Fetal programming of cardiovascular function through exposure to maternal undernutrition

Simon C. Langley-Evans

Nutritional Biochemistry, University of Nottingham, Sutton Bonington, UK

A substantial and robust body of epidemiological evidence indicates that prenatal dietary experience may be a factor determining cardiovascular disease risk. Retrospective cohort studies indicate that low birth weight and disproportion at birth are powerful predictors of later disease risk. This prenatal influence on non-communicable disease in later life has been termed programming. Maternal nutritional status has been proposed to be the major programming influence on the developing fetus. The evidence from epidemiological studies of nutrition, fetal development and birth outcome is, however, often weak and inconclusive. The validity of the nutritional programming concept is highly dependent on experimental studies in animals. The feeding of low-protein diets in rat pregnancy results in perturbations in fetal growth and dimensions at birth. The offspring of rats fed low-protein diets exhibit a number of metabolic and physiological disturbances, and are consistently found to have high blood pressure from early postnatal life. This experimental model has been used to explore potential mechanisms of programming through which maternal diet may programme the cardiovascular function of the fetus. Indications from this work are that fetal exposure to maternally-derived glucocorticoids plays a key role in the programming mechanism. Secondary to this activity, the fetal hypothalamic–pituitary–adrenal axis may stimulate renin–angiotensin system activity, resulting in increased vascular resistance and hypertension.

Pregnancy: Fetal programming: Hypertension

Early-life origins of human cardiovascular disease

Cardiovascular disease, CHD and diabetes have for a long time been linked to adult lifestyle factors such as diet, smoking and obesity. As reviewed by Willett (1994), the consumption of a high-fat diet, low in starch and fibre, appears to substantially raise individual risk of cardio-vascular mortality. Recent work suggests that nutritional influences encountered much earlier in life may be of equal importance in determining cardiovascular disease risk.

An ever increasing and apparently robust body of epidemiological evidence indicates that prenatal dietary experience may play a pivotal role in this respect. Initial studies identified an association between CHD prevalence among middle-aged men and infant mortality early in the twentieth century (Barker, 1994). This work indicated that adverse factors experienced in early life either resulted in death, or adaptations which contributed to disease risk in later life.

Such findings were extended through large follow-up studies of cohorts across the UK (Barker, 1994). These studies provided evidence of associations between low birth weight and adult risk of CHD, hypertension and diabetes. Further investigations have led to a re-evaluation of the early work, and it is now apparent that subtle deviations in the fetal growth pattern leading to disproportion at birth (thinness, large head circumference in proportion to length, small abdominal circumference) are more powerful predictors of later disease risk. Support for the work in the UK has come from across the globe, and it seems that the influence of early life on adult health is as much present in developing countries, such as India and Jamaica (Yajnik *et al.* 1995; Forrester *et al.* 1996; Stein *et al.* 1996), as it is in more affluent countries such as the UK, USA, Sweden and Australia (Barker, 1994; Rich-Edwards *et al.* 1995; Moore *et al.* 1996; Leon, 1999).

These observations have led to the postulation of what has become widely known as the 'Barker hypothesis'. This

hypothesis asserts that adverse factors encountered during fetal life have the dual effect of perturbing prenatal growth patterns and establishing a pre-susceptibility to major disease states in adult life.

Metabolic programming

The observations of associations between indices of fetal growth and later risk of cardiovascular disease have been attributed to 'programming' or 'imprinting'. Programming was originally defined by Lucas (1992), as a permanent response to an insult or stimulus, experienced during a critical period of development. The developing fetus goes through a number of critical developmental periods when organogenesis and differentiation take place. These periods of rapid growth, development and maturation are stages where the fetus is vulnerable should variations in nutrient or O_2 supply, or other perturbations to the environment occur. The immediate issue for the fetus is survival. Adaptations to ensure survival may, at critical points in development, result in long-term or permanent changes to organ morphology, metabolic functions, endocrine functions and physiology.

Barker and colleagues (Barker, 1994) propose that the primary programming influence experienced by the human fetus is maternal nutrition. The central tenet of the Barker hypothesis is that less than optimal maternal nutritional status, as opposed to clinically significant malnutrition, provides sufficient insult to the developing fetus to programme an increased susceptibility to major disease states.

In support of this argument there are a number of studies, which suggest links between maternal diet and weight at birth in human subjects. Godfrey et al. (1996) studied a population of pregnant women in Southampton and noted that a high intake of sucrose in early pregnancy and a low protein intake in late pregnancy exerted slight but significant ($P < 0.01$) influences on both birth and placental weight at full term. In a much larger study of Jamaican women, weight at birth and thinness at birth appeared to be related to indices of maternal nutritional status before pregnancy, such as height, weight and BMI (Thame et al. 1997). Similar influences of variables such as maternal stature and BMI on fetal growth and later cardiovascular indices have been reported by Forsen et al. (1999). The findings of the Godfrey et al. (1994) study of 10-year-old Jamaican children suggested that blood pressure was related to maternal haemoglobin concentrations and triceps skin-folds in pregnancy. These findings appear consistent with a role for maternal nutritional status in programming cardiovascular function. Similarly, Campbell et al. (1996) reported that in a retrospective cohort of British men in their mid-forties, blood pressure was highest in those individuals whose mothers consumed a high-carbohydrate low-protein diet in pregnancy. The validity of the dietary data in this study may, however, be questionable.

Despite the evidence in favour of the Barker hypothesis, it is true to say that the evidence supporting a role for maternal nutrition in determining fetal growth patterns in well-nourished populations such as the UK is weak. Historical evidence from the Dutch Hunger Winter of 1944 (Ravelli et al. 1998), from the Leningrad siege of 1942 (Antonov, 1947) and the long-term (UK) Medical Research Council surveys of nutrition in The Gambia (Prentice et al. 1987), indicate that major famine and variation in food supply actually produce relatively minor effects on birth weight. The New York intervention trial, in which impoverished women were provided with nutritional supplements during pregnancy, produced worse birth outcomes than were noted among the non-supplemented population (Rush et al. 1980).

A recent study of pregnant women in Portsmouth indicated that placental and birth weights were not related to maternal intakes of any macronutrients (Mathews et al. 1999). Vitamin C intake in early pregnancy was related to birth weight, but late pregnancy intakes of neither vitamin C nor any other micronutrient were related to weight at birth. Doyle et al. (1992) reported that nutrition may have a more marked impact on birth weight in socially-disadvantaged populations, but a randomised controlled supplementation trial beginning in the second trimester of pregnancy showed no effect on birth weight.

