Sex differences in developmental programming models

Catherine E Aiken1,2 and Susan E Ozanne2

1Department of Obstetrics and Gynaecology, The Rosie Hospital and NIHR Cambridge Comprehensive Biomedical Research Centre, University of Cambridge, PO Box 223, Cambridge CB2 0SW, UK and 2Metabolic Research Laboratories, Institute of Metabolic Science, Addenbrooke’s Hospital, University of Cambridge, Cambridge CB2 0QQ, UK

Correspondence should be addressed to C E Aiken at Department of Obstetrics and Gynaecology, The Rosie Hospital and NIHR Cambridge Comprehensive Biomedical Research Centre, University of Cambridge; Email: cema2@cam.ac.uk

Abstract

The theory of developmental programming suggests that diseases such as the metabolic syndrome may be ‘programmed’ by exposure to adverse stimuli during early development. The developmental programming literature encompasses the study of a wide range of suboptimal intrauterine environments in a variety of species and correlates these with diverse phenotypic outcomes in the offspring. At a molecular level, a large number of variables have been measured and suggested as the basis of the programmed phenotype. The range of both dependent and independent variables studied often makes the developmental programming literature complex to interpret and the drawing of definitive conclusions difficult. A common, though under-explored, theme of many developmental programming models is a sex difference in offspring outcomes. This holds true across a range of interventions, including dietary, hypoxic, and surgical models. The molecular and phenotypic outcomes of adverse in utero conditions are often more prominent in male than female offspring, although there is little consideration given to the basis for this observation in most studies. We review the evidence that maternal energy investment in male and female conceptuses may not be equal and may be environment dependent. It is suggested that male and female development could be viewed as separate processes from the time of conception, with differences in both timing and outcomes.

Reproduction (2013) 145 R1–R13

Introduction

The theory of the Developmental Origins of Health and Disease (DoHaD) suggests that diseases such as the metabolic syndrome may be ‘programmed’ during development and early post-natal life. The theory suggests that insults to the conceptus during development, for example, suboptimal maternal nutrition, may manifest as type 2 diabetes, heart disease or hypertension in later life (Hales & Barker 1992, Stanner et al. 1997, Ravelli et al. 1998). Many developmental programming studies have found that male and female offspring exhibit different phenotypes following insults in utero (Table 1). This observed difference in outcomes between male and female offspring has led many researchers to target their efforts exclusively on offspring of one sex (Vieau et al. 2007). Despite wide recognition of the importance of offspring sex in developmental programming outcomes, the basis for the observed sexual dimorphism in ‘programming’ of the metabolic syndrome is not understood. It is unclear what aspects of the differences between male and female development give rise to the differential susceptibility to programming insults. Table 1 demonstrates that although there are patterns suggesting differential susceptibilities to certain outcomes, for example, male sensitivity to nephrogenic insult (Woods et al. 2005, Loria et al. 2007) and female sensitivity to placental disruption (Gallou-Kabani et al. 2010, Mao et al. 2010), these are not consistently observed between different models and between different species.

