Birth Weight Predicts Risk of Cardiovascular Disease Within Dizygotic but Not Monozygotic Twin Pairs
A Large Population-Based Co-Twin–Control Study

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Background—The widely reported inverse association between birth weight and risk of cardiovascular disease (CVD) has sparked theories about early life determinants of adult disease. Within-twin-pair analysis provides a unique opportunity to investigate whether factors shared within twin pairs influence the association.

Methods and Results—In a population-based cohort of like-sexed twins with known zygosity born in Sweden from 1926 to 1958, disease-discordant twin pairs were identified through linkage to the National Inpatient and Cause of Death registers between 1973 and 2006. Co-twin–control analyses were performed on twins discordant for cardiovascular disease (n=3884), coronary heart disease (n=2668), and stroke (n=1372). Overall, inverse associations between birth weight and risk of cardiovascular diseases were seen within dizygotic but not monozygotic twin pairs. In dizygotic twins, the odds ratios for a 1-kg within-pair increase in birth weight were 0.74 (95% confidence interval, 0.56 to 0.98) for coronary heart disease and 0.57 (95% confidence interval, 0.37 to 0.88) for stroke. Conversely, no statistically significant associations were found within monozygotic twins (for coronary heart disease: odds ratio, 1.10; 95% confidence interval, 0.73 to 1.68; for stroke: odds ratio, 0.92; 95% confidence interval, 0.48 to 1.80).

Conclusions—We found an association between birth weight and risk of cardiovascular disease within disease-discordant dizygotic but not monozygotic twin pairs. This indicates that the association between birth weight and cardiovascular disease could be a result of common causes, and that factors that vary within dizygotic but not monozygotic twin pairs may help identify them. (Circulation. 2011;123:2792-2798.)

Key Words: birth weight cardiovascular diseases fetal development stroke twins

Since the emergence of reports of a link between birth weight and cardiovascular mortality, fetal growth (approximated by birth weight or birth weight for gestational age) has been found to be inversely associated with coronary heart disease and stroke,1–3 as well as risk factors such as hypertension and non–insulin-dependent diabetes mellitus.4,5 The early focus on the role of maternal nutrition paved the way for sophisticated evolutionary theories of developmental plasticity in which the fetus is programmed in preparation for the outside world (as signaled by the nutritional supply line from the mother).6 Although this field of research has moved on to consider prenatal exposures that are potentially independent of fetal growth,7 the underlying mechanisms of the original findings remain to be explained. Impaired fetal growth may result in structural changes that ultimately affect cardiovascular disease (CVD) risk. Alternatively, factors that influence fetal growth may also be involved in CVD development. Growth in utero is determined by many factors, including fetal and maternal genes,8 which may also be linked to cardiovascular pathogenesis and thereby explain the association.

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Assessing associations within twin pairs can help identify potential influence from shared common causes of fetal growth and common complex diseases. Factors that are shared by twin siblings include early environment and, to a varying extent, common genes. Potential differences between dizygotic and monozygotic twins could help us gain further insights into what factors are influential. Investigations of disease-discordant twins of the Swedish Twin Registry have shown attenuation of fetal growth associations within monozygotic twin siblings for non–insulin-dependent diabetes mellitus,9 but not for hypertension.10 In a previous small study of twins discordant for acute myocardial infarction, there was no difference in mean birth weight between cases and co-twins, but further within-pair analysis including strat-
accurately in 95% and 99%, respectively. Weight at birth is a common cause of death, and coronary heart disease (CHD) was combined for a disease. The Cause of Death Register only. For each outcome, Table 1 presents the diagnosis codes included from each revision of the International Classification of Disease and the total number of cases identified (number of cases identified from the Cause of Death Register in parentheses).

The establishment of this cohort of the Swedish Twin Register was approved by the Swedish Data Inspection Board (DNR 3083–74, 7254–95). The data collection necessary for this study was approved by the ethics committee at Karolinska Institutet (DNR 00–410).

Table 1. Definition and Distribution of Cardiovascular Disease Groups

<table>
<thead>
<tr>
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<th>Cardiovascular Disease</th>
<th>Coronary Heart Disease</th>
<th>Stroke</th>
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<tbody>
<tr>
<td></td>
<td>10th revision 120–125, 160–169, 645</td>
<td>120–125</td>
<td>160–162</td>
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Study samples, n

- Twins at risk in 1973: 23,455
- Total cases: 2548 (204)
- Pairs discordant for disease: 1942 (172)

ICD indicates International Classification of Diseases. Numbers in parentheses indicate the number of cases identified from the Cause of Death Register.

