Pregnancy Complications and Offspring Cardiovascular Health

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***Abstract*—Large scale epidemiological studies have established that pregnancy complications, such as preterm birth and preeclampsia, have an important impact on cardiovascular disease risk in the offspring. Emerging data also suggest they are associated with other health outcomes, such as respiratory disease and pregnancy disorders. Detailed imaging and laboratory phenotyping studies have defined cardiovascular features that may underlie these associations and are identifiable in those born to complicated pregnancies, even when they reach adult life. These patterns include altered cardiac shape and function, microvascular dysfunction and disrupted angiogenic capacity. A broad range of experimental models have now been established, which replicate such problems as preterm birth, fetal hypoxia and maternal obesity, in order to investigate both mechanisms and potential therapeutics. This report summarises a selection of findings from epidemiological, experimental, human and basic science studies, reported within the last few years, which exemplify the rapid development of this field. The data highlights the attraction of pathological pregnancy as a major developmental research paradigm, that allows clear identification of those affected and, potentially, highly tractable targets to alter their cardiovascular disease risk.**

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I. Introduction

Realisation that pregnancy complications may be of central importance to associations between early life and later cardiovascular health has opened a new paradigm in developmental origins research. Offspring born following pregnancy complications, in particular preeclampsia and preterm birth, appear to carry a substantial increased risk of cardiovascular disorders during the first 30 years of life [1–5]. Their high incidence of, for example, early-onset hypertension suggests this area of research may have immediate clinical relevance [2, 4, 6–10]. Understanding mechanistic links between vascular disorders in mother and offspring may also open opportunities for insights into familial predisposition to cardiovascular diseases.

Research contributions to this field have occurred, independently, across a broad range of diverse specialties including epidemiology, cardiology, neonatology, obstetrics, physiology and the basic sciences. Therefore, a symposium was held to provide a rare opportunity for key researchers to present epidemiological and birth linkage studies, clinical follow-up and experimental models and engage in cross-disciplinary discussions. The objectives were to: discuss how pregnancy complications such as preterm birth and preeclampsia could associate with long-term cardiovascular changes and determine the likely clinical impact; gain new insights into quantification of cardiovascular phenotype using animal models, physiological tests and imaging modalities from newborn infants to young adults; and discuss priorities for future observational, experimental and interventional research.

II. Epidemiological evidence for links between pregnancy complications and offspring cardiovascular health

A. Hypertensive disorders of pregnancy and offspring health in ALSPAC

Between April 1991 and December 1992, more than 14,000 pregnant women were recruited into the Avon Longitudinal Study of Parents and Children (ALSPAC), also known as the 'Children of the 90s' study. Based at the University of Bristol, UK, the women (some of whom had two pregnancies or multiple births during the recruitment period), the children arising from the pregnancy, and their partners have been followed up intensively over two decades. One of the early findings was that patterns of blood pressure during pregnancy

differ, with women who develop preeclampsia having a more rapid increase from 30 weeks than those who develop gestational hypertension or had pre-existing essential hypertension. This distinct pattern of blood pressure therefore may identify women with a specific increased risk of severe hypertensive pregnancy disorders. The researchers went on to investigate the association between hypertensive disorders of pregnancy and offspring cardiovascular risk. In the offspring, at age 9 years [11], 11 years [12], and 17 years [5], both preeclampsia and gestational hypertension were associated with increased systolic and diastolic blood pressure.

Interestingly, maternal blood pressure at eight weeks gestation positively associated with offspring blood pressure in childhood. This may reflect a familial susceptibility to hypertension or the eight week measure may still not be early enough to differentiate pre-pregnancy from pregnancy-related factors. Associations between offspring blood pressure and maternal preeclampsia, but not gestational hypertension or essential hypertension, were also attenuated when adjusted for birth weight and gestational age, which suggests other markers of severity of pregnancy complications need to be considered in understanding risk of offspring hypertension. Interestingly, pregnancy hypertensive disorders did not relate to other measures of offspring cardiometabolic or vascular health at age 11 years or 17 years consistent with a specific hypertensive phenotype. Future studies will allow further exploration of whether the association with blood pressure is causal, such as through a direct intrauterine effect, as well as whether it is unique to preeclampsia or all hypertensive disorders of pregnancy.

