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Frontiers in Research Review:

ISH Early Origins of Hypertension Workshop: Early Life Exposure and Development of a Healthy Heart

Developmental programming: Variations in early growth and adult disease

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SUMMARY

1. Suboptimal conditions *in utero* are associated with the development of adult-onset diseases in offspring. Uteroplacental insufficiency in rats is a well-established animal model used to mimic and study the effects of developmental insults relevant to countries of abundant nutrient supply. However, wide-ranging outcomes for the offspring are apparent between the different investigators that use this model and also between cohorts generated in our laboratory.

2. We aimed to explore the reasons for variability in rat models of uteroplacental insufficiency between different investigators and also between our own animal cohorts. We suggest differences in growth and disease development reflect uniqueness in susceptibility and highlight the complexity of interactions between genetic potential and environmental exposures.

3. The impact of adverse exposures *in utero* has been described as having far-reaching effects that extend well beyond the first, directly exposed generation. However, the resulting phenotypes are not consistent between generations. This suggests that programmed effects are established *de novo* in each generation and challenges the prediction of disease.

4. Characterization of growth and disease in the numerous rat models has led to our understanding of the impact of early life experiences on adult health. In order to drive the development of preventative and/or treatment strategies, future studies should focus on identifying the initial cause(s) of uteroplacental insufficiency, including genetic origins and the influence of poor diets.

Key words: animal models, early life exposures, trans-generational effects.

INTRODUCTION

Intrauterine growth restriction, which occurs in approximately 8% of pregnancies worldwide, is associated with perinatal morbidity and mortality and the development of adult-onset diseases.[1,2] Therefore, the fetal environment is considered a critical determinant of long-term health. In the first instance, human epidemiological studies were performed across well-nourished settings in the UK, whereby detailed birth records were traced back to almost 70 years.[1–7] A range of morphometric measurements, including weight and stature, from birth to early infancy were identified as independent determinants of later-life disease risk. These initial discoveries prompted many researchers worldwide to elucidate the mechanisms underlying these links through the use of animal models, including sheep and rats. These models have included, but are not limited to, exposure to maternal undernutrition (e.g. low protein and global caloric restriction),[8,9] maternal glucocorticoid exposure[10,11] and placental insufficiency (reduced uterine perfusion pressure, uteroplacental insufficiency, placental embolization).[12–14] The use of maternal undernutrition and glucocorticoid exposure models has proved invaluable with respect to defining the 'windows' of development (prenatal and early postnatal) that are susceptible to adverse exposures. However, uteroplacental insufficiency has been regarded as the

"the fetal environment is a determinant of health"

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major cause of low birth weight in nations of abundant nutrient supply and is the focus of the present review.

VARIABILITY IN GROWTH AND DISEASE

For a number of years our group and others have been studying the effects of uteroplacental insufficiency through the generation of rat models. Most of these involve ligation of the uterine vessels during late gestation to restrict blood flow to the uteroplacental bed, but otherwise the pregnancy is generally regarded as uncomplicated.[13,15–17] The offspring, particularly males, often present with growth, metabolic and cardiorenal defects, which can include obesity, impaired glucose tolerance, hypertension and deficits in cell numbers.[18] However, as indicated in Table 1, individual effects are variable between, and even within, the same animal model(s). In addition, a rat model that has physiological characteristics similar to those seen in pre-eclampsia has been developed whereby both the uterine vessels and abdominal aorta are ligated after mid-gestation and maternal hypertension and proteinuria become apparent.[14] Certainly, differences in growth and disease between models are conceivable and may be due to any number of factors, including the strain and sex studied, gestational period and the severity of surgical ligation and the age at which the animals are studied (Fig. 1). Animal studies have investigated the impact of uteroplacental insufficiency using similarly aged, relatively young dams and so differences in maternal age are unlikely to be a confounding influence. However, we have recently reported compromised pregnancy outcomes associated with advanced maternal age (reduced litter size and birth weight).[19] Given the increasing rates of pregnancy in women aged 39–44 years,[20] the use of older mothers should be considered to study the contribution of this factor to the offspring's risk of later disease. It is also worth recognizing the influence of postnatal exposures in modifying the direct effects of prenatal programming, which may explain the variability we observe between studies.

