**REVIEWs**

**Epidemiological challenges in studying the fetal origins of adult chronic disease**

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The relatively new research field of the fetal origins of adult disease has matured greatly in the past decade. Recent systematic overviews provide strong evidence that robust inverse epidemiological associations exist between birthweight and later blood pressure/hypertension and glucose intolerance/type 2 diabetes. Consistent associations are also evident for the inverse associations of birthweight with coronary heart disease incidence and mortality. Studies with high follow-up rates and adjustment for social and economic variables have to a large extent mitigated earlier criticisms that observed associations were highly subject to confounding and selection bias. Many questions remain, however, about the contributing roles of postnatal growth and development, underlying biological mechanisms, and the importance of the epidemiological findings to public health. This review outlines current challenges—stated in the form of propositions—that epidemiologists in this field currently face.

1. The Lifecourse Approach Helps PutFetal Influences into Perspective

The lifecourse approach to chronic disease posits that fetal life is one important period of development, but not the only one, in determining susceptibility to adult chronic disease. Many studies have now shown that associations of birth size with later cardiovascular outcomes are modified by body mass index in adulthood or, indeed, in childhood. The highest risk is typically among adults who were born small and become overweight during childhood or adulthood. Examples of outcomes that fit this profile include insulin resistance in Indian 8-year-old boys and girls, blood pressure levels among 50-year-old Swedish men, and incidence of coronary heart disease among middle to older age Welsh men. In another example, body mass index at age 11 appeared to modify the association of ponderal index at birth and a decrease in ponderal index from 6 to 18 months of age with ischaemic heart disease in the first 7 years of life. In contrast, in a small group of Chinese adults, both lower ponderal index at birth and an increase in ponderal index from 6 to 18 months of life were related to increased systolic blood pressure level at age 30 years. Moreover, in two recently reported studies from the UK, catch up growth in the first 6 months of life was not clearly related to young adult blood pressure at all, although birthweight was. In another study, growth patterns exemplified by lower birthweight followed by reduced growth until age 1 seemed to confer the highest risk for ischaemic heart disease later in life, but the analysis was confined to men. These disparate results call for additional serial growth data to determine which periods of growth contribute most to the observed interactions between fetal and postnatal size in determining health outcomes.

A second issue is that catch up growth traditionally refers to the first 6–12 months after birth, not later growth. The standard interpretation is that catch up growth represents realignment of one’s genetic growth potential after intrauterine growth restriction. One advantage of this interpretation is that it allows for fetal growth restriction at any birthweight—even large fetuses can be growth restricted relative to their genetic potential—thus obviating the need for arbitrary definitions such as ‘small for gestational age’. This distinction is important in the fetal origins of adult disease, since observed associations of birthweight with later outcomes generally span the entire birthweight spectrum, not just at the ‘low birthweight’ end. The major problems, however, are in the measurement of catch up growth. Some investigators resort to a post hoc, empirical definition, using the observed infant growth patterns relative to birthweight. A second possibility is that mid-parental height gives a good
estimate of the child’s growth potential, but usually for height, not relative weight, and the estimation is better after 2 years of age than in infancy. Third, while needing confirmation, recent data raise the possibility that levels of maternal insulin-like growth factor 1 during pregnancy may reflect fetal growth restriction. A final issue with measuring catch up growth is that even small increases from influences occurring at various points in infancy, childhood, and later. They will also benefit from a clear definition of catch up growth for its traditional meaning of realignment to genetic potential during infancy.

2. Adjustment for Achieved Body Mass Index, Despite Being Controversial, Is Often Helpful

While the points above concern effect modification by achieved body size, this section addresses adjustment for it. Some epidemiological associations, for example the inverse relationship of birthweight with blood pressure, appear stronger—or in some studies, appear only—after statistical adjustment for adult (or childhood) body mass index. How, or even whether, to make such adjustments is controversial. Some of the controversy seems to emanate from the fact that epidemiologists from a variety of fields—perinatal, cardiovascular, paediatric—with different lexicons, are converging on the same research topics. Also, perinatal epidemiologists have traditionally been concerned with avoiding ‘low birthweight’. In fact, increasing birthweight at this end of the spectrum, primarily by avoiding preterm births, would reduce neonatal and infant adverse outcomes. From this perspective, birthweight is an entity in and of itself, with aetiological significance. In contrast, in the field of fetal origins of adult disease, birthweight is not a monolith. Not all determinants of birthweight are salient, so increasing birthweight is not necessarily helpful. Further, this new paradigm is concerned with the entire spectrum of birthweight, rather than just the lower parts of the distribution. Adjusting for later body size is therefore appropriate; it can elucidate underlying aetiological pathways.

