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REVIEW

Nutritional programming of disease: unravelling the mechanism

Simon C. Langley-Evans

Division of Nutritional Sciences, School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough, UK

Abstract

Nutritional programming is the process through which variation in the quality or quantity of nutrients consumed during pregnancy exerts permanent effects upon the developing fetus. Programming of fetal development is considered to be an important risk factor for non-communicable diseases of adulthood, including coronary heart disease and other disorders related to insulin resistance. The study of programming in relation to disease processes has been advanced by development of animal models, which have utilized restriction or over-feeding of specific nutrients in either rodents or sheep. These consistently demonstrate the biological plausibility of the nutritional programming hypothesis and, importantly, provide tools with which to examine the mechanisms through which programming may occur. Studies of animals subject to undernutrition *in utero* generally exhibit changes in the structure of key organs such as the kidney, heart and brain. These appear consistent with remodelling of development, associated with disruption of cellular proliferation and differentiation. Whilst the causal pathways which extend from this tissue remodelling to disease can be easily understood, the processes which lead to this disordered organ development are poorly defined. Even minor variation in maternal nutritional status is capable of producing important shifts in the fetal environment. It is suggested that these environmental changes are associated with altered expression of key genes, which are responsible for driving the tissue remodelling response and future disease risk. Nutrition-related factors may drive these processes by disturbing placental function, including control of materno-fetal endocrine exchanges, or the epigenetic regulation of gene expression.

Key words cardiovascular disease; metabolic syndrome; nutrition, pregnancy; programming.

Introduction

Modern patterns of disease are strongly related to patterns of nutrition in the population. The major killers in all developed countries are coronary heart disease, stroke and cancer and these are growing in prevalence in all parts of the world, as increasing wealth and global trade change dietary patterns in countries where the population used to subsist on simpler and largely plant-based diets. Risk of cardiovascular disease and cancer is strongly influenced by obesity and overweight, and hence the consumption of energy dense, high-sugar, high-fat diets. Although for cancer the contribution of specific dietary patterns is proving difficult to elucidate (WCRF, 2007), it has been overwhelmingly shown that cardiovascular disease is promoted by diets rich in saturated fatty acids and low in complex carbohydrates (Willett, 2006).

As scientists and lay people we accept that our adult lifestyle, comprising diet and physical activity, smoking habits and alcohol consumption, is one of the main determinants of our long-term health and well-being. This is of course only a part of the story and recent decades have brought huge leaps forward in terms of understanding how these lifestyle factors interact with the genome and allow inherited factors to modulate that risk of disease. For example, adults who carry the TT variant of the C677T polymorphism of methyltetrahydrofolate reductase (MTHFR) are more vulnerable to cardiovascular disease, but only if they consume a diet that is low in folic acid (Klerk et al. 2002). It is becoming increasingly clear that these interactions of genes and environment begin to shape health and disease at much earlier stages of life. For example, being breastfed protects children from early-onset obesity (Arenz et al. 2004) and hence may reduce risk of related disorders (Martin et al. 2005). Disease in adulthood is in fact the product of continual exposures to protective and disease-promoting

Correspondence
Simon C. Langley-Evans, Division of Nutritional Sciences, School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough, LE12 5RD, UK.
E: Simon.Langley-Evans@Nottingham.ac.uk

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factors throughout the lifespan. Exposures at early life stages may be particularly important, as the plasticity of growing and developing tissues means that they shape the way in which the body responds to later challenges. This review will set out the evidence that nutrition-related factors in fetal life impact upon risk of diseases that do not manifest for 40–60 years post-exposure.

The concept of nutritional programming

The term programming describes the 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. There are many examples of this process observable within the natural world. One that is frequently cited describes the mechanisms that determine sex in crocodilians. Alligators and crocodiles lack sex chromosomes (Milnes et al. 2002). Their eggs are laid into earth mounds within which exists a temperature gradient. At most temperatures within the nest the embryos will develop into females, whilst within a very specific range of 4–5 °C the embryos are more likely to become males. These effects are explained by the fact that the temperature of the egg determines the expression of genes encoding aromatases that are responsible for synthesis of the sex steroids. These then govern the physiological development of the embryos (Milnes et al. 2002). Under the definition provided above, this is clearly an example of programming, as the stimulus is temperature, the critical phase of development lies within the embryonic period and the effect of the exposure is permanent.

Mammalian systems are also subject to programming during development. Programming is a feature of the plasticity of cell lines during embryonic and fetal life. For most types of cell, plasticity is a short-lived characteristic, being only a feature of the embryonic and fetal stages. These are the critical developmental phases that are of greatest interest in the context of nutrition and human health and disease. The capacity to programme mammalian systems during early life can be demonstrated as dramatically as the example of reptilian temperature-dependent sex determination. Treatment of newborn female rats with testosterone in the first few days of life perturbs their lifelong reproductive function. Regions of the hypothalamus that control the reproductive axis and archetypal female reproductive behaviours are remodelled to resemble the male brain and the female rats are rendered sterile (Arai & Gorski, 1968). The critical period in which this androgenizing treatment is effective is relatively short, but the effects are permanent.

In humans such precise experiments can obviously not be recreated and demonstrations of programming effects are often less convincing. Normal human physiology can, however, be shown to respond to cues in the environment that prevails during early development (Gluckman & Hanson, 2004). During the Second World War the Japanese military considered the performance of their soldiers in hotter climates, in relation to the areas of Japan that they were recruited from. This led to the discovery that the number of sweat glands is set soon after birth and cannot then be further adjusted. When individuals were born in cooler climates they activated a smaller number of sweat glands than did individuals who were born in warmer climates. The response to the environment during the early postnatal period therefore brings about permanent changes to physiology. These adaptations allow the individual born in a warm climate to be optimally adapted for life in that climate, whilst the individual from a cold climate will cope less well with the heat.

The observation that the developing mammal has the ability to respond to environmental cues, or physiological insults, changes the way in which we should think about the developmental process. Mammalian development clearly is not entirely gene-led and is not purely a matter of activating and switching off the expression of genes, in well-ordered sequences. Adaptive responses triggered by changes in the environment, as signalled through the maternal system, can change the profile of genes that are expressed at any given stage of development, with profound and irreversible effects upon the physiology of the fetus. Environmental influences upon apparently genetically determined processes can be most simply observed by looking at the effects of maternal physiology and experience upon fetal growth. The growth trajectory of a fetus is largely determined by the genes inherited from both the mother and the father, but evolution has provided mechanisms through which the genetically determined growth rate can be constrained in response to maternal cues. Classic experiments using embryo transfers in horses and cattle show that the size of the mother is a primary factor governing fetal growth. When Shetland mares carry the foals resulting from Shire horse $\times$ Shetland pony crosses, the genetically large offspring are born at a size similar to the pure Shetland (Walton & Hammond, 1938). This form of constraint ensures maternal survival, as it prevents the development of a fetus that will become too large to pass through the birth canal.

Similar observations can be made in human pregnancies, where it is clear that very diverse signals can constrain fetal growth. Factors which signal under-privilege or other indicators of a less than optimal environment are generally associated with lower weights at birth among human babies. Socioeconomic class of the mother is more strongly predictive of birth weight than any other single factor (Bibby & Stewart, 2004). Social class is, however, only a crude proxy indicator of maternal nutritional status, family income, smoking habits, and access to healthcare services. Several of these factors, for example smoking, are known to influence fetal growth in their own right. Slowing of fetal growth appears to be a common response to any factor that may threaten the integrity of human pregnancy

or the health of the mother, including undernutrition, maternal infection, multiple pregnancy and major psychological trauma (Brown & Carlson, 2000; Smits et al. 2006).

Placing these concepts into the general context of the aetiology of disease that was discussed at the start of this review, it can be asserted that the early life exposures that might manifest as constrained growth are part of a battery of factors that are disease-promoting. These factors include disease-susceptibility genes, adverse early life exposures, a high-risk adult lifestyle (poor diet, sedentary lifestyle, smoking) and ageing. Individual risk will be determined by the interactions of these factors and the influences of protective genes, optimal early life development and lower-risk adult lifestyle. Fetal growth constraint is possibly just a readily assessed marker of the early life exposure to adverse environmental cues. Whilst acknowledging that more subtle changes to fetal physiology might occur, markers of fetal growth retardation (low birthweight, disproportion at birth) have been most widely used as the basis of the evidence that human disease states are subject to early life programming influences.

Epidemiological evidence for programming of human disease

Some of the earliest evidence to support a role for the fetal environment in determining risk of adult disease came from studies of retrospective cohorts in England. Barker and colleagues published a series of studies of a cohort of men and women who had been born in the county of Hertfordshire between 1911 and 1933 (Barker et al. 1989, 1990, 1993a; Hales et al. 1991). Initial findings showed that, among men, risk of death from coronary heart disease was inversely related to weight at birth, with those men who were born at less than 5.5 lbs being at double the risk of those who were in excess of 9.5 lbs at birth. Risk factors for coronary heart disease were similarly related to birth weight, with low birth weight predicting higher blood pressure, higher circulating clotting factors, and impaired glucose tolerance. Indeed men of lower weight at birth were at massively increased risk of type-2 diabetes and the metabolic syndrome (Hales et al. 1991; Barker et al. 1993a).

Observations linking lower birth weight to disease later in life were extended by studies of other cohorts that had gathered more detailed information about infant characteristics at birth. Greater risk of cardiovascular disease and metabolic syndrome was shown to be associated with reduced abdominal circumference (Martyn et al. 1995), a large head circumference in relation to body length (Barker et al. 1993b), and relative thinness (low weight in proportion to body length) at birth (Barker et al. 1992). All of these measurements provide evidence that intrauterine growth was disproportionate and constrained in an uneven manner. Several of the observations made using retrospective cohorts were verified through consideration of more contemporary populations. For example, glucose intolerance in Indian children was found to be related to birth characteristics (Yajnik et al. 1995), whilst thinness at birth predicted greater blood pressure and other physiological markers in English infants (Fall et al. 1995). Demonstrating these associations in young children was important, as it showed that programmed changes to physiology manifest almost immediately and do not depend upon postnatal factors for their expression.