Simmons *et al.* reported that male rat offspring from mothers who experienced surgical uterine vessel ligation during late pregnancy exhibited reductions in pancreatic β-cell mass and developed obesity and overt diabetes by 26 weeks of age.[15] Performed more than a decade ago, using Sprague-Dawley rats, this was one of the first animal models to reflect a metabolic profile that was characteristic of growth-restricted humans. Styrud *et al.* also used Sprague-Dawley rats and reported similar reductions in β-cell mass in male and female growth-restricted offspring, but glucose tolerance was normal at 12 weeks of age.[16] Differences in the glucose profile suggest that age-related increases in insulin demand may be necessary to reveal abnormalities in glycaemic control under states of β-cell deficiency. Conversely, using Wistar-Kyoto rats we have shown that growth-restricted males exhibit similar reductions in β-cell mass but do not develop diabetes even at 24 weeks of age.[21,22] One important distinction may be the rat strain, given the suggestion that Sprague-Dawley rats have a greater propensity for the development of metabolic diseases than Wistar rats.[23–26] However, the study by Nusken *et al.*[17] was performed using Wistar Unilever rats and, although pancreatic β-cell mass was not measured, growth-restricted male offspring developed impaired glucose tolerance and elevated glycosylated haemoglobin (HbA1c) levels by 15 weeks of age. In that study, the smallest six offspring from ligated litters were compared with the heaviest 12 offspring from sham-exposed litters.[17] Although likely to introduce selection bias, this approach remains valid if the question being asked lies purely in the effect of birth weight *per se* (rather than the effect of uteroplacental insufficiency).

"differences between cohorts are intriguing"

In all studies by other investigators in Table 1, litter size was manipulated to ensure uniformity across the groups[14–17] and, in some studies, pups were fostered onto unoperated mothers from birth until weaning.[15,17] Importantly, we have shown that reducing litter size impairs maternal mammary morphology and lactation and the subsequent postnatal growth and health of the offspring,[21,27,28] thus introducing an additional variable. Furthermore, cross-fostering growth-restricted pups onto sham-operated mothers completely prevented the development of hypertension and deficits in nephron endowment and partially ameliorated the impaired glucose tolerance that was otherwise evident in pups fostered onto another dam exposed to uterine vessel ligation.[29,30] These studies highlight the critical importance of lactation, particularly in altricial species such as rats, whereby much of their organ development persists into the early postnatal life. This early postnatal period in rats is likely to reflect late gestation in human pregnancies. As such, pups should remain with their natural mother throughout lactation in order to minimize environmental changes during this critical period of development.

Among our recent studies, despite the same experimental procedures undertaken to induce uteroplacental insufficiency and no culling or cross-fostering of pups at birth, we report a wide range of deficiencies in birth weight (from –10% to 32%), postnatal growth patterns (remain smaller or catch up) and disease outcomes in offspring (Table 1). It appears that a lack of catch-up growth and the development of hypertension in adulthood are associated with a greater degree of growth restriction at birth.[19,28] The differences in birth weight between cohorts of the same animal model are intriguing and challenge the comparisons between studies. Although we cannot eliminate the possibility that the magnitude of mechanical restriction of blood flow differed between the studies, the consistent and

Table 1 Differences in first generation (F1) growth and disease outcomes between animal studies of uteroplacental insufficiency