Sex differences in rodent models of developmental programming

Sex differences in response to developmental programming stimuli have been reported across a range of model organisms. Direct comparisons between male and female offspring have been made predominantly in rats (Langley-Evans et al. 1996, Ozaki et al. 2001, Khan et al. 2003, Nivoit et al. 2009, Reverte et al. 2011), but sex-specific differences are also reported in mice (Gallou-Kabani et al. 2010, Vickers et al. 2011; see Table 1). There is evidence that male rat offspring are more susceptible to developmentally programmed hypertension than female offspring in studies where the sexes are directly compared (Langley-Evans et al. 1996, Kwong et al. 2000, Dodic et al. 2002, Alexander 2003, Woods et al. 2005, Maloney et al. 2011). This effect is
Table 1 Selected studies demonstrating a sex difference in developmentally programmed outcome in various species.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Main result</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal models</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male-specific outcomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Langley-Evans et al. (1996)</td>
<td>Low-protein diet gives elevated blood pressure in male offspring relative to females</td>
<td>Rat</td>
</tr>
<tr>
<td>Kwong et al. (2000)</td>
<td>Low-protein diet increases blood pressure in male offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>Ozaki et al. (2001)</td>
<td>Nutrient restriction (30% reduction) increases blood pressure and vascular dysfunction more in male offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>Dodic et al. (2002)</td>
<td>Dexamethasone exposure gives increased blood pressure more in male offspring</td>
<td>Sheep</td>
</tr>
<tr>
<td>Franco Mdlo et al. (2002)</td>
<td>Nutrient restriction (50%) reduces eNOS expression in aorta of male offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>Alexander (2003)</td>
<td>Reduced uterine perfusion causes sustained increase in blood pressure in male offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>Ortiz et al. (2003)</td>
<td>Antenatal dexamethasone injection persistently elevates blood pressure in male offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>Hemmings et al. (2005)</td>
<td>Hypoxia gives increased myogenic tone in male offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>McMullen &amp; Langley-Evans (2005)</td>
<td>Low-protein diet gives elevated blood pressure, which is glucocorticoid dependent only in male offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>Woods et al. (2005)</td>
<td>Low-protein diet gives elevated blood pressure and reduced nephron number in male offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>Zambrano et al. (2005)</td>
<td>Isoaloric low-protein diet during lactation affects insulin sensitivity more in second-generation male offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>Gilbert et al. (2007)</td>
<td>Nutrient restriction impairs nephrogenesis in male offspring</td>
<td>Sheep</td>
</tr>
<tr>
<td>Loria et al. (2007)</td>
<td>Early post-natal ANGII blockade increases proteinuria and impairs urine concentrating ability in male offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>Mueller &amp; Bale (2007)</td>
<td>Chronic variable stress exposure in early gestation decreases learning ability in male offspring</td>
<td>Mouse</td>
</tr>
<tr>
<td>Sinclair et al. (2007)</td>
<td>Periconceptual reduction in methionine and B vitamins gives impaired blood pressure in male offspring</td>
<td>Sheep</td>
</tr>
<tr>
<td>Nivoit et al. (2009)</td>
<td>High-fat and sugar diet increases insulin resistance in male offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>Tang et al. (2009)</td>
<td>Prenatal betamethasone exposure decreased glomerular filtration rate and sodium excretion in male offspring</td>
<td>Sheep</td>
</tr>
<tr>
<td>Micke et al. (2010)</td>
<td>Low-protein diet in the first trimester gives heavier male offspring in young adult life</td>
<td>Cow</td>
</tr>
<tr>
<td>Reverte et al. (2011)</td>
<td>Early post-natal ANGII blockade followed by high-protein diet leads to renal insufficiency in male offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>Maloney et al. (2011)</td>
<td>Methyl-deficient maternal diet periconceptually impairs glucose homoeostasis in male offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>Female-specific outcomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lee &amp; Rivier (1996)</td>
<td>Ethanol exposure leads to exaggerated HPA stress response in young female offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>Khan et al. (2003)</td>
<td>High-fat diet increases blood pressure in female offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>Wilcoxon et al. (2003)</td>
<td>Ethanol exposure leads to decreased placental 11β-hydroxysteroid dehydrogenase-2 levels and left ventricular hypertrophy in female offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>Manikkam et al. (2004)</td>
<td>Prenatal testosterone exposure provokes post-natal catch-up growth in female offspring only</td>
<td>Sheep</td>
</tr>
<tr>
<td>Porter et al. (2007)</td>
<td>High-salt diet preconceptually increases stress response in female offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>Padmavathi et al. (2009)</td>
<td>Maternal zinc deficiency impairs glucose-induced insulin secretion in male but not female offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>Mao et al. (2010)</td>
<td>High-fat and low-fat diet produce up-regulation of placental genes more marked in female offspring</td>
<td>Mouse</td>
</tr>
<tr>
<td>Gallou-Kabani et al. (2010)</td>
<td>High-fat diet leads to DNA hypomethylation in female placenta only</td>
<td>Mouse</td>
</tr>
<tr>
<td>Rodriguez et al. (2011)</td>
<td>Betamethasone exposure reduces associative learning more in female offspring</td>
<td>Baboon</td>
</tr>
<tr>
<td>Vickers et al. (2011)</td>
<td>Fructose exposure gives decreased placental weight, increased plasma leptin and glucose in female offspring</td>
<td>Rat</td>
</tr>
<tr>
<td>Epidemiology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male-specific outcomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zaren et al. (2000)</td>
<td>Maternal smoking decreases birth weight and head circumference proportionally more in male offspring</td>
<td>Human</td>
</tr>
<tr>
<td>Goldenberg et al. (2006)</td>
<td>Placentas from preterm deliveries showed more lymphohistiocytic infiltration in male offspring</td>
<td>Human</td>
</tr>
<tr>
<td>Haley et al. (2006)</td>
<td>Prenatal exposure to alcohol increases the heart rate of female infant offspring more than males, but with the males showing a greater increase in plasma cortisol</td>
<td>Human</td>
</tr>
<tr>
<td>Mingrone et al. (2008)</td>
<td>Male offspring of obese mothers have higher levels of insulin secretion in response to GTT</td>
<td>Human</td>
</tr>
<tr>
<td>Female-specific outcomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roseboom et al. (2001)</td>
<td>Higher rates of obesity in female offspring conceived during famine</td>
<td>Human</td>
</tr>
<tr>
<td>Clifton (2005)</td>
<td>Maternal asthma in pregnancy causes altered placental glucocorticoid metabolism and growth in female offspring</td>
<td>Human</td>
</tr>
<tr>
<td>Stark et al. (2009)</td>
<td>Antenatal betamethasone administration increased placental 11β-hydroxysteroid dehydrogenase-2 activity in female offspring and this was positively correlated with mean arterial blood pressure</td>
<td>Human</td>
</tr>
</tbody>
</table>