Epidemiological studies from the Scandinavian countries have been especially useful in demonstrating programming effects of early life upon disease risk. Two cohorts from Helsinki, Finland, have enabled researchers to investigate the interaction of prenatal and post-natal factors. Among Helsinki men and women born between 1924 and 1944 the greatest risk of developing coronary heart disease and diabetes was associated with being thin at birth and relatively fat in later childhood (Ericksson et al. 2002a). This has prompted the suggestion that fetal growth retardation followed by rapid 'catch-up growth' in childhood is a major risk factor for disease (Eriksson et al. 2002b).

The above examples show how health and disease in adult life are related to factors that impact upon rates of growth in fetal life. It is widely inferred that these relationships are best explained by variation in maternal nutrition. Figure 1 shows the fetal origins of adult disease hypothesis and shows the central role of less than optimal maternal nutrition as one of the drivers of programming processes. However, epidemiological support for this hypothesis is rather tenuous as there are few data to directly link exposure to undernutrition to later ill-health. This weakness is to some extent offset by studies of the Dutch Hunger Winter, which occurred towards the end of the Second World War. The Nazi blockade of food rations to Western Holland in retaliation for strikes by railway workers, resulted in widespread hunger over a period of 6 months. At the height of the famine the adult ration delivered only 500–600 kcal per day. Birth weights among babies affected by famine in late gestation were approximately 250 g lower than those of babies born before or conceived after the famine (Roseboom et al. 2001). Surprisingly, and rather in contrast to the fetal origins of adult disease hypothesis, the babies whose mothers were caught by famine in the first trimester of gestation, were heavier at birth than the population average before and after the period of famine. Follow-up studies of the babies born during the Dutch famine do, however, strongly support a role for nutrition in programming disease risk. Adults exposed to the famine *in utero* suffered more ill-health than their contemporaries whose mothers had not been affected by the famine. Exposure to famine in early gestation was associated with greater prevalence of coronary heart disease, with raised concentrations of circulating lipids, blood-clotting factors and more obesity than in individuals who were not exposed to the famine (Roseboom et al. 2001).

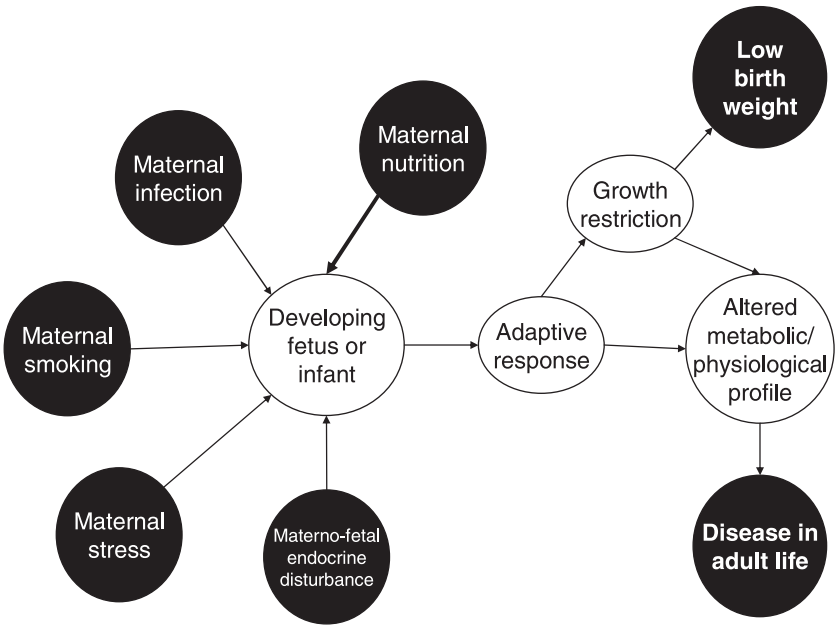

Fig. 1 The fetal origins of adult disease hypothesis. Adverse environmental cues from the mother are signalled to the developing fetus. The prevailing conditions may be sufficiently harsh to result in the loss of the pregnancy, or alternatively the fetus may mount adaptive responses to ensure immediate survival. One of these responses may be a slowing of growth that will ultimately result in lower weight at delivery. Other aspects of the adaptive response, which may be localized to specific organs and tissue types, will serve to modify physiology and metabolism and hence tissue functions. The trade-off for overcoming the challenge in fetal life may be increased risk of disease later in life.

Animal models provide proof of principle

The epidemiological studies described above are highly suggestive of an association between early life nutrition and later disease risk, but remain a long way off establishing the causality of any such link. Work in this area has been subjected to robust criticism and concerns have been raised about the fact that retrospective analyses of these historical cohorts are inevitably confounded by many different factors, about failures to correct adequately analyses for confounders and about the possibility of a publication bias in the nutritional programming literature (Joseph & Kramer, 1996; Paneth et al. 1996; Huxley et al. 2002). Most importantly, the link between maternal nutrition and fetal growth is far from clear cut and the majority of studies of women living in developed countries show little or no association between maternal intakes of energy, macro- and micronutrients and infant birth weight (Mathews et al. 1999; Langley-Evans & Langley-Evans, 2003). A few studies have shown direct associations between measured intakes of nutrients in pregnancy and risk factors for disease in the resulting offspring. For example, follow-up of a study of nutrition in pregnancy conducted in Aberdeen in the 1940s, showed that elevated blood pressure in middle-aged men was predicted by a low intake of animal protein if the mothers were consuming a high-carbohydrate diet (Campbell et al. 1996). Similarly, a US study has shown that blood pressure in infants was related to maternal calcium intake in pregnancy (Gillman et al. 2004).

Such studies are far from convincing, however, and even the remarkable findings of the definitive 'natural experiment' of the Dutch Hunger Winter can be questioned. During the Dutch famine, women still had access to black-market foodstuffs so the severity of undernutrition may have been patchy. Moreover, the stress of wartime and the Occupation may explain some of the findings attributable to undernutrition (Stein, 2004). It is also overly simplistic to assume that maternal intake is a good indicator of the nutritional environment experienced by the fetus. Intake is just one element of the fetal supply line, which is also related to maternal nutrient stores and body composition, maternal age, maternal physical activity and energy expenditure, blood flow to the placenta, efficiency of placental nutrient transfer and the action of hormones such as the insulin-like growth factors, which regulate nutrient partitioning between maternal and fetal compartments (Harding, 2004).

Epidemiology may be a particularly poor tool for the study of nutritional programming. It is clear that intervention studies to explore the issue would be unethical and impractical. Properly designed prospective cohort studies will, by definition, require five or six decades of follow-up to consider any possible association between maternal nutrition, body composition or other factors, and the development of disease. Animal studies therefore become the only practical way forward for this area of research. Work with suitable models not only allows the plausibility of the programming hypothesis to be demonstrated, it also permits measurement of invasive endpoints, consideration of programming effects across the full lifespan, and evaluation of the possibility of intergenerational effects of undernutrition in pregnancy (Langley-Evans et al. 2006).

Programming in animals

Studies of nutritional programming using animal models have been ongoing since the early 1990s and one of the

Table 1 Animal models of nutritional programming. A diverse series of experiments have been performed in rodents and large animals to demonstrate the biological plausibility of disease programming through modification of maternal or fetal nutrition

General approach	Specific intervention	Species used
Induction of low birth weight	Global nutrient restriction	Rat, sheep
	Uterine ligation	Rat, guinea pig
Over-nutrition of mother	Induction of maternal obesity	Rat, mouse
	High-fat, high-cholesterol die	Rat, rabbit
	High protein diet	Rat
Undernutrition of mother	Micronutrient deficiency (Ca, Fe, Na, Zn)	Rat, mouse
	Macronutrient restriction (protein)	Rat, mouse

most striking features of the body of literature that has accumulated since then is the consistency of effects (Langley-Evans, 2006). The models that have been developed are immensely varied and have largely been based on rats and mice, or sheep. Within these species it has been possible to examine the impact of variation in the maternal diet at different stages of gestation, and then consider the impact upon the developing fetus and the mature offspring that are generated. The range of nutritional insults that have been utilized is diverse and can be loosely grouped into models that seek to restrict global nutrition (i.e. all nutrients consumed by the mother), macronutrient intake or micronutrient intake, or models that seek to interfere with delivery of nutrients to the fetus without impacting on maternal diet, or models that attempt to deliver an excess of nutrients (Table 1).

Initially many of these experiments were developed to model the low birth weight hypothesis in its simplest form. It is well established that restricting maternal food intake, or ligating a uterine artery (Nüsken et al. 2008), during rodent pregnancy, will lead to fetal growth retardation, so this global undernutrition approach seemed a natural way of considering cross-species correlates of the kind of observations noted with cohorts such as the Hertfordshire cohort. Woodall and colleagues (Woodall et al. 1996) initially published studies of the offspring of rats subject to a severe food restriction during pregnancy. Rat dams fed just 30% of normal rations produced offspring with markedly lower birth weight, and these animals went on to develop elevated blood pressure by 7 months of age. Moreover, if the offspring subject to fetal growth retardation were fed a hypercaloric, obesity-promoting diet in their adult life, they showed a greater propensity for fat gain, which appeared to be partly explained by programming effects upon levels of energy expenditure physical activity (Vickers et al. 2003).

One of the major criticisms of the global nutrient restriction models is that they have little relationship to contemporary problems in human nutrition. Other experimental approaches have sought to examine nutritional programming effects independently of any impact of the maternal diet upon fetal growth rates, instead focusing on common nutrient deficiencies and problems in human populations. Iron deficiency anaemia is the most prevalent micronutrient deficiency on a global scale (Stoltzfus, 2003). It is estimated that approximately two thirds of the world population suffers some degree of iron deficiency, and pregnant women in both developed and developing countries are particularly at risk. The feeding of an iron-deficient diet to female rats prior to and during pregnancy results in elevated blood pressure in their offspring, an endpoint that is associated with changes in cardiac development during the fetal period (Gambling et al. 2003; Andersen et al. 2006). Studies of the offspring of iron-deficient dams also show that fatty acid metabolism is subject to long-term programming effects (Zhang et al. 2005).