Figure 1 demonstrates this argument, using the example of blood pressure as an outcome. If it is true that (1) birthweight is positively related to later body mass index; (2) birthweight is inversely related to later blood pressure—the association of primary interest, and (3) body mass index is positively related to contemporaneous blood pressure (a well-known relationship), then the prenatal factors explaining pathways #1 and #2 (labelled Factors A and Factors B, respectively) are almost surely different. Adjusting for body mass index could elucidate some of the factors underlying the inverse birthweight-blood pressure association, and is thus potentially useful.

One empirical example that serves to illustrate this point is the relationship between birthweight and development of type 2 diabetes. Unadjusted for attained body mass index, the shape of the relationship is a ‘U’ or reverse ‘J’, with increased risk at both ends of the birthweight spectrum. But this observation obscures the fact that there are probably two underlying mechanisms. At high birthweights, maternal gestational diabetes is causing obesity and excess diabetes risk in the offspring. Adjusting for attained body mass, however, reveals an inverse and linear association across the birthweight spectrum, reflecting a second pathway with reduced risk at higher birthweights, a pattern sometimes attributed to the influence of a ‘thrifty phenotype’.

3. Social and Economic Factors Are Not Just Confounders

Many decades span the time from birth until cardiovascular diseases declare themselves. Most of these diseases are related to both lower birthweight and poorer socioeconomic circumstances in adulthood. Thus controlling for these circumstances seems necessary to isolate prenatal influences. On the other hand, for some social class is stable across generations. Under these conditions, the same socioeconomic factors that affect postnatal life may affect prenatal life as well, and could thus determine the putative exposures under study. In this view, the social and economic factors are part of the causal pathway, both before and after birth.

For others, social circumstances change over time. Depending on the disorder, the place, and the era, societal factors can have impact in different points in the lifespan. In the UK in the
late 20th century, for example, low childhood social class is more strongly associated with risk of stroke than is adult social class. For coronary heart disease, however, childhood and adulthood social factors appear to play equal roles.27

The challenges for future analyses are to consider the explanatory as well as confounding effects of social and economic factors; and to take into account changes in these factors across the lifespan. As these effects are being clarified, it will be important to assess how such societal factors affect physiological mechanisms underlying the epidemiological phenomena. Some factors may operate through their associations with behavioural factors. For example, maternal diet may differ by social class. In contrast, other societal factors may directly affect biological responses; for example, accumulated stress may influence the hypothalamic-pituitary-adrenal axis independent of lifestyle choices. Alternatively, the two kinds of pathways may actually work in concert with each other. In rodent models, stress during gestation causes reduced levels of maternal licking/grooming of her offspring, which are in turn related to levels of offspring stress reactivity through effects on systems that regulate activity of corticotropin-releasing hormone.28

4. Fetal Growth and Size at Birth Are Not Synonymous
In studies that can separate length of gestation from overall birthweight, most observed associations between birthweight and later cardiovascular outcomes are not due to length of gestation.29 Epidemiologists have called the remaining factor in birthweight ‘fetal growth’. However, fetal size at birth, adjusted for length of gestation, is only a snapshot of the trajectory of fetal growth throughout gestation.

Some, but clearly not all, epidemiological studies have found stronger associations with later outcomes for thinness or shortness at birth, or even abdominal or other body circumferences, rather than birthweight itself. Authors have hypothesized trimester-specific perturbations of growth of the fetus or particular organs to explain these phenomena.30 Whether such hypotheses have merit cannot be fully addressed with crude measures of fetal size at birth, considering not only the measurement error inherent in length and circumference measures, but also the non-specificity of even accurate measures at birth for determining fetal growth trajectories.31,32

Ongoing animal studies of fetal growth will continue to inform the epidemiology. In addition, one way studies of humans can be refined is to take advantage of serial ultrasound measures to measure fetal growth parameters throughout pregnancy. For example, preliminary data from one study suggested that femur length at 24 weeks was more strongly inversely associated with blood pressure at age 6 years than were either head or abdominal circumferences.33 In that study, however, changes in ultrasound measurements from 18 to 38 weeks were not clearly related to the outcome.

