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Workshop on 'Nutritional models of the developmental origins of adult health and disease'

Nutritional models of the developmental programming of adult health and disease

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The ability to not only replicate but also extend the findings from both historical epidemiological studies and contemporary cohorts of the developmental programming of later disease are critical if the mechanisms by which early diet impacts on later disease are to be fully understood. To date, a plethora of models have been established, with the range including global changes in dietary input, imbalanced diets and diets deficient in single nutrients. Key factors in translating these findings to the human situation are the pronounced differences in the relative growth and development between large and small mammals from the time of conception through pregnancy, lactation and weaning. This disparity is reflected in the very different nutritional requirements between species and the substantial divergence between rodents and large animals in the ontogeny of many of the organ systems that are nutritionally regulated. For example, hypothalamic circuitry is much more developed in species with a long gestation and offspring are born with a mature hypothalamic–pituitary axis in sheep and man compared with mice and rats. Similarly, nephron number is established towards the end of gestation in large mammals compared with the lactational period in rats. These types of differences will impact on the ability of individual and combined nutritional interventions to reset developmental processes, and may be further compounded by the gender of a fetus. The challenge for future work in this exciting and dynamic area of research is to utilise these marked comparative differences to generate imaginative nutritional interventions in order to improve the viability, health and well-being of the offspring.

Pregnancy: Growth: Lactation: Metabolism

Research into 'the developmental origins of adult health and disease' has made considerable progress over the past two decades, but a clear consensus on the exact nutrients involved and their mechanisms remains to be established. This problem relates, in part, to the long-term developmental time frame in which changes in metabolism and cardiovascular function occur. At the same time, the discipline has had to contend with the dual challenges of integrating findings from lifetime epidemiological studies that have largely focused on birth weight and its relationship (or otherwise) with adult disease and the ability to incorporate these results into appropriate nutritional intervention studies using animal models. It is now established that there are many potential influences on offspring outcome, including maternal body composition, age and parity, genetic constitution and macro- and micronutrient intake and handling, size, shape and number of offspring, their gender and type of lactational diet. Thus, it is not unexpected that as knowledge of the intricacies involved expands, a substantive number of publications have, in retrospect, been misleading because of inappropriate statistical analysis and/or mismatches in the different groups (of which some key examples will be summarised).

In the present brief review an attempt will be made to provide an overview of some of the major challenges for both human and animal studies, in conjunction with

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optimal experimental paradigms that may be utilised in future research aimed at elucidating the precise mechanisms by which changes in the maternal diet during reproduction can impact on the lifetime health of resulting offspring. Particular emphasis will be given to the applicability of the animal model that has been utilised and how similarities in the reproductive process may enable its best use in future examinations of the nutritional programming of adult health and disease.

Human models: historical and contemporary models of fetal programming

The majority of the early work conducted by David Barker and colleagues utilised data from historical cohorts born in the 1930s and benefitted from meticulous birth records, often kept by the same individual over long periods of time[1]. This approach has enabled clear relationships between not only the size and shape of an infant at birth but also its accompanying placental mass and later hypertension to be established[1]. Subsequently, the recruitment of long-term historical records from Finland has enabled longitudinal studies of infant growth to be related to adult insulin sensitivity[2]. More recently, the use of nutritional interventions of preterm formula in randomised controlled studies has emphasised the impact of inappropriate growth in early infancy on later disease risk[3]. However, with more contemporary studies and the rise in both childhood and adult obesity it is now apparent that the translation of findings from such studies into current lifestyle interventions is very difficult. This situation results, at least in part, from the complexity of the biological processes involved and the challenge of effecting lifestyle changes in activity and dietary intake. At the same time, the causes of the ongoing epidemic of obesity, and the predicted increase in associated renal diseases and CVD, are multifactorial[4] and may be either exacerbated, or reduced, by early dietary exposure[5].