The prevailing concerns about the nutrition of women in developed countries are focused more on nutritional excess rather than deficiency of specific nutrients. Surprisingly, whilst experiments which restrict the nutrition of pregnant animals almost universally result in high blood pressure, glucose intolerance, insulin resistance and a greater propensity to become obese (Langley-Evans, 2006), experiments where the nutritional manipulation is in the opposite direction have almost exactly the same outcomes. The feeding of diets which are high in fat during rat pregnancy is associated with elevated blood pressure and vascular endothelial dysfunction in the resulting offspring (Khan et al. 2003, 2005). Fetal exposure to a high protein diet has been shown to induce defects of energy expenditure that lead to obesity (Daenzer et al. 2002).

Maternal obesity may also be an important programming influence with major relevance to modern societies. Obesity changes many aspects of the environment experienced by the fetus, including the quality and quantity of nutrients delivered across the placenta and the endocrine milieu. Obese women are, of course, at greater risk of most complications of pregnancy, including gestational diabetes, pre-eclampsia and pre-term delivery (Jensen et al. 2003). Bayol and colleagues sought to examine the impact of feeding a 'junk-food' diet, comprising highly palatable human foods, to rats during pregnancy and

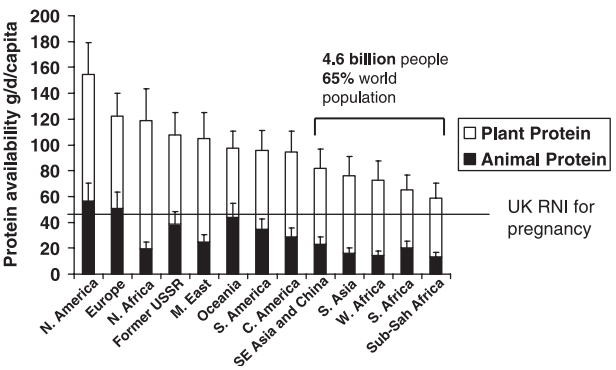

Fig. 2 Protein consumption is highly variable on a worldwide scale. Data shows per capita availability of protein from plant and animal sources calculated from the 2004 Food and Agriculture Organization, global food balance sheets. Intakes of animal protein and lower quality plant protein sources are shown for countries grouped into regions defined by the World Health Organization. The most densely populated regions (SE Asia and China, S Asia) can be seen to have less protein available than richer regions (N America and Europe). The UK RNI is the Reference Nutrient Intake, which defines the level of protein intake for pregnancy at which deficiency in a population is highly unlikely. Although all regions have protein availability above this figure, actual intakes are likely to be lower as food balance sheets overestimate the amount actually consumed and cannot differentiate between sub-groups in the population (e.g. rich vs. poor, urban vs. rural).

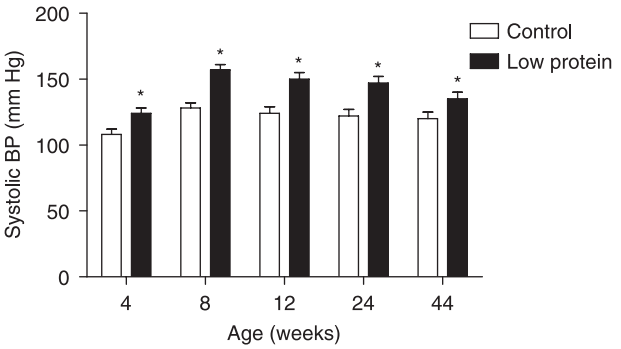

Fig. 3 Systolic blood pressure is elevated in the offspring of rats fed a low protein diet in pregnancy. Pregnant rats were fed an 18% casein control diet or a 9% casein low protein diet throughout gestation. At birth all dams were fed the same standard chow diet, which was also used to wean the offspring. Blood pressure was measured using an indirect tail-cuff method. Data are shown as mean ± SE for $n = 5–12$ observations per group at each time point. * indicates a significant difference between control and low protein-exposed animals ($P < 0.05$). Data compiled from Langley & Jackson (1994) and Langley-Evans & Jackson (1995).

lactation. Offspring exposed to such a dietary pattern were themselves more obese than controls (Bayol et al. 2007). Samuelsson et al. (2008), induced obesity in mice several weeks prior to mating and then studied the impact of maternal obesity upon several endpoints in their offspring. Mice exposed to the obese maternal environment had higher blood pressure, greater adiposity and insulin resistance in comparison with controls.

Low protein diets and rodent pregnancy

Work in our laboratory has focused upon maternal protein restriction during rat and mouse pregnancy. This experimental model is by far the best characterized and most widely studied of all of the animal models of nutritional programming. As shown in Fig. 2, availability of protein varies considerably between regions across the world. In all, 65% of world population are at risk of protein intakes below the UK Reference Nutrient Intake for pregnancy and rely heavily on lower quality, plant protein sources. Within developed countries there is considerable variation in maternal protein intake, and younger mothers in lower socioeconomic groups are more likely to consume lower protein intakes (Langley-Evans & Langley-Evans, 2003).

The feeding of a low protein (LP) diet in rat pregnancy produces only subtle effects on fetal growth (Langley-Evans et al. 1996a) and consistently induces persistent high blood pressure (Langley & Jackson, 1994) and impairments of renal development (Langley-Evans et al. 1999) in the offspring. LP-exposed offspring are hypertensive relative to control animals from as early as weaning (Langley-Evans et al. 1994) and this effect persists throughout their adult lives (Langley-Evans & Jackson, 1995, Fig. 3). Changes in blood pressure appear to be related to dysfunction of the renin-angiotensin system. Studies have shown elevated activity of angiotensin converting enzyme and altered renal expression of the angiotensin II receptors (Sherman & Langley-Evans, 1998, 2000; McMullen et al. 2004). Treatment of LP-exposed offspring with angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists during the suckling period appears to reverse the programming effect of the maternal diet (Sherman & Langley-Evans, 2000).

Rats exposed to LP diets *in utero* have a shorter lifespan (Aihie-Sayer et al. 2001) and show evidence of disturbed glucose homeostasis (Fernandez-Twinn et al. 2005), vascular dysfunction (Torrens et al. 2006), increased susceptibility to oxidative injury (Langley-Evans & Sculley, 2005), impaired immunity (Calder & Yaqoob, 2000), altered feeding behaviour (Bellinger et al. 2004) and increased central fat deposition (Bellinger et al. 2006). Studies of the impact of fetal protein restriction upon longevity and mechanisms of ageing in the rat have been particularly enlightening. Rats exposed to a maternal low protein diet during fetal development show little evidence of metabolic abnormalities at 9 months of age, although it is established that blood pressure is elevated and there are renal abnormalities well before this stage. By 18 months of age, however, the rats develop hepatic steatosis, have normoglycaemia but raised plasma insulin (indicating insulin resistance), are profoundly hypertriglyceridaemic and are hypercholesterolaemic

(Erhuma et al. 2007). Work by Ozanne and colleagues has yielded similar findings, despite differences in the precise nature of the dietary restriction protocol applied in early life. Rats exposed to a maternal low protein diet throughout the fetal and suckling periods develop insulin resistance resulting from insulin signalling defects, but only in old age (males at 18 months and females at 21 months) (Ozanne et al. 2003; Fernandez-Twinn et al. 2005). Together, these observations suggest that protein undernutrition in early life is able to programme an insulin-resistant phenotype which develops with ageing. As with the human metabolic syndrome, the combined influence of fetal undernutrition and ageing produces rats that are insulin resistant, hypertensive, at risk of obesity and exhibit microalbuminuria.

The programming effects of a low protein diet in rat pregnancy are also noted in mice. This creates exciting opportunities for the investigation of programming using the full range of molecular tools that are available, including transgenic strains. Our laboratory has used this approach to study nutritional programming of atherosclerosis, a problem that cannot be investigated in rats, which are atherosclerosis-resistant. The apoE*3 Leiden mouse is a strain that carries a naturally occurring mutation in the human *ApoE* gene, on a C57Bl/6J background (Groot et al. 1996). This results in impaired clearance of lipoproteins from the plasma, raised plasma lipid levels and greater susceptibility to developing atherosclerosis when the mice are fed diets rich in cholesterol. Whilst other transgenic mouse strains, for example the LDL receptor knockout mouse, develop atherosclerosis even when fed a standard chow diet, the ApoE*3 Leiden mouse develops lesions only when fed a cholesterol-rich diet. This makes this strain ideal for studies evaluating the influence of diet upon atherosclerosis and coronary heart disease. Feeding a low protein diet to wild-type mice crossed with ApoE*3 Leiden males, resulted in ApoE*3 Leiden offspring that, when fed an atherogenic diet from weaning, developed atherosclerotic lesions that were more than twice as large as those exhibited by mice exposed to a protein-replete diet in fetal life (Yates et al. 2008).

Unravelling the mechanism of programming

Tissue remodelling

Normal tissue development in mammalian systems involves the key processes of proliferation and differentiation. All organs and tissues develop from a relatively small pool of progenitor cells in the embryo. These progenitor lineages are expanded in organogenesis during a proliferative phase. The precise timing of this proliferative phase will vary between tissues. Some organs such as the heart are formed very early in development, whereas others, for example the kidney, begin to grow at later stages. Proliferation is followed by a differentiation phase, during which the pool of cells formed earlier takes on the characteristic morphology and functions associated with the organ. The simplest explanation for how nutrition might programme physiology and function of organs is that the maternal response to under- or overnutrition interferes with either the proliferation or differentiation steps in tissue and organ development (Brameld et al. 1998).

The impact of disruption to either process would be expected to yield characteristic changes within a tissue. Adverse conditions during proliferation would not impact upon the differentiation of the cells within a tissue, and the affected organ would be expected to be smaller (fewer cells in total), but with a normal profile of cell types. In contrast, a tissue subject to adverse conditions during differentiation would be of normal size but would have an altered profile of cell types and potentially fewer functional units. Examples of both scenarios can be clearly seen in animal models of nutritional programming.

