CHAPTER 3

Studies of Twins:
What Can They Tell Us about the Developmental Origins of Adult Health and Disease?

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Abstract

There is still limited understanding of the causal pathways underlying the observed association between exposures during fetal life and later health and disease in humans. Without better understanding we cannot estimate public health implications and assess the potential for intervention.

Study of twins should help us understand more about the role of factors shared by both twins versus factors affecting the individual fetus, the role of genetic factors, the role of placental factors, and which aspects or consequences of postnatal growth are associated with increased risk of later cardiovascular disease.

Generalisability of data from twin studies is open to question, but there is evidence that birth size—cardiovascular disease risk associations are similar in twins to those generally observed in singletons, suggesting that similar causal pathways are involved and study of twins will be informative.

There is an extensive body of literature relating to the association between size at birth and risk of later disease " the fetal origins of adult disease hypothesis",[1] and increasing understanding that growth throughout the developmental phase of life may be important. However, understanding of the underlying causal pathways is still limited and we need to understand these before we can estimate public health implications and assess the potential for intervention.

We consider:

1. Possible causal pathways underlying these observations.
2. How study of twins might shed light on these causal pathways.
3. Whether findings in twins are generalisable.

Possible Causal Pathways

Figure 1 shows some of the possible pathways and factors that may be involved in links between early development and risk of later disease.

Maternal Factors

Some maternal factors (A) will not affect later health. Others (B) may influence fetal growth and birth size, leading to risk of later disease. In other cases (C) gestational exposures may

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Early Life Origins of Health and Disease, edited by E. Marelyn Wintour and Julie A. Owens.

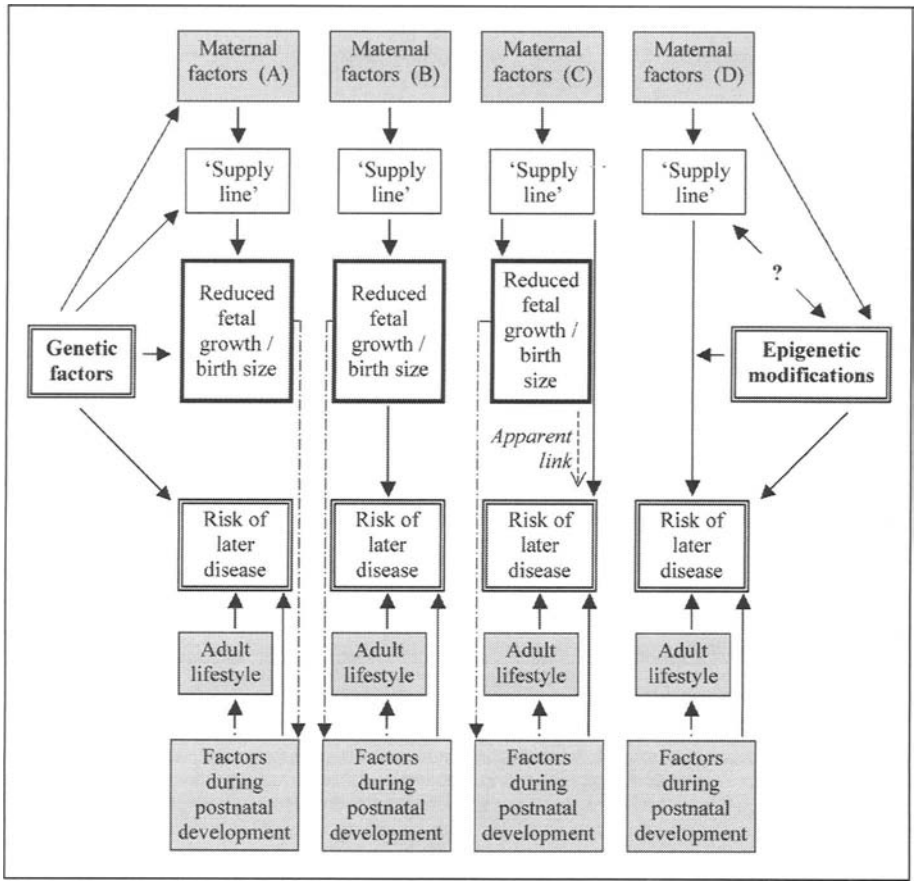

Figure 1. Pathways that may be involved in links between early development and later disease risk. Shading denotes factors that are currently potentially modifiable.

influence both fetal growth and disease risk, so there is an apparent link between size at birth and risk of later disease. Finally, some factors may influence disease risk with no influence on birth size.[2,3] Maternal factors may be of any of these types, and it is possible that the same exposure could act in more than one of these four different ways, depending on the timing, severity and duration of the exposure, and the risk factor or disease outcome examined. Evidence from the Dutch famine provides some support for this concept (summarised in ref. 2).

