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The influence of maternal protein nutrition on offspring development and metabolism: the role of glucocorticoids

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The consequences of sub-optimal nutrition through alterations in the macronutrient content of the maternal diet will not simply be reflected in altered neonatal body composition and increased mortality, but are likely to continue into adulthood and confer greater risk of metabolic disease. One mechanism linking manipulations of the maternal environment to an increased risk of later disease is enhanced fetal exposure to glucocorticoids (GC). Tissue sensitivity to cortisol is regulated, in part, by the GC receptor and 11 β -hydroxysteroid dehydrogenase (11 β -HSD) types 1 and 2. Several studies have shown the effects of maternal undernutrition, particularly low-protein diets, on the programming of GC action in the offspring; however, dietary excess is far more characteristic of the diets consumed by contemporary pregnant women. This study investigated the programming effects of moderate protein supplementation in pigs throughout pregnancy. We have demonstrated an up-regulation of genes involved in GC sensitivity, such as GC receptor and 11 β -HSD, in the liver, but have yet to detect any other significant changes in these piglets, with no differences observed in body weight or composition. This increase in GC sensitivity was similar to the programming effects observed following maternal protein restriction or global undernutrition during pregnancy.

Maternal protein: Glucocorticoids: Liver: Programming: Pigs

Maternal nutrition and programming of adult disease

It is now well established that a sub-optimal environment *in utero* can have pronounced effects on the development of the fetus and thus confer greater risk of disease in later life. The process whereby a stimulus or insult at a sensitive or critical period of development has long-term effects is termed programming. This was first investigated by the

retrospective cohort studies of Barker *et al.* during the late 1980s who established that individuals with low birth weight, who were short or thin at birth, or who were small in relation to the placental size, were at increased risk of metabolic disease such as hypertension and impaired glucose tolerance in adulthood^(1,2).

It is presumed that low birth weight and disproportionate body size are indicative of a lack of nutrients and/or

Abbreviations: 11 β -HSD, 11 β -hydroxysteroid dehydrogenase; GC, glucocorticoid; GR, glucocorticoid receptor.

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oxygen during gestation, reflecting adaptations that the fetus has made to sustain its normal development⁽³⁾. The highest prevalence of impaired glucose tolerance and diabetes was seen in individuals who were lean at birth and became obese as adults suggesting that it is actually the mismatch between the pre- and postnatal environment that causes these effects⁽²⁾. This is further supported by later studies using the sheep as a model for maternal under-nutrition, which have shown similar detrimental outcomes on the metabolism of the offspring who are adequately nourished, with or without changes in birth weight⁽⁴⁻⁶⁾.

Animal models for nutritional programming

Animal models are required to examine the underlying physiological, biochemical and molecular mechanisms behind the nutritional programming of offspring disease in a manner that would be unethical in human subjects. One advantage of such models is that the effect of nutrition can be assessed independently of confounding factors such as genetics, other environmental factors and social status under precisely controlled conditions.

Various species have been used as models to study the effects of nutrition on fetal programming, the most common being the rat and the sheep⁽⁷⁾. Although rodents are small, inexpensive and are ideal in multivariate experiments, there are numerous differences between rodents and human subjects. One of the major limitations is that rodents are altricial animals, born with an undeveloped brain and endocrine system, with significant maturation of organs during the weaning period.

Due to the differences in the digestive system of sheep and human subjects (sheep are ruminants), sheep have been used primarily to investigate the effects of global undernutrition during pregnancy rather than specific macronutrient manipulations such as low protein or high fat. In contrast to the rat, sheep have a similar rate of pre- and postnatal growth to human subjects, and only produce one or two offspring, weighing between 3 and 6 kg, not unlike human subjects⁽⁷⁾.

Primates are the ideal animal models due to their similarities to human subjects, but long lifespan, expensive housing and ethical considerations limit their use⁽⁸⁾.

In recent years, the pig has been more widely used as an animal model for human disease and is particularly useful for nutritional studies because of the similarities to human subjects in terms of the physiology and anatomy of the digestive system⁽⁹⁻¹¹⁾. The digestive functions of each segment of the gastrointestinal tract are similar with comparable enzyme activities and organ secretions⁽¹¹⁾. In addition, the endocrine and paracrine control of gastrointestinal tract growth, motility and overall function, appear to be similar. Finally, both pigs and human subjects are able to utilise some fibre as a source of energy due to the fermentation that occurs in the large intestine⁽¹¹⁾.