Methods

Study Setting and Participants

The study was performed within a cohort of like-sexed twins born between 1926 and 1958 who were included in the population-based Swedish Twin Register (n=37,194). Zygosity has been determined in 32,539 of these twin pairs by self reports of similarity in a paper-based questionnaire sent to all like-sexed twins in 1972 to 1973 (83% response rate), and again in a telephone interview during 1998 to 2000. The questions were found to determine zygosity accurately in 95% and 99%, respectively. Weight at birth is routinely recorded by midwives/physicians at delivery. Through a nationwide search, official birth records were retrieved, with birth weight data available in 80% of all like-sexed twins with known zygosity. For comparisons within twin pairs to be valid, it is critical that each twin has been assigned his or her (and not the co-twin’s) birth characteristics. To minimize potential misclassification of birth characteristics within twin pairs, only twins for whom birth order could be ascertained (requiring twins either to have been baptized at birth or to have answered in agreement questions about birth order in the telephone interview) were considered (n=23,689). In this sample, the mean birth weight discordance between like-sexed twin siblings was 350 g (range, 0 to 2250 g) in dizygotic twins and 304 g (range, 0 to 2420 g) in monozygotic twins.

Outcomes

Through the Swedish personal registration number, twins were followed in national registries of hospitalizations, causes of death, and population statistics (immigration and emigration from 1969 and 1961, respectively). The Inpatient Register, which started in 1964, has 85% coverage from 1983 and full coverage of all private and public hospitalizations in Sweden from 1987 on. Information includes dates of admission and discharge and up to 8 discharge diagnoses coded according to the International Classification of Disease. The Cause of Death Register (computerized and considered reliable from 1961) contains all deaths of Swedish residents; the coverage of cause of death certificates is 99%. Time at risk was defined from the beginning of 1973 (when the twins were 15 to 47 years old) until incident disease, migration, death, or end of follow-up on December 31, 2006. Cerebrovascular disease and coronary heart disease (CHD) were combined for a relatively strict definition of overall CVD. Stroke and CHD were also considered separately, and stroke was further categorized according to subtype (ischemic, hemorrhagic, or not specified). Because of the potentially fatal outcome of these diseases, we also included the few cases that were identified through the Cause of Death Register only. For each outcome, Table 1 presents the diagnosis codes included from each revision of the International Classification of Disease and the total number of cases identified (number of cases identified from the Cause of Death Register in parentheses).

The Co-Twin–Control

In a matched case-control study, analyses made conditional on the matching factors will produce estimates that are independent of the same. In a co-twin control analysis, the matching takes place at the twin-pair level; thus, estimates of effect will be independent of all factors that are shared by the twins of the twin pair. All twin siblings share early environment and, to a varying extent, genetic factors. Originating from 2 separate fertilizations, dizygotic twin siblings are genetically related like any full siblings, whereas monozygotic twin siblings originate from the same fertilized egg (zygote) and share genetic setup. A difference in the within-pair effect between dizygotic and monozygotic twins will reflect an influence of factors shared within monozygotic but not dizygotic twin pairs.

Co-twin–control selection was performed for each of the 3 main outcomes. Eligible pairs were those in which both twins were at risk of the disease until the date of case diagnosis. Table 1 presents the population at risk and the total number of cases identified per outcome. The final study samples amounted to 1942 pairs discordant for CVD (1194 male and 748 female), 1334 pairs discordant for CHD (884 male and 450 female), and 686 pairs discordant for stroke (388 male and 298 female).

Statistical Analysis

We used conditional logistic regression in the LOGISTIC procedure in SAS version 9.219 to estimate risk of CVD according to birth weight within twin pairs. To investigate the nature of the association, birth weight was first categorized into <2500, 2500 to 2999, and >3000 g, and the highest category was set as reference. Then, to test for a potential linear trend, the median birth weight of each category was used as a linear predictor. Finally, with indications of linear trends, we also assessed the effect of birth weight as a continuous covariate. In a 1:1 matched sample like this, the conditional logistic regression estimates the effect (change in log-odds of the outcome) of the difference between the 2 observations in the strata. Thus, a continuous measure of birth weight estimates a potential linear effect of the within-pair difference in birth weight. Statistical hypotheses were evaluated at the 2-sided 5% level of significance.
The main aim of the paired analysis was to investigate potential differences in effect between zygotic groups; therefore, we performed all analyses stratified according to zygosity. To test for a statistically significant difference in effect between dizygotic and monozygotic twin pairs, an interaction term between birth weight and zygosity was tested in models including all twins.

