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Intrauterine Programming and Postnatal Hypertension

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ABSTRACT

The adult phenotype is known to be dependent on environmental signals operating during intrauterine development. This study reviews the effect and the mechanisms of intrauterine programming-induced hypertension. Low birth weight impairs endothelial dependent and non-endothelial dependent vasodilatation by decreasing endothelial NO synthase (eNOs) and eNOs activity, increasing pressor response to angiotensin II, increasing apoptosis in fetal heart and increasing sympathoadrenergic activity. It also leads to permanent structural alterations of the kidneys with a high tendency to cardiorenal disease.

Key words: Intrauterine programming, hypertension, nitric oxide, angiotensin, kidney

INTRODUCTION

Insult during pregnancy causes an adaptation, which improves the chance for survival of the fetus. These adaptations may be vascular, metabolic or endocrine. They permanently change the function and the structure of the body in adult life. In combination with adverse adult lifestyle risk factors, these adaptations increase the risk for adult diseases such as hypertension, type-II diabetes and hypercholesterolemia.

Causes of intrauterine programming: In mammals, programming by internal and external cues during early postnatal life is well established. It is the concept that the adult phenotype also depends on environmental signals operating during intrauterine development that has recently received the most attention. Since fetal growth and development depend primarily on nutrient and oxygen supply (Harding and Johnson, 1995; Gomez, 1990), the association between low birth weight and adult phenotype has been linked to poor nutrition and oxygenation during early life. However, since nutrient and O_2 availability invariably affect the endocrine environment, the role of hormones as programming signals has also been examined in humans and experimental animals (Fowden and Forhead, 2004).

Nutritional programming: Prenatal nutritional programming of postnatal physiological functions has been demonstrated experimentally in a wide variety of laboratory and farm animals by manipulating the availability of macronutrients and micronutrients during development (Buchanan and Kjos, 1999; Fowden and Forhead, 2006). A range of techniques has been used to reduce the fetal availability of macronutrients used for tissue accretion (Bertram and Hanson, 2001). These include calorie restriction, isocalorific protein deprivation, placental restriction and

reductions in umbilical and/or uterine blood flows. All of these procedures impair fetal growth and lead to abnormalities in cardiovascular, metabolic and endocrine function both before and after birth (McMillen and Robinson, 2005). Altered postnatal physiological function has also been observed after maternal fat feeding and dietary manipulation of specific micronutrients, such as minerals (calcium, iron), cofactors (folic acid, taurine) and vitamins (A and D) (Armitage *et al.*, 2004). In some instances, the programming effects of macronutrient restriction can be ameliorated by supplementation of the altered diet with single cofactors and amino acids (Armitage *et al.*, 2004). Indeed, it may be the balance of micronutrients and macronutrients that is more important in programming than the absolute amount of nutrient *per se*.

Studies have shown that ligation of a uterine horn in pregnant guinea pigs restrict fetal growth. This resulted in severe fetal growth restriction and relative hypertension in the restricted pups. In 1994, Langley and Jackson reported that feeding low-protein diets (60-120 g protein kg^{-1} diet) to pregnant rats resulted in an increase in blood pressure in the offspring, compared with control animals. This low-protein diet (the Southampton low-protein diet) is composed of casein, sucrose, starch and corn oil and is supplemented with vitamins, minerals and methionine. When dams were fed the low-protein diet for single weeks during pregnancy, the magnitude of the effect of the diet on postnatal blood pressure was greatest when the low-protein diet was consumed during the last week of gestation (Langley-Evans, 2001). Subsequent studies using a low-protein diet (6%) from the 12th day of gestation or a low-protein (9%) diet throughout gestation also reported that the offspring developed hypertension by the 8th and 21st week of life respectively (Manning and Vehaskari, 2001; Vehaskari *et al.*, 2001; Woods and Rasch, 1998). The hypertension associated with prenatal exposure to a low-protein diet appears to be a consequence of an increased peripheral resistance as the pulse rate tends to be lower and there is no cardiac hypertrophy, suggesting that cardiac output is not elevated (Jackson *et al.*, 2002). Interestingly, a balanced reduction (a 50-70% decrease) in maternal nutrient intake produces a less consistent effect on later blood pressure than does the specific restriction of maternal protein intake (Holemans *et al.*, 1999). Furthermore, when different low-protein diets have been compared, it was demonstrated that they elicited different programming effects on blood pressure in the offspring (Langley-Evans *et al.*, 1996, 1999).

Programming by alteration in Oxygen (O_2): Several of the techniques used to induce fetal under-nutrition also reduce fetal O_2 delivery. In rats, chronic hypoxia during pregnancy causes disproportionate intrauterine growth restriction (IUGR) and leads to abnormalities in cardiovascular function in the adult offspring (Chang *et al.*, 1984; De Grauw *et al.*, 1986). Similarly, in human populations, the hypobaric hypoxia in high altitude is associated with reduced birth weight and asymmetric growth retardation (Giussani *et al.*, 2005). However, the developmental changes induced by hypoxia may also have a nutritional component, because high altitude populations are often impoverished and chronic hypoxia during pregnancy in experimental animals reduces maternal food intake (De Grauw *et al.*, 1986; Giussani *et al.*, 2005). The relative contributions of fetal hypoxemia and under-nutrition to programming in the circumstances have been investigated experimentally by pair-feeding pregnant rats at normal PO_2 and by using chick embryos. In avian species, the specific developmental effects of O_2 deprivation can be studied *in vivo* without the confounding effects of a hypoxic placenta and/or reduced maternal dietary intake. Both of these approaches show that hypoxia alone during early development can program the cardiovascular system and more especially, the blood vessels (Fowden *et al.*, 2005). In the chick

embryo, hypoxia during the mid to late incubation period exaggerates response to periarterial sympathetic nerve stimulation and downregulates nitric oxide-dependent dilator function in the vessels of the adult birds (Fowden *et al*., 2005).

Endocrine programming: Hormones have an important role in regulating normal growth and development in utero and their concentrations and bioactivity change in response to many of the environmental challenges known to cause intrauterine programming (Fowden and Forhead, 2004). Under-nutrition, hypoxemia and stress can alter both maternal and fetal concentrations of many hormones including growth hormone (GH), insulin-like growth factors (IGFs), insulin, glucocorticods, catecholamines, leptin, thyroid hormones and placental hormones such as the eicosandoid, sex steroids and placental lactogen. Because some of these hormones cross the placenta, the fetal endocrine response to adverse conditions reflects the activity of both maternal and fetal endocrine glands and depends on the type, duration, severity and gestational age at onset of the insult (Fowden *et al*., 2005). In general, suboptimal intrauterine conditions lower anabolic hormone levels and increase catabolic hormone concentrations in the fetus (Fowden, 1995). These endocrine changes then affect fetal growth and development either directly or indirectly by altering the delivery, uptake and metabolic fate of nutrients in the fetoplacental tissues (Fowden, 1995). Certainly, direct manipulation of glucocorticoid androgen and thyroid hormone levels in utero alters fetal development and has long term consequences on cardiovascular, reproductive and metabolic function (Fowden *et al*., 1998; Rhind *et al*., 2001). Of the hormones known to regulate fetal development, it is the glucorticoids that are most likely to have widespread programming effects in utero (Bertram and Hanson, 2001). They are growth inhibitory and affect development of all the tissues and organ systems that are at increased risk of adult pathophysiology when fetal growth is impaired (Fowden *et al*., 1998). In rats, guinea pigs and sheep, fetal overexposure to either endogenous or exogenous glucocorticoids leads to hypertension, glucose intolerance and abnormalities in Hypothalamic-Pituitary-Adrenal (HPA) function after birth (Matthews, 2000; Seckl, 2004). In addition, the programming effects of under-nutrition can be prevented by abolishing maternal glucocoticoid synthesis by adrenalectomy or metyrapone treatment (Langley-Evans, 2001). Glucocorticoids can therefore, program tissues in utero and may also mediate the programming effects of nutritional and other environmental challenges during pregnancy (Fowden and Forhead, 2004).

Critical period of intrauterine programming: Exposure to a period of maternal restriction during different stages of gestation results in the programming of high blood pressure in a range of species. However, the mechanisms underlying these programming events are likely to be different, multifactorial and depend on the relative influence of fetal nutrient deprivation and the maternal and fetal neuroendocrine adaptations to under-nutrition during critical periods of organogenesis and development of fetal cardiorenal function (Mcmillen and Jeffery, 2005).

Environmental insults can cause programming at many stages during development (Fowden *et al*., 2005). During the periconceptual and preimplantation periods, nutrient, O_2 and hormone levels affect development of the oocyte and blastocyts, with consequences for the distribution of cell between the trophoblast and inner cell mass (Fowden *et al*., 2005). Exposure to excess progesterone and metabolites, such as urea, especially during these periods leads to enhanced birth weight in sheep and pigs (McEvoy *et al*., 2001). In addition, dietary restriction during the periconceptional period has also been shown to shorten gestation and cause

hypertension and abnormal hypothalamic-pituitary-adrenal (HPA) function in adult sheep (Kumarasamy *et al*., 2005). Developmental changes arising before implantation are likely to affect many cell lineages, although adaptations later in gestation, such as upregulation of placental nutrient and O_2 transport, may compensate for the early defects and normalize birth weight. Once placentation has begun, the programming effects of environmental signals may be mediated via changes insults program the placenta per se remains unknown.

During organogenesis, environmental insults may cause discrete structural defects that permanently reduce the functional capacity of the organ. If the insult occurs during gametogenesis, reproductive potential of the next generation may be impaired (Rhind *et al*., 2001). During the phase of rapid fetal growth, insults, which alter the supply, uptake and utilization nutrients, will influence tissue growth and may switch the cell cycle from proliferation to differentiation with adverse consequences for total cell number (Harding and Johnson, 1995; Fowden *et al*., 1998; Kind *et al*., 1995). Since, different fetal organs grow at different rates, the timing of the insult is important in determining the tissue specificity of the programmed effects (Fowden *et al*., 2005). In late gestation, there is a period of fetal maturation during which many tissues undergo structural and functional changes in preparation for extrauterine life (Fowden *et al*., 1998). These processes are often glucocorticoid-independent and can be activated prematurely by early glucocorticoid exposure (Fowden *et al*., 1998).

Although, the sequence of developmental changes is broadly similar in all mammalian species, there are differences in their precise timing between animals (Fowden *et al*., 1998). In artificial species that are immature at birth (e.g. rodents and rabbits), in utero continue to develop after birth (Fowden *et al*., 1998). The period of developmental plasticity, therefore, extends after birth in these species in contrast to precocial species (e.g., human, sheep, pig) that are more physiologically mature at birth (Fowden *et al*., 1998). Consequently, postnatal environmental changes, particularly before weaning, may ameliorate or exaggerate the morphological and functional changes programmed in utero (Ross and Desai, 2005). Ultrasound studies have shown that in the longitudinal plane of fetal kidneys was similar in small- and appropriate-for gestational age groups. However, growth in the anterior-posterior, transverse and circumference planes of the kidneys was significantly slower in the small-for-gestational-age group after 26wk gestation (Mcmillen and Jeffery, 2005). Differences in growth rate in the two groups were most marked between the 26th and 34th week of gestation and persisted until delivery when the anterior-posterior diameter was significantly smaller in the small-for-gestational group (Konje *et al*., 1996).

Mechanism of intrauterine programming: Intrauterine programming can occur at any level within the affected physiological system and may involve structural and functional changes in genes, cells, tissues and even whole organs (Fowden *et al*., 2005). These changes may be isolated or widespread events with either discrete or cumulative effects on development depending on the nature and timing of the programming stimulus (Gluckman and Hanson, 2005; McMillen and Robinson, 2005).