Studies were included only if the results of both sexes were reported in the original paper. Studies were included if the effect of a developmental programming insult was of greater statistical significance in one sex or was seen only in one sex. For animal models, only papers that specified an intended developmental programming intervention were included.
seen in rats with a range of interventions including antenatal dexamethasone administration (Alexander et al. 2003, Ortiz et al. 2003), prenatal low-protein diet (Langley-Evans et al. 1996, Kwong et al. 2000, Woods et al. 2005) and uterine hypoperfusion (Alexander 2003). The effect is not entirely consistent, however, as a maternal high-fat diet has been reported to elevate blood pressure in female offspring only (Khan et al. 2003). The attenuation of the endothelial response to acetylcholine induced by this model was, however, present in both male and female offspring. In keeping with the predominantly male hypertensive effects of prenatal insults on development, studies have found more impaired renal function in male than in female rat offspring (Woods et al. 2005, Gilbert et al. 2007, Loria et al. 2007, Reverte et al. 2011). Focusing on studies where the sexes are compared directly within the same model, it has been suggested that an anatomical basis for impaired renal function can be seen in a direct reduction of the number of nephrons in male offspring (Woods et al. 2005). The greater susceptibility of male renal development to adverse stimuli is also demonstrated with post-natal intervention, when exposure to angiotensin II receptor antagonists causes decreased nephron number in both male and female rats but decreased glomerular filtration rate and papillary volume only in males (Saez et al. 2007).

Impaired glucose tolerance and insulin resistance are also a common finding in rat offspring in developmental programming studies. This feature has been noted in both male and female offspring in various models (Vickers et al. 2000, Petry et al. 2001, Simmons et al. 2001, Seckl 2004, Ozanne et al. 2005). Where the sexes are directly compared, the effect is observed in either the male (Nivoit et al. 2009) or the female (Vickers et al. 2011) offspring. It has further been suggested that impaired insulin sensitivity is a male-specific finding in second-generation rat offspring when the dams of the F0 generation are exposed to isocaloric low-protein diet during lactation (Zambrano et al. 2005). Conflicting results on offspring outcomes may be an effect related to the precise nature and timing of the intervention. Variations in the type of exposure, developmental age at which it was applied and age at which outcomes were measured could all contribute to the differences in outcomes observed. In the absence of further studies directly comparing offspring outcomes, systematic conclusions cannot be drawn. Despite the preponderance of developmental programming papers measuring outcomes related to hypertension, renal development and impaired glucose tolerance, other sex-specific differences in rodent studies specifically linked to developmental programming are reported including learning ability (Mueller & Bale 2007), myogenic tone (Hemnings et al. 2005) and endothelial function (Franco Mdo et al. 2002). Studies of mouse models that have measured effects of developmental programming on the placenta in both male and female offspring have reported that the placentas of female offspring are more readily affected by prenatal insults (Wilcoxon et al. 2003, Gallou-Kabani et al. 2010, Mao et al. 2010). It may be hypothesised that female offspring are more protected from in utero insults by the relative ease with which the placenta adapts in the face of adverse conditions. The relative malleability of the female placenta is also observed in other species, including the rat, where ethanol exposure in utero leads to decreased 11β-hydroxysteroid dehydrogenase-2 activity in female offspring (Wilcoxon et al. 2003), and in human studies, where placental up-regulation of 11β-hydroxysteroid dehydrogenase-2 activity in response to antenatal steroid administration occurs specifically in female offspring (Stark et al. 2009). 11β-Hydroxysteroid dehydrogenase-2 is associated with improved physiological stability, particularly in infants born preterm, and it is therefore unsurprising to note that levels are decreased by a physiological insult such as alcohol consumption and increased by antenatal steroid administration (which confers advantage in neonatal outcome; Stark et al. 2009).

**Sex differences in larger mammals and humans**

Sex-specific differences in offspring outcomes from developmental programming models are also demonstrated in farm animals, mainly sheep (Dodic et al. 2002, Manikkam et al. 2004, Gilbert et al. 2007, Sinclair et al. 2007, Tang et al. 2009) and cows (Micke et al. 2010). A male offspring-predominant hypertensive effect, similar to that demonstrated in rat models, has been observed in sheep exposed to excess steroids in utero (Dodic et al. 2002) and in sheep fed a low-methionine and vitamin B diet in the periconceptional period (Sinclair et al. 2007). In keeping with rodent data suggesting a greater sensitivity of the developing renal system to developmental insult in males than in females (Woods et al. 2005, Saez et al. 2007), studies in sheep have also demonstrated that prenatal steroid exposure reduces glomerular filtration rate in male offspring (Tang et al. 2009) and nephrogenesis is impaired in male offspring exposed to nutrient restriction (Gilbert et al. 2007).

Growth patterns are addressed in larger mammals with the phenomenon of catch-up growth observed in young adult male cows when exposed to low-protein diet in the third trimester, with no significant difference in the carcass weights of females with the same exposure (Micke et al. 2010). However, prenatal testosterone exposure in sheep caused intrauterine growth retardation (IUGR) in both sexes, but catch-up growth was reported in female offspring only (Manikkam et al. 2004).