The "Supply Line" from Mother to Fetus

Blood supply to the uterus and placental development, health and function are important factors in the fetal supply line and in the human it is likely that intrauterine growth restriction relates to placental abnormality or insufficiency rather than maternal nutritional factors.[4] The placenta transports oxygen and nutrients from the maternal to the fetal circulation, metabolises key nutrients that are then supplied to the fetus (e.g., amino acids, especially glycine, and the active form of vitamin D) and produces hormones like placental lactogen and growth hormone that may influence fetal and maternal nutritional supply.[5] It also acts as an important barrier between maternal and fetal circulations, with enzymes that reduce fetal exposure to, for example, maternal glucocorticoids[6] and androgens,[7] as well as some xenobiotics.[8]

Post-natal factors, including nutrition and lifestyle factors, are clearly important determinants of health.[9] There has been recent interest in the role of postnatal growth rate as a determinant of later overweight/obesity, other cardiovascular risk factors, or adult cardiovascular disease. However, there is some inconsistency in the now extensive literature on this subject. For example, men with CHD had similar birthweight but lower weight at a year than others in a British cohort[10] and in a Swedish cohort low weight gain in the first year was associated with increased risk of CHD.[11] Conversely there are publications demonstrating that accelerated growth in infancy is disadvantageous,[12-14] and increased growth or size during childhood or adolescence are disadvantageous.[15-18]

Genetic Factors

Genetic endowment may be linked to both birth weight and later disease risk, so that there is an apparent link between birth weight and disease risk.[19-21] Since the placenta is part of the conceptus, genes affecting fetal growth could potentially act via an influence on placental development. There is also some evidence of interaction between genes and birth size. For example, interaction has been observed between birthweight and (i) angiotensin converting enzyme (ACE) polymorphism with respect to insulin response to glucose load,[22] (ii) apolipoprotein (apo) E genotypes and plasma lipid levels,[23] (iii) polymorphism of the peroxisome proliferator-activated receptor (PPAR)-gamma2 gene and insulin sensitivity and metabolism,[24] (iv) K121Q polymorphism of the plasma cell glycoprotein-1 gene and type 2 diabetes and hypertension[25] and (v) vitamin D receptor genotype and adult bone size and mineral density.[26] Mechanisms underlying such interactions are not understood.

Epigenetic Factors

Epigenetic modifications (discussed elsewhere in this book, Chs. 6, 7) represent a potential but largely unproven mechanism for programming the fetus or developing child in response to environmental factors.

DNA is associated with proteins called histones to form a complex substance known as chromatin. DNA methylation or histone acetylation/deacetylation alter the structure of chromatin to silence (switch off) genes without changing DNA sequence. Such modifications are described as epigenetic.[27] Imprinted genes are switched on or off according to parent of origin so are functionally haploid (only one copy is functional) so they are vulnerable to genetic or epigenetic mutations, or to epigenetic modification in response to environmental factors.[28] However, imprinting defects in humans generally cause clinically recognisable abnormal phenotypes, such as Prader-Willi and Beckwith-Wiedemann syndromes.

Evidence has emerged recently that heritable epigenetic changes in expression of nonimprinted genes may result from early nutritional or other exposures and could potentially affect disease risk.[29-31] In rats there is also evidence relating to early postnatal exposures to maternal behaviour.[32] Whether imprinting can similarly be modified by environmental factors has been little investigated, though circumstantial and animal evidence suggest this is a possibility.[33,34]

How Study of Twins Might Shed Light on the Underlying Causal Pathways

A natural starting point is the question of whether an association between birth size and later disease risk is seen in populations of twins. If there is no such association, then study of twins is unlikely to be helpful. A negative relationship between birth weight and blood pressure is seen in most (but not all) studies of twins,[35-46] as in studies of singletons, though there are a number of problems with comparing results of the various studies, including differences in statistical methodology that can affect both regression coefficients and 95% confidence intervals. In a recent large study of twins[47] the association between birth weight and risk of type 2 diabetes was similar in magnitude to that seen in populations of singletons.[48]

