Adverse prenatal environment and kidney development: implications for programming of adult disease

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Abstract

The 'developmental origins of health and disease' hypothesis suggests that many adult-onset diseases can be attributed to altered growth and development during early life. Perturbations during gestation can be detrimental and lead to an increased risk of developing renal, cardiovascular, metabolic, and neurocognitive dysfunction in adulthood. The kidney has emerged as being especially vulnerable to insult at almost any stage of development resulting in a reduction in nephron endowment. In both humans and animal models, a reduction in nephron endowment is strongly associated with an increased risk of hypertension. The focus of this review is twofold: i) to determine the importance of specific periods during development on long-term programming and ii) to examine the effects of maternal perturbations on the developing kidney and how this may program adult-onset disease. Recent evidence has suggested that insults occurring around the time of conception also have the capacity to influence long-term health. Although epigenetic mechanisms are implicated in mediating these outcomes, it is unclear as to how these may impact on kidney development. This presents exciting new challenges and areas for research.

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Introduction: the developmental origins of health and disease

The concept of developmental programming of adult-onset disease first evolved through an extensive series of epidemiological studies linking a low birth weight with disease risk in adulthood (Barker 2007). Early investigations, which have since been confirmed in a multitude of studies worldwide, found strong associations between birth weight and risk of developing cardiovascular (Barker & Osmond 1986) and metabolic diseases (Hales & Ozanne 2003). Indeed, over the past 10–15 years, the list of diseases that can be 'programed' has been extended to include osteoporosis, kidney disease, lung disease, and some mental illnesses (Gluckman *et al.* 2008). It is now appreciated that a low birth weight, used as a surrogate marker of poor nutrition *in utero*, is not essential for programming of adult-onset disease (Moritz *et al.* 2009), but rather that outcomes are graded across the entire birth weight spectrum (Barker *et al.* 2012). Independent of growth *in utero*, early postnatal growth and adult lifestyle factors can modify disease onset and progression (Eriksson *et al.* 1999). This is proposed to occur through alterations in the normal development of organs and homeostatic mechanisms leading to an increased risk of adult disease (Barker *et al.* 2012).

A number of animal models have consequently been established to elucidate specific alterations caused by

maternal perturbations, which include calorie/protein restriction, exposure to glucocorticoids, and placental insufficiency, that induce a suboptimal *in utero* environment and program adult-onset disease (Moritz *et al.* 2008, 2009, O'Sullivan *et al.* 2012, Gallo *et al.* 2013). This has allowed interrogation of underlying mechanisms contributing to the development of disease. Whilst these studies have reported that a suboptimal *in utero* environment can impair the development of many organs including the heart and brain, the kidney has been identified as being particularly vulnerable. This vulnerability may present as a reduction in kidney weight but more importantly also in a reduction in the number of nephrons (Moritz *et al.* 2008). As all nephrons are formed before birth in the human, this congenital nephron deficit is permanent and has been strongly correlated with an increased risk of hypertension (Keller *et al.* 2003) and renal disease in later life (Luyckx *et al.* 2013).

Whilst dietary deficiencies may affect most or all of pregnancy, many perturbations occur for a limited duration during a particular developmental period leading to the concept of 'critical windows' during which development of specific organ systems may be altered. Organogenesis, the period of rapid fetal growth in late gestation, and lactation, have been identified as critical periods of sensitivity. Interestingly, the kidney has been shown to be vulnerable to insult at multiple time

points throughout gestation as well as postnatally, although, as discussed below, the early stages of renal development appear to be most susceptible. An area now receiving considerable attention is the long-term impact of an adverse periconceptional environment. Even in developed countries where women have ready access to contraceptives, it is estimated that 40–45% of pregnancies are unplanned (Naimi *et al.* 2003). The period around the time of conception and early embryonic development represents a time when a woman may unknowingly expose her conceptus to an adverse environment.

The focus of this review is thus twofold: to focus on the importance of different developmental periods as potentially critical determinants of programming and secondly to examine the effects of maternal perturbations on the kidney and how this may program adult-onset disease (particularly hypertension). Whilst the periconceptional period represents a highly sensitive period of development, studies to date have mainly explored nutritional insults and potential renal outcomes have yet to be fully explored. The paucity of studies in this area highlights a strong need for continuing research.