The Barker hypothesis has been vigorously challenged on a number of grounds. Bartley et al. (1994) demonstrated that low birth weight was more likely in families of low socio-economic status, and that these same socio-economic indicators of cardiovascular risk tend to persist throughout childhood and into adult life. Kramer & Joseph (1996), among the most vociferous critics of the Barker hypothesis, have pointed out inconsistencies in findings between some of the epidemiological studies. It is suggested that selection bias in large retrospective cohorts has been inappropriately ignored, and it is claimed that statistical analyses have ignored unmeasured but important confounders such as genetics and social class. Importantly, some studies have found no evidence that characteristics at birth are predictive of later cardiovascular risk (Matthes et al. 1994).

Nutritional programming in animals

The lack of a clear and unambiguous impact of maternal nutritional status on indices of fetal growth measured at birth represents a major potential flaw in the Barker hypothesis. The validity of the nutritional programming concept is in fact only sustained through experiments which have made use of nutritional manipulations in pregnant animals. One of the first such studies specifically directed at the Barker hypothesis was the experiment of Persson & Jansson (1993), in which pregnant guinea-pigs had one uterine horn ligated to restrict the nutritional supply of pups within that horn. The outcome of the experiment was severe fetal growth retardation accompanied by relative hypertension, in comparison with pups from the non-ligated horn.

Kind et al. (1999) also studied guinea-pigs as a possible model for nutritional programming. Restriction of food intake to 85 % of the ad libitum intake produced smaller offspring, which exhibited exaggerated responses to cholesterol loading. Most other work in the field has focused on the rat as a working model. The most basic approach has been global food restriction in rat pregnancy. A very severe (70 %) maternal food restriction, whilst leading to severe growth retardation in fetal life, produced only minor, but

significant ($P < 0.05$), increases in blood pressure for the offspring (Woodall *et al*. 1996). Inconsistent effects of maternal diet on blood pressures of rat offspring were also reported by Crowe *et al*. (1995). Fe restriction in rat pregnancy produced offspring which in early postnatal life had lower blood pressures than offspring of rats fed an Fe-replete diet. These Fe-restricted offspring developed relative hypertension later in life.

The most widely reported and best characterised animal model of the Barker hypothesis has utilised the feeding of low-protein diets in rat pregnancy. These studies were based on early observations that feeding diets containing only 40–60 g protein/kg diet (the pregnant rat has a requirement for 120 g protein/kg diet and most standard laboratory rat diets contain 180–200 g protein/kg) produces severe reductions in offspring weight at birth (Zeman & Stanbrough, 1969; Hastings-Roberts & Zeman, 1977).

Langley & Jackson (1994) reported that the feeding of diets containing 60, 90 or 120 g protein/kg diet to pregnant rats elevated blood pressure in the resulting offspring, compared with control animals, despite all rat dams being transferred to the same standard laboratory chow diet at littering. Subsequent studies concentrated on the long-term effects of fetal exposure to a maternal diet containing 90 g protein/kg (maternal low-protein (MLP) diet). The effects of this diet have now been characterised in considerable detail. The growth of fetuses exposed to the MLP diet is disturbed (Levy & Jackson, 1993; Langley-Evans & Nwagwu, 1998). When rat dams are fed MLP throughout pregnancy their fetuses are larger than those of rats fed the control diet from day 14 of gestation through to day 20 (Langley-Evans *et al*. 1996*b*). This growth-enhancing effect of MLP is accentuated if the diet is fed for a relatively short period, between 0 and 7 days of gestation (Langley-Evans & Nwagwu, 1998). Placental size largely mirrors fetal growth and MLP tends to produce an enlarged placenta by day 20 of gestation.

The large day-20 fetuses of rats fed MLP undergo major retardation of growth over the last 2d of gestation (days 21 and 22). By full term they tend to be of low or low–normal birth weight compared with control pups (Langley-Evans *et al*. 1996*b*). The late gestation growth retardation appears to selectively affect the growth of truncal organs such as the liver and lungs. Increases in body length are also impaired by MLP over this time, but the growth of the brain appears to be spared. The postnatal growth of the MLP-exposed animals also appears to be slightly altered by their intra-uterine experience (Langley & Jackson, 1994; Langley-Evans *et al*. 1996*e*). MLP-exposed males and females generally grow more rapidly between birth and 4 weeks of age, but once mature tend to be smaller than control animals of equivalent age.

The observed differences in weight gain between MLP-exposed rats and control animals exposed to a protein-replete (180 g protein/kg) diet *in utero* are also apparent in terms of the size of some major organs. Whilst liver, heart and lung size appear to be unaffected by MLP feeding, MLP-exposed rats have smaller spleens and kidneys (Langley-Evans *et al*. 1996*b*). These kidneys also differ in shape, being short in relation to weight and wide in relation to length (Langley-Evans *et al*. 1999; Nwagwu *et al*. 2000).

Body composition has not been fully assessed in MLP-exposed animals, but adipose depots in discrete sites appear heavier, suggesting an increased fat content (Jackson *et al*. 1996). Anguita *et al*. (1993) and Petry *et al*. (1997) also propose that increased risk of obesity may stem from prenatal exposure to undernutrition. Analysis of MLP carcasses using dual-energy X-ray absorptiometric scanning indicates a significantly lower ($P < 0.05$) bone mineral content than that in control animals (SC Langley-Evans and C Cooper, unpublished results) and Roach *et al*. (1999) have reported abnormalities in the bone morphology of MLP-exposed animals.

In addition to altered morphology and growth patterns, fetal exposure to MLP induces long-term changes to physiology and metabolism. These changes include altered regulation of the γ-glutamyl enzyme pathway (Langley-Evans *et al*. 1995) resulting in increased susceptibility to oxidant-induced lung injury (Langley-Evans *et al*. 1997). There is evidence of abnormal glucose handling and insulin responses (Langley *et al*. 1994*a*; Pickard *et al*. 1996), and the MLP-exposed rat appears to have impaired inflammatory responses (Langley *et al*. 1994*b*), impaired T killer cell function and reduced lymphocyte proliferative responses (Calder & Yaqoob, 2000). Ultimately, exposure to low-protein diets in fetal life appears to shorten overall lifespan (Hales *et al*. 1996; Sayer *et al*. 2001).