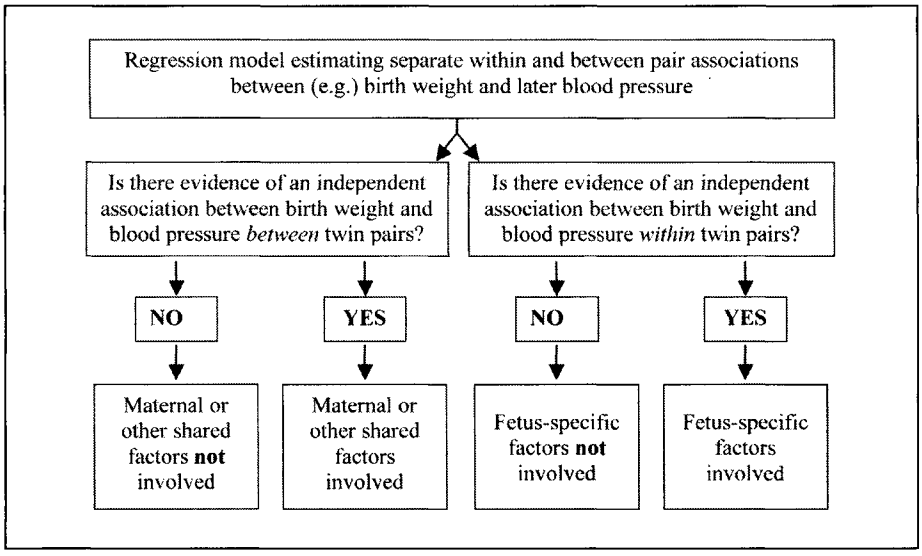

Figure 2. Between versus within pair analyses.

The Role of Shared Factors

We suggested comparing the association between birth weight (or birth weight standard deviation score for gestation and sex) and cardiovascular risk factors in twins treated as individuals, with that estimated within pairs.[35] Within pairs analysis assesses whether pair difference in birth weight is related to pair difference in cardiovascular risk, and so controls perfectly for all shared factors (identified and unidentified). If an association is seen in the cross-sectional analyses (which essentially ignore the cotwin's data) but is not seen or is substantially reduced in within-pair analyses then one could conclude that shared factors were involved in the underlying causal pathways. Conversely if it remains, we can conclude that factors affecting the individual fetus are involved.

However, the statistical issues involved in making these arguments precise are more involved than we have space to cover adequately here. Twins are effectively "clusters" within a dataset and there are a number of ways in which recognising the paired structure of the data can be incorporated into the analysis. When treating twins as individuals, one at least needs to recognise the lack of independence between cotwins, since this is necessary to obtain valid estimates of precision (standard errors) and statistical significance. Gains in efficiency of estimation can also be obtained by using generalised estimating equations (GEE) or other varieties of multilevel modelling.[41,43] However, a broader issue is that the simple "treated-as-individuals" regression model can be generalised to a form that estimates two distinct coefficients which more explicitly represent "within-pair" and "between-pair" regression effects.[44,47,49,50] The coefficient for the "between pairs" association may be interpreted to assess the role of shared factors whereas the "within pair" coefficient assesses the role of factors affecting the individual fetus (Fig. 2). This is the most appropriate statistical approach, permitting direct assessment of the relative strength of these associations.

Shared Factors will include:

- Maternal factors, including maternal nutrition, lifestyle factors, hormonal and metabolic status
- Paternal factors, almost always shared[51]
- Placental imprinting (apart from stochastic differences, the frequency of which are not known, or any resulting from environmental exposures)

- DNA sequence in monozygotic (MZ or "identical") twins
- Many factors in the postnatal environment

Factors influencing the individual fetus will include:

- The "supply line" to the individual fetus
- Genetic factors in dizygotic (DZ or "nonidentical") twins

Comparison of within and between Pair Differences in Monozygotic and Dizygotic Twins

It is clear that genetic factors are shared in MZ twins but differ in DZ twins. Thus if an association seen between individual twins is substantially weaker when estimated in a paired analysis within MZ twin pairs than it is when estimated within DZ twin pairs, the most obvious conclusion is that genes are involved in the underlying causal pathway. However, the possibility of greater sharing by MZ twins of other factors in the feto-placental unit should be considered.

