Strength and Anthropometric Measures in Identical and Fraternal Twins: No Evidence of Masculinization of Females with Male Co-Twins

David Gaist,[1,2] *Lise Bathum,*[3] *Axel Skytthe,*[1,2] *Tina Kold Jensen,*[4] *Matt McGue,*[5] *James W. Vaupel,*[6] *and Kaare Christensen*[1,7]

Sharing of intrauterine environment in twins of opposite sex has been hypothesized to result in masculinization of the female twin. We tested this hypothesis by comparing strength (maximum hand-grip pressure) and various anthropometric measures in a newly established survey panel comprising 4,314 middle-aged twins identified through a Danish population-based twin registry. Sex- and zygosity-specific mean values of handgrip strength, height, weight, body mass index, and waist circumference were highly comparable between fraternal twins of opposite sex and fraternal twins of same sex. Our results provide no support for the hypothesis of masculinization of female twins from opposite sex twin pairs. (Epidemiology 2000; 11:340–343)

Keywords: twins, gender, anthropometry, muscle strength, masculinization, phenotype.

In several species of litter-bearing mammals, intrauterine positioning predicts postnatal characteristics.[1] Female animals gestated between two male fetuses are exposed to increased levels of androgens; this has been shown to result in masculinization of the female fetus with regard to a broad range of morphological and behavioral postnatal characteristics.[2–5]

Human twins can be identical (MZ), fraternal of the same sex (DZss), or fraternal of the opposite sex (DZos). Pregnancy with DZos is the human analogy to the intrauterine position phenomenon in litter-bearing animals. The female DZos fetus shares the intrauterine environment with a male fetus and is potentially exposed to higher androgen levels produced by the male co-twin. This exposure has been offered as an explanation for the higher degree of masculinization of female DZos observed in some studies.[6–8]

In the present twin study, we focused on strength and body composition measures, phenotypes that are known to depend on intrauterine positioning in animals,[1,2,4] and that are also important predictors of human morbidity and disability.[9,10] We used data from a large newly established panel of middle-aged identical and fraternal twins identified in a population-based registry.

Subjects and Methods

We identified pairs of twins born between 1931 and 1952 through the Danish Twin Registry.[11] The twins had responded to a brief mailed questionnaire (response rate 77%) in 1997 and had declared their willingness to participate in future studies (90% of responders). This questionnaire included items on similarity of the twins, based on which we assigned zygosity, a method found to result in misclassification rates of less than 5%.[12] Within each of the 22 birth cohorts, we randomly identified 40 pairs of each zygosity (MZ, DSss, and DZos). Half of the retrieved MZ and DZss twin pairs were female. Owing to insufficient numbers of MZ pairs in the birth cohorts of 1933, 1934, and 1936, we retrieved an additional 11 MZ pairs from the birth cohorts of 1931 and 1935. A total of 2,640 pairs were identified. Shortly before the survey we obtained demographic information on these twins from a continuously updated nation-wide population register, the Danish Civil Registration System. Ninety-one twins (1.7%) had died or emigrated in the 2-year period pre-

From the [1]The Danish Twin Registry, University of Southern Denmark, Odense University, [2]Epidemiology, Institute of Public Health, University of Southern Denmark, Odense University, [3]Department of Clinical Biochemistry, Odense University Hospital, [4]Department of Epidemiology and Public Health, Imperial College of Medicine at St. Mary's, United Kingdom & Department of Environmental Medicine, University of Southern Denmark, Odense University, Odense, Denmark; [5]Department of Psychology, University of Minnesota, Minneapolis, MN; [6] Max Planck Institute for Demographic Research, Rostock, Germany; [7]Danish Center for Demographic Research, Odense, Denmark.

Address reprint requests to: David Gaist, Epidemiology, Institute of Public Health, University of Southern Denmark, Odense University, Sdr. Boulevard 23 A, 5000 Odense C, Denmark.

This study and the activities of the Danish Center for Demographic Research are supported by a grant from the Danish National Research Foundation and Sygekassernes Helsefond Grant 11/2 12–97.

Submitted July 6, 1999; final version accepted October 12, 1999.