Perturbations resulting in developmental programming: the importance of timing

In the human, poor placental function or maternal under nutrition (including micronutrient deficiencies) are the most likely causes of growth restriction and both are associated with an increased risk of adult disease in offspring including cardiovascular and metabolic diseases (Elmadfa & Meyer 2012, Barker & Thornburg 2013). In the last decade, fetal exposure to excess nutrients, often associated with maternal obesity and/or diabetes, has also been identified as a risk factor for adult-onset disease (Ma *et al.* 2013). This has very important implications for the future health of Western nations where the rates of obesity are increasing dramatically. A major challenge is determining if the development of disease is due to a prenatal exposure occurring at a particular time in gestation. Epidemiological studies of offspring conceived/born during the Dutch winter famine suggested that those born to mothers exposed to the famine during the first trimester of pregnancy had increased rates of obesity, altered lipid profiles, and cardiovascular disease. By contrast, those exposed in mid-gestation were more likely to develop renal dysfunction whilst exposure to the famine during late gestation often caused a low birth weight (Schulz 2010). Recent studies have shown that relatively short periods of fasting, such as for Ramadan, can cause alterations in maternal body composition and placental weight which were dependent upon the stage of pregnancy (Alwasel *et al.* 2011).

Animal models enable researchers to invoke a maternal perturbation at specific times of gestation to determine times of developmental sensitivity. Protein restriction throughout pregnancy in the rat results in offspring that are born small, develop high blood pressure (Woods *et al.* 2001), impaired renal function (Alwasel *et al.* 2012), glucose intolerance (Ozanne *et al.* 1996), and impaired fertility (Zambrano *et al.* 2005), suggesting that sustained nutritional deficits throughout pregnancy can elicit a wide variety of long-term abnormalities (Woods *et al.* 2001, 2005). Short-term infusions of glucocorticoids during early- or mid-gestation culminate in hypertension in both sheep (Dodic *et al.* 2002) and rat offspring respectively (Ortiz *et al.* 2003, Singh *et al.* 2007a). Interestingly, the outcome is highly dependent upon the timing of exposure as the same level of glucocorticoid exposure did not result in hypertension when administered at other stages of development (Dodic *et al.* 1999, Ortiz *et al.* 2003). Models of uteroplacental insufficiency have been developed to explore the long-term effects of reduced placental function in late gestation in the rat, which coincides with the period of rapid fetal growth. This procedure results in fetal growth restriction and male offspring develop elevated blood pressure (Wlodek *et al.* 2005, 2007), impaired glucose tolerance (Siebel *et al.* 2008), and vascular dysfunction (Tare *et al.* 2012). Collectively, these studies suggest that adult-onset disease may follow either an acute or a chronic prenatal insult but outcomes may depend upon the timing of exposure. As discussed below, these differential outcomes may in part relate to the stage of kidney development, suggesting that it is the relative developmental stage of organs that is important rather than the gestational age *per se*.

Renal development is susceptible to prenatal challenges

Although many of the animal studies described above vary in the timing, duration, type, and severity of the insult to the developing fetus, a strong common finding across most of these models has been impairments in renal development (Table 1; Moritz *et al.* 2008). This has led to speculation that impaired renal development may be a common factor contributing to the programming of disease. During development, three distinct urinary excretory organs are formed: the first two, the pronephros and mesonephros are temporary organs that precede the formation of the permanent kidney, the metanephros. The metanephros commences growth around day 30 in the human when the ureteric bud buds off the Wolffian duct and invades the surrounding metanephric mesenchyme. Through branching morphogenesis, the ureteric tree is formed, completing the scaffold for the future nephrons. Molecular signals from

Table 1 Summary of kidney adaptations following maternal insults across various time periods.

