Animal (2010), **4:7**, pp 1075–1083 © The Animal Consortium 2010
doi:10.1017/S1751731110000479

animal

Nutritional regulation of fetal growth and implications for productive life in ruminants

M. E. Symonds†, S. P. Sebert and H. Budge

Early Life Nutrition Research Unit, Respiratory Biomedical Research Unit, Academic Child Health, School of Clinical Sciences, University Hospital, Nottingham, NG7 2UH, United Kingdom

(Received 10 September 2009; Accepted 17 February 2010; First published online 23 March 2010)

The maternal nutritional and metabolic environment is critical in determining not only the reproductive success but also the long-term health and viability of the offspring. Changes in maternal diet at defined stages of gestation coincident with different stages of development can have pronounced effects on organ and tissue function in later life. This includes adipose tissue for which differential effects are observed between brown and white adipose tissues. One early, critical window of organ development in the ruminant relates to the period covering uterine attachment, or implantation, and rapid placental growth. During this period, there is pronounced cell division within developing organelles in many fetal tissues, leading to their structural development. In sheep, a 50% global reduction in caloric intake over this specific period profoundly affects placental growth and morphology, resulting in reduced placentome weight. This occurs in conjunction with a lower capacity to inactivate maternal cortisol through the enzyme 11β-hydroxysteroid dehydrogenase type 2 in response to a decrease in maternal plasma cortisol in early gestation. The birth weight of the offspring is, however, unaffected by this dietary manipulation and, although they possess more fat, this adaptation does not persist into adulthood when they become equally obese as those born to control fed mothers. Subsequently, after birth, further changes in fat development occur which impact on both glucocorticoid action and inflammatory responses. These adaptations can include changes in the relative populations of both brown and white adipocytes for which prolactin acting through its receptor appears to have a prominent role. Earlier when in utero *nutrient restricted (i.e. between early-to-mid gestation) offspring are exposed to an obesogenic postnatal environment; they exhibit an exaggerated insulin response, which is accompanied by a range of amplified and thus, adverse, physiological or metabolic responses to obesity. These types of adaptations are in marked contrast to the effect of late gestational nutrient restriction, which results in reduced fat mass at birth. As young adults, however, fat mass is increased and, although basal insulin is unaffected, these offspring are insulin resistant. In conclusion, changes in nutrient supply to either the mother and/or her fetus can have profound effects on a range of metabolically important tissues. These have the potential to either exacerbate, or protect from, the adverse effects of later obesity and accompanying complications in the resulting offspring.*

Keywords: pregnancy, growth, adipose tissue, hormones

Implications

The nutritional environment in which the fetus and newborn grow and develop has the potential to have long-term effects on body composition as well as metabolic health of the offspring. These outcomes are highly dependent on the timing of the nutritional intervention and thus reflect the specific organ system that is most rapidly developing at each time point. Adipose tissue development is particularly sensitive throughout this period and there is clearly the potential to promote both brown and white adiposity that may either benefit or adversely affect the offspring.

Introduction

Diet during pregnancy is one important modifiable factor that can have a substantial influence on the viability and body composition of the newborn. This is usually considered to have the largest impact in late gestation when the absolute rate of growth of the fetus is greatest (Symonds *et al.*, 2007). As such, total nutrient requirements through the final 10 weeks of pregnancy were carefully calculated 30 years

† E-mail: michael.symonds@nottingham.ac.uk

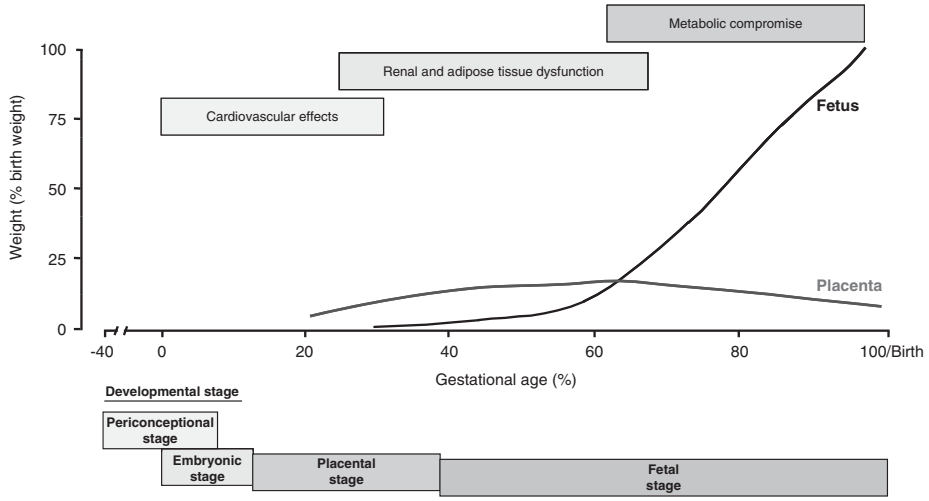

Figure 1 Summary of the different long-term effects in the offspring after maternal nutrient restriction at defined stages of the reproductive cycle in sheep.

ago and provide a widely used standard by which to define the feeding requirements for sheep production (Agricultural Research Council 1980). A major factor that, therefore, determines the total metabolizable energy requirements is fetal number (Agricultural and Food Research Council, 1993). The need for this type of information was highlighted by the potential losses in the neonatal period as a result of starvation and hypothermia being greatest in small sized offspring (Symonds and Lomax, 1992). These individuals are likely to be characterized as having depleted fat stores in conjunction with an increased surface area to volume ratio, thus subjecting them to much greater thermal demands. Interestingly, in some breeds of sheep, these potential risks have been reduced over the past 20 years as global temperatures have risen (Hellmann *et al.*, 2008). Consequently, more small sized offspring have survived into adulthood resulting in mean adult body weight actually decreasing over the same period (Ozgul *et al.*, 2009).

This review will, therefore, consider the relative impact of changes in maternal dietary intake at different stages of the reproductive cycle to highlight how nutrition can have variable effects on the offspring which are primarily dependent on the developmental process targeted. It will focus on studies in sheep, as these have been the most widely investigated largely because of their extensive use for investigating the fetal or developmental origins of adult health and disease. Indeed, investigations of this type are now the main type of ruminant research funded, emphasizing the current scarcity of funding for large animal research (Roberts *et al.*, 2009).

Critical windows in development and the impact of changes in maternal diet

The main emphasis of recent research into the impact of maternal diet on reproduction in ruminants has focused upon the impact of global reductions in food intake (Symonds and Budge, 2009). These types of studies have addressed the more widespread concerns relating to inadequate maternal nutrition, rather than excess food intake which may now be more of a problem within the developed world. Although, its relevance to ruminant production is not always direct, it does emphasize the potential long-term outcomes that may have particular relevance to selecting animals for later sheep production particularly 'replacement ewes'.

