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Symposium on 'Frontiers in adipose tissue biology'

Early programming of adipose tissue function: a large-animal perspective

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The emerging role of adipose tissue as a dynamic endocrine organ with an extent of anatomical and physiological plasticity has generated numerous studies linking early-life events with long-term alterations in adipose tissue structure and function. Coupled with increasing rates of human obesity, which cannot be explained without some genetic component, the role of early programming of adipose tissue may provide an insight into potential mechanisms. The developmental origins of health and disease hypothesis investigates the potential association between a compromised fetal and postnatal environment and later disease, such as obesity and type 2 diabetes, in the offspring. A number of animal models have been developed to examine potential mechanisms that drive these physiological changes, including rodent and large-mammal models that provide mechanistic insights into the epidemiological findings. *In utero* challenges such as under- or over-provision of nutrients, placental insufficiency and glucocorticoid infusion, as well as postnatal nutritional challenges, can all result in the long-term programming of adipose tissue abundance and function. A range of hormones, enzymes, transcription factors and other metabolic signalling molecules have been implicated in adverse adipose tissue development, including leptin, glucocorticoids, members of the PPAR family, fatty acid-binding proteins and adipokines. The long-term structural and physiological consequences associated with these molecular and cellular changes are less well described. The experimental models, potential mechanisms and regulators of the early programming of adipose tissue in large mammalian species will be summarised in the present review.

Growth: Nutrition: Maternal diet: Development

Developmental origins hypothesis

Programming is defined as a process through which exposure to environmental stimuli or insults during critical phases of development brings about permanent changes to the physiology or metabolism of the organism. The embryonic, fetal and early postnatal periods represent critical stages of development during which programming of body systems can occur[1]. Thus, there is great potential for the *in utero* and early postnatal environment to impact on future physiological and biochemical systems, including adipose tissue.

The link between birth weight and adult disease came initially from epidemiology highlighting that rates of CVD are highest in 'poorer' more-socially-deprived locations and in lower-income groups in the UK[2]. However, variations in adulthood lifestyle choices such as diet and smoking could not explain these differences in CVD. Investigation of the rates of infant mortality 60 years previously in the same geographical locations has demonstrated the highest infant mortality in the areas that 50 or 60 years later show the highest rates of CVD[2,3]. By inferring that infants in socially-deprived areas in the 1930s had experienced a suboptimal *in utero*

Abbreviations: DOHaD, developmental origins of health and disease; FABP, fatty acid-binding protein; UCP, uncoupling protein.
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or postnatal environment, particularly poor nutrition, a link was developed between an adverse intrauterine environment and the risk of CVD in adult life. Several subsequent large-scale epidemiological studies that used cohorts in Hertfordshire (UK), Preston (UK) and Helsinki (Finland) as well as analysis of historical data from the 1946 Dutch famine have confirmed that nutrient restriction during gestation increases the incidence of several disorders, including obesity[4–6]. Researchers investigating the developmental origins of health and disease (DOHaD) hypothesis concurrently began to study the potential associations between a suboptimal fetal and/or postnatal environment and have found that several pathologies such as obesity and diabetes are increased in the offspring[7,8]. The finding that suboptimal conditions in utero can lead to obesity will come as no surprise to farmers who have known for many years that 'runt' pigs grow more slowly and produce pork containing excess fat[9]. There are numerous experimental studies now investigating the impact of early-life events on later health.

As described in other presentations from the 'Frontiers in adipose tissue biology' symposium, over the past 15 years adipose tissue has emerged as a dynamic endocrine organ with an extent of anatomical and physiological plasticity[10,11]. Epidemiological and animal studies have determined that obesity and type 2 diabetes are pathologies programmed by adverse events in utero or in early postnatal life[4,12–14]; therefore, it is unsurprising that adipose tissue is a prime target. There are several features of adipose tissue that make it a highly-programmable tissue: plasticity and/or expansion capacity; cell number set in early life; insulin signalling; obesity; adipokine production; type 2 diabetes; energy storage and regulation; depot specificity.