The kidneys of rats exposed to low protein diets *in utero* provide a good example of remodelling. On gross examination there is little difference between the kidneys of control and low protein-exposed animals and the organs are of similar size. However, determination of nephron number reveals a marked reduction of the order of 30–40% in the nutrient-restricted animals (Langley-Evans et al. 1999; Vehaskari et al. 2001). Similar effects on nephrogenesis are noted in sheep subjected to global undernutrition *in utero* (Gopalakrishnan et al. 2005). A reduction in the number of functional units, whilst maintaining normal tissue mass and cell number, strongly suggests a major influence of undernutrition during the differentiation of the specialized structures. In humans, nephron number has been correlated with weight at birth (Hughson et al. 2003). Populations in which there is a high degree of poverty are noted to manifest differences in renal morphometry. Among Australian aboriginal populations, for example, rates of chronic renal failure are more than 20-fold more common than in the Caucasian population and renal volume (a proxy for nephron number) tends to be lower (Singh & Hoy, 2004).

The brain is also a target for remodelling in response to variation in maternal nutrition. Fetal exposure to a maternal low protein diet results in a reduced density of capillaries within the cerebral cortex of mature offspring (Bennis-Taleb et al. 1999). When low protein feeding was extended to cover both the fetal and suckling periods in rats, there were profound changes to the gross structure of the brain. Hypothalamic centres involved in appetite regulation were enlarged, suggesting changes during proliferation, but differentiation was also clearly affected as neurons expressing peptides (galanin and neuropeptide Y) responsible for regulating appetite were present at much lower densities (Plagemann et al. 2001). Given the role of the hypothalamus in homeostasis, even relatively subtle

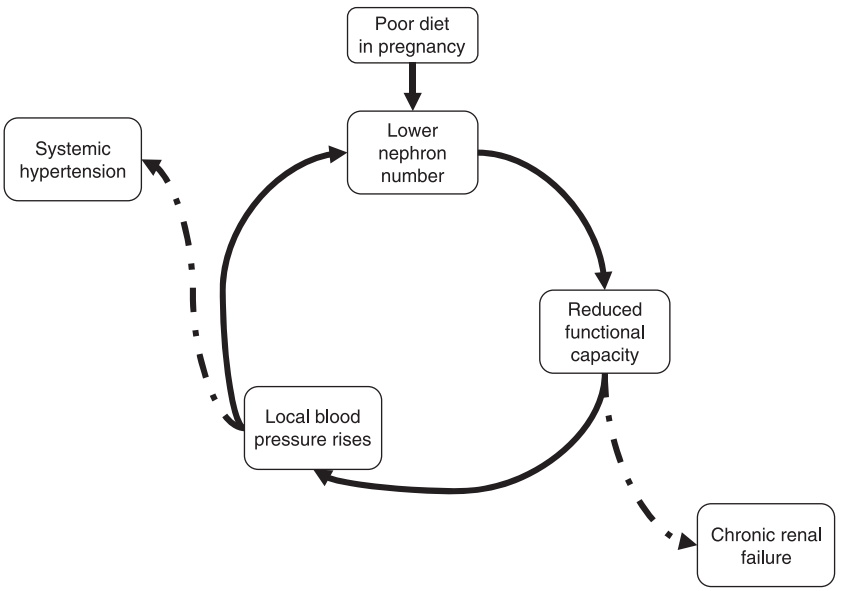

Fig. 4 Programming of low nephron number is a potential driver of hypertension and renal disease. Lower nephron number resulting from undernutrition during fetal development cannot be recovered in postnatal life as nephrogenesis is complete by birth in humans and most other species. This results in reduced functional capacity for the kidneys, forcing increases in local blood pressure to maintain glomerular filtration and fluid homeostatic functions. Rising pressures cause further damage and loss of nephrons and the kidney enters a vicious cycle of rising pressures and progressive tissue injury. Eventually, function will be degraded and systemic blood pressure will rise and the individual may enter a state of renal failure. Adapted from Mackenzie & Brenner (1995).

changes, of the kind identified by these studies, could have huge repercussions for a wide range of basic physiological and metabolic functions. The pancreas from rats exposed to low protein diets during pregnancy and lactation also shows evidence of both disordered proliferation and differentiation. Undernutrition reduces the number of functional units (β-cells and islets of Langerhans) present. The islets that are present are smaller and have a reduced vascular supply due to a reduction in the density of the pancreatic capillary network (Snoeck et al. 1990; Dahri et al. 1991).

Remodelling of the heart has not been as clearly demonstrated as in the tissues described above. However, variation in the size of the heart has been noted in offspring exposed to maternal undernutrition. Increased heart size was noted in cultured day 10.5 rat embryos from female rats fed iron-deficient diets for 4 weeks before mating and throughout pregnancy (Andersen et al. 2006). In contrast, exposure to a low protein diet in fetal development impairs heart growth in late gestation. This is in keeping with a general impairment of truncal growth that is noted in low protein-exposed fetuses. Neonates exposed to protein restriction *in utero* exhibit thinning of the left ventricular wall, and their hearts contain fewer cardiomyocytes (Cheema et al. 2005). This suggests that remodelling similar to that noted in other organs is taking place. Crucially, this remodelling appears to promote left ventricular hypertrophy in adult life (Tappia et al. 2005). Male rats subject to protein undernutrition in fetal life are more vulnerable to the effects of ischaemia–reperfusion (Elmes et al. 2007).

The remodelling processes described above are almost certainly the proximal causes of the disease phenotypes that are programmed by nutrition. Changes to the structure and function of tissues and organs will drive disordered physiology through a variety of means. For example, in the kidney it is believed that chronic renal disease and hypertension are products of the process shown in Fig. 4. A programmed deficit of nephrons leads to a vicious cycle in which rising local blood pressures are required to maintain renal perfusion and fluid homeostasis, and yet in turn these higher pressures promote further nephron loss. However, all such processes are secondary to the true drivers of programming. The most important questions to be addressed remain unanswered. We do not yet fully understand which process or processes are responsible for remodelling of tissue development.

The role of the placenta

The placenta might conceivably play a major role in mediating the programming effects of maternal under- or overnutrition. Placental factors are inevitably involved to some extent, as perturbations in the maternal environment must be transmitted across the placenta to affect the fetus. The relationship between placental function and fetal nutrition is complex. The nutrients that are transferred from the maternal to the fetal compartment may not reflect actual maternal intakes as some, for example amino acids, can be synthesized *de novo* within the placenta (Cleal & Lewis, 2008). In some cases, fetal demand for energy and nutrients might be met by mobilization of maternal stores and transfer by placenta. In other cases, low maternal intakes of some nutrients, for example folic acid, may disproportionately impact upon the fetus as the maternal requirements will take priority. Moreover, the ability of the placenta to provide the fetus with the substrates it requires will depend upon the quality of the placentation, the size of the placenta, the demands of the placenta itself for nutrients, and the extent of the

maternal adaptations to pregnancy that should increase cardiac output to support placental perfusion (Harding, 2004).

The contribution of the placenta to the maternal–fetal nutrient supply line is therefore difficult to assess in the context of programming. It is conceivable, however, that deficits or imbalances in the quality or quantity of maternal nutrition could contribute to programming simply by restricting fetal substrate supply. The impact of unsuccessful placentation upon fetal growth, for example, can be readily observed in conditions such as pre-eclampsia. Here, problems with the invasion of the placenta during the early stages of its development not only contribute to the hypertensive disorder experienced by the mother. The reduced placental perfusion that accompanies pre-eclampsia is also associated with fetal growth retardation (Poston, 2006).

In addition to the contribution to the maternal–fetal supply line, the placenta is a major source of endocrine signals. Whilst some of these play a role in maintaining the pregnancy, others are likely to be involved in modulating the fetal growth rate and the maturation of the brain and other organs within the fetus. For example, the placenta secretes corticotrophin releasing hormone, which is known to have an important role in the maturation of the fetal hypothalamic–pituitary adrenal axis and, via production of glucocorticoids from the fetal adrenal, the maturation of the lungs. Many of the hormones produced by the placenta serve to control the flow of blood through the placenta and hence optimize the delivery of substrates to the fetal tissues. Maternal undernutrition and other stressors may modulate patterns of placental hormone secretion and hence alter the uterine environment experienced by the fetus, compromise the integrity of the placenta itself, alter gestation length and modify fetal growth rates.

Despite these putative effects, the role of the placenta in programming has not been investigated in great depth, either in humans or in any of the animal models. It is apparent, however, that the placenta is highly sensitive to variation in the maternal diet. We have consistently shown that feeding a low protein diet in rat pregnancy initially promotes an increased rate of placental growth (Langley-Evans et al. 1996a). Placentas from rats culled at 14 days' gestation, for example, were larger when mothers were fed a low protein diet, particularly if the undernutrition was targeted at the first 7 days of pregnancy (Langley-Evans & Nwagwu, 1998). By day 20 gestation, differences in placental weight associated with protein restriction are not apparent, but the fetal : placental ratio differs from that of controls. Observations of this sort are suggestive of effects of undernutrition upon placentation, but are far from convincing.