Rat strain/ reference	Ligation date	Study age	Sex	Birth growth deficit	Postnatal growth	Metabolic phenotype	Blood pressure phenotype	Renal phenotype
Sprague-Dawley[15]	E19	26 weeks	♂	15%	Caught up at 7 weeks and overweight thereafter	Impaired glucose tolerance from 7 weeks, elevated fasting insulin at 7–15 weeks and β-cell deficit from 15 weeks	–	–
Sprague-Dawley[14]	E14 (+ aorta clip)	12 weeks	♂	12%	Remained smaller	–	Hypertension at 4–12 weeks (carotid artery catheter)	–
			♀	12%	Remained smaller	–	Hypertension at 4–8 weeks (carotid artery catheter)	Normal renal function
Sprague-Dawley[16]	E16	12 weeks	♂	10%	Caught up at 1 week	Normal glucose tolerance but elevated fasting insulin and β-cell deficit	–	–
			♀	10%	Caught up at 1 week	Normal glucose tolerance and fasting insulin but β-cell deficit	–	–
WU[17]	E19	30 weeks	♂	30%	Caught up at 1 week	Impaired glucose tolerance, reduced fasting insulin at 7 weeks and elevated HbAlc from 15 weeks	–	Normal renal function
WKY[21]*	E18	24 weeks	♂	27%	Remained 10% smaller	Normal glucose tolerance and HOMA-IR	–	–
			♀	22%	Remained 8% smaller	Normal glucose tolerance but increased HOMA-IR	–	–
WKY[28]*	E18	22 weeks	♂	26%	Remained 10% smaller	–	Hypertension (tail-cuff)	Nephron deficit and glomerular hypertrophy (stereology)
WKY[22]*	E18	24 weeks	♂	17%	Caught up by 20 weeks	Normal glucose tolerance but increased HOMA-IR and β-cell deficit	–	–
WKY[39,50]*	E18	16 weeks	♀	10%	Caught up by 16 weeks	Normal glucose tolerance but reduced fasting insulin and β-cell deficit	Normal blood pressure (tail-cuff) but increased uterine and renal artery stiffness	Normal nephron number, glomerular size (stereology) and renal function
WKY[19]*	E18	12 months	♀	19–21%	Remained 6–10% smaller	Normal glucose tolerance and fasting insulin	Hypertension (tail-cuff)	Normal renal function
WKY[31,40]*	E18	12–13 months	♀	17%	Remained 4% smaller	Normal glucose tolerance and β-cell mass but reduced HOMA-IR	Normal blood pressure (tail-cuff and tail artery catheter)	Normal renal function

*Studies from our laboratory that include only growth-restricted animals that were not subjected to any postnatal challenge (i.e. cross-fostering, pregnancy).
E, embryonic day; HOMA-IR, homeostasis model assessment of insulin resistance; WKY, Wistar-Kyoto; WU, Wistar Unilever; –, not measured.

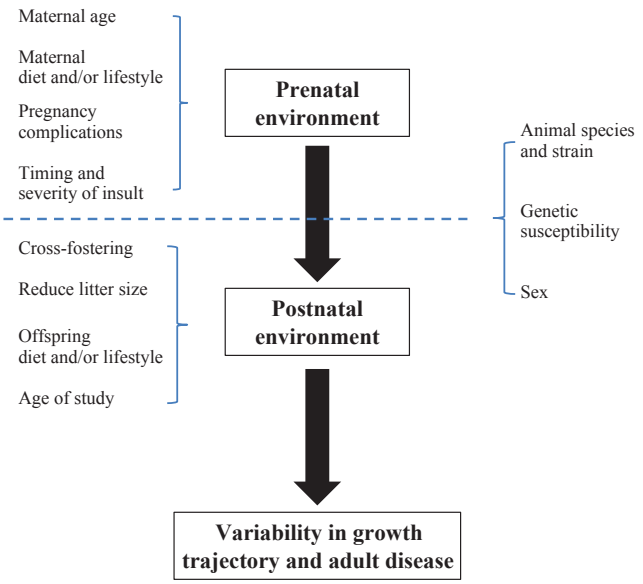

Fig. 1 Numerous variables can affect the prenatal and/or postnatal environment, contributing to the variability in growth trajectory and disease outcomes.

narrow variation in pup survival (litter size ~ 4–6) argues against this as a variable. Furthermore, there is no pattern with respect to the year the study was undertaken that may reflect changes in the maternal and/or offspring environment, such as food and water composition, diurnal cycles, room temperature and/or humidity.

