

Cardiovascular Mortality in Twins and the Fetal Origins Hypothesis

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The intrauterine growth patterns for twins are characterized by normal development during the first two trimesters and reduced growth during the third trimester. According to the fetal origins hypothesis this growth pattern is associated with risk factors for cardiovascular morbidity and mortality. We studied cause-specific mortality of 19,986 Danish twin individuals from the birth cohorts 1870–1930 followed from 1952 through 1993. Despite the large sample size and follow-up period we were not able to detect any difference between twins and the general population with regard to all-cause mortality or cardiovascular mortality. Hence, the intrauterine growth retardation experienced by twins does not result in any “fetal programming” of cardiovascular diseases. There is still an important role for twins (and other sibs) to play in the testing of the fetal origins hypothesis, namely in studies of intra-pair differences, which can assess the role of genetic confounding in the association between fetal growth and later health outcome.

The fetal origins hypothesis (the “Barker hypothesis”) states that fetal under-nutrition in middle to late gestation, which leads to disproportionate fetal growth, influences the risk of cardiovascular diseases, diabetes mellitus, and a number of other diseases (Barker, 1998). The typical growth pattern for twins is normal development during the first two trimesters and reduced growth during the third trimester (MacGillivray et al., 1988). According to the fetal origins hypothesis (Barker, 1995) such a growth pattern leads to elevated blood pressure and raised serum low density lipoprotein cholesterol and plasma fibrinogen concentration.

This raises a question important to twin researchers: Does the reduced growth pattern in the last trimester make twins more vulnerable in adult life with an increased risk of cardiovascular diseases, diabetes mellitus and other diseases that are suspected to be “programmed”? If so, then twin studies may be a poor model for studying these diseases because the causal field of the diseases could be different from that of singletons.

If twins are “programmed” due to the considerable growth retardation during the third trimester, one could expect an increased mortality and especially an increased cardiovascular mortality in adulthood for twins compared to the general population. Both a Swedish and our earlier Danish twin study found similar mortality patterns among twins and singletons in adulthood (Christensen et al., 1995; Vågerö and Leon, 1994). However, the Swedish twin study (Vågerö and Leon, 1994) had a limited follow-up period

with no distinction between monozygotic (MZ) and dizygotic (DZ) twins (the latter having slightly higher birth weights on average), and our Danish twin study (Christensen et al., 1995) did not include causes of death.

The present study used data on cause-specific mortality of 19,986 Danish twin individuals from the birth cohorts 1870–1930 followed-up from January 1, 1952 until December 31, 1993. We compared the mortality pattern of MZ and DZ twins as well as the mortality pattern of all twins together (including twins of unknown zygosity) and the general Danish population with respect to all-cause mortality and cardiovascular death.

Material and Method

The Danish Twin Registry

This registry was established in 1954 as the first nationwide twin registry in the world (Hauge, 1981; Hauge et al., 1968). The birth registers from all 2,200 parishes of the relevant calendar years were manually scrutinized to identify all twin births. Through regional population registers (in operation since 1924) and other public sources, a search was made for the twins, or whenever needed, their closest relatives. As soon as a twin was traced, a questionnaire was sent to him or her. If neither of the partners was alive, a questionnaire was sent to the closest relative. Specific questions about the degree of similarity between the partners of a pair were included in the questionnaire to assess zygosity in like-sexed twins. For twins dying or emigrating at an early age it was impossible to obtain reliable data to be used in zygosity classification. Consequently, pairs were not followed-up if one or both partners died or emigrated before age 6. Nearly all the twins surviving past age 15 have been traced; untraced twins are almost entirely twins who died or emigrated in childhood, although the date of death or emigration is unknown. The validity of zygosity classification based on answers to mailed questionnaires has been evaluated by comparison with the results of later blood group determinants and the misclassification rate has been found to be less than 5% (Hauge, 1981).

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The validity of the Danish twin panel of like-sexed cohorts born 1881–1920 has been evaluated and found to be very high (Holm, 1983). Of special importance for mortality studies was the observation that year of death was correctly coded in 99% of the twins, i.e., only 1% were coded as alive when in fact they were dead. During the last decade, matching twins with the Danish Person and Death Registers has reduced this 1% error rate to almost zero.

Follow-up Period and Cause of Death

The data set contains records of twins who were born between January 1, 1870 and December 31, 1930, and deceased after January 1, 1952. Individuals were followed-up to December 31, 1993. Altogether, 9,329 male twin individuals and 10,657 female twin individuals were included. 296 individuals with unknown cause of death during the follow-up period were excluded. Around two thirds of the study population died during the follow-up period (Table 1).