Fat cell turnover

One of the most striking features of adipose tissue and its role in obesity is that cell number is set early in life. Recent work has demonstrated convincingly that fat cell number, but not volume, is set during childhood and adolescence[15]. Earlier work that informed the latter research had indicated that the development of fat depots of obese children differ both quantitatively and qualitatively from that of non-obese children of the same age[16]. Obese children display marked increases in fat cell size, up to non-obese adult values, early in life at a time when non-obese subjects display no change or reduced lipid content. These findings strongly suggest that the foundations for excessive adiposity are laid down during infancy. Furthermore, it is suggested that there are certain time intervals that have important consequences for ultimate adulthood cellularity and size of fat depots[16]. Continuation of the time course of adipose tissue study into adulthood has confirmed that adipose tissue cellularity is set in childhood and adolescence, as adipocyte number remains stable through adulthood[15]. Interestingly, cell number, but not volume, appears to be irreversible when weight is lost after bariatric surgery[15].

Animal models

In order to produce offspring in which to study adipose tissue, experimenters must produce a suboptimal in utero or postnatal environment. There are several different methodologies employed by researchers in the DOHaD field, including placental restriction (sheep, rodent), glucocorticoid infusion (sheep, rodent, avian species), nutritional manipulation (sheep, pig, rodent, non-human primate) and natural birth-weight variation (pig; for a review of these methodologies, see McMullen & Mostyn[17]). Such in utero challenges can result in the long-term programming of adipose tissue abundance and function. A range of hormones, enzymes, transcription factors and other metabolic signalling molecules have been implicated in adverse adipose tissue development, including leptin, glucocorticoids, members of the PPAR family, fatty acid-binding proteins (FABP) and adipokines[18,19]. For the purpose of the present review only evidence from two large-animal models, sheep and pig, will be considered.

Evidence from porcine studies

Low-, normal- and high-birth-weight pigs. There is overwhelming evidence from agriculture to demonstrate that small (or 'runt') piglets grow more slowly and become 'fat' adults[9,20]. In an investigation of a cohort of low-, middle- and heavy-birth-weight piglets up to 180 d postnatal age it was found that the low-birth-weight piglets grow more slowly but contain more perirenal adipose tissue and intramuscular fat than the middle-birth-weight piglets[21].

Several experimental studies have now compared the effects of physiological and biochemical outcomes in low- and normal-birth-weight piglets on adipose tissue in later life. Low-birth-weight piglets have been demonstrated to have significantly higher back fat at 12 months ($P<0.05$)[22]. However, despite the increase in fat depth, male pigs at 12 months of age have reduced plasma leptin and there are no associations between leptin and back fat[22]. Gender-specific effects of birth weight and other in utero and postnatal challenges are a common feature in DOHaD studies, with many experimental findings being gender, and also age, specific. The same cohort of low-birth-weight pigs have impaired glucose tolerance at 12 months of age[21].

Although the early epidemiological studies of DOHaD focused on nutrient restriction and/or socio-economic background linked to a low birth weight, offspring with high birth weight may also be at risk from obesity and other diseases in later life; the 'U'-shaped curve[23]. On this basis, adipose tissue from piglets with low, normal or high birth weights were investigated on days 7 and 14 of age (neonatal period). It was found that uncoupling proteins (UCP) 2 and 3, which have a number of cellular roles including energy regulation and inflammation, are reduced in adipose tissue from low-birth-weight piglets on day 7, but not day 14[24]. In addition, UCP3 is positively related to plasma leptin in normal-sized pigs but negatively correlated in low- and high-birth-weight piglets on day 14, suggesting reduced activation[25]. Although the study only investigated the neonatal effects of low birth weight, a loss
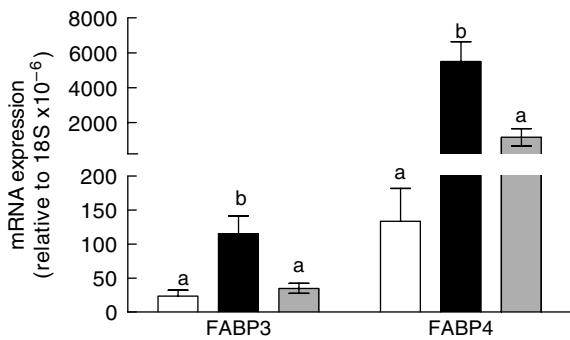
Fig. 1. Adipose tissue gene expression on day 7 of postnatal age in low-body-weight (□), normal-body-weight (■) and high-body-weight (▨) piglets. Values are means with their standard errors represented by vertical bars for five animals per group. [a,b]Means with unlike superscript letters were significantly different for each gene ($P<0.05$). BW, birth weight; FABP, fatty acid-binding protein. (Adapted from Williams et al.[26].)
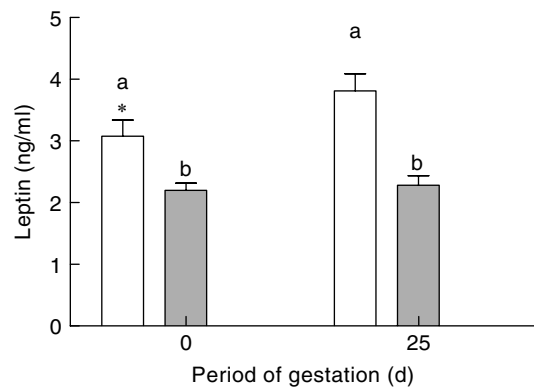