Of greatest interest within the scope of the present review is the effect of the MLP diet on blood pressure. The offspring of rats fed the MLP diet consistently exhibit raised systolic blood pressure by the time of weaning at 4 weeks of age. The magnitude of the effect varies between 7 and 30 mmHg (Langley & Jackson, 1994; Langley-Evans *et al*. 1994, 1996*a*, 1999). No differences between males and females have been noted. Typically, the elevated blood pressure is accompanied by a lower heart rate (Nwagwu *et al*. 2000). The hypertensive effect of MLP is not dependent on blood pressure changes in the mother during pregnancy, and it has been demonstrated that MLP has no effect on blood pressure in mature animals (Langley-Evans *et al*. 1994). Although some of the effects of MLP on growth and organ size appear to be related to the maternal plane of nutrition before conception (Langley-Evans & Nwagwu, 1998), the hypertensive effect is entirely independent of the prepregnancy diet (Langley-Evans *et al*. 1996*a*). The timing of MLP feeding, similarly, is not critical in the programming of raised blood pressure. Feeding MLP for single weeks of pregnancy produces raised blood pressure in the offspring, although the magnitude of the effect is greatest if MLP feeding is targeted to the final week of gestation (days 15–22; Langley-Evans *et al*. 1996*e*).

In contrast, the low-protein diet (Hope Farms diet) employed by Hales *et al*. (1996) and Iglesias-Barreira *et al*. (1996) for studies of nutritional programming of glucose metabolism and insulin resistance does not appear to have major effects on cardiovascular development (Langley-Evans, 2000). This diet differs from the MLP diet of Langley & Jackson (1994) in several respects. The MLP diet supplies extra methionine to avoid S deficiency associated with a casein-based diet, provides fat as maize oil (100 g/kg diet) and carbohydrate as sucrose–starch (1:2, w/w). The Hope Farms diet contains only 30 g fat/kg, in the form of

soyabean oil, and provides carbohydrate mostly in the form of glucose. These differences in diet composition and long-term effects on the offspring of rats fed these diets in pregnancy strongly suggest that the main nutritional programming influence on the fetus is the overall balance of nutrients in the maternal diet and not low protein *per se*. Rees *et al.* (2000) have argued that imbalances in specific amino acids associated with MLP feeding result in metabolic disturbance which may programme cardiovascular health.

This assertion of nutrient imbalance is supported by the study of Langley-Evans (1996), which demonstrated that the effects of the low-protein diet on fetal growth and later blood pressure could be largely duplicated by alterations to the fat source. Substituting 90 g coconut oil/kg diet, plus 10 g maize oil/kg diet for the typical 100 g maize oil/kg diet, at 180 g/kg diet level of protein, produced offspring with the same elevated blood pressure as offspring of rats fed a low-protein diet.

As will be detailed later, the experiments with low-protein diets in rat pregnancy have allowed important progress to be made in defining the mechanisms which link maternal nutritional status to raised blood pressure in later life. Recent findings have demonstrated that maternal nutrition in the period before the fertilized rat embryo has implanted may also be critical in programming cardiovascular health. Rat pups exposed to low-protein diets for just the first 4·25 d of gestation had significantly elevated ($P < 0.05$) blood pressures by the age of 4 weeks (Kwong *et al.* 2000). Further studies of the effects of maternal protein restriction on the development of the early embryo will be facilitated by the recent duplication of rat studies in the mouse. Feeding of low-protein diets throughout mouse pregnancy, as in the rat, produces offspring of low birth weight, with raised blood pressures from the age of 8 weeks (Dunn *et al.* 2001).

Mechanisms of programming

Glucocorticoids

Glucocorticoids have long been recognised as potent hormones in fetal tissues. Evidence from animals and human subjects indicates that these steroids have a growth-retarding influence and the capacity to have major developmental effects through the regulation of a wide range of genes (Liggins, 1969; Reinisch *et al.* 1978). This effect is exploited clinically, for example, in the dexamethasone treatment of women in premature labour in order to enhance fetal lung maturation. Studies of the fetal sheep indicate that intravenous glucocorticoid infusions raise blood pressure *in utero* (Tangalakis *et al.* 1992).

During normal fetal development circulating concentrations of glucocorticoids (cortisol in human subjects, corticosterone in rats) are very low relative to concentrations seen in the maternal system. A natural surge in fetal glucocorticoids is observed in late gestation in many species, and is associated with the maturation of the fetal adrenal (Arishima *et al.* 1977). Separation of the fetal circulation from glucocorticoids of maternal origin is considered crucial to the normal development of the fetal hypothalamic–pituitary–

adrenal axis, and also ensures that the correct developmental pattern of gene expression is followed (Chatelain *et al.* 1980). This separation is achieved through the activity of 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) located in the placenta (Edwards *et al.* 1996). This enzyme converts active glucocorticoids to inactive forms, and hence acts as a gatekeeper, preventing glucocorticoids from the maternal circulation from flooding the fetal system.

The synthetic glucocorticoid dexamethasone is a poor substrate for 11βHSD2, and crosses the placenta freely. Treatment of pregnant rats with dexamethasone induces hypertension in the resulting offspring, in a manner analogous to the MLP model (Benediktsson *et al.* 1993). Similarly the treatment of pregnant rats with an inhibitor of 11βHSD2 (carbenoxolone) has a blood-pressure programming effect (Lindsay *et al.* 1996). This finding has led to the proposal that fetal exposure to abnormally high levels of glucocorticoids may play a key role in the programming of cardiovascular disease (Edwards *et al.* 1993). This hypothesis is strongly supported by the observation that placentas from rats fed a low-protein diet in pregnancy have lower 11βHSD2 activity (Langley-Evans *et al.* 1996*c*) and expression (SC Langley-Evans and CB Whorwood, unpublished results) than placentas from rats fed a control diet.

Further support derives from studies using carbenoxolone and another pharmacological agent, metyrapone. Treatment of rats fed a protein-replete control diet with carbenoxolone induced hypertension in their offspring which was equivalent to that produced by MLP (Langley-Evans, 1997*a*). Metyrapone is an inhibitor of maternal glucocorticoid synthesis, and effectively pharmacologically adrenalect-omises treated animals. Metyrapone treatment of pregnant rats fed the MLP diet resulted in offspring which did not develop raised blood pressure (Langley-Evans *et al.* 1996*d*). Corticosterone replacement of metyrapone-treated pregnant rats on the MLP diet restored the hypertensive effect of the diet (Langley-Evans, 1997*b*). Nutritionally-induced hypertension thus depends on maternal glucocorticoid production, and appears to be mediated through increased access of glucocorticoids to fetal tissues, via the placenta. No direct measurements of increased glucocorticoid concentrations have been obtained from fetal tissues or circulation, but the activities of glucocorticoid-inducible enzymes in liver and brain were noted to be elevated in offspring of MLP-fed rats at birth and at day 20 of gestation (Langley-Evans & Nwagwu, 1998).