Genes: The associations between birth weight and later risk of degenerative disease may be due to a direct genetic link between intrauterine growth and disease susceptibility inherited at conception (Young, 2001). In part, this reflects the survival value of genes selected for reduced fetal growth yet rapid postnatal growth and fuel storage during evolution in populations subjected to periodic under-nutrition (Bateson *et al*., 2004). The observation that mutations in the pancreatic glucokinase gene result in reduced fetal insulin secretion, lower birth weight and adult glucose

intolerance indicates that fetal growth and disease susceptibility can be linked through a single gene (Hattersley and Tooke, 1999). However, these monogenic disorders are rare and cannot explain the variation in disease risk across the normal birth weight (Fowden *et al.*, 2005). The discordant disease risks in monozygotic twins of discrepant birth weights compared with the concordant risks when birth weight is similar also shows that different adult phenotypes can develop from the same genotype when intrauterine growth is compressed (Phillips, 1993).

During development, DNA can be modified epigenetically to alter gene expression without a change in DNA sequence (Vickaryous and Whitelaw, 2005). In mammals, these modifications occur primarily by DNA methylation and/or posttranslational alterations to the histones packaging the chromatin. In turn, these conformational changes alter the interaction between DNA and its regulatory proteins at the promoters, with effects on gene activation and repression (Waterland and Carza, 1999). DNA methylation is particularly important in genomic imprinting, the process by which one allele of a gene is silenced in a parent-of-origin manner. Imprinting has a major role in early mammalian development and many of the 80 known imprinted genes are involved in controlling placental and/or fetal growth.

Epigenetic, therefore, provides a molecular mechanism for programming that links genes, the prenatal environmental intrauterine growth and subsequent susceptibility to disease (Fowden *et al.*, 2005).

There are two main periods of epigenetic modification: gametogenesis and early embryogenesis. These are the times of widespread demethylation and resetting of the epigenetic marks by de novo availability of methyl donors and cofactors essential for one-carbon metabolism, such as folate and glutathione (Waterland and Carza, 1999). Once established, the epigenetic marks are stable mitotically and create unique, lineage-specific patterns of gene expression and/or silencing. Epigenetic modifications can also occur later in development as changes in DNA methylation and imprint status have been observed during the perinatal period in several species (Waterland and Carza, 1999). Both nutritional and endocrine factors have been shown to influence the epigenotype (Waterland and Carza, 1999). Glucocorticoid administration causes DNA demethylation associated with increased gene expression of a hepatic aminotransferase in rats during the perinatal period (Thomassin *et al.*, 2001). In neonatal rat kidney, uteroplacental insufficiency induced by uterine artery ligation during late gestation causes hypomethylation of p53, a gene involved in apoptosis (Pham *et al.*, 2003; Ghosh *et al.*, 2001). When methyl donor availability is altered by a low-protein diet during pregnancy, the pattern of hepatic DNA methylation is changed in both the fetus and adult offspring (Rees *et al.*, 2000). Addition of folate or methyl donors, such as glycine and taurine, to the low-protein diet restores normal patterns of DNA methylation and prevents the abnormalities in adult cardiovascular function that are programmed by the unsupplemented diet (Armitage *et al.*, 2004).

Environmental challenges may, therefore, cause programming by altering gene expression via several mechanisms (Fowden *et al.*, 2005). These include loss of imprinting, differential promoter usage and upregulation or downregulation of expression from the active allele(s) mediated epigenetically or via transcription factors (Waterland and Carza, 1999) . Certainly in several genes with multiple mRNA transcripts, the relative glucocorticoid exposure in a tissue-specific manner, with potential consequences for protein translation in fetal tissue (Fowden, 2003).

Cell: Changes in transcription induced by environmental challenges lead to altered protein synthesis and permanent changes in cellular protein abundance (Fowden *et al.*, 2005). These proteins include receptors, ion channels, transporters, enzymes, growth factors, cytoarchitectural

proteins, binding proteins and components of several intracellular signaling pathways (Fowden *et al.*, 2005). In particular, there are changes in the adenylyl cyclase and insulin signaling pathways in cells, such as adipocytes, hepatocytes and myocytes, in response to nutrient and O_2 deprivation, which persist after birth and influence adult metabolic activity (Ozane and Hales, 2002; McMillen and Robinson, 2005). Similarly, prenatal and hypoxemia induce permanent changes in the abundance of membrane and nuclear receptors for several hormones including glucocorticoids, catecholamines, insulin, Intrauterine Growth Factor (IGF), Growth Hormone (GH), leptin and prolactin (Fowden and Forhead, 2004; Fowden *et al.*, 2005; McMillen and Robinson, 2005). Overall, these changes in protein synthesis alter cell metabolism and growth and modify sensitivity to subsequent challenges.

Environmentally induced changes in cell physiology can occur at any point in development but are more likely to have long-term consequences during the formation of cell lineages and when cells are differentiating during late gestation in preparation for extrauterine life (Fowden *et al.*, 2005). In addition, when cells are growing at their maximum rate, undernutrition and hypoxemia may reduce cell proliferation directly by limiting substrate availability for tissue accretion and energy generation. Indeed, restriction of uterine blood flow near term is known to reduce DNA synthesis in fetal cells with a high proliferation rate in utero (Asano *et al.*, 1997). Suboptimal intrauterine condition, therefore, induce changes in the function, number and size of cells by altering proliferation, clonal selection and apoptotic remodeling of cell populations (Fowden *et al.*, 2005).

Tissues of organs: The changes in cell structure and function, in turn, alter the morphology and physiology of tissues and organs as a whole and if the insult occurs at the time of organogenesis, the changes may be severe and lead to a permanent developmental deficit (Fowden *et al.*, 2005). For instance, glucocorticoid administration to pregnant ewes for 2 days when the mesonephric kidney is developing at the end of the first month of gestation causes a permanent reduction in nephron number and leads to hypertension in the adult offspring by suboptimal conditions in utero, can also influence postnatal physiological functions. For example, there are changes in the relative proportions of different cell types in the pancreatic islets, liver and skeletal muscles after IUGR that are associated with adult insulin resistance, glucose intolerance and hypertension (Ozane and Hales, 2002; Holemans *et al.*, 2003; McMillen and Robinson, 2005). In organs like the placenta, early changes in development at the gene (imprinting), cell (transporter abundance) and tissue (vascularity) levels may impair fetal nutrient and O_2 delivery throughout gestation with implications for tissue programming long after the original insult (Godfrey, 2002; Reik *et al.*, 2003).

Systems: The postnatal physiological abnormalities and observed after suboptimal intrauterine conditions are multifactorial and involve several organs and endocrine systems and the severity of outcome is, therefore, determined by the number of organs and systems adversely affected by the intrauterine insult, which, in turn, depends on the nature and duration of the insult in relation to the stage of development (Barker, 1994; Gluckman and Hanson, 2005). In sheep, for instance, maternal glucocorticoid treatment early in gestation leads to hypertension but not glucose intolerance, whereas treatment late in gestation has the opposite effects in the adult offspring (Moritz *et al.*, 2002a, 2003; Fowden and Forhead, 2004). In some systems, a specific trigger, or a second challenge, is required postnatally to unmask the intrauterine programming (Fowden *et al.*, 2005). In the heart, an ischemic challenge in adulthood is required to expose the underlying abnormalities in development induced by prenatal hypoxia or cocaine exposure. In several

physiological systems, sex-linked differences in intrauterine programming do not appear until puberty when the onset of gonadal steroidogenesis uncovers physiological abnormalities in peripheral tissues or in the hypothalamic-pituitary-gonadal axis itself (Rhind *et al.*, 2001). However, in the majority of physiological systems studies, the adverse consequences of intrauterine compensatory adaptations in other tissues and organ systems fail (McMillen and Robinson, 2005).

Role of birth weight: One important issue that has been flagged in the discussion of the importance of the fetal origins of adult disease is the contribution of low birth weight to the burden of adult disease and the extent to which birth weight is a useful measure or surrogate of fetal growth (Rasmussen, 2001). Term is defined as a pregnancy which least 37-41weak and 2% of term babies are of low birth weight, i.e., weight <2,500 g. Infants can also be characterized as small or large for gestational age in the context of a larger reference population (Roberts and Lancaster, 1999). Being small for gestational age is defined as having a birth weight and/or length at least two standard deviations below the mean for gestational age in the reference population (Lee *et al.*, 2003). While, these definitions identify infants that are relatively small at birth, they do not distinguish the different growth patterns by which infants may have arrived at their weight at birth. Importantly, some small babies have achieved their genetic growth potential and are simply in the lower tail of the birth weight distribution, whereas other babies may have experienced a prenatal nutritional deficit which slowed their growth but still have a birth weight that falls within the normal portion of the birth weight distribution.

Studies found that lower birth weight and weight at 1 year of age were associated with an increased risk of death from cardiovascular disease and stroke in a cohort of men and women born in Hertfordshire, England between 1911 and 1930 (Barker *et al.*, 1989a; Osmond *et al.*, 1993). Early criticisms of the association of death rate from coronary heath disease with lower birth weight raised questions about completeness of follow-up (Joseph and Kramer, 1996). Although, it was recognized that loss of subjects from cohorts was inevitable with the requirement for follow-up over many decades (Kramer, 1998). This criticism was largely answered in a study of 15,000 Swedish men and women with 97% follow-up over a period of more than 50 years. Studies show that the death rates from ischemic heart disease were increased in individuals in the lower quartiles of birth weight compared with those in the highest quartile. Also of note was the Nurses study in the United States which includes 121,700 women who have been followed since 1976. In a retrospective study of more than 70,000 women, there were strong negative trends between self-reported birth weight and nonfatal coronary heart disease and stroke (Rich-Edwards *et al.*, 1997). The association of cardiovascular death and birth weight has now been reported in many (Frankel *et al.*, 1996a, b; Martyn *et al.*, 1996; Stein *et al.*, 1996), but not all studies (Hulman *et al.*, 1998), particularly those involving twins.

The importance of environmental influences that impair fetal and infant growth was raised when it was reported that men with the lowest weights at birth and at 1 year of age had the highest rates of ischemic heart disease. Although the death rate increased with decreases in birth weight in both males and females, the effect of reduced infant growth on subsequent cardiovascular disease was present in men only (Osmond *et al.*, 1993). It was suggested that promotion of fetal and infant growth might reduce cardiovascular deaths and a special emphasis was given to the importance of promoting growth in the first year of life of boys who weighed <7.5 Ibs. (3.5 kg) at birth (Barker *et al.*, 1989b). Although, low weight gain in the first year of life was associated with increased cardiovascular mortality, it has also been shown in a cohort study among Finish men that

increased weight gain as early as 3 years of age is also associated with increased cardiovascular mortality (Eriksson *et al.*, 2001, 2002a). Men who died were obese with above average Body Mass Index (BMI) at all ages from 7 to 15 years of age (Eriksson *et al.*, 2002b). These effects may be greatest in males born to mothers who were obese (Forsen *et al.*, 1997). Short birth length of female infants followed by increased in height was also associated with increased risk of death from coronary heart disease (Forsen *et al.*, 1999). These women tended to have tall mothers (Forsen *et al.*, 1999), which suggested that prenatal growth of these female infants was constrained. Thus, the most adverse cardiovascular disease risk profile is found in men and women who were small at birth but became obese in adult life (Eriksson and Forsen, 2002) and the effects of adult BMI on high blood pressure, type 2 diabetes and insulin resistance were greater in individuals of low birth weight (Frankel *et al.*, 1996a, b). Whatever the mechanisms, the possibility of a biological link between birth weight and adult BMI complicates interpretation of studies that link birth weight and adult BMI complicates interpretation of studies that link birth weight and adult BMI to other adult outcomes such as cardiovascular disease, hypertension and insulin resistance. Whilst many authors adjust for current BMI when evaluating associations between birth weight and adult outcomes, it has been argued that there may be statistical implications with the approach that should be taken into consideration when interpreting the interaction between birth weight BMI and cardiovascular or metabolic health comes (Lucas *et al.*, 1999; Waterland and Carza, 1999; Burke *et al.*, 2004).