Nutrient requirements during infancy, growth, reproduction and lactation are similar between man and pig⁽¹⁰⁻¹²⁾. In addition, the neonatal pig is comparable to the human infant with respect to the stage of development and function of several organ systems. The large litter size,

which allows for multiple comparisons based on birth weight, and the high postnatal growth rate also makes the pig an attractive candidate for nutritional intervention studies.

Low-protein maternal diets

Following the findings by Barker *et al.*⁽¹⁻³⁾, proof of principle was required in order to establish the cellular and molecular mechanisms behind programming effects. Due to the importance of protein in growth and development, many studies, particularly in rats, focused on the effects of maternal protein restriction on the offspring.

Protein restriction during pregnancy in rats has been shown in some studies to produce low-birth-weight offspring with higher blood pressure in early adulthood⁽¹³⁻¹⁵⁾. In addition, in the longer term, these offspring exhibit reduced insulin sensitivity and perturbed TAG metabolism, as indicated by raised plasma cholesterol and TAG, although birth weight was unaffected⁽¹⁶⁾. Interestingly, these changes have been demonstrated to take place despite no alterations in overall energy intake of the mothers^(14,16), or differences in body weight or fat mass of the offspring⁽¹⁶⁾.

The role of glucocorticoids

The precise mechanisms linking maternal malnutrition, particularly low-protein diets, to metabolic disease in the offspring are still unknown. However, studies in both sheep and rats have strongly suggested that glucocorticoids (GC) play a key role^(13,15,17). It has been proposed that fetal overexposure to maternal GC may trigger programming events *in utero* that establish persistent increases in GC hormone action in the offspring throughout life, although this is yet to be consistently established^(15,17).

GC excess has been linked to the clinical observations associated with the metabolic syndrome^(18,19). For example, patients with Cushing's disease who have increased secretion of cortisol, normally due to a pituitary tumour, can develop abdominal obesity, hypertension, hyperlipidaemia and insulin resistance⁽¹⁸⁾. Also, clinical administration of GC to treat acute and chronic inflammatory diseases has been associated with similar adverse metabolic effects⁽¹⁹⁾. It is therefore possible that programmed alterations in GC sensitivity may play a role linking maternal nutrient availability, fetal growth and metabolic disease risk.

Cortisol, the principal GC in human subjects, sheep and pigs, but not rodents, is regulated by the activity of the hypothalamic-pituitary-adrenal axis, a neuro-endocrine feedback loop, and is secreted in response to stress or low levels of circulating cortisol (Fig. 1)⁽²⁰⁾. Individual sensitivity to GC are highly regulated at a tissue level by intracellular expression of the GC receptor (GR), and the enzymes 11 β -hydroxysteroid dehydrogenase (11 β -HSD) types 1 and 2 at the level of gene transcription (Fig. 1)⁽²⁰⁾. The two isoforms of 11 β -HSD are located in the endoplasmic reticulum and are responsible for the tissue-specific inter-conversion of the less biologically active

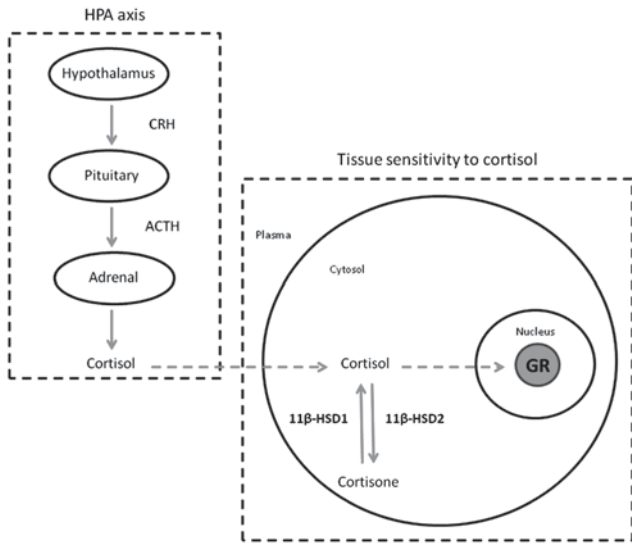


Fig. 1. Diagrammatic representation of cortisol secretion via the hypothalamic–pituitary–adrenal (HPA) axis and its regulation at a tissue-specific level. CRH, corticotrophin releasing hormone; ACTH, adrenal corticotrophic hormone; 11β-HSD, 11β-hydroxysteroid dehydrogenase; GR, glucocorticoid receptor.

cortisone to cortisol. 11β-HSD1 behaves as an 11-oxo-reductase, catalysing the conversion of cortisone to bioactive cortisol⁽²¹⁾. Conversely 11β-HSD2 acts as an 11-oxo-dehydrogenase and catalyses the opposite reaction⁽²¹⁾. The two isozymes are products of two different genes and have distinct tissue distributions, with 11β-HSD1 expressed primarily in the liver, adipose tissue, kidney and brain, and 11β-HSD2 mainly in the kidney and salivary glands^(22,23).