Primary factors determining newborn birth weight are fetal number in conjunction with maternal parity (Gardner *et al.*, 2007). Consequently, first time mothers will produce smaller offspring and usually have single rather than multiple fetuses. Then, as the number of pregnancies increases, both fetal number and weight rises. These adaptations are mediated, in part, by the changes in maternal physiology and uterine function after completion of a first pregnancy, in conjunction with more subtle changes in body composition and endocrine sensitivity (Hyatt *et al.*, 2010).

The critical stages of pregnancy after conception are summarized in Figure 1 and cover the peri-conceptional period up to the time of rapid embryo development, followed by the establishment of the placenta as morphogenesis of the fetus occurs enabling the formation of the fetal circulation and all essential organ systems. Subsequently, during late gestation, important adaptations in placental function occur as its total mass declines (Heasman *et al.*, 1998). These enable the substantial increase in nutrient requirements by the rapidly growing fetus to be met in conjunction with an appreciable mobilization of maternal fat stores, which is, itself, dependent on the prevailing nutritional and thermal environment (Symonds and Clarke, 1996). For individuals that have been subjected to a previous period of more extreme nutritional deprivation, this adaptation may be severely compromised leading to the termination of pregnancy (Bloomfield *et al.*, 2003) as discussed further below. A final important component of the global nutritional requirements at this stage include mammary gland development, which is obviously necessary to ensure sufficient milk is supplied to the newborn (Agricultural Research Council, 1980).

Maternal diet prior to pregnancy and its potential contribution to preterm labor

The extent to which a reduction in maternal food intake before conception has a direct effect on the reproductive cycle, as opposed to an indirect influence as a result of the pronounced changes in the maternal metabolic and hormonal environment, remains an area of debate. This controversy is emphasized by the very variable reproductive effects of reducing maternal food intake from at least 60 days before mating (McMillen and Robinson, 2005). In a comparatively small subgroup of sheep, in which a reduction in body weight was greatest before conception, this was accompanied by preterm labor (Bloomfield *et al.*, 2003). Interestingly, that this finding has not been confirmed by any other groups (Edwards and McMillen, 2002; Budge *et al.*, 2004) to date, may be related to differences in breed of sheep, body composition or even in the time of year in which such experiments are undertaken. Irrespective of the very different reproductive outcomes, a reduction in maternal food intake either before, or after, pregnancy results in a pronounced reduction in plasma concentration of counter-metabolic hormones particularly cortisol (Bispham *et al.*, 2003; Jaquiery *et al.*, 2006). As such the lack of a persistent increase in maternal plasma cortisol suggests that a reduction in food availability of this magnitude is within that normally experienced by the animal. It also reinforces the point that maternal nutrient restriction is not equivalent to a biological and/or psychological stress (Budge *et al.*, 2007), which may be due in part to the fact that food (i.e. roughage) is actually available throughout the majority of the day in these studies. The net effect is to promote the mobilization of maternal fat stores and maintenance of glucose production that would be important for supporting either the rapidly dividing embryo and/or the growing placenta (Symonds and Clarke, 1996).

It is not normal agricultural practice to suddenly restrict maternal food intake before conception, as this will reduce both ovulation and rates of conception, thereby having the net effect of reducing the mother's fertility. This type of adaptation would be particularly detrimental in lowland breeds of sheep for which twin or triplet bearing pregnancies are the norm. The long-term impact of improving the overall plane of nutrition of the mother before conception is emphasized when comparing fetal number in highland breeds of sheep in which simply bringing them down to a lowland pasture for one season before mating can result in a majority bearing twin, as opposed to singleton, pregnancies (M. E. Symonds unpublished).

The impact of maternal diet on growth and metabolism of the placenta

After uterine attachment that in the sheep occurs between approximately 22 and 28 days gestation, a reduction in maternal diet can restrict overall growth of the placenta, although the magnitude of this response is strongly influenced by the timing of any nutritional intervention (Yiallourides *et al.*, 2009). Interestingly, in sheep, reducing maternal food intake from the time of mating up to the period when placental growth has commenced in both the maternal caruncle and adjoining fetal cotyledon (i.e. 0 to 30 days gestation), or continuing up to the time at which fetal growth becomes exponential (i.e. 0 to 110 days gestation) (Reynolds *et al.*, 2005), has no effect on either placental or fetal weight in later gestation (Yiallourides *et al.*, 2009). A decrease in maternal food intake from $\sim$28 days gestation continuing up to the end of the period of maximal placental growth that is 80 days gestation, does restrict placental mass primarily as a consequence of reducing growth of the placental component (Heasman *et al.*, 1998). At the same time, this results in a reduction in epithelial cell proliferation, which is most likely mediated by the increased glucocorticoid sensitivity exhibited by the placenta, which persists up to term (Gnanalingham *et al.*, 2007). This, in turn, appears to be primarily a consequence of the reduction in maternal plasma cortisol which persists throughout the period of nutrient restriction (Bispham *et al.*, 2003) and which, in conjunction with a reduction in both gene expression and activity of the enzyme 11β-hydroxysteriod dehydrogenase type 2, would be expected to enhance glucocorticoid sensitivity within the placenta (Whorwood *et al.*, 2001). However, although placental growth is initially restricted, the normal decrease in placental mass that occurs between mid and late gestation in the sheep fails to occur which means that previously nutrient restricted mothers have larger placenta at term and there are no negative effects on fetal growth in the second half of gestation (Heasman *et al.*, 1998). It is thus possible that placental efficiency is subsequently enhanced in mothers that were previously nutrient restricted (Fowden *et al.*, 2009). Indeed, the growth of some fetal organs, including the kidney and adipose tissue, can be enhanced (Whorwood *et al.*, 2001; Bispham *et al.*, 2003; Brennan *et al.*, 2005).

