Fetal Growth and the Fetal Origins Hypothesis in Twins — Problems and Perspectives

David I.W. Phillips[1], Michael J. Davies[2] and Jeffrey S. Robinson[2]

[1] *MRC Unit(University of Southampton), Southampton General Hospital, UK*
[2] *Department of Obstetrics and Gynaecology, University of Adelaide, Australia*

Although there is substantial evidence from studies of singletons that small size at birth is linked with long-term adverse health effects, until recently little was known as to whether these associations extend to twins. A review of published studies suggests that at present there is little consistent evidence that birthsize in twins is associated with increased morbidity or morality. While, these findings may reflect methodological limitations, it is also argued that they arise as a consequence of the substantially different biology of fetal growth in twins.

There is now substantial evidence that suboptimal fetal growth as indicated by low birthweight in babies born at term is linked with a higher prevalence of adult cardiovascular disease and increased risks of type 2 (non-insulin-dependent) diabetes, hypertension and the metabolic syndrome (Barker, 1998). This evidence has given rise to the fetal origins hypothesis which suggests that fetal undernutrition in mid to late gestation programs cardiovascular and metabolic disease. However, the fetal origins hypothesis was developed on the basis of studies of singleton pregnancies and until recently almost nothing was known as to whether the associations between birthsize and adult outcomes were present in twins. Interest in studying twins has been stimulated by the possibility that twins may be at greater risk from the long-term effects of reduced fetal growth. This is because the fetal growth of twins falls below that of singletons especially in late gestation and as a consequence are substantially smaller at birth than singletons. Furthermore, it has been suggested that twin studies could shed light on the underlying mechanisms, particularly discriminating between the influence of the early environment and the influence of genetic factors. In this paper, we consider a number of issues that may limit the utility of the twin model for examining the fetal origins hypothesis. In particular it will be argued that the although size at birth can be influenced by several factors operating individually or in concert, in the case of twins there is a much greater role for maternal constraint on fetal growth than for singletons.

Do Twins Have a Greater Prevalence of Metabolic and Cardiovascular Disease?

Twins are on average more than 900g lighter than singleton babies at birth. Although they have a shorter gestation, their weight at birth is still smaller than singletons of similar gestational age. Twin pregnancies are also associated with increased rates of fetal death, including increased rates of miscarriage, and a higher perinatal mortality rate. A number of studies have sought, therefore, to determine whether twins surviving to adulthood have a higher mortality. Vågerö and Leon (1994) studied the mortality of 6612 male Swedish twins and found no evidence of an increased mortality from coronary heart disease compared with the general population. Similar conclusions were reached in a study of the Danish twin register, (Christensen et al., 1995) and in the US Veterans study (Hrubec & Neel, 1981) mortality was in fact lower than expected in twins. There is less information on morbidity. However, with regard to important CHD risk factors, a recent study from a prospective cohort in New Zealand has reported lower blood pressure in twins than singletons at the age of 8 years (Williams & Poulton, 1999).

Another approach has been to compare the mortality experience of monozygous compared with dizygous twins. Monozygous twins have lower birthweight and evidence of a more adverse intrauterine environment than dizygous twins. This occurs because a high proportion of monozygous twins share a placenta and therefore compete for nutrients. The differences in birthweight which amount to between 100 and 200g are not large but in the context of the low birthweight of twins represent a significant reduction in birthsize. Furthermore, monozygous twins have higher perinatal mortality rate and higher rates of congenital abnormalities. Whether monozygous twins have a greater frequency of metabolic and cardiovascular disease in adult life than dizygous twins has been specifically examined in the Danish Twin study (Christensen et al., 1995). No difference in overall mortality between monozygous and dizygous

Address for correspondence: Prof. DIW Phillips PhD FRCP, MRC Unit, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, The United Kingdom. E-mail: diwp@mrc.soton.ac.uk

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twins was found, while middle-aged monozygous twins had somewhat lower mortality. In contrast a study of glucose tolerance in 125 monozygous and 178 dizygous twins showed that during an oral glucose tolerance test the monozygous twins had higher incremental insulin concentrations — presumptive evidence of insulin resistance (Poulsen et al., 1999).

Taken together these studies suggest that there is little evidence of significantly higher rates of cardiovascular disease in twins. Why should this be so given the solidity of the evidence linking birthsize and disease in singletons?

Are Twins Different?

Some of the difficulties in achieving consistent and interpretable results with twin studies are methodological. Studies of twins are harder to carry out than studies of singletons as both members of the pair need to be found and interviewed. Many twin studies, particularly those carried out in later adult life, are severely limited by censoring of twin-pairs so that those studied may be poorly representative of the original cohort. Few twin studies are linked with birth registers and much of the birthweight data is based on recalled birth weights which may be less reliable in twins: Identical twins may be mixed up during the difficult obstetric conditions associated with a twin delivery.