11β-HSD2 is highly expressed in fetoplacental tissues and is thought to play a key role in protecting the fetus from overexposure to maternal cortisol^(13,17,24–26). In the rat, the effects of the maternal low-protein diet on reducing offspring birth weight and programming of hypertension and dysregulation of glucose metabolism are potentially mediated by the inhibition of placental 11β-HSD2^(13,15,16,27). This is also observed in human studies where a positive relationship between placental 11β-HSD2 activity and fetal weight has been identified⁽²⁵⁾.

Studies in both rats and sheep have shown that maternal diet programmes increased GC sensitivity at a tissue-specific level in both the fetal, neonatal and adult offspring, probably due to the reduction in placental 11β-HSD2^(15,17). In sheep, GR expression is increased in the adrenals, liver, lungs, perirenal adipose tissue and kidney of neonatal offspring born to ewes which were nutrient restricted during early-mid-gestation (Table 1)⁽¹⁷⁾. In addition, there was a 50% reduction in 11β-HSD2 expression in all tissues in which this key enzyme was found to be abundant, such as in the kidneys and adrenals⁽¹⁷⁾. 11β-HSD1 expression was unaffected by maternal diet, except in perirenal adipose tissue, where there was a 2-fold increase in mRNA abundance⁽¹⁷⁾. Importantly, these effects were observed without any significant alterations in the fetal metabolic or endocrine environment^(28,29). Similar findings were shown in a study on rats in which dams were protein restricted throughout gestation (Table 1)⁽¹⁵⁾. These offspring were

Table 1. Summary of effects of a maternal low-protein diet or nutrient restriction during pregnancy on offspring development

Source	Species and diet composition	Placenta		Offspring			Phenotype
		Size	11β-HSD2	Birth weight	Age analysed	GC sensitivity	
Bertram <i>et al.</i> ⁽¹⁵⁾	Rats throughout pregnancy C: 18% protein LP: 9% protein	n/a	↓	↓	Birth to 5 months	↑GR (peripheral tissues) ↓11β-HSD2 (kidney)	↑ Blood pressure
Ehruma <i>et al.</i> ⁽¹⁶⁾	Rats throughout pregnancy C: 18% protein LP: 9% protein	n/a	n/a	n/a	1, 19 and 18 months	n/a	No difference in BW ↑ Insulin resistance ↑ Liver TAG
Langley-Evans <i>et al.</i> ^(13,14)	Rats throughout pregnancy C: 18% protein LP: 9% protein	↑	↓	↓	7 weeks	n/a	No difference in BW ↑ Blood pressure ↑ Kidney weight
Whorwood <i>et al.</i> ⁽¹⁷⁾	Sheep, days 28–77 of gestation C: 110% requirements NR: 50% requirements	↑	↓	No difference	Fetal (day 77) and birth	↑GR and ↓11β-HSD2 (peripheral tissues)	

C, control; LP, low-protein; ↓, decreased in low-protein or nutrient-restricted gestational groups; GR, glucocorticoid receptor; 11β-HSD, 11β-hydroxysteroid dehydrogenase; ↑, increased in low-protein or nutrient-restricted gestational groups; BW, body weight; NR, nutrient restricted.

shown to have decreased expression of 11 β -HSD2 in the kidney from birth until adulthood (5 months of age), with no effect on 11 β -HSD1 expression⁽¹⁵⁾. In addition, GR mRNA and protein expression were increased in peripheral tissues, such as the kidneys and lungs, from both late fetal (day 20) and neonatal offspring up to 12 weeks of age⁽¹⁵⁾. Therefore, this suggests that an increase in GC sensitivity in the offspring due to sub-optimal maternal nutrition is associated with an increased risk of metabolic disease^(15,17).