Fig. 2. Serum leptin concentrations in two breeds of pregnant sow: Meishan–Landrace (□); Yorkshire–Landrace (▨). Values are means with their standard errors represented by vertical bars. [a,b]Means with unlike superscript letters were significantly different between breeds ($P<0.05$). Mean value was significantly different from that at 25 d of gestation: *$P<0.05$. (Adapted from Guay et al.[37].)

of the relationship between leptin and UCP3 may promote adipose tissue deposition. However, at this early stage of development there is no difference in adipose tissue TAG content. Low-birth-weight piglets do possess markedly more adipocytes[26], in keeping with the earlier findings[15] and suggesting an increased capacity for adipose tissue expansion and lipid accumulation. FABP3 and 4 (Fig. 1) and PPARγ are down regulated in low- and high-birth-weight piglets on day 7, but not day 14 in adipose tissue. Conversely, FABP3 and 4 are down-regulated in low- and high-birth-weight piglets in skeletal muscle on day 14. The reduced expression of PPARγ2 in adipose tissue of the low- and high-birth-weight piglets suggests delayed adipocyte development. This finding is partly confirmed by histological data, as the low-birth-weight piglets, but not the high-birth-weight piglets, have more adipocytes per visual field on day 7[26]. The reduction in FABP4, the 'adipose tissue'-specific FABP, may be protective against the development of metabolic syndrome, as a number of recent studies suggest FABP4 is central to its onset through interaction with the insulin signalling and inflammatory pathways[27–29]. A protective effect in this case seems unlikely, given the well-described 'U'- or 'J'-shaped relationship between birth weight and obesity and the metabolic syndrome in human studies[23,30], but cannot be excluded because these animals have not been studied into adulthood.

The Meishan breed. Early programming of adipose tissue in the Meishan breed of pig may provide protection against hypothermia and hypoglycaemia in this ancient oriental breed. The comparatively low birth weight of Meishan piglets compared with commercial lean breeds, associated with a high litter number, is not detrimental to offspring survival as the mortality rate is lower than that of commercial breeds[31]. Adipose tissue PPARγ expression during the first week of life is reduced in Meishan piglets compared with that of piglets of a commercial breed[32] and the expression of adiponectin and its receptor (adiponectin receptor 1) are below the level of detection at ≤21 d of age[33]. A greater percentage of plurilocular adipocytes has been reported in Meishan piglets at ≤30 d

of age compared with a lean breed[34]. Taken together these findings suggest that adipose tissue development is delayed in Meishan piglets, which may have a beneficial impact on nutrient partitioning and energy regulation during the neonatal period. Although older but prepubertal Meishan pigs (i.e. aged 80 d) have increased lipogenic potential compared with Large White pigs, the opposite has been found after puberty at 100 d, when several fat depots exhibit less lipogenic potential[35]. These findings suggest that the mechanisms responsible for high fat accretion occur after weaning but before puberty[34,36].