However, these studies have given little consideration to the biology of twinning and fetal growth in twins. Consideration of these additional factors suggests that low birthweight or small size at birth in twins is unlikely to have the same significance as low birthweight in singletons. There is a small but increasing body of evidence that maternal factors constraining the size of the fetus act disproportionately in twins compared to singletons and as a consequence small size at birth in twins is unlikely to have the same significance as small size in singletons. The powerful effects of maternal constraint have been well-documented by cross-breeding experiments between animals of vastly different genetic potentials for growth (Walton & Hammond, 1938). Hence, there is operating in multiple pregnancies a potentially important confounder of relationships between birthsize and disease. Put simply, twins may be small at birth for different reasons to those individuals who are small and go on to develop metabolic or cardiovascular disease in adult life. This, of course raises the question as to how being small due to different reasons can have differing consequences, and what are the particular characteristics of the fetal environment that give rise to both small size and ill-health in the long term. The fetal origins hypothesis suggests that the links between reduced fetal growth and adult disease result from the persistence of metabolic and endocrine adaptations to growth retardation in late gestation. It is possible that fetal and placental growth differs in twins in a way that may minimise the risk of undernutrition during late gestation. Although there is a consensus that twin growth falls below that of singletons during the third trimester, there is evidence that twins may downregulate their growth rate earlier in gestation. Of the few studies that have studied mid-term fetal growth in twins some (Divers & Hemsell, 1979; Leveno et al., 1979) but not all (Grennert et al., 1978; Socol et al., 1984) suggest that twins are smaller than singletons as early as 16 weeks of gestation. These findings are supported by studies in sheep which suggest that multiple pregnancy is associated with enhanced placental growth during the first half of gestation (up to day 70; Heasman et al., 1998). In fetal lambs early downregulation of fetal growth protects against growth retardation induced by undernutrition in later gestation and it is therefore possible that the same process occurs in twins thereby protecting them against nutrient restriction in later pregnancy (Harding et al., 1992). These adaptive mechanisms may also operate in discordant pairs. Recently published data suggests that the frequency of discordant growth in twins is related to the total birthweight of the twins such that large intra-pair differences are more evident when the total birthweight of the twins is lower. This suggests that growth discordance is an adaptive mechanism imposed by a limited uterine milieu and it may be that failure of this mechanism leads to adverse outcomes (Blickstein et al., 2000).

Other evidence that the prenatal growth of twins differs from that of singletons derives from detailed anthropometric studies of twin size at birth. These studies suggest that twin growth in late gestation is symmetrical — there is proportionate slowing of weight, head size and length toward term. In contrast fetal programming effects in singleton babies are typically observed in association with disproportion in head size, length or weight which are markers of lack of nutrients or oxygen at specific stages of gestation.

Another line of evidence suggesting that twin growth is different comes from intergenerational studies of birthweight. It has been established in experimental animals that the animals born growth restricted in turn have smaller offspring than controls, even under conditions of ad libitum feeding. Ounstead and Ounstead (1986) found that mother's birthweight was predictive of the intrauterine growth of their babies such that women who were small at birth tended to have smaller babies than average. Subsequent studies found that the mother's birthweight was related to maternal stature, prepregnancy weight and her reproductive performance including pregnancy weight gain, babies' intrauterine growth and birthweights, gestational duration and the rate of neonatal complications. These findings suggest that the prenatal growth of a fetus is not only determined by nutrient and oxygen supply but also by factors which are related to mothers early environment and rate of fetal growth. These transgenerational effects on birthweight are not observed in twins. In 1985 Magus showed that women who were born as one of a twin pair had babies with birthweights similar to those of singleton babies (Magnus et al., 1985). This effect was also observed in monozygous twins. Women who were monozygous twins and who differed in birthweight by at least 300g had babies of similar birthweight (Emanuel et al., 2001). Although the mechanistic basis of these observations is not understood, these findings again suggest that the factors which determine size at birth in twins differ fundamentally from those operating in singletons.

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Do Birthweight Differences Between Twins Correlate With Long Term Differences in Outcomes?