Interpretation of such differences has proved challenging in practice. Findings to date have been somewhat contradictory for blood pressure (e.g., IJzerman's[38] versus Christensen's findings[41]) and there is only one reported study of angina[52] and of acute myocardial infarction,[53] suggesting genetic factors are involved in the relationship between coronary heart disease and birth weight.

In the large study of birth weight and type 2 diabetes, the between pair association in MZ and DZ twins was of similar magnitude and statistically significant in both cases, suggesting a role for shared factors.[47] However, the within pair association appeared to be reduced more (relative to the between pair association) in DZ than in MZ twins. The mechanism for this, if it reflected a real effect, is not obvious. It should be remembered here that around two thirds of MZ twins will share a placenta, whereas DZ twins have separate placentas (see Fig. 3, originally published in ref. 35). This study did not have information on chorionicity (placentation) but these findings may point to a possible key role for the placenta.

The Role of Postnatal Growth

Within pair analyses in large cohorts present an opportunity to estimate the role of aspects of postnatal growth as determinants of later health, without confounding by gestation length and largely unconfounded by external factors influencing child growth. These include parental eating habits, activity level and lifestyle (likely to influence similar factors in the child), as well as attitudes to child feeding and rearing.[54,55]

Comparison of Twins with Singletons

In general, twins have lower birth weight than singletons. This is partly because of higher risk of preterm delivery, and partly because twins are on average smaller for gestational age than singletons.[56] It might therefore be expected that twins, as a group, would have higher risk of cardiovascular disease than singletons. Studies to date have demonstrated no difference,[57-62] other than in one small study.[63,64] If the larger studies are generally correct, this suggests that birth weight per se is not involved in the observed associations, and that whatever causes the general constraint on intrauterine growth of twins is not involved in causal pathways underlying the association between size at birth and risk of later cardiovascular disease. However, it is clear from Iliadou's study[47] that among twins there is a similar association between birth weight and type 2 diabetes to that seen in the general population.[48]

A note of caution is that 'ecological' gross twin-singleton comparisons have not taken into account all relevant confounding factors so that inferences about birth weight-related pathways (or different postnatal growth) can only be made with limited confidence.

Another implication of these findings is that accelerated post-natal growth per se may not be related to risk of later cardiovascular disease. Twins in general have accelerated growth relative to singletons. Wilson followed twin children to age 9 and showed that they "caught up" to singletons over that period.[65,66] Study of postnatal growth in twins versus singletons may provide important clues as to what aspect or consequence of accelerated post-natal growth is involved in programming of later cardiovascular risk.

