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PROCEEDINGS

Metabolic programming in pregnancy: studies in animal models

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The developmental origins of adult disease hypothesis

Epidemiological studies of many large populations indicate that non-communicable diseases in adulthood are related to factors in fetal life or during infancy [1]. Low birthweight or disproportion at birth are associated with increased risk of cardiovascular disease and type-II diabetes. These associations have been explained in terms of developmental programming, which is the process through which insults or stimuli during early life exert permanent effects upon organ development, physiology and metabolism [18]. Variation in the nutrient supply during fetal has been proposed as the major programming stimulus that determines risk of disease in adulthood.

Studies of human cohorts have mainly required the use of retrospective cohorts and this has raised serious issues regarding control for confounding factors, selection bias and measurement bias [11]. Moreover, within relatively well-nourished populations variation in maternal nutrient intakes are noted to have little impact upon patterns of fetal growth or birthweight [17], which has led some observers to question the plausibility of the developmental origins of adult disease hypothesis. Experimental studies employing small animal species (e.g. rat, mouse or guinea pig) or larger species (pig and sheep) have, however, clearly demonstrated the biological plausibility of nutritional programming [25]. A diverse range of nutritional manipulations in pregnancy have been shown to programme profound changes in tissue morphology, physiology and metabolism and often these changes occur independently of fetal growth retardation [25].

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Animal models of cardiovascular programming

Although the approaches used to manipulate the maternal diet in order to test the developmental programming hypothesis have been varied, cardiovascular changes in the resultant offspring are a near universal outcome. Global nutrient restriction during pregnancy (controlled reductions of maternal nutrient intake) in rodents [36] and in sheep [9] produces small changes in blood pressure in the adult offspring, which often only manifest at maturity. Similar delays in the appearance of blood pressure changes are noted with the feeding of high-saturated fat diets [15] or iron deficient diets [8] to pregnant rats. In contrast the marked increase in blood pressure and variation in vascular reactivity to vasoconstrictors and relaxants that follows intrauterine protein restriction [7, 16] tends to manifest very early in the postnatal period (Fig. 1).

One of the major drawbacks of working with rodent species is the fact that these are generally resistant to the development of atherosclerosis. Thus, whilst nutritional programming of cardiovascular risk factors is demonstrable, the development of coronary heart disease is difficult to model in animals. Ongoing studies with transgenic lines which develop vascular lesions in response to high fat-high cholesterol diets, for example the apo E*3 Leiden strain [10], will yield important data in this area over the next few years

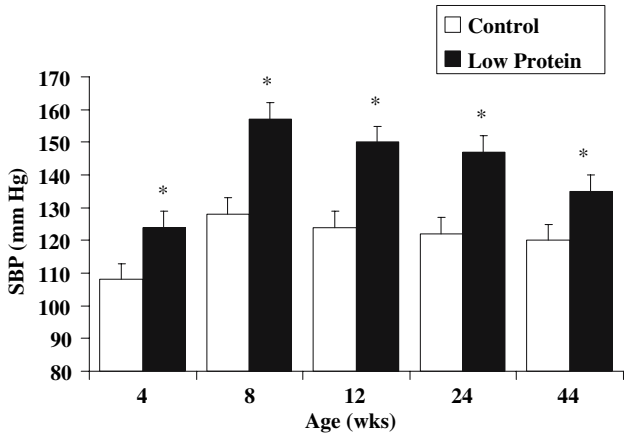

Fig. 1 Systolic blood pressure (*SBP*) of rats exposed to a maternal low-protein diet in utero. Fetal undernutrition promotes a significant elevation of blood pressure that is manifest from as early as weaning (4 weeks) and which persists throughout the life of the animal. $*P < 0.05$ between groups

Glucose intolerance and insulin resistance

A number of studies have investigated the impact of manipulating the maternal diet upon glucose metabolism in the associated offspring, and these studies indicate that there are sensitive periods for programming in both the prenatal and suckling periods. Studies of the effects of low-protein diets generally find that the response to a glucose load is enhanced in young adult animals, but that with ageing insulin resistance develops [29]. High-fat diets in pregnancy also programme insulin resistance in older rat offspring [33]. However, iron deficiency in utero is not associated with impaired glucose tolerance, indicating that, in contrast to cardiovascular programming, the dietary insults promoting disturbances of glucose and insulin may involve specific nutrients [8].

