The Influence of Zygosity and Chorion Type on Fat Distribution in Young Adult Twins Consequences for Twin Studies

Ruth J.F. Loos[1,2], Gaston Beunen[2], Robert Fagard[3], Catherine Derom[1], and Robert Vlietinck[1]

[1]*Faculty of Medicine, Center for Human Genetics*
[2]*Faculty of Physical Education and Physiotherapy, Department of Sport and Movement Sciences*
[3]*Faculty of Medicine, Hypertension and Cardiovascular Rehabilitation Unit, Katholieke Universiteit Leuven.*

An adverse intra-uterine environment has been associated with abdominal fat distribution in singletons. Twins often have a low birth weight and a short gestation. Therefore, they may have an increased risk to develop abdominal obesity. Furthermore, monozygotic monochorionic twins (MZ MC) have a larger intra-pair birth weight difference compared to monozygotic dichorionic twins (MZ DC). If adult anthropometry is programmed in utero, this may affect the intra-pair correlations in adulthood and, consequently, also the results from the classic twin method to estimate genetic and environmental influences. In the present study, we compared the absolute values, the intra-pair differences, and the intra-pair correlations of body mass, height, BMI, and abdominal fat distribution of 424 MZ MC, MZ DC and dizygotic (DZ) twin pairs (aged 18–34 yrs). DZ, MZ DC and MZ MC twins did not differ for most anthropometric characteristics. Only MZ women tended ($p = 0.03$) to accumulate more abdominal fat compared to DZ twins. Overall, the contribution of zygosity and chorion type to adult anthropometry was rather low (≤ 1.7%). Although the intra-pair birth weight difference of MZ MC pairs (10.5% in men, 12.3% in women) was significantly larger compared to that of MZ DC pairs (6.9% and 9.2% resp.), the intra-pair differences in adult anthropometry were similar for both MZ twin types. Also the intra-pair correlations of MZ MC and MZ DC pairs were strikingly alike, suggesting no significant influence of the prenatal environment on adult concordance. In conclusion, the substantial difference in the prenatal environment of MZ MC and MZ DC twins did not result in a difference in intra-pair concordance of adult anthropometry and fat distribution. Therefore, we suggest that the chorion type of MZ twins does not bias the twin design and the estimation of the genetic contribution to adult anthropometry.

The increasing prevalence of overweight and obesity over the last 30 years poses major public health concerns in most industrialized countries. It has been suggested that the intra-uterine environment may 'program' the development of adult obesity (Dietz, 1994). Several studies showed that people who were heavy at birth tended to be more obese as adults (Ravelli et al., 1976; Seidman et al., 1991). Others found that those who were light at birth tended to have more abdominal or truncal fat later in life (Law et al., 1992; Barker et al., 1997; Malina et al., 1996), which is a major risk factor for many health related complications (Björntop, 1997).

As the intra-uterine growth of twins is characterized by a marked deceleration during the last trimester of pregnancy (Naeye et al., 1966), they can be considered as a group at risk for abdominal fat accumulation. A limited capacity in the maternal/placental supply line has been postulated as the underlying cause of this fetal growth retardation, rather than 'crowding in the uterus' (Gruenwald, 1970; Bleker et al., 1995; MacGillivray, 1983).

Furthermore, according to the zygosity and the number of the placental membranes, three types of twins that differ substantially in prenatal environment can be distinguished: (1) dizygotic (DZ), (2) monozygotic dichorionic (MZ DC), and (3) monozygotic monochorionic (MZ MC). Since the placenta is formed from chorionic tissue, DZ and MZ DC twins have two placentas that can fuse during development. Both members of a MZ MC pair always share one placenta, which not only results in competition for a limited food supply but also enables vascular anastomoses between the circulations of the two fetuses. Indicators of the more adverse intra-uterine environment of MZ MC twins are the significantly lower birth weight, the shorter gestation, and the higher perinatal mortality and morbidity (Corey et al., 1979; Gruenwald, 1970; Loos et al., 1998; Naey et al., 1966; Ramos-Arroyo et al., 1988). Furthermore, the intra-pair birth weight difference of MZ MC twin members is much more pronounced than that of MZ DC pairs (Blickstein et al., 1999). Therefore, the MZ MC twins in particular may be more vulnerable for prenatal programming compared to MZ DC and DZ twins.