Mice fed low protein diets in pregnancy also manifest gross differences in placentation, but these effects appear different to those noted in rats. At day 14.5 gestation, pregnant mice fed low protein diets had smaller fetuses but placentas of similar size to controls (Rutland et al. 2007). By day 18.5, both fetal and placental weight were reduced. Stereological examination of placental structure revealed changes in the lengths of maternal and fetal blood vessels within the labyrinthine layers of the placenta (Fig. 5). Low protein feeding was associated with perturbation of the expression of cadherin and β-catenin in the vascular endothelium. These adhesion molecules are regulators of junctional integrity and permeability (Dejana, 1996). Their relative absence from junctional sites in the low protein-exposed placenta may indicate that undernutrition may render the placenta more permeable. Put together, the reduction in length of the labyrinthine vessels and the down-regulation of the adhesion molecules suggests that maternal undernutrition causes vascular dysfunction in the placenta. This could certainly contribute

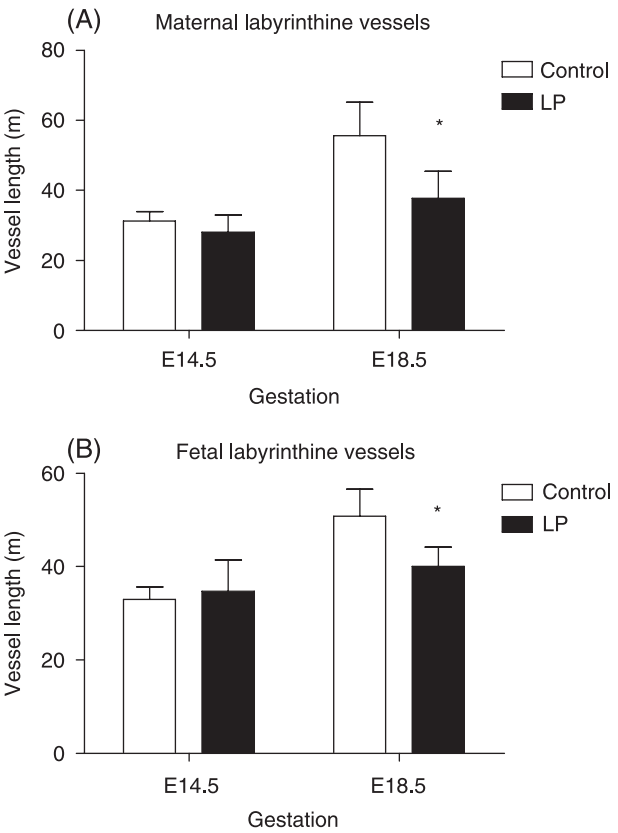

Fig. 5 Maternal low protein diets in mouse pregnancy impair the development of blood vessels in the labyrinthine layers of the placenta. Pregnant mice were fed an 18% casein (Control) or 9% casein (low protein) diet from conception until sacrifice at either day 14.5 or 18.5 of gestation. Stereological analysis revealed differences in the length of maternal and fetal vessels in later pregnancy. (A) Maternal vessel length; (B) fetal vessel length. Data are shown as mean ± SE for $n = 5$ observations at each time point. * indicates a significant difference between control and low protein fed groups ($P < 0.05$). Data redrawn from Rutland et al. (2007).

to disordered maternal–fetal substrate supply and hence drive fetal tissue remodelling.

A series of studies by Powell and Jansson have shown that expression of placental glucose and amino acid transporters and hence the supply of substrates to the fetus may be modulated by maternal nutritional status. Jansson et al. (2006) reported that low protein feeding in rats down-regulated the placental system A amino acid transporter by as much as 80% in mid-gestation and suggested that this effect may have been driven by lower concentrations of insulin, leptin and IGF-1 in the maternal circulation. In contrast, treatment of pregnant rats with six injections of glucose (2 g kg^{-1} body weight to induce hyperglycaemia) on days 10 and 11 of gestation, did not alter expression of the system A or system L amino acid transporter, but despite this did reduce the capacity of placentas to transport [^{14}C]methyl-aminoisobutyric acid, a function mediated by system A (Ericsson et al. 2007). This suggests that maternal metabolic control in early pregnancy could be an important determinant of feto-placental growth and placental function later in pregnancy.

Glucocorticoids and programming

In addition to acting as the conduit for nutrient exchange between mother and fetus and as a source of hormones, the placenta acts as a critical barrier, preventing the access of potentially harmful substances to the fetal compartment. For substances in the aqueous phase, this is relatively simple as only those for which transporters exist will be able to cross the placenta. For fat-soluble materials, movement to the fetal compartment should be free and unimpeded, but it is clear that the placenta has systems in place to regulate the entry of at least some compounds.

The glucocorticoids are steroid hormones which are important mediators of stress responses and metabolic functions. Like all steroid hormones, binding of hormones to glucocorticoid receptors can initiate the transcription of gene targets through binding of receptor complexes to glucocorticoid response elements. These are widespread within the genome. There is a very large concentration gradient for glucocorticoids across the placenta, which can be as great as 1000 : 1, indicating that the placenta actively prevents them from passing from mother to fetus. This function is carried out by 11β-hydroxysteroid dehydrogenase 2 (11βHSD2), which in placenta converts active glucocorticoids such as cortisol to inactive forms (Edwards et al. 1996). The presence of 11βHSD2 ensures that the fetal tissues are exposed to only low levels of glucocorticoid. This enables the fetal hypothalamic–pituitary–adrenal axis to develop independently of maternal influences and, importantly, ensures that fetal gene expression is not overly influenced by factors that impact upon maternal glucocorticoid secretion.

Glucocorticoids are routinely used in obstetric practice to promote maturation of the fetal lungs when preterm delivery is likely. Antenatal glucocorticoids promote secretion of pulmonary surfactant. These therapeutic uses circumvent the barrier function of 11βHSD2 as the administered hormones are synthetic glucocorticoids such as dexamethasone and betamethasone, which are poor substrates for the enzyme. Studies of rodents and sheep suggest that fetal exposure to these hormones can have potent programming effects upon renal development and later blood pressure (Benediktsson et al. 1993; Celsi et al. 1998; Dodic et al. 2002). The strong similarities between the phenotypes associated with antenatal glucocorticoid exposure and nutritional insults have prompted consideration that undernutrition may act as a stressor that alters the endocrine cross-talk between mother and fetus. Indeed, the feeding of a low protein diet in rat pregnancy resulted in reduced expression and activity of placental 11βHSD2 (Langley-Evans et al. 1996b). As shown in Fig. 6, this has led to the hypothesis that undernutrition promotes overexposure of the fetal tissues to glucocorticoids of maternal origin, and this drives tissue remodelling and the development of the programmed phenotype (Langley-Evans, 1997a).

Support for this hypothesis can be derived from experiments that have sought to blockade or mimic the relationship between maternal undernutrition and placental 11βHSD2. When rat dams fed low protein diets are treated with metyrapone in early to mid-gestation, effectively performing a pharmacological adrenalectomy, their offspring fail to develop the programmed phenotype of hypertension and renal insufficiency (McMullen & Langley-Evans, 2005). Replacement doses of corticosterone restore the programming effect of the diet. The programming effects of protein restriction are therefore, to some extent, dependent upon an intact hypothalamic–pituitary–adrenal axis in the mother. Inhibition of 11βHSD2 using carbenoxolone during late gestation programmes raised blood pressure in the offspring of rats fed a control diet (Langley-Evans, 1997b).

The processes through which glucocorticoid overexposure promotes widespread programming of physiology, metabolism and disease risk are not well understood. There are many reports of glucocorticoid target genes being up-regulated in a range of tissues in animals exposed to undernutrition *in utero* (Langley-Evans et al. 1996c; Desai et al. 1997; McMullen et al. 2004), but in almost all cases these observations have been made in adult animals and therefore well downstream of primary programming events. However, glucocorticoids are known to bring about the maturation of tissues, favouring differentiation over proliferation. It may therefore be possible that in the context of tissue remodelling they lead to production of smaller organs with an altered profile of functional units. Certainly in the kidney, even very brief exposure to synthetic glucocorticoids reduces nephron number (Dodic et al. 2002).

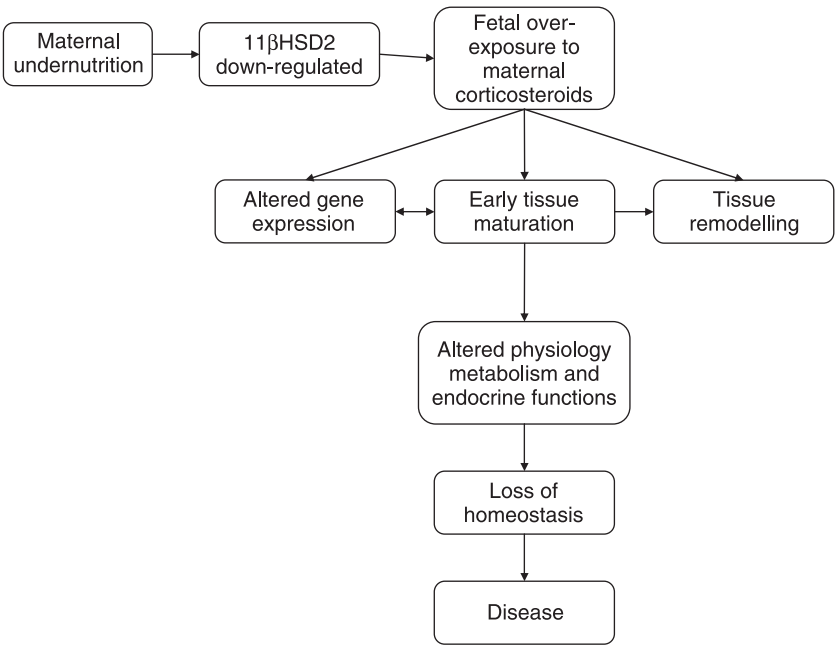

Fig. 6 The glucocorticoid programming hypothesis. Overexposure of fetal tissues to glucocorticoids of maternal origin may play a central role in nutritional programming. 11β-hydroxysteroid dehydrogenase 2 (11βHSD2) has a barrier role in placenta, preventing movement of active corticosteroids from the maternal to the fetal compartment. Nutritional down-regulation of this enzyme can be postulated to have a major impact on organ development.

Epigenetic regulation of gene expression

The expression of genes is known to be regulated by a number of epigenetic factors that can silence or switch on transcription through modulating access of the transcriptional machinery to the DNA strands. DNA methylation and histone acetylation essentially control the tightness of the coiling of DNA around the histone proteins within the chromosome, with methylation leading to gene silencing and acetylation promoting transcription (Bird, 2002). DNA methylation patterns have been shown to be stably inherited and may therefore allow phenotypic traits, acquired as a result of nutritional programming, to be passed on to any offspring. Undernutrition during embryonic, fetal or even early postnatal development may irreversibly modify DNA methylation. In this way, even a brief period of sub-optimal nutrition can alter gene expression over a long period and compromise normal physiology and metabolism.

The major interest in a role for DNA methylation in nutritional programming was largely initiated by the work of Waterland & Jirtle (2003). Experiments using the Agouti mouse, in which a yellow coat colour is determined by the overexpression of a single gene locus (Avy), demonstrated that nutritional factors in fetal life can modify expression of genes. The over-expression of this gene is due to hypomethylation of CpG islands in the Avy locus. When yellow mice were supplemented with folic acid, vitamin B12, choline chloride and betaine, the methylation of Avy was increased in the offspring. This resulted in more mice with an intermediate brown coat colour being born to the supplemented mothers.