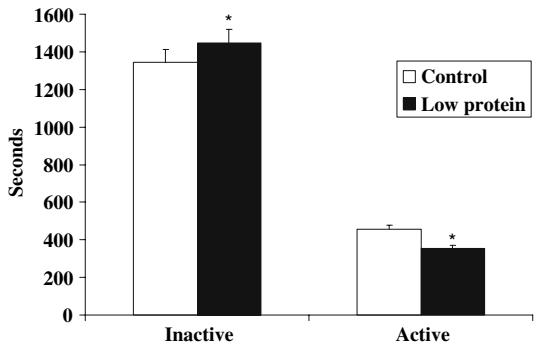

Fig. 2 Programming of locomotor activity. Rats exposed to a maternal low-protein diet during fetal development had locomotor activity determined at 13 months of age using an infrared array system. The data shows seconds spent active or inactive during a 30-min period during the daylight. $*P < 0.05$ between groups [4]

Programming of appetite and obesity

Epidemiological data regarding the programming of obesity is somewhat equivocal. Whilst it seems likely that early infancy is a sensitive period for establishing lifelong risk of adiposity and possibly appetite [14], data relating to fetal life is conflicting. Most studies show that weight at birth is positively related to BMI in adulthood, although there are some exceptions to this that show an inverse relationship. Interestingly data from the wartime Dutch Hunger Winter indicate that undernutrition in the first trimester of pregnancy may programme later obesity in both men and women [32].

In animals it appears that undernutrition programmes an enhanced propensity for fat deposition, but this propensity requires the additional insult of a high-fat diet in postnatal life. The timing of this postnatal challenge may be critical. In rats subject to protein restriction or global nutrient restriction in utero the introduction of a high-fat diet at weaning leads to a greater degree of obesity than is noted in the offspring of rats fed a control diet [13, 34]. However, studies in which high-fat feeding is introduced in adult life do not show the same finding. Programming of obesity in animal models is also apparent when excess nutrients are available to the developing offspring. Plagemann et al. [31], have demonstrated that overnutrition of suckling rats leads to hyperphagia and obesity in adult life.

The programming of obesity appears to be associated with effects of maternal nutrition upon both appetite and energy expenditure. The offspring of rats subject to 70% food restriction in pregnancy exhibit both hyperphagia and reduced locomotor activity [35]. Similarly the offspring of rats fed low-protein diets in pregnancy have reduced locomotor activity, although the effects are small and dependent upon the timing of maternal nutrient restriction [4] (Fig. 2). When offered a low-fat standard laboratory chow diet such animals are *hypophagic*, but when provided with a choice of fat-, protein- or carbohydrate-rich foods become hyperphagic and show an increased preference for fat [3] (Fig. 3).

Intergenerational programming

There is some evidence from studies of animals to suggest that the effects of undernutrition in fetal life may be transmissible through several generations. Beach et al. [2] fed zinc deficient diets in mouse pregnancy and noted that effects upon the immune system of the offspring persisted for three generations. Similarly (Fig. 4), the effects of a maternal low-protein diet on blood pressure in rats can be transmitted to a second generation [19]. Further studies are required to investigate such phenomena in more detail. The