Because the co-twin-control design encompasses matching on parental, pregnancy, and early life factors, all estimated effects of birth weight on risk of CVD were independent of gestational age, birth year, and gender. Information on adult factors not shared by the co-twin was available from the 1973 questionnaire (eg, smoking, socioeconomic status, and adult body mass index). However, after careful consideration of their potential role in the association between within-pair deviance in birth weight and adult risk of CVD, we refrained from including any of these factors in the final analyses. Although unquestionably potential determinants of CVD, these factors were found to be a likely effect of (adult body mass index), or not associated with (smoking, socioeconomic status in adulthood), a within-pair difference in birth weight. Conditioning on the latter should not be of importance for the validity of the effect of interest, whereas conditioning on the former could introduce a spurious association between exposure and outcome (through unmeasured common causes of the intermediate factor and the outcome, eg, physical activity).

### Results

Table 2 shows the within-pair effects of birth weight and risks of CVD, CHD, and stroke in like-sexed dizygotic twins and monozygotic twins. Overall, there was an inverse association between birth weight and risk of CVD within dizygotic (P for linear trend=0.005) but not monozygotic (P for linear trend=0.82) twin pairs (Table 2). For CVD overall, a 1-kg increase in birth weight from the co-twin corresponded to an odds ratio of 0.73 (95% confidence interval [CI], 0.57 to 0.92) in dizygotic twins, whereas no statistically significant association was seen in monozygotic twins (odds ratio, 0.93; 95% CI, 0.65 to 1.32).

Separate analyses of CHD and stroke revealed similar patterns of inverse linear associations in dizygotic (P for linear trend=0.06 and 0.005 for CHD and stroke, respectively) but not monozygotic (P for linear trend=0.68 and 0.46, respectively) twin pairs (Table 2). In dizygotic twins, corresponding effects for a 1-kg increase in birth weight from the co-twin were 0.74 (95% CI, 0.56 to 0.98) and 0.57 (95% CI, 0.37 to 0.88) for CHD and stroke, respectively. In contrast, within monozygotic twin pairs, no statistically significant associations were seen between birth weight and risk of CHD (odds ratio, 1.10; 95% CI, 0.73 to 1.68) or stroke (odds ratio, 0.92; 95% CI, 0.48 to 1.80).

We also had the ability to stratify analyses according to subtypes of stroke (Table 3), but interpretations of these analyses were slightly hampered by reduced statistical power. Nevertheless, for ischemic stroke, the same pattern of an inverse linear association was obvious within dizygotic but not monozygotic twins (odds ratio, 0.49; 95% CI, 0.28 to 0.88 within dizygotic twins; and odds ratio, 0.98; 95% CI, 0.41 to 2.33 within monozygotic twins). For hemorrhagic stroke, however, estimates were imprecise for both dizygotic and monozygotic twins, precluding interpretation of potential effects in either group.

Finally, tests of statistically significant difference in effect of birth weight on risk of disease between like-sexed dizygotic and monozygotic twins (from an interaction term between birth weight and zygosity) yielded P=0.27 for CVD, P=0.18 for CHD, and P=0.06 for ischemic stroke. Stratified analyses and tests for interaction between birth weight and sex were hampered by low statistical power and did not support any appreciable differences in effect of birth weight on risks of CVD between male and female subjects (data not shown).