Methylation may be important in the context of disease programming. Sinclair et al. (2007) fed sheep a diet that was deficient in the methyl donors, folic acid, vitamin B12 and methionine for a period of 8 weeks prior to conception and for the first 6 days of pregnancy. Male offspring from these ewes were insulin resistant and had elevated blood pressure. Restriction landmark genome scanning showed that 4% of 1400 CpG islands in the fetal liver were differentially methylated. The majority of these loci were either hypomethylated or totally demethylated. This finding is in keeping with studies in rats that have suggested that exposure to maternal low protein diets also leads to hypomethylation and therefore overexpression of certain genes (Lillycrop et al. 2005, 2007, 2008).

Lillycrop and colleagues have reported that low protein feeding in rat pregnancy results in the adult offspring overexpressing the peroxisome proliferator activated receptor-α (PPARα) and glucocorticoid receptor in this manner (Lillycrop et al. 2005). Furthermore, changes to histone acetylation accompanying hypomethylation of the glucocorticoid receptor (GR_{10}) promoter further facilitate transcription. There is some evidence that the expression of DNA methyltransferase 1, the enzyme responsible for maintenance of DNA methylation patterns that are set in the embryonic period, may be down-regulated by the maternal low protein diet (Lillycrop et al. 2007). Availability of folic acid as a donor of methyl groups for methylation may also be of importance in these processes. Supplementation of maternal low protein diets with high-dose folate supplements can prevent expression of the physiological phenotypes and also appears to normalize the DNA methylation patterns (Lillycrop et al. 2005, 2007; Torrens et al. 2006).

The findings from these sheep and rat studies suggest that prenatal undernutrition can impact upon epigenetic regulation of gene expression. These changes may be a

direct cause of the metabolic phenotype associated with exposure to undernutrition, by modifying tissue development and the long-term expression of key genes. In this context PPARα provides an interesting example. Like Lillycrop et al. (2005) we have shown in our laboratory that the maternal low protein diet results in hepatic overexpression of this gene during early adulthood (Erhuma et al. 2007). The downstream target genes for this transcription factor result in a obesity-resistant phenotype with apparently increased insulin sensitivity. With ageing, however, PPARα expression declines, and in older rats exposed to low protein diets *in utero* it is expressed at only half the level seen in control animals. This change is accompanied by a dramatic change in phenotype to an obesity-prone state, accompanied by hepatic steatosis and insulin resistance. It is possible that this change is driven by a phenomenon called epigenetic drift. This process occurs during ageing, and can promote DNA hypo- and hypermethylation. The basis of age-related epigenetic drift is that there is a progressive decline in expression of DNA methyltransferase-1 (Casillas et al. 2003; Fraga & Esteller, 2007). This leads to passive demethylation of the whole genome. In some tissues the response to this may be up-regulation of DNA methyltransferase-3b, leading to hypermethylation of CpG islands in gene promoters (Casillas et al. 2003; Fraga & Esteller, 2007). This mechanism has already been linked to the development of human cancers. The possibility that it explains the age-related emergence of some programmed phenotypes has not yet been explored experimentally.

Future priorities

Work with animal models of nutritional programming has clearly demonstrated the plausibility of the hypothesis that nutrition-related factors in human pregnancy could be related to disease in adulthood. The effects of even very brief periods of under- or overnutrition during pregnancy have been neatly demonstrated, and despite the huge diversity in the experimental approaches that have been used, the range of phenotypes displayed by the offspring is remarkably narrow. With evidence firmly pointing to cardiovascular disease, obesity and metabolic disorders as conditions that are influenced by programming *in utero*, there is no longer any need for observational studies that characterize the effects of manipulating maternal nutrition and all attention must focus firmly on understanding the mechanisms that link nutritional factors to disease processes. In addressing this priority there are a number of key issues that should be addressed:

1 *There needs to be a systematic and unbiased search for programming mechanisms.* The nutritional programming field as a whole needs to recognize that, as yet, there is no definitive mechanism which is known to drive programming. Even the glucocorticoid and epigenetic hypotheses describe processes that are likely to be secondary to other changes induced by signals related to maternal nutrition. Animal studies of programming tend to characterize the downstream phenotypes that are observed in adult offspring and hence will focus on processes that may mediate the exact pathology or metabolic consequences of programming. Many of these processes are likely to be secondary phenomena and do not explain the fundamental basis of programming. Broad assumptions have been drawn about observed changes in the expression of single genes or specific pathways that have been selected on the basis of their plausible involvement in development of metabolic or vascular phenotypes. Close association with the phenotype actually increases the likelihood that observed effects are secondary events. It seems likely that changes in the expression of a small number of 'gatekeeper' genes and/or their protein products at critical phases of development will have a major impact on cell type and number within tissues, and hence responsiveness to homeostatic signals, gene expression and tissue function. A systematic and unbiased search for these gatekeepers should be a high priority for the field. The diversity of animal models with similar phenotypes may provide a mechanistic tool to conduct such a search. For example, the low protein model and the iron deficiency model of rat pregnancy both yield offspring with elevated blood pressure (Langley & Jackson, 1994; Gambling et al. 2003). Proteomic and DNA microarray comparisons of the two models, timed to appropriate stages in fetal or embryonic life, would therefore identify the genes, proteins and pathways that are differentially expressed in both of the undernourished groups. These would be putative gatekeepers that would merit further study.

2 *Studies need to consider the whole life-course of the model species.* Most work with large and small animal models of programming has involved a nutritional manipulation during pregnancy, with follow-up of the offspring generally only in early adult life. Our experience with studies of longevity revealed that programmed phenotypes can change dramatically with ageing (Erhuma et al. 2007). Work that seeks to confirm any putative mechanism must therefore address this issue and make measurements very early in life (e.g. during the embryonic or fetal period when exposure is occurring) and at points in adult life right through to senescence.

3 *Studies must confirm the functional significance of gene or protein changes*. It is likely that any systematic screen for candidate genes, proteins and pathways that are responsible for mediating programming effects will yield a large number of putative targets. Care will need to be taken to demonstrate the true functional significance of any up- or down-regulation of these targets using appropriate pharamacological agonists and antagonists, transgenic animals and other technologies. Where epigenetic mechanisms are invoked it will be critical to demonstrate

that the often small changes in methylation or histone acetylation reported for particular target gene promoters are actually functionally significant. For example, Lillycrop et al. (2008), reported that methylation of the PPARα promoter was down-regulated by 26% in offspring of rats fed a low protein diet during pregnancy. Studies in the far more advanced cancer field suggest that differential methylation needs to be of the order of two-fold or more up- or down- to have a physiological impact.

4 *Studies need to have a robust statistical design and power.* In stark contrast to the epidemiological literature, studies of the animal models of programming often report findings from relatively small numbers of animals and have been criticized on the basis of poorly conducted statistical analyses (Walters & Edwards, 2004). Pooling of datasets and expertise between different groups using similar animal models would be of huge benefit in driving understanding of nutritional programming forward.

5 *Can we intervene?* Having identified the contribution that nutritional programming effects make to the development of disease, and with work to discover the underpinning mechanisms in progress, the development of novel therapeutic and disease prevention strategies is a key priority. Interventions to offset programming of disease might include improvements in the quality of diets for pregnant women, identification of individuals at risk of adult disease based upon screening of maternal and birth characteristics, or administration of novel therapeutics during childhood to target gatekeeper or related processes. There are already examples of these kinds of interventions in animal models. For example, folate or glycine supplementation of a low protein diet during rat pregnancy can offset the programming effect of the protein undernutrition (Jackson et al. 2003, Torrens et al. 2006). Administration of angiotensin II receptor antagonists in the suckling period ameliorates the programming effect of undernutrition, but has harmful effects on unaffected animals (Sherman & Langley-Evans, 2000).

6 *Can programming effects persist over multiple generations?* This issue also relates to the importance of being able to intervene to offset the impact of programming. If maternal undernutrition can exert major effects upon fetal development and disease risk, the whole field is of major significance to public health. The impact of this magnifies exponentially if there is any chance that programmed effects could be transmitted from one generation to another. In populations such as India and China, where for generations the quality of the diet for most of the population has been poor, intergenerational programming would mean that the nutritional/economic transition associated with greater affluence may produce a sharp decline in malnutrition-related disease to be followed by a century (two to three generations) of unavoidable metabolic disease. There is some evidence that immune function can be programmed for up to three generations in mice (Beach et al. 1982), and our own studies of low protein diets in rat pregnancy indicate transmission of high blood pressure to a second generation via both maternal and paternal lines (Harrison & Langley-Evans, 2008).

Conclusion

The prevailing nutritional environment during fetal development exerts powerful and long-lasting effects upon physiology and metabolism. Adaptive responses to less-than-optimal nutrition may play an important role in the aetiology of human disease. Understanding of the mechanisms through which this nutritional programming occurs is still at an early stage. However, the key questions to be addressed have been largely identified and progress towards identifying strong candidates for future preventative and therapeutic strategies is likely to be rapid.

References

Aihie Sayer A, Dunn R, Langley-Evans S, Cooper C (2001) Prenatal exposure to a maternal low protein diet shortens life span in rats. *Gerontology* **47**, 9–14.

Andersen HS, Gambling L, Holtrop G, McArdle HJ (2006) Maternal iron deficiency identifies critical windows for growth and cardiovascular development in the rat postimplantation embryo. *J Nutr* **136**, 1171–1177.

Arai Y, Gorski RA (1968) Critical exposure time for androgenization of the developing hypothalamus in the female rat. *Endocrinology* **82**, 1010–1014.

Arenz S, Rückerl R, Koletzko B, von Kries R (2004) Breast-feeding and childhood obesity – a systematic review. *Int J Obes Relat Metab Disord* **28**, 1247–1256.

Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ (1989) Weight in infancy and death from ischaemic heart disease. *Lancet* **ii**, 577–580.