If the prenatal programming hypothesis is valid for twins, the prenatal differences between the three types of twins may have consequences for genetic studies. The twin design has been frequently applied to estimate the relative contribution of genes and environment to a variety of traits, including body composition and fat distribution, by

Address for correspondence: Ruth Loos, Katholieke Universiteit Leuven, Faculteit voor Lichamelijke Opvoeding en Kinesitherapie, Tervuursevest 101, B – 3001 Leuven, BELGIUM. Email: ruth.loos@med.kuleuven.ac.be

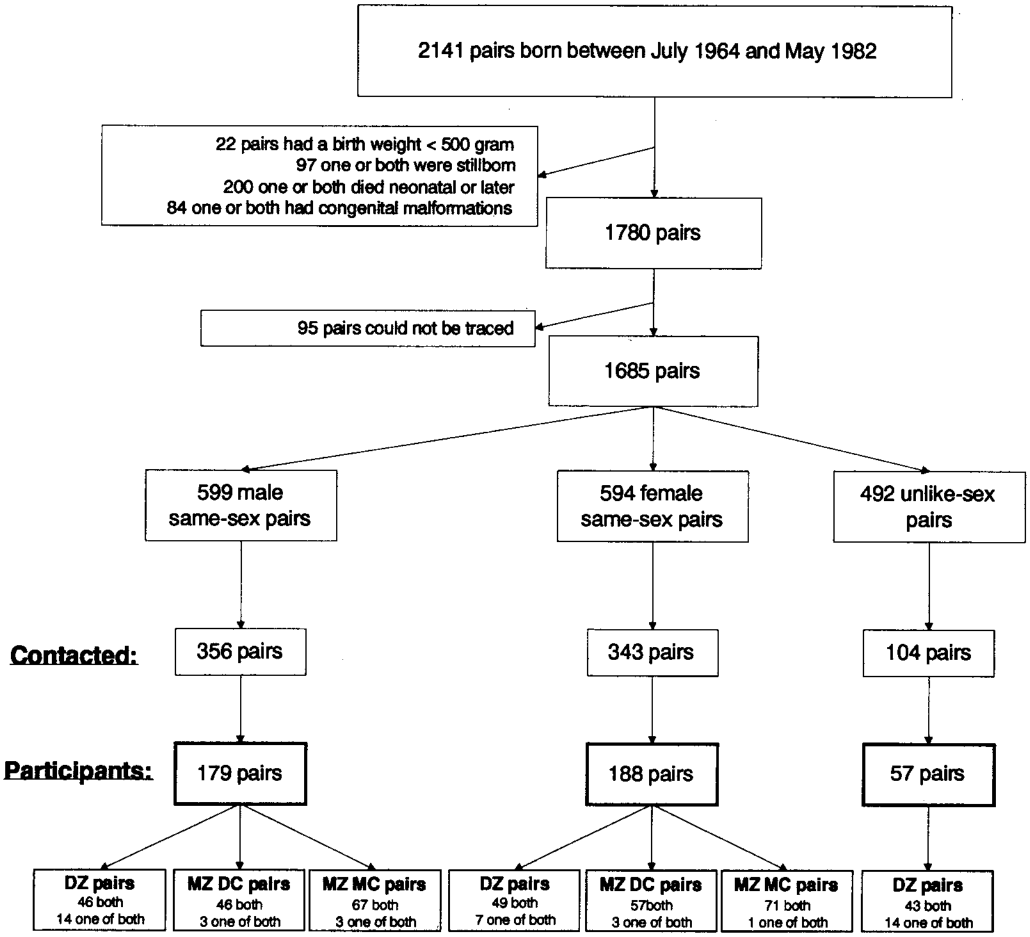

Figure 1

Flow chart illustrating the allocation of subjects.

comparing the concordance or correlation in MZ and DZ twins (Maes et al., 1997). A greater degree of similarity between members of MZ pairs than between members of DZ pairs suggests the presence of genetic influences. The basic assumption of the twin design is that MZ MC, MZ DC, and DZ twins experience a similar pre- and postnatal environment. However, this assumption has been questioned in the light of the prenatal programming hypothesis (Corey et al., 1979; Gruenwald, 1970; Phillips, 1992; Phillips, 1993). As the prenatal environment of MZ MC twins differs substantially from that of MZ DC and DZ twins, Phillips argued that the classic twin study might be an unreliable method of estimating the genetic component of traits in which the prenatal environment is thought to play a role. He suggested modifying the design of twin studies by excluding the MZ MC pairs, leaving MZ DC and DZ twins with a more similar prenatal environment.

The purpose of the present study was to evaluate the usefulness of the twin design to estimate the genetic influence to adult fat distribution in case prenatal programming is present. Therefore, two hypotheses were examined. (1) MZ twins, particularly the MZ MC ones, experience a more adverse prenatal environment. According to the prenatal programming hypothesis they will have a more adverse fat distribution in adult life as compared to DZ twins. (2) Although genetically identical, the intra-pair birth weight difference in MZ MC pairs is more pronounced than in MZ DC pairs. This may result in larger intra-pair differences and in reduced intra-pair correlations of fat distribution in adult life between the members of MZ MC twins compared to MZ DC twins.

Methods

Participants

All twins were randomly selected from the East Flanders Prospective Twin Survey (EFPTS). This population-based survey prospectively registers all twins born in the Belgian Province of East Flanders since 1964. It is characterized by its extensive collection of perinatal data at birth and placental examination within 24 hours after delivery. A detailed description of the EFPTS has been given previously (Loos et al., 1998).