Human studies in developmental programming are by necessity limited to observational and epidemiological data; however, these do suggest that adverse conditions during pregnancy have sexually dimorphic
effects. The developmental insults studied are wide-ranging, including maternal smoking (Zaren et al. 2000), antenatal steroid administration (Stark et al. 2009), famine (Roseboom et al. 2001), asthma (Clifton 2005) and obesity (Mingrone et al. 2008). These reflect the results of animal models in showing effects on placental function more in female offspring (Roseboom et al. 2001, Clifton 2005, Stark et al. 2009), but growth restriction, increased insulin secretion and plasma cortisol preferentially in males (Zaren et al. 2000). These reflect the results of animal models in showing effects on placental function more in female offspring (Roseboom et al. 2001, Clifton 2005, Stark et al. 2009), but growth restriction, increased insulin secretion and plasma cortisol preferentially in males (Zaren et al. 2000, Haley et al. 2006, Mingrone et al. 2008).

In attempting to explain the difference in outcomes for male and female offspring, parallels may be drawn from fields other than developmental biology and molecular biology, such as evolutionary biology. Evolutionary biology traditionally views mammalian males as the more ‘costly’ sex, and it may therefore not be energy-efficient for a mother to invest heavily in long-term protection of male conceptuses (Rickard et al. 2007, Mathews et al. 2008). This type of effect may explain a number of apparently anomalous results; for instance, female infants of mildly asthmatic mothers demonstrate a degree of IUGR, but this is ‘rescued’ after maternal exposure to inhaled steroids. The growth of male infants on the other hand is unaffected by mild asthma, but males have a high rate of in utero mortality and severe IUGR if an acute exacerbation of asthma occurs (Clifton 2005).

Sexual dimorphism in terms of energy expenditure and investment in conceptuses may be viewed as a species-level survival advantage to preserve precious resources at times of environmental stress in order to gain maximum chance of overall species survival. Adaptations in the offspring somatic tissues to environmental stresses that are costly in terms of energy investment would therefore be made preferentially to female offspring, whose overall physiology and reproductive tracts must be protected to ensure that they can reproduce and withstand the stresses of pregnancy and lactation, whereas the male has only to protect his germ cells and be fit to mate. This kind of adaptive mechanism could be manifested in the changes described in rat models in Table 1, i.e. lack of protection for male offspring in terms of renal tract development, and susceptibility to hypertensive disease (Langley-Evans et al. 1996, Kwong et al. 2000, Ozaki et al. 2001, Alexander 2003, Woods et al. 2004, Loria et al. 2007), where the somatic development of the male offspring is ‘killed’ to save maternal resources. Female offspring on the other hand seem to have more placental adaptations to adverse conditions (Wilcoxon et al. 2003, Vickers et al. 2011), which might reflect attempts to buffer and adapt to stressors, in order to preserve female conceptuses in optimal condition and maximise the chances of successful pregnancy and raising of a litter. The adaptive response to some interventions may not be clear-cut, for example to maternal hyperglycaemia, where this may reflect a nutritionally replete environment or a maternal pathology that would compromise the well-being of the pregnancy. It is clear that for propagation of the species, there must be survival of both male and female offspring to reproductive years, but developmental programming could reflect subtle differences in the long-term investment in each sex. Nonetheless, male conceptuses do survive in utero and post-natally despite harsh conditions, and there is clearly capacity to postulate that counter-mechanisms have evolved to ensure survival of both sexes. On this basis, we propose that females may be more sensitive and adaptable to the intrauterine environment, whereas males may only be vulnerable and respond to the harshest of pre- and post-natal environments. The evidence for the basis of a sex difference is examined on both a species and an individual conceptus level, in an attempt to gain insight into the phenomenon.

**Evolutionary aspects of sexual dimorphism in developmental programming**

Mammalian sex differences may be considered on three levels – primary reproductive characteristics; secondary differences in males and females not directly linked to reproduction (for example differences in muscle mass; Wells et al. 2010); and species-level differences in behaviours, hierarchies and mating strategies (Bouissou 1983, Mank 2009). The first and second of these differences are the areas with which developmental biology is primarily concerned; however, it may be illuminating to additionally consider the third. Sexual dimorphism is observed throughout the animal kingdom and is dependent on sex-limited gene expression (Williams & Carroll 2009). Patterns of sex-limited gene expression and sex determination transcription factors are broadly similar in mammalian species but diverge across other classes and phyla (Williams & Carroll 2009). From an evolutionary biology point of view, the energy invested by parents in male vs female offspring is thought to be influenced by the condition of the parents and the post-natal environment faced by the offspring (Trivers & Willard 1973, Koskela et al. 2009).