Barker DJ, Bull AR, Osmond C, Simmonds SJ (1990) Fetal and placental size and risk of hypertension in adult life. *BMJ* **301**, 259–262.

Barker DJ, Godfrey KM, Osmond C, Bull A (1992) The relation of fetal length, ponderal index and head circumference to blood pressure and the risk of hypertension in adult life. *Paediatr Perinat Epidemiol* **6**, 35–44.

Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM (1993a) Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* **36**, 62–67.

Barker DJ, Osmond C, Simmonds SJ, Wield GA (1993b) The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. *BMJ* **306**, 422–426.

Bayol SA, Farrington SJ, Stickland NC (2007) A maternal 'junk food' diet in pregnancy and lactation promotes an exacerbated taste for 'junk food' and a greater propensity for obesity in rat offspring. *Br J Nutr* **98**, 843–851.

Beach RS, Gershwin ME, Hurley LS (1982) Gestational zinc deprivation in mice: persistence of immunodeficiency for three generations. *Science* **218**, 469–471.

Bellinger L, Lilley C, Langley-Evans SC (2004) Prenatal exposure to a maternal low-protein diet programmes a preference for high-fat foods in the young adult rat. *Br J Nutr* **92**, 513–520.

Bellinger L, Sculley DV, Langley-Evans SC (2006) Exposure to undernutrition in fetal life determines fat distribution, locomotor activity and food intake in ageing rats. *Int J Obes (Lond)* **30**, 729–738.

Benediktsson R, Lindsay RS, Noble J, Seckl JR, Edwards CR (1993) Glucocorticoid exposure in utero: new model for adult hypertension. *Lancet* **341**, 339–341.

Bennis-Taleb N, Remacle C, Hoet JJ, Reusens B (1999) A low-protein isocaloric diet during gestation affects brain development and alters permanently cerebral cortex blood vessels in rat offspring. *J Nutr* **129**, 1613–1619.

Bibby E, Stewart A (2004) The epidemiology of preterm birth. *Neuro Endocrinol Lett* **25** (Suppl. 1), 43–47.

Bird A (2002) DNA methylation patterns and epigenetic memory. *Genes Dev* **16**, 6–21.

Brameld JM, Buttery PJ, Dawson JM, Harper JM (1998) Nutritional and hormonal control of skeletal-muscle cell growth and differentiation. *Proc Nutr Soc* **57**, 207–217.

Brown JE, Carlson M (2000) Nutrition and multifetal pregnancy. *J Am Diet Assoc* **100**, 343–348.

Calder PC, Yaqoob P (2000) The level of protein and type of fat in the diet of pregnant rats both affect lymphocyte function in the offspring. *Nutr Res* **20**, 995–1005.

Campbell DM, Hall MH, Barker DJ, Cross J, Shiell AW, Godfrey KM (1996) Diet in pregnancy and the offspring's blood pressure 40 years later. *Br J Obstet Gynaecol* **103**, 273–280.

Casillas MA, Lopatina N, Andrews LG, Tollefsbol TO (2003) Transcriptional control of the DNA methyltransferases is altered in aging and neoplastically-transformed human fibroblasts. *Mol Cell Biochem* **252**, 33–43.

Celsi G, Kistner A, Aizman R, et al. (1998) Prenatal dexamethasone causes oligonephronia, sodium retention, and higher blood pressure in the offspring. *Pediatr Res* **44**, 317–322.

Cheema KK, Dent MR, Saini HK, Aroutiounova N, Tappia PS (2005) Prenatal exposure to maternal undernutrition induces adult cardiac dysfunction. *Br J Nutr* **93**, 471–477.

Cleal JK, Lewis RM (2008) The mechanisms and regulation of placental amino acid transport to the human foetus. *J Neuroendocrinol* **20**, 419–426.

Daenzer M, Ortmann S, Klaus S, Metges CC (2002) Prenatal high protein exposure decreases energy expenditure and increases adiposity in young rats. *J Nutr* **132**, 142–144.

Dahri S, Snoeck A, Reusens-Billen B, Remacle C, Hoet JJ (1991) Islet function in offspring of mothers on low-protein diet during gestation. *Diabetes* **40** (Suppl. 2), 115–120.

Dejana E (1996) Endothelial adherens junctions: implications in the control of vascular permeability and angiogenesis, *J Clin Invest* **98** 1949–1953.

Desai M, Byrne CD, Zhang J, Petry CJ, Lucas A, Hales CN (1997) Programming of hepatic insulin-sensitive enzymes in offspring of rat dams fed a protein-restricted diet. *Am J Physiol* **272**, G1083–G1090.

Dodic M, Abouantoun T, O'Connor A, Wintour EM, Moritz KM (2002) Programming effects of short prenatal exposure to dexamethasone in sheep. *Hypertension* **40**, 729–734.

Edwards CR, Benediktsson R, Lindsay RS, Seckl JR (1996)11 beta-Hydroxysteroid dehydrogenases: key enzymes in determining tissue-specific glucocorticoid effects. *Steroids* **61**, 263–269.

Elmes MJ, Gardner DS, Langley-Evans SC (2007) Fetal exposure to a maternal low-protein diet is associated with altered left ventricular pressure response to ischaemia-reperfusion injury. *Br J Nutr* **98**, 93–100.

Erhuma A, Salter AM, Sculley DV, Langley-Evans SC, Bennett AJ (2007) Prenatal exposure to a low-protein diet programs disordered regulation of lipid metabolism in the aging rat. *Am J Physiol* **292**, E1702–E1714.

Ericsson A, Säljö K, Sjöstrand E, et al. (2007) Brief hyperglycaemia in the early pregnant rat increases fetal weight at term by stimulating placental growth and affecting placental nutrient transport. *J Physiol* **581**, 1323–1332.

Eriksson JG, Lindi V, Uusitupa M, et al. (2002a) The effects of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-gamma2 gene on insulin sensitivity and insulin metabolism interact with size at birth. *Diabetes* **51**, 2321–2324.

Eriksson JG, Forsen T, Tuomilehto J, Jaddoe VW, Osmond C, Barker DJ (2002b) Effects of size at birth and childhood growth on the insulin resistance syndrome in elderly individuals. *Diabetologia* **45**, 342–348.

Fall CH, Pandit AN, Law CM, et al. (1995) Size at birth and plasma insulin-like growth factor-1 concentrations. *Arch Dis Child* **73**, 287–293.

Fernandez-Twinn DS, Wayman A, Ekizoglou S, Martin MS, Hales CN, Ozanne SE (2005) Maternal protein restriction leads to hyperinsulinemia and reduced insulin-signaling protein expression in 21-mo-old female rat offspring. *Am J Physiol*. **288**, R368–R373.

Fraga MF, Esteller M (2007) Epigenetics and aging: the targets and the marks. *Trends Genet* **23**, 413–418.

Gambling L, Dunford S, Wallace DI, et al. (2003) Iron deficiency during pregnancy affects postnatal blood pressure in the rat. *J Physiol* **552**, 603–610.

Gillman MW, Rifas-Shiman SL, Kleinman KP, Rich-Edwards JW, Lipshultz SE (2004) Maternal calcium intake and offspring blood pressure. *Circulation* **110**, 1990–1995.

Gluckman PD, Hanson MA (2004) Living with the past: evolution, development, and patterns of disease. *Science* **305**, 1733–1736.

Gopalakrishnan GS, Gardner DS, Dandrea J, et al. (2005) Influence of maternal pre-pregnancy body composition and diet during early-mid pregnancy on cardiovascular function and nephron number in juvenile sheep. *Br J Nutr* **94**, 938–947.

Groot PH, van Vlijmen BJ, Benson GM, et al. (1996) Quantitative assessment of aortic atherosclerosis in APOE*3 Leiden transgenic mice and its relationship to serum cholesterol exposure. *Arterioscler Thromb Vasc Biol* **16**, 926–933.

Hales CN, Barker DJ, Clark PM, et al. (1991) Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ* **303**, 1019–1022.

Harding J (2004) Nutritional basis for the fetal origins of adult disease. In *Fetal Nutrition and Adult Disease* (eds Langley-Evans SC), pp. 21–54. CABI, Wallingford, UK.

Harrison M, Langley-Evans SC (2008) Intergenerational programming of impaired nephrogenesis and hypertension in rats following maternal protein restriction during pregnancy. *Br J Nutr* in press.

Hughson M, Farris AB, Douglas-Denton R, Hoy WE, Bertram JF (2003) Glomerular number and size in autopsy kidneys: the relationship to birth weight. *Kidney Int* **63**, 2113–2122.

Huxley R, Neil A, Collins R (2002) Unravelling the fetal origins hypothesis: is there really an inverse association between birthweight and subsequent blood pressure? *Lancet* **360**, 659–665.

Jackson AA, Dunn RL, Marchand MC, Langley-Evans SC (2003) Increased systolic blood pressure in rats induced by a maternal low-protein diet is reversed by dietary supplementation with glycine. *Clin Sci (Lond)* **103**, 633–639.

Jansson N, Pettersson J, Haafiz A, et al. (2006) Down-regulation of placental transport of amino acids precedes the development of intrauterine growth restriction in rats fed a low protein diet. *J Physiol* **576**, 935–946.

Jensen DM, Damm P, Sørensen B, et al. (2003) Pregnancy outcome and prepregnancy body mass index in 2459 glucose-tolerant Danish women. *Am J Obs Gynecol* **189**, 239–244.

Joseph KS, Kramer MS (1996) Review of the evidence on fetal and early childhood antecedents of adult chronic disease. *Epidemiol Rev* **18**, 158–174.

Khan IY, Taylor PD, Dekou V, et al. (2003) Gender-linked hypertension in offspring of lard-fed pregnant rats. *Hypertension* **41**, 168–175.

Khan IY, Dekou V, Douglas G, et al. (2005) A high-fat diet during rat pregnancy or suckling induces cardiovascular dysfunction in adult offspring. *Am J Physiol* **288**, R127–R133.

Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ, Schouten EG; MTHFR Studies Collaboration Group (2002) MTHFR 677C→T polymorphism and risk of coronary heart disease: a meta-analysis. *JAMA* **288**, 2023–2031.