B. Linkage studies of pregnancy complications in women born preterm

Boivin *et al* addressed a novel question of whether those born following pregnancy complications, specifically those born preterm, had, themselves, a higher risk of developing complications such as preeclampsia, gestational hypertension and gestational diabetes [10]. They studied the relation between preterm birth and later pregnancy complications, independent of intrauterine growth restriction, among women born in Quebec, Canada between 1976 and 1995, who delivered at least one newborn between 1987 and 2008. They also studied whether there was a dose-response relationship with women born more preterm having a greater risk of developing gestational diabetes, gestational hypertension, preeclampsia and eclampsia. The percentage of women with at least one pregnancy complication, at least once, during the study period increased significantly with decreasing gestational age at their own birth ($<$ 32 weeks = 19.9%; 32 to 36 weeks = 13.2%; 37 to 42 weeks = 11.7%, $P < 0.001$). After adjustment for various factors, including birth weight for gestational age, the odds of pregnancy complications associated with preterm birth was elevated by 1.95 fold (95% confidence interval: 1.54-2.47) among women born before 32 weeks gestation and 1.14 fold (95% confidence interval: 1.03-1.25) among those born at 32 to 36 weeks gestation relative to women born at term (37 to 42 weeks gestation). The results prompted the authors to suggest that, to understand the likely risk of pregnancy complications for women who present for obstetric care, the obstetrician should take into account the pregnancy history of the women herself.

III. Cardiac, vascular and metabolic changes in human offspring in relation to pregnancy complications

A. Vascular structure in preterm offspring and the natural history of hypertension in the young

Rate of elastin synthesis falls rapidly following birth, with little to no synthesis or turnover in adult tissue [13]. The early transition from the *in utero* to *ex utero* environment that occurs in preterm-born infants therefore is likely to lead to a premature change in the rate of connective tissue development before complete vascular maturation. In addition, the additional haemodynamic insult that the *ex utero* physiology imposes on the underdeveloped vascular system might be expected to lead to abnormal macrovascular development. Modern neonatal care has led to improved survival in extreme preterm birth and, as such, McEniery *et al* studied the vascular consequences of extreme prematurity in 11-year-olds born at or before 25 completed weeks of gestation in the EPICure study [14]. 219 extremely preterm-born adolescents ($<$ 26 weeks gestation) and 270 classmates born term ($\geq$ 37 weeks gestation), matched for age, gender and ethnic group, were included in the study. Haemodynamic measures were included at a later stage, and thus 70 of the preterm-born and 91 of the term-born adolescents had valid measurements. Interestingly, arterial pressure wave reflections were significantly elevated in the extremely preterm-born children ($P < 0.001$). However, arterial stiffness did not differ between groups (P=0.10) [15–17] and McEniery *et al* hypothesised that the differences in arterial wave reflection result from alterations in function of smaller preresistance and resistance vessels rather than larger elastic arteries [18–20].

B. Intrauterine growth restriction and cardiac structure in childhood

Intrauterine growth restriction (IUGR) affects 5-10% of newborns and is associated with increased cardiovascular mortality in adulthood. Crispi *et al* designed a prospective cohort study that included 80 case subjects with IUGR and 120 control subjects with birth weight appropriate for gestational age identified in fetal life and followed up into childhood.20 Cardiovascular assessment was performed at age five years [21]. Compared with control subjects, children with IUGR showed cardiac structural and functional changes, with a more globular heart, reduced longitudinal motion, decreased stroke volume and increased heart rate. The researchers have also shown in animal models that IUGR offspring have shorter sarcomere length and an altered intracellular myocardial structure, with a looser packing of mitochondria and increased cytosolic space between mitochondria and myofilaments [22]. Together, these findings suggest that IUGR induces primary cardiac changes that could explain the increased predisposition

to cardiovascular disease in adult life. As a result, further work to explore therapeutic strategies that have beneficial effects on cardiac remodeling in children with IUGR are likely to be of interest to reduce cardiovascular risk in this population.