Time	Breed	Maternal insult	Renal morphological changes	Kidney gene changes	Kidney function, blood pressure, and metabolic changes	References
Periconceptional/perigestational/oocyte maturation E0–E4.25 E-3.5–E0	Rat Mouse	Low-protein diet Low-protein diet	↑ Female NN (28.1%)		↑ Male SBP ↑ Male SBP ↑ Female SBP from 21 weeks Vasodilation attenuated ↓ Female kidney:BW ratio ↑ SBP (Emb-LPD and LPD) ↓ Female heart size (Emb-LPD and LPD). Male attenuated vascular responses (Emb-LP and LPD) ↑ ACE activity Inverse relationship between fetal kidney weight and maternal weight loss ↑ Fetal BP in twins but not singletons Normal responsiveness to Ang II infusion ↑ Male MAP Methylation changes Males Insulin resistance	Kwong <i>et al.</i> (2000) Watkins <i>et al.</i> (2008b) Watkins <i>et al.</i> (2008a, 2010) MacLaughlin <i>et al.</i> (2010) Edwards & McMillen (2002) Sinclair <i>et al.</i> (2007)
E0–E3.5 (Emb-LPD) or E0-term (LPD)	Mouse	Low-protein diet				
E-6 weeks to E7	Sheep	70% of control diet		↑ <i>IGF1</i> mRNA ↓ <i>IGF1R</i> mRNA ↑ <i>HSD11B2</i> mRNA		
E-60 to E7	Sheep	70% of energy requirements				
E-8 weeks to E6	Sheep	Methyl-deficient diet				
Early–mid-gestation E0–E65	Sheep	Protein restricted	↓ NN ↑ GV		↑ Urinary albumin:creatinine ratio	Lloyd <i>et al.</i> (2012)
E26–E28	Sheep	Dexamethasone (DEX) or cortisol (CORT)	↓ DEX (25%), CORT (40%) ↑ GV	↑ M angiogenic markers ↑ Subunits of ENaC and Na ⁺ –K ⁺ –ATPase ↑ Fetal renal <i>AGTR1</i> (<i>AT1</i>) and <i>AGTR2</i> (<i>AT2</i>) mRNA ↑ Fetal/postnatal <i>AGTR1</i> (<i>AGTR1A</i>) and <i>AGTR2</i> expression ↓ Fetal and ↑ postnatal <i>AGTR1</i> (<i>AGTR1B</i>) expression ↑ <i>BMP4</i> , <i>TGFβ1</i> , gremlin, and <i>BAX</i>	Fetus: altered renal response to angiotensin II. Offspring: ↑ MAP	Dodic <i>et al.</i> (2002) and Moritz <i>et al.</i> (2011)
E14–E15	Rat	Corticosterone	↓ Male (21%), female (19%) ↑ Mean GV and corpuscle volumes ↓ NN ↑ GV and corpuscle volume		↑ MAP	Singh <i>et al.</i> (2007a)
E23–E26 – term is 39 days Late gestation E18-term	Spiny mouse	Dexamethasone			↑ Male HR for 24 h after surgery	Dickinson <i>et al.</i> (2007)
E7–E22 All of gestation and/or postnatal E0-term	Rat Rat	Uteroplacental insufficiency (Res) and reduced litter size (Red) Ethanol Low-protein diet	↓ Red (36%), rest (27%) ↓ Male NN (25%), ↑ male GV	↓ M renin	↑ SBP Red at 9 weeks and Red and Rest at 22 weeks ↑ Water intake/urine output ↑ Male MAP ↓ GFR normalized to kidney weight ↓ Male kidney:BW ratio ↔ Female MAP and GFR ↑ MAP ↑ GFR in LP offspring particularly under high-salt challenge diet	Wlodek <i>et al.</i> (2008) Knee <i>et al.</i> (2004) Woods <i>et al.</i> (2001, 2004, 2005) Hoppe <i>et al.</i> (2007)
E-2 weeks–lactation–postnatal	Rat	Low-protein diet	↓ Male NN (31%) ↓ GV and corpuscle volume			

SBP, systolic blood pressure; MAP, mean arterial pressure; HR, heart rate; GFR, glomerular filtration rate; NN, nephron number; GV, glomerular volume; BW, body weight.

the ureteric tree cause cells of the metanephric mesenchyme to condense and form the complex nephron structure (Moritz *et al.* 2008). This process of nephrogenesis in the permanent metanephric kidney is complete by ~36 weeks in the human and, therefore, although a growth-restricted infant may experience 'catch-up' growth postnatally, the kidney cannot form any more nephrons. It is imperative to note that the timing of renal development differs amongst species with rats and mice not commencing development of the metanephric kidney until mid-gestation and nephrogenesis continuing for some days after birth (Singh *et al.* 2012). This may be taken into consideration when interpreting the findings from animal studies.