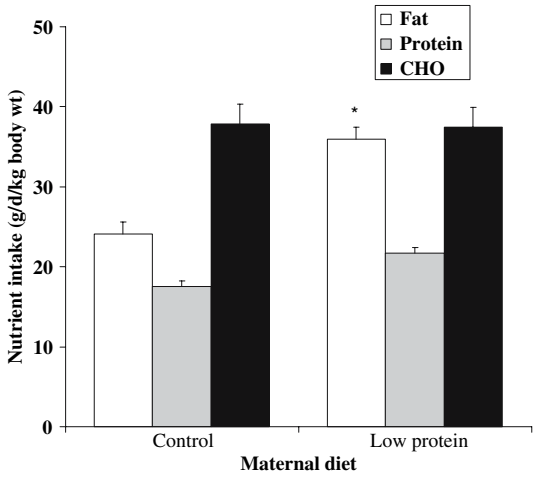

Fig. 3 Macronutrient self-selection by rats exposed to undernutrition in utero. The offspring of pregnant rats fed a low-protein diet throughout pregnancy were provided with a choice of fat-, protein- or carbohydrate-rich food sources. Macronutrient intake was calculated and the low protein exposed animals were found to consume significantly ($*P < 0.05$) more fat than control rats of the same age [3]

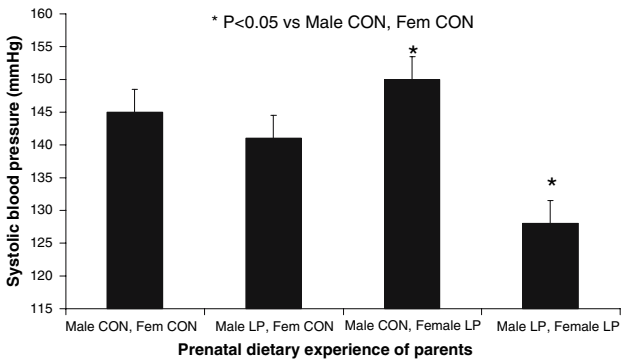

Fig. 4 Evidence of intergenerational effects on maternal protein restriction upon blood pressure. Pregnant rats were fed either control (*CON*) or Low Protein (*LP*) diets in utero. Offspring were all fed the same standard diet and were mated to produce a second generation without further dietary change. Blood pressure of this second generation was measured at 8 weeks of age. The resulting offspring had raised blood pressure. This F1 generation were crossed to yield the F2. Low-blood pressures were noted in the offspring of low protein exposed females mated with low protein exposed males, but the hypertensive phenotype was transmitted by these females if mated with a control male ($*P < 0.05$) [19]

fact that acquired metabolic or disease phenotypes can be transmitted between generations suggests that the process through which programming occurs must impact upon heritable material. Such observations therefore provide a strong clue that epigenetic modification of gene expression by nutritional factors during early development is at least one of many programming mechanisms. The existence of intergenerational effects following relatively brief exposures to undernutrition could also be of profound importance in terms of public health. In populations undergoing a nutritional transition (e.g. India and Brazil), the consequences of many generations of poor nutrition, followed by the switch to the dietary patterns of more affluent nations may be felt for many decades.

Mechanisms of utritional programming

It is apparent that a wide variety of nutritional manipulations in rodent pregnancy produce a relatively narrow and consistent range of responses in the mature offspring. This suggests that whilst there may be more than one mechanism linking variation in nutrition to eventual physiological and metabolic phenotypes, it is likely that there are a few common mechanisms exerting these effects irrespective of the precise nature of the nutritional insult.

Tissue remodelling

The simplest process through which nutrition could programme long-term function of organs is likely to involve alteration to the structure of organs. All organs essentially develop from small pools of embryonic progenitor cells. These cell lines go through phases of proliferation and differentiation to produce the mature organ with all of its' specialist cell types. Nutritional insults that impact upon the developing organ during either or both of these phases will have the potential to remodel the structure of that organ. Reductions in cell number or changes in cell type will have the capacity to limit the number of functional structures within the organ, to alter the patterns of gene expression within those structures and to change the cell–cell signalling pathways that regulate the actions of the organ.