The glucocorticoid dependence of MLP-induced hypertension appears to extend beyond the fetal period. Surgical adrenalectomy of 4-week-old rats exposed to MLP *in utero* restored their blood pressure to levels seen in control animals of the same age (Gardner *et al.* 1997*a*). Corticosterone replacement reversed the effect of adrenalectomy. Although the hypertension of MLP-exposed rats requires corticosteroid action to maintain it in early postnatal life, there is no evidence of high circulating levels of glucocorticoids. Assessment of the hypothalamic–pituitary–adrenal axis activity through the light cycle suggests that MLP-exposed rats have low–normal corticosterone concentrations throughout the day and night (Langley-Evans *et al.* 1996*a*). Unusually, the normal diurnal variation in corticotrophin appears to be absent.

In the absence of high hormone levels it has been inferred that MLP-exposed rats are hypersensitive to normal concentrations. This inference is confirmed by observations of high activities of glucocorticoid-inducible enzymes in livers (tyrosine aminotransferase) and regions of the brains (glutamine synthetase and glycerol-3-phosphate dehydrogenase) of these animals (Langley-Evans *et al.* 1996*a*). Hypersensitivity appears to be conferred by raised numbers and expression of classical glucocorticoid receptors in key target tissues (N Copin, C Bertram, S Buck, SC Langley-Evans and CB Whorwood, unpublished results).

A role for glucocorticoids in the programming of hypertension is also supported by studies of human populations. Stewart *et al.* (1995) report that, as in the rat, low birth weight is associated with low activity of 11βHSD2 in the placenta. Clark *et al.* (1996) demonstrated an inverse association between birth weight and excretion of cortisol metabolites in children. More recently, Phillips *et al.* (2000) reported the findings of three studies of adult men and women, which indicated that fasting cortisol concentrations were higher in individuals of low birth weight. Furthermore, cortisol concentrations correlated with adult blood pressure.

Fetal exposure to glucocorticoids and ensuing hypothalamic–pituitary–adrenal axis adjustments may thus underpin processes leading to growth retardation and major alterations to structures and metabolic functions in fetal life. Their effects persist into postnatal life, and impact on health and well-being. Glucocorticoids may link directly to the increases in hypertension noted in MLP-exposed rats. As seen in the sheep fetus, these hormones can raise blood pressure *in utero* (Tangalakis *et al.* 1992). In mature animals glucocorticoids stimulate the entry of Ca into vascular smooth muscle cells, promoting vasoconstriction and increases in peripheral resistance (Kornel *et al.* 1995). Glucocorticoids may also act indirectly on blood pressure. The renin–angiotensin system, in particular, is subject to regulation by corticosteroids at several levels (Langley-Evans, 1997*c*).

The kidney

The fetal kidney appears to be extremely vulnerable to the effects of growth retardation. Studies of growth-retarded human infants indicate that the kidneys are disproportionately affected relative to the other organs (Konje *et al.* 1996). In particular, Hinchcliffe *et al.* (1992) demonstrated that in stillborn infants growth retardation reduced the renal reserve, or number of functional nephrons in the kidneys. Nephron number, which is established before birth, is a highly variable factor in human subjects, with a range from $300\,000$ to 1×10^6 per kidney (Mackenzie & Brenner, 1995).

MacKenzie & Brenner (1995) postulated that reduced renal reserve established in fetal life could promote an irreversible progression towards renal dysfunction and hypertension. Kidneys with lower nephron numbers maintain their haemodynamic and excretory functions through increases in local vascular resistance and blood pressure. Increased pressures within nephrons will lead to a progressive deterioration and loss of nephrons. The subsequent rise in pressure necessary to maintain function will then promote further nephron loss and hence a vicious cycle

of rising pressure and advancing renal failure is established. Ultimately, the increases in single nephron pressure and vascular resistance will translate into elevated systemic blood pressure.

In the rat a number of studies have demonstrated that fetal exposure to growth-retarding agents decreases nephron number (Hall & Zeman, 1968; Zeman, 1968; Lelievre-Pegorier *et al.* 1993; Merlet-Benichou *et al.* 1994). Severe maternal protein restriction was shown by Merlet-Benichou *et al.* (1994) to produce a significant ($P < 0.01$) nephron deficit in 2-week-old offspring, and nephron number was correlated with birth weight. Using the much-less-severe MLP model, Langley-Evans *et al.* (1999) demonstrated a significant ($P < 0.05$) nephron deficit in low-protein-exposed offspring at maturity. This study also highlighted that, in keeping with overall fetal growth, early nephrogenesis appeared to be enhanced by a low-protein diet, but that the last 2 d of gestation impacted on the development of functional units in the kidney. Targeting protein restriction to the final 7 d of rat pregnancy had the greatest impact on renal morphometry in the offspring. Rees & Hay (1998) noted up regulation of a growth arrest gene in the fetal rat kidney following protein restriction.

In addition to structural adaptations within the kidney, the MLP diet exerts a programming effect on renal function. Nwagwu *et al* (2000) reported that 4-week-old rats exposed to MLP *in utero* were unable to maintain the glomerular filtration rate at the same level as control animals, despite increased systemic blood pressure. This functional deficit resolved as the animals aged, but the resolution appeared to be accompanied by a progressive deterioration of renal function, as evidenced by rising blood urea-N concentrations and urinary albumin excretion. Sherman *et al.* (1999) report increased urinary excretion of vasoactive prostaglandins and lower activity of the renal prostaglandin-metabolizing enzyme, prostaglandin dehydrogenase. These findings mirror those of Mercuri *et al.* (1979) and of studies of spontaneously-hypertensive rats (Narce *et al.* 1992), and suggest intrauterine programming of genes involved both in prostaglandin synthesis and degradation.