We randomly contacted 803 pairs, aged 18 to 34 years, by using an envelope system. Eventually, 424 pairs (overall response of 52.8%) agreed on participating in the Prenatal Programming Twin Study (PPTS). Of 45 pairs only one of both members participated and one male subject was excluded because of severe scoliosis. To assure equally distributed groups we stratified for birth year, zygosity and chorion type. Figure 1 illustrates the allocation of the subjects into detail. The twins gave informed consent and the project was approved by the Local Committee of Medical Ethics.

Zygosity and Chorion Type

Zygosity was determined through sequential analysis based on sex, fetal membranes, umbilical cord blood groups (ABO, Rh CcDEe, MNSs, Duffy, Kell), placental alkaline phosphatase and DNA fingerprints (Derom et al., 1985). Unlike-sex twins and same-sex twins with at least one different genetic marker were classified as DZ; MC twins were classified as MZ. For all same-sex dichorionic twins with the same genetic markers a probability of monozygosity was calculated using a lod-score method (Vlietinck, 1986). All MZ DC twins reached a probability of monozygosity of at least 0.99.

Measurements

Perinatal data were registered within 48 hours after birth. Birth weights were obtained from the obstetric records and gestational age was reported by the obstetrician and was calculated as the number of completed weeks of pregnancy, based on the last menstrual period. For 46 pairs, gestational age was reported by the mother, because no data were available from the obstetric record. Gestational age was missing for 9 pairs.

Between February 1997 and April 2000, all twins visited our research center for a two-hour examination, which took place in the morning. Anthropometric measurements were performed by two trained researchers according to standardized procedures. The intra-class correlation for inter-observer reliability reached .93–.99. Subjects were measured barefoot and lightly clothed. Standing height (cm) was measured with a Harpenden fixed stadiometer and body mass (kg) on a balance scale (SECA, Hamburg, Germany), respectively to the nearest 0.1 cm and 0.1 kg. Waist and hip circumference were taken with a flexible steel tape to the nearest 0.1 cm. Waist circumference was taken between the costal margin and the iliac crest, and hip circumference at the widest part of the hips, generally at the level of the greater trochanters. Five skinfolds were taken, in duplicate, to 0.1 mm accuracy with a Harpenden skinfold calliper at biceps, triceps, subscapula, supra-iliaca and calf. We calculated BMI (kg/m^2) as a measure for overall body composition and waist-to-hip-ratio as a measure of abdominal fat distribution. For clarity waist-to-hip-ratio was expressed as a percentage. The two trunk (subscapula, supra-iliaca) and three extremity (triceps, biceps, calf) skinfolds were summed to assess the respective subcutaneous fatness. Intra-pair differences in anthropometry were calculated as the difference between the heaviest at birth minus the lightest at birth.

Statistical Analyses

Twins were considered both as individuals and as members of twin pairs for particular analyses. First, we performed an analysis of variance between DZ, MZ DC, and MZ MC pairs to test whether the anthropometry differed between the three twin types. Covariance analysis was applied to estimate the relationship (slope) of birth weight and gestational age with adult anthropometry in addition to the influence of zygosity and chorion type.

In a second series of analyses, we compared the intra-pair concordance of MZ MC and MZ DC pairs. As MZ twins are genetically identical, any intra-pair birth weight difference can be attributed to differences in the prenatal environment. In case of DZ twins, intra-pair differences result from both genetic and environmental differences. To avoid complicating the inferences, only the intra-pair differences of MZ pairs were compared. According to the prenatal programming hypothesis, we hypothesized that as the intra-pair birth weight difference increases also the intra-pair difference in adult anthropometry would increase, and this in favor of the heaviest at birth. Given the more pronounced intra-pair birth weight difference in MZ MC pairs, we examined whether the intra-pair differences in adult anthropometry differed between MZ DC and MZ MC twins by means of a t test. Accordingly, we tested whether the intra-pair correlations in adult anthropometry were affected by characteristics of the intra-uterine environment. Intra-pair correlations were compared after a Fisher z-transformation. Pair-wise analyses were restricted to pairs of whom both members had participated. Adjustments for adult age, birth weight and gestational age were performed by linear regression.

We log-transformed the sum of skinfolds before the analysis because of its skewed distribution. Tables represent geometric means, and in the regression analysis, the standardized slope is given, which indicates the number of standard deviation changes in sum of skinfolds with a standard deviation change in birth weight. Data analyses were performed using SAS version 6.12 (SAS institute inc, 1997). All p-values are two-sided and were considered statistically significant if they were < 0.05.

Results

Birth weight, gestational age, and age at examination are given in Table 1. At birth, MZ MC twins, men and women respectively, weighed on average 92 g and 83 g less than MZ DC twins, who weighed on average 89 g and 67 g less than DZ twins. Accordingly, gestation of MZ MC twins lasted 5 days (0.7 wks) less than that of DZ twins. Only in men, gestational age of MZ DC twins was significantly less compared to DZ twins. Birth weights and gestational age of the participants were representative for that of the East-Flanders twin population born between July 1964 and May 1982.

