Human Reproduction, Vol.28, No.10 pp. 2865–2871, 2013
Advanced Access publication on August 6, 2013 doi:10.1093/humrep/det283

human reproduction

ORIGINAL ARTICLE *Reproductive epidemiology*

Birth size and age at menarche: a twin perspective

Kaspar Sørensen[1,*], Anders Juul[1], Kaare Christensen[2,3,4], Axel Skytthe[2], Thomas Scheike[5], and Tina Kold Jensen[1,2]

[1]Department of Growth and Reproduction GR, Copenhagen University Hospital, Rigshospitalet Section 5064, Blegdamsvej 9, Copenhagen Ø DK-2100, Denmark [2]The Danish Twin Registry, University of Southern Denmark, Odense, Denmark [3]Department of Clinical Biochemistry and Pharmacology, Odense University Hospital, Odense, Denmark [4]Department of Clinical Genetics, Odense University Hospital, Odense, Denmark [5]Department of Biostatistics, University of Copenhagen, Copenhagen, Denmark

*Correspondence address. E-mail: kasparsoerensen@gmail.com

Submitted on January 16, 2013; resubmitted on June 8, 2013; accepted on June 13, 2013

STUDY QUESTION: Do birthweight (BW) and co-twin sex influence the age at menarche in twins?

SUMMARY ANSWER: BW, but not co-twin sex, was associated with age at menarche in twins. However, BW was not associated with age at menarche after controlling for genetics and shared rearing environment.

WHAT IS KNOWN ALREADY: Nutritional deprivation during critical developmental periods can trigger long-term effects on health. A small size at birth has been associated with early age at menarche in singletons. However, the relative influence of genetics and environmental factors on this association remains unresolved.

STUDY DESIGN, SIZE, DURATION: In total, 2505 twin pairs were included in this cohort study.

PARTICIPANTS/MATERIALS, SETTING, METHODS: All participants were recruited from the Danish Twin Register. Data on the age at menarche were collected by questionnaire and combined with data on BW, birth length (BL) and gestational age (GA) from the Danish Medical Birth register. The BW for GA standard deviation score (BW-SDS) was calculated.

MAIN RESULTS AND THE ROLE OF CHANCE: BW-SDS [hazard ratio (HR) 0.96; 95% confidence interval (CI): 0.93–0.00], $P = 0.04$], but not BW, BL or GA ($P \geq 0.15$), was positively associated with age at menarche in all twins after adjustment for zygosity and year of birth. However, BW-SDS was not associated with menarcheal age within twin pairs (HR 1.01; 95% CI: 0.91–1.12, $P = 0.88$). No differences were found in the age at menarche or birth size between twin girls from same sex and twin girls from opposite-sex pregnancies. Heritability of menarcheal age and BW were estimated to be 0.61 (95% CI: 0.38–0.84) and 0.27 (95% CI: 0.18–0.38), respectively. Both BW and menarcheal age were influenced by genetic and environmental factors.

LIMITATIONS, REASONS FOR CAUTION: A limitation of this study is recall bias on the age at menarche. It is also not clear how these results should be extrapolated to the non-twin population.

WIDER IMPLICATIONS OF THE FINDINGS: lower BW for GA is associated with earlier age at menarche in twin girls. However, the lack of within-pair differences in menarcheal age between even markedly BW-discordant twins indicates that this association is governed by environmental or genetic factors shared by both twins. Thus, within-pair differences in intrauterine nutritional factors leading to discordant growth do not seem to influence timing of menarche.

STUDY FUNDING/COMPETING INTEREST(S): The authors have nothing to declare. Departmental funds were used to support all authors during the study period and preparation.

Key words: birthweight / menarche / twins / sex

Introduction

Nutritional deprivation during critical developmental periods can trigger long-term effects on health (Gluckman *et al.*, 2008). Epidemiological studies have repeatedly shown that markers of nutritional restraint *in utero*, such as low birthweight (BW) or relative thinness at birth, are associated with increased risk of cardiovascular disease and type 2 diabetes in adulthood (Barker *et al.*, 1989, 1993). In twins, the prevalence of

cardiovascular disease does not differ from the general population (Vagero and Leon, 1994; Christensen *et al.*, 2001). In addition, within-pair differences in adult cardiovascular risk markers in BW discordant monozygotic (MZ) twins have shown diverging results (Vagero and Leon, 1994; Poulsen *et al.*, 2009; Frost *et al.*, 2012) indicating that genetic and/or environmental confounding may partly explain the association between intrauterine growth restriction and cardiovascular risk.