The Trivers–Willard hypothesis (Trivers & Willard 1973) states that in a species where there is a sex-based difference in reproductive success, mothers with plentiful resources will be able to invest in the sex with a reproductive disadvantage, whereas mothers facing an adverse environment will preferentially produce offspring of the sex with a greater chance of reproductive success. If developmental programming is considered as the molecular mechanism by which this differential investment in offspring sex can be realised, then the sexually dimorphic results of the various animal models of developmental programming may be better understood.
The application of the underlying principles of the Trivers–Willard hypothesis to the developmental programming hypothesis has several attractive elements. In particular, the inherent assumptions in both models are remarkably similar: first that maternal ‘condition’ relates to fetal ‘condition’, secondly that the effects ‘programmed’ by the in utero environment persist into adult life and thirdly that the effects of advantageous conditions should be greater in the more reproductively variable sex (Trivers & Willard 1973). Detecting a sex ratio skew in laboratory models of developmental programming is not straightforward, as many models cull litters to a standardised number of males and females, or a set low number of single sex offspring in order to maximise the plane of nutrition (Khan et al. 2003, Loria et al. 2007, Chen et al. 2008, Tarry-Adkins et al. 2010) but do not report pre-culling litter size or gender distributions. Interestingly, however, a few studies have shown that the sex ratio in rodent models can be altered by changing maternal diet (Rosenfeld et al. 2003, Rosenfeld & Roberts 2004), and a similar effect has been noted with calorie restriction (Meikle & Drickamer 1986) and increased fats in the maternal diet (Fountain et al. 2008). It has been demonstrated that a mouse model utilising a maternal high-fat diet, which may be taken as a proxy for a nutrient-rich plentiful environment, produced litters skewed towards excess males (male fraction 0.67) whereas a low-fat diet in the same animals skewed the ratio towards excess female offspring (male fraction 0.39) (Rosenfeld et al. 2003). Calorie restriction in a mouse model gave results, consistent with the idea of a sex ratio skewed towards female offspring by acute dietary stress (Meikle & Drickamer 1986), but the sex ratio is not significantly different after a long period of calorie restriction. This adds weight to the idea of an adaptive process in response to environmental stress.

The idea of maternal enhancement of the fitness of one sex over the other depending on the post-natal environment that the offspring will face is consistent with the differences observed in developmental programming models. Some of the sex differences described in the developmental programming literature may be more easily explained if viewed with respect to the evolutionary basis rather than focusing on the molecular mechanism. If the Trivers–Willard hypothesis is re-written in terms more usually accessible to developmental biologists, it postulates that the external environment is signalled to the conceptus by the mother via the uterine environment. This signalling influences the phenotype of offspring produced to maximise chances of species survival. This theory equates to the DoHaD hypothesis and is a useful framework for considering the apparent complexity of the observed sex differences in developmental programming phenotypes. More particularly, the Trivers–Willard hypothesis specifies sexual dimorphism as a key feature of the changes produced by environmental pressures. The weight of evidence for such an effect in the developmental programming literature implies that a similar import should be attached to considering offspring sex when reviewing and designing developmental programming studies.

Evidence for the Trivers–Willard effect is controversial in epidemiological studies of human populations, but several studies have found evidence of skewed sex ratios linked to environmental conditions (Andersson & Bergstrom 1998, Kanazawa 2007, Cameron & Dalerum 2009). The situation for monotocous as opposed to polytocous mammals may differ substantially. If a Trivers–Willard effect does exist for monotocous mammals, including humans, it may also be more difficult to detect in population studies, as there is no scope for a subtle shift in sex ratio within a single pregnancy.

**Mechanistic aspects of sexual dimorphism in developmental programming**

While it is useful to consider developmental programming at a species level, and to gain insight from an evolutionary perspective into the reasons behind the utility of a developmental programming effect, it is also necessary to consider how sex-specific differences in development occur at an individual level.

Three major factors give rise to sex differences in development: differences in patterns of development (genetic, transcriptional and morphological), differences in timing of development and the influence of steroid hormone exposure during life in utero and post-natally. These factors combine to produce mature adult organisms that are sexually dimorphic in terms of anatomy, physiology, reproductive capacity and behaviour (Fig. 1).

**Genetic and morphological differences in development**

**Evidence from rodent models**

From the earliest stages of preimplantation development, the sex of the embryo influences both growth patterns and survival. In the mouse, cultured individual blastomeres from the two-cell embryo yield a higher rate of successful male than female conceptuses (Sato et al. 1995). Cultured preimplantation murine male embryos show a faster rate of growth in vitro than do female embryos (Valdivia et al. 1993). Fetal sex is determined by the presence or absence of a Y chromosome bearing the Sry gene. In the mouse, this gene is responsible for the onset of sexual dimorphism between embryonic (E) days 10.5–12.5. Sexual differentiation in the male initially involves the interaction of SRY with SF1 to stimulate (via SOX9) Sertoli cell development in the genital ridges, which subsequently differentiate into testes (Sekido & Lovell-Badge 2008). However, there are other important central and peripheral regions expressing SRY, which
contribute to the development of a phenotypically male or female neonate, including heart, liver and kidneys, but particularly the brain and adrenal glands (Kopsida et al. 2009). Cells derived from day 10.5 mouse embryos and grown in culture show similar patterns and rates of cell division irrespective of chromosomal sex but differ in their sensitivity to applied insults (e.g. ethanol) with female lineages showing higher rates of cell death (Penaloza et al. 2009).

It is possible that female feto-placental units are more responsive to adverse uterine environments at an early stage. For instance, maternal high-fat diet during pregnancy appears to directly influence placental methylation patterns only in female murine offspring (Gallou-Kabani et al. 2010). This finding is consistent with the increased sensitivity of gene expression profiles in female placentas to a maternal high-fat diet (Mao et al. 2010). The decreased incidence of adverse outcomes in female fetuses compared with males could be the consequence of a greater environmental adaptability, and hence protection, by placental physiology.