C. Long-term cardiac and vascular impact of preterm birth

Lewandowski *et al* have developed a program of work to determine the impact of preterm birth and related perinatal events on the cardiovascular system in young adulthood [6, 7, 18–20]. 102 individuals born preterm between 1982 and 1985 have been prospectively followed since recruitment at birth to randomised feeding regimes. Using cardiovascular magnetic resonance and computational atlas formation, it was demonstrated that preterm birth is associated with a unique cardiac phenotype. Preterm-born young adults have an increase in left ventricular mass, which is inversely related to gestational age and independent of variation in blood pressure [6]. They also have shorter left ventricles, smaller internal left ventricular cavity diameters, and a displaced left ventricular apex compared to term-born controls. Furthermore, preterm-born young adults show distinct differences in left ventricular function related to preterm birth, which is significantly worse in those whose mothers had preeclampsia.

Young adults born preterm were also shown to have smaller right ventricles with increased mass [7]. The relative impact of preterm birth appears to be greater on the right ventricle than for the left ventricle. Importantly, the reduction in right ventricular systolic function is such that 21% of young adults born preterm have ejection fractions below the lower limit observed in adults born at term. A better understanding of the mechanisms underlying these changes in cardiac structure and function, and their clinical impact on later cardiac disease development, will help determine possible pathways to prevent future disease in the growing cohort of adults born preterm.

More recently, the researchers have shown that preterm-born individuals have increased circulating antiangiogenic markers in young adulthood and, of these, variation in sENG and sFlt1 are proportional to both resting and ambulatory blood pressure levels [19]. Of particular interest are the associations between sENG and blood pressure, which appear to be specifically related to degree of prematurity, rather than other perinatal factors, and mediated by differences in microvascular structure and function. sFlt1 levels are largely related to pregnancy-induced hypertension as a cause for preterm birth. Whether this pathway provides a modifiable target for future prevention strategies to delay the development of hypertension in individuals born preterm is of significant future interest.

D. Long-term lipid deposition, adiposity and metabolic risk in preterm offspring

The third trimester of pregnancy is a period of rapid adipose tissue distribution. Uthaya *et al* measured anthropometric indices and quantified subcutaneous, intraabdominal and total adipose tissue volumes using whole-body magnetic resonance imaging in 38 infants born at less than 32 weeks gestation, when they reached term, and 29 term-born infants [23]. Preterm-born infants at term, though significantly lighter and shorter than term-born infants, had increased relative adiposity, increased intra-abdominal adipose tissue, and decreased subcutaneous adipose tissue.

Interestingly, in young adults aged 18-27 years, the researchers have shown that, although not different from term-born counterparts in external physique, preterm-born individuals have significantly increased whole-body adiposity, altered adipose tissue partitioning, increased intrahepatocellular and intramyocellular lipids, and differences in the urinary metabolome, including elevated methylamines and acetylglycoproteins and lower hippurate [24]. More recently, the researchers have investigated determinants of ectopic lipid deposition in preterm-born individuals, given the known health significance and associations between elevated intrahepatocellular lipids, insulin resistance and adiposity in adult populations [25]. Importantly, they have shown that components of the preterm diet, particularly early lipid intake, may be relevant to the development of intrahepatocellular lipids at term age.

Determining the components of early nutrition that drive intrahepatocellular lipid deposition will be essential, as will establishing whether nutritional intervention strategies can improve long-term outcomes. Particularly as the researchers also performed a meta-analysis that confirms that preterm-born individuals have an increase in blood pressure in later life [4]. The meta-analysis included 27 studies, comprising a combined total of 17,030 preterm-born and 295,261 term-born adults, and showed that preterm birth is associated with significantly higher systolic and diastolic blood pressure in young adulthood.