At the time of the examination, female DZ and MZ MC twins were older than MZ DC twins. This can be attributed to the sample conformation. At the beginning of the study, the subjects were selected by envelope system stratified by age, starting with to oldest age group. In November 1998, we chose to stratify additionally for zygosity and chorion type. Otherwise, we would end with a too small MZ DC group to allow comparisons between the three twin types. In the following two years, proportionally more MZ DC twins were contacted, mainly of younger age.

Table 1

Birth weight, Gestational Age and Age at Examination According to Zygosity and Chorion type for Men and Women.

	mean	*SD*	mean	*SD*	mean	*SD*	*p*	ANOVA
MEN	DZ (*n* = 156)		MZ DC (*n* = 95)		MZ MC (*n* = 137)			
Birth weight (gram)	2680	482	2591	541	2499	406	*< 0.01*	*DZ > MZ MC*
Gestational age (wks)*	37.5	2.4	36.7	2.5	36.8	2.2	*< 0.01*	*DZ > MZ DC, MZMC*
Age at examination (yrs)	26.2	4.8	25.1	4.9	25.4	4.5	0.13	
WOMEN	DZ (*n* = 155)		MZ DC (*n* = 117)		MZ MC (*n* = 143)			
Birth weight (gram)	2560	474	2493	535	2410	443	0.03	*DZ > MZ MC*
Gestational age (wks)**	37.4	2.3	37.4	2.7	36.7	2.5	0.02	*DZ, MZ DC > MZ MC*
Age et examination (yrs)	26.1	4.7	24.2	4.8	25.9	4.4	< 0.01	*DZ, MZ MC > MZ DC*

* missing: *n* = 3 for DZ

** missing: *n* = 3 for DZ, *n* = 4 for MZ DC, *n* = 6 for MZ MC

Anthropometry According to Zygosity and Chorion Type

Because of significant ($p < 0.02$) correlations between age and anthropometry (0.15–0.47), anthropometric characteristics were adjusted for age when DZ, MZ DC and MZ MC twins were compared (Table 2a and 2b). In men, we observed no significant differences between the three twin types. In women, body mass, height, and BMI were similar, but MZ DC twins had significantly larger trunk skinfolds compared to DZ twins. Also MZ MC twins tended to have more truncal fat, however this was not significant ($p = 0.12$). For waist-to-hip ratio, closely related to trunk skinfolds, similar tendencies were observed ($p = 0.06$). Because birth weight and gestational age differed according to zygosity and chorion type, we adjusted adult anthropometry for these two perinatal characteristics. This, however, did not change our findings, i.e. only for trunk skinfolds in women, zygosity and chorion type contributed significantly to the variance, though only 1.6%. Moreover, the contribution of birth weight and gestational age to the variance of body mass was more important than of zygosity and chorion type.

Intra-Pair Differences of Monozygotic Twins

Table 3 represents the intra-pair difference of birth weight and adult anthropometry between the heaviest and lightest twin at birth. The intra-pair birth weight difference of MZ MC was significantly larger compared to that of MZ DC twins. However, this did not result in larger intra-pair differences in adult anthropometry of MZ MC compared to MZ DC. On the contrary, the intra-pair difference of waist-to-hip ratio of men was even larger in MZ DC than in MZ MC twins. Adjusting adult anthropometry for birth weight and gestational age did not change these findings.

Intra-Pair Correlations of Monozygotic Twins

The intra-pair correlation of birth weight was significantly lower for MZ MC than for MZ DC pairs. In accordance with the intra-pair differences, the intra-pair correlations of adult anthropometry did not differ between MZ MC and MZ DC pairs. Adjusting adult anthropometry for birth weight and gestational age did not change these findings.

Discussion

To our knowledge, this is the first study that examined the influence of zygosity and chorion type as an indicator of the intra-uterine environment in respect to the prenatal programming hypothesis. We showed that the more adverse prenatal environment of MZ twins, especially that of MZ MC ones, did not result in a less favorable adult body composition compared to DZ twins. All though, we cannot neglect that MZ women tended to have slightly more abdominal fatness compared to DZ women. The more adverse prenatal environment of MZ MC compared to MZ DC twins is reflected also in the intra-pair birth weight difference, which was significantly larger in MZ MC pairs. Nevertheless, in adult life, the intra-pair differences in anthropometry were similar for both, MZ DC and MZ MC pairs. Concordantly, also the intra-pair correlations were strikingly alike, which suggests that the concordance between the members of a twin pair was not affected by differences in the prenatal environment.

Twins are frequently used to estimate the genetic contribution to a trait by comparing the intra-pair concordance of MZ and DZ pairs. A higher concordance between the members of MZ pairs than between members of DZ pairs is taken as evidence for a genetic influence. The basic assumption of the twin method is that the environment is similar for all twin types. However, because the prenatal environment is substantially different between MZ DC and MZ MC twins, this assumption may be violated. As a growing number of epidemiological and animal studies support the prenatal programming hypothesis (Barker, 1998), the usefulness of the twin method has been questioned (Gruenwald, 1970; Corey et al., 1979; Phillips, 1992; Phillips, 1993).

