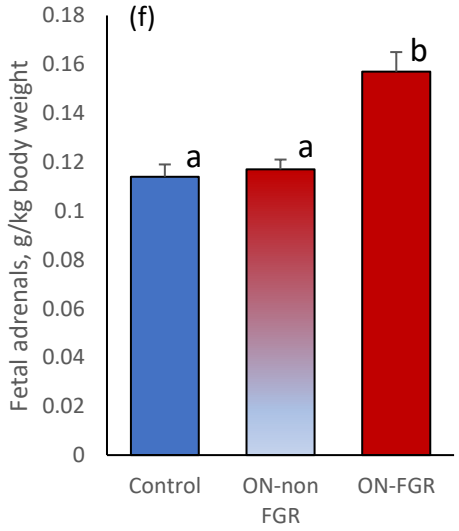

(f)
Fetal adrenals, g/kg body weight
a
a
b
Control
ON-non FGR
ON-FGR

Figure 3

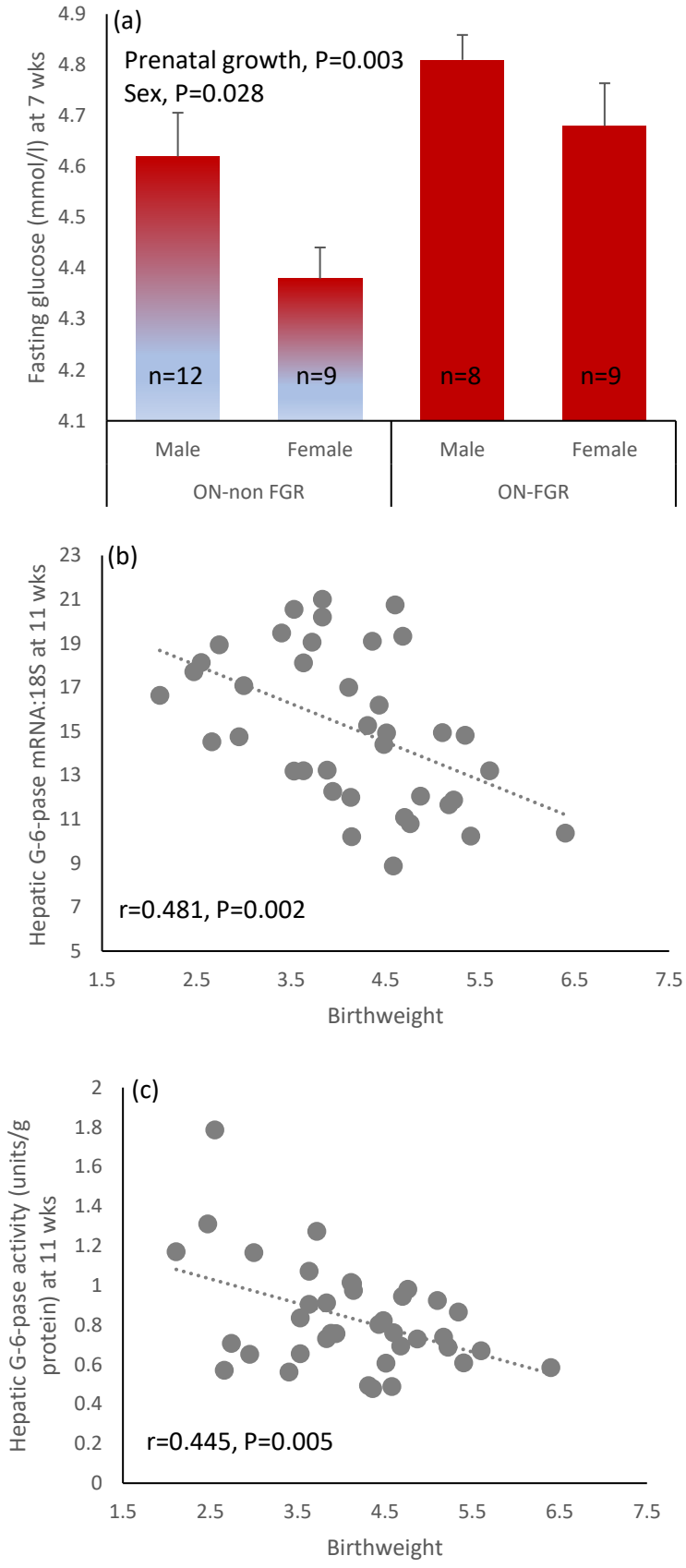
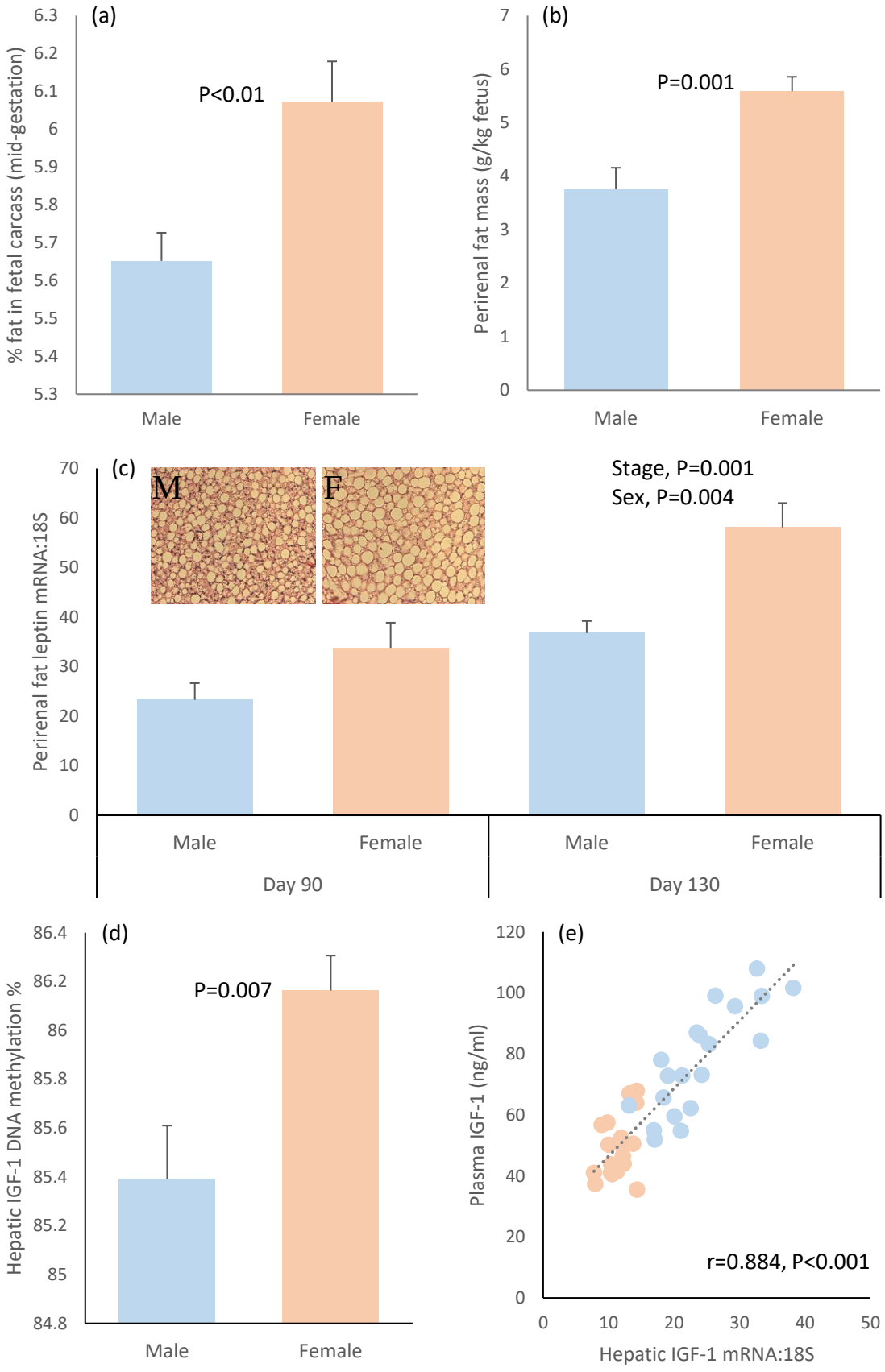