There are numerous examples of tissue remodelling following a programming insult in rodents. The kidney, for example, appears vulnerable to protein restriction and in the rat even brief periods of undernutrition can elicit decreases in nephron number of up to 30% (Fig. 5). The reduction in the number of functional units within the kidney is observed with little discernible change in organ weight, suggesting that differentiation has been disrupted thereby producing structures other than nephrons [23]. Similarly, within the hypothalamus undernutrition altered the volume of key centres involved in appetite control and reduced the density of neurons staining for neuropeptide Y and galanin [30]. Remodelling of hypothalamic structure could have a huge impact upon most physiological systems and organs within the body due to the role of this brain region as an integrator of homeostasis.

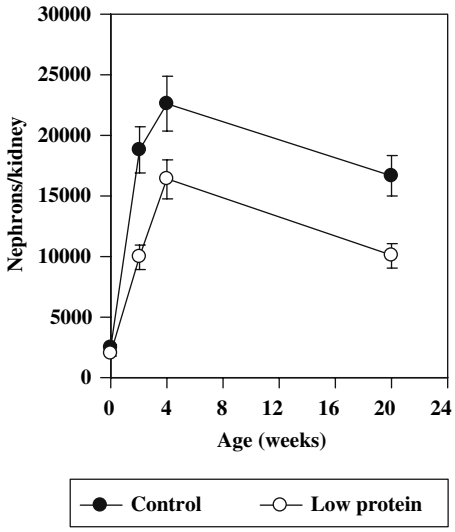

Fig. 5 Nephron number is significantly ($P < 0.05$) reduced in rats exposed to a maternal low-protein diet in utero. The decline in nephron number with ageing is likely to result in earlier renal failure and associated pathology in the prenatally undernourished rats at an earlier age than is seen in control animals

Role of glucocorticoids

In humans and animals there is a gradient of active glucocorticoid concentrations across the placenta. The fetal tissues are protected from the massive excess of corticosteroids in maternal circulation by the placental isoform of 11β-hydroxysteroid dehydrogenase (11βHSD2). This gatekeeper enzyme converts active glucocorticoids to inactive forms, thereby ensuring the autonomy of the fetal hypothalamic-pituitary-adrenal axis and preventing glucocorticoid activation of genes that promote early maturation of tissues. Studies of rats fed low-protein diets in pregnancy have shown that placental 11βHSD2 is down-regulated by undernutrition [22] (Fig. 6) and similar effects are noted in nutrient restricted sheep [27].

In addition to this evidence that the placental control of maternofetal endocrine cross-talk is disrupted by undernutrition, it is apparent that the glucocorticoids may directly mediate some of the observed programming effects of nutrition. Whilst the effects of maternal protein restriction on offspring blood pressure can be duplicated by administering an inhibitor of 11βHSD2 [21], the opposite effect can also be demonstrated. Treatment of protein restricted rat dams with an inhibitor of glucocorticoid synthesis protects their fetuses from nutritionally programmed hypertension [20].

Recent studies have indicated that the effects of glucocorticoids in relation to fetal undernutrition may be sex-specific. Whilst nutrition can regulate the long-term expression of angiotensin-II receptors in the rat kidney, a possible underlying cause of renal tissue remodelling, the effect is seen in females only and is glucocorticoid independent. In males programming of blood pressure is glucocorticoid-dependent [26].

DNA methylation and epigenetic programming

The expression of genes may be silenced by epigenetic mechanisms such as DNA methylation or histone acetylation. The window of opportunity for the establishment of patterns of DNA methylation is believed to lie entirely within the developmental stage of life. The pattern of methylation is believed to be fixed beyond this point and so factors including variation in nutrient supply that impact upon the process in early life may have permanent effects on gene expression [37].

