Intrauterine environmental and genetic influences on the association between birthweight and cardiovascular risk factors: studies in twins as a means of testing the fetal origins hypothesis

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Summary

Evidence has accumulated that low birthweight is associated with several risk factors for cardiovascular disease. However, it is not known whether or not these associations are due to a programmed response to intrauterine malnutrition or genetic factors influencing both birthweight and cardiovascular risk factors. Twin studies offer a unique opportunity to distinguish between intrauterine and genetic origins of the association between birthweight and cardiovascular risk. In our twin cohort, low birthweight was associated with insulin resistance, lower HDL and shorter height within both dizygotic and monozygotic twin pairs, suggesting that these associations are, at least in part, independent of genetic factors. In contrast, low birthweight was associated with blood pressure, total and LDL cholesterol, fibrinogen and sympathetic activation within dizygotic twin pairs, but not within monozygotic twin pairs. These differences between dizygotic and monozygotic twins suggest that these associations are, at least in part, due to genetic factors. Therefore, both intrauterine environmental and genetic factors appear to play a role in the association between birthweight and cardiovascular risk factors. In the future, strategies may be developed targeted at improving or preventing impaired intrauterine growth. However, the effects of interventions that comprise changes in environment within the normal range may be limited due to the possible important role of genetic factors.

Introduction

Evidence has accumulated that low birthweight is associated with an increased risk of cardiovascular disease.[1] This association may be mediated by the association of low birthweight with several established risk factors for cardiovascular disease, such as blood pressure, insulin resistance, diabetes, plasma lipids and fibrinogen.[1] The fetal origins hypothesis suggests that the association between birthweight and cardiovascular risk is due to a programmed response to intrauterine malnutrition.[1] According to this hypothesis, improvements in intrauterine nutrition may prevent disease in later life. This would have major implications for public health. However, it has been suggested that the association between birthweight and cardiovascular disease may be due to confounding by an adverse environment that is related to small size at birth and later cardiovascular disease.

Socio-economic status in particular may have an important impact on lifestyle choices that are related to both low birthweight[2] and cardiovascular risk (factors).[3] For example, reduced size at birth may simply be a marker for poor maternal socio-economic circumstances that predict relative deprivation of the offspring in adult life, which leads in turn to an increased risk of cardiovascular disease. Although a few studies have shown that adjustment for lifestyle factors, such as smoking, employment, alcohol consumption and exercise, had little effect on the association between birthweight and cardiovascular disease,[1] influences of other (unknown) factors cannot be excluded in these studies. In addition, it is not possible to adjust for these

factors completely, because they cannot be measured without error.

Another alternative explanation for the association between birthweight and cardiovascular disease is confounding by genetic factors. Both birthweight and cardiovascular disease are influenced by genetic factors. Therefore, the genetic factors influencing both birthweight and adult cardiovascular disease may be responsible for the association.[4] In other words, a genotype responsible for cardiovascular disease may itself cause restricted fetal growth *in utero*. In this case, nutrition-induced changes in fetal growth may not prevent the development of cardiovascular disease.

Testing the fetal origins hypothesis

The epidemiological studies linking low birthweight with cardiovascular risk do not provide evidence for a causal relationship, and mechanistic tests of the fetal origins hypothesis are useful. Only in experimental settings can we investigate whether the association of one variable with another is causal. An ideal experimental setting creates circumstances across which only one factor affecting the outcome of interest varies. In humans, such a setting is usually achieved in a randomised trial. However, in humans a randomised trial with interventions to influence birthweight is difficult to perform for several reasons. Firstly, it is not known what intervention(s) should be used to increase birthweight. Secondly, it should be remembered that, although any intervention in a fetus may have large benefits for disease in later life, unexpected adverse effects may also develop. Thirdly, the results of these trials will be definitive only after sufficient clinical cardiovascular diseases have developed in the study group, which means a study duration of at least 50 years.

Usually, the term experiment is restricted to situations in which circumstances are manipulated by the investigator. However, intrapair comparisons in twins (and to a lesser extent in siblings) provide a unique opportunity to mimic a scientific experiment. The influence of one factor (in this case birthweight) on the outcome of interest (cardiovascular risk factors) can be investigated independent of many other factors (such as socio-economic and genetic factors), because the influence of these factors is eliminated within pairs. Therefore, twins can be considered as an 'experiment of nature'.

Confounding by socio-economic factors can be diminished by comparing siblings, as all children within a family have been born from the same mother and father, and socio-economic factors are thus approximately the same. If socio-economic factors do not play a role in the association between birthweight and cardiovascular risk factors, one would expect that the sibling with the lowest birthweight will also have the highest level of the cardiovascular risk factor compared with the sibling with the highest birthweight. In addition, differences in birthweight should be inversely associated with differences in the risk factor of interest. However, if socio-economic factors do play a role, then these associations would be diminished within the paired siblings as compared with unpaired analyses. To our knowledge, only a few studies have used the comparison of siblings to investigate the fetal origins hypothesis.[5,6] These studies thus suggest that the association of IQ,[5] height and weight[6] with birthweight are independent of parental socio-economic factors.