(a)
Prenatal growth, P=0.003
Sex, P=0.028
Fasting glucose (mmol/l) at 7 wks
4.9
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
n=12
n=9
n=8
n=9
Male
Female
Male
Female
ON-non FGR
ON-FGR
(b)
Hepatic G-6-pase mRNA:18S at 11 wks
23
21
19
17
15
13
11
9
7
5
r=0.481, P=0.002
1.5
2.5
3.5
4.5
5.5
6.5
7.5
Birthweight
(c)
Hepatic G-6-pase activity (units/g protein) at 11 wks
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
r=0.445, P=0.005
1.5
2.5
3.5
4.5
5.5
6.5
7.5
Birthweight

Figure 4


(a)
P<0.01
% fat in fetal carcass (mid-gestation)
6.3
6.2
6.1
6
5.9
5.8
5.7
5.6
5.5
5.4
5.3
Male
Female
(b)
P=0.001
Perirenal fat mass (g/kg fetus)
7
6
5
4
3
2
1
0
Male
Female
(c)
M
F
Stage, P=0.001
Sex, P=0.004
Perirenal fat leptin mRNA:18S
70
60
50
40
30
20
10
0
Male
Female
Male
Female
Day 90
Day 130
(d)
P=0.007
Hepatic IGF-1 DNA methylation %
86.4
86.2
86
85.8
85.6
85.4
85.2
85
84.8
Male
Female
(e)
Plasma IGF-1 (ng/ml)
120
100
80
60
40
20
0
r=0.884, P<0.001
0
10
20
30
40
50
Hepatic IGF-1 mRNA:18S