These findings appear to be paralleled in human subjects of lower weight at birth. Sherman (1999) noted that excretion of prostaglandin E_2 by 10-year-old children was greater in those of low ponderal index (weight/length^3; i.e. thin at birth). Similarly 10-year-old children who were of low birth weight or who were thin at birth excrete more albumin in their urine (Marchand *et al.* 2001), which is suggestive of increased glomerular permeability. Studies of Australian aboriginal populations (Hoy *et al.* 1998) also indicate a contribution of intrauterine factors to a high prevalence of renal disease.

The renin–angiotensin system

The components of the renin–angiotensin system are key elements of blood-pressure control systems. At the systemic level, and also at the level of most major organ systems, the generation of the vasoconstrictor hormone angiotensin II results in increased vascular resistance and elevation of blood pressure. Within the kidney, for example, this process allows increases in the perfusion pressures across glomeruli

in order to maintain the glomerular filtration rate and haemodynamic function.

Studies of the renin–angiotensin system in MLP-exposed rats, although very general in nature, are suggestive of disturbances associated with prenatal undernutrition. Adult rats exposed to MLP diets *in utero* tend to have elevated concentrations of angiotensin II in the circulation, but this effect of fetal undernutrition is not statistically significant or consistent (Langley-Evans & Jackson, 1995; Langley-Evans *et al*. 1996*e*). This finding is surprising, as the activity of angiotensin converting enzyme, which generates angiotensin II from its precursor angiotensin I, is consistently elevated by fetal exposure to MLP in all tissues which have been studied to date (Langley-Evans & Jackson, 1995). This effect is noted in fetal rats, neonates and adults alike (Sherman, 1999).

Gardner *et al*. (1997*b*) investigated the possibility that hypertension in MLP-exposed rats may result from increased sensitivity to angiotensin II. Intravenous injections of angiotensin II in increasing stepwise doses indicated that the pressor response to the peptide was greater and more prolonged in MLP-exposed rats, but only at lower doses. This finding led to the proposal that MLP-induced hypertension may result from an increased concentration of angiotensin II receptors in key tissues. In the absence of raised hormone concentrations, this increased receptor concentration would tend to raise blood pressure by amplifying the effects of normal or even low hormone concentrations. AR Trowern, RC Sherman, C Bertram, R Dunn, DS Gardner, SC Langley-Evans and CB Whorwood (unpublished results) found evidence to support this hypothesis. Expression of the angiotensin receptor was significantly elevated ($P < 0.05$) in the kidneys of 8-week-old rats exposed to MLP *in utero*, although no maternal-diet-related difference in expression was observed in neonates.

Langley-Evans & Jackson (1995) first demonstrated that the relative hypertension induced by fetal exposure to an MLP diet could be successfully treated by using the inhibitor of the renin–angiotensin system captopril. Captopril is one of the angiotensin converting enzyme inhibitor class of drugs, and is widely used in the treatment of human hypertension. Mature adult rats exposed to MLP *in utero* showed lowered blood pressure during 1 week of captopril treatment, and on withdrawal of the drug their blood pressures increased back to pretreatment levels. In contrast, treatment with captopril for 2 weeks during the preweaning period appeared to produce an irreversible effect (Sherman & Langley-Evans, 1998). Offspring of MLP-fed rats received oral angiotensin converting enzyme inhibitor from 2 to 4 weeks of age, and at 8 weeks beyond the cessation of treatment had similar blood pressures to those of the offspring of rats fed a control (180 g casein/kg) diet. A similar effect of early angiotensin converting enzyme inhibitor treatment has been noted in rats of the spontaneously-hypertensive strain (Harrap *et al*. 1994).

Studies of the effects of angiotensin converting enzyme inhibitors on programmed hypertension were broadly supportive of a role for the renin–angiotensin system in the programming mechanism linking an imbalance of maternal nutrition to later cardiovascular disease. Captopril is, however, a drug that is non-specific in its mode of action,

and the possibility also remained that any anti-hypertensive drug could produce a similar effect when administered preweaning. Sherman & Langley-Evans (2000) administered nifedipine, a Ca channel antagonist to assess the contribution of an anti-hypertensive with virtually no effect on renin–angiotensin functions. MLP-exposed offspring did not respond to this drug, and blood pressures remained significantly above ($P < 0.01$) those of control rats. Losartan, a specific angiotensin II receptor antagonist, when administered to MLP-exposed offspring significantly reduced ($P < 0.01$) blood pressure, and in the same way as captopril had an irreversible effect when used preweaning. These data strongly suggest that the renin–angiotensin system plays a key role in the development of programmed hypertension, and that this mechanism operates to some extent in the early postnatal period in addition to during fetal life.

In addition to providing insight into the mechanisms of programming, the early effects of drugs targeted to the renin–angiotensin system may open up new approaches to the treatment of hypertension in human subjects. Harrap (1998) suggests that treatments tailored to critical periods in childhood, combined with strategies to ensure balanced nutrition in fetal life, may provide a way forward in anti-hypertensive therapy.

Overview

Experimental studies of nutritional programming using animal models provide major support for the hypothesis proposed by Barker and colleagues (Barker, 1994), and have enabled the first steps to be made towards identifying the mechanisms of metabolic programming. The broad range of animal studies employed to date suggest that a deficit of no single nutrient or group of nutrients is responsible for prenatal programming effects. Instead, it seems clear that any major imbalance of nutrients within the maternal diet may set in train fetal adaptations, and that the occurrence of such adaptations during critical periods of development results in permanent modification of physiology and metabolism. Very early exposure to nutrient imbalance may, as suggested by Kwong *et al*. (2000), produce embryonic adaptations which will later impact on the growth trajectory of the fetus. Later undernutrition appears to result in the down regulation of 11βHSD in the placenta. Evidence from the MLP rat model and the studies of Seckl and colleagues (for review, see Edwards *et al*. 1996) would suggest that this process is a central event in programming, with an impact on many fetal organ systems. The ensuing overexposure of fetal tissues to glucocorticoids would be expected to produce growth retardation. Renal adaptation to the growth-retarding effects of steroids would appear to involve a reduction in the renal reserve (Celsi *et al*. 1997), which in the older animal will lead to elevated systemic blood pressure and earlier renal failure.

A second consequence of fetal glucocorticoid exposure appears to be an early-life hypersensitivity to glucocorticoid action and altered regulation of the hypothalamic–pituitary–adrenal axis. Even if only transient, this effect is sufficient to activate the renin–angiotensin system. Consequent elevation of blood pressure in early postnatal life may then be reinforced by structural changes within the arteries. Work

with the MLP rat model indicates that animals exposed to low-protein diets *in utero* exhibit vascular smooth muscle cell hypertrophy and reduced elastin content as early as the age of weaning (SC Langley-Evans, CN Martyn and SE Greenwald, unpublished results).