The magnitude of endocrine and related adaptations within the placenta after shorter term reductions in maternal food intake ($<$30 days) is strongly dependent on the stage of gestation in which it is introduced as summarized in Table 1. Under these conditions, adaptations within the mother and/or within the placenta appear to minimize any structural changes in the placenta or alterations in glucocorticoid action (Yiallourides *et al.*, 2009). The period of maternal nutrient restriction that has the greatest endocrine effect within the placenta after maternal nutrient restriction is, therefore, seen when this is targeted to between 66 and 110 days that is commencing at the time of maximal growth. In addition to increased glucocorticoid sensitivity, gene expression for the insulin-like growth factor II receptor is raised together with markers of lipid metabolism (Yiallourides *et al.*, 2009) of which peroxisome-activated receptor (PPAR)γ has a primary role (Nunn *et al.*, 2007). It is, therefore, possible that, as in the placenta of other species in which PPARγ modulates lipid uptake and metabolism (Schaiff *et al.*, 2006), a comparable effect is seen in the sheep. Increased action of uncoupling protein (UCP)2 in the

Table 1 *Summary of the influence of stage of gestation on the effect of maternal nutrient restriction on placental weight, cell proliferation and glucocorticoid action in sheep.*

Stage of gestation (days)	Change in placental weight at mid gestation (% control)	Effect on cell proliferation	Effect on glucocorticoid action
0 to 30	6	None	None
0 to 110	10	None	None
28 to 80	−56	Reduced	Increased
31 to 65	0	None	None
66 to 110	0	Reduced	Increased

Based on Gnanalingham *et al.*, 2007; Yiallourides *et al.*, 2009

Table 2 *Summary of the effect of maternal nutrient restriction between early-to-mid gestations on the primary symptoms of the metabolic syndrome after adolescent onset obesity in the sheep*

Characteristic of the metabolic syndrome	Adverse adaptation in offspring born to nutrient-restricted mothers	Reference
Plasma insulin	Raised	(Sebert *et al.*, 2009)
Adipose tissue dysfunction	Insulin resistance and appearance of crown-like structures	(Sharkey *et al.*, 2009)
Ectopic lipid accumulation	Raised	(Chan *et al.*, 2009)
Hypertension	Accelerated increase in blood pressure with age	(Symonds *et al.*, 2009b)

placenta during maternal nutrient restriction would promote mitochondrial fatty acid oxidation while limiting glucose metabolism. UCPs are able to uncouple the oxidation of fatty acids by affecting the electron transport chain from ATP synthesis (Echtay *et al.*, 2000). Fatty acids also affect the expression of UCPs by acting as ligands for PPARs, with the main products of fatty acid metabolism that is acetate and β-hydroxybutyrate being readily transferred across the placenta (Hull *et al.*, 1979). These could, therefore, be used as an alternative fuels to glucose for fetal metabolism and lipogenesis (Herrera, 2002). Clearly, further studies are required to establish the role of lipid metabolism in the sheep placenta after changes in the maternal diet and the extent to which this impacts on nutrient supply to the fetus.

Effect of maternal nutrient restriction between early-to-mid gestation on fetal growth and organ development

One of the primary effects of maternal nutrient restriction either before, or during, pregnancy is a reduction in plasma concentration of catabolic hormones including cortisol, thyroid hormones and insulin. These adaptations ensure that the maternal plasma glucose concentration is maintained and, thus, fetal growth is not compromised. The endocrine sensitivities of a large number of fetal organs are, however, reset particularly with regard to glucocorticoid action. As in the placenta (see above), this response is mediated by an increase in both gene expression for the glucocorticoid receptor and reduced action of the enzyme 11β-hydroxysteriod dehydrogenase type 2 which occurs in a range of newborn tissues including heart, kidney, liver and lungs (Whorwood *et al.*, 2001). These changes would be predicted to enhance glucocorticoid action even in the absence of any change in plasma cortisol (Gardner *et al.*, 2006). These have no immediate adverse effect on the offspring and persist in some tissues into juvenile life when offspring are raised in an outdoor-free living environment. Under these conditions, their body composition remains the same as those born to control fed mothers and they show no signs of metabolic or cardiovascular compromise (Gopalakrishnan *et al.*, 2005).

When these animals are raised under an obesogenic environment with comparable thermal and photoperiod conditions that is inside within a barn in which their activity is reduced (by 75% compared with free-living animals maintained within an adjacent field), they show a majority of the adverse symptoms of the metabolic syndrome as summarized in Table 2. These include an accelerated increase in resting blood pressure (Symonds *et al.*, 2009b), greatly enhanced ectopic lipid accumulation (Chan *et al.*, 2009) and insulin resistance (Sebert *et al.*, 2009). Although, such disease symptoms are not obviously relevant to agricultural practice, they illustrate the added value of using sheep as a model for human metabolic disease.

Increased maternal food intake from mid gestation and its effect on adipose tissue development and function

Increasing the amount of food provided to pregnant sheep from the time at which placental mass has peaked can have positive effects in promoting fetal growth and repartitioning of nutrients between skeletal muscle and adipose tissue growth (Budge *et al.*, 2000). The magnitude of this response is dependent, in part, on the amount of feed consumed in the earlier stages of gestation (Bispham *et al.*, 2005). One of the major adaptations is to increase the abundance of brown

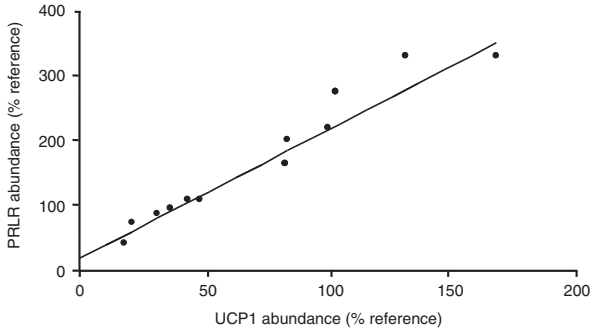

Figure 2 Linear correlation between abundance of the brown adipose tissue specific uncoupling protein (UCP)1 and the abundance of the prolactin receptor (PRLR) over the first month of postnatal life in sheep. (Based upon Pearce *et al.*, 2005).

adipose tissue, clearly benefiting the newborn's ability to effectively adapt to the cold exposure of the extra-uterine environment (Budge *et al.*, 2000). Brown adipose tissue is uniquely characterized as possessing UCP1 which enables the rapid generation of heat through the uncoupling of electron transport from oxidative phosphorylation (Cannon and Nedergaard, 2004). One hormone that can act to promote brown adipose tissue function in the newborn is prolactin (Pearce *et al.*, 2005). Plasma prolactin concentrations are positively correlated with gestational age in infants (Lucas *et al.*, 1990), and in the sheep fetus there is an increase in both prolactin gene expression and circulating prolactin concentrations during the last 10 to 15 days of gestation to peak at birth (term, 147 ± 3 days gestation) (Gluckman *et al.*, 1983). Prolactin acts through its receptor of which there are at least two forms, the long and short form (Bignon *et al.*, 1997). In the fetal sheep, changes in gene expression and protein abundance of the prolactin receptor, both with gestational age and the maternal nutritional environment, are key factors determining both the initial appearance in UCP1, as well as changes in its activity around the time of birth (Symonds *et al.*, 1998 and 2001).