### Discussion

In this large population-based twin sample, birth weight was found to be inversely associated with risk of CVD within dizygotic but not monozygotic twin pairs. Similar to previous findings in singletons, associations were seen for both CHD and ischemic stroke, but only within dizygotic twin pairs. The findings indicate that the apparent association between birth weight and CVD could be a result of common causes and that factors that vary within dizygotic but not monozygotic twin pairs may help identify them.
An association between birth weight and/or fetal growth and cardiovascular morbidity and mortality has been established in various populations.\textsuperscript{1–5} From the early theories of maternal nutrition and the paradigm shift to an evolutionary perspective and programming,\textsuperscript{9} the true underlying mechanisms have proved difficult to unravel. In humans, most attempts involve linking impaired fetal growth to indicators of different pathways of cardiovascular pathogenesis, including endothelial function,\textsuperscript{21} sympathetic nerve activity,\textsuperscript{22} β-cell function,\textsuperscript{23} and low-grade inflammation.\textsuperscript{24} Exploring the effects of causes or components of fetal growth has proved more complicated, at least in humans. Although animal experiments have provided important insights, most studies involve manipulations of the maternal diet, which may or may not lead to fetal growth impairment or low birth weight.\textsuperscript{10} It has consequently been argued that programming mechanisms may be independent of fetal growth and that the study of growth may be inappropriate in the first place.\textsuperscript{10} However, apart from natural experiments like the Leningrad siege\textsuperscript{25} and the Dutch famine,\textsuperscript{26} findings to support fetal programming in humans rest mainly on associations with birth weight and/or fetal growth, and the origin of these associations remains unresolved. The findings of the present study indicate that further insights could be gained by understanding which factors are uniquely shared within twin pairs. Classic twin studies often assume that monozygotic and dizygotic twins share the same environment. This assumption of equal environment may not be plausible, particularly in prenatal life. Differences in sharing experience in utero are mainly a consequence of placentation. Roughly all dizygotic twins share the same environment. This assumption proposes a common genetic background for fetal growth and CVD. Fetal genes are responsible for more than one third of fetal growth and CVDs are under genetic influence.\textsuperscript{32} The genetic perspective has further been put forward in the fetal insulin hypothesis, which proposes a common genetic background for fetal growth and insulin resistance.\textsuperscript{33} Indeed, allelic variations in genes involved in insulin regulation (INS, IGF1, and ADCY5) have been found to be associated with low birth weight and risk of non–insulin-dependent diabetes mellitus and myocardial infarction.\textsuperscript{6,34,35} Recently, common polymorphisms in the promoter region of PONI (encoding paraoxonase-I, which pro-

\begin{table}
\centering
\caption{Within-Pair Effect of Birth Weight on Stroke Subtypes in Like-Sexed Twins}
\begin{tabular}{llccc}
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 & & & & \\
 & & Ischemic Stroke & Hemorrhagic Stroke & \\
 & & Cases & Co-Twins & OR (95\% CI) & Cases & Co-Twins & OR (95\% CI) \\
\hline
DZ & & & & & \\
\leq 2499 g & 111 & 88 & 2.04 (1.06–3.90) & 39 & 37 & 1.59 (0.66–3.80) \\
2500–2999 g & 98 & 116 & 1.02 (0.61–1.70) & 46 & 40 & 1.57 (0.79–3.12) \\
\geq 3000 g & 64 & 69 & Reference & 29 & 37 & Reference \\
Total & 273 & 273 & \textit{P for trend}=0.036 & 114 & 114 & \textit{P for trend}=0.309 \\
Per 1000-g increase & 0.49 (0.28–0.88) & 0.51 (0.23–1.13) & \\
\hline
MZ & & & & & \\
\leq 2499 g & 72 & 74 & 0.49 (0.18–1.39) & 43 & 44 & 1.56 (0.44–5.51) \\
2500–2999 g & 44 & 49 & 0.50 (0.20–1.26) & 37 & 30 & 1.89 (0.75–4.78) \\
\geq 3000 g & 25 & 18 & Reference & 16 & 22 & Reference \\
Total & 141 & 141 & \textit{P for trend}=0.32 & 96 & 96 & \textit{P for trend}=0.543 \\
Per 1000-g increase & 0.98 (0.41–2.33) & 0.74 (0.25–2.24) & \\
\hline
\end{tabular}
\end{table}
tects low-density lipoproteins from oxidative modification), previously linked to oxidative stress and risk of CVD, were found to be determinants of occurrence of small for gestational age.

Inference about potential genetic influence has also been sought from studying CVD occurrence in parents and grandparents in relation to offspring birth weight. With respect to CVD morbidity and mortality, associations with low birth weight in offspring have been shown in both parents, but predominantly in mothers. Some interpret the stronger association between offspring’s birth weight and CVD in mothers compared with fathers as favoring fetal programming mechanisms rather than potential genetic influence. Others point to the double role of maternal genes, arguing that their influence on fetal growth is not only through genetic inheritance (fetal genetic effect), but also through the provided intrauterine environment (maternal genetic effect). Although more speculative, there are also potential explanations suggesting a role of imprinting, mitochondrial DNA, and X-linked inheritance.