One consistent theme that is apparent from both historical and contemporary studies is that changes in nutrition at specific stages of pregnancy can have very different outcomes[6]. This finding is not unexpected as different organs have critical and precise developmental windows that may be compromised, or enhanced, and thereafter permanently set for the rest of that individual's life. Importantly, adaptations of this type appear not only to be dependent on the period in which the mother's diet is altered but also the diet to which she is rehabilitated[7]. One fundamental consideration is that the self-limitation in food intake between early-to-mid gestation that occurs commonly as a result of the nausea affecting approximately 90 % of pregnant women in the UK may be directly linked to Western diets[8]. The extent to which this factor relates to changes in placental function and/or fetal growth remains less clear, but there is a need to match global pre- and postnatal nutritional requirements in order to avoid accelerated growth and the concomitant increased risk of later obesity and metabolic complications[9]. Importantly, however, intergenerational acceleration mechanisms do not appear to make an important contribution to levels of raised childhood BMI within the population[10].

The lactational environment and postnatal development

A further area requiring consideration is the relationship between the maternal diet in late pregnancy, its impact on mammary gland development and milk production and whether the infant is breast-fed or formula-fed[11]. The higher macronutrient content of formula feed compared with breast milk, in conjunction with its fixed composition throughout a feed (unlike breast milk, the composition of which changes with time), will impact on the nutrient supply to the infant. It is therefore necessary to consider not only short-term but also long-term advantages of breast-feeding in relation to appetite regulation[12]. The type of lactational diet also impacts on other behavioural aspects, including sleep–awake activity cycles[13], so that extended breast-feeding may not only be beneficial in developing countries[14]. Other confounding factors such as social class and smoking during pregnancy further determine postnatal diet[15].

In summary, the relationship between nutrient supply during key stages of development from the time of conception to weaning is highly complex and requires careful in-depth consideration. It is necessary to conduct detailed animal experiments in a range of species in order to elucidate the mechanisms involved, be they epigenetic or related processes[16].

Animal models of nutritional programming

The main animal models that have been utilised to date to investigate the impact of maternal diet on long-term programming have been rats and sheep[17]. These animals obviously have very different developmental patterns in the relationship between placental and fetal growth, maturity at birth and milk composition[18]. The advantage of utilising rats is their very short gestational length. However, the type of diet they normally consume in the wild is very different from that fed to housed laboratory animals in which semi-purified diets are the norm. Such diets provide substantially greater differences in nutrients to pregnant rats than to controls and, therefore, should be considered 'pharmacological' as opposed to 'physiological' as they are outside the normal distribution of wild-type diets. For example, high-fat diets utilised to date in rat studies contain four times as much fat when compared with control diets and are at risk of being deficient in micronutrients. Not surprisingly, when fed a diet so rich in fat, maternal food intake is reduced[19]. In addition, rats exhibit coprophagia, which has a substantial effect on nutrient flux and the ability to experimentally manipulate the intake of specific nutrients.

Appreciable placental growth necessarily continues up to term in the rat to meet the much higher protein demands for fetal growth compared with that of man or sheep[20]. In large mammals, however, the maximal period of placental growth is early in pregnancy in order to meet the greatly increased nutrient requirements of the fetus in late gestation when its growth is exponential[21]. Furthermore, rats produce large litters whereas sheep and man only normally produce one (or two) offspring of comparable birth weight per pregnancy.