Given that many of our disease outcomes are relatively subtle (i.e. mild hypertension and no overt diabetes or kidney dysfunction), differences in phenotypes may not be so surprising. Obtaining statistical differences in certain end-points, such as blood pressure, in two cohorts of 10 animals per group may simply come down to chance variation. This is supported by the fact that growth-restricted female littermates allocated to two different studies in our laboratory were discordant for the emergence of hypertension at 11–12 months of age.[19,31] It is worth noting that for all our studies only one offspring per sex is randomly allocated per study, whereas others select multiple pups from the same litter to make up the final sample size.[16,17] Thus, we have potentially introduced a wider genetic pool into each group, such that differences in fetal, and perhaps maternal, susceptibility to unfavourable pregnancy exposures is most certainly a discerning factor for our mild phenotypes. Although Wistar-Kyoto rats are an inbred strain and unlikely to have wide genetic variation between litters, future studies may consider the use of gene array technology to identify differences between offspring that go on to develop specific diseases compared with those that do not.

PROGRAMMED EFFECTS ARE NOT STABLY TRANSMITTED BETWEEN GENERATIONS

Evidence from both humans and animals exposed to undernutrition suggests that adverse prenatal exposures are not limited to affecting the first, directly exposed generation, but can impact on a number of generations.[32] At the end of World War II, the Dutch hunger winter allowed researchers to investigate the immediate and transgenerational effects of maternal undernutrition in humans. Individuals exposed to famine during prenatal development had an increased risk of developing cardiovascular disease and diabetes.[33–35] In the offspring of prenatally famine-exposed females, poorer health outcomes, including neonatal adiposity and autoimmune diseases, were reported, but there was no increase in the incidence of cardiovascular or metabolic diseases.[36] The recurrence of pre-eclampsia in women who themselves or their partner were born to pre-eclamptic mothers has been reported.[37] However, no human study has investigated the transgenerational effects of uteroplacental insufficiency *per se*.

"there is no consistency between generations"

Recently, using our rat model of uteroplacental insufficiency, we demonstrated normal birth weight and a biphasic postnatal growth trajectory in second generation (F_2) offspring from low birth weight mothers (slowed followed by accelerated until catch up).[38] The F_2 male and female offspring demonstrated blunted first-phase insulin response to a glucose load at 24 weeks, associated with reduced β-cell mass in males and increased β-cell mass in females.[38] In age-matched first generation (F_1) growth-restricted males, one study reported normal first-phase insulin secretion,[21] whereas another found reduced first-phase insulin secretion and deficits in β-cell mass.[22] The F_1 growth-restricted females consistently presented with normal first-phase insulin secretion at 16 weeks,[39] 24 weeks[21] and 12 months of age,[40] but β-cell mass was reduced at 16 weeks[39] and normal at 13 months of age.[40] In addition, F_1 nephron deficits observed postnatally in male[28] and female[39] growth-restricted offspring were not transmitted to the second generation.[41] However, the hypertensive phenotype was exacerbated in F_2 offspring compared with directly exposed F_1 growth-restricted adult males (difference in average systolic blood pressure between control and experimental groups: +16 mmHg in F_2 vs +9 mmHg in F_1). This observation was particularly surprising given previous reports of similar[42,43] or 'diluted'[44–46] transgenerational effects in different models. Thus, there appears to be no consistency in the adult phenotype between generations, which makes the prediction of disease a challenging task.

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Early life insults, through epigenetic modifications, allow for the induction of a large array of phenotypes from a single genotype.[47] In particular, modifications in cytosine methylation in the promoter region of genes that silence transcription and chromatin remodelling (methylation, acetylation and phosphorylation of histone proteins) may underlie the link between early life exposures and adult health outcomes.[48] Recently, others have demonstrated that hepatic epigenetic changes in response to a maternal low-protein diet were not stable between generations.[49] Taken together with the fact that programmed phenotypes often lack consistency between generations, it is likely that epigenetic alterations are established *de novo* in each generation. This may occur as a result of specific anatomical or physiological constraints in the growth-restricted mother, including small uterine size, raised blood pressure, hyperglycaemia and/or glucocorticoid excess that induce their own set of epigenetic modifications to somatic tissues of the offspring. Recently, we reported mild hyperglycaemia but normal uterine size, blood pressure and uterine artery stiffness in growth-restricted pregnant rats.[39,50] Although disrupted maternal glycaemic control is known to affect offspring health,[51,52] we cannot be certain, based on the evidence described previously, that maternal responses to pregnancy were similar for those that generated the F_2 offspring that we studied to adulthood.[38,41] However, at the time of the study, we were interested in investigating transgenerational effects without the confounding influence of maternal stress imposed through performing physiological measurements.[53] In support of this phenomenon, we reported that low maternal birth weight induced F_2 fetal growth restriction only when pregnant females were exposed to physiological stressors during late gestation (restrained tail-cuff blood pressure recording, intraperitoneal glucose tolerance test and 24 h in a metabolic cage).[39]