In addition, the dearth of data linking reduced gestational age at birth with adult health outcomes is chiefly a function of the historical data available to date, not necessarily because the link is absent. When most of the participants in the currently published long-term studies were born, relatively few preterm infants survived. To what extent gestational age predicts adult outcomes is an unresolved question, but an important one. In general, determinants of preterm birth and fetal growth are not the same.34

5. Some Determinants of Fetal Growth Will Be Red Herrings
While the observed epidemiological associations appear to implicate fetal growth as a major factor, overall fetal size at birth is an amalgam of many determinants. Some of these determinants may be related to susceptibility to adult disease, and others may not. Conversely, some prenatal determinants of adult outcomes may not be related to fetal growth. While investigations of fetal growth are likely to reveal some underlying processes, the need exists to investigate more directly prenatal determinants of postnatal outcomes, irrespective of effects on birthweight.

One example of size at birth potentially being a good proxy for an underlying causal pathway derives from the hypothesis that a congenital nephron deficit underlies predisposition to hypertension in adult life.35 A recent study shows that kidney volume is smaller in adults who were thinner at birth, even after adjustment for current body size.36 In contrast, a potential example of a prenatal exposure causing postnatal disease without effects on birthweight is the newly described association of paracetamol use by mothers in late pregnancy and risk of asthma in their preschool age offspring.37 Maternal smoking during pregnancy is a good example of a prenatal exposure whose deleterious effect on fetal growth is well-known but whose influence on long-term offspring health is uncertain. Perhaps investigation of the seemingly paradoxical protective effect of smoking against the development of pre-eclampsia38 will offer insights.

6. Both Genes and Environment Are Important. Even Distinction Between the Two Is Difficult
Many critics of the ‘fetal origins hypothesis’ have taken to task the epidemiological studies for not accounting for possible genetic explanations. Although rare genetic mutations do not explain the findings of population-based studies, they may provide a paradigm for understanding. For example, a rare mutation in the glucokinase gene causes drastically reduced birthweight and offspring insulin resistance.39 No analogous common allelic variation has yet been discovered, but it is possible that some exist to help explain the observed phenomena.

In addition, maternal, paternal, and fetal genes may all play roles. While inherited fetal gene expression can underlie susceptibility to disease, it is also likely that the maternal genome codes for substances that affect the fetal environment. As such, what is genetic for the mother is environmental for the fetus. One example of this principle is that it appears that the maternal thermolabile variant of methylenetetrahydrofolate reductase may be associated with the risk of neural tube defects in the offspring, even in the face of a normal fetal genotype.40

This consideration also highlights the close relationship between genes and environment and the need to examine interactions between them. Not only can maternal gene expression alter the fetal environment, but it is also possible that the maternal gestational environment can affect fetal gene expression.
For example, in a mouse model, feeding the mother foods with different quantities of methyl donors can alter offspring coat colour through modification of gene expression. More substantial progress in humans will occur when investigators can assess interactions among environmental factors and candidate genes as their identities become available. Ultimately, there will be probably be less distinction between genes and environment than most researchers currently consider.

7. Maternal Nutrition Does Not Equal Fetal Nutrition

Scientists and the public have been confused by statements about ‘undernutrition’ explaining the observed epidemiological phenomena. Except at the extremes of intake, maternal energy and macronutrient intake have relatively little impact on birthweight. In addition, little or no firm evidence suggests that maternal intake of total energy or macronutrients during pregnancy, or maternal body composition, have substantial impact on offspring risk of chronic disease. In fact, some empirical results seem diametrically opposed to each other. For example, results of one abstract presented at the 2001 World Congress on the fetal origins of adult disease suggested that a high dietary protein/carbohydrate ratio in the third trimester was associated with lower offspring blood pressure, whereas the authors of the next presentation observed higher blood pressures among offspring of mothers consuming a relatively high protein, low carbohydrate diet in late pregnancy.