**Evidence from large mammal and human studies**

There is evidence that development of male and female bovine conceptuses may be differently influenced by early culture conditions. In bovine blastocysts, the expression of antivirals in culture medium is twice as high in female conceptuses as in males, due to increased interferon τ levels (Larson et al. 2001). This difference has been postulated to give survival advantage to female embryos (Walker et al. 2009). In bovine conceptuses cultured in vitro, increasing glucose concentrations in the culture medium led to an increased proportion of male vs female blastocysts surviving in culture to the blastocyst stage (Gutierrez-Adan et al. 2001, Larson et al. 2001). Thus, in the bovine preimplantation embryo, before expression of any overt sex-specific morphology, survival may already be influenced in a sex-dependent manner by the interaction of genetic factors and environmental conditions. By contrast, however, a study of in vitro development of murine embryos in elevated glucose concentrations failed to demonstrate any sex-specific effects, although total and trophectoderm cell numbers were reduced in all conceptuses (Bermejo-Alvarez et al. 2012). There is insufficient data available to draw conclusions on whether glucose concentrations may influence development in vitro in a sex-specific manner in other species, but given the difference in the findings of bovine and murine studies, it may be expected that this will prove to be a species-specific effect.

In humans, male sex is a significant risk factor for adverse motor and cognitive outcomes in premature infants and for survival in neonatal intensive care units (Stevenson et al. 2000), with 20% reduced survival of male preterm infants relative to females (Vatten & Skjaerven 2004). Recent evidence regarding the differing outcomes of male and female premature births suggests that feto-placental immune function may be sexually dimorphic and may play a key role in influencing survival. Premature (<32 weeks) male neonates are more likely to have a placenta showing evidence of chronic inflammation and decidual lymphoplasmacytic cell infiltration (Ghidini & Salafia 2005, Goldenberg et al. 2006), whereas female placentas appear better protected from inflammatory insult. The other major difference influencing early life survival, and highlighted in many studies, is the degree of peripheral vasodilatation of the microvasculature, which is considerably lower in female neonates (Stark et al. 2008). This difference confers a considerable survival benefit as it enables better cardiovascular stability and may be linked to the observations on immune function, via

**Figure 1** Sex differences arising in male and female development from conception to the sexually dimorphic adult.
levels of circulating cytokines. These adaptations in the female fetus require increased energy expenditure on the feto-placental unit, which may be proportionally more costly to the mother in keeping with the Trivers–Willard hypothesis.

Timing of development

Human males and females are known to undergo development at different rates, both in utero and post-natally up until the post-pubertal stage (Pedersen 1980, Davis et al. 1993, de Onis et al. 2009). Growth velocity may be critical to the extent to which an individual is affected by an insult at any particular time point. A fetus growing faster in utero can be considered to have a greater effective exposure to a given insult than one that undergoes fewer cell cycles during the period of exposure. This may be the basis for the observation from human studies that male infants of diabetic mothers show an increased incidence of fetal macrosomia (weight at birth >4000 g, leading to increased risk of adverse events at delivery and neonatal outcomes; Di Renzo et al. 2007), despite being exposed to the same level of hyperglycaemia for the same absolute time period as their female counterparts.

Sex differences in temporal development have been postulated from the time of conception itself, with the proposition that human male conceptuses are more likely to result at the extremes of the fertile period (James 2001, 2008). However, the empirical evidence for this proposition is contentious (Gray et al. 1998). The theory has been advanced that conception at either end of the fertile window results in less ‘fit’ embryos, purporting to explain why a higher number of spontaneously aborted conceptuses are male and advances an idea of male ‘fragility’ extending through life (Kraemer 2000). Sexual dimorphism is also a feature of early post-implantation growth in mammalian conceptuses (Pedersen 1980). Later post-morphogenesis in utero growth is also faster in human males with an average higher birth weight and also a greater spread of birth weights (Clifton 2010); however, levels of β human chorionic gonadotrophin, which are responsible for maternal recognition and response to the pregnancy, are greater with a female than a male fetus (Cowans et al. 2009). Ultrasonographic measurements of human growth in utero shows that the slower rate of growth of the female fetus becomes significant at 28 weeks of gestation and increases towards term (Parker et al. 1984).

Timing of sexual development

Sexual maturity is reached earlier in females than in males, an effect that is preserved across a range of species from mice to humans, and may be correlated with expression of kisspeptin in the hypothalamus (Clarkson & Herbison 2006, Kauffman 2010). There is a marked difference in developmental programming of offspring phenotypes when measured before and after puberty. For example, in prenatal exposure to maternal ethanol consumption (Lee & Rivier 1996), immature rat males and females show a dimorphic hypothalamo-pituitary axis (HPA) response to immune stimuli, but post-puberty, both show exaggerated ACTH release to the same stimuli. Spurious results may be obtained using experimental protocols that take measurements at an absolute time point after birth in young adult animals and which consequently fail to take account for the later sexual maturation of males, thereby observing an effect that reflects pubertal status rather than primarily a sex difference.

In considering the applicability of these results to a human population, a further level of complexity is added by the suggestion that a prenatal insult may itself influence the timing of puberty, for example children born at very low birth weight, whether at term or prematurely, show early puberty, up to 2 years in advance of their normal birth weight peers (Wehkalampi et al. 2011). Hence, not only may the measured phenotype be influenced by the timing of the measurement but also by the nature and extent of the insult applied.