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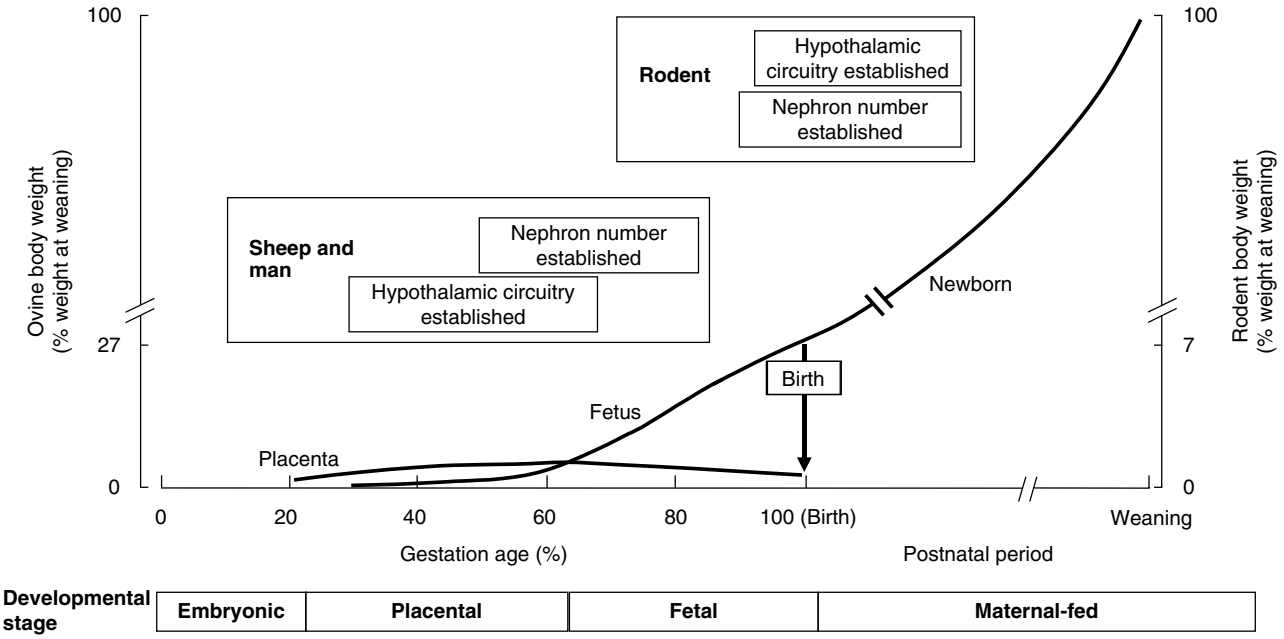

Fig. 1. Summary of the ontogenic differences in development and maturation of the hypothalamus and kidney in relation to pre- and postnatal growth trajectories between large (including man) and small animals.

Comparative differences in organ development

Another substantive difference between rats and man (and rats and sheep) is that the development and maturation of the hypothalamic–pituitary system occurs after birth during the neonatal period in rats compared with during late gestation in sheep and man. Thus, any nutritional intervention that stops at birth in the rat will have substantially different outcomes compared with those extending into the neonatal period[9]. Other organs that continue to develop postnatally in the rat, compared with prenatally in sheep and man, include the kidney and brown adipose tissue[6] (Fig. 1). For example, in those species born with a mature hypothalamic–pituitary axis after a long gestation, such as in sheep and man, the majority of the appetite-controlling networks appear to be in place by mid- to late gestation[22]. This position is clearly in contrast to that in small mammals in which there is substantial development during the lactational period as the hypothalamic axis matures. It is thus not unexpected that leptin administration has such a marked effect on this process[23] and related processes[24,25], which are not seen when given to the late-gestation fetus or neonatal sheep[26,27]. Indeed, there is no postnatal leptin surge in either sheep[28] or man[29].

Nephron development occurs primarily after birth, as does brown fat, which in sheep and man was originally thought to be completely lost in the postnatal period[30], but is retained throughout the life cycle in the rodent. As such, brown adipose tissue has a fundamental impact on the energy balance of adult rats because it uniquely possesses uncoupling protein 1 and is thus able to rapidly generate large amounts of heat[31]. Indeed, brown fat has a pivotal role in the regulation of thermogenesis, with a thermogenic potential of 300 W/g whilst all other tissues generate approximately 1 W/g[32]. Not unexpectedly, loss of uncoupling protein 1 is accompanied by an increase in fat mass, making it difficult to dissociate the dietary and related effects on changes in fat mass from adaptations in uncoupling protein 1 in this species[33]. Recently, indirect measurements in patients with cancer suggest that brown fat may be retained in small amounts in these individuals[34]. Its location is, however, primarily in the neck region[35,36] and whether it has any functional importance in energy balance remains to be established.

Methodological considerations in the interpretation of metabolic programming

There have been two major problems with rat studies utilised in the assessment of long-term cardiovascular outcomes. First, in many studies blood pressure has only been measured using the tail-cuff technique in restrained and heated animals during the day when they are normally inactive, being nocturnal animals (for example, see Langley-Evans[37]; Kwong *et al.*[38]). It is recognised that this approach can give very different results when compared with blood pressure measurements obtained using telemetry[39]; as such, it is only recommended for use in obviously hypertensive animals in which the technique was originally validated[40]. Thus, in the majority of studies in which it has been used in this area the tail-cuff technique is not informative in relation to the modest differences in blood pressure recorded in such normotensive rats. This factor may explain why in more recent studies the offspring born to dams fed a low-protein high-carbohydrate diet through pregnancy show either no difference or a reduction in blood pressure when measured using either telemetry or an indwelling arterial catheter[41,42]. Comparable findings are seen in offspring born to dams in which