Interestingly, milk leptin of Meishan sows is lower than that of commercial sows, suggesting that in the pig milk leptin does not reflect maternal fat stores or serum leptin[37,38]. Milk leptin is, however, positively related to piglet growth rate, girth and body, gut, heart and spleen weight in Meishan piglets, suggesting a growth-promoting effect[38]. Despite milk leptin not reflecting adiposity, serum leptin is associated with backfat thickness in early pregnancy in Meishan–Landrace sows and increases significantly ($P<0.05$) up to day 25 of gestation (Fig. 2), when leptin receptor (total and long form) mRNA expression in homogenates prepared from whole fetuses is higher in Meishan–Landrace pigs than in Yorkshire–Landrace pigs[37].

The *in utero* factors that regulate Meishan adipose tissue are as yet unknown, but may reflect differences in glucocorticoid signalling[39,40]. Plasma cortisol concentrations of Meishan fetuses are 30% greater than those of age-matched white cross-bred (Yorkshire × Landrace × Large White × Chester White) fetuses, despite similar plasma adrenocorticotropin and cortisone[39]. At 6 weeks old Meishan pigs exhibit higher circulating cortisol concentrations under basal conditions yet demonstrate a reduced response to a stressful experience[40]. Furthermore, 7-week old Meishan piglets exhibit an enhanced response to adrenocorticotropin infusion compared with Large White piglets[41]. These increased basal and stimulated cortisol concentrations are likely to be a result of differential regulation in the Meishan adrenal gland, particularly
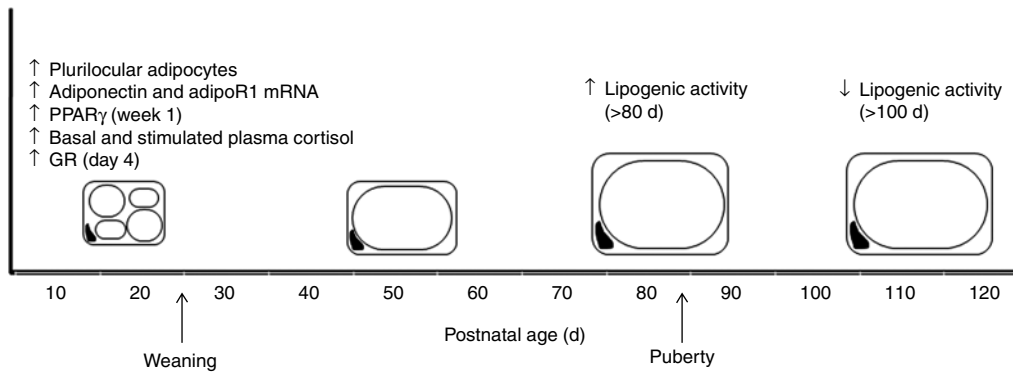
Fig. 3. Summary of early-life differences in adipose tissue of Meishan pigs in comparison with a typical 'commercial' lean porcine breed[34,37,38,62]. ↑, Increased; ↓, decreased; GR, glucocorticoid receptor; adiporR1, adiponectin receptor 1.
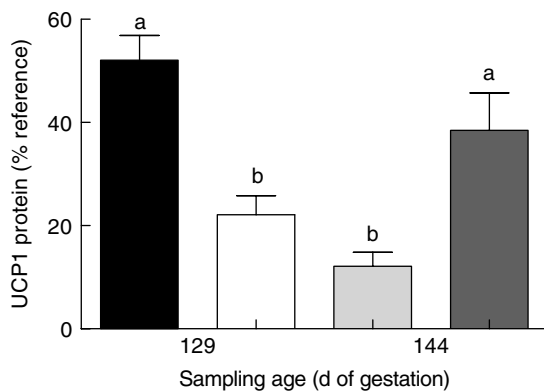

Fig. 4. Uncoupling protein (UCP) 1 protein abundance in perirenal adipose tissue from fetal sheep that were infused for 5 d with cortisol (■) or saline (9 g NaCl/l; □), adrenalectomised (Ax; ▨) or sham-operated (sham; ▨). Values are means with their standard errors represented by vertical bars. [a,b]Means with unlike subscript letters were significantly different between groups (P<0·05). (Adapted from Mostyn et al.[47]).