Death status, age at death and cause of death were obtained from the Civil Registration System, the Danish Cause-of-Death Register, the Danish Cancer Registry (founded in 1942), and other public registers in Denmark. 'Cause of death' was coded according to the International Classification of Diseases (ICD, sixth, seventh and eighth editions), and two groups of causes of death are considered here: 'heart disease' (i.e. all cardiovascular diseases minus stroke) and stroke (ICD 6 and 7: 400–468, 330–334; ICD 8: 390–429 & 440–458, 430–438).

Survival Analysis

Detailed lifetime information is directly available for nearly all of the Danish twins with exact date of death. At the population level, however, this information is only available as age at death in years. To overcome this limitation in the data source, lifetimes of the twins were truncated to full years to make data from both populations comparable. The Kaplan-Meier estimator (Kaplan & Meier, 1958) was used to calculate life expectancy for all groups separately. The Log-Rank test was performed to detect differences in the mortality pattern of MZ and DZ twins (all-cause and cause-specific mortality), and of all Danish twins combined and the general population (all-cause mortality).

Information about cause-specific mortality of the general Danish population is available for 5 year age groups

20–24, 25–29, 30–34, ..., 80–84 since 1952 from the WHO Mortality Data Base. Five year mean death rates were calculated on a cohort basis with $m_x = D_x / (P_x - D_x / 2 - C_x / 2)$, where D_x is the number of deaths in age group x , C_x is the number of censored observations in age group x and P_x is the population at risk at age x . Because of the information available on cause-specific mortality in the general Danish population (5 year intervals), sign tests based on m_x values were used to detect significant differences between the cause-specific mortality pattern of all twins and the general population. Sign tests provide a robust non-parametric procedure for testing differences in mortality rates.

Analyses were made with the standard statistical software package SPSS (SPSS 1989–1997). All tests were two-tailed and $p < 0.05$ was considered significant.

Results

All-cause Mortality

For both male and female MZ twins, life expectancy is higher than for the respective DZ twins and twins of unknown zygosity (76.0 (95% CI: 75.6–76.5) and 79.8 (79.3–80.3) vs. 75.0 (74.7–75.4) and 79.3 (79.0–79.7) (DZ twins) and 74.8 (74.1–75.5) and 77.5 (77.0–78.0) (UZ twins)). For the same (left truncated and right censored) cohort of the Danish population, life expectancy is 75.1 for males and 79.1 for females. Analysing lifetimes of twins and the Danish population born between January 1, 1870 and December 31, 1930 and deceased after January 1, 1952 the Log-Rank test indicates no significant differences between the lifetimes of all Danish twins combined and the Danish population for both males and females, respectively. The advantage of MZ twins compared with DZ twins is significant for males (see Table 2).

The sex specific hazard functions among MZ and DZ twins and the general Danish population are shown in Figure 1. The figures underline that mortality among twins and the general population is nearly identical.

Cause Specific Mortality

For the Danish population and all twins together cause-specific death rates for heart disease, stroke, and all-cause mortality are shown in Figures 2–4 for males and females, respectively. Confidence intervals were calculated using the binomial formula. The analysis showed no significant

Table 1

Status for the 19,986 Danish Twins born 1870–1930 Followed Up From January 1, 1952 Until December 31, 1993

Number of persons	Males			Females			Total
	MZ	DZ	UZ	MZ	DZ	UZ	
Deaths							
— heart disease	706	1268	391	603	1093	489	4550
— stroke	167	324	113	209	361	184	1358
— other	919	1703	458	831	1619	597	6127
all causes	1792	3295	962	1643	3073	1270	12035
Alive	988	1816	476	1404	2658	609	7951
Total	2780	5111	1438	3047	5731	1879	19986

Note: MZ = Monozygotic, DZ = Dizygotic, UZ = Unknown zygosity

Table 2

Comparison of Mortality for Twins and the General Population (Log-rank Test and Sign Test)

	Males		Females	
	MZ vs DZ	Twins vs DK	MZ vs DZ	Twins vs DK
	p-value	p-value	p-value	p-value
heart disease	0.20* (+)	0.11* (++)	0.52* (-)	0.69* (---)
Stroke	0.11* (+)	1.00* (---)	0.86* (-)	0.51* (---)
All causes	0.01* (+)	0.13* (++)	0.09* (+)	0.74* (++)