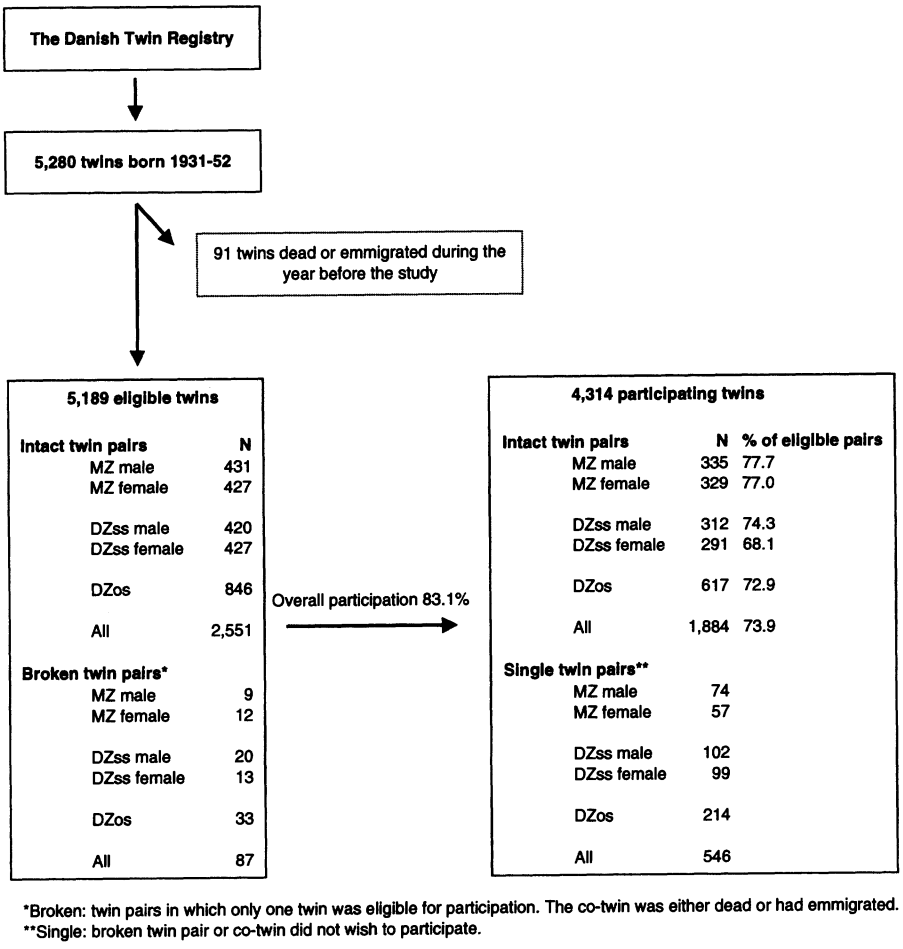

FIGURE 1. Eligibility and participation in the study of middle-aged Danish twins.

ceding the study, leaving 5,189 twins eligible for participation in the study.

Interviews were conducted by a total of 100 interviewers from the Danish National Institute of Social Research, an approach previously used in twin research projects.[13] The survey, which lasted on average 1½ hours, comprised a questionnaire, tests of cognitive and physical functioning, and sampling of DNA.

All interviewers received a detailed training program by a physician and were closely monitored in the 6-month period (October 1998 through March 1999) during which the interviews were completed. The twins were considered not possible to contact only when at least three unsuccessful attempts to contact them at their residence were made. To avoid interviewer bias, which would inflate twin similarity, twins from a pair were never interviewed by the same interviewer. A pilot study testing all procedures resulted in minor changes only.

We compared participants and non-participants with regard to age, gender, urban residency, and marital status using data from the Civil Registration System. Height in centimeters and weight in kilograms were self-reported and were used to calculate the body mass index (weight/height^2 kg/m^2). With a tape measure, the interviewers took two measurements of the subjects' waist circumference between the lowest rib margin and the iliac crest and recorded the values to the closest 0.1 cm. The average of the two measures was used in this analysis. For grip strength, we identified the maximum value among the recorded measurements (three for each hand for 97.8% of the sample).