Publication of the early epidemiological studies led many to attempt to verify experimentally the link between reduced birth size and later disease risk size at birth is readily manipulated in experimental animals by altering maternal nutrition in pregnancy. For example, reducing the proportion of protein in the diet of pregnant rats results in offspring which have reduced size at birth and also elevated blood pressure. A variety of experimental approaches to reduce maternal nutrition in pregnancy have led to similar observation in rats (Woodall *et al.*, 1996) and guinea pigs (Persson and Jansson, 1992). Thus, there is no doubt that changes in maternal nutrition in animals can both change size at birth and also permanently alter (program) aspects of the physiology of the offspring in a way that is consistent with the disease susceptibility observed in human studies. Fetal growth in late gestation is normally limited by maternal size and her capacity to supply nutrients to her fetus, a phenomenon known as maternal constraint. Thus fetal growth in late gestation is normally regulated by fetal nutrient supply (Gluckman and Hanson, 2005). Since nutrition has such a central role in the regulation of fetal growth, it is a good candidate for a programming stimulus, holding a central role in the link between size at birth and subsequent disease risk.

Target for programming

Endothelium: Endothelial-dependent vasodilation is mediated by a combination of Nitric Oxide (NO), prostacyclin and endothelium-derived hyperpolarization factors that act directly to reduce vascular smooth muscle tone (Mcmillen and Jeffery, 2005). Several studies have reported that either endothelial-dependent or endothelial-independent vasodilation is impaired and that flow-mediated dilation is decreased in low-birth-weight individuals at the third month of age, in later childhood or in early adult life (Goodfellow *et al.*, 1998; Martin *et al.*, 2000; Goh *et al.*, 2001; Leeson *et al.*, 2001). Offspring from rats that were undernourished (30% global reduction) during the first 18 days of pregnancy had higher blood pressure from the 60th days after birth and the maximal vasoconstriction response to phenylephrine or to norepinephrine was reduced in the isolated femoral arteries for these pups at the 20th days of age, although these differences did not

persist into adulthood (Ozaki *et al*., 2001a). These results are consistent with studies in offspring from dams fed a globally restricted diet (50% reduction during the second half or pregnancy) (Brawley *et al*., 2003a) in which there was no effect of the prenatal undernutrition on the vasoconstrictor responses of the peripheral arteries, suggesting that there is no alteration in the adrenoceptor-medicated constriction pathway (Mcmillen and Jeffery, 2005). When rats were fed 50% of the normal intake diet for the whole of pregnancy, endothelium intact aortic rings from the offspring at the 14th week of age showed increased responses to nerepinephrine (Franco *et al*., 2002). In these animals, there was also decreased vasodilation of the aortic rings to acetylcholine (Franco *et al*., 2002). Intrauterine undernutrition resulted in decreased gene expression for endothelial NO synthase (eNOs) in male aortae and a reduction in eNOs activity in both male and female offspring (Franco *et al*., 2002). In this model, there was also increased superoxide synthesis in small mesenteric arteries, which could contribute to reduced endothelial-dependent dilatation through reduction of NO or tissue oxidative damage (Mcmillen and Jeffery, 2005).

In male offspring of dams fed a low-protein diet throughout pregnancy, vascular relaxation induced by the endothelium-independent vasodilator sodium nitroprusside and a phosphodiesterase type 3 inhibit were reduced (Brawley *et al*., 2003a; Benediktsson *et al*., 1993). Reduced vasodilation may be a potential mechanism underlying the elevated systolic blood pressure observed in this model (Brawley *et al*., 2003a). This mechanism appears to be generalizable to other key vessels as maternal protein restriction is also associated with diminished NO-dependent relaxation of cerebral microvasculature, which is predominantly due to decreased soluble guanyl cyclase activity and cGMP levels (Lamireau *et al*., 2002). It has also been shown that dietary protein restriction reduces the acetylcholine sensitivity and relaxation in small mesenteric arteries from the pregnant dams compared with control dams (Koumentaki *et al*., 2002; Brawley *et al*., 2004). Glycine supplementation to pregnant dams fed a protein-restricted diet reversed this vascular dysfunction and the impaired release of NO from the vessels (Brawley *et al*., 2004). It was found that exposure to a low-protein diet in utero did not decrease the levels of eNOs mRNA, which indicates that changes in eNOS protein levels, eNOS activity and/or substrate deficiency account for the endothelial dysfunction (Brawley *et al*., 2004). Glycine supplementation normalizes the elevated blood pressure observed in the adult offspring of pregnant rats fed a low-protein diet (Jackson *et al*., 2002). The molecular mechanisms by which changes in the fetal and postnatal vasculature are programmed by alterations in maternal nutrition are not understood, although as discussed, maternal homocysteine metabolism may ultimately affect the degree of fetal DNA methylation and disturb embryonic vasculogensis (Steegers-Theunissen and Steegers, 2003). Endothelial cell phenotypes are dependent on a range of factors including laminar flow and vascular endothelial growth factor (Gale and Yancopoulos, 1999; Topper and Gimbrone, 1999) and undernutrition during pregnancy may alter the phentotypic modulation by these factors resulting in permanent changes in endothelial and vascular smooth muscle differentiation. Recent data also suggest that dietary protein restriction in pregnancy programs vasolidator dysfunction in isolated resistance arteries of female offspring when they become pregnant, which may in turn lead to "transgenerational" programming (Torrens *et al*., 2003). It has been speculated that such programming may be dependent on the modification of the genomic DNA in the offspring through imprinting mechanisms, although it is also possible that vascular dysfunction present in the offspring of low-protein-exposed dams may influence vascular function in subsequent generations through a nongenetic mechanism (Brawley *et al*., 2003b). Maternal diets that are rich in fat may also result in endothelial dysfunction in offspring resulting in reduced endothelium-dependent

dilation in mesenteric resistance vessels (Khan *et al*., 2003). Interestingly, although rats maintained on the same fat-rich diet that they were exposed to during pregnancy had raised blood pressure, they had improved endothelium-dependent vasolidation, when compared with animal adapted to a control diet in utero and then exposed to the fat-rich diet in adult life (Khan *et al*., 2004). It was considered that this study provided evidence that predictive adaptive responses prevent endothelial diet but do not prevent development of raised blood pressure (Khan *et al*., 2004). One concern in relation to the studies of offspring of protein-restricted pregnant dams has been that dysfunction of the vascular endothelium may be a consequence, rather than a cause, of the hypertension (Mcmillen and Jeffery, 2005). Accordingly, there have been a number of studies of manipulation of nutrition of the pregnant ewe during early, mid and late gestation to study the direct influence of undernutrition on vascular function, before the emergence of hypertension (Mcmillen and Jeffery, 2005). In a study, ewes were nutritionally restricted by 30% (global restriction) or by 30% of the recommended protein intake (protein restricted) for 12 days before and 70 days after conception (Nishina *et al*., 2003). The femoral vascular response to norepinephrine, the maximal relaxation and sensitivity to acetylcholine were all reduced in protein restricted fetuses at the 70th day of gestation. These data are consistent with the observation that a 50% global restriction of maternal nutrient intake across the same periconceptual window resulted in a blunted endothelial-dependent and non-endothelial-dependent vasodilation at the 130th day of gestation (Ozaki *et al*., 2001b). When dexamethasone is administered to pregnant ewes as 3 weekly courses (4 injections of 2 mg 12-h intervals), starting at 103 days gestation, there was an enhanced sensitivity to the endothelial vasoconstrictor, endothelin-1, abnormal endothelium-depend relaxation and normal endothelium-independent relaxation in femoral arteries at the 120th day of gestation (Molnar *et al*., 2002). When ewes were allowed to give birth following the maternal dexamethasone treatment in late pregnancy, it was found that whilst arterial blood pressure was not elevated in the lambs at 5 month of age, the vascular sensitivity to endothelium-independent vasodilatation (Molnar *et al*., 2003). It was speculated that after prenatal dexamethasone treatment, there would be a limited ability to increase compensatory vasodilatation after 5 month of age, resulting in the eventual emergence of hypertension in these animals (Molnar *et al*., 2003). Whether the levels of glucocorticoids and their vascular consequences are relevant to the changes in endogenous glucocorticoids measured following maternal undernutrition at different stages of pregnancy or in response to placental insufficiency is not clear. However, any persistent or programmed vascular effect are important in the context of the administration of single or repeat does of synthetic glucocorticoids to pregnant women in threatened preterm labor.

Renin-Angiotensin System (RAS) and vascular smooth muscle: It is important to distinguish between the effects of maternal or fetal nutrition on the intrarenal and circulating (RAS) as these systems do not necessarily change in parallel. The pressor response to increasing doses of angiotensin II is greater an more prolonged in rats exposed to a low-protein diet in utero showed lowered blood pressure after treatment with the angiotensin converting enzyme (ACE) inhibitors, captopril (Langley-Evans and Jackson, 1995) or enalapril (Manning and Vehaskari, 2001) and this effect was reversible (Langley-Evans and Jackson, 1995). Treatment with captopril from 2-4 weeks after birth during the pre-weaning period also produced an irreversible decrease in blood pressure in protein-restricted offspring (Sherman and Langley-Evans, 1998). Losartan, a specific angiotensin 1 receptor (AT1R) antagonist, also reduced blood pressure in a similar irreversible manner when administered at 2-4 weeks after birth in rats exposed to a low-protein diet in utero

and these data suggest that there may be an upregulation of the AT1R during the early postnatal period which plays a role in the development of the programmed hypertension (Sherman and Langley-Evans, 2000). This upregulation could occur within the vasculature, kidney, brain or heart and in this context, it is interesting that glucocorticoids are known to increase AT1R expression at different peripheral sites including the vasculature (Sato *et al*., 1994). As discussed in the previous section, a low-protein diet during pregnancy is associated with a decrease in intrarenal RAS activity and treatment of newborn rat with losartan during the first 12 days of life results in a decreased number of nephrons and adult hypertension. This suggest that the development of both intrarenal and circulating RAS could be perturbed as a consequence of the maternal low-protein diet during pregnancy and that there must be two critical periods during which a decrease in the circulating RAS or activation of AT1R (before birth and between 2-4 weeks after birth) can result in hypertension. Blood pressure responses to increasing doses of angiotensin (Ang) II, but not norepinephrine, were increased in fetal sheep after a 48 h cortisol infusion at around the 125th day of gestation (Tangalakis *et al*., 1992). Intrafetal infusion of cortisol also results in an increased expression of angiotensin AT1 receptor mRNA within the fetal heart (right and left atrium and right ventricle) (Segar *et al*., 1995) and there is a greater hypotensive effect after blockade of AT1R in fetal sheep that have been infused with cortisol (Forhead *et al*., 2000). Maternal undernutrition (a 50% reduction in nutrient intake) during late gestation also results in an increase in maternal plasma concentrations of cortisol and an associated increase in fetal arterial blood pressure and in the pressor responses to increasing doses of Ang II (Edwards and McMillen, 2001). Thus increased exposure to cortisol during fetal life may result in an increased sensitivity to the vasoconstrictor actions of Ang II through either an increase in the expression of the AT1R or changes in the postreceptor-mediated events within the vascular smooth muscle. Alternatively, there may be an enhanced central action of Ang II resulting in enhanced stimulation of sympathetic tone and concomitant of vagal tone.

In species like human and the sheep, the response of the faetal hypothalamo-pituitary-adrenal (HPA) axis to intrauterine stressor may play an important role in the adaptive responses of the fetal cardiovascular system to the prevailing suboptimal intrauterine environment. Cordocentesis studies have shown that plasma cortisol concentrations are higher in intrauterine growth restricted human fetuses than in normally grown fetuses at 18-38 weeks of gestation (Economides *et al*., 1988). Repeated injections of betamethasone into pregnant ewes between 104 and 125 days of gestation do not result in an increase in blood pressure in the offspring between 6 and 12 month of age (Moss *et al*., 2001). It is clear that exposure of the fetus to high concentrations of synthetic or maternal glucocorticoids during the period of nephrogenesis in the rat (late gestation and first week after birth) and sheep (26-27 days of gestation) result in an increase in arterial blood pressure in the offspring. It is possible that the fetal glucocorticoid responses to placental restriction or maternal undernutrition in the sheep during late gestation are examples of important fetal adaptations to an adverse intrauterine environment which do not result in an increase in arterial blood pressure that persists into postnatal life.