High-protein diets

Despite the tendency for dietary protein intake to exceed recommended values in the western world, particularly in younger women⁽³⁰⁾, investigations into the effects of a high-protein diet during pregnancy on offspring development are limited^(31–35). At present, there is insufficient evidence in human subjects that a high protein intake or protein supplementation during pregnancy affects offspring birth weight or postnatal growth due to conflicting outcomes of epidemiological studies^(31,32).

In rats, a reduction in offspring birth weight, similar to that observed with protein restriction, has been demonstrated when dams were fed an isoenergetic, high-protein diet (40%) throughout gestation⁽³³⁾. These offspring showed accelerated postnatal growth and by the age of 9 weeks had increased fat mass in comparison with those born to mothers fed an adequate amount of protein (20%) during gestation⁽³³⁾. In contrast, other rat studies with similar levels of protein supplementation, have not demonstrated any effects on offspring birth weight^(34,35), but have shown an increase in blood pressure of the male pups by 4 weeks of age⁽³⁴⁾.

We have previously reported that maternal protein supplementation throughout pregnancy in the sow, increases offspring pre-weaning mortality with no effects on litter size or piglet birth weight⁽³⁶⁾. The reasons for this increase in mortality are unclear, but it could be linked to constipation in sows because of the reduced fibre content of the protein supplemented diets, through the removal of sugarbeet pulp, enabling the diets to be balanced for energy. Constipation in sows is a common problem in the pig industry and has been associated with a number of adverse outcomes including farrowing problems, mastitis and failure of milk let down, all of which could increase piglet mortality⁽³⁷⁾.

Despite some similarities between maternal protein restriction and supplementation investigations in rats^(33,34), our recent study was the first to investigate the effects of protein supplementation (16.3% v. 12.3% control) on GC sensitivity. The study demonstrated that offspring born to sows fed a protein-supplemented diet throughout pregnancy exhibited an increase in gene expression of both GR and 11 β -HSD1 in the liver at 1 week⁽³⁸⁾ and 6 months of age (K Almond, P Bikker, M Lomax, M E Symonds and A Mostyn, unpublished results), suggesting that these animals were more sensitive to GC. These results are similar to the effects observed in offspring born to mothers who were protein restricted or under-nourished during pregnancy^(15,17). It is possible that this is due to enhanced transfer of maternal GC to the fetus, resulting in an

increase in GC sensitivity in the peripheral tissues of the offspring^(15,17). In addition to the increase in both GR and 11 β -HSD1 gene expression with maternal protein supplementation, the liver weight (taken as a percentage of body weight) of these animals were increased at 6 months of age (K Almond, P Bikker, M Lomax, M E Symonds and A Mostyn, unpublished results). The reasons for this are as yet unclear, and investigations are still ongoing, as both lipid and glycogen content were unaffected by maternal diet (K Almond, P Bikker, M Lomax, M E Symonds and A Mostyn, unpublished results).

Despite these differences in liver weight and gene expression, at present, no other differences or adverse health effects have been demonstrated in the variables measured in these offspring at 6 months of age.

Conclusions

The consequences of sub-optimal nutrition through alterations in the macronutrient content of the maternal diet will not simply be reflected in altered neonatal body composition and increased mortality, but are likely to continue into adulthood and confer greater risk of metabolic disease. The mechanisms behind this nutritional programming are beginning to be elucidated, with particular focus on GC action. The liver is of key importance in these studies due to its primary role in metabolism and maintaining whole body energy balance.

This study investigated the programming effects of protein supplementation in pigs throughout pregnancy. We have demonstrated an up-regulation of genes involved in GC sensitivity, such as GR and 11 β -HSD1, in the liver, but have yet to detect any other significant changes in these piglets, with no differences observed in body weight or composition. This increase in GC sensitivity was similar to the programming effects observed following maternal protein restriction or global undernutrition during pregnancy^(15,17). Taken together, these findings suggest that the type of nutritional insult pregnancy is not important and that maternal under- and over-nutrition may cause similar programming effects. Therefore, these findings could have important implications in determining the programming effects of maternal diet on human disease risk.

The importance of these findings for the pig industry are not clear, as no phenotypic differences were observed between offspring of protein supplemented, compared to control fed mothers; however, protein supplementation significantly increased offspring mortality. It is currently unknown whether meat quality would be affected by the alterations in maternal dietary protein, although further work is currently being carried out to investigate the effects on muscle quality to validate this. However, this study may have important implications if these offspring were to become breeding stock and were allowed to grow and develop past 6 months of age.

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