Fig. 5. Plasma insulin (ng/ml; □) and NEFA (mmol/l; ▨) from offspring of nutrient-restricted (NR) or control ewes that were reared in an obesogenic postnatal environment. Values are means with their standard errors represented by vertical bars. [a,b]Means with unlike subscript letters were significantly different between groups (P<0·05). (Adapted from Sebert et al.[53].)

via regulators of steroidogenesis[41]. The reported increased plasma cortisol levels, along with greater glucocorticoid receptor gene expression observed in adipose tissue from 4-d-old Meishan piglets, may represent a potential for greater lipid uptake[38]. Fig. 3 summarises the early-life programming of adipose tissue in the Meishan breed.

Evidence from ovine studies

A number of *in utero* and postnatal challenges have been utilised to investigate the early-life programming of adipose tissue, including glucocorticoid infusion, suboptimal *in utero* nutrition (either through dietary or placental manipulation) and a postnatal obesogenic environment.

Glucocorticoid administration. Glucocorticoid infusion has been utilised in many studies of the DOHaD hypothesis, in particular in small animals in which a suboptimal maternal diet is linked to overexposure of the fetus or neonate to glucocorticoids[42–44]. However, the effect of a suboptimal maternal diet in a large-animal species has divergent responses in relation to maternal and offspring
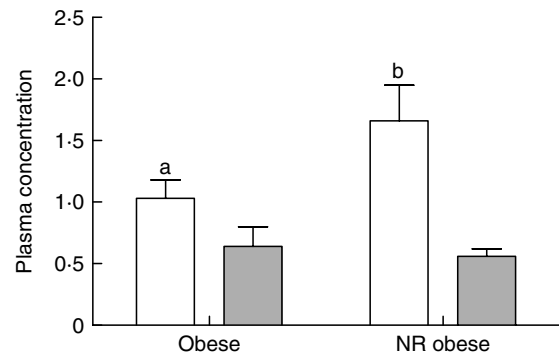
glucocorticoid concentrations, which are dependent on the type and timing of the nutritional challenge[42]. Studies of the effects of late-gestational glucocorticoid infusion on the growth of the fetus have demonstrated that the prepartum cortisol surge may be responsible for the normal decline in fetal growth rate observed towards term in the sheep[45]. Furthermore, glucocorticoid infusion has a pronounced impact on adipose tissue in the sheep when delivered near term. Maternal administration of the synthetic glucocorticoid dexamethasone on day 138 of gestation (term is approximately 147 d) enhances UCP1 abundance in perirenal adipose tissue (the most abundant adipose tissue depot in the fetal and newborn lamb) as well as thermogenic potential in prematurely-delivered sheep (day 140 of gestation)[46]. Infusing cortisol for 5 d to near-term (day 129 of gestation) fetal sheep produces an increase in UCP1 protein and UCP2 gene expression in perirenal adipose tissue. These early-life programming effects increase protein and mRNA for UCP1 to values comparable with those of a fetus at day 144 of gestation (Fig. 4), confirming another role for cortisol in the preparation of fetal adipose tissue for life after birth[47].