Note: * log-rank test # sign test

MZ = Monozygotic, DZ = Dizygotic, DK = general population

(+) Mortality is lower among MZ twins compared with DZ twins

(-) Mortality is higher among MZ twins compared with DZ twins

(++) Mortality is lower among twins compared with the general population

(---) Mortality is higher among twins compared with the general population

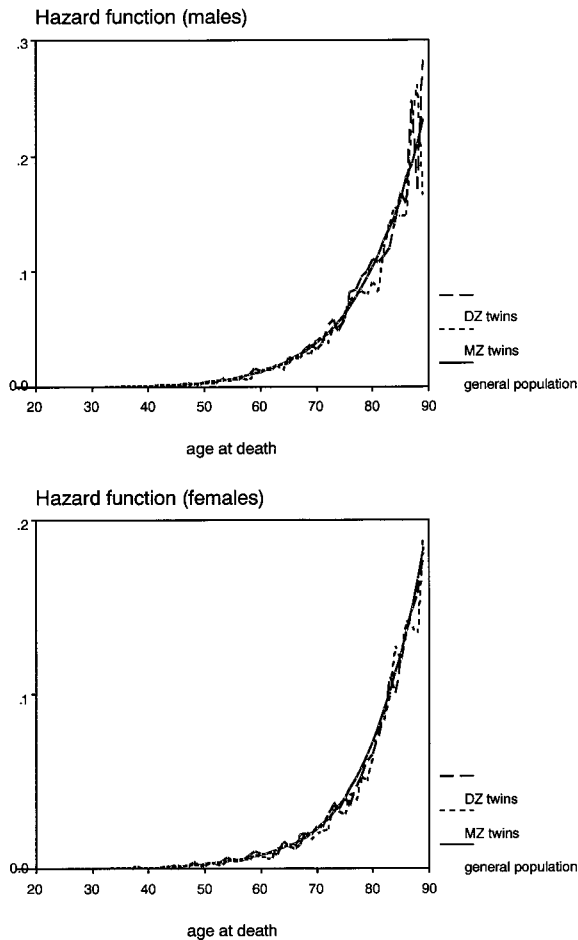


Figure 1.

Mortality in twins and in the general population in Denmark, still alive on January 1, 1952. Mortality was calculated for cohorts born 1870–1930.

MZ = monozygotic, DZ = dizygotic.

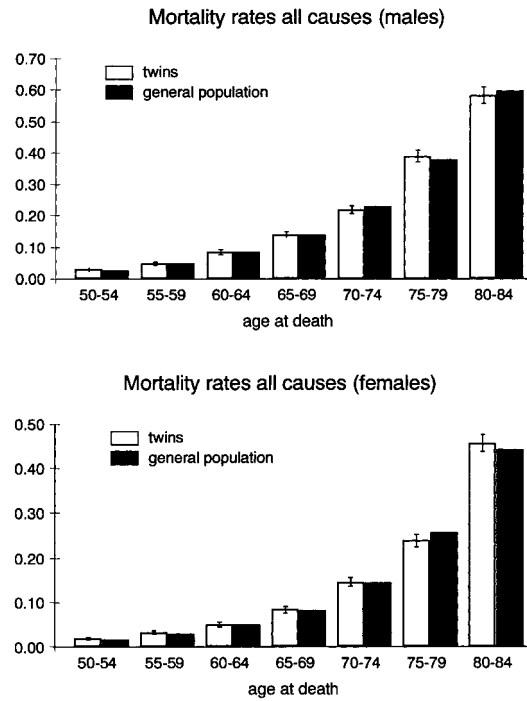


Figure 2.

Mortality rates of all causes for twins and the general population. The bars for twin mortality rates include 95% confidence intervals for comparisons with the mortality rates of the general population.

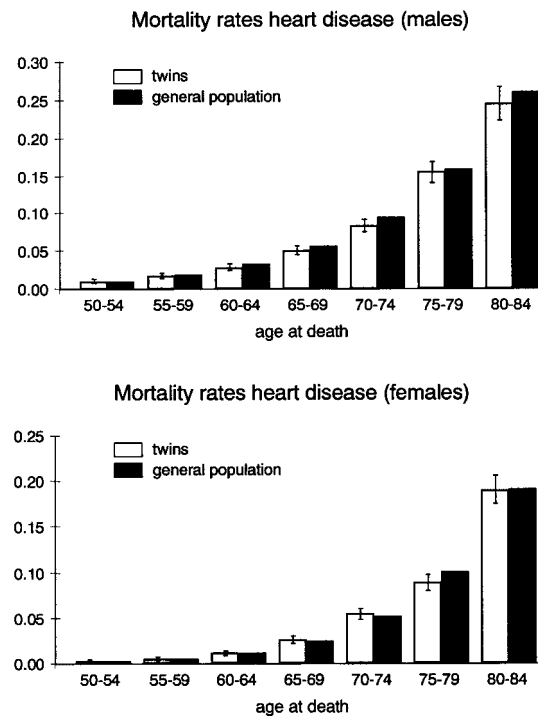


Figure 3.