This comparison between siblings can be taken one step further by investigating twin pairs.[7] Similar to singleton siblings, the members of a twin pair are raised in the same family and therefore the same socio-economic class. Twins also share maternal hormonal and nutritional status and other aspects of maternal health, which can change over time and therefore between pregnancies. In addition, twin pairs provide a unique tool to investigate the influence of genetic factors.

In monozygotic twins, who are genetically identical, the influence of genetic factors on the differences within a twin pair is excluded. In dizygotic twins, who share on average half of their genes, the influence of genetic factors is reduced, but not excluded. If genetic factors do not play a role in the association between birthweight and cardiovascular risk factors, one would expect that, both for dizygotic and for monozygotic twins, the twin with the lowest birthweight from each pair will also have the highest level of the cardiovascular risk factor compared with the co-twin with the highest birthweight. In addition, inverse associations between intrapair differences in birthweight and intrapair differences in the risk factor should exist both in dizygotic and in monozygotic twins. However, if genetic factors do play a role, these associations would exist only within dizygotic twins, and not within monozygotic twins. In this case, within dizygotic twins, unfavourable genetic factors will cause growth

restriction and cardiovascular disease in one twin member, but not in the co-twin who does not have the unfavourable genetic factors. In monozygotic twins, both twin members have the same set of genes, so they both have the same unfavourable or favourable genetic factors.

Birthweight and cardiovascular risk factors in adolescent twin pairs

To examine whether the association between birthweight and cardiovascular risk factors is explained by intrauterine or genetic factors, we have studied the association of birthweight with several cardiovascular risk factors in a group of adolescent dizygotic and monozygotic twin pairs. This study is part of a larger project carried out at the Department of Biological Psychology at Vrije University in which cardiovascular risk factors were studied in a group of adolescent twin pairs and their parents. Opposite-sex dizygotic twin pairs were excluded because of the effects of sex differences within a pair on both birthweight and cardiovascular risk factors.

A total of 53 dizygotic and 61 monozygotic twin pairs were eligible for analysis. Intrapair differences in birthweight were negatively associated with differences in blood pressure in dizygotic twins, but not in monozygotic twins. (regression coefficient: –5.7 mmHg/kg [95% confidence interval –10.4, –1.0] vs. –0.1 mmHg/kg [–5.4, 5.2]).[8] Similarly, birthweight was associated with indicators of sympathetic activity within dizygotic twin pairs, but not within monozygotic twin pairs.[9] In a subgroup of this twin cohort, we found that intrapair differences in birthweight were negatively associated with differences in insulin resistance in both dizygotic twins and monozygotic twins (unpublished data). This association was statistically significant within monozygotic twins. Low birthweight was associated with high total and LDL cholesterol within dizygotic twin pairs (–0.49 mmol/L per kg [–0.89, –0.08]; $P = 0.02$ and –0.51 mmol/L per kg [–0.90, –0.13]; $P = 0.01$ respectively), but with low total and LDL cholesterol within monozygotic twin pairs (+0.32 mmol/L per kg [0.03, 0.62]; $P = 0.03$ and +0.23 mmol/L per kg [–0.03, 0.49]; $P = 0.08$ respectively).[10] In addition, low birthweight was associated with high fibrinogen within dizygotic twin pairs (–0.25 g/L per kg [–0.49, –0.01], $P < 0.05$), but not within monozygotic twin pairs (+0.16 g/L per kg [–0.12, 0.45], $P = 0.3$).[11] On the other hand, intrapair differences in birthweight were positively associated with differences in HDL cholesterol in both dizygotic and monozygotic twins (+0.04 mmol/L per kg [–0.10, 0.16]; $P = 0.6$ and +0.11 mmol/L per kg [–0.00, 0.23]; $P = 0.05$ respectively).[10] Finally, intrapair differences in birthweight were significantly associated with differences in height in both monozygotic and dizygotic twins in adolescence (+4.3 cm/kg [1.0, 7.5] and +2.8 cm/kg [1.4, 4.1] respectively).[12]

Discussion

In our twin studies, lower birthweight was associated with insulin resistance, lower HDL and shorter height within monozygotic twin pairs, suggesting that these associations are, at least in part, independent of genetic factors. In contrast, lower birthweight was not associated with blood pressure, total and LDL cholesterol and fibrinogen within monozygotic twin pairs, suggesting that these associations are, at least in part, due to genetic factors.