While maternal undernutrition, placental restriction, or exposure to an increase in exogenous, maternal, or fetal glucocorticoids during late gestation may be associated with an enhanced activation of the RAS, there is little evidence that maternal undernutrition during the perconceptional period or synthetic glucocorticoids treatment in early gestation result in a hypertension that is dependent on an enhanced activation of the circulating RAS. While mean arterial pressure was higher during late gestation in twin fetuses of ewes that were undernourished

during the preconception period (60 days preceding and first week after conception), there was no difference in the fetal blood pressure responses to either increasing doses of angiotensin II or to captopril (Edwards and McMillen, 2002). Similarly, in animals that had been exposed to dexamethasone treatment at 26-28 days gestation, there was no difference in the circulating plasma levels of renin, angiotensinogen, ACE, Ang I and Ang II, in the fetal or postnatal arterial blood pressure responses to increasing doses of Ang II or to short-term blockade of peripheral AT1R between glucocorticoid-and non-glucocorticiod-treated groups (Dodic *et al.,* 1998; Moritz *et al.*, 2002b; Peers *et al.*, 2001). In this model however, there was an upregulation of the gene expression for AT1R in the medulla oblongata and for angiotensinogen in the hypothalamus at 130 days of gestation and for AT1R in the medulla oblongata at 7 years of age, suggesting that a permanent alteration in the brain RAS might be implicated in the programming of adult hypertension (Wintour *et al.*, 2003).

The heart

Cardiomyocyte hyperplasia, hypertrophy and apoptosis: One target for prenatal programming of cardiac function is the final number of cardiomyocytes within the heart. Growth of the vertebrate heart during embryonic and fetal life is characterized by hyperplasia of myocardial cell. These cells increase in number to a value characteristic for each species. In rodents, binucleation of cardiomyocytes occurs over postnatal days 4-12 (Oparil *et al.*, 1984; Li *et al.*, 1996, 1999; Soonpaa *et al.*, 1996) after which myocardial cells lose the capability of dividing and further growth of the heart is due to myocardial cell hypertrophy and nonmuscle cell hyperplasia (Mcmillen and Jeffery, 2005). This hypertrophic growth result in a 30-to 40-fold increase in volume of individual myocardial cells during normal postnatal growth and maturation. The transition from hyperplastic to hypertropic growth is related to formation of binucleated myocardial cells as a result of karyokinesis without cytokinesis (Oparil *et al.*, 1984).

Whereas the impact of a range of intrauterine perturbations, including uteroplacental ligatioin, global nutrient restriction and low-protein diets on nephrogenesis has been intensively studied, there have been fewer studies on the associated changes in cardiomyocyte number or size in the heart of the growth-restricted fetus (Mcmillen and Jeffery, 2005). In pregnant rats exposed to chronic hypoxia (10.5% O_2) between days 15 and 21 of gestation, there was an increase in the percentage and size of binucleated myocytes and an increase in apoptotic cells in the fetal heart (Bae *et al.,* 2003). Chronic hypoxia also resulted in an increase in 1-adrenoreceptors and a decrease in heat shock proteins and it was speculated that these changes may also play an important role in apoptosis in the fetal heart (Bae *et al.*, 2003). The increased cell death in the fetal heart following exposure to hypoxia may lead to cardiac hypertrophy, resulting in the asymmetric enlargement of the fetal heart in hypoxic animals. These changes in cardiomyocyte development appear to confer no immediate adaptive benefit, rather they may represent a disruption in the normal pattern of cardiomyocyte development, which renders the postnatal heart more vulnerable in situations where an increased cardiac work load is required. Following prenatal hypoxia there is also an upregulation of 2-adrenoceptor expression and isoproterenol sensitivity (Heydeck *et al.*, 1994), which may serve as a compensatory mechanism and maintain left ventricular function at the resting level. In the heart of the normal fetal sheep, myocyte size, intercapillary distance and myocyte myofibrillar and mitochondrial volume densities increase, whereas capillary density, the myocyte-to-capillary ratio and the myocyte matrix volume density decrease with increasing gestational age (Smolich *et al.*, 1989). Before 110 days gestation, cardiac growth appeared to be due

to myocyte hyperplasia, as approximate myocyte numbers and ventricular free wall weight increased at the same rate. After 110 days gestation, the approximate myocyte number per gram ventricular free wall weight decreased, which suggests that myocyte hypertrophy as well as hyperplasia, was occurring in association with the appearance of a greater proportion of binucleated cells after that time (Burrell *et al*., 2003). By 4-6 weeks of age, there was marked hypertrophy of myocytes and an apparent reduction in myocyte number (Burrell *et al*., 2003). It has been shown that the ratio of binucleated to mononucleated cells can be altered by environmental factors during fetal life. For example, an experimental systolic pressure load applied to the right ventricle in fetal life. For example, an experimental systolic pressure load applied to the right ventricle in fetal animals stimulates cardiomyocyte hypertrophy, hyperplasis and binucleation simultaneously, increasing the percentage of myocytes with two nuclei and the total number of myocytes (Barbera *et al*., 2000). Thus, changes in the pattern of placental function or maternal nutrition which increase fetal blood pressure may result in a change in the development profile of cardiomyocytes. In sheep maternal nutrient restriction (50% global nutrient restriction) between the 28 and 78th day of gestation alters the expression of a range of genes that have been implicated in either cardiac hypertrophy or inhabitation of cardiac remodeling. A number of circulating and intracardiac agents and growth factors have been implicated in the regulation of cardiomyocyte that Ang II stimulates hyperplastic growth among mononucleate myocytes, whereas phenylephrine, but not Ang II, stimulates hypertrophy of the binucleate myocytes (Sundgren *et al*., 2003a). When Intrauterine Growth Factor (IGF) -I is over expressed in the mouse heart using a transgenic approach, heart weight is increased, as is heart weight-to-body weight ratio, apparently due to an increase in cardiomyocyte number rather than cell size (Reiss *et al*., 1996). It has also been shown in the sheep fetus that IGF-I activation of the IGF-I receptor leads to an increase in heart weight due to hyperplasia rather than hypertrophy (Sundgren *et al*., 2003b). The author of this study speculated that an important consequence of fetal growth restriction and an association in the total number of cardiomyocytes at birth with associated risks for heart disease in adult life (Sundgren *et al*., 2003b). Again, a development deficit in cardiomyocyte number may be part of the overall biological trade off for a fetus in a situation where a poor substrate supply requires it to grow more slowly and may limit the subsequent functional capacity of the heart to respond to a range of postnatal demands.

Myocardial growth and vascularization: The impact of chronic hypoxia on myocardial growth and vascularization appears to vary depending on the mechanism used to achieve maternal and or fetal hypoxia. Capillary tubes in the embryonic myocardium are initially formed by vasculogenesis from precursor cells that migrate from the liver region (Mcmillen and Jeffery, 2005). The primary coronary plexus develops as a result of capillaries coalescing into microvessels and the formation of new vessels from preexisting capillaries (Thompson, 2003). The process of coronary vessel development is influenced by growth factors, extracellular matrix and the mechanical forces of the beating heart (Mcmillen and Jeffery, 2005). In fetal sheep whose mother were exposed to high altitude between 30 and 139 days of gestation, long-term hypoxia did not increase either capillary volume density or capillary-to-fiber ration, or cardiac fiber hypertrophy in the fetal heart. It was suggested that ventricular hypertrophy is required for angiogenesis to occur. In fetal sheep exposed to chronic anemia (isovolemic hemorrhage for at least 10 days during late gestation), there is an increase in the fetal heart-to-body weight ratio and the minimal capillary diameter was increased and the intercapillary distance was decreased in both right and left ventricles of anemic

fetuses compared with controls (Martin *et al.*, 1998). Furthermore, there was an increase in vascular endothelial growth factor (VEGF) mRNA and protein and in hypoxia-inducible factor 1 (HIF1) protein in the ventricular tissue from the anemic fetuses (Martin *et al.*, 1998). Thus, in chronic fetal anemia there is evidence that cardiac hypertrophy stimulates HIF1 and VEGF expression to result in an increase in myocardial vascularization (Martin *et al.*, 1998).

There is evidence that coronary conductance can be modified in utero by anemia (high flow and fetal hypoxemia) and that this remodeling persists into adulthood. In fetal sheep exposed to chronic anemia (isovolemic hemorrhage for at least 10 days during late gestation), remodeling of the coronary vasculature occurs and there is a significant increase in coronary conductance. In these animals after birth, whilst resting coronary blood flow is normal, there is an increase in maximal coronary conductance at 28 week of age. In contrast, there was no effect of in utero anemia on the vascular reactivity of the mesenteric arteries in these animals (Mcmillen and Jeffery, 2005). This provides evidence that the oxygen and hemodynamic environment during fetal life may alter the architecture of the coronary circulation for life. These authors reported that animals exposed to a 3-week period of induced anemia in utero responded differently to acute hypoxemia when tested under general anesthesia at 28 week of age (Broberg *et al.*, 2003). In response to acute hypoxemia, there was a doubling of the left ventricular end-systolic elastance in animal that had been exposed to in utero anemia, but not in control animals (Broberg *et al.*, 2003). Thus, the responses to in utero anemia appear to confer both immediate and predictive adaptive benefit.

Glucocorticoids and cardiac programming: There is emerging evidence that prenatal exposure to glucocorticoids may also alter subsequent cardiac development. Sheep that become hypertensive after exposure to 2 days of dexamethasone treatment at the end of the first month of gestation have a significant increase in cardiac output at 40 weeks old (Dodic *et al.*, 1999). The increase in cardiac output is due to an increase in stroke volume, rather than heart rate and there is also a rightward shift of the baroreflex curve in these animals. These animals also show left ventricular (LV) hypertrophy, a reduced cardiac functional reserve and increased LV type 1 collagen content in adult life (Dodic *et al.*, 2002a,b). In rats, there is also a significant downregulation of certain protein expression and an upregulation of glucose transporter (GLUT) -1 protein expression in the hearts of adult male offspring of dexamethasone-treated mother (Langdown *et al.*, 2001a) and it has been proposed that such changes may be linked with changes in cardiac fuel selection in later life (Langdown *et al.*, 2001b).

There is therefore emerging evidence that intrauterine substrate restriction and associated changes in neuroendocrine, hemodynamic, metabolic and growth factors may alter patterns of cardiomyocyte hyperplasia, apoptosis and hypertrophy, myocardial development, fuel selection and vascularization and therefore result in programmed changes in cardiac structure and function.