The reversibility of the programmed hypertension demonstrated by anti-hypertensive treatment in the first 4 weeks of life (Sherman & Langley-Evans, 2000) adds further evidence to the hypothesis that programming of hypertension involves an interaction of prenatal and early postnatal factors. The prenatal diet establishes a hormonal milieu which favours the development of hypertension. Early postnatal hypertension then transforms the morphology of resistance arteries and maintains raised blood pressure even after correction of endocrine and metabolic abnormalities.

Future directions

The next decade of research in this field promises to be both exciting and fruitful. In the preceding 10 years remarkable progress was made, largely through the efforts of a handful of research groups in the UK, Australia, New Zealand and Belgium. These groups have jealously guarded their pre-eminent position, and consequently all major funding opportunities. Despite this situation, the number of new groups becoming involved in such studies is increasing at an exponential rate, particularly in the USA.

It is clear that in the coming years the emphasis needs to shift away from large-scale epidemiological studies towards more detailed experimental approaches. To determine the mechanisms of nutritional programming in human subjects, it will first be necessary to explore possible parallels between the animal studies and human disease states. As demand is likely to grow for some form of human intervention trial, timed in pregnancy and aimed at the prevention of cardiovascular disease, it will become necessary to define the nature of the nutrients which may determine fetal growth patterns and long-term health. Until such research has been completed, the assertions made by Barker and colleagues (Barker, 1994) are difficult to justify, and will contribute very little to the prevention and treatment of disease.

References

Anguita RM, Sigulem DM & Sawaya AL (1993) Intrauterine food restriction is associated with obesity in young rats. *Journal of Nutrition* **123**, 1421–1428.

Antonov AN (1947) Children born during the siege of Leningrad in 1942. *Journal of Pediatrics* **30**, 250–259.

Arishima K, Nakama K, Monkava Y, Hashimot Y & Eguchi Y (1977) Maternal–fetal interrelations of plasma corticosterone concentrations at the end of gestation in the rat. *Journal of Endocrinology* **72**, 239–240.

Barker DJP (1994) *Mothers, Babies and Disease in Later Life*. London: BMJ Publishing Group.

Bartley M, Power C, Davey-Smith G & Shipley M (1994) Birthweight and later socioeconomic disadvantage: evidence from the 1958 British cohort study. *British Medical Journal* **309**, 1475–1478.

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

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

Campbell DM, Hall MH, Barker DJP, Cross J, Shiell AW & Godfrey KM (1996) Diet in pregnancy and the offsprings blood pressure 40 years later. *British Journal of Obstetrics and Gynaecology* **103**, 273–280.

Celsi G, Kistner A, Eklof AC, Ceccatelli S, Aizman R & Jacobson S (1997) Inhibition of renal growth by prenatal dexamethasone and the programming of blood pressure in the offspring. *Journal of the American Society for Nephrology* **8**, A1360.

Chatelain J, Dupouy J-P & Allaume P (1980) Fetal-maternal adrenocorticotropin and corticosterone relationships in the rat: effects of maternal adrenalectomy. *Endocrinology* **106**, 1297–1302.

Clark PM, Hindmarsh PC, Shiell AW, Law CM, Honour JW & Barker DJP (1996) Size at birth and adrenocortical function in childhood. *Clinical Endocrinology* **45**, 721–726.

Crowe C, Dandekar P, Fox M, Dhingra K, Bennet L & Hanson MA (1995) The effects of anaemia on heart, placenta and body weight, and blood pressure in fetal and neonatal rats. *Journal of Physiology* **488**, 515–519.

Doyle W, Wynn AHA, Crawford MA & Wynn SW (1992) Nutritional counselling and supplementation in the second and third trimester of pregnancy: a study in a London population. *Journal of Nutrition and Medicine* **3**, 249–256.

Dunn RL, Langley-Evans SC, Jackson AA & Whorwood CB (2001) Hypertension in the mouse following intrauterine exposure to a maternal low-protein diet. *Proceedings of the Nutrition Society* **60**, 51A.

Edwards CRW, Benediktsson R, Lindsay RS & Seckl JR (1993) Dysfunction of placental glucocorticoid barrier: link between fetal environment and adult hypertension. *Lancet* **341**, 355–357.

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

Forrester TE, Wilks RJ, Bennett FI, Simeon D, Osmond C, Allen M, Chung AP & Scott P (1996) Fetal growth and cardiovascular risk factors in Jamaican schoolchildren. *British Medical Journal* **312**, 156–160.

Forsen T, Eriksson JG, Tuomilehto J, Osmond C & Barker DJP (1999) Growth in utero and during childhood among women who develop coronary heart disease: longitudinal study. *British Medical Journal* **319**, 1403–1407.

Gardner DS, Jackson AA & Langley-Evans SC (1997a) Maintenance of maternal diet-induced hypertension in the rat is dependent upon glucocorticoids. *Hypertension* **30**, 1525–1530.

Gardner DS, Jackson AA & Langley-Evans SC (1997b) Prenatal undernutrition alters postnatal vascular sensitivity to angiotensin II. *Clinical Science* **95**, Suppl. 39, 14P.

Godfrey KM, Forrester T, Barker DJP, Jackson AA, Landman JP, Hall JStE, Cox V & Osmond C (1994) The relation of maternal nutritional status during pregnancy to blood pressure in childhood. *British Journal of Obstetrics and Gynaecology* **101**, 398–403.

Godfrey KM, Robinson S, Barker DJP, Osmond C & Cox V (1996) Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. *British Medical Journal* **312**, 410–414.

Hales CN, Desai M, Ozanne SE & Crowther NJ (1996) Fishing in the stream of diabetes: from measuring insulin to the control of fetal organogenesis. *Biochemical Society Transactions* **24**, 341–350.

Hall SM & Zeman FJ (1968) Kidney function of the progeny of rats fed a low protein diet. *Journal of Nutrition* **95**, 49–56.

Harrap SB (1998) Preventing adult disease: windows of opportunity. *Clinical Science* **94**, 337–338.

Harrap SB, Mirakian C, Datodi SR & Lever AF (1994) Blood pressure and lifespan following brief ACE inhibitor treatment in young Spontaneously Hypertensive rats. *Clinical and Experimental Pharmacology and Physiology* **21**, 125–127.