Mortality rates of heart disease for twins and the general population. The bars for twin mortality rates include 95% confidence intervals for comparisons with the mortality rates of the general population.

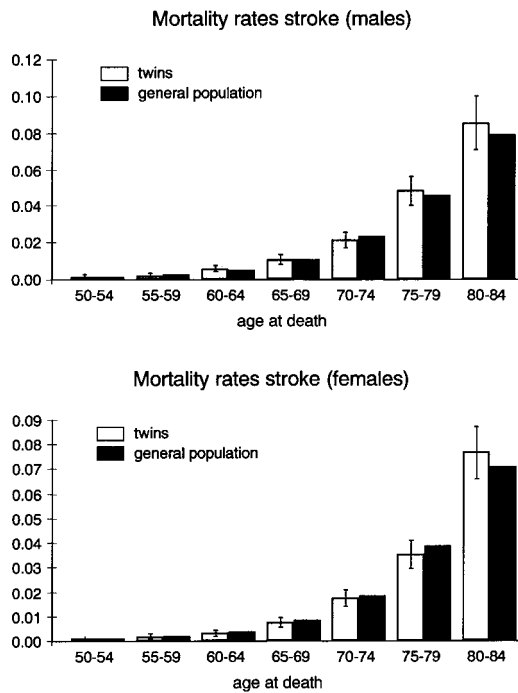


Figure 4.

Mortality rates of stroke for twins and the general population. The bars for twin mortality rates include 95% confidence intervals for comparisons with the mortality rates of the general population.

difference between death rates of all twins together and the general population for either all-cause mortality, or mortality related to heart disease and stroke (see Table 2). Additionally, a comparison of the mortality pattern of MZ and DZ twins does not indicate any differences except for all-cause mortality in males where MZ had a slightly lower mortality than DZ twins ($p = 0.01$).

Discussion

This follow-up of nearly 20,000 twin individuals born 1870–1930 has failed to show any increased general mortality or cardiovascular mortality in adulthood among twins compared to the general population. The only differences found in the large sample were the slightly lower mortality among MZ twins and a higher mortality among the twins with unknown zygosity. The latter finding was expected as a reason for being without a known zygosity could be early death. It should be noticed, however, that when we consider all twins vs. the general Danish population there are no differences in general mortality or cardiovascular mortality.

Phillips (1993) has argued that even small birth weight differences in twins could reflect important differences in intrauterine conditions important for the programming of diseases later in life, because the mean birth weight among twins is already considerably lower than in singletons. On the other hand, Barker (1995) has argued that twin mortality is interesting but irrelevant for the programming hypothesis, because twins “are a mixture of proportionately and disproportionately small babies”. Naturally,

not all twins (or for that sake low birth weight singletons) have the same growth pattern. However, what is more important is that the typical growth pattern for twins is normal development during the first two trimesters and reduced growth during the third trimester (MacGillivray et al., 1988). According to the fetal origins hypothesis (Barker, 1995) such a growth pattern leads to elevated blood pressure and raised serum low density lipoprotein cholesterol and plasma fibrinogen concentration - conditions associated with an increased risk of cardiovascular morbidity and mortality.

Phillips (1995) has argued that ultrasound evidence suggests that twins down regulate their growth rate early in gestation, possibly during the first trimester and that setting a low growth trajectory early in pregnancy protects the fetus against the effects of undernutrition later in pregnancy. The ultrasound evidence Phillips refer to is a study by Leveno et al. (1979), which included 75 cases with sonar cephalometry performed in middle gestation (between 16 and 28 weeks) and none from the first trimester. This study showed that among twins the average biparietal diameter was 3–4 mm smaller than among singletons. Surprisingly, the study did not show any increase in the difference with gestational age, which could have been expected based on the well documented third trimester growth retardation among twins (Campbell & Samphier, 1988). Leveno et al. (1979) also emphasise that their finding of a growth difference between twins and singletons early in pregnancy is in contrast to previous ultrasound and birth weight studies.