IV. Experimental studies

A. Heart disease link to fetal hypoxia and oxidative stress

During pregnancy, one of the common features across pregnancy complications is the presence of fetal hypoxia typically related to the placental dysfunction seen in conditions such as preeclampsia, chorioamnionitis, placental abruption and praevia. Alternatively, direct interruption of blood supply to the fetus such as occurs with cord compression, abnormal presentation or prolonged labour can lead to fetal hypoxia exposure. Acute hypoxia promotes redistribution of cardiac output in the fetus and has led to the concept of the 'Selfish Brain' so that there are chronic reductions in cerebral vascular tone and increases in peripheral vascular resistance [26] [27] [28] [29]. The response of the fetal tissue to this redistribution is to generate an imbalance between pro- and anti-oxidant defences in areas of hypoxia and, it is speculated, lead to assymetric IUGR. To investigate the role of fetal hypoxia in the programming of cardiovascular disease and whether any long-term effects are reversible a series of experiments have been performed in rats exposed to chronic hypoxia during pregnancy. In this model, it was found that those offspring exposed to maternal hypoxia (14% O_2 from day 6 of pregnancy had altered appearances of the fetal heart and aorta that could be rescued if antioxidants were given to the mother at the same time as hypoxia exposure. Mechanistic studies of vascular responses in femoral artery segments mounted in vitro with wire myography and exposed

to phenylephrine and metacholine, similarly, demonstrated persistent reductions in vascular responses distant from the pregnancy in offspring exposed to hypoxia. Again this could be rescued by simultaneous antioxidant provision [30].

Translation of these findings to human pregnancies requires larger animal model studies. This is being facilitated by work in Cambridge, UK on the ovine model, which has been made possible due to a purpose built in vivo materno-fetal whole animal hypoxia chamber. In this facility, wireless pressure and flow data can be acquired. In proof of principle of the ability of this infrastructure to provide information on modification of fetal and maternal physiology in response to hypoxia, work has demonstrated that melatonin and vitamin C can increase umbilical blood flow via nitric oxide dependent mechanisms, with Vitamin C leading to an increase in transplacental partial pressure gradient for oxygen [31]. Further work has used an experimental protocol of altered chamber pO_2, proven with maternal blood gas analysis, to demonstrate chronic fetal hypoxia is induced and leads to IUGR in this model. Furthermore, in adulthood, the sheep offspring exposed to hypoxia have increased mean arterial pressure, left ventricular free wall size and altered aortic collagen content, which is prevented with co-administration of vitamin C during pregnancy. Wire myography studies confirm that in this ovine model differences in vascular responses, as observed in the rat model, also persist into adulthood.

In conclusion, prenatal hypoxia may be an important trigger for the early origins of cardiovascular disease that exerts an impact on cardiovascular disease due to alterations in oxidant balance in utero. Furthermore, in experimental models, effects of hypoxia on cardiac and vascular structure and function can be reversed when there is co-administration of vitamin C to the mother during hypoxia exposure.

B. Transient neonatal high oxygen exposure and cardiovascular changes in early adult life

Preterm-born neonates are known to have decreased antioxidant defences and, upon delivery, are exposed to high oxygen concentrations compared to in utero conditions. As such, Yzydorczyk *et al* first tested the hypothesis that neonatal oxidative injury causes long-term vascular damage and leads to hypertension, with related damage to the renal system [32]. To do this, Sprague-Dawley rat pups were exposed to transient high oxygen levels in early neonatal life and were followed up into adulthood. The experiments demonstrated abnormal vessel structure and function in those exposed to high oxygen levels compared to controls, including: microvascular rarefaction; vascular oxidative stress, demonstrated by eNOS uncoupling and increased expression of NADPH oxidase [33]; increased vascular stiffness; vascular remodeling [34]; and vascular dysfunction, demonstrated by increased vasomotor response to angiotensin II [32]. In addition, high oxygen exposed rats had impaired nephrogenesis and nephropenia [35]. The authors concluded that these changes in vascular and renal physiology likely participate in the observed hypertension in adults.

The researchers have since extended their model to determine whether transient neonatal high oxygen exposure alters heart development, determining the programming of cardiac dysfunction [36]. Bertagnolli *et al* first demonstrated that transient neonatal exposure leads to cardiac hypertrophy, impaired myocardium contractility, diastolic dysfunction and impaired myocardium relaxation in adulthood [36]. Adult rats exposed to high oxygen also displayed an increased susceptibility to develop heart failure under angiotensin II infusion. Cardiac alterations induced by high oxygen exposure impair the heart capacity to adapt to pressure overload in adulthood, determining rapid progression to heart failure. Interestingly, the authors also demonstrated that cardiac remodeling and dysfunction are present prior to elevation of blood pressure in rats exposed to neonatal high oxygen. Finally, it was shown that adult rats have increased cardiac fibrosis, enhanced ratio of angiotensin II type 1 to type 2 (AT1/AT2) receptors expression, and upregulation of senescence-associated proteins. The research team is now investigating whether specific pharmacological treatments are able to prevent cardiac alterations induced by neonatal high oxygen exposure.