Intrauterine Growth Restriction (IUGR) and the sympathoadrenal system: Studies shows a direct relationship between adult pulse rate and birth weight. Although, the resting pulse rate is an imperfect index of activity of the Sympathetic Nervous System (SNS) activity established in utero may be one mechanism linking small size with increased blood pressure in adult life. It is well established that during acute hypoxemia, blood flow to the brain, heart and adrenal glands is increased and that blood flow to the gastrointestinal, renal and peripheral vascular beds decreases (Hanson, 1997). This redistribution of fetal cardiac output is also maintained with prolonged hypoxemia in pregnancy presumably as a consequence of the action of vasoactive hormones

(Hanson, 1997). Clearly, the redistribution of the fetal cardiac output as an adaptation to chronic hypoxemia is of critical importance for the maintenance of the relative growth and optimal function of key fetal organs including the heart and the brain which occurs in placentally restricted fetuses (McMillen *et al.*, 2001). The vasoconstrictor responses to acute fetal hypoxemia and asphyxia are reduced by sympathectomy and adrenergic blockade (Jones *et al.*, 1998; Giussani *et al.*, 2005), but it is not yet clear whether the redistribution of fetal cardiac output during chronic hypoxemia is dependent on an increase in fetal sympathetic activity and whether this has longer term consequences for the programming of hypertension. This is important in considering whether the fetal neuroendocrine adaptations to the suboptimal intrauterine environment are simply immediately adaptive or whether there is any predictive response that alters the development of the sympathetic innervation of the peripheral vasculature and persists into postnatal life. Plasma norepinephrine concentrations are significantly higher in chronically hypoxemic, growth-restricted fetal sheep than their control counterparts between 110 and 140 days of gestation and for every 1 mm Hg decrease in arterial PO_2, norepinephrine increases by 0.4 pmol mL^{-1} during basal conditions in both placentally restricted and control fetal sheep (Simonetta *et al.*, 1997). Studies of total body catecholamine kinetics before and after birth in spontaneously hypoxemic fetal lambs indicate that such increase in circulating catecholamine levels are related to an increased sympathoadrenal activity (Smoich and Esler, 1999). One possibility is that placental restriction and the presence of chronic hypoxemia throughout late gestation is a stimulus for hyperinnervation of fetal vessels and tissues by sympathetic, postganglionic neurons. Alternatively, low PO_2, or other factors associated with placental restriction, reflexly stimulates catecholamine synthesis and secretion in developing sympathetic neurons. Ruijtenbeek *et al.* (2003a) have used the chicken embryo as a suitable model for studying the effects of hypoxia on cardiovascular development as it is possible to study the effects of adverse conditions in this model without the interference of maternal endocrine, metabolic, or cardiovascular factors. Chronic exposure of the chick embryo to moderate hypoxia led to growth restriction, increased arterial sympathetic innervation and reduced endothelial vasodilatory function at embryonic day 19 (Ruijtenbeek *et al.*, 2003a, b), while there was an increase in sympathetic nerve density and neuronal norepinephrine reuptake at embryonic day 19. These effects were not present when chickens exposed to hypoxia in vivo were studied at 3-4 week postnatal age (Ruijtenbeek *et al.*, 2003b). This may be because sympathetic neuronal development in this model can continue past the hatching stage. In this model, mean arterial pressure and heart rate at 14-15 week of age were also not affected by chronic *in vivo* hypoxia, but isolated femoral arteries were more sensitive to electrical stimulation and pharmacological stimulation of periarterial sympathetic nerves (Ruijtenbeek *et al.*, 2003b).

In the growth-restricted sheep fetus, although there is an inverse relationship between circulating norepinephrine and arterial PO_2, there is a direct relationship between plasma epinephrine concentrations and arterial and arterial PO_2 and between the level of expression of the epinephrine-synthesizing enzyme, phenylethanolamine-N-methyltransferase (PNMT), in the fetal adrenal and PO_2 (Adams *et al.*, 1998). In the pig, plasma norepinephrine, but not epinephrine, concentrations are increased in low-birth-weight animal at 3 month of age (Poore and Fowden, 2003). In 12-month-old male pigs, the norepinephrine response to hypoglycemia was reduced, whereas the epinephrine response to hypoglycemia was increased in animals of low birth weight (Poore and Fowden, 2003). In contrast, in 12-month-old female pigs, basal epinephrine concentrations were reduced and there was no increase in the epinephrine response to hypoglycemia in the low-birth-weight group. The authors suggested that the sex-related differences

in catecholamine secretion may be associated with the failure of low-birth-weight female to catch up in body weight by 12 month of age (Poore and Fowden, 2003). Thus, there appears to be a dissociation of the responses of the separate components of the sympathoadrenal system to a restriction of fetal substrate supply, which may have cardiovascular and metabolic consequences in postnatal life.

The kidney and intrarenal angiotensin system: It has been proposed that maternal undernutrition may lead to permanent structural alteration within the kidneys that contribute to a propensity for adult cardiorenal disease. Brenner and colleagues (Brenner and Chertow, 1994) originally proposed the theory that essential hypertension, including that associated with intrauterine growth restriction, is a consequence of a reduced total of nephrons, leading to sodium retention. This theory was developed further by the proposal that reduction of nephron number is followed by increasing single-nephron GFR and where increased pressure within single nephrons is sustained, focal glomerulosclerosis occurs, resulting in nephron loss (Mcmillen and Jeffery, 2005). The individual therefore enters a cycle in which mean arterial pressure continues to rise to maintain hemodynamic function with progressive and irreversible renal injury (Mackenzie and Brenner, 1995; Mackenzie *et al*., 1996; Brenner and Mackenzie, 1997). In the human, nephrogenesis is completed by 32-34 weeks and therefore, a nephron deficit present at birth would persist through life (Mcmillen and Jeffery, 2005). Two studies have reported that the nephron number in still-born intrauterine growth restricted (IUGR) human fetuses was significantly lower than in their normally grow counterparts (Hinchliffe *et al*., 1992; Manalich *et al*., 2000) and that there was a correlation between nephron number and increased glomerular volume with birth weight (Manalich *et al*., 2000). Furthermore, ultrasound glomerular studies have shown that growth in the longitudinal plane of fetal kidneys was similar in small- and appropriate-for-gestational-age group after 26th week of gestation (Mcmillen and Jeffery, 2005). Differences in growth rate in the two group were most marked between the 26th and 34th weeks and persisted until delivery when the anterior-posterior diameter was significantly smaller in the small-for-gestational-age group (Konje *et al*., 1996). These authors suggested that the 26-34 weeks of gestation could be the critical period during which the insult that leads to in utero programming for the development of adult hypertension occurs (Konje *et al*., 1996).

A recent study showed that in humans, the number of nephrons ranged from 227, 327 to 1,825, 380 and that there was a strong correlation with birth weight so that it was estimated that the number of nephrons increased by 257, 426 for each kilogram increase in birth weight (Hughson *et al*., 2003). Thus a glomerular origin of hypertension may extend across the normal range of birth weights and not be confined to growth-restricted individuals.

In the rat, nephrogenesis begins at around day 12 of gestation and is not complete until 8 days after birth and involves rapid remodeling of structures and apoptosis (Koseki *et al*., 1992; Coles *et al*., 1993). Recently, it has been demonstrated in a model of uteroplacental insufficiency, induced by bilateral uterine ligation of the pregnant rat at 19 days of gestation, that there was a significant reduction in glomeruli number in the full-term fetal kidneys (Pham *et al*., 2003). These studies are consistent with the finding that uteroplacental insufficiency reduced nephron numbers by up to 30% in the rat, rabbit and piglets and in these animals, the decrease in nephron number was associated with a parallel drop in GFR (Merlet-Benichou *et al*., 1994; Bassan *et al*., 2000; Bauer *et al*., 2002).

A model of maternal protein restriction throughout pregnancy has also been shown to produce a significant deficit in nephron number in the offspring in early postnatal and adult life (Vehaskari *et al*., 2001), a decrease in renal function (GFR/Kidney weight) (Woods *et al*., 2001a) and an increase in plasma sodium concentration, which has been suggested to be a result of primary sodium retaining state as a consequence of a shift in the pressure natriuresis curve to the right (Manning and Veshakari, 2001). Interestingly, a low protein diet throughout pregnancy programs an increased expression of the Glucocorticoid Receptor (GR) and corticosteroid responsive Na+-K+-ATPase 1- and 1-subunits in the kidney, liver and lung in the offspring (Bertram *et al*., 2001). There is also evidence that there is upregulation of two sodium transporters, the thick ascending limb bumetanide-sensitive Na+-K+-2Cl- contransporter (BNSC 1) and the distal convoluted tubule thiazide-sensitive Na+-Cl- contransporter (TSC) before and during the early phase of the development of hypertension in this model (Manning *et al*., 2002). The prenatal programming of the Na+-K+- ATPase expression in these of gene expression in these offspring may therefore contribute to sodium retention and mediate a glucocorticoid-dependent programming of hypertension. In the rat, prenatal dexamethasone for 2-days periods in late gestation results in a reduction in glomerular number, glomerulosclerosis and hypertension (Ortiz *et al*., 2001, 2003). Adult rats given dexamethasone on days 15 and 16 had more glomeruli with glomerulosclerosis than control rats. Male rats that received prenatal dexamethasone on days 13 and 14, 15 and 16 and 17 and 18 of gestation had elevated blood pressures at 6 mo of age, but the group treated on days 13 and 14 of gestation did not have a reduction in glomerular number, suggesting that a reduction in glomerular number is not the sole cause for the development of hypertension (Mcmillen and Jeffery, 2005).

In sheep, the permanent metanephric kidney completes nephrongesis by 130 days of gestation (Moritz and Wintour, 1999), which is well before birth (term dexamethasome (0.48 mg h^{-1}, for 48 h at 26-28 days of gestation), also have a significantly lower nephron number and an increase glomerular volume in adult life (Wintour *et al*., 2003). While intrauterine growth restriction may be associated with a decrease in nephron number and an increase glomerular volume in adult life (Wintour *et al*., 2003). While intrauterine growth restriction may be associated with a decrease in nephron number, it is clear that loss of a kidney in either early or late adult life is not necessarily associated with an effect on renal functional reserve or blood pressure (Chevalier, 1982; Makipernaa *et al*., 1991; Fehrman-Ekohlm *et al*., 2001). In contrast, a reduction of nephron number occurring during the period of active neprogenesis is associated with changes in postnatal renal function and elevations in arterial blood pressure (Mcmillen and Jeffery, 2005). Unilateral renal agenesis in humans results in a variable reduction in GFR in the adult, which, is correlated with elevations in arterial blood pressure (De Santo *et al*., 1997). Similarly in rats, removal of the kidney in the first day of life result in hypertension in the adult (Woods *et al*., 2001b) and unilateral nephrectomy at the 100th day of gestation in the sheep, in which nephrogenesis is complte by the 130th days results in compensatory nephrogenesis in utero and a reduced GFR and an elevated blood pressure in female offspring by 6 month of age (Douglas-Denton *et al*., 2002; Moritz *et al*., 2002c). It has therefore suggested that some aspects of compensatory growth and/or nephrogenesis that occur during a critical window in development are essentially maladaptive leading the subsequent development of hypertension (Dodic *et al*., 2002a, b). The response of the intrarenal renin-angiotesin system (RAS) has been implicated in this aspect of programming of postnatal hypertension (Mcmillen and Jeffery, 2005).

CONCLUSIONS

This review documents the role of intrauterine programming in postnatal secondary hypertension via structural and functional changes in genes, cells, tissues and organs with consequent discrete or cumulative effects on development depending on the nature and timing of the programming stimulus.

The role of intrauterine programming in postnatal secondary hypertension has been linked to the low-birth weight- induced impairment of endothelial NO synthase and eNOs activity, increase pressor response to angiotensin II, increase apoptosis in fetal heart, increase sympathoadrenergic activity and permanent alteration of the kidneys.

REFERENCES

Adams, M.B., I.D. Phillips, G. Simonetta and I.C. McMillen, 1998. Differential effects of increasing gestational age and placential restriction on tyrosine hydroxylase, phenylethanolamine N-methyltransferase and proenkephalin A mRNA levels in the fetal sheep adrenal. J. Neurochem., 71: 394-401.

Armitage, J.A., I.Y. Khan, P.D. Taylor, P.W. Nathaniels and L. Poston, 2004. Developmental programming of the metabolic syndrome by maternal nutritional imbalance: How strong is the evidence from experimental models in mammals. J. Physiol., 561: 355-377.

Asano, H., V.K. Han, J. Homa and B.S. Richardson, 1997. Tissue DNA synthesis in the pre-term ovine fetus following 8 h of sustained hypoxemia. J. Soc. Gynecol. Investing, 4: 236-240.