"dysfunction may stem from a Westernized diet"

The view of birth weight as a predictor of adult health outcomes may be too simplistic. This is supported by the numerous studies in a range of species, including sheep, rats and mice, demonstrating that short-term maternal glucocorticoid exposure induced hypertension in directly exposed F_1 offspring in the absence of growth deficiencies.[54–56] Therefore, the coexistence of low birth weight and adulthood disease, if present, is likely to result from distinct changes in response to adverse stimuli. Small size at birth, as a stand-alone determinant of adverse prenatal exposures, may therefore underestimate the influence of the pregnancy environment to the growing prevalence of chronic diseases in adulthood. This has major implications for the use of animal models in this field, because the majority of researchers aim to induce low birth weight.

CAUSES OF UTEROPLACENTAL INSUFFICIENCY

Through the use of animal models we have uncovered the complexity of this field and generated a plethora of unanswered questions. The impact of early life insults on adult health is certainly apparent, but the specific changes and magnitude of effects are what remain unclear. Continuing the study of offspring pathophysiology, including specific cellular pathways that have been compromised by prenatal insults, may lead to the development of tailored therapies. However, preventing the incidence of prenatal contributions to later life disease risk by identifying the original source(s) of uteroplacental insufficiency is of great interest to the field. Human pregnancies that result in offspring who, regardless of birth weight, go on to develop metabolic and/or cardiovascular diseases that are unexplained by genetics or obvious lifestyle challenges could be retrospectively screened for and associated with circulating factors in the mother and/or abnormalities during the pregnancy. This is no doubt an extensive task and it will be years from now that we gain any useful information from such a study.

Given that the percentage of low birth weight babies (defined as < 2500 g) in Australia has increased by 19.6% in the past 30 years,[57] we cannot discount the possibility that uteroplacental dysfunction, although largely of genetic origin,[58,59] stems partly from a Westernized diet. Certainly, others have shown that maternal high-fat feeding programs metabolic pathologies in rodent offspring that may be mediated by alterations in placental nutrient transfer.[60,61] In addition, in non-human primates, a maternal high-fat diet was associated with impaired uterine blood flow and increased expression of placental inflammatory cytokines that were independent of maternal obesity.[62] However, the mechanisms underlying how diet can influence placental function remain unclear. Indeed, saturated fats and processed foods are extremely high in a group of sugar modifications known as advanced glycation end-products (AGE). Pre-eclamptic women have elevated serum levels of AGE, as well as increased placental protein expression of AGE and its receptor RAGE, compared with healthy pregnant women.[63] Thus, it is likely that much overlap exists between adverse maternal diets and the incidence of uteroplacental insufficiency. Future studies should consider investigating the direct effects of a poor diet on uteroplacental function and fetal development to help trace back to the potential origins(s) of programming effects.

CONCLUSIONS

There is no doubt that animal models have enhanced our understanding of the influence of prenatal and early postnatal exposures on our long-term health. However, the variability in disease outcomes is wide ranging, even when the initial cause is

thought to be the same. This may be due to differences in the strain studied, the timing and severity of the insult (mid-, late or post-pregnancy) and/or early postnatal exposures (changes to the lactation environment). However, even when the same experimental model and techniques for measurement are used, disparities in birth weight, postnatal growth and disease development are discernible. More recently, the impact of adverse exposures *in utero* have been described as having far-reaching effects that extend well beyond the first, directly exposed generation. Organ-specific changes occur in the absence of perpetuating growth deficiencies and only sometimes mimic the phenotype of the exposed parent(s). These data highlight the complexity of interactions between our genetic potential and environment, and make for the translation of findings an incredibly challenging task. Finally, shifting our focus to the initial cause(s) of uteroplacental insufficiency, including genetic origins and the influence of poor diets, may prove invaluable to the progression of research in this field.

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