Results

Eighty-five percent of male and 81.3% of female eligible twins participated (Figure 1). Compared with non-participants, participants were slightly younger, more frequently married, and lived less frequently in urban areas (Table 1). These differences remained when the sample was stratified by zygosity (results not shown). Pair-wise

TABLE 1. Characteristics of Participants and Non-Participants in the Study of Middle-Aged Danish Twins (N = 5,189)

	Both Twins Participants N = 3,768	Both Twins Non-Participants N = 366	One Twin Participant* N = 546	One Twin Non-Participant† N = 509	Participants* N = 4,314	Non-Participants† N = 875
Age,‡ mean (SD)	56.9 (6.3)	57.9 (6.3)	57.1 (6.4)	56.6 (6.4)	56.9 (6.3)	57.2 (6.4)
Male, %	50.7	37.7	52.6	49.1	51.0	44.3
Urban residency,§ %	26.0	28.1	30.6	33.0	26.6	31.0
Married, %	77.2	73.0	75.6	69.4	77.0	70.9

* Includes 62 twins from twin pairs where only one twin was eligible for participation.
† Includes 25 twins from twin pairs where only one twin was eligible for participation.
‡ As of January 1, 1999.
§ Defined as residency in borough with 500 or more inhabitants per km^2.

TABLE 2. Mean Values (Standard Deviations) of Handgrip Strength and Body Composition Components by Gender and Zygosity in 4,223 Middle-Aged Danish Twins

	Identical Twins	Fraternal Twins Same Sex	Fraternal Twins Opposite Sex
Male			
N	734	709	722
Age, years	56.9 (6.4)	56.8 (6.4)	57.0 (6.3)
Height, cm	176.1 (6.5)	176.5 (6.5)	176.7 (7.0)
Weight, kg	80.4 (11.3)	81.3 (11.7)	80.7 (11.5)
Body mass index, kg/m^2	25.9 (3.2)	26.1 (3.3)	25.9 (3.3)
Waist circumference, cm	97.8 (9.7)	98.6 (10.3)	98.0 (9.6)
Maximum handgrip, kg	47.4 (8.2)	47.9 (8.2)	48.2 (8.7)
Female			
N	703	655	700
Age, years	56.9 (6.4)	56.7 (6.3)	56.9 (6.3)
Height, cm	164.0 (5.8)	164.8 (5.8)	164.9 (6.2)
Weight, kg	65.4 (10.7)	65.9 (11.7)	66.0 (11.0)
Body mass index, kg/m^2	24.3 (3.9)	24.2 (3.9)	24.3 (3.9)
Waist circumference, cm	85.1 (11.7)	85.1 (11.9)	85.1 (11.8)
Maximum handgrip, kg	27.1 (5.7)	28.2 (5.8)	27.8 (6.0)

participation was greater for MZ pairs (77%) than DZ pairs (72%).

After exclusion of twins with missing values on variables of interest, the sample was reduced to 4,223 twins (97.9%). The mean age of these subjects was 56.9 years (SD = 6.3) and varied 0.3 years or less in sex and zygosity strata (Table 2). Mean values and standard deviations for grip strength and body composition were virtually identical for MZ, DZss, and DZos in both males and females. In particular, the mean values of female DZos for all measures differed by less than 1% from the corresponding means for other female twins (MZ and DZss), and the differences were not consistently in one direction.

Discussion

Animal studies provide strong support for masculinization of female fetuses placed between males *in utero*. Nevertheless, the results of this study are not consistent with the hypothesis of masculinization of human female DZos. In this large sample of twins, members of DZos pairs were strikingly similar to MZ and DZss of the same sex with respect to an objective measure of strength and anthropometric measures.

Previous studies of the potential masculinization of human female DZos have yielded conflicting results with respect to physical and physiologic variables. Boklage reported an intermediate gender phenotype with regard to craniofacial growth for DZos twins.[14] McFadden reported a masculinized pattern of spontaneous otoacoustic emissions in female DZos compared with female DZss and female non-twins.[6] Studies of birth weight have yielded conflicting results.[15–17] In a sample of Australian female twins, height and some items related to reproductive functions showed minor effects consistent with a masculinization of female DZos.[18] In a large Danish study, fecundability measured by waiting time to first ever pregnancy did not differ by zygosity or sex of co-twin.[19]

Studies of behavioral traits, such as sensation seeking[7] and attitudes,[8] seem to show more consistent results in favor of a more masculine phenotype in DZos. However, the effects of rearing (female DZos growing up with a brother) and intrauterine exposures cannot be adequately discerned in these studies. Physical and physiological measures may in fact also be confounded by the effects of psychosocial rearing in DZos twins, an effect we would expect to be less pronounced in a Danish setting, which is characterized by minimal class variability and sexism. If such confounding is present, however, we would expect it to bias female DZos phenotypes toward a greater similarity with male phenotypes. Clearly this is not the case in the present study.