Anthropometry According to Zygosity and Chorion Type

So far, only few studies have examined the influence of zygosity, as an indicator of the prenatal environment, in the light of the prenatal programming of adult disease (Christensen et al., 1995; Levine et al., 1994; Petersen et al., 1997; Vågerö & Leon, 1994; Williams & Poulton, 1999). The strength of the present study is that we could examine

Table 2a

Age Adjusted Anthropometric Characteristics (*Mean, SD*) of Male DZ, MZ DC and MZ MC Twins, With and Without Adjusting for Birth Weight and Gestational Age.

MEN	DZ (*n* = 156)		MZ DC (*n* = 95)		MZ MC (*n* = 137)		zygosity/chorion type		birth weight (kg)			gestational age (wks)		
n adjusted for age and	mean	*SD*	mean	*SD*	mean	*SD*	*p*	r^2 (%)	slope	*p*	r^2 (%)	slope	*p*	r^2 (%)
Body mass (kg)	71.6	11.6	70.3	7.6	70.8	10.1	0.57	0.3						
BW, GESTAGE	71.5	11.2	69.9	7.3	71.3	9.6	0.42	0.5	*7.64*	*< 0.001*	*14.2*	*–1.23*	*< 0.001*	*4.6*
Height (cm)	178.6	6.3	178.2	7.1	177.9	5.7	0.64	0.3						
BW, GESTAGE	178.5	6.0	178.0	6.7	178.2	5.3	0.79	0.1	*5.78*	*< 0.001*	*11.4*	*–0.78*	*< 0.001*	*5.1*
BMI (kg/m²)	22.4	3.1	22.2	2.5	22.4	2.8	0.76	0.1						
BW, GESTAGE	22.4	3.0	22.1	2.5	22.4	2.8	0.66	0.2	*0.96*	*0.01*	*1.4*	*–0.19*	*0.01*	*1.3*
Waist-to-hip ratio (%)	82.7	4.8	83.8	5.1	83.6	4.8	0.12	1.1						
BW, GESTAGE	82.7	4.7	83.8	5.1	83.6	4.8	0.14	1.0	–0.15	0.83	0.0	–0.02	0.90	0.0
Trunk skinfolds (mm)	22.2	11.8	23.1	10.0	22.9	13.5	0.65	0.2						
BW, GESTAGE	22.2	11.9	23.3	9.9	22.9	13.6	0.59	0.3	–0.21*	0.76	0.0	0.03*	0.68	0.0
Extremity skinfolds (mm)	20.1	10.4	19.5	9.3	20.7	11.1	0.55	0.3						
BW, GESTAGE	19.9	10.4	19.7	9.2	20.9	11.2	0.50	0.4	0.00*	0.95	0.0	0.03*	0.64	0.1

The slope of relation gives the change in anthropometric characteristics per kg increase in birth weight and per week increase in gestational age, r^2 gives the % of variance explained by birth weight and gestational age.

* Standardized slope: indicates the number of standard deviation changes in sum of skinfolds with a standard deviation change in birth weight.

BW: birth weight GESTAGE: gestational age *n* = number of subjects

Table 2b

Age Adjusted Anthropometric Characteristics (*Mean, SD*) of Female DZ, MZ DC and MZ MC Twins, With and Without Adjusting for Birth Weight and Gestational Age.

WOMEN	DZ (n = 155)		MZ DC (n = 117)		MZ MC (n = 143)		zygosity/chorionicity		birth weight (kg)			gestational age (wks)		
n adjusted for age and	mean	*SD*	mean	*SD*	mean	*SD*	*p*	r^2 (%)	slope	*p*	r^2 (%)	slope	*p*	r^2 (%)
Body mass (kg)	60.9	10.3	61.4	10.3	60.8	10.7	0.87	0.1						
BW, GESTAGE	61.1	10.2	62.1	10.0	60.1	8.7	0.27	0.7	*3.67*	*< 0.01*	*1.9*	*–0.64*	*0.01*	*1.5*
Height (cm)	166.3	6.2	165.6	6.1	165.1	6.2	0.25	0.7						
BW, GESTAGE	166.1	6.2	165.7	5.8	165.2	5.9	0.44	0.4	*4.84*	*< 0.001*	*8.2*	*–0.47*	*< 0.01*	*2.0*
BMI (kg/m²)	22.0	3.5	22.4	3.4	22.3	3.9	0.66	0.2						
BW, GESTAGE	22.1	3.6	22.6	3.4	22.1	3.3	0.45	0.4	0.04	0.92	0.0	–0.11	0.23	0.3
Waist-to-hip ratio (%)	72.5	4.3	73.7	4.1	73.3	4.4	0.06	1.4						
BW, GESTAGE	72.6	4.3	73.8	4.0	73.2	4.3	0.06	1.4	–0.06	0.91	0.0	–0.13	0.24	0.3
Trunk skinfolds (mm) **	27.7	12.2	31.2	13.2	29.7	12.2	*0.03*	*1.7* *MZ DC > DZ*						
BW, GESTAGE	27.9	12.3	31.5	13.1	29.4	12.1	*0.04*	*1.6* *MZ DC > DZ*	–0.11*	0.10	0.6	–0.03*	0.56	0.1
Extremity skinfolds (mm)	42.1	17.0	42.1	16.9	43.8	17.4	0.69	0.2						
BW, GESTAGE	42.5	16.9	42.9	16.2	42.5	17.1	0.96	0.0	0.01*	0.90	0.0	*–0.14**	*0.02*	*1.4*

The slope of relation gives the change in anthropometric characteristics per kg increase in birth weight and per week increase in gestational age, r^2 gives the % of variance explained by birth weight and gestational age.