Effects on nephron number

A human kidney contains on average ~1 million nephrons, but the range in a normal human population varies considerably (Hughson *et al.* 2006). Nephron number is most accurately determined by counting glomeruli using unbiased stereological methods that at the current time can only be done on kidneys obtained at autopsy. Although this makes it difficult to assess nephron endowment routinely in humans, studies have demonstrated that growth-restricted infants have small kidneys with fewer nephrons (Hinchliffe *et al.* 1992). There is a strong association between birth weight and nephron number with ~300 000 more nephrons for every kilogram increase in birth weight (Hughson *et al.* 2006). This suggests in human babies that any sub-optimal *in utero* environment that slows fetal growth may result in a congenital nephron deficit. Nephron endowment has been explored in the animal models of programming described above and as shown in the table,

results in a nephron deficit of up to 30–40%. Consistent with a reduction in nephron endowment, animal studies have demonstrated alterations in molecular pathways that regulate renal development. Insults such as dexamethasone (Dickinson *et al.* 2007, Singh *et al.* 2007b) or alcohol exposure (Gray *et al.* 2010) alter mRNA expression levels of factors controlling branching morphogenesis, thereby suggesting inhibition of ureteric branching in the developing kidney.

How does a low nephron number contribute to disease?

A low nephron number has been most strongly associated with a risk for increased blood pressure. Brenner *et al.* (1988) proposed some years ago that hypertension was directly related to glomerular number and this was demonstrated in a carefully controlled study where individuals with clinically diagnosed hypertension had approximately half the number of nephrons compared with a control population (Keller *et al.* 2003). The 'Brenner hypothesis' suggests that a congenital nephron deficit results in an inability to maintain normal fluid and electrolyte balance (particularly sodium) resulting in volume expansion, glomerular hyperfiltration, and systemic hypertension (see Fig. 1). Animal models have tested aspects of this hypothesis. Late-gestation ovine fetuses and young lambs demonstrate augmented expression of renal sodium channels following early-gestation maternal glucocorticoid exposure (Moritz *et al.* 2011). Moreover, increased renal protein levels of the bumetanide-sensitive Na–K–2Cl cotransporter (SLC12A1 (BSC1)) and the thiazide-sensitive Na–Cl cotransporter (SLC12A3 (TSC)) have been observed in offspring following maternal low-protein diet throughout pregnancy (Manning *et al.*

Figure 1 A schematic representation depicting a pathway through which a multitude of prenatal perturbations may result in hypertension. The original Brenner hypothesis, suggesting a congenital nephron deficit predisposes to hypertension, has been adapted to include postnatal factors which may modify disease progression including aging and lifestyle factors.

2002). This is speculated to result in increased sodium retention and volume expansion and be a contributing factor to adult-onset hypertension. The renin–angiotensin system (RAS) is also implicated in some of these programming outcomes. Specifically, the RAS is often suppressed during renal development, potentially contributing to the nephron deficit, followed by a compensatory increase in activity in postnatal life (Moritz *et al.* 2010). Given the integral role of the RAS in fluid balance and blood pressure control in the adult, long-term upregulation of this system, combined with the low nephron endowment, provides a highly plausible pathway through which prenatal insults may result in hypertension. The reasons for this high susceptibility of the developing kidney to insult are not known but are likely related to the way the kidney functions *in utero*. Although the fetal kidney is functional and produces urine (Moritz *et al.* 2008), which is essential for amniotic fluid production, fetuses with renal agenesis can grow to term. This is because, during development, much of the fetal fluid and electrolyte balance is controlled by the placenta. It has been suggested that when challenged by nutrient availability, the growing fetus preferentially maintains supply to the brain and heart at the expense of organs such as the kidney (Barker *et al.* 2012).

It should be noted that a nephron deficit in isolation does not always result in disease outcomes, suggesting that other factors may contribute to disease susceptibility. Indeed, many children born with one kidney (unilateral renal agenesis), or have one removed in early life (thereby removing half the nephrons), do not develop overt hypertension (for review, see Lankadeva *et al.* (2014)). Increases in blood pressure can also be dissociated from nephron deficits in some animal models of glucocorticoid exposure (Ortiz *et al.* 2003) and in spontaneously hypertensive rats (Black *et al.* 2004). Whilst the prenatal perturbation increases the risk of dysfunction, disease does not occur in all individuals and outcomes are dependent upon factors such as the sex of the offspring (males in general being more susceptible than females) (Woods *et al.* 2001, 2005), age (elevated blood pressure may only develop with age), and postnatal lifestyle. The inclusion of a secondary insult or 'second hit' to exacerbate an underlying phenotype is commonly used in the field as a way to mimic postnatal lifestyle factors in a human population that may exacerbate underlying predispositions. For example, following uteroplacental insufficiency in rats, female offspring do not develop overt signs of dysfunction, however, with aging or during pregnancy (Gallo *et al.* 2012), renal and metabolic dysfunctions are unmasked. Similarly, offspring from dams exposed to protein restriction, during pregnancy were more vulnerable to secondary renal insult from advanced glycation products (Zimanyi *et al.* 2006).