Reproductive aging in developmental programming

In comparing phenotypes during later life, very few developmental programming studies address ambient sex steroid levels at the time of sampling, yet a very different outcome may be observed depending on whether females still have the cardioprotective effect of oestrogen. Female rodents demonstrate cycle irregularities at around 9–12 months of age (Sokol et al. 1999, Spencer et al. 2008), eventually becoming acyclic and entering ‘estropause’ at 12–18 months of age (Van Kempen et al. 2011). The key differences between menopause and estropause may be understood in terms of circulating oestrogen levels, which fall dramatically in human menopause, but are maintained at a higher ambient level in rodents (Maffucci & Gore 2006). Many developmental programming studies have sampling time points at around estropause (9–12 months; Remmers et al. 2008) and yet do not consider whether the effects of sex steroids on female tissues are consistent at this age, or indeed whether the results would have relevance to a human population.

At the later extreme of life, aging and longevity have been studied in the developmental programming literature, particularly with respect to telomere length (Jennings et al. 1999, Tarry-Adkins et al. 2008). It is known that telomere length in both the renal cortex and the medulla of male rats is shorter than in females and that telomere shortening is associated with increased markers of cellular senescence (Tarry-Adkins et al. 2006). In male rats, it has also been shown that prenatal
exposure to a maternal low-protein diet in rats shortens telomeres prematurely in a number of organs (Tarry-Adkins et al. 2008), but the effect is less well studied in females.

In humans, differences in the lifespan of males and females are well recognised, despite the lack of a demonstrated difference in telomere length at birth (Okuda et al. 2002). Women have greater telomere lengths in later life than men in peripheral blood leukocytes (Benetos et al. 2001), and this difference may be linked to oestrogen-dependent promotion of telomerase activity (Kyo et al. 1999). European women undergo menopause at a median age of 54 years (Dratva et al. 2009) and thereafter their general propensity to diseases of the metabolic syndrome changes considerably. A sex difference in developmental timing thus extends throughout life and is worthy of consideration in the design of developmental programming studies when planning both exposure and measurement of phenotypic outcomes. Spurious results may be obtained when comparing males and females at the same absolute time from conception, but failing to take account of the different maturational stage that this represents. Particularly in obtaining tissue samples from young adult animals around the advent of sexual maturity, a sex difference in pubertal stage is not often considered. Similarly, in studies that involve senescent tissue, the normal rate of tissue aging in male and female tissues and hence differences in for example telomere length and markers of oxidative damage should be considered.

**Exposure to sex steroids**

Increased steroid exposure in utero has been demonstrated to affect fetal and placental metabolism, nutrient transfer and endocrine function (Milley 1996, Timmerman et al. 2000, Challis et al. 2002). Fetal glucocorticoid exposure is a strong candidate for involvement in programming of the metabolic syndrome and has been shown to affect development in a range of organisms (Seckl 2008). It is unclear whether exposure to sex steroids may have similar adverse effects. Evidence from human populations suggests that the prevalence of insulin insensitivity and polycystic ovarian syndrome is higher in the presence of excess androgen hormones (Veiga-Lopez et al. 2008, Moulana et al. 2011), and it has been suggested that exposure to excess androgen in childhood influences the development of the metabolic syndrome and polycystic ovarian syndrome (Idkowiak et al. 2011). In adult humans, male subjects show an increased propensity to at least some aspects of the metabolic syndrome compared with females, possibly as a primary effect of ambient sex steroid levels (Regitz-Zagrosek et al. 2006). Epidemiological evidence shows that the sex difference in risk for cardiovascular disease narrows considerably after the menopause and that oophorectomy in rats can narrow this gap in incidence at an earlier age (Ojeda et al. 2007, 2008).

From a developmental point of view, male fetuses are exposed to higher levels of androgens in utero compared with female fetuses (Barry et al. 2010). In the human fetus, it has been demonstrated that the testes can synthesise androgens from LDL-cholesterol by gestational week 10 (Carr et al. 1983). It is difficult to determine with accuracy fetal testosterone levels, particularly in the human where fetal and maternal testosterone serum levels are poorly correlated (Scott et al. 2009). The essential ‘masculinisation programming window’ – when androgen levels become significant in fetal serum – occurs shortly before the time at which definitive male fetal phenotypic characteristics appear, corresponding to E15.5–17.5 in the rat (Welsh et al. 2008). If the molecular-level sex differences in rat developmental programming models are ascribed to the difference in fetal testosterone exposure, it would be reasonable to expect that sex differences should also become detectable at around E15.5–17.5, or at least not detectable significantly before this time in the rat model. In female sheep offspring, it has been demonstrated that in utero administration of testosterone to the mother produces pancreatic dysregulation and changes in liver metabolism (Hogg et al. 2011).