Bae, S., Y. Xiao, G. Li, C.A. Casiano and L. Zhang, 2003. Effect of maternal chronic hypoxic exposure during gestation on apoptosis in fetal heart. Am. J. Physiol. Heart Circ. Physiol., 285: H983-H990.

Barbera, A., G.D. Giraud, M.D. Reller, J. Maylie, M.J. Morton and K.L. Thornburg, 2000. Right ventricular systolic pressure load alters myocyte maturation in fetal sheep. Am. J. Physiol. Regul. Inter. Comp. Physiol., 279: R1157-R1164.

Barker, D.J., C. Osmond, J. Golding, D. Kuh and M.E. Wadsworth, 1989a. Growth in utero, blood pressure in childhood and adult life and mortality from cardiovascular disease. Bone Miner J., 298: 564-567.

Barker, D.J., C. Osmond, P.D. Winter, B.M. Margetts and S.J. Simmonds, 1989b. Weight in infancy and death from ischaemic heart disease. Lancet, 2: 577-580.

Barker, D.J.P., 1994. Mothers, Babies and Disease in Later Life. BMJ Publishing Group, London, pp: 180.

Bassan, H., L.L. Trejo, N. Kariv, M. Bassan and E. Berger *et al.*, 2000. Experimental intrauterine growth retardation alters renal development. Pediatr. Nephrol., 15: 192-195.

Bateson, P., D. Barker, T. Clutton-Brock, D. Debal and B. D'Udine *et al.*, 2004. Developmental plasticity and human health. Nature, 430: 419-421.

Bauer, R., B. Walter, K. Bauer, R. Klupsch, S. Patt and U. Zwiener, 2002. Intrautrine growth restriction reduces nephron number and renal excretory function in newborn piglets. Actal. Physiol. Scand., 176: 83-90.

Benediktsson, R., R.S. Lindsay, J. Noble, J.R. Seckl and C.R. Edwards, 1993. Glucocorticoid exposure in utero: New model for adult hypertension. Lancet, 341: 339-341.

Bertram, C., A.R. Trowern, N. Copin, A.A. Jackson and C.B. Whorwood, 2001. The maternal diet during pregnancy programs altered expression of the glucocorticoid receptor and type 211-hydroxysteroid dehydrogenase: Potential molecular mechanisms underlying the programming of hypertension in utero. Endocrinology, 142: 2841-2853.

Bertram, C.E. and M.A. Hanson, 2001. Annual models and programming of metabolic syndrome. Br. Med. Bull., 60: 103-121.

Brawley, L., L. Poston and M.A. Hanson, 2003a. Mechanisms underlying the programming of small artery dysfunction: Review of the model using low protein diet in pregnancy in the rat. Arch. Physiol. Biochem., 111: 23-25.

Brawley, L., S. Itoh, C. Torrens, A. Barker, C. Bertram, L. Poston and M. Hanson, 2003b. Dietary protein restriction in pregnancy induces hypertension and vascular defects in rat male offspring. Pediar. Res., 54: 83-90.

Brawley, L., C. Torrens, F.W. Anthony, S. Itoh and T. Wheeler *et al.*, 2004. Glycine rectifies vascular dysfunction induced by dietary protein imbalance during pregnancy. J. Physiol., 554: 497-504.

Brenner, B.M. and G.M. Chertow, 1994. Congenital oligonephropathy and the etiology of adult hypertension and progressive renal injury. Am. J. Kidney Dis., 23: 171-176.

Brenner, B.M. and H.S. Mackenzie, 1997. Nephron mass as a risk factor for progression of renal disease. Kidney Int. Suppl., 63: S124-S127.

Broberg, C.S., G.D. Garcia, J.M. Schultz, K.L. Thornburg, A.R. Hohimer and L.E. Davis, 2003. Fetal anemia leads to augmented contractile response to hypoxic stress in adulthood. Am. J. Physiol. Regul. Integr. Comp. Physiol., 285: R649-R655.

Buchanan, T.A. and S.L. Kjos, 1999. Gestational diabetes: Risk or myth. J. Clin. Endocrinol. Metab., 84: 1854-1857.

Burke, V., L.J. Beilin, K.V. Blake, D. Doherty and G.E. Kendeall *et al.*, 2004. Indicators of fetal growth do not independently predict blood pressure in 8-year old Australians: A prospective cohort study. Hypertension, 43: 208-213.

Burrell, J.H., A.M. Boyn, V. Kumarasamy, A. Hsieh, S.I. Head and E.R. Lumbers, 2003. Growth and maturation of cardiac myocytes in fetal sheep in the second half of gestation. Anat. Rec. A Discov. Mol. Cell Evol. Biol., 274: 952-961.

Chang, J.H.T., J.C. Rutledge, D. Stoops and R. Abbe, 1984. Hypobaric hypoxia-induced intrauterine growth retardation. Biol. Neonate, 46: 10-14.

Chevalier, R.L., 1982. Functional adaptation to reduced renal mass in early development. Am. J. Physiol. Renal. Physiol., 242: F190-F196.

Coles, H.S.R., J.F. Burne and M.C. Raff, 1993. Large-scale normal cell death in the developing rat kidney and its reduction by epidermal growth factor. Development, 118: 777-784.

De Grauw, T.J., R. Meyers and W.J. Scott, 1986. Fetal growth retardation in rats from different levels of hypoxia. Biol. Neonate, 49: 85-89.

De Santo, N.G., P. Anastasio, L. Spitali, D. Santoro, D. Capodicasa, E. Cirillo and G. Cappasso, 1997. Renal reserve is normal in adults born with unilateral renal agenesis and is not related to hyperfiltration or renal failure. Miner Electrolyte Metab., 23: 283-286.

Dodic, M., E.M. Wintour and J.P. Coghlam, 1998. An early prenatal exposure to excess glucocorticoid leads to hypertensive offspring in sheep. Clin. Sci., 94: 149-155.

Dodic, M., A. Peers, J.P. Coghlan, C.N. May, E. Lumbers, Z. Yu and E.M. Wintour, 1999. Altered cardiovascular haemodynamics and baroreceptor-heart rate reflex in adult sheep after prenatal exposure to dedamethasone. Clin. Sci., 97: 103-109.

Dodic, M., K. Moritz, I. Koukoulas and E.M. Wintour, 2002a. Programmed hypertension: Kidney, brain or both. Trends Endocrinol. Metab., 13: 403-408.

Dodic, M., V. Hantzis, J. Duncan, S. Rees and I. Koukoulas *et al.*, 2002b. Programming effects of short prenatal exposure to cortisol. FASEB J., 16: 1017-1026.

Douglas-Denton, R., K.M. Moritz, J.F. Bertram and E.M. Wintour, 2002. Compensatory renal growth after unilateral nephrectomy in the ovine fetus. J. Am. Soc. Nephrol., 13: 406-410.

Economides, D.L., K.H. Nicolades, E.A. Linton, L.A. Perry and T. Chard, 1988. Plasma cortisol and adrenocorticotropin in appropriate and small for gestational age fetuse. Fetal. Ther., 3: 158-164.

Edwards, L.J. and I.C. McMillen, 2001. Maternal undernutrition increases arterial blood pressure in the sheep fetus during late gestation. J. Physiol., 533: 561-570.

Edwards, L.J. and I.C. McMillen, 2002. Periconceptional nutrition programs development of the cardiovascular system in the fetal sheep. Am. J. Physiol. Regul. Integr. Comp. Physiol., 283: R669-R679.

Eriksson, J.G., T. Forsen, J. Tuomilehto, C. Osomond and D.J.P. Barker, 2001. Early growth and coronary hearth disease in later life: Longitudinal study. Bone Miner J., 322: 949-953.

Eriksson, J.G. and T.J. Forsen, 2002. Childhood growth and coronary heart disease in later life. Ann. Med., 34: 157-161.

Eriksson, J.G., T. Forsen, J. Tuomilehto, P.D. Winter, C. Osmond and D.J. Barker, 2002a. Catch-up growth in childhood and death from coronary heart disease in later life. Ann. Med., 34: 157-161.

Eriksson, J.G., T. Forsen, J. Tuomilehto, V.W. Jaddoe, C. Osmond and D.J.P. Barker, 2002b. Effect of size at birth and childhood groeth on the insulin resistance syndrome in elderly individuals. Diabetologia, 45: 342-348.

Fehrman-Ekohlm, I., F. Duner, B. Brink, G. Tyden and C.G. Elinter, 2001. No evidence of accelerated loss of kidney function in living kidney donors: Results from a cross-sectional follow-up. Transplantation, 72: 444-449.

Forhead, A.J., P.F. Broughton and A.L. Fowden, 2000. Effect of cortisol on blood pressure and the renin-angiotensin system in fetal sheep during late gestation. J. Physiol., 526: 167-176.

Forsen, T., J.G. Eriksson, J. Tuomilehto, K. Teramo and D.J.P. Barker, 1997. Mothers weight in pregnancy and coronary heart disease in a cohort of finish men: Fellow up study. Bone Miner J., 315: 837-840.

Forsen, T., J.G. Eriksson, J. Tuomilehto, C. Osmond and D.J.G. Barker, 1999. Growth in utero and during childhood among women who develop coronary heart disease: Longitudinal study. Bone Miner J., 319: 1403-1407.

Fowden, A.L. and A.J. Forhead, 2004. Encocrine mechanisms of intrauterine programming. Reproduction, 127: 515-526.

Fowden, A.L. and A.J. Forhead, 2006. Nutritional basis of the fetal origins of adult disease. Reproduction, 120: 510-516.

Fowden, A.L., 1995. Endocrine regulation of fetal growth. Reprod. Fertil. Dev., 7: 351-363.

Fowden, A.L., J. Li and A.J. Forhead, 1998. Glucocorticoids and the preparation for life after birth: Are there long-term consequences of the life insurance. Proc. Nutr. Soc., 57: 113-122.

Fowden, A.L., 2003. The insulin-like growth factors and fetoplacental growth. Placenta, 24: 803-812.

Fowden, A.L., D.A. Giussani and A.J. Forhead, 2005. Endocrine and metabolic programming during intrauterine development. Early Hum. Dev., 81: 723-734.

Franco, M.C., R.M.P. Arruda, A.P. Dantas, E.M. Kawamoto and Z.B. Fortes *et al.*, 2002. Intrauterine undernutrition: Expression and activity of the endothelial nitric oxide synthase in male and female adult offspring. Cardiovasc. Res., 56: 145-153.

Frankel, S., P. Elwood, P. Sweetnam, J. Yarnell and G.D. Smith, 1996a. Birthweight, adult risk factors and incident coronary heart disease the caerphilly study. Public Health, 110: 139-143.

Frankel, S., P. Elwood, P. Sweetnam, J. Yarnell and G.D. Smith, 1996b. Birthweight, body-mass index in middle age and incident coronary heart disease. Lancet, 348: 1478-1480.

Gale, N.W. and G.D. Yancopoulos, 1999. Growth factors acting via endothelial cell-specific receptor tyrosine kinases: VEGFs, angiopoietins and ephrins in vascular development. Genes Dev., 13: 1055-1066.

Ghosh, P., D. Bitsanis, K. Ghebremeskel, M.A. Crawford, L. Poston, 2001. Abnormal aortic fatty acid composition and small artery function in offspring of rats fed a high-fat diet in pregnancy. J. Physiol., 533: 815-822.

Giussani, D.A., J.A.D. Spencer and M.A. Hanson, 2005. Fetal Cardiovascular Reflex Responses to Hypoxaemia: Fetal Matric Evolution, Development and Disease. Cambridge University Press, Cambridge, UK.

Gluckman, P. and M. Hanson, 2005. The Fetal Matrix: Evolution, Development and Disease. 1st Edn., Cambridge University Press, Cambridge, ISBN-13: 9780521542357, pp: 272.

Godfrey, K.M., 2002. The role of the placenta in fetal programming-a review. Placenta, 23: S20-S27.