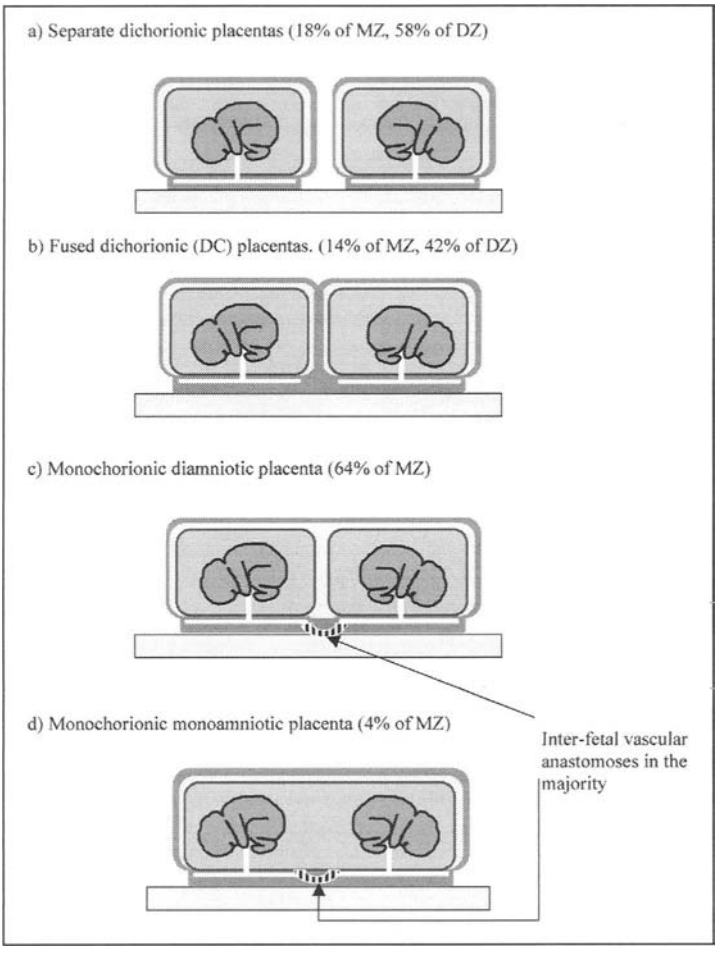

Figure 3. Different types of twin chorionicity (placentation). MZ = monozygotic. DZ = dizygotic.

The Role of the Placenta

Birth weight discordance is common in twin pairs, suggesting that within pairs the intrauterine environment can differ, and confirming that aspects of placental structure or function are likely to be important determinants of fetal growth. There is an important opportunity, not yet exploited, to use twins to study the role of the placenta. Maternal factors are perfectly controlled and postnatal environment is much more similar than that of unrelated singletons. Furthermore, both maternal and postnatal factors are better controlled than between siblings. Twins therefore provide a particular opportunity to investigate the role of placental differences (in structure, function, disease and possibly epigenetic modifications) in terms of both birth size and later health. In the case of monozygotic twins genetic factors will also be controlled.

Around two thirds of MZ twins are monochorionic (share one placenta), and it seems intuitively unlikely that epigenetic modifications will differ between two parts of one placenta. However, this has not been investigated and several studies have reported discordance for Beckwith-Wiedemann syndome in MZ twin pairs,[67] some monochorionic.[68,69]

Large prospective twin studies with good biological sample collection will be needed to elucidate the role of placental factors.

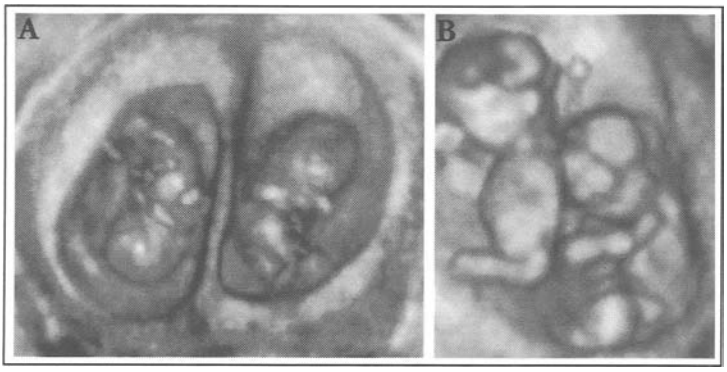

Figure 4. 3D ultrasound scans of twins at 12 weeks of gestation. A) Dichorionic twins (courtesy of Dr. Martin Metzenbauer, Donauspital, Vienna, Austria). B) Monochorionic diamnionic twins (courtesy of Dr. Philippa Ramsay, Ultrasound for Women, Sydney Adventist Hospital, Australia).

Are Findings in Twins Generalisable?

There are important differences between twin and singleton pregnancies, and some researchers believe that we cannot extrapolate from twins to the largely singleton population[48,70,71] because of "the substantially different biology of fetal growth in twins".[70] If this were true it might shed doubt on aspects of evidence from studies of multifetal species like rodents. Furthermore, and as we have indicated elsewhere, fetal growth per se may not be in the causal pathways linking gestational factors to later health, and in any case if it were, the public health implications of the association would be limited.[2]

Issues regarding generalisability of twin data are:

1. **Shorter median gestation.**
   Evidence on the relationship between gestation length and cardiovascular disease or its risk factors is inconclusive and most recent large studies (with reliable gestation length estimates) failed to demonstrate an association independent of birth size.[72-77] There is insufficient information to determine whether gestation length modifies the relationship between birth size and later health.
2. **Bias in volunteer twin cohorts.**
   Representative sampling is not a requirement for aetiologic studies.[78] A cohort with a wide spread of exposures (risk factors) to ensure adequate control of any known confounding factors that might obscure associations of interest is the main requirement, and this requirement is generally met in twin cohorts.
3. **Recalled birth weights in some twin cohorts.**
   Recalled birth weight has also been used in some singleton cohort studies, for example the Caerphilly Study.[79] We do not know whether there is a systematic difference in recall accuracy between twin and singleton cohorts.
4. **Zygosity and chorionicity (placentation).**
   In one study MZ twins had a more adverse lipid profile and higher fasting plasma glucose and insulin concentrations than DZ. twins,[80,81] though these findings await confirmation in other cohorts. Conversely, in another study mortality among female dizygotic twins was 1.77 times higher than among monozygotic twins at age 30 - 59 years.[82] However, the issue of chorionicity (shared placenta versus separate placentae in MZ twins, see Figs. 3, 4), was not taken into account by any of these studies because of lack of information.
   Two thirds of MZ twins share a placenta and there are vascular communications between their circulations. If circulating factors mediate the relationship between intrauterine compromise and later health, then such factors could pass from a compromised twin to its uncompromised cotwin, thus blunting the association between birth weight and later health

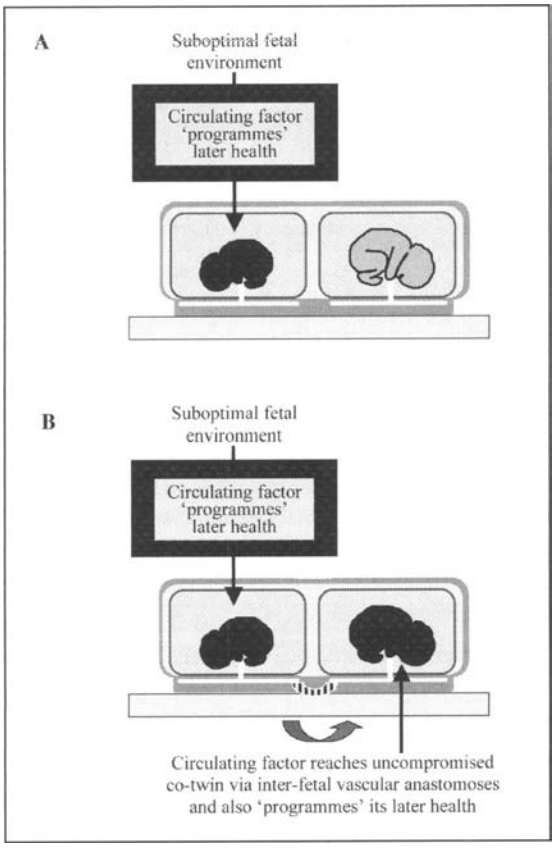

Figure 5. Monochorionic twins: potential for circulating factors from compromised twin to 'programme' otherwise uncompromised co-twin.

in the "uncompromised" twin (Fig. 5). This remains speculative and Iliadou's findings (of at least as strong within pair association between birth weight and type II diabetes in MZ versus DZ twins) suggest such 'blunting' is unlikely, at least in the programming of later risk of type II diabetes.

5. **Gender mix.**
   It has been suggested that exposure of a female twin to testosterone from a male cotwin might affect her later health.[70] There is little good evidence to support this,[83-94] though it is noteworthy that there are no data on cardiovascular disease or its risk factors. Many investigators have avoided this issue by studying only same-sex pairs.[37,38,40,42-45]
6. **Maternal subfertility and assisted reproduction technology.**
   Assisted reproduction technology (ART) is associated with an increased twinning rate[95] and there is evidence of a small influence on human fetal growth.[96] No study to date has investigated cardiovascular outcomes in relation to ART or maternal subfertility but there are reports of imprinting defects in children conceived by ART, in particular by ICSI (intracytoplasmic sperm injection).[97] Whether these defects relate to the causes of infertility or to the ICSI procedure (or both) is not known. However, there is evidence that abnormal spermatogenesis (leading to low sperm counts) is associated with an increase in defective methylation of the *H19* locus,[98] potentially leading to the presence of two inactive *IGF2* genes in the placenta, with implications for embryo development.

Summary

Study of twins should help us understand more about:

- The role of shared factors versus factors affecting the individual fetus.
- The role of genetic factors and possibly epigenetic ones.
- The role of placental factors.
- Which aspects or consequences of postnatal growth are associated with increased risk of later cardiovascular disease.

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