C. Altered angiogenesis and renal function in low birthweight and preterm infants

Offspring of complicated pregnancies typically exhibit vascular dysfunction in both experimental and human studies [15]. A reduction in function and number of circulating endothelial colony forming cells (ECFCs) - important for the maintenance of endothelial responses, in part, due to their role in endothelial repair - has been found to be associated with a range of different cardiovascular diseases including diabetes, renal failure, atherosclerosis and preeclampsia. Analysis of cord blood from pregnancies with different complications including IUGR and preterm birth has highlighted that ECFC number and function are also altered in the fetal circulation in these conditions [37] [38]. In low birthweight preterm infants, there is a striking reduction in angiogenic potential of ECFCs and they are switched towards an angiostatic phenotype. Recently, preterm birth has been associated with ECFC dysfunction due to a stress-induced premature senescence driven by decreased expression of SIRT1, which can be salvaged with resveratrol treatment [39]. Circulating factors may be of importance in this ageing-associated senescence, as ECFC proliferation and migration is also significantly reduced when ECFCs are cultured with serum from low birthweight neonates. This serum has also been found to have low VEGF concentrations [40]. These studies have introduced for the first time the concept that pregnancy complications are associated with changes in angiogenic capacity of the offspring. Whether, and how, these biological differences relate to future risk of cardiovascular disease requires further study.

D. Programming of cardiovascular dysfunction by maternal diet-induced obesity

Obesity represents a major health problem with a dramatic rise in obesity across the globe. As a result, an increasing proportion of women are now obese during pregnancy. Follow up studies of offspring born to obese mothers suggest this

phenomenon is associated with increased risk of cardiovascular disease. In a Finish cohort of 3302 males [38], risk of cardiovascular mortality increased with maternal BMI, and in the Aberdeen maternity dataset of 37709 offspring, maternal obesity was associated with both the number of cardiovascular events and risk of premature death [41]. Animal models of maternal overfeeding support a direct causal mechanism for these associations. In a maternal overfeeding ovine model [42], the offspring had increased fetal cardiac weight, fibrosis and stress signalling with reduced contractility. Rodent models based on high fat feeding during pregnancy, as well as throughout lactation, have offspring with increased blood pressure and metabolic disorders [43] [44] [45] [46]. A specific rodent model of maternal obesity is possible in which dams are fed a high fat, high sugar diet from prior to mating and through pregnancy and lactation. This is associated with up to 40% increase in fat mass in the dams. By eight weeks of age [47], the offspring, despite similar body weight and composition, have significantly greater heart weight (127mg vs 154mg) and are hyperinsulinaemic, with reduced cardiac insulin receptor activity.

When does this cardiac hypertrophy develop? Serial measurements of rodent cardiac size following delivery indicate heart weight is already increased in both the left and right ventricle by 3 weeks of age and occurs due to an increase in cardiomyocyte cell area alongside re-expression of cardiac fetal genes including NPPB, MYH7, MYH6 and ACATA1. In an isolated heart perfusion study of 12 week old offspring significant changes in cardiac function are evident with abnormal chronotropic and ionotropic responses. These changes appear to relate to an alteration in sympathetic:parasympathetic ratio, with a sympathetic dominance according to the expression of beta-1 adrenergic receptor compared to acetylcholine receptor protein. The cardiac dysfunction appears to be driven by altered SERCA-linked calcium handling, similar to that observed in a mouse model of dilated cardiomyopathy, which is currently the subject of an on-going clinical gene therapy trial of SERCA replacement to restore cardiac function. These experiments support the hypothesis that maternal obesity leads to pathological cardiac changes in offspring. Future intervention studies such as use of metformin or exercise in obese mothers may provide an opportunity to explore these associations further in humans and determine whether these changes are reversible [48, 49].