Monozygous twins are frequently dissimilar in birthweight as a result of unequal partitioning of nutrients and blood supply between the twins. A number of studies have sought associations between within-pair difference in birthweight and adult outcomes. Because of the genetic identity of MZ twins, associations between within-pair differences in birthsize and adult outcomes cannot have a genetic basis and must be due to environmental differences between the twins. Hence it was proposed that twins could provide a means of distinguishing between genetic and environmental explanations of the epidemiological associations. In addition, by investigating within-pair differences parental confounding factors (such as smoking and socio-economic class) are controlled for in twins. Studies of extreme differences in birthweight in MZ pairs have shown that the smaller twin at birth remained the smaller in height, weight and head circumference into adult life (Babson & Phillips, 1973). More recently, an analysis based on the Danish Twin Register identified 14 MZ twin pairs who were discordant for type 2 diabetes and showed that the diabetic twins had lower birthweight than the non-diabetic co-twins (Poulsen et al., 1997) with similar findings in a Italian study (Bo et al., 2000). Two recent studies have used the same approach in studying blood pressure (Dwyer et al., 1999; Poulter et al., 1999). Although not statistically significant, both studies found a tendency for the MZ twin who was lightest at birth to have the highest systolic blood pressure later in life. However, two more recent studies cast some doubt on these findings failing to find associations between within-pair differences in birthweight and adult outcomes in monozygous twins (Baird et al., 2001; IJzerman et al., 2000). Undoubtedly, some of the difficulties are due to the relatively small size and limited power of the studies carried out so far. Few of these studies, however, have considered the possibility that twins are not independent of each other and the body of evidence that the growth and development of a twin may by influenced by its co-twin.

The Influence of the Co-twin

There is a large body of research based on studies of litter bearing rodents which shows that the intrauterine position that a male or female fetus occupies in relation to its siblings influences the hormonal milieu in which it matures and its eventual phenotype (vom Saal, 1989). Male fetuses occupying intrauterine positions between two males have greater blood concentrations of testosterone than their brothers who develop between females. Similarly, female fetuses located between male fetuses have higher testosterone concentrations than do their sisters located between female fetuses. Studies using isotopically labelled steroids suggest that this effect is a result of the secretion of androgens by male fetuses diffusing through the amniotic fluid and across fetal membranes to an adjacent fetus. These differing levels of exposure to androgens affect both the behaviour and morphology of the adult rodent (Gandelman et al., 1977). For example, female mice who have developed adjacent to male mice are more aggressive, have longer oestrus cycles, are less attractive to male mice and have a shorter reproductive life than their sisters who developed between female mice.

Studies in humans suggest that similar phenomena occur in opposite-sexed twins. These studies suggest that there is a degree of masculinization of women who had a male co-twin and include differences in the auditory system (Mcfadden, 1993), personality and measures of disinhibition or adventure seeking (Resnick et al., 1993). They also suggest that there are morphological differences including alterations in craniofacial growth (Boklage, 1985) and the size of the crowns of the molar teeth (Dempsey et al., 1999). Although not proven these effects are presumed to be due to diffusion of steroid hormones from one fetus to the other either directly through the fetal membranes and amniotic fluid or indirectly via the maternal circulation.

These observations suggest that the growth and development of a twin may be modified by its co-twin. The principal agents by which a twin may be influenced by a co-twin are most likely to be steroid hormones (including both gonadal steroids and glucocorticoids) which are extremely lipid soluble and readily diffuse within biological tissues where they have potent influences on growth and development. There is increasing evidence that the secretion of steroid hormones can be programmed during development and that they may be mediators of the effects of restricted fetal growth (Phillips et al., 1998; Reynolds et al., 2001). If steroid programming is an important mediator of the long-term effects of fetal growth restriction, this has important significance for the interpretation of twin studies. In the case of a monozygous pair where there is unequal partitioning of nutrients or placental blood supply, the endocrine changes and stress response of the smaller fetus may programme the larger fetus and would tend to minimise subsequent differences between the twins. This may explain why studies correlating differences in birthweight between twin pairs with adult outcomes have been rewarded with limited success.

Conclusion

Recently, a number of studies examining aspects of the fetal origins hypothesis that have been based on twins have been published. These have followed two types of study design. Some have sought to determine whether being a twin *per se* is associated with a higher prevalence of cardiovascular and metabolic disease in adult life. Others have investigated whether naturally occurring, differences in fetal growth between pairs of monozygous and dizygous twins at birth are linked with long-term differences in health outcomes. The results of these studies have been inconclusive. There is little evidence that twins have increased morbidity or mortality in comparison with singletons. Furthermore, follow-up studies of growth differences between twin pairs have produced contradictory results with some but not other studies finding evidence that birthweight differences between twin pairs correlate with adult outcomes. These data contrast with the strength and consistency of the evidence linking small size at birth in singleton babies. Although these results would appear at first to undermine the fetal origins hypothesis, we suggest that

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they are entirely predictable in terms of what we know about the biology of twinning and fetal growth in twins. This evidence suggests that of potential mechanisms that operate to constrain fetal growth, there is at least one powerful factor operating with greater influence in twins, and that these factors are not importantly linked to an increase in the prevalence of metabolic or cardiovascular disease in adult life. Likewise studies of the long-term effects of within-pair differences in birthweight between twin pairs may be limited by twin-twin interactions. Clearly, the interpretation of twin studies is complex and it is difficult to see how the contributions of fetal programming, genetic influences and other factors these will be resolved unless studies are carried out where there is much more detail about the prenatal growth patterns and data on the form of placentation (e.g. whether the twins are mono or dichorionic and the presence of vascular communications between the twins).

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