For potential nongenetic inheritance, the situation becomes more complex, mainly because of the heterogeneity of this concept, which encompasses any mode of transgenerational transmission of phenotypic variation independently of the genetic code. Suggested mediators include epigenetic, nutritional, behavioral, and environmental variation. Notably, epigenetic modulation has been put forward as a compelling mechanism for phenotypic discordance in dizygotic twins. It has been suggested, however, that the epigenetic effects of maternal nutrition occur already in the preimplantation embryo. Epigenetic modulation from individually experienced exposures would lead to phenotypic discordance in both dizygotic and monozygotic twins. It has further been proposed that the underlying genotype may modify the programming effects of early life exposures. If susceptibility to early life exposures depends on underlying genotype, only susceptible individuals would contribute to the observed associations. This would also be the case in twins; ie, only pairs in which the exposed twin was also genetically susceptible would contribute to a within-pair association. It follows that for this scenario to be compatible with the present findings, there would have to be a systematic difference in the distribution of genetic susceptibility between dizygotic and monozygotic twins; notably, monozygotic twins would have to be significantly less genetically susceptible to programming in utero compared with dizygotic twins.

In the present study, we were able to follow a population-based sample of twins 15 to 47 years of age with respect to cardiovascular outcomes for >30 years. Zygosity was established with high validity and good coverage. We also took measures to reduce misclassification of birth weights within twin pairs, which is of utmost importance for the validity of within-pair analysis. In addition, because twin siblings have the same gestational length, mother, socioeconomic status before adulthood, and, to a varying extent, genes, these factors were all accounted for in within-pair analysis. Matching precluded the evaluation of zygosity on risk of CVD in this sample, but in the cohort in which this study was nested, age- and gender-standardized rates of CVD were the same in dizygotic and monozygotic twins. Within-pair comparisons can also be evaluated in a cohort setting, eg, by estimating the effect of the individual deviance from the twin-pair mean birth weight. The co-twin–control design is limited to disease-discordant pairs but lends itself easily to the evaluation of nonlinear effects and conceptual interpretation (with the disease-free co-twin used as the control to the case).

Findings in twin studies are commonly questioned concerning their representativeness of the general population. It may be especially important to consider whether differences in prenatal environment between twins and singletons. Because of spatial and nutritional constraints, twins generally have shorter gestation and lower birth weights for gestational age compared with singletons. Yet, there appears to be no difference between twins and singletons with respect to cardiovascular mortality in adulthood. However, there are many reports of associations between birth weight and adult diseases in twin samples, similar to those widely reported in singleton populations. Taken together, these findings indicate that the general growth constraint of twinning (owing to sharing of space and supply line) may not influence adult disease risks, whereas factors that make twins experience different growth (from other twins and/or their co-twin) could. Such factors could be fetal growth determinants also common for singletons, such as gestational length and maternal, socioeconomic, and genetic factors. We believe that, although the twin experience in utero is different from that of singletons, understanding whether fetal growth differences within dizygotic and monozygotic twin siblings influence the risk of CVD makes an important contribution to the understanding of early life influence on adult disease. The present findings do not support an association between birth weight and risk of CVD in the absence of genetic variation.

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Disclosures

None.
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CLINICAL PERSPECTIVE

Weight at birth has consistently been shown to predict risk of cardiovascular disease (CVD). When considering birth weight as a proxy for the fetal environment, this has prompted theories of early life determinants of adult disease. Although this field of research has come to include exposures that may be independent of birth weight, the underlying mechanisms of the original association remain to be elucidated. Birth weight has many determinants, which, if also involved in CVD development, could explain the association between the two. Since twin siblings share early environment and a varying degree of genetic factors, assessing the association between birth weight and risk of CVD within twin pairs offers unique possibilities to explore the influence from these factors. In this study, we identified 1942 twin pairs discordant for CVD in a population-based cohort of like-sexed twins born in Sweden 1926–1958. Birth weight was found to be inversely associated with risk of CVD within dizygotic, but not monozygotic twin pairs. Similar to previous findings in singletons, associations were seen for both coronary heart disease and ischemic stroke, but only within dizygotic twin pairs. Our study thus lends no support for an association between birth weight and CVD in the absence of genetic differences. We believe these findings make an important contribution to the understanding of early life influence on adult disease, by indicating that the association between birth weight and CVD could be a result of common causes.
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