Hastings-Roberts MM & Zeman FJ (1977) Effects of protein deficiency, pair-feeding, or diet supplementation on maternal, fetal and placental growth in rats. *Journal of Nutrition* **107**, 973–982.

Hinchcliffe SA, Lynch MRJ, Sargent PH, Howard CV & van Zelzen D (1992) The effect of intrauterine growth retardation on the development of renal nephrons. *British Journal of Obstetrics and Gynaecology* **99**, 296–301.

Hoy WE, Mathews JD, McCredie DA, Pugsley DJ, Hayhurst BG, Rees M, Kile E, Walker KA & Wang Z (1998) The multidimensional nature of renal disease: Rates and associations of albuminuria in an Australian Aboriginal community. *Kidney International* **54**, 1296–1304.

Iglesias-Barreira V, Ahn M-T, Reussens B, Dahri S, Hoet JJ & Remacle C (1996) Pre- and postnatal low protein diet affect pancreatic islet blood flow and insulin release in adult rats. *Endocrinology* **137**, 3797–3801.

Jackson AA, Langley-Evans SC & McCarthy HD (1996) Nutritional influences in early life upon obesity and body proportions. In *Origins and Consequences of Obesity. CIBA Foundation Symposium* no. 201, pp. 407–435 [WPT James and G Shaper, editors]. Chichester, West Sussex: John Wiley and Sons.

Kind KL, Clifton PM, Katsman AI, Tsiounis M, Robinson JS & Owens JA (1999) Restricted fetal growth and the response to dietary cholesterol in the guinea pig. *American Journal of Physiology* **277**, R1675–R1682.

Konje JC, Bell SC, Morton JJ, De Chazal R & Taylor DJ (1996) Human fetal kidney morphometry during gestation and the relationship between weight, kidney morphometry and plasma active renin concentration at birth. *Clinical Science* **91**, 169–175.

Kornel L, Prancan AV, Kanamarlapudi N, Hynes J & Kuzianik E (1995) Study on the mechanisms of glucocorticoid-induced hypertension: glucocorticoids increase transmembrane Ca^{2+} influx in vascular smooth muscle in vivo. *Endocrine Research* **21**, 203–210.

Kramer MS & Joseph KS (1996) Enigma of fetal/infant-origins hypothesis. *Lancet* **348**, 1254–1255.

Kwong WY, Wild AE, Roberts P, Willis AC & Fleming TP (2000) Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. *Development* **127**, 4195–4202.

Langley SC, Browne RF & Jackson AA (1994a) Altered glucose tolerance in rats exposed to maternal low protein diets in utero. *Comparative Biochemistry and Physiology* **109**A, 223–229.

Langley SC & Jackson AA (1994) Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diet. *Clinical Science* **86**, 217–222.

Langley SC, Seakins M, Grimble RF & Jackson AA (1994b) The acute phase response of adult rats is altered by in utero exposure to maternal low protein diets. *Journal of Nutrition* **124**, 1588–1596.

Langley-Evans SC (1996) Intrauterine programming of hypertension: nutrient interactions. *Comparative Biochemistry and Physiology* **114**A, 327–333.

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

Langley-Evans SC (1997b) Hypertension induced by fetal exposure to a maternal low protein diet, in the rat, is prevented by pharmacological blockade of glucocorticoid synthesis. *Journal of Hypertension* **15**, 537–544.

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

Langley-Evans SC (2000) Critical differences between two low protein diet protocols in the programming of hypertension in the rat. *International Journal of Food Sciences and Nutrition* **51**, 11–17.

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

Langley-Evans SC, Gardner DS & Jackson AA (1996b) Association of disproportionate growth of fetal rats in late gestation with raised systolic blood pressure in later life. *Journal of Reproduction and Fertility* **106**, 307–312.

Langley-Evans SC & Jackson AA (1995) Captopril normalises systolic blood pressure in rats with hypertension induced by fetal exposure to maternal low protein diets. *Comparative Biochemistry and Physiology* **110**A, 223–228.

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

Langley-Evans SC, Phillips GJ, Benediktsson R, Gardner DS, Edwards CRW, Jackson AA & Seckl JR (1996c) Protein intake in pregnancy, placental glucocorticoid metabolism and the programming of hypertension. *Placenta* **17**, 169–172.

Langley-Evans SC, Phillips GJ, Gardner DS & Jackson AA (1996d) Role of glucocorticoids in programming of maternal diet-induced hypertension in the rat. *Journal of Nutritional Biochemistry* **7**, 173–178.

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, Phillips GJ & Jackson AA (1997) Fetal exposure to a maternal low protein diet alters the susceptibility of the young adult rat to sulfur dioxide-induced lung injury. *Journal of Nutrition* **127**, 202–209.

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

Langley-Evans SC, Welham SJM, Sherman RC & Jackson AA (1996e) Weanling rats exposed to maternal low protein diets during discrete periods of gestation exhibit differing severity of hypertension. *Clinical Science* **91**, 607–615.

Langley-Evans SC, Wood S & Jackson AA (1995) Enzymes of the gamma-glutamyl cycle are programmed in utero by maternal nutrition. *Annals of Nutrition and Metabolism* **39**, 28–35.

Lelievre-Pegorier M, Euzet S & Merlet-Benichou C (1993) Effect of fetal exposure to gentamicin on phosphate transport in young rat kidney. *American Journal of Physiology* **265**, F807–F812.

Leon DA (1999) Fetal growth and later disease: epidemiological evidence from Swedish cohorts. In *Fetal Programming: Influences on Development and Disease in Later Life*, pp. 12–29 [PMS O'Brien, T Wheeler and DJP Barker, editors]. London: RCOG Press.

Levy L & Jackson AA (1993) Modest restriction of dietary protein during pregnancy in the rat: fetal and placental growth. *Journal of Developmental Physiology* **19**, 113–118.

Liggins GC (1969) Premature delivery of foetal lambs infused with glucocorticoids. *Journal of Endocrinology* **45**, 515–523.

Lindsay RS, Lindsay RM, Edwards CRW & Seckl JR (1996) Inhibition of 11α-hydroxysteroid dehydrogenase in pregnant rats and the programming of blood pressure in the offspring. *Hypertension* **27**, 1200–1204.

Lucas A (1992) Programming by nutrition in man. In *Early Diet, Later Consequences*, pp. 24–28 [D Conning, editor]. London: British Nutrition Foundation.