The present study and the previous Swedish study (Vågerö & Leon, 1994) provide strong evidence for the absence of a “programming effect” for twins. Hence, results from twin studies of cardiovascular diseases are likely to be valid also for the general population. Of course it cannot be excluded that the effect of selection imposed by high perinatal mortality for twins (only the “strongest” twins survive) and the programming effect are equal in size and thereby make the general mortality pattern for twins similar to that of the general population. However, the similarity in adult mortality for twins and the general population is consistent over birth cohorts with different infant and childhood mortality and therefore it is unlikely to be an artefact of selection.

There is still an important role to play for twins in testing the fetal programming hypothesis. There is little doubt that an association exists between fetal growth and a number of later life health outcomes such as blood pressure or cardiovascular mortality. The key question is, however, whether it is fetal nourishment or other factors such as genes or socio-economic conditions that cause the associations. Recent studies suggest that socio-economic confounding cannot explain the association between fetal growth and cardiovascular mortality (Leon et al., 1998), but few studies have evaluated the influence of genetic confounding.

The potential for genetic confounding has been illustrated by Dunger et al. (1998) who showed that variation in the insulin gene (INS VNTR) is associated with fetal growth. Based on studies of fetal insulin secretion and monogenic diseases, Hattersley and Tooke (1999) recently proposed that genetically determined insulin resistance con-

tributes substantially to the association of low birth weight with diabetes, hypertension and vascular diseases and named this hypothesis the 'fetal insulin hypothesis'.

We recently tested the potential influence of genetic confounding on the association between birth weight and systolic blood pressure (Christensen et al., 2001), which is the best documented association between fetal growth and later life health outcome (Taylor et al., 1997). The effect of genetic confounding was evaluated by analysing individual twin data as well as intra-pair differences in birth weight and systolic blood pressure. This approach enables controlling for the effect of all genetic factors in monozygotic pairs and on average half of the genetic factors in dizygotic pairs as well as environmental maternal effects. We found that the association between birth weight and blood pressure attenuated when genetic factors were controlled in agreement with a recent study of 114 adolescent twins by Ijzerman et al. (2000) and further supported by a family study by Melander et al. (1999). This suggests an important contribution of genetic factors to the association between fetal growth and systolic blood pressure. However, other twin studies have suggested that the association between birth weight and diseases in adult life is independent of genetic influence (Bo et al., 2000; Poulsen et al., 1999; Poulter et al., 1999). Baird et al., (2001) have argued that genetic determinants for fetal growth and adult outcome are not likely to be prevalent or powerful, but still the available data on this topic are sparse. Therefore future studies of intra-pair differences in twins and ordinary sibs are important for assessing the magnitude of genetic confounding in the association between fetal growth and later life health outcomes.

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A N N O U N C E M E N T

NWO/Spinoza A W A R D

Dorret Boomsma to receive NWO/Spinoza Award

The Dutch Organisation for Scientific Research (NWO) has awarded its annual NWO/Spinoza award to Dorret Boomsma for her internationally acclaimed work in the fields of Behaviour Genetics and Twin Research. The ceremony with a testimonial of the laureates will be held in January 2002.

The NWO/Spinoza award, named after the Dutch philosopher Baruch de Spinoza (1632–1677), is the most prestigious award in the Dutch Science Community and has been nicknamed the national 'Nobel Prize' (the 1995 laureate Gerard 't Hooft went on to receive "the" Nobel Prize).

Dorret Boomsma was nominated by the Chancellors of the Vrije Universiteit on grounds of her excellent work on the statistical methodology of twin family studies and the successful use of a wide scope of psychophysiological phenotypes to support the links between genes and behavior. The laureate's work is held to meet the highest international standards and she is considered a worthy ambassador of Dutch science. Her relentless efforts have, furthermore, made the Dutch Twin Registry into one of the largest and most complete in the world, with 30,000 twin pairs enrolled.

Boomsma's research team has made significant contribution to the genetics of cognitive development and brain function, personality and psychopathology, nicotine and alcohol dependence, and stress and health. In structural equation modeling, Boomsma actively engaged in the development of the first multivariate models, power-analyses for QTL detection, and more recently the extension of the twin design with one or more siblings.

With this award, the fields of Behaviour Genetics and Twin Research receive a major boost in the Netherlands. The award comes with 3.3 million Dutch guilders (or 1.5 million Euro) that the laureate may spend to set up a research project of her choice. More importantly, the award is certain to attract the finest talent from the ranks of graduate and post-graduate students to enter our field.

Twin Research is proud to congratulate one of its associate editors and strong supporters on this eminent award, which brings great credit both to her and to the field.

Dorrett **BOOMSMA**



C O N G R A T U L A T I O N S