In utero dietary challenges. Suboptimal nutrition during gestation has been demonstrated in several species to
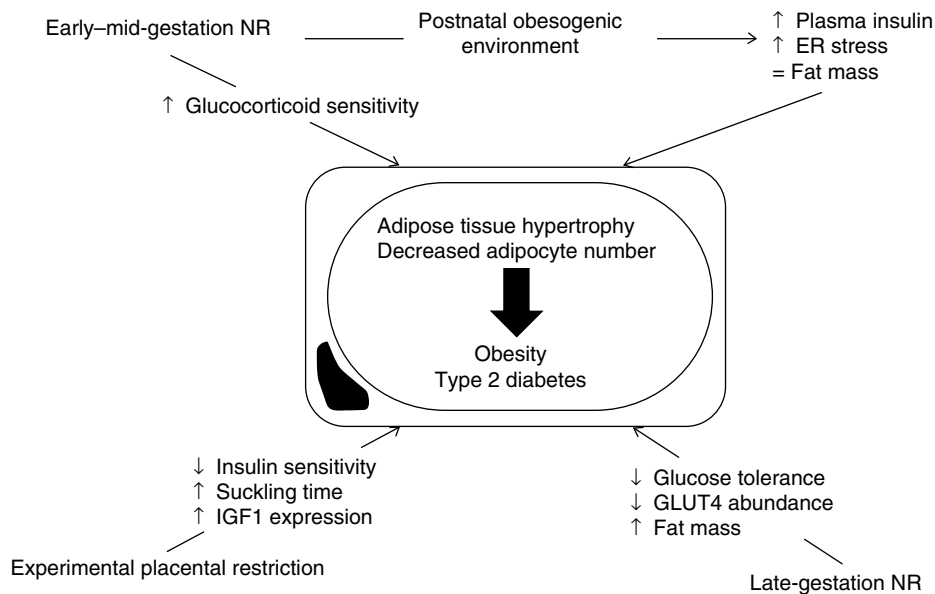
Fig. 6. Summary of adipose tissue programming effects in sheep subject to suboptimal *in utero* nutrition (either through dietary or placental manipulation) or a postnatal obesogenic environment in comparison with control animals[49,52,54,59,63]. ↑, Increased; ↓, decreased; = , similar; NR, nutrient-restricted; ER, endoplasmic reticulum; IGF1, insulin-like growth factor 1.

impact on offspring adiposity and can range from over- or undernutrition to placental restriction.

Studies of maternal nutritional restriction have played a substantial role in DOHaD research; many have demonstrated effects on adipose tissue[19]. For example, offspring sampled at day 140 of gestation from ewes that have experienced nutrient restriction during early–mid gestation (day 28–day 80 of gestation), which is coincident with the period of maximal placental growth, exhibit increased perirenal adipose tissue at birth[48]. This adipose tissue also displays alterations in glucocorticoid signalling at day 140 of gestation and at 6 months postnatally[49]. Gene expression of the glucocorticoid receptor and 11β-hydroxysteroid dehydrogenase 1 are up regulated in perirenal adipose tissue from offspring of sheep that have experienced suboptimal nutrition during early–mid gestation; however, 11β-hydroxysteroid dehydrogenase 2 (which inactivates cortisol to cortisone) is reduced[49]. Cortisol has consistently been implicated in both obesity and the metabolic syndrome, which is predictable given the physiological traits observed in Cushing's syndrome[50]. However, there is little evidence for raised plasma cortisol in obesity[51]. Although plasma cortisol *per se* is not consistently elevated with increased fat mass in human subjects, tissue sensitivity to glucocorticoids is increased via alterations in the enzymes and receptors that regulate local levels[49]. Tissue sensitivity to cortisol is regulated by the enzyme 11β-hydroxysteroid dehydrogenase and the glucocorticoid receptor. Taken together these results suggest that these offspring, who have experienced suboptimal nutrition *in utero* early in gestation, may be at a higher risk of developing obesity and thus metabolic syndrome, which is in keeping with human findings from epidemiological studies[6]. The findings of increased glucocorticoid sensitivity in these ovine offspring are similar to those observed

in Meishan piglets, which are known to develop into obese adults.

In contrast, maternal nutrient restriction during late gestation (day 110–day147 of gestation), which is coincident with the period of maximal fetal growth and a parallel rise in fetal fat depots, results in reduced adiposity in the offspring. At days 1 and 30 postnatally offspring adipose tissue mRNA expression of glucocorticoid receptor and 11β-hydroxysteroid dehydrogenase 1 is lower and that of 11β-hydroxysteroid dehydrogenase 2 is higher in the nutrient-restricted group[49]. This adaptation may initially be protective against the development of visceral obesity or may simply represent a response to the reduced nutrient supply. However, the offspring have greater relative fat mass and impaired glucose tolerance at 1 year, which is associated with a reduction in GLUT4 protein abundance in adipose tissue[52]. Again, this animal work complements the findings of human epidemiological studies. The Dutch Famine data highlighted that nutrient restriction during discrete periods of gestation leads to differential effects on body systems, with late-gestational nutrient restriction affecting intermediary metabolism (in particular glucose–insulin homeostasis) and increasing the risk of the offspring developing type 2 diabetes[6].