* Standardized slope: indicates the number of standard deviation changes in sum of skinfolds with a standard deviation change in birth weight.

BW: birth weight GESTAGE: gestational age *n* = number of subjects

Table 3

Intra-pair Difference in Birth Weight and Anthropometric Characteristics (Calculated as the Heaviest at Birth Minus the Lightest at Birth) for MZ DC and MZ MC Twin Pairs.

	MALE PAIRS					FEMALE PAIRS				
	MZ DC (*n* = 46)		MZ MC (*n* = 67)		MZDC vs MZMC	MZ DC (*n* = 57)		MZ MC (*n* = 71)		MZDC vs MZMC
intra-pair difference	mean	*SD*	mean	*SD*	*p*	mean	*SD*	mean	*SD*	*p*
Birth weight (%)	*6.88*	*6.90*	*10.49*	*7.20*	*< 0.01*	*9.16*	*6.44*	*12.25*	*9.49*	*0.04*
Adult anthropometry:										
Body mass (kg)	–0.21	4.26	1.17	5.47	0.15	1.40	7.16	0.78	7.28	0.63
Height (cm)	0.73	1.92	0.89	2.20	0.68	1.02	2.41	0.77	2.12	0.53
BMI (kg/m^2)	–0.23	1.41	0.16	1.71	0.21	0.25	2.53	0.08	2.59	0.70
Waist-to-hip-ratio (%)	*–1.31*	*3.74*	*0.20*	*3.42*	*0.03*	–0.01	3.12	0.31	3.87	0.62
Trunk skinfolds (mm)	–0.60	7.19	0.21	9.46	0.62	1.55	11.07	–0.70	10.91	0.26
Extremity skinfolds (mm)	–0.48	5.47	1.38	7.71	0.16	1.59	13.41	0.58	12.57	0.66

MZ DC vs MZ MC: comparison by *t* test.

Table 4

Intra-pair Correlation in Birth Weight and Anthropometric Characteristics of MZ DC and MZ MC Twin Pairs.

	MALE PAIRS			FEMALE PAIRS		
	MZ DC (*n* = 46)	MZ MC (*n* = 67)	MZDC vs MZMC	MZ DC (*n* = 57)	MZ MC (*n* = 71)	MZDC vs MZMC
	r	*r*	*p*	*r*	*r*	*p*
Birth weight	0.87	0.67	< 0.01	0.84	0.60	< 0.01
Adult anthropometry:						
Body mass	0.87	0.87	0.95	0.79	0.79	0.98
Height	0.96	0.92	0.07	0.91	0.94	0.32
BMI	0.87	0.85	0.61	0.78	0.80	0.76
Waist-to-hip-ratio	0.77	0.80	0.74	0.78	0.64	0.15
Trunk skinfolds	0.82	0.80	0.77	0.77	0.73	0.30
Extremity skinfolds	0.82	0.78	0.64	0.73	0.77	0.65

MZ DC vs MZ MC: comparison by *t* test, after Fisher z-transformation.

the influence of chorion type, besides that of zygosity. Since the prenatal environment of MZ MC and MZ DC twins differs substantially, this distinction is of great importance when studying the prenatal programming hypothesis. MZ MC twins have more often a peripheral umbilical cord insertion and always share one placenta, which may result in a hampered maternal/placental supply of nutrients (Bleker et al., 1979; Bleker et al., 1995; Loos et al., 2001; MacGillivray, 1983). MZ DC and DZ twins seem to experience a similar prenatal environment with respect to chorion type. However, fusion of the placentas has a significantly greater impact on birth weight in MZ DC compared to DZ twins (Loos et al., 2001). In spite of these differences in prenatal environment, we found no significant influence of zygosity or chorion type on adult body mass, height, or BMI. Birth weight and gestational age contributed much more to the variance, which suggests that the perinatal environment is of importance in the programming of adult height and body mass, irrespective of zygosity and chorion type.

Central fat deposition, more than overall obesity, has been shown to be a strong risk factor for cardiovascular disease and type 2 diabetes mellitus (Björntop, 1997). MZ women had a little more truncal fat accumulation compared to DZ twins. Rather surprisingly, it were the MZ DC twins that had the highest values, whereas, according to the prenatal programming hypothesis, we would expect the MZ MC twins to have so. Although similar tendencies were present for waist-to-hip ratio in men, the differences were not significant. As abdominal fatness substantially increases with age (Lahti-Koski et al., 2000), it might be that the influence of zygosity and chorion type amplifies as well. More and, especially, longitudinal twin studies may elucidate this potential age effect. Overall, the contribution of zygosity and chorion type to the variance of the anthropometric characteristics was rather small.