The extent to which an increase in prolactin receptor abundance may determine the rate of loss of UCP1 after birth has yet to be established. Given the close relationship between the rate of decline in both UCP1 and prolactin receptor (Figure 2), it is likely to delay this process. This may have practical implications because, after birth when UCP1 is rapidly lost, there is a period of pronounced hyperplasia within the same perirenal-abdominal fat depot, which adopts primarily white adipose tissue characteristics (Clarke *et al.*, 1997b). This type of transformation within one fat depot, may be very different to that seen in small mammals in which it, has been suggested that brown and white fat have very different origins (Timmons *et al.*, 2007). In these species, however, the main site of brown fat is within the intrascapular region which does not undergo the same type of transition to white fat after birth but is instead retained as brown fat throughout the life cycle (Cannon and Nedergaard, 2004).

In utero determinants of fat distribution and function: potential role of photoperiod

Recent studies have highlighted the importance of early life events in determining further aspects of adipocyte function and distribution (Budge *et al.*, 2009). This includes the finding that white adipocyte progenitor cells become committed to the adipose lineage during the late fetal/early postnatal period, and that there is also a marked expansion of this cellular pool because of the proliferation during postnatal life (Tang *et al.*, 2008). Furthermore, it has also been suggested that brown adipocytes have the same lineage as skeletal myoblasts, a process that may be regulated by bone morphogenetic protein acting through PRD1-BF1-RIZ1 homologous domain containing 16 gene (Seale *et al.*, 2008; Tseng *et al.*, 2008). Alternatively, there may be two types of brown adipose tissue cells that are either positive or negative for Myf-5 (Enerback, 2009) and, thus, are either 'normal' or 'recruitable' brown adipose tissue and whose origin may be set very early in development. Ultimately, this would provide a common mechanism relating brown adipocyte and muscle development, differing significantly from white adipocytes, and is in accord with the distinct differences in myogenic gene expression found between brown and white cells. This is also in accord with some phenotypic similarities observed between brown adipocytes and skeletal muscle (Timmons *et al.*, 2007). The main regulators of this process remain to be established but it is notable that prolactin receptor knockout mice not only show reduced UCP1 abundance at birth (Viengchareun *et al.*, 2008) but also less fat as adults (Freemark *et al.*, 2001). It has yet to be established whether the same plethora of factors that have been implicated in the regulation of brown fat development in rodents have the same role in large mammals including ruminants. Given the strong link between maternal diet, prolactin receptor abundance and UCP1 (Figure 3) may provide a mechanism by which to promote brown fat abundance both at birth and in the longer term.

The potential importance of the established relationship between the prolactin receptor and brown fat function to both fetal growth and development, but also to longer term fat development, is highlighted by the recent finding of brown fat in adult humans (Virtanen *et al.*, 2009) and for which a strong seasonal influence has been demonstrated (Au-Yong *et al.*, 2009). It is established that one of the major hormones that responds to photoperiod is prolactin (Bassett *et al.*, 1988) (Goldman *et al.*, 1981) which also increases with day length (Steger *et al.*, 1983). Studies in adult hamsters have also indicated an important role for melatonin in mediating the effect of day length on brown adipose tissue function (Heldmaier *et al.*, 1981). The melatonin receptor is present on fetal adipose tissue, however, melatonin seems to inhibit the lipolytic actions of noradrenaline on fetal adipose tissue, at least when studied *in vitro* at $\sim$130 days of gestation under hypothermic conditions (Torres-Farfan *et al.*, 2008) (i.e. 37°C compared with the normal fetal body temperature of $\sim$40°C (Power, 1989)).

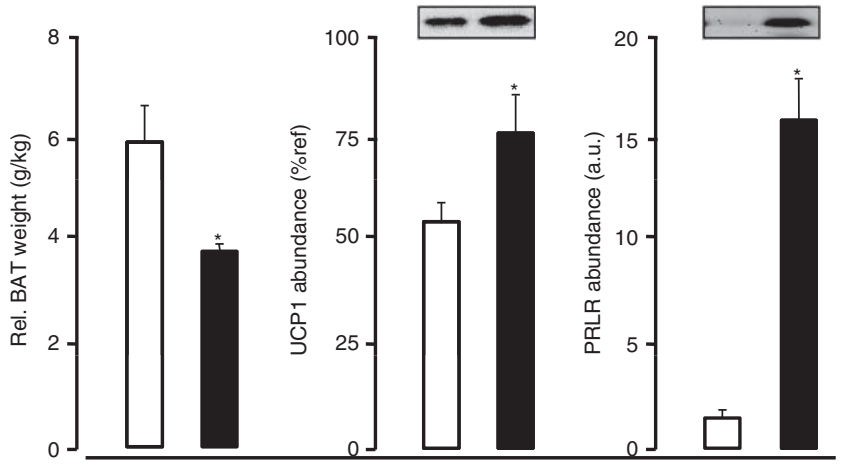

Figure 3 Effect of increased maternal food intake over the final half of gestation on relative fat mass, the brown adipose tissue specific uncoupling protein (UCP)1 and the abundance of the prolactin receptor (PRLR) in the newborn sheep. Mothers were either fed to 100% of total metabolisable energy requirements (open boxes) or *ad libitum* (i.e. 150% of metabolisble energy requirements; closed boxes). (Based upon Budge *et al.*, 2000).

The extent to which photoperiod, as opposed to ambient temperature, is a primary regulator of brown adipose tissue function remains uncertain as, although photoperiod can determine brown adipose tissue activity irrespective of ambient temperature, its effect is enhanced in the cold (Klingenspor *et al.*, 1989; Wiesinger *et al.*, 1989). In mammals that naturally show a seasonal change in body composition according to day length, exposure to short days for only 5 weeks promotes brown adipose tissue function and is accompanied by marked fat mobilization (Demas *et al.*, 2002). However, in this model the effect declines with the duration of exposure that may be related to compensatory changes in food intake.

Prolactin administration also promotes the loss of UCP1 in lactating rats (Chan and Swaminathan, 1990) and prolactin secretion can be temperature sensitive (Vigas *et al.*, 2000). The prolactin receptor is essential for brown adipose tissue function in the newborn (Viengchareun *et al.*, 2008), in which its direct stimulation promotes brown adipose tissue thermogenesis (Pearce *et al.*, 2005). Indeed, during normal development, an increase in prolactin receptor abundance could be a key stage in fetal brown adipose tissue development (Symonds *et al.*, 1998). In this regard, photoperiod is the primary determinant of plasma prolactin in maternal and thus fetal circulation with values more than a 100 fold different between pregnant sheep (and their fetuses) maintained under long daylight ($>$16 h) compared with short daylight ($<$9.5 h) (Bassett *et al.*, 1988). Clearly, future studies are necessary to establish the role of photoperiod on fetal development and how this may interact with maternal diet.