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Table 1. Summary of studies into nutritional programming in which outcome measures are potentially confounded by a mismatch between groups in their composition of offspring from singleton and twin pregnancies

Nutritional intervention	Composition of control group	Composition of nutritionally-manipulated group	Reported effect of nutritional intervention	Potential confounder	Reference
Maternal nutrient restriction (50% of the intake of controls) between 28 and 80 d gestation	Three singletons and six twins	Seven singletons and two twins	Raised blood pressure, increased fat mass and altered glucose handling	Significantly more singletons and fewer twins in the nutrient-restricted group (all males were castrated)	Gilbert *et al.*[54], Ford *et al.*[55]
Increased maternal food intake (50% greater than controls) from 110 d gestation (term 147 d)	Four singletons and eight twins	Seven singletons and two twins	Transient increase in food intake over the first 3 weeks of lactation	Significantly more singletons and fewer twins in the well-fed group	Muhlhausler *et al.*[56]
Growth rate of offspring reduced by 15% between 12 and 25 weeks after birth	Seven singletons and seven twins (plus two additional groups of the same mix born to mothers nutrient restricted over the first 30 d gestation)	Three singletons and seven twins	Cardiovascular and renal dysfunction only in the group with mismatched twins and singletons	Significantly greater proportion of twin offspring compared with singleton offspring in the intervention group. Also, female offspring excluded	Cleal *et al.*[57]

food intake is reduced by 50% through pregnancy compared with controls[43]. A further confounding factor in many of these early studies has been a failure to consider the dam as the experimental unit rather than her offspring[38], which is now recommended as standard practice[44]. There also appears to be a marked divergence in the long-term outcomes between genders in rats that is primarily linked to the faster, as well as continued, growth of males compared with females[45].

Despite the fact that sheep are ruminants, they have proved invaluable in enabling the understanding of the nutritional and endocrine regulation of placento–fetal development. As in man, the primary metabolic substrate for fetal metabolism is glucose, for which GLUT 1 is the main placental regulator[46]. Glucose is thus transported across the placenta by active diffusion determined by its concentration in maternal blood[47]. In addition, not only does kidney development show a very similar ontogeny in sheep and man, but the distribution of total nephrons across the adult population is comparable[48]. It is also feasible to obtain very consistent blood pressure recordings in the offspring using arterial cannulation whilst the animal is standing freely with continual access to its diet[49]. At the same time, there is no discernable difference in blood pressure control or glucose regulation between genders when measured in intact adult sheep[48,50].

Mismatched offspring and later outcomes?

Another confounding factor that has become apparent in a number of recent studies using sheep is a mismatch between the numbers of singletons and twins between nutritional groups. This mismatch has introduced erroneous conclusions, which is not unexpected given the pronounced differences in outcomes of human newborns that are also dependent on fetal number[51]. Appropriate statistical correction (as in the case of rat studies) for this factor is therefore necessary[44]. Some of the more prominent examples of such mismatch between control and intervention groups are summarised in Table 1. Indeed, this mismatch is further confounded by using both twins from the same mother for whom the lactational environment will be further altered. In contrast, when one twin is reared alone by its mother, its growth rate matches that of a singleton, as does its later blood pressure control and glucose homeostasis[50,52].

It is now clear that future studies in the area will need to take into account not only changes in diet through pregnancy but also the relative contributions of gender, litter size, type and duration of lactation as well as weaning diets. These considerations will undoubtedly increase the time necessary to conduct such studies as well as their cost, but it is imperative that with the plethora of nutritional models already established these factors are optimised in order to elucidate the main pathways involved rather than pursuing models that may have little translational relevance[53].

Conclusion

In summary, appropriate nutritional interventions are now available that can examine relevant short- and long-term outcomes and determine how contemporary diets impact on fat deposition, metabolic homeostasis and cardiovascular control in a consistent and validated manner. The completion of such studies may enable the determination of the optimum nutrition in terms of quantity and quality that may ultimately improve the health of future generations.

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