Goh, K.L., A.C. Shore, M. Quinn and J.E. Tooke, 2001. Impaired microvascular vasodilatory function in 3-month-old infants of low birth weight. Diabetes Care, 24: 1102-1107.

Gomez, R.A., 1990. Molecular biology of components of the rennin-angiotensin system during development. Pediatr. Nephrol., 4: 421-423.

Goodfellow, J., M.F. Bellamy, S.T. Gorman, M. Brownlee and M.W. Ramsey *et al.*, 1998. Endothelial function is impaired in fit young adults of low birth weight. Cardiovasc. Res., 40: 600-606.

Hanson, M.A., 1997. Do we now understand the control of the fetal circulation?. Eur. J. Obstet. Gynecol. Reprod. Biol., 75: 55-61.

Harding, J.E. and B. Johnson, 1995. Nutrition and fetal growth. Reprod. Fertil. Dev., 7: 539-547.

Hattersley, A.T. and J.E. Tooke, 1999. The fetal insulin hypothesis: An alternative explanation of the association of low birthweight with and vascular disease. Lancet, 353: 1789-1792.

Heydeck, D., J. Roigas, C. Roigas, B. Papies and A. Lun, 1994. The catecholamine sensitivity of adult rats is enhanced after prenatal hypoxia. Biol. Neonate, 66: 106-111.

Hinchliffe, S.A., M.R. Lynch, P.H. Sergent, C.V. Howard and D. van Velzen, 1992. The effect of intrauterine growth retardation on the development of renal nephrons. Br. J. Obstet. Gynaecol., 99: 296-301.

Holemans, K., R. Gerber, K. Meurrens, F. De Clerck, L. Poston and F.A. van Assche, 1999. Maternal food restriction in the second half of pregnancy affects vascular function but not blood pressure of rat female offspring. Br. J. Nutr., 81: 73-79.

Holemans, K., L. Aerts and F.A. van Assche, 2003. Lifetime consequence of abnormal fetal pancreatic development. J. Physiol., 547: 11-20.

Hughson, M., A.B. Farris, R. Dounglas-Denton, W.E. Hoy and J.F. Bertram, 2003. Glomerular number and size in autopsy kidneys: The relationship to birth weight. Kidney Int., 63: 2113-2122.

Hulman, S., H. Kushner, S. Katz and B. Falkner, 1998. Can cardiovascular risk be predicted by newborn, childhood and adolescent body size: An examination of longitudinal data in urban African Americans. J. Pediatr., 132: 90-97.

Jackson, A.A., R.L. Dunn, M.C. Marchand and S.C. Langley-Evans, 2002. Increased systolic blood pressure in rats induced by maternal low-protein diet is reversed by dietary supplementation with glycine. Clin. Sci., 103: 633-639.

Jones, C.T., M.M. Roebuck, D.W. Walker and B.M. Johnston, 1998. The role of the adrenal medulla and peripheral symphatic nerves in the physiological responses of the fetal sheep to hypoxia. J. Dev. Physiol., 10: 17-36.

Joseph, K.S. and M.S. Kramer, 1996. Review of the evidence on fetal and early childhood antecedents of adults chronic disease. Epidemiol. Rev., 18: 158-174.

Khan, I.Y., P.D. Taylor, V. Dekou, P.T. Seed and L. Lakasin *et al.*, 2003. Gender-linked hypertension in offspring of lard-fed prenant rats. Hypertension, 41: 168-175.

Khan, I., V. Dekou, M. Hanson, L. Poston and P. Taylor, 2004. Predictive adaptive responses to maternal high-fat diet prevent endothelial dysfunction but not hypertension in adult rat offspring. Circulation, 110: 1097-1102.

Kind, K.L., J.A. Owens, J.S. Robinson, K.J. Quinn and P.A. Grant *et al.*, 1995. Effect of restriction of placental growth on expression of IGFs in fetal sheep: Relationship to fetal growth, circulating IGFs and binding proteins. J. Endocrinol., 146: 23-34.

Konje, J.C., S.C. Bell, J.J. Morton, R. de Chanzal and D. Taylor, 1996. Human fetal kidney morphometry during gestation and the relationship between weight, kidney morphometry and plasma active rennin concentration at birth. Clin. Sci., 91: 169-175.

Koseki, C., D. Herzlinger and Q. Al-Awqati, 1992. Apoptosis in metanephric development. J. Cell Biol., 119: 1327-1333.

Koumentaki, A., F. Anthony, L. Poston and T. Wheeler, 2002. Low-protein diet impairs vascular relaxation in virgin and pregnant rats. Clin. Sci., 102: 553-560.

Kramer, M.S., 1998. A comparison of laboratory and epidemiologic approaches to pediatric research. Pediatr. Res., 43: 711-718.

Kumarasamy, V., M.D. Mitchell, F.H. Bloomfield, M.T. Oliver, M.E. Campbell and J.R.G. Challis, 2005. Effects of periconceptual undernutrition on the initiation of parturition in sheep. Am. J. Physiol. Regul. Integr. Comp. Physiol., 288: R67-R72.

Lamireau, D., A.M. Nuyt, X. Hou, S. Bernier and M. Beauchamp *et al.*, 2002. Altered vascular function in fetal programming of hypertension. Stroke, 33: 2992-2998.

Langdown, M.L., M.J. Holness and M.C. Sugden, 2001a. Early growth retardation induced by excessive exposure of glucocorticoid in utero selectively increases cardia GLUT 1 protein expression and Akt/protein kinase B activity in adulthood. J. Endocrinol., 169: 11-22.

Langdown, M.L., N.D. Smith, M.C. Sugden and M.J. Holness, 2001b. Excessive glucocorticoid exposure during late intrauterine development modules the expression of cardiac uncoupling proteins in adult hypertensive male offspring. Pflugers Arch., 442: 248-255.

Langley-Evans, S.C. and A.A. Jackson, 1995. Captopril normalizes systolic blood pressure in rats eith hypertension–induced by fetal exposure to maternal low-protein diets. Comp. Biochem. Physiol. A. Physiol., 10: 223-228.

Langley-Evans, S.C., G.J. Philips, R. Benediksson, D.S. Gardner, C.R.W. Edwards, A.A. Jackson and J.R. Seckl, 1996. Protein intake in pregnancy, placental, glucocorticoid metabolism and the programming of hypertension in the rat. Placenta, 17: 169-172.

Langley-Evans, S.C., S.J. Welham and A.A. Jackson, 1999. Fetal exposure to a maternal low protein diet impairs nephrongensis and promotes hypertension in the rat. Life Sci., 64: 965-974.

Langley-Evans, S.C., 2001. Fetal programming of cardiovascular function through exposure to maternal undernutrition. Proc. Nutr. Soc., 60: 505-513.

Lee, P.A., S.D. Chernausek, A.C. Hokken-Koelega and P. Czernichow, 2003. International small for gestation age advisory board consensus development conference statement: Management of short children born small for gestational age. Pediatrics, 111: 1253-1261.

Leeson, C.P.M., M. Kattenhorn, R. Morley, A. Lucas and J.E. Deanfield, 2001. Impact of low birth weight and cardiovascular rsik factors on endothelial function inearly adult life. Circulation, 103: 1264-1268.

Li, F., X. Wang, J.M. Capaso and A.M. Gerdes, 1996. Rapid transition of cariac myocytes from hyperthesia to hypertrophy during postnatal development. J. Mol. Cell Cardiol., 28: 1737-1746.

Li, G., Y. Xiano, J.L. Estrella, C.A. Ducsay, R.D. Gilbert and L. Zhang, 1999. Effect of fetal hypoxia on heart susceptibility to ischemcia and reperfusion injuryin the adult rat. J. Soc. Gynecol. Investing, 10: 265-274.

Lucas, A., M.S. Fewtrell and T.J. Cole, 1999. Fetal origins of adult disease-the hypothesis revisited. Bone Miner J., 319: 245-249.

Mackenzie, H.S. and B.M. Brenner, 1995. Fewer nephrons at birth: A missing link in the etiologic of essential hypertension. Am. J. Kidney Dis., 26: 91-98.

Mackenzie, H.S., E.V. Lawler and B.M. Brenner, 1996. Congenital oligonephropathy: The fetal flaw in esseinal hypertension. Kidney Int. Suppl., 55: S30-S34.

Makipernaa, A., O. Koskimies, J. Jasskelainen, A.M. Teppo and M.A. Siimes, 1991. Renal growth and function 11-28 years after treatment of Wilms tumour. Eur. J. Pedatr., 150: 444-447.

Manalich, R., L. Reyes, M. Herrera, C. Melendi and I. Fundora, 2000. Relationship between weight at birth and the number and size of renal glomeruli in humans: A histomorphometric study. Kidney Int., 58: 770-773.

Manning, J. and V.M. Vehaskari, 2001. Low birth weight-associated adult hypertension in the pat. Pedatr. Nephrol., 16: 417-422.

Manning, J., K. Beutler, M.A. Kneper and V.M. Vehaskari, 2002. Upregulation of renal BSC1 and TSC in prenatally programmed hypertension. Am. J. Physiol. Renal. Physiol., 283: F202-F206.

Martin, C., A.Y. Yu, B.H. Jiang, L. Davis, D. Kimberly, A.R. Hohimer and G.L. Semenza, 1998. Cardiac hypertrophy in chronically anemic fetal sheep: Increased vascularization is associated with increased myocardial expression of vascular endothelial growth factor and hypoxia-inducible factor 1. Am. J. Obstet. Gynecol., 178: 527-534.

Martin, H., J. Hu, G. Gennser and M. Norman, 2000. Impaired endothelial function and increased carotid stiffness in 9-year-old children with low birthweight. Circulation, 102: 2739-2744.

Martyn, C.N., D.J.P. Barker and C. Osmond, 1996. Mothers pelvic size, fetal growth and death from stroke and coronary heart disease in men in the UK. Lancet, 348: 1264-1268.

Matthews, S.G., 2000. Antenatal glucocorticoids and programming of the developing CNS. Pediatr. Res., 47: 291-300.

McEvoy, T.G., J.J. Robinson and K.D. Sinclair, 2001. Developmental consequwnces of embryo and cell manipulation in mice and farm animnals. Reproducation, 122: 507-518.

McMillen, I.C. and J.S. Robinson, 2005. Developmental orignins of the metabolic syndrome: Prediction, plasticity and programming. Physiol. Rev., 85: 571-633.

McMillen, I.C., M.B. Adams, J.T. Ross, C.L. Coulter and G. Simonetta *et al.*, 2001. Fetal growth restriction: Adaptations and consequences. Reproduction, 122: 195-204.

McMillen, I.C. and S.R. Jeffery, 2005. Developmental origin of metabolic syndrome: Prediction, plasticity and programming. Physiol. Rev., 85: 571-633.

Merlet-Benichou, C., T. Gilbert, M. Muffat-Joly, M. Lelievre-Pegoreir and B. Leroy, 1994. Intrauterine growth retardation leads to permanent nephron deficit in the rat. Pediatr. Nephrol., 8: 175-180.

Molnar, J., M.J.M. Nijland, D.C. Howe and P.W. Nathanielsz, 2002. Evidence for microvascular dysfunction after prenatal dexamethasone at 0.7, 0.75 and 0.8 gestation in sheep. Am. J. Physiol. Regul. Integr. Comp. Physiol., 283: R561-R567.

Molnar, J., D.C. Howe, M.J.M. Nijland and P.W. Nathanielsz, 2003. Prenatal dexamethasone leads to both endothelial dysfunction and vasodilatory compensation in sheep. J. Physiol., 547: 61-66.

Moritz, K.M. and E.M. Wintour, 1999. Functional development of the meso and metanephros. Pediatr. Nephrol., 13: 171-178.

Moritz, K., A. Butkus, V. Hantzis, A. Peers, E.M. Wintour and M. Dodic, 2002a. Prolonged low-dose dexamethasone, in early gestation, has no long-term deleterious effect on normal ovine fetuses. Endocrinology, 143: 1159-1165.