The influence of temperature on fetal development

One of the best examples of the potential influence of the thermal environment on fetal development comes from studies that have examined the impact of chronic maternal cold exposure induced by winter shearing (Symonds *et al.*, 1995). This procedure is often utilized by lowland farmers to increase stocking rates close to the time of lambing when all sheep are barn-housed to aid animal husbandry during lambing. At the same time, the beneficial effects on reproductive performance are only seen in twin or triplet bearing sheep (Symonds and Lomax, 1992). The primary effect of this challenge to both the mother and the fetus is to induce chronic maternal adaptations to the cold that result in enhanced secretion and/or action of thyroid hormones, noradrenaline and growth hormone, whereas the action of insulin is reduced (Symonds *et al.*, 1988 and 1986). The magnitude of these adaptations is dependent, in part, on the prevailing ambient temperature. Overall, the net effect of these long-term changes in energy metabolism within the mother is that her ability to mobilize and then utilize her own fat stores is increased, thereby preventing the late gestational decline in plasma glucose that often occurs in unshorn mothers (Symonds *et al.*, 1988).

The beneficial effects on the fetus and newborn include not only an increase in brown fat but also a larger liver with greater glycogen stores (Symonds *et al.*, 1992; Clarke *et al.*, 1997a). This means that newborn is better adapted not only to meet the thermal challenge of the extra-uterine environment but also to improve the thyroid and respiratory function (Symonds *et al.*, 1993; Clarke *et al.*, 1997a). These effects then persist through at least the first month of life, so that even when the offspring are artificially reared on a comparatively low plane of nutrition, they still deposit more fat in which brown adipose tissue characteristics are retained (Symonds *et al.*, 1992). In those offspring that are reared by their mothers, growth rate is increased through lactation primarily as a result of increased milk production (Symonds *et al.*, 1990).

Future studies into epigenetic control mechanisms

Now it appears that those individuals exposed to an inadequate diet during very early development may exhibit a range of adaptations that not only relate to up or down-regulation

of gene expression but also this could include epigenetic modifications (Symonds *et al*., 2009a). To date, all of the experimental evidence relating to this process has been largely confined to studies using rodents with extreme dietary modifications such as consumption of a hypermethylating diet through pregnancy and lactation (Waterland and Jirtle, 2003) that contains a ~9-fold increase in choline and folic acid, ~3 times or more increase in methionine and ~60 times or more increase in vitamin B12 compared with control diets (Reeves, 1997). The one exception involved sheep that were conceived in elderly (i.e. cull-ewes) fed with a diet that was lacking in sulphur and cobalt with result that the recipients were depleted in sulphur containing amino acids, specific B group vitamins (vitamin B_{12} and folate), as well as having reduced plasma glucose (Sinclair *et al*., 2007). Subsequently, only after 6 days of exposure to this embryonic environment, all blastocysts were transferred to sheep fed with a standard diet. As such, it is not possible to fully dissociate the suggested adverse outcomes from either maternal age, diet, embryo transfer or accelerated postnatal growth. In addition, the high embryo loss rate of ~45% (Sinclair *et al*., 2007) may exaggerate the reported outcomes as this is much greater than is seen in normal agricultural practice, especially when using a breed of sheep that normally produces singleton fetuses.

An alternative experimental manipulation that has been used to compromise fetal growth in the rodent is that of uterine artery ligation in late gestation which reduces uterine artery blood flow by ~50% (Simmons *et al*., 1993) and results in substantial intra-uterine growth retardation (Wigglesworth, 1974). When these offspring are cross-fostered onto control dams they show rapid catch-up growth, an adaptation that is likely to be as important in determining the subsequent adverse outcomes as the preceding growth restriction *in utero* (Symonds, 2007). As adults, these growth manipulated offspring exhibit type 2 diabetes that is associated with progressive epigenetic silencing of the homeobox 1 transcription factor *Pdx1* which is critical for pancreatic β-cell function and development (Park *et al*., 2008). Nevertheless, during the neonatal period, the reduction in *Pdx1* expression could be reversed *in vitro* by inhibition of histone deacetylase action. Potential epigenetic effects may well be extended to a number of other tissues and cells including the regulation of intracellular energy locus, as recently shown *in vitro* (by using human neonatal skin fibroblasts), at least under conditions of 0 or very high (i.e. 1000 mg/l) glucose concentration (Murayama *et al*., 2008).

The extent to which such mechanisms operate under the range of normal circulating plasma glucose, particularly during early development, is important as it has also been shown that glucose metabolism is closely linked to chromatin modification and global transcription control (Wellen *et al*., 2009). This involves the production of acetyl-coenzyme A from glucose and ATP-citrate lyase, which is a key intermediate in regulating energy metabolism within mitochondria, the cytoplasm and the nucleus. It is now necessary to translate these *in vitro* findings of a novel regulatory mechanism that produces a substrate for chromatin modification to the *in vivo* situation (Rathmell and Newgard, 2009). This is especially the case for the fetus in which changes in plasma glucose are much more subtle, but can have much greater and long-term effects (Symonds *et al*., 2007). For example, it has been shown that exposure to the Dutch Famine during the end of World War II can result in subtle changes in the methylation status of the insulin-like growth factor II receptor as determined in genomic DNA extracted from blood samples of the survivors aged ~60 years (Heijmans *et al*., 2008). Whether this is a direct nutritional effect remains to be established as, interestingly, such an adaptation was only seen in those offspring that were actually conceived during the famine and not in those that were exposed to the famine during the final trimester and who were smaller at birth. It remains to be seen whether the types of adaptations to targeted reductions, or increases, in the maternal diet are accompanied by comparable epigenetic adaptations that ultimately underpin the long-term outcomes.

In conclusion, changes in the amount or composition of feed consumed by the mother from the time of ovulation through to lactation have the potential to significantly reset the growth trajectory of a majority of fetal organs and tissues. Ultimately, this will determine size at birth which in conjunction to other external stimuli, such as temperature and/or photoperiod, will not only determine size at birth but long term health and survival. An increased understanding of these processes may be particularly important over the next 10 years or so as the gradual changes in the planet's temperature and the effects of climate change start to have further impact on agricultural production.