A further consideration in polytocous organisms is the effect of differing levels of intrauterine sex steroid exposure in the female offspring, dependent on intrauterine position. Those females that developed in utero between two male fetuses are known as ‘2M females’ and have higher testosterone exposure than ‘0M females’, which develop between two female fetuses (vom Saal & Bronson 1980). Testosterone exposure of 2M females has been shown to affect their subsequent reproductive function (Vom Saal & Moyer 1985) and sexually dimorphic preoptic area development (Pei et al. 2006). The consideration of intrauterine position applies much less frequently to larger mammal and human populations that are not litter-bearing, but the differences in physiology observed in 2M females adds weight to the hypothesis that in utero exposure to sex steroids may profoundly influence developmental programming.

Much less work has gone into understanding the effects of female sex hormone exposure in utero, but it has been shown that sex differences in insulin sensitivity may be influenced by the effect of oestrogen as a leptin sensitiser on the fetal brain (Clegg et al. 2003, 2006).

**Sex-specific differences in epigenetic regulation**

Sex-specific differences in epigenetic modulations are associated with developmentally programmed phenotypes in animal models (fully reviewed in Dunn et al. (2011)). These effects are proposed to extend to second generations of offspring in rats (Morgan & Bale 2011) and can be induced by a variety of adverse stimuli,
including maternal dietary and stress interventions (Dunn et al. 2011).

In the female preimplantation embryo, there exists a period during which transcription occurs from both X chromosomes, between the start of transcription from the embryonic genome and X inactivation. The absolute extent of this window is species specific, lasting in the mouse between the eight-cell and blastocyst stages (Gardner et al. 2010). The result of a period of expression from both X chromosomes is a proteome considerably different from that of the male (Epstein et al. 1978). Differential expression of over 600 X-linked genes has been shown in the mouse (Kobayashi et al. 2006), including dose-dependent up-regulation of hypoxanthine–guanine phosphoribosyltransferase (Kratzer & Gartler 1978) and glucose 6-phosphate dehydrogenase (Williams 1986). Persistence of these proteomic changes after X inactivation could account for ongoing differences in male and female growth patterns, as well as potentially determining their responsiveness to developmental insults.

Applying these considerations to larger mammals and humans is more challenging – the timing and mechanism of X inactivation is not well conserved across mammalian species (Okamoto & Heard 2009). The precise timing of X inactivation in human embryos is yet to be fully determined (van den Berg et al. 2009). It remains uncertain how the contribution of X inactivation to developmental programmed offspring phenotypes should be interpreted, particularly in view of the fact that not all genes on the X chromosome are silenced at X inactivation (Brown & Greally 2003). In humans, up to 15% of genes carried on the X chromosome appear to escape inactivation and hence a gene dosage effect remains a plausible explanation for sex differences in developmental programming even after X inactivation has occurred (Brown & Greally 2003).

Conclusions

In the foregoing discussion, it is assumed that the sex differences in developmental programming models are the result of the differential ability of the male and female fetus to respond to a particular stress; however, a further question, which is difficult to address experimentally, is whether the mother’s response to a given stress is influenced by the sex of fetus she is carrying. Developmental programming stresses are not normally applied to the fetus directly, but particularly in the case of dietary interventions are mediated by the maternal physiology. It could therefore be postulated that all fetuses respond identically to the same insult, but the insult is buffered by the mother in a way that depends on the sex of the fetus. This is a major point requiring clarification via further experimental work.

In view of the major temporal, spatial and biochemical differences between male and female development, both pre- and post-natally, it may be argued that the sexes should be treated by researchers as separate models. In designing experiments, it is the goal of most researchers to use the fewest animals and least resources practicable to fully address their question. This leads to a paucity of studies in which sexual dimorphism is built into the model, and yet this is a pertinent issue when applying the results of developmental programming studies to designing diagnostic, preventative and therapeutic measures.

Declaration of interest

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

Funding

S E Ozanne is a British Heart Foundation Senior Fellow.

References

Alexander BT 2003 Placental insufficiency leads to development of hypertension in growth-restricted offspring. Hypertension 41 457–462. (doi:10.1161/01.HYP.000005448.95913.3D)


Chen JH, Jones RH, Tarry-Adkins J, Smith NH & Ozanne SE 2008 Adverse effects of reduced oxygen tension on the proliferative capacity of rat...


Fogg K, Wood C, McNeilly AS & Duncan WC 2011 The in utero programming effect of increased maternal androgens and a direct fetal intervention on liver and metabolic function in adult sheep. PLOS ONE 6 e24877. (doi:10.1371/journal.pone.0024877)


James WH 2008 The variations in human sex ratio at birth with time of conception within the cycle, coatal rate around the time of conception, duration of time taken to achieve conception, and duration of gestation: a synthesis. Journal of Theoretical Biology 255 199–204. (doi:10.1016/j.jtbi.2007.06.016)


Khanyi, Taylor PD, Dekou V, Seed PT, Lakasing L, Graham D, Dominiczak AF, Hanson MA & Poston L 2003 Gender-linked hypertension in ovariase of lard-fed pregnant rats. Hypertension 41 168–175. (doi:10.1161/01.HYP.0000047511.97879.FC)


Kratzer PG & Gartler SM 1978 HGPRT activity changes in preimplantation mouse embryos. Nature 274 500–503. (doi:10.1038/274500a0)


Received 21 December 2011
First decision 2 February 2012
Revised manuscript received 1 October 2012
Accepted 15 October 2012