To our knowledge, no studies have examined anthropometry according to zygosity and chorion type and only few reported results on cardiovascular mortality (Christensen et al., 1995; Vågerö & Leon, 1994), blood pressure (Levine et al., 1994; Williams & Poulton, 1999), and insulin dependent diabetes mellitus (Petersen et al., 1997). None of these studies found differences between MZ and DZ twins (Christensen et al., 1995; Levine et al., 1994; Petersen et al., 1997) and only Petersen et al. (Petersen et al., 1997), who studied insulin dependent diabetes mellitus, found a higher prevalence of islet cell autoantibodies in twins than in singletons.

Intra-Pair Differences in Monozygotic Twins

The intra-pair birth weight difference in MZ MC pairs was significantly more pronounced than in MZ DC pairs, which was reflected in a lower intra-pair correlation for MZ MC than for MZ DC pairs. The reasons for the larger intra-pair difference in MZ MC pairs are not fully understood and several explanations have been proposed, including vascular anastomoses that, rather exceptionally, may lead to the twin-to-twin transfusion syndrome (Pridjian et al., 1991), greater competition for nutrients and unequal sharing of the single placenta (Machin, 1997; Pridjian et al, 1991). However, the prenatal disparity between MZ MC and MZ DC twins did not result in larger intra-pair differences in adult anthropometry of MZ MC pairs, as would be predict from the prenatal programming hypothesis. On the contrary, in men we even found a larger intra-pair difference in waist-to-hip ratio of MZ DC compared to MZ MC pairs. Concordant with the intra-pair differences, also the intra-pair correlations of MZ MC and MZ DC pairs were strikingly similar.

For diseases of metabolic etiology, Phillips (1992) hypothesized that the concordance in MZ pairs could be overestimated because of the shared intra-uterine circulation of MZ MC pairs. Given the fact that abdominal fat distribution is under hormonal control (Björntop, 1996), this is a valid assumption. However, no tendencies towards higher MZ MC intra-pair correlations were apparent. When we adjusted for birth weight, to control for a possible ‘lowering’ of the intra-pair correlations caused by birth weight discordances, still no difference between the adult intra-pair correlation of MZ MC and MZ DC pairs emerged. To our knowledge, no other study has compared adult anthropometry of MZ MC and MZ DC pairs.

In conclusion, the substantial difference in prenatal environment of MZ MC and MZ DC twins did not result in a difference in intra-pair concordance of adult anthropometry and fat distribution. Therefore, we suggest that for adult anthropometry and fat distribution MZ MC twins can be included in the classic twin study and that the influence of the prenatal environment is not large enough to bias the results of this design. More studies comparing the different twin types should be performed to confirm our results.

Acknowledgments

We are grateful to all the twins who took part in the study and to Kathleen Gysen, Hilde Van Gucht, and Nathalie Muermans who carried out the fieldwork.

The Prenatal Programming Twin Study was funded by the National Fund for Scientific Research Belgium (nr. 3.0269.97). The East Flanders Prospective Twin Survey is partly supported by the Association for Scientific Research in Multiple Births. Ruth Loos is supported by a grant from the Fund for Scientific Research, Flanders (Belgium). Robert Fagard is holder of the Prof. A. Amery Chair in Hypertension Research, founded by M.S.D.-Belgium.

References

Barker, D. J. P. (1998). *Mothers, babies and health in later life.* Edinburgh: Churchill Livingstone.

Barker, M., Robinson, S., Osmond, C., & Barker, D. J. P. (1997). Birth weight and body fat distribution in adolescent girls. *Archives of Disease in Childhood, 77,* 381–383.

Björntop, P. (1996). The regulation of adipose tissue distribution in humans. *International Journal of Obesity and Related Metabolic Disorders, 20,* 291–302.

Björntop, P. (1997). Body fat distribution, insulin resistance, and metabolic disease. *Nutrition, 13,* 795–803.

Bleker, O. P., Breur, W., & Huidekoper, B. L. (1979). A study of birth weight, placental weight and mortality of twins as compared to singletons. *British Journal of Obstetrics and Gynaecology, 86,* 111–118.

Bleker, O. P., Wolf, H., & Oosting, J. (1995). The placental cause of fetal growth retardation in twin gestations. *Acta Geneticae Medicae et Gemellologiae, 44,* 103–106.

Blickstein, I., Goldman, R. D., Smith-Levitin, M., Greenberg, M., Sherman, D., & Rydhström, H. (1999). The relation between inter-twin birth weight discordance and total twin birth weight. *Obstetrics & Gynecology, 93,* 113–116.