Acknowledgments

The authors would like to acknowledge the support of the European Union Sixth Framework Program for Research and Technical Development of the European Community – The Early Nutrition Programming Project (FOOD-CT-2005-007036) and the Nottingham Respiratory Biomedical Research Unit in their research.

References

Agricultural and Food Research Council 1993. Technical committee on responses to nutrients. Report no. 9. Technical committee on responses to nutrients. Report no. 9. CAB International, Wallingford, UK.

Agricultural Research Council 1980. Requirements for energy. The nutritional requirements of ruminant livestock. Commonwealth Agricultural Bureau, Slough, UK.

Au-Yong IT, Thorn N, Ganatra R, Perkins AC and Symonds ME 2009. Brown adipose tissue and seasonal variation in humans. Diabetes 58, 2583–2587.

Bassett JM, Bomford J and Mott JC 1988. Photoperiod: an important regulator of plasma prolactin concentration in fetal lambs during late gestation. Quarterly Journal of Experimental Physiology 73, 241–244.

Bignon C, Binart N, Ormandy C, Schuler LA, Kelly PA and Djiane J 1997. Long and short forms of the ovine prolactin receptor: cDNA cloning and genomic analysis reveal that the two forms arise by different alternative splicing mechanisms in ruminants and in rodents. Journal of Molecular Endocrinology 19, 109–120.

Bispham J, Gopalakrishnan GS, Dandrea J, Wilson V, Budge H, Keisler DH, Broughton Pipkin F, Stephenson T and Symonds ME 2003. Maternal endocrine adaptation throughout pregnancy to nutritional manipulation: consequences for maternal plasma leptin and cortisol and the programming of fetal adipose tissue development. Endocrinology 144, 3575–3585.

Bispham J, Gardner DS, Gnanalingham MG, Stephenson T, Symonds ME and Budge H 2005. Maternal nutritional programming of fetal adipose tissue development: differential effects on messenger ribonucleic acid abundance for uncoupling proteins and peroxisome proliferator-activated and prolactin receptors. Endocrinology 146, 3943–3949.

Bloomfield FH, Oliver MH, Hawkins P, Campbell M, Phillips DJ, Gluckman PD, Challis JR and Harding JE 2003. A periconceptional nutritional origin for noninfectious preterm birth. Science 300, 606.

Brennan KA, Gopalakrishnan GS, Kurlak L, Rhind SM, Brooks AN, Rae MT, Olson DM, Stephenson T and Symonds ME 2005. Impact of maternal undernutrition and fetal number on glucocorticoid, growth hormone and insulin-like growth factor receptor mRNA abundance in the ovine fetal kidney. Reproduction 129, 151–159.

Budge H, Bispham J, Dandrea J, Evans E, Heasman L, Ingleton PM, Sullivan C, Wilson V, Stephenson T and Symonds ME 2000. Effect of maternal nutrition on brown adipose tissue and its prolactin receptor status in the fetal lamb. Pediatric Research 47, 781–786.

Budge H, Edwards LJ, McMillen IC, Bryce A, Warnes K, Pearce S, Stephenson T and Symonds ME 2004. Nutritional manipulation of fetal adipose tissue deposition and uncoupling protein 1 abundance in the fetal sheep; differential effects of timing and duration. Biology of Reproduction 71, 359–365.

Budge H, Stephenson T and Symonds ME 2007. Maternal nutrient restriction is not equivalent to maternal biological stress. Current Drug Targets 8, 888–893.

Budge H, Sebert S, Sharkey D and Symonds ME 2009. Adipose tissue development, nutrition in early life and its impact on later obesity. Proceedings of the Nutrition Society 68, 321–326.

Cannon B and Nedergaard J 2004. Brown adipose tissue: Function and significance. Physiological Reviews 84, 277–359.

Chan E and Swaminathan R 1990. Role of prolactin in lactation-induced changes in brown adipose tissue. American Journal of Physiology – Regulatory, Integrative and Comparative Physiology 27, R51–R56.

Chan LL, Sebert SP, Hyatt MA, Stephenson T, Budge H, Symonds ME and Gardner DS 2009. Effect of maternal nutrient restriction from early to mid gestation on cardiac function and metabolism after adolescent-onset obesity. American Journal of Physiology – Regulatory, Integrative and Comparative Physiology 296, R1455–R1463.

Clarke L, Bryant MJ, Lomax MA and Symonds ME 1997a. Maternal manipulation of brown adipose tissue and liver development in the ovine fetus during late gestation. British Journal of Nutrition 77, 871–883.

Clarke L, Buss DS, Juniper DS, Lomax MA and Symonds ME 1997b. Adipose tissue development during early postnatal life in ewe-reared lambs. Experimental Physiology 82, 1015–1017.

Demas GE, Bowers RR, Bartness TJ and Gettys TW 2002. Photoperiodic regulation of gene expression in brown and white adipose tissue of Siberian hamsters (phodopus sungorus). American Journal of Physiology – Regulatory, Integrative and Comparative Physiology 282, R114–R121.

Echtay KS, Winkler E and Klingenberg M 2000. Coenzyme Q is an obligatory cofactor for uncoupling protein function. Nature 408, 609–613.

Edwards LJ and McMillen IC 2002. Impact of maternal undernutrition during the periconceptional period, fetal number, and fetal sex on the development of the hypothalamo-pituitary adrenal axis in sheep during late gestation. Biology of Reproduction 66, 1562–1569.

Enerback S 2009. The origins of brown adipose tissue. The New England Journal of Medicine 360, 2021–2023.

Fowden AL, Sferruzzi-Perri AN, Coan PM, Constancia M and Burton GJ 2009. Placental efficiency and adaptation: endocrine regulation. The Journal of Physiology 587, 3459–3472.

Freemark M, Fleenor D, Driscoll P, Binart N and Kelly P 2001. Body weight and fat deposition in prolactin receptor-deficient mice. Endocrinology 142, 532–753.

Gardner DS, Van Bon BW, Dandrea J, Goddard PJ, May SF, Wilson V, Stephenson T and Symonds ME 2006. Effect of periconceptional undernutrition and gender on hypothalamic-pituitary-adrenal axis function in young adult sheep. Journal of Endocrinology 190, 203–212.

Gardner DS, Buttery PJ, Daniel Z and Symonds ME 2007. Factors affecting birth weight in sheep: maternal environment. Reproduction 133, 297–307.

Gluckman PD, Marti-Henneberg C, Kaplan SL and Grumbach MM 1983. Hormone ontogeny in the ovine fetus: XIV. The effect of 17 beta-estradiol infusion on fetal plasma gonadotropins and prolactin and the maturation of sex steroid-dependent negative feedback. Endocrinology 112, 1618–1623.