V. Approaches for prevention

Prevention of cardiovascular disorders in offspring of complicated pregnancies is at present poorly managed. For some time, the designs of trials during pregnancy have been focused on the concept that increases in birthweight and growth should be the primary aim. A recent commentary suggested ?For doctors, the pertinent question is whether these early effects can be modified. Can we identify interventions that might improve the early life environment and promote ?normal? growth trajectories[50]? However, a systematic review of RCTs designed to reduce growth restriction suggested they were able, on average, to increase birth weight by 100 grams compared to the 1kg change in weight required to decrease systolic blood pressure by 1 to 3 mmHg [51].

It is possible, from a lifecourse perspective, that a timely early intervention that generates a small change in body size may have a larger impact on later disease [52]. However, this remains to be proven. To judge the impact of early interventions in this life course approach alternative outcome measures may be required, which go beyond fetal growth and judge modification of underlying vascular changes. One possibility is to use intermediate phenotypes of disease such as endothelial dysfunction or accumulation of vascular damage, as characterised by arterial wall thickness. These vascular measures are known to differ in early life and those born small for gestational age have an increased maximum aortic intima media thickness (IMT) compared to control subjects (810 micrometres vs 743 micrometres, P=0.02) [53].

One potential therapy of interest is dietary omega-3 fatty acids [54, 55]. Omega-3 fatty acids are known to improve vascular health in high risk groups such as smokers and those with metabolic disorders, although meta-analysis of RCTs for cardiovascular prevention have suggested there is no overall benefit [56]. However, it is possible response differs with birthweight. In the Childhood Asthma Prevention Study (CAPS) study, in which infants and children were randomised to omega-3 supplementation, carotid IMT was related to birthweight only in those who did not receive supplements, suggesting supplements may have modified this association [57]. There is also evidence that progression of carotid IMT over a six year period is inversely proportional to the amount of omega-3 fatty acids consumed each day by study participants, but only if they had evidence of IUGR [58]. Blood pressure was also lower in those with IUGR who had higher intakes of eicosapentaenoic acid and docosahexaenoic acid [59].

A 50% difference in daily intake of alpha linolenic acid (plant-derived omega-3 fatty acid), equivalent to the omega-3 present in 1 to 2 walnuts, was associated with a 0.05 mm reduction in carotid IMT [60]. If extrapolated to other cardiovascular prevention RCTs, this reduction in IMT is seen with 4 years of statin use or 8 years of anti-hypertensive treatment in adults. Omega-3 fatty acids may therefore represent a potential candidate to be included for recommendation in cardiovascular prevention guidelines for those born small for gestational age. The role of supplementation in other pregnancy complications remains unknown.

VI. Conclusions

Pathological pregnancy as the focus of research to identify tractable targets to alter risk of cardiovascular disease in mother and offspring is, arguably, the most exciting new paradigm in developmental origins disease research to emerge within the last two decades. Stratifying prevention advice based on pregnancy history may have immediate relevance to the clinic. For example, 1 in 5 of those born following a pregnancy complicated by preeclampsia or whose mother had pregnancy hypertension severe enough to result in preterm birth, exhibit blood pressures within a hypertensive range by the age of 20 years3. Pregnancy complications may also

be of value for identification of those with other adverse health outcomes, in particular risk of developing pregnancy complications themselves. Those who wish to study the impact of pathological pregnancy on the offspring have had access to a range of experimental models, from small to large animals, and have clear clinical guidelines to define at risk populations in human cohorts. The emerging research from these studies has now identified specific alterations in vascular and cardiac structure and function, with intriguingly consistent differences in angiogenesis and microvascular development. Remaining areas to be clarified include the relative importance of preterm birth versus preeclampsia versus other pregnancy disorders, such as IUGR, in determining offspring phenotype. Furthermore, the specific role of in utero exposures, such as hypoxia or maternal obesity, versus familial predisposition is still under exploration. The next phase of work will need to consider the most appropriate interventions to modify phenotype but, when established, it should be possible to apply these approaches easily in young human populations to reduce cardiovascular risk.

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