Langley-Evans SC (1997a) Intrauterine programming of hypertension by glucocorticoids. *Life Sci* **60**, 1213–1221.

Langley-Evans SC (1997b) Maternal carbenoxolone treatment lowers birthweight and induces hypertension in the offspring of rats fed a protein-replete diet. *Clin Sci (Lond)* **93**, 423–429.

Langley-Evans SC (2006) Developmental programming of health and disease. *Proc Nutr Soc* **65**, 97–105.

Langley SC, Jackson AA (1994) Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diets. *Clin Sci (Lond)* **86**, 217–222.

Langley-Evans SC, Jackson AA (1995) Captopril normalises systolic blood pressure in rats with hypertension induced by fetal exposure to maternal low protein diets. *Comp Biochem Physiol* **110**, 223–228.

Langley-Evans AJ, Langley-Evans SC (2003) Relationship between maternal nutrient intakes in early and late pregnancy and infants weight and proportions at birth: prospective cohort study. *J R Soc Health* **123**, 210–216.

Langley-Evans SC, Nwagwu M (1998) Impaired growth and increased glucocorticoid-sensitive enzyme activities in tissues of rat fetuses exposed to maternal low protein diets. *Life Sci* **63**, 605–615.

Langley-Evans SC, Sculley DV (2005) Programming of hepatic antioxidant capacity and oxidative injury in the ageing rat. *Mech Ageing Dev* **126**, 804–812.

Langley-Evans SC, Phillips GJ, Jackson AA (1994) In utero exposure to maternal low protein diets induces hypertension in weanling rats, independently of maternal blood pressure changes. *Clinical Nutrition* **13**, 319–324.

Langley-Evans SC, Gardner DS, Jackson AA (1996a) Association of disproportionate growth of fetal rats in late gestation with raised systolic blood pressure in later life. *J Reprod Fertil* **106**, 307–312.

Langley-Evans SC, Phillips GJ, Benediktsson R, et al. (1996b) Protein intake in pregnancy, placental glucocorticoid metabolism and the programming of hypertension in the rat. *Placenta* **17**, 169–172.

Langley-Evans SC, Gardner DS, Jackson AA (1996c) Maternal protein restriction influences the programming of the rat hypothalamic-pituitary-adrenal axis. *J Nutr* **126**, 1578–1585.

Langley-Evans SC, Welham SJ, Jackson AA (1999) Fetal exposure to a maternal low protein diet impairs nephrogenesis and promotes hypertension in the rat. *Life Sci* **64**, 965–974.

Langley-Evans SC, Lilley C, McMullen S (2006) Maternal protein restriction and fetal growth: lack of evidence of a role for homocysteine in fetal programming. *Br J Nutr* **96**, 578–586.

Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC (2005) Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. *J Nutr* **135**, 1382–1386.

Lillycrop KA, Slater-Jefferies JL, Hanson MA, Godfrey KM, Jackson AA, Burdge GC (2007) Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications. *Br J Nutr* **97**, 1064–1073.

Lillycrop KA, Phillips ES, Torrens C, Hanson MA, Jackson AA, Burdge GC (2008) Feeding pregnant rats a protein-restricted diet persistently alters the methylation of specific cytosines in the hepatic PPARalpha promoter of the offspring. *Br J Nutr*, in press.

Mackenzie HS, Brenner BM (1995) Fewer nephrons at birth: a missing link in the etiology of essential hypertension? *Am J Kidney Dis* **26**, 91–98.

Martin RM, Ben-Shlomo Y, Gunnell D, Elwood P, Yarnell JW, Davey Smith G (2005) Breast feeding and cardiovascular disease risk factors, incidence, and mortality: the Caerphilly study. *J Epidemiol Commun Health* **59**, 121–129.

Martyn CN, Barker DJ, Jespersen S, Greenwald S, Osmond C, Berry C (1995) Growth in utero, adult blood pressure, and arterial compliance. *Br Heart J* **73**, 116–121.

Mathews F, Yudkin P, Neil A (1999) Influence of maternal nutrition on outcome of pregnancy: prospective cohort study. *BMJ* **319**, 339–343.

McMullen S, Langley-Evans SC (2005) Maternal low-protein diet in rat pregnancy programs blood pressure through sex-specific mechanisms. *Am J Physiol* **288**, R85–R90.

McMullen S, Gardner DS, Langley-Evans SC (2004) Prenatal programming of angiotensin II type 2 receptor expression in the rat. *Br J Nutr* **91**, 133–140.

Milnes MR, Roberts RN, Guillette LJ (2002) Effects of incubation temperature and estrogen exposure on aromatase activity in the brain and gonads of embryonic alligators. *Environ Health Perspect* **110**, 393–396.

Nüsken KD, Dötsch J, Rauh M, Rascher W, Schneider H (2008) Uteroplacental insufficiency after bilateral uterine artery ligation in the rat: impact on postnatal glucose and lipid metabolism and evidence for metabolic programming of the offspring by sham operation. *Endocrinology* **149**, 1056–1063.

Ozanne SE, Olsen GS, Hansen LL, et al. (2003) Early growth restriction leads to down regulation of protein kinase C zeta and insulin resistance in skeletal muscle. *J Endocrinol* **177**, 235–241.

Paneth N, Ahmed F, Stein AD (1996) Early nutritional origins of hypertension: a hypothesis still lacking support. *J Hypertens* **14**, S121–S129.

Plagemann A, Harder T, Rake A, Melchior K, Rohde W, Dorner G (2001) Hypothalamic nuclei are malformed in weanling offspring of low protein malnourished rat dams. *J Nutr* **130**, 2582–2589.

Poston L (2006) Endothelial dysfunction in pre-eclampsia. *Pharmacol Reports* **58 Suppl**, 69–74.

Roseboom TJ, van der Meulen JH, Ravelli AC, Osmond C, Barker DJ, Bleker OP (2001) Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. *Mol Cell Endocrinol* **185**, 93–98.

Rutland CS, Latunde-Dada AO, Thorpe A, Plant R, Langley-Evans S, Leach L (2007) Effect of gestational nutrition on vascular integrity in the murine placenta. *Placenta* **28**, 734–742.

Samuelsson AM, Matthews PA, Argenton M, et al. (2008) Diet-induced obesity in female mice leads to offspring hyperphagia,

adiposity, hypertension, and insulin resistance: a novel murine model of developmental programming. *Hypertension* **51**, 383–392.

Sherman RC, Langley-Evans SC (1998) Early administration of angiotensin-converting enzyme inhibitor captopril, prevents the development of hypertension programmed by intrauterine exposure to a maternal low-protein diet in the rat. *Clin Sci (Lond)* **94**, 373–381.

Sherman RC, Langley-Evans SC (2000) Antihypertensive treatment in early postnatal life modulates prenatal dietary influences upon blood pressure in the rat. *Clin Sci* **98**, 269–275.

Sinclair KD, Allegrucci C, Singh R, et al. (2007) DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. *Proc Natl Acad Sci USA* **104**, 19351–19356.

Singh GR, Hoy WE (2004) Kidney volume, blood pressure, and albuminuria: findings in an Australian aboriginal community. *Am J Kidney Dis* **43**, 254–259.

Smits L, Krabbendam L, de Bie R, Essed G, van Os J (2006) Lower birth weight of Dutch neonates who were in utero at the time of the 9/11 attacks. *J Psychosom Res* **61**, 715–717.

Snoeck A, Remacle C, Reusens B, Hoet JJ (1990) Effect of a low protein diet during pregnancy on the fetal rat endocrine pancreas. *Biol Neonate* **57**, 107–118.

Stein AD (2004) Birthweight and the development of overweight and obesity. In *Fetal Nutrition and Adult Disease* (eds Langley-Evans SC), pp. 195–210. CABI, Wallingford, UK.

Stoltzfus RJ (2003) Iron deficiency: global prevalence and consequences. *Food Nutr Bull* **24**, S99–S103.

Tappia PS, Nijjar MS, Mahay A, Aroutiounova N, Dhalla NS (2005) Phospholipid profile of developing heart of rats exposed to low-protein diet in pregnancy. *Am J Physiol* **289**, R1400–R1406.

Torrens C, Brawley L, Anthony FW, et al. (2006) Folate supplementation during pregnancy improves offspring cardiovascular dysfunction induced by protein restriction. *Hypertension* **47**, 982–987.

Vehaskari VM, Aviles DH, Manning J (2001) Prenatal programming of adult hypertension in the rat. *Kidney Int* **59**, 238–245.

Vickers MH, Breier BH, McCarthy D, Gluckman PD (2003) Sedentary behavior during postnatal life is determined by the prenatal environment and exacerbated by postnatal hypercaloric nutrition. *Am J Physiol* **285**, R271–R273.

Walters E, Edwards RG (2004) Further thoughts regarding evidence offered in support of the 'Barker hypothesis'. *Reprod Biomed Online* **9**, 129–131.

Walton A, Hammond J (1938) The maternal effects on growth and conformation in Shire horse-Shetland pony crosses. *Proc R Soc Lond B Biol Sci* **125**, 311–335.

Waterland RA, Jirtle RL (2003) Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* **23**, 5293–5300.

Willett WC (2006) Trans fatty acids and cardiovascular disease – epidemiological data. *Atherosclerosis* **7**, 5–8.

Woodall SM, Johnston BM, Breier BH, Gluckman PD (1996) Chronic maternal undernutrition in the rat leads to delayed postnatal growth and elevated blood pressure of offspring. *Ped Res* **40**, 438–443.

World Cancer Research Fund (2007) *Food, Nutrition, Physical Activity and the Prevention of Cancer*. London: WCRF.

Yajnik CS, Fall CH, Vaidya U, et al. (1995) Fetal growth and glucose and insulin metabolism in four-year-old Indian children. *Diabet Med* **12**, 330–336.

Yates Z, Tarling EJ, Langley-Evans SC, Salter AM (2008) Maternal undernutrition programmes atherosclerosis in the Apo E*3 Leiden mouse. *Br J Nutr*, in press.

Zhang J, Lewis RM, Wang C, Hales N, Byrne CD (2005) Maternal dietary iron restriction modulates hepatic lipid metabolism in the fetuses. *Am J Physiol* **288**, R104–R111.