Gnanalingham MG, Williams P, Wilson V, Bispham J, Hyatt MA, Pellicano A, Budge H, Stephenson T and Symonds ME 2007. Nutritional manipulation between early to mid-gestation: effects on uncoupling protein-2, glucocorticoid sensitivity, IGF-I receptor and cell proliferation but not apoptosis in the ovine placenta. Reproduction 134, 615–623.

Goldman BD, Matt KS, Roychoudhury P and Stetson MH 1981. Prolactin release in golden hamsters: photoperiod and gonadal influences. Biology of Reproduction 24, 287–292.

Gopalakrishnan GS, Gardner DS, Dandrea J, Langley-Evans SC, Pearce S, Kurlak LO, Walker RM, Seetho IW, Keisler DH, Ramsay MM, Stephenson T and Symonds ME 2005. Influence of maternal pre-pregnancy body composition and diet during early-mid pregnancy on cardiovascular function and nephron number in juvenile sheep. British Journal of Nutrition 94, 938–947.

Heasman L, Clarke L, Firth K, Stephenson T and Symonds ME 1998. Influence of restricted maternal nutrition in early to mid gestation on placental and fetal development at term in sheep. Pediatric Research 44, 546–551.

Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, Slagboom PE and Lumey LH 2008. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proceedings of the National Academy of Sciences of the United States of America 105, 17046–17049.

Heldmaier G, Steinlechner S, Rafael J and Vsiansky P 1981. Photoperiodic control and effects of melatonin on nonshivering thermogenesis and brown adipose tissue. Science 212, 917–919.

Hellmann JJ, Byers JE, Bierwagen BG and Dukes JS 2008. Five potential consequences of climate change for invasive species. Conservation Biology 22, 534–543.

Herrera E 2002. Implications of dietary fatty acids during pregnancy on placental, fetal and postnatal development – a review. Placenta 23(suppl. A), S9–S19.

Hull D, Elphick MC and Broughton PF 1979. The transfer of fatty acids across the sheep placenta. Journal of Developmental Physiology 1, 31–45.

Hyatt MA, Keisler DH, Budge H and Symonds ME 2010. Maternal parity and its effect on adipose tissue deposition and endocrine sensitivity in the postnatal sheep. Journal of Endocrinology 204, 173–179.

Jaquiery AL, Oliver MH, Bloomfield FH, Connor KL, Challis JR and Harding JE 2006. Fetal exposure to excess glucocorticoid is unlikely to explain the effects of periconceptional undernutrition in sheep. Journal of Physiology 572, 109–118.

Klingenspor M, Klaus S, Wiesinger H and Heldmaier G 1989. Short photoperiod and cold activate brown fat lipoprotein lipase in the Djungarian hamster. American Journal of Physiology – Regulatory, Integrative and Comparative Physiology 26, R1123–R1127.

Lucas A, Baker BA and Cole TJ 1990. Plasma prolactin and clinical outcome in preterm infants. Archives of Diseases in Childhood 65, 977–983.

McMillen IC and Robinson JS 2005. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiological Reviews 85, 571–633.

Murayama A, Ohmori K, Fujimura A, Minami H, Yasuzawa-Tanaka K, Kuroda T, Oie S, Daitoku H, Okuwaki M, Nagata K, Fukamizu A, Kimura K, Shimizu T and Yanagisawa J 2008. Epigenetic control of rDNA loci in response to intracellular energy status. Cell 133, 627–639.

Nunn AV, Bell J and Barter P 2007. The integration of lipid-sensing and anti-inflammatory effects: how the PPARs play a role in metabolic balance. Nuclear Receptors 5, 1.

Ozgul A, Tuljapurkar S, Benton TG, Pemberton JM, Clutton-Brock TH and Coulson T 2009. The dynamics of phenotypic change and the shrinking sheep of St. Kilda. Science 325, 464–467.

Park JH, Stoffers DA, Nicholls RD and Simmons RA 2008. Development of type 2 diabetes following intrauterine growth retardation in rats is associated with progressive epigenetic silencing of Pdx1. Journal of Clinical Investigation 118, 2316–2423.

Pearce S, Budge H, Mostyn A, Genever E, Webb R, Ingleton P, Walker AM, Symonds ME and Stephenson T 2005. Prolactin, the prolactin receptor and

uncoupling protein abundance and function in adipose tissue during development in young sheep. Journal of Endocrinology 184, 351–359.

Power G 1989. Biology of temperature: the mammalian fetus. Journal of Developmental Physiology 12, 295–304.

Rathmell JC and Newgard CB 2009. Biochemistry. A glucose-to-gene link. Science 324, 1021–1022.

Reeves PG 1997. Components of the AIN-93 diets as improvements in the AIN-76A diet. The Journal of Nutrition 127, 838S–841S.

Reynolds LP, Borowicz PP, Vonnahme KA, Johnson ML, Grazul-Bilska AT, Wallace JM, Caton JS and Redmer DA 2005. Animal models of placental angiogenesis. Placenta 26, 689–708.

Roberts RM, Smith GW, Bazer FW, Cibelli J, Seidel GE Jr, Bauman DE, Reynolds LP and Ireland JJ 2009. Research priorities. Farm animal research in crisis. Science 324, 468–469.

Schaiff WT, Barak Y and Sadovsky Y 2006. The pleiotropic function of PPAR gamma in the placenta. Molecular and Cellular Endocrinology 249, 10–15.

Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scime A, Devarakonda S, Conroe HM, Erdjument-Bromage H, Tempst P, Rudnicki MA, Beier DR and Spiegelman BM 2008. PRDM16 controls a brown fat/skeletal muscle switch. Nature 454, 961–967.

Sebert SP, Hyatt MA, Chan LL, Patel N, Bell RC, Keisler D, Stephenson T, Budge H, Symonds ME and Gardner DS 2009. Maternal nutrient restriction between early-to-mid gestation and its impact upon appetite regulation following juvenile obesity. Endocrinology 150, 634–641.

Sharkey D, Gardner DS, Fainberg HP, Wilson V, Sebert S, Bos P, Bell R, Symonds ME and Budge H 2009. Maternal nutrient restriction during pregnancy differentially alters the unfolded protein response in adipose and renal tissue of obese juvenile offspring. The FASEB Journal 23, 1314–1324.

Simmons RA, Flozak AS and Ogata ES 1993. The effect of insulin and insulin-like growth factor-I on glucose transport in normal and small for gestational age fetal rats. Endocrinology 133, 1361–1368.