The participation rate in the present study was high. Non-participation was not highly selected, but showed patterns recognized from other studies: a tendency for lower participation of fraternal twins, persons who were not married, and urban area dwellers. Interestingly, males were more likely to participate in this as well as in previous Danish twin studies,[13,20] a pattern opposite to what we expected from the literature.[21]

In conclusion, the data of this large study of twins using both objective measurements and self-reported data provide no evidence of a more masculinized phenotype in female DZos.

References

1. vom Saal SF. Sexual differentiation in litter-bearing mammals: influence of sex of adjacent fetuses *in utero*. J Anim Sci 1989;67:1824–1840.
2. Kinsley C, Miele J, Wagner CK, Ghiraldi L, Broida J, Svare B. Prior intrauterine position influences body weight in male and female mice. Horm Behav 1986;20:201–211.
3. Kinsley C, Miele J, Konen C, Ghiraldi L, Svare B. Intrauterine contiguity influences regulatory activity in adult female and male mice. Horm Behav 1986;20:7–12.
4. Forger NG, Galef BGJ, Clark MM. Intrauterine position affects motoneuron number and muscle size in a sexually dimorphic neuromuscular system. Brain Res 1996;735:119–124.
5. Khan MZ, Foley GL. Retrospective studies on the measurements, karyotyping and pathology of reproductive organs of bovine freemartins. J Comp Pathol 1994;110:25–36.
6. McFadden D. A masculinizing effect on the auditory systems of human females having male co-twins. Proc Natl Acad Sci USA 1993;90:11900–11904.
7. Resnick SM, Gottesman II, McGue M. Sensation seeking in opposite-sex twins: an effect of prenatal hormones? Behav Genet 1993;23:323–329.
8. Miller EM, Martin N. Analysis of the effect of hormones on opposite-sex twin attitudes. Acta Genet Med Gemellol (Roma) 1995;44:41–52.
9. Rantanen T, Guralnic JM, Foley D, Masaki K, Leveille S, Curb JK, White L. Midlife hand grip strength as a predictor of old age disability. JAMA 1999;281:558–560.
10. Lean MEJ, Han TS, Seidell JC. Impairment of health and quality of life in people with large waist circumference. Lancet 1998;351:853–856.
11. Kyvik KO, Christensen K, Skytthe A, Harvald B, Holm NV. The Danish Twin Register. Dan Med Bull 1996;43:467–470.
12. Hauge M. The Danish Twin Register. In: Mednick SA, Baert AE, Backman BP, eds. Prospective longitudinal research: An empirical basis for the primary prevention of psychosocial disorders. London: Oxford University Press, 1981;218–221.
13. Christensen K, Holm NV, McGue M, Corder L, Vaupel JW. A Danish population-based twin study on general health in the elderly. J Aging Health 1999;11:49–64.

14. Boklage CE. Interactions between opposite-sex dizygotic fetuses and the assumptions of Weinberg difference method epidemiology. Am J Hum Genet 1985;37:591–605.
15. Corey LA, Nance WE, Kang KW, Christian JC. Effects of type of placentation on birthweight and its variability in monozygotic and dizygotic twins. Acta Genet Med Gemellol (Roma) 1979;28:41–50.
16. Glinianaia SV, Magnus P, Harris JR, Tambs K. Is there a consequence for fetal growth of having an unlike-sexed cohabitant *in utero*? Int J Epidemiol 1998;27:657–659.
17. Orlebeke JF, van Baal GC, Boomsma DI, Neeleman D. Birth weight in opposite sex twins as compared to same sex dizygotic twins. Eur J Obstet Gynecol Reprod Biol 1993;50:95–98.
18. Loehlin JC, Martin NG. A comparison of adult female twins from opposite-sex and same-sex pairs on variables related to reproduction. Behav Genet 1998;28:21–27.
19. Christensen K, Basso O, Kyvik KO, Juul S, Boldsen J, Vaupel JW, Olsen J. Fecundability of female twins. Epidemiology 1998;9:189–192.
20. Andersen-Randberg K, Christensen K, Jeune B, Skytthe A, Vasegaard L, Vaupel JW. Activity of daily living among elderly and oldest-old in Denmark. Age Ageing 1999;28:373–377.
21. Lykken DT, McGue M, Tellegen A. Recruitment bias in twin research: the rule of two-thirds reconsidered. Behav Genet 1987;17:343–362.