The difference in the birthweight–blood pressure relationship between dizygotic and monozygotic twin pairs is in accordance with several other twin studies,[13–15] and suggests that genetic factors may play an important role in the association between birthweight and blood pressure. The intrapair association between birthweight and insulin resistance adds to the findings of two previous twin studies that demonstrated that monozygotic diabetic twins have a lower birthweight than their non-diabetic co-twins.[16,17] In addition, our findings are in accordance with a recent study that showed that, within monozygotic twin pairs, differences in birthweight were related to differences in insulin resistance, as measured with the euglycaemic hyperinsulinaemic clamp technique.[18] Therefore the association between these two variables may be, at least in part, due to intrauterine factors.

Analyses of glucose metabolism and blood pressure in the Dutch famine birth cohort were also compatible with the results from these twin studies. Intrauterine exposure to famine was related to changes in glucose metabolism,[19] but not to changes in blood pressure.[20] Interestingly, a similar pattern has also been observed in the rat model of intrauterine nutrition induced by uterine artery ligation, a model that may be more relevant to the human situation than animal models of maternal undernutrition.[21] Uterine artery ligation was related to a diminished glucose tolerance,[21,22] but not to an elevated blood pressure.[21] In conclusion, the

results from twin studies, the Dutch famine and also ligation studies in rats provide evidence for an intrauterine non-genetic influence on the association between size at birth and glucose metabolism, whereas genetic factors may, at least in part, explain the link between size at birth and blood pressure.

It could be argued that differences in birthweight in twins are a poor model for differences in birthweight in singletons. For example, intrauterine growth in twins is different from that in singletons.[23,24] However, the association between birthweight and blood pressure in the overall sample of our twin cohort (–1.9 mmHg per kg increase of birthweight) was remarkably similar to the well-established association in singletons (approximately –2 mmHg per kg increase of birthweight).[25] The same holds true for the size of the association of birthweight with serum lipids[10] and later height in the overall sample of twins, which were similar to the size of the associations of birthweight with serum lipids[26,27] and height[28,29] in singletons. In addition, differences in birthweight within twin pairs have been associated with differences in many variables that have been related to birthweight in singletons, such as blood pressure,[8,13–15] diabetes,[16,17] insulin resistance,[18] serum lipids,[10] fibrinogen,[11] myocardial infarction[30] and height.[12,31] Although intrauterine growth in twins may be different from that in singletons, the associations between birthweight and cardiovascular risk in twins suggest that birthweight in twins is relevant to the development of cardiovascular disease, and that differences in birthweight in twins can be used as a model for differences in birthweight in singletons.

It could further be argued that the association between intrapair differences in dizygotic twins cannot be compared to intrapair differences in monozygotic twins to study the influence of genetic factors, as around two-thirds of monozygotic twins are monochorionic (i.e. share a placenta), whereas all dizygotic twins are dichorionic (i.e. have separate placentas). Therefore, besides genetic factors, intrauterine factors may also differ between dizygotic and monozygotic twins and may be the cause of any observed differences in the intrapair association of birthweight. However, we consider it unlikely that the differences in the intrapair associations in dizygotic vs. monozygotic twins are due to intrauterine differences. First, the overall associations of birthweight with all investigated variables were similar in dizygotic and monozygotic twins. Second, the results of two previous studies suggest that chorionicity did not influence the intrapair association between birthweight and blood pressure.[15,32] Furthermore, it should be noted that intrapair differences in birthweight in monozygotic twins have been related to within-pair differences in HDL cholesterol,[10] insulin sensitivity,[18] diabetes[16,17] and height,[12,31] demonstrating that the twin study design in general is quite capable of showing that intrauterine factors can influence adult outcome.

In the intrapair analyses in our twins, we have found evidence for a genetic influence on the association of birthweight with blood pressure, sympathetic activity, total cholesterol, LDL cholesterol and fibrinogen. However, it should be appreciated that evidence for a genetic influence does not exclude the possibility for additional non-genetic intrauterine factors. In addition, we cannot exclude that the size of this genetic effect is quite small. A similar reasoning applies to our findings of evidence for an intrauterine influence on the association of birthweight with insulin resistance, HDL cholesterol and height. Although the results within the monozygotic twins provide evidence for a non-genetic influence, we cannot exclude that the size of this effect is quite small. In addition, we cannot exclude the influence of additional genetic factors. Larger studies and meta-analyses are necessary to investigate the relative contributions of genetic and intrauterine influences more precisely.

In conclusion, both intrauterine environmental and genetic factors appear to play a role in the association between birthweight and cardiovascular risk factors. In the future, strategies may be developed targeted at improving or preventing impaired intrauterine growth. However, the effects of interventions that comprise changes in environment within the normal range may be limited due to the possible important role of genetic factors.

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