Sinclair KD, Allegrucci C, Singh R, Gardner DS, Sebastian S, Bispham J, Thurston A, Huntley JF, Rees WD, Maloney CA, Lea RG, Craigon J, McEvoy TG and Young LE 2007. DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. Proceedings of the National Academy of Sciences of the United States of America 104, 19351–19356.

Steger RW, Bartke A, Goldman BD, Soares MJ and Talamantes F 1983. Effects of short photoperiod on the ability of golden hamster pituitaries to secrete prolactin and gonadotropins in vitro. Biology of Reproduction 29, 872–878.

Symonds ME 2007. Integration of physiological and molecular mechanisms of the developmental origins of adult disease: new concepts and insights. Proceedings of the Nutrition Society 66, 442–450.

Symonds ME and Budge H 2009. Nutritional models of the developmental programming of adult health and disease. Proceedings of the Nutrition Society 68, 173–178.

Symonds ME and Clarke L 1996. Nutrition-environment interactions in pregnancy. Nutrition Research Reviews 9, 135–148.

Symonds ME and Lomax MA 1992. Maternal and environmental influences on thermoregulation in the neonate. Proceedings of the Nutrition Society 51, 165–172.

Symonds ME, Bryant MJ and Lomax MA 1986. The effect of shearing on the energy metabolism of the pregnant ewe. British Journal of Nutrition 56, 635–643.

Symonds ME, Bryant MJ and Lomax MA 1988. Metabolic adaptation during pregnancy in winter-shorn sheep. Journal of Agricultural Science, Cambridge 111, 137–145.

Symonds ME, Bryant MJ and Lomax MA 1990. Metabolic adaptation during lactation in winter-shorn sheep. Journal of Agricultural Science 114, 201–205.

Symonds ME, Bryant MJ, Clarke L, Darby CJ and Lomax MA 1992. Effect of maternal cold exposure on brown adipose tissue and thermogenesis in the neonatal lamb. Journal of Physiology 455, 487–502.

Symonds ME, Lomax MA, Kenward MG, Andrews DC and Johnson P 1993. Effect of the prenatal maternal environment on the control of breathing during non-rapid-eye-movement sleep in the developing lamb. Journal of Developmental Physiology 19, 43–50.

Symonds ME, Bird JA, Clarke L, Gate JJ and Lomax MA 1995. Nutrition, temperature and homeostasis during perinatal development. Experimental Physiology 80, 907–940.

Symonds ME, Phillips ID, Anthony RV, Owens JA and McMillen IC 1998. Prolactin receptor gene expression and foetal adipose tissue. Journal of Neuroendocrinology 10, 885–890.

Symonds ME, Mostyn A and Stephenson T 2001. Cytokines and cytokine-receptors in fetal growth and development. Biochemical Society Transactions 29, 33–37.

Symonds ME, Stephenson T, Gardner DS and Budge H 2007. Long-term effects of nutritional programming of the embryo and fetus: mechanisms and critical windows. Reproduction Fertility and Development 19, 53–63.

Symonds ME, Sebert SP, Hyatt MA and Budge H 2009a. Nutritional programming of the metabolic syndrome. Nature Reviews Endocrinology 5, 604–610.

Symonds ME, Stephenson T and Budge H 2009b. Early determinants of cardiovascular disease: the role of early diet in later blood pressure control. The American Journal of Clinical Nutrition 89, 1518S–1522S.

Tang W, Zeve D, Suh JM, Bosnakovski D, Kyba M, Hammer RE, Tallquist MD and Graff JM 2008. White fat progenitor cells reside in the adipose vasculature. Science 322, 583–586.

Timmons JA, Wennmalm K, Larsson O, Walden TB, Lassmann T, Petrovic N, Hamilton DL, Gimeno RE, Wahlestedt C, Baar K, Nedergaard J and Cannon B 2007. Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. Proceedings of the National Academy of Sciences of the United States of America 104, 4401–4406.

Torres-Farfan C, Valenzuela FJ, Mondaca M, Valenzuela GJ, Krause B, Herrera EA, Riquelme R, Llanos AJ and Seron-Ferre M 2008. Evidence of a role for melatonin in fetal sheep physiology: direct actions of melatonin on fetal cerebral artery, brown adipose tissue and adrenal gland. Journal of Physiology 586, 4017–4027.

Tseng YH, Kokkotou E, Schulz TJ, Huang TL, Winnay JN, Taniguchi CM, Tran TT, Suzuki R, Espinoza DO, Yamamoto Y, Ahrens MJ, Dudley AT, Norris AW, Kulkarni RN and Kahn CR 2008. New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. Nature 454, 1000–1004.

Viengchareun S, Servel N, Feve B, Freemark M, Lombes M and Binart N 2008. Prolactin receptor signaling is essential for perinatal brown adipocyte function: a role for insulin-like growth factor-2. PLoS ONE 3, e1535.

Vigas M, Celko J and Koska J 2000. Role of body temperature in exercise-induced growth hormone and prolactin release in non-trained and physically fit subjects. Endocrine Regulation 34, 175–180.

Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerback S and Nuutila P 2009. Functional brown adipose tissue in healthy adults. The New England Journal of Medicine 360, 1518–1525.

Waterland RA and Jirtle RL 2003. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Molecular and Cellular Biology 23, 5293–5300.

Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR and Thompson CB 2009. ATP-citrate lyase links cellular metabolism to histone acetylation. Science 324, 1076–1080.

Whorwood CB, Firth KM, Budge H and Symonds ME 2001. Maternal undernutrition during early- to mid-gestation programmes tissue-specific alterations in the expression of the glucocorticoid receptor, 11b-hydroxysteroid dehydrogenase isoforms and type 1 angiotensin II receptor in neonatal sheep. Endocrinology 142, 1778–1785.

Wiesinger H, Heldmaier G and Buchberger A 1989. Effect of photoperiod and acclimation temperature on nonshivering thermogenesis and GDP-binding of brown fat mitochondria in the djungarian hamster phodopus s. Sungorus. Pflugers Arch 413, 667–672.

Wigglesworth JS 1974. Fetal growth retardation. Animal model: uterine vessel ligation in the pregnant rat. American Journal of Pathology 77, 347–350.

Yiallourides M, Sebert SP, Wilson V, Sharkey D, Rhind SM, Symonds ME and Budge H 2009. The differential effects of the timing of maternal nutrient restriction in the ovine placenta on glucocorticoid sensitivity, uncoupling protein 2, peroxisome proliferator-activated receptor-gamma and cell proliferation. Reproduction 138, 601–608.