Christensen, K., Vaupel, J. W., Holm, N. V., & Yashin, A. I. (1995). Mortality among twins after age 6: Fetal origins hypothesis versus twin method. *British Medical Journal, 310,* 432–436.

Corey, L. A., Nance, W. E., Kang, K. W., & Christian, J. C. (1979). Effects of type of placentation on birth weight and its variability in monozygotic and dizygotic twins. *Acta Geneticae Medicae et Gemellologiae, 28,* 41–50.

Derom, C., Bakker, E., Vlietinck, R., Derom, R., Thiery, M., & Pearson, P. (1985). Zygosity determination in newborn twins using DNA variants. *Journal of Medical Genetics, 22,* 279–282.

Dietz, W. H. (1994). Critical periods in childhood for the development of obesity. *American Journal of Clinical Nutrition, 59,* 955–959.

Gruenwald, P. (1970). Environmental influences on twins apparent at birth: A preliminary study. *Biology of the Neonate, 15,* 79–93.

Lahti-Koski, M., Pietinen, P., Mannisto, S., & Vartiainen, E. (2000). Trends in waist-to-hip ratio and its determinants in adults in Finland from 1987 to 1997. *American Journal of Clinical Nutrition, 72,* 1436–1444.

Law, C. M., Barker, D. J. P., Osmond, C., Fall, C. H. D., & Simmonds, S. J. (1992). Early growth and abdominal fatness in adult life. *Journal of Epidemiology and Community Health, 46,* 184–186.

Levine, R. S., Hennekens, C. H., & Jesse, M. J. (1994). Blood pressure in prospective population based chort of newborn and infant twins. *British Medical Journal, 308,* 298–302.

Loos, R., Derom, C., Vlietinck, R., & Derom, R. (1998). The East Flanders Prospective Twin Survey (Belgium): A population-based register. *Twin Research, 1,* 167–175.

Loos, R. J. F., Derom, C., Derom, R., & Vlietinck, R. (2001). Birthweight in liveborn twins — the influence of the umbilical cord insertion and fusion of placenta. *British Journal of Obstetrics and Gynaecology,* (in press).

MacGillivray, I. (1983). Determinants of birthweight in twins. *Acta Geneticae Medicae et Gemellologiae, 32,* 151–157.

Machin, G. A. (1997). Velamentous cord insertion in monochorionic twin gestation: An added risk factor. *Journal of Reproductive Medicine, 42,* 785–789.

Maes, H. H., Neale, M. C., & Eaves, L. J. (1997). Genetic and environmental factors in relative body weight and human obesity. *Behavior Genetics, 27,* 325–351.

Malina, R. M., Katzmarzyk, P. T., & Beunen, G. (1996). Birth weight and its relationship to size attained and relative fat distribution at 7 to 12 years of age. *Obesity Research, 4,* 385–390.

Naey, R. L., Benirschke, K., Hagstrom, J. W., & Marcus, C. C. (1966). Intrauterine growth of twins as estimated from liveborn birth-weight data. *Pediatrics, 37,* 409–416.

Petersen, J. S., Kyvik, K. O., Bingley, P. J., Gale, E. A. M., Green, A., Dyrberg, T., & Beck-Nielsen, H. (1997). Population based study of prevalence of islet cell autoantibodies in monozygotic Danish twin pairs with insulin dependent diabetes. *British Medical Journal, 314,* 1575–1579.

Phillips, D. I. W. (1992). Can twin studies assess the genetic component in type 2 (non-insulin-dependent) diabetes mellitus ? *Diabetologia, 36,* 471–472.

Phillips, D. I. W. (1993). Twin studies in medical research: Can they tell us whether diseases are genetically determined ? *Lancet, 341,* 1008–1009.

Pridjian, G., Nugent, C., & Barr, M. (1991). Twin gestation: Influence of placentation on fetal growth. *American Journal of Obstetrics and Gynecology, 165,* 1394–1401.

Ramos-Arroyo, M. A., Ulbright, T. M., Yu, P-L., & Christian, J. C. (1988). Twin study: Relationship between birth weight, zygosity, placentation, and pathologic placental changes. *Acta Geneticae Medicae et Gemellologiae, 37,* 229–238.

Ravelli, G-P., Stein, Z. A., & Susser, M. (1976). Obesity in young men after famine exposure in utero and early infancy. *New England Journal of Medicine, 295,* 349–353.

Seidman, D. S., Laor, A., Gale, R., Stevenson, D. K., & Danon, Y. L. (1991). A longitudinal study of birth weight and being overweight in late adolescence. *American Journal of the Diseased Child, 145,* 782–785.

Vågerö, D., & Leon, D. (1994). Ischeamic heart disease and low birth weight: A test of the fetal-origins hypothesis from the Swedish Twin Registry. *Lancet, 343,* 260–263.

Vlietinck, R. (1986). Determination of the zygosity of twins. Unpublished doctoral dissertation. K.U.Leuven.

Williams, S., & Poulton, R. (1999). Twins and maternal smoking: Ordeals for the fetal origins hypothesis ? A cohort study. *British Medical Journal, 318,* 897–900.