Early age at menarche has been associated with an increase in the risk of cardiovascular disease and type 2 diabetes in adulthood (Jacobsen *et al.*, 2009; Lakshman *et al.*, 2009). In girls, intrauterine growth restriction as well as low BW and relative thinness within the normal BW range has all been associated with an earlier onset of puberty and menarche in most studies (Bhargava *et al.*, 1995; Ibanez *et al.*, 2000; Adair, 2001; Veening *et al.*, 2004; Tam *et al.*, 2006; Sloboda *et al.*, 2007), although not consistently (Maisonet *et al.*, 2010). In addition, rapid infant growth and weight gain (Ong *et al.*, 2009) and childhood adiposity (Adair, 2001; Lee *et al.*, 2007) predict early pubertal timing independent of birth size.

Although the above literature indicates strong effects of both pre- and post-natal environment on menarcheal age, birth size, infant and childhood growth and adiposity as well as the age at menarche are all strong heritable traits (Kaprio *et al.*, 1995; Kirk *et al.*, 2001; Beardsall *et al.*, 2009; Demerath *et al.*, 2007; Ong *et al.*, 2009; Elks *et al.*, 2010) some of which may be influenced by common genes. Young maternal age at menarche has been shown to predict lower BW (Scholl *et al.*, 1989), increased early post-natal growth and higher childhood BMI as well as earlier menarcheal age in female offspring (Ong *et al.*, 2007). Thus, the association between BW, post-natal growth and pubertal timing may be of partly genetic rather than solely of environmental origin. However, the relative contribution of genetics and environment to this relationship has never been tested in a suitable twin population.

Prenatal androgen excess may programme reproductive and metabolic functions in females (Padmanabhan *et al.*, 2006). Effects on fetal development and subsequent behaviour indicative of increased masculinization have been reported in girls from opposite-sex twin pregnancies (Luke *et al.*, 2005). Studies in rodents have shown delayed maturation, reduced sexual attractiveness to males and shorter reproductive lifespans in females developing adjacent to males (Ryan and Vandenbergh, 2002). In humans, reduced reproductive fitness in females from male–female twin pairs has been reported (Lummaa *et al.*, 2007). However, these findings have not been replicated (Christensen *et al.*, 1998; Medland *et al.*, 2008). In parallel with the results in rodents, later age at menarche has been reported in females from opposite-sex dizygotic (DZ) compared with same-sex DZ twin pairs (Kaprio *et al.*, 1995).

The aim of the present study was to evaluate the influence of (i) BW and (ii) co-twin sex on age at menarche in a large cohort of healthy twin girls.

Methods and Materials

Subjects

All participants were recruited from The Danish Twin Register. This twin population has previously been described in detail (Kyvik *et al.*, 1995). In 1994 a questionnaire survey, including questions on the age at menarche (in years and months), zygosity and medical history, was conducted among all known twins born in Denmark from 1953 to 1982. In total, 29 433 twins returned a questionnaire, corresponding to a response rate of 86.2% (or 73% of the total Danish twin population). From the Danish Medical Birth Registry, data were available on sex, birth order, birth length (BL), BW and gestational age (GA). In the present study we selected the twin pairs born in the period from 1973 to 1982, corresponding to the period with complete ascertainment of twin pairs born in Denmark. In addition, data on GA was registered in the Danish Medical Registry from 1973 onwards. In total, 2505 twin pairs aged 12.0–22.0 were included: 733 female MZ, 625 were female dizygotic (SSDZ) and 1147 opposite-sex dizygotic (OSDZ) twin pairs. Data were unavailable on age at menarche in 115 (27 MZ, 27 SSDZ and 59 OSDZ) twin girls. All twins were reared with their respective twin sister or brother. No participants had a known prior or current medical history that could influence pubertal timing. Sex-specific BW standard deviation scores (BW-SDS) adjusted for GA were calculated according to the twin BW reference by Glinianaia *et al.* (2000).