Postnatal dietary challenges. The influence of a secondary 'insult' on ovine offspring who have experienced maternal nutrient restriction during early–mid gestation has been investigated by restricting offspring physical activity and providing *ad libitum* feed. From weaning to 1 year of age offspring from control and nutrient-restricted sheep were raised in an obesogenic environment that produces a 60% reduction in physical activity compared with pasture-reared sheep[53,54]. At 1 year of age offspring that have experienced *in utero* nutrient restriction have a total body, subcutaneous and visceral fat weight similar to that of

the animals born of control-fed mothers and reared in the obesogenic environment. The nutrient-restricted offspring do, however, exhibit a reduction in daily food intake when individually housed, an adaptation that is accompanied by increased plasma insulin, but not NEFA (Fig. 5). Indeed, raised plasma insulin may represent the primary adaptation promoting excess storage of nutrients in these offspring.

There is evidence to suggest that metabolic syndrome and obesity disrupt endoplasmic reticulum function and cause protein misfolding, which can trigger the misfolded protein response, as documented by several reviews linking tissue stress responses to the metabolic syndrome[55,56]. In an attempt to alleviate stress within the endoplasmic reticulum, insulin signalling pathways are inhibited through activation of c-Jun N-terminal kinase 1[57], which impairs systemic glucose regulation[56]. Emerging evidence from the obese offspring born to nutrient-restricted mothers demonstrates an increase in phosphorylated c-Jun N-terminal kinase protein in perirenal adipose tissue, suggesting a down-regulation of insulin signalling[54]. Several other aspects of the unfolded protein response, inflammation and infiltration of pro-inflammatory macrophages are enhanced in obese offspring born to nutrient-restricted mothers[54]. This outcome may represent an *in utero* programming effect whereby total adipocyte number is reduced, thus increasing its susceptibility for cellular hypertrophy[58].

Experimental placental restriction. Experimental restriction of placental growth results in reduced birth weight, increased early postnatal growth and increased adiposity in the offspring at 6 weeks of age[59] and impairs insulin sensitivity. Feeding behaviour is also reset at 2 weeks of age, at least in the 90 min after 1 h of fasting, when placental-restriction offspring exhibit a longer suckling time, which is predictive of catch-up growth and increased central adiposity, at least up to 6 weeks of age. The molecular adaptations that produce these physiological responses in response to placental restriction may be set in fetal life. A reduction in insulin-like growth factor 1 and leptin mRNA expression has been demonstrated in perirenal adipose tissue from placental-restriction fetuses at day 140–day 145 of gestation[60], which could impair adipocyte proliferation and differentiation, thereby potentially increasing their susceptibility for hypertrophy in later life[58]. A reduction in leptin may simply reflect a reduction in lipid stores of the fetal perirenal adipose tissue[61]. Together these findings suggest that offspring from an ovine model of intrauterine growth restriction display features that may predispose to obesity in later life, but to date have not demonstrated any long-term complications.

The various models of early-life programming of adipose tissue in the sheep are summarised in Fig. 6.

Conclusion

In summary, suboptimal conditions *in utero* have the potential to alter adipose tissue development and impact on long-term function and health of the offspring. The evidence from large-animal studies can inform both animal and clinical science; for example, runt piglets that have been cross-fostered to obtain greater milk intake become fatter than runts who remain with their litter[9], highlighting the problems of rapid postnatal growth in low-birth-weight offspring. The molecular differences in Meishan piglets that provide improved neonatal mortality may provide an insight into potential strategies for improving survival in other breeds. Given that discrete periods of gestational nutrient restriction have been shown to have negative impacts on future adipose tissue function, maternal diet during pregnancy should be optimised to meet fetal requirements and avoid adverse effects in later life. Research into the emerging role of adipose tissue as a dynamic and endocrine organ is likely to yield further evidence for early programming of this tissue.

Acknowledgements

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