Critical periods for renal development

A maternal low-protein diet administered throughout pregnancy resulted in offspring with reduced nephron endowment and hypertension. This was also observed when the dietary restriction was imposed during the second (but not first) half of gestation, corresponding to the period of nephrogenesis (Woods *et al.* 2004). Short-term glucocorticoid exposure in different species (sheep, rat, mouse, and spiny mouse) has demonstrated that maternal exposure specifically during early kidney development can consistently reduce nephron number although the relative period of gestation is remarkably different. In each species, glucocorticoid was administered for 2–3 days during a time when the metanephric kidney was in the very early stages of development. In the sheep, this was relatively early in pregnancy (days 26–28 where term is 150 days, Dodic *et al.* (2002)), when compared with the rat (day 14/15 out of a 22-day pregnancy, Singh *et al.* (2007a)), the mouse (days 12–14 of a 19-day gestation, O'Sullivan *et al.* (2013)), and the Spiny mouse (the exposure was mid-gestation: days 21–23 of a 39-day pregnancy, Dickinson *et al.* (2007)). These findings suggest that early impairment of renal development cannot be restored later in gestation, even if the insult is removed. Late renal development is also a period of susceptibility as nephron number is increasing exponentially at this time. Uteroplacental insufficiency induced in late pregnancy or a modest reduction in litter size at birth (which causes maternal stress and altered lactation) can both decrease nephron endowment in the rat (Wlodek *et al.* 2007, 2008). This presumably affects the final few branching events of the ureteric tree. In contrast to an insult in early development, nephron endowment could be restored following a late-gestation perturbation. Cross-fostering offspring from a dam that underwent uteroplacental insufficiency onto a control dam resulted in almost complete restoration of nephron endowment and prevention of hypertension in adult life (Wlodek *et al.* 2007).

Exceptions to the finding of a reduced nephron endowment have been observed when an insult was administered in the early postnatal period. Overnutrition during lactation (due to severe litter reduction) was shown to increase nephron number in rats (Boubred *et al.* 2007). Despite the increased nephron number, this insult resulted in offspring with elevated blood pressure. Similarly, a maternal low-protein diet specifically during oocyte maturation in mice (−3.5 days until mating) resulted in female offspring with smaller kidneys relative to body weight but paradoxically an increased nephron number (Watkins *et al.* 2008b). To our knowledge, this is the only study to assess nephron endowment following a periconceptional exposure, and further investigation is required. These findings suggest that insults at this very early time point of development may program disease by different mechanisms to gestational exposures.

Alternately, it may be that the additional nephrons formed do not function entirely normally and the increase in nephron endowment in fact represents dysregulated renal development. This raises many important questions as to whether insults around the time of conception can also program adult-onset disease. The second part of this review will focus on this question.

Human periconceptual exposures

The period immediately before and following conception potentially represents a period of exposure to a range of perturbations some of which may be modified upon recognition of pregnancy. For example, 22.5% of women report an episode of drinking during the first month of gestation but not later in pregnancy (Ethen *et al.* 2009), whilst many women alter their diet or begin taking dietary supplements when knowingly pregnant (Elmadfa & Meyer 2012). Studies into long-term health of babies from assisted reproductive technologies have shown that children born following IVF have higher systolic and diastolic blood pressure, as well as higher fasting blood glucose levels (Ceelen *et al.* 2008). This suggests that manipulation of the embryo around the time of conception can alter development and can have long-lasting effects.

Animal models of periconceptual exposure

The only perturbation studied to any large extent during the periconceptual period is altered maternal nutrition with a limited number of studies in other relevant models such as ethanol exposure and methyl-deficient diets. The data are primarily derived from either rodent (see Watkins *et al.* (2008a,b, 2010)) or sheep models (Edwards & McMillen (2002), MacLaughlin *et al.* 2007, 2010, Todd *et al.* 2009, Lie *et al.* 2013, Nicholas *et al.* 2013). As shown in the Table 1, the timing of the periconceptual insult may include oocyte maturation and the preimplantation period.