The methylation of DNA is catalysed by the DNA methyltransferases and utilises S-adenosylmethionine as the principal methyl donor. As a result DNA methylation is strongly linked to two biochemical pathways; the folate cycle and the methionine–homocysteine cycle [28]. Studies of the impact of low-protein diets in rat pregnancy have

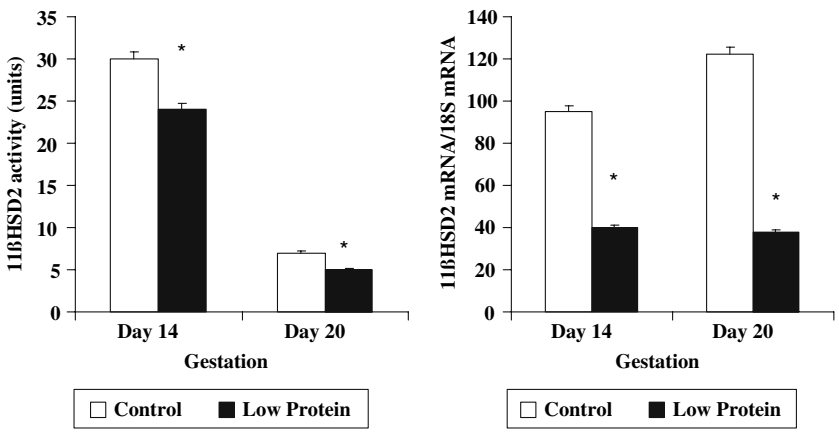

Fig. 6 Placental 11βHSD 2 expression in the rat. The feeding of a maternal low-protein diet down-regulates both gene expression and enzyme activity (*$P < 0.05$) [5, 22]

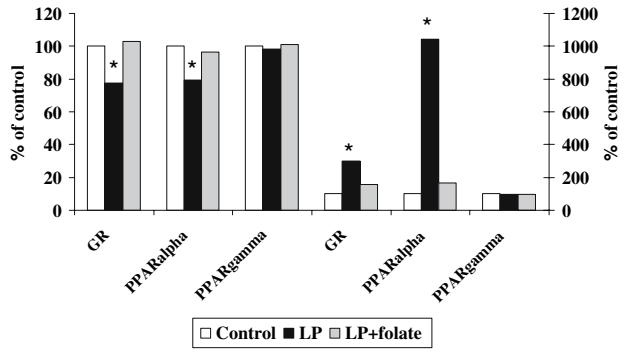

Fig. 7 Gene expression and methylation status in rat liver. Pregnant animals were fed control or low-protein diets throughout gestation [24]. A third group were fed the low-protein diet, with supplemental folate. Methylation status and gene expression were assessed in the livers of the adult offspring. Methylation of the glucocorticoid receptor (*GR*) gene and PPAR alpha was sensitive to maternal dietary factors and accordingly the expression of these genes was increased. PPAR gamma methylation and expression were not influenced by the maternal diet ($*P < 0.05$) [24]

generally used casein as the sole protein source, and as such have required additional dietary methionine to provide requirements for sulphur. This may promote disturbances of the methionine–homocysteine cycle that impact upon the provision of methyl donors for methylation of DNA. This view is supported by evidence from a number of studies in which the MLP diet is supplemented with folic acid [24], or with glycine [12]. These generally show that the effects of low-protein feeding can be reversed. Lillycrop et al. [24], have shown that MLP feeding impacted upon both the expression and methylation status of specific genes (Fig. 7). However, in contrast Bogdarina et al. [6] found no evidence that maternal protein restriction impacted on the methylation of the promoter for hepatic glucokinase, despite programmed changes in gene expression.

Conclusion

Animal studies show that even very short periods of nutrient restriction or excess at any stage of fetal development can exert powerful effects upon long-term health and well-being. These findings are wholly consistent with the concept that all disease is related to cumulative experience across the lifespan. The interindividual variation in response to nutrients is not wholly explained by nutrient–gene interactions and overall disease risk is determined by the nutritionally programmed phenotype, which can also impact upon gene expression and upon the nature of the metabolic and physiological response to the prevailing environment. This adds to the complexity of designing approaches to personalised nutrition.

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