In contrast, what is often termed ‘fetal nutrition’ is likely to be vital. The oxygen and nutrients that support fetal growth and development rely on the entire nutrient supply line, beginning with maternal consumption and body size, but extending to uterine perfusion, placental function, and fetal metabolism. Interruptions of the supply line at any point could result in programming the fetus for future risk of cardiovascular disease. Some of the confusion arises because a common animal model for interrupting the supply line is to alter maternal gestational intake. Interrupting the supply line at other points, say by underperfusing the placenta, can have analogous effects. One should use the animal experimental data only as models, as they are intended.

The placenta is likely to be a key to understanding associations of fetal growth with adult health, owing to its inherent endocrine function as well as its metabolic and transfer capacities. The lack of good animal models for placentation has hampered understanding of the pathways by which the fetus receives nutrients and oxygen from the mother, and what processes alter these pathways. Possible ways to study placental function in population-based studies include placental morphological pathology, as well as using specimens of placenta, maternal prenatal blood or other biological specimens, and umbilical cord blood to measure markers of altered blood flow, endocrine and transport characteristics, or activity of specific enzymes. These biological specimens can also be used to investigate other mechanisms underlying the observed epidemiological findings, including maternal and fetal endocrine pathways, inflammatory and immune function, and changes in the central and peripheral nervous system, among others.

Still, researchers should not dismiss the possibility that maternal dietary intake could be an important supply line component in humans. In investigating maternal dietary impact, investigators should concentrate on pathways that could involve specific foods and micronutrients in addition to energy and macronutrient intake, which appear to be relatively unimportant on their own. For example, recent data from 27 pregnant women suggest that while overall gestational protein intake is unimportant, relative partitioning from protein oxidation to deposition, perhaps related to micronutrient factors, is associated with birthweight. Relevant examples from perinatal epidemiology include that fatty acids of the n-3 family prolong gestation and reduce the incidence of pre-term birth, and inadequate folic acid intake causes neural tube defects. Future research may reveal that fatty acids, folate, or other nutrients may also be involved in programming of chronic disease risk.

8. New Epidemiological Study Designs Will Help

Many of the extant epidemiological findings have emanated from historical cohort studies, taking great advantage of serendipitous clinically recorded perinatal variables. The next generation of studies, however, should overcome the limitations of the earlier studies. These limitations include lack of research quality measures, limited information on covarying factors, and absence of biological specimens. Although no one study can address all methodological issues, three types of studies will be especially valuable in the coming years:

1. New prospective studies of pregnant women (including ones that even start pre-conceptionally) that follow their offspring over time, collecting biological samples to explore mechanisms, as well as research quality epidemiological data. These have the advantage of collecting detailed prospective data and examining childhood cardiovascular risk factors and diseases such as asthma, but must wait decades for adult disease outcomes to occur.

2. Follow-up of adult cohorts in which biological samples and prospectively collected epidemiological data are available from the past. These have the advantage of research quality data and emerging disease outcomes, but may lack important exposure or covariate information, such as maternal diet during pregnancy.

3. Follow-up into childhood or adulthood of randomized controlled trials conducted during pregnancy. These have the advantage of isolating an environmental ‘insult’ during pregnancy and so can come close to proving that programming occurs. However, this type of study is limited to examining a single or limited number of exposures.

9. To Make Policy Recommendations Now Regarding Fetal Programming Is Dangerous

Strong evidence exists for programming in humans. First is the robust epidemiological findings of associations between size at birth and later health outcomes. Second is the experimental animal evidence that in utero environmental insults produce lifelong alterations in metabolism, physiology, and pathology,
whether or not the exact perturbations have analogy in human systems.

One area of great importance is implications for developing countries undergoing the epidemiological transition from infectious disease to chronic disease, chiefly cardiovascular disease, as well as the ‘nutrition’ transition from an active, low-calorie lifestyle to a sedentary, high-calorie lifestyle.\textsuperscript{56} Available data indicate that a lower birthweight combined with higher attained body mass confers the highest risk for cardiovascular disease. Successive generations of individuals in developing countries are likely to have ever larger proportions with that high-risk profile. Efforts to prevent development of obesity in areas undergoing the epidemiological and nutritional transitions are paramount.

It is premature to make policy recommendations regarding fetal programming. Since birthweight is only a marker for undernutrition in young children and that high-risk countries undergoing the epidemiological transition are likely to have ever larger proportions with that high-risk profile. Efforts to prevent development of obesity in areas undergoing the epidemiological and nutritional transitions are paramount.

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