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

Marchand MC, Dunn RL, Jackson AA & Langley-Evans SC (2001) Programming of blood pressure and renal structure in rats exposed to nitrogen-supplemented maternal low protein diets. *Proceedings of the Nutrition Society* **60**, 139A.

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

Matthes JWA, Lewis PA, Davies DP & Bethel JA (1994) Relation between birth weight at term and systolic blood pressure in adolescence. *British Medical Journal* **308**, 1074–1077.

Mercuri O, de Tomas ME & Itarte H (1979) Prenatal protein depletion and delta 9, delta 6 and delta 5 desaturase. *Lipids* **14**, 822–825.

Merlet-Benichou C, Gilbert T, Muffat-Joly M, Lelievre-Pegorier M & Leroy B (1994) Intrauterine growth retardation leads to a permanent nephron deficit in the rat. *Pediatric Nephrology* **8**, 175–180.

Moore VM, Miller AG, Boulton TJC, Cockington RA, Hamilton Craig I, Magarey AM & Robinson JS (1996) Placental weight, birth measurements and blood pressure at age 8 years. *Archives of Disease in Childhood* **74**, 538–541.

Narce M, Poisson J-P, Belleville J & Chanusot B (1992) Depletion of delta 9 desaturase (*EC* 1.14.99.5) enzyme activity in growing rats during dietary protein restriction. *British Journal of Nutrition* **68**, 627–637.

Nwagwu MO, Cook A & Langley-Evans SC (2000) Evidence of progressive deterioration of renal function in rats exposed to a maternal low protein diet *in utero*. *British Journal of Nutrition* **83**, 79–85.

Persson E & Jansson T (1993) Low birth weight is associated with elevated adult blood pressure in the chronically catheterized guinea-pig. *Acta Physiologica Scandinavica* **115**, 195–196.

Petry CJ, Ozanne SE, Wang CL & Hales CN (1997) Early protein restriction and obesity independently induce hypertension in year old rats. *Clinical Science* **93**, 147–152.

Phillips DIW, Walker BR, Reynolds RM, Flanagan DEH, Wood PJ, Osmond C, Barker DJP & Whorwood CB (2000) Low birth weight predicts elevated plasma cortisol concentrations in adults from 3 populations. *Hypertension* **35**, 1301–1306.

Pickard CL, McCarthy HD, Browne RF & Jackson AA (1996) Altered insulin response to a glucose load in rats following exposure to a low protein diet *in utero*. *Proceedings of the Nutrition Society* **55**, 44A.

Prentice AM, Cole TJ, Foord FA, Lamb WH & Whitehead RG (1987) Increased birthweight after prenatal dietary supplementation of rural African women. *American Journal of Clinical Nutrition* **46**, 912–925.

Ravelli ACJ, van der Meulen JHP, Michels RPJ, Osmond C, Barker DJP, Hales CN & Bleker OP (1998) Glucose tolerance in adults after exposure to the Dutch Famine. *Lancet* **351**, 173–177.

Rees WD & Hay SM (1998) The effect of maternal protein deficiency on the expression of the growth arrest specific gene 6 (gas6) in the fetal kidney. *Biochemical Society Transactions* **667**, 70.

Rees WD, Hay SM, Brown DS, Antipatis C & Palmer RM (2000) Maternal protein deficiency causes hypermethylation of DNA in the livers of rat fetuses. *Journal of Nutrition* **130**, 1821–1826.

Reinisch JM, Simon NG & Karwo WG (1978) Prenatal exposure to prednisone in humans and animals retards intra-uterine growth. *Science* **202**, 436–438.

Rich-Edwards J, Stampfer M, Manson J, Rosner B, Colditz G, Willett W, Speizer F & Hennekens C (1995) Birthweight, breast-feeding and the risk of coronary heart disease in the nurses health study. *American Journal of Epidemiology* **141**, S78.

Roach HI, Langley-Evans SC & Cooper C (1999) Protein deficiencies during pregnancy affect skeletal development in the offspring. *Journal of Bone and Mineral Research* **14**, Suppl. 1, S394.

Rush D, Stein Z & Susser M (1980) A randomized controlled trial of prenatal nutritional supplementation in New York City. *Pediatrics* **65**, 683–697.

Sayer AA, Dunn RL, Langley-Evans SC & Cooper C (2001) Intra-uterine exposure to a maternal low protein diet shortens lifespan in rats. *Gerontology* **47**, 9–14.

Sherman RC (1999) The role of the renin-angiotensin system in the fetal programming of hypertension. PhD Thesis, University of Southampton.

Sherman RC, Jackson AA & Langley-Evans SC (1999) Long term modification of the excretion of prostaglandin E2 by fetal exposure to a maternal low protein diet in the rat. *Annals of Nutrition and Metabolism* **43**, 98–106.

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. *Clinical Science* **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. *Clinical Science* **98**, 269–275.

Stein CE, Fall CHD, Osmond C, Cox V & Barker DJP (1996) Fetal growth and coronary heart disease in South India. *Lancet* **348**, 1269–1273.

Stewart PM, Whorwood CB & Mason JI (1995) Type 2 11α-hydroxysteroid dehydrogenase in fetal and adult life. *Journal of Steroid Biochemistry and Molecular Biology* **55**, 465–471.

Tangalakis K, Lumbers ER, Moritz KM, Towstoless MK & Wintour EM (1992) Effect of cortisol on blood pressure and vascular reactivity in the ovine fetus. *Experimental Physiology* **77**, 709–717.

Thame M, Wilks RJ, McFarlane-Anderson N, Bennett FI & Forrester TE (1997) Relationship between maternal nutritional status and infants weight and body proportions at birth. *European Journal of Clinical Nutrition* **51**, 134–138.

Willett WC (1994) Diet and health: What should we eat? *Science* **264**, 532–537.

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. *Pediatric Research* **40**, 438–443.

Yajnik CS, Fall CHD, Vaidya U, Pandit AN, Bavdekar A, Bhat DS, Osmond C, Hales CN & Barker DJP (1995) Fetal growth and glucose and insulin metabolism in four-year-old Indian children. *Diabetic Medicine* **12**, 330–336.

Zeman FJ (1968) Effects of maternal protein restriction on the kidney of the newborn young of rats. *Journal of Nutrition* **94**, 111–117.

Zeman FJ & Stanbrough EC (1969) Effect of maternal protein deficiency on cellular development in the fetal rat. *Journal of Nutrition* **99**, 274–282.