Moritz, K.M., E.M. Wintour and M. Dodic, 2002b. Fetal uninemphrectomy leads to postnatal hypertension and compromised renal function. Hypertension, 39: 1071-1076.

Moritz, K.M., K. Johnson, R. Douglas-Denton, E.M. Wintour and M. Dodic, 2002c. Materanl glucocorticoid treatment programs alterations in the rennin-angiostenin system of the ovine fetal kidney. Endocrinology, 143: 4455-4463.

Moritz, K.M., M. Dodic and E.M. Wintour, 2003. Kidney development and the fetal programming of adult disease. Bioessays, 25: 212-220.

Moss, T.J.M., D.M. Sloboda, L.C. Gurrin, R. Harding, J.R.G. Challis and J.P. Newnham, 2001. Programming effects in sheep of prenatal growth restriction and glucocortidoids exposure. Am. J. Physiol. Regul. Integr. Comp. Physiol., 281: R960-R970.

Nishina, H., L.R. Green, H.H. McGarrigle, D.E. Noakes, L. Poston and M.A. Hanson, 2003. Effect of nutritional restriction in early pregnancy on isolated femoral artery function in mid-gestation fetal sheep. J. Physiol., 553: 637-647.

Oparil, S., S.P. Bishop and F.J.Jr. Clubb, 1984. Myocardial cell hypertrophy or hyperplasia. Hypertension, 6: III-38-III-43.

Ortiz, L.A., A. Quan, A. Weinberg and M. Baum, 2001. Effect of prenatal dexamethasone on rat renal development. Kidney Int., 59: 1663-1669.

Ortiz, L.A., A. Quan, F. Zarzar, A. Weinberg and M. Baum, 2003. Effect of prenatal dexamethasone programs hypertension and renal injury in the rat. Hypertension, 41: 328-334.

Osmond, C., D.J.P. Barker, P.D. Winter, C.H.D. Fall and S.J. Simmonds, 1993. Early growth and death from cardiovascular disease in women. Bone Miner J., 307: 1519-1524.

Ozaki, T., H. Nishina, M.A. Hanson and L. Poston, 2001a. Dietary restriction in pregnant rats causes gender-related hypertension and vascular dysfunction in offspring. J. Physiol., 530: 141-152.

Ozaki, T., P. Hawkins, H. Nishina, C. Steyn, L. Poston and M.A. Hanson, 2001b. Effects of undernutrition in early pregnancy on systemic small artery function in late-gestation fetal sheep. Am. J. Obstet. Gynecol., 183: 1301-1307.

Ozane, S.E. and C.N. Hales, 2002. Early programming of glucose-insulin metabolism. Trends Endocrinol. Metab., 13: 368-373.

Peers, A., D.J. Campbell, E.M. Wintour and M. Dodic, 2001. The peripheral reni-angiotensin system is not involved in the hypertension of sheep exposed to dexamethasone. Clin. Exp. Pharmacol. Physiol., 28: 306-311.

Persson, E. and T. Jansson, 1992. Low birth weight is associated with elevated adult blood pressure in the chronically catheterized guinea-pig. Acta Phsysiol. Scand., 145: 195-196.

Pham, T.D., N.K. MacLennan, C.T. Chiu, G.S. Laksana, J.L. Hsu and R.H. Lane, 2003. Uteroplacental insufficiency increases apoptosis and alters p53 gene methylation in the full term IUGR rat kidney. Am. J. Physiol. Regul. Inter. Comp. Physiol., 285: R962-R970.

Phillips, D.I., 1993. Twin studies in medical research: Can they tell us whether diseases are genetically determined. Lancet, 341: 1008-1009.

Poore, K.R. and A.L. Fowden, 2003. The effect of birth weight on hypothalamo-pituitary-adrenal axis function in juvenile and adult pigs. J. Physiol., 547: 107-166.

Rasmussen, K.M., 2001. The fetal origins hypothesis: Challenges and opportunities of maternal and child nutrition. Ann. Rev. Nutr., 21: 73-95.

Rees, W.D., S.M. Hay, D.S. Browth, C. Antipatic and R.M. Palmer, 2000. Maternal protein deficiency causes hypermethylation of DNA in livers of rat fetuses. J. Nutr., 130: 1821-1826.

Reik, W., M. Constancia, A. Fowden, N. Anderson and W. Dean *et al.*, 2003. Regulation of supply and demand for maternal nutrient in mammals by imprinted genes. J. Physiol., 547: 35-44.

Reiss, K., W. Cheng, A. Ferber, J. Kajstura and P. Li *et al.*, 1996. Over expression of insuling-like growth factor-1 in the heart is coupled with myocyte proferation in transgenic mice. Proc. Natl. Acad. Sci., 93: 8630-8635.

Rhind, S.M., M.J. Rae and A.N. Brooks, 2001. Effects of nutrition and environmental factors on the fetal programming of the reproductive axis. Reproduction, 122: 205-214.

Rich-Edwards, J., M.J. Stampfer, J.E. Manson, B. Rosner and S.E. Hankinson *et al.*, 1997. Birth weight and risk of cardiovascular disease in cohort of women followed up since 1976. Bone Miner J., 315: 396-400.

Roberts, C.L. and P.A. Lancaster, 1999. Australian national birthweigh percentiles by gestational age. Med. J. Aust., 170: 114-118.

Ross, M.G. and M. Desai, 2005. Gestational programming: population survival effects of drought and famine during pregnancy. Am. J. Physiol. Regul. Integr. Comp. Physiol., 288: 25-33.

Ruijtenbeek, K., C.G. Kessels, B.J. Janssen, N.J. Bitsch and G.E. Fazzi *et al.*, 2003a. Chronic moderate hypoxia during in ovo development alters arterial reactivity in chickens. Pflugers Arch., 447: 158-167.

Ruijtenbeek, K., L.C. Kessels, J.G.R. De Mey and C.E. Blanco, 2003b. Chronic Moderate hypoxia and protein malnutrition both induce growth retardation, but have distinct effects on arterial endothelium-dependent reactivity in the chicken embryo. Pediatr. Res., 53: 573-579.

Sato, A., H. Suzuki, Y. Nakazato, H. Shibata, T. Inagami and T. Saruta, 1994b. Increased expression of vascular angiotensin II type 1A receptor gene in glucocorticoid-induced hypertension. J. Hypertens., 12: 511-516.

Seckl, J.R., 2004. Prenatal glucocorticoids and long-term programming. Eur. J. Endocrinol., 151: U49-U62.

Segar, J.L., K. Bedell, W.V. Page, J.E. Mazursky, A.M. Nuyt and J.E. Robillard, 1995. Effect of cortisol on gene expression of the rennin-angiotensin system in fetal sheep. Pediatr. Res., 37: 741-746.

Sherman, R.C. and S.C. Langley-Evans, 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., 94: 373-381.

Sherman, R.C. and S.C. Langley-Evans, 2000. Antihypertensive treatment in early postnatal life modulates prenatal dietary influences upon blood pressure in the rat. Clin. Sci., 98: 269-275.

Simonetta, G., A.K. Rouke, J.A. Owens, J.S. Robinson and I.C. McMillen, 1997. Impact of placental restriction on the development of the sympathoadrenal system. Pediatr. Res., 42: 805-811.

Smolich, J.J., A.M. Walker, G.R. Campbell and T.M. Adamson, 1989. Left and right ventricular myocardial morphometry in fetal, neonatal and adult sheep. Am. J. Physiol. Heart Circ. Physiol., 257: HI-H9.

Smoich, J.J. and M.D. Esler, 1999. Total body catecholamine kinetics before and after birth in spontaneously hypoxemic fetal lambs. Am. J. Physiol. Regul. Integr. Comp. Physiol., 46: R1313-R1320.

Soonpaa, M.H., K.K. Kim, L. Pajak, M. Franklin and L.J. Field, 1996. Cardiomyocyte DNA synthesis and binucleation during murine development. Am. J. Physiol. Heart Circ. Physiol., 271: H2183-H2189.

Steegers-Theunissen, R.P.M. and E.A.P. Steegers, 2003. Nutrient-gene interactions in early pregnancy: A vascular hypothesis. Eur. J. Obstet. Gynecol. Reprod. Biol., 106: 115-117.

Stein, C.E., C.H.D. Fall, K. Kumaran, C. Osmond, V. Cox and D.J.P. Barker, 1996. Fetal growth and coronary heart disease in South India. Lacnet, 348: 1269-1273.

Sundgren, N.C., G.D. Giraud, J.M. Schultz, M.R. Lasarev, P.J.S. Stork and K.L. Thornburg, 2003a. Extracellularsignal-regulated kinase and phosphoinositol-3 kinase mediate IGF-1 induced proliferation of fetalsheep cardiomyocytes. Am. J. Physiol. Regul. Integr. Comp. Physiol., 285: R1481-R1489.

Sundgren, N.C., G.D. Giraud, P.J. Stork, J.G. Maylie and K.L. Thornburg, 2003b. Angiotensin II stimulates hyperplasia but not hypertrophy in immature ovine cardiomyocytes. J. Physiol., 548: 881-891.

Tangalakis, K., E.R. Lumbers, K.M. Moritz, M.K. Towstoless and E.M. Wintour, 1992. Effect of cortisol on blood pressure and vascular reactivity in the ovine fetus. Exp. Physiol., 77: 709-717.

Thomassin, H., M. Flavin, M.L. Espinas and T. Grange, 2001. Glucocorticoid-induced DNA demethylation gene memory during development. EMBO J., 20: 1974-1983.

Thompson, L.P., 2003. Effects of chronic hypoxia on fetal coronary responses. High Altitude Med. Biol., 4: 215-224.

Topper, J.N. and M.A. Gimbrone, 1999. Blood flow and vascular gene expression: Fluid shear stress as a modulator of endothelial phetoype. Mol. Med. Today, 5: 40-46.

Torrens, C., L. Brawley, A.C. Barker, S. Itoh, L. Poston and M.A. Hanson, 2003. Maternal protein restriction in the rat impairs resistance artery but not conduit artery function pregnant offspring. J. Physiol., 547: 77-84.

Vehaskari, V.M., D.H. Aviles and J. Manning, 2001. Prenatal programming of adult hypertension in the rat. Kidney Int., 59: 238-245.

Vickaryous, N. and E. Whitelaw, 2005. The role of the early embryonic environment on epigeotype and phenotype. Reprod. Fertil. Dev., 17: 335-340.

Waterland, R.A. and C. Garza, 1999. Potential mechanisms of metabolic imprinting that lead to chronic disease. Am. J. Clin. Nutr., 69: 179-197.

Wintour, E.M., K. Johnson, I. Koukoulas, K. Moritz, M. Tersteeg and M. Dodic, 2003. Programming the cardiovascular system, kidney and the brain: A review. Placenta, 24: S65-S71.

Woodall, S.M., B.M. Johnson, B.H. Breier and P.D. Gluckman, 1996. Chronic maternal undernutrition in the rat leads to delayed postnatal growth and elevated blood pressure of offspring. Pediatr. Res., 40: 438-443.

Woods, L.L. and R. Rasch, 1998. Perinatal ANG II programs adult blood pressure, glomerular number and renal function in rats. Am. J. Physiol. Regul. Integr. Comp. Physiol., 275: R1593-R1599.

Woods, L.L., D.A. Weeks and R. Rasch, 2001a. Hypertension after neonatal uninephrectomy in rats precedes glomerular damage. Hypertension, 38: 337-342.

Woods, L.L., J.R. Inderfinger, J.R. Nyengaard and R. Rasch, 2001b. Maternal protein restriction suppresses the newborn rennin-angiotensin system and programs adult hypertension in rats. Pediatr. Res., 49: 460-467.

Young, L.E., 2001. Imprinting of genes and the Barker hypothesis. Twin Res., 5: 307-317.