Rodent models

The periconceptual model utilizing maternal low-protein diets in the rodent allows separation of periconceptual and preimplantation influences by breaking the period of exposure down into key time points including oocyte maturation (Egg-LPD) or embryonic development (Emb-LPD). These short-term periods of nutrient restriction have been compared with a low-protein or control diet throughout pregnancy. Following a low-protein diet around conception, maternal control of insulin and glucose was impaired in association with changes in amino acid levels in both the maternal and blastocyst environment. Furthermore,

changes in trophoblast, inner cell mass, and total cell numbers were observed (Kwong *et al.* 2000, Watkins *et al.* 2008a,b, Eckert *et al.* 2012). These changes in the maternal milieu highlight the importance of understanding the environment around the time of conception.

Offspring phenotypes have been explored following periconceptual challenges. Reductions in heart and kidney weights relative to body weight were shown, though tended to be sexually dimorphic (Kwong *et al.* 2000, Watkins *et al.* 2008a,b). Increases in systolic blood pressure were demonstrated following a period of protein restriction through either the period of oocyte maturation (−3.5 until mating) or the preimplantation (0–3.5/4.5 days of gestation) period in both the mouse and rat (Kwong *et al.* 2000, Watkins *et al.* 2008a,b). As shown in the Table 1, these outcomes are similar to those following a low-protein diet throughout pregnancy (Woods *et al.* 2004). Preimplantation low-protein diet exposure also caused attenuated vascular responsiveness and changes in the RAS activity (Watkins *et al.* 2010).

Sheep models

Using a maternal diet containing 70% of required energy from 6 weeks before mating until day 7 of pregnancy, late gestation twin fetuses had increased blood pressure (Edwards & McMillen 2002). Kidneys of undernourished fetuses were of a similar weight to control at day 50 of pregnancy, though they exhibited increased expression of renal *IGF1* and *HSD11B2* mRNA but decreased *IGF1R* mRNA expression (MacLaughlin & McMillen 2007, MacLaughlin *et al.* 2010), suggesting that renal development may be affected. In late gestation, fetuses of ewes that were undernourished around conception had elevated insulin concentrations and altered abundance of factors regulating adipogenesis and lipogenesis in the perirenal fat. Undernutrition during the preimplantation period decreased the abundance of phosphorylated mTOR, which was increased following periconceptual undernutrition in ewes carrying twins (Lie *et al.* 2013). These studies highlight that changes in visceral and brown fat, evident during fetal life, may contribute to the development of insulin resistance in adulthood, which has been found in adult sheep offspring following periconceptual undernutrition (Todd *et al.* 2009).

Altered maternal nutrition around conception in obese mothers is now being explored, which is of clinical relevance as obese women are advised to lose weight to improve the chances of becoming pregnant. Overnutrition to induce obesity was followed by a period of restricted nutrition around conception in an ovine model. All embryos were then transferred into control ewes for the remainder of pregnancy. Increased total fat was found in female offspring at 4 months of age following an environment of overnutrition without restriction, whilst this effect was ameliorated by a period

of restriction (Rattanatrak et al. 2010). An increased responsiveness to stress was shown in female lambs, as well as changes in methylation patterns following a period of restriction during the periconceptional period (Zhang et al. 2010). Altered nutrition around conception resulted in epigenetic changes in the insulin signaling pathway, involving hepatic micro RNAs. Hepatic *MIR29B* (*miR-29b*), *MIR107* (*miR-107*), and *MIR103* (*miR-103*) were upregulated in overweight ewes, though following restriction, only the change in *MIR103* (*miR-103*) persisted (Nicholas et al. 2013).

Other clinically relevant perturbations include periconceptional alcohol exposure that resulted in changes in E- and N-cadherin expression and slowed development of embryos (Coll et al. 2011). Periconceptional alcohol exposure has also been shown to impair placental development that may contribute to fetal growth restriction (Gardebjer et al. 2014). A methyl-deficient diet during this period of development has been shown to change immune function, glucose metabolism, cardiovascular function and methylation in sheep offspring (Sinclair et al. 2007). It is important to note that many of the studies to date have focused on embryonic/fetal outcomes and relatively young offspring. As highlighted above, the importance of studying aged or aging offspring is evident as many of the outcomes of the prenatal environment are not seen until later in life and are often only apparent after exposure to lifestyle influences.

Potential mechanisms underlying developmental programming

The emerging picture suggests that multiple gestational insults may alter growth trajectories and increase the chances of obesity, insulin resistance, and cardiovascular disease later in life through programmed changes in the fetus, which include changes to nephron endowment. The question remains as to the underlying mechanisms through which this occurs. As already discussed, although the perturbations can be diverse, the outcomes are remarkably similar suggesting converging mechanisms to impact on key organ systems. Moreover, these programming events are clearly related to inappropriate nutrient supply and as recent data using models of periconceptional nutrient restriction have suggested, they may be determined before implantation. The preimplantation period represents a critical time of susceptibility, as this is a period in which the embryo undergoes a series of critical developmental events, which set up its future growth trajectory. These include zygotic genome activation (Zeng & Schultz 2005) as well as morphological (Johnson 2009) and metabolic differentiative events with the outcome being blastocyst formation and the establishment of the two primary cell lineages, the inner cell mass and the cells of the trophectoderm. Nutrient availability impacts not only on

the ability of the newly fertilized zygote to form a blastocyst but also on the number of cells that form the progenitor pool in the blastocyst. These are the progenitor cells of the embryo proper and the placental membranes respectively. Significantly, one of the early determinants of developmental potential and fetal size at birth is reported to be the number of cells specifically in the inner cell mass at day 4 (Kaye & Gardner 1999, Kwong et al. 2000, Fleming et al. 2004). Whilst the molecular mechanisms involved in the regulation of embryonic cell number in response to nutrient perturbations are elusive, these may include mTor signaling (Eckert et al. 2012) as well as the hexosamine signaling pathways (Pantaleon et al. 2010).

Evidence also supports a role for epigenetic mechanisms underlying programming, affecting growth of numerous tissues to alter susceptibility to hypertension, and other adult-onset diseases. Studies in sheep show that clinically relevant reductions in folate and methionine around the time of conception lead to widespread changes in methylation (mostly hypomethylation), which lead to increased adiposity, insulin resistance, reduced immune function, and high blood pressure (Sinclair et al. 2007). The preimplantation period is a critical developmental checkpoint because it is one of the two points during development where epigenetic reprogramming occurs, the other being in primordial germ cells at about the time of migration to the genital ridges (Seisenberger et al. 2013). This epigenetic reprogramming involves the erasure of DNA methylation and repressive histone modifications (such as H3K4) and their resetting following *de novo* methylation after compaction (Reik 2007). This allows for cell fate plasticity and makes way for new generations to develop and grow into adults on the basis of their genetic make-up and their developmental environment. However, some epigenetic information does spill over into the next generation giving rise to transgenerational cellular memory (Lange & Schneider 2010) of an adverse environment in the absence of the original environmental trigger. Interestingly, however, it is the epigenetic paternal inheritance that is erased (Reik 2007). Whilst it is not clear whether hypomethylation is the basis for all developmental programming phenomena, data from mice suggest that *in vitro* culture does indeed alter levels of critical modifiers of the epigenome such as the *de novo* DNA methyl transferase *Dnmt3l* during this stage in development (Kafer et al. 2011) to potentially impact on *de novo* genome-wide methylation following resetting of epigenetic marks.

Although speculative, differential establishment of the methylation landscape in the placental progenitor pool (trophectoderm cells) may also underlie the sexually dimorphic nature of these perturbations, given that the endocrine placenta is poised to be a key mediating tissue in responding to a dynamic and changing maternal environment. Sex differences in how this tissue develops

and therefore responds to the same maternal milieu are likely to contribute to the altered susceptibility of male and female fetuses to long-term programming outcomes. This may be related to expression of key imprinted growth factors such as IGF2 that may be differentially expressed in male versus female placentae or differences in placental structure as a result of differences in nutrient handling.

Summary

The programming of adult-onset disease, particularly hypertension, is strongly linked with impaired renal development. Experimental evidence suggests that the kidney is vulnerable following a range of maternal insults across gestation resulting in a congenital nephron deficit. Poor lifestyle choices and aging, in combination with the reduced nephron endowment, render an individual more susceptible to the development of disease. However, few studies have explored the renal and long-term health consequences of exposures occurring around the time of conception. Insults at this time can alter early nutrient supply to the blastocyst resulting in epigenetic changes in both the embryo and placenta. Future studies focusing on a range of periconceptional lifestyle factors will determine the long-term outcomes but, more importantly, provide insight into the mechanisms involved. This knowledge is essential to allow preventative strategies or potential interventions to be implemented.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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