Statistics

Descriptive characteristics are presented as medians and 2.5th and 97.5th percentiles. For the non-censored data, Mann–Whitney *U*-tests were used for non-parametric comparisons between groups and Spearman Rho tests were used for non-parametric correlations.

Approximately 10% of the girls were pre-menarcheal when the questionnaire survey was carried out. We therefore considered the timing of menarche as right-censored survival data. The regression modelling was done using marginal Cox's regression survival models with cluster-corrected estimates of the standard errors giving estimates of the effect on the population level. The Cox's regression model was validated for possible interactions among covariates and proportional hazards assumption.

To describe the correlations within twins, we used random effects survival models. We considered the two-stage modelling with Cox's marginals (Glidden, 2000; Martinussen and Scheike, 2006) using the timereg package of R. These results were consistent with those based on standard frailty modelling. The fitted model therefore had the same marginal models for both MZ and DZ twins, which was also validated by formal statistical tests. The first cohort had almost no censorings and the observed correlation within this cohort was also fully consistent with the model-based estimates. The variance of the random effects described the correlation present in the survival times. We compared the correlation for MZ and DZ twins by a statistical comparison of these variances. As a consequence of the fitted survival model, we computed the model-based estimates of the Pearson's correlations for MZ and DZ twins, respectively. These correlations for MZ and DZ twins were converted into the amount of variation due to genes assuming an underlying additive structure of the different sources of variation by the following formula:

$$\sigma^2(X) = \sigma^2(a) + \sigma^2(c) + \sigma^2(\mathrm{e}),$$

with $\sigma^2(X)$ denoting the total variation, $\sigma^2(a)$ the shared genetic variation, $\sigma^2(c)$ the shared environmental variation and $\sigma^2(\mathrm{e})$ the non-shared environmental variation. The correlation among MZ twins was assumed to be:

$$\frac{\sigma^2(a) + \sigma^2(c)}{\sigma^2(X)}$$

and the correlation among DZ twins was assumed to be

$$\frac{(1/2)\sigma^2(a)+\sigma^2(c)}{\sigma^2(X)}.$$

Note that this is based on the assumptions that the shared ($\sigma^2(c)$) and the non-shared environmental effects (σ^2(e)) are the same within MZ and DZ twin pairs, respectively. To test if the genetic component is 0, we tested if the underlying random effects of the DZ and MZ twins were the same, thus performing a test that is not on the boundary of the parameter space. Similarly, to test if the shared environmental effect was 0, we made a formal test for the ratio of the two correlations being 1/2, again performing a test that is not on the boundary of the parameter space.

The genetic variance was thus estimated as twice the differences in the Pearson's correlations for the MZ and DZ twins. This estimate is valid for both an ACE and AE decomposition of variances that were both consistent with the data. In addition, similar conclusions were obtained by using an inverse probability of censoring weighted approach directly on the observed data using standard variance component modelling. Considering only the non-censored part of the menarche data also lead to similar results, but this reduced the data considerably by only including the birth years prior to 1977. These analyses were done using the mets package of R. The decomposition of BW into genetic and shared environmental effects was done using standard variance component modelling using the mets package of R.

The paired analyses were done fitting Cox's regression models stratified on twin pair (Holt and Prentice, 1974). This approach leads to subject-specific regression effects. One difficulty for the paired analyses is the potential correlation in the predictors that may lead to very low power.

The age at menarche was subject to digit preference leading to an overestimation of the underlying random effects and subsequent estimates of correlation. To deal with the digit preference, when assessing the correlation, random noise was added assuming that the true age of menarche occurred up to plus minus half a year within the observations that were reported in whole years. We tried various procedures for this and the results were robust for the choices made. In addition, the right censoring of the data, most pronounced in the last cohorts, also led to severe overestimation of the correlation between twins if ignored. Standard errors for the correlations and heritability were obtained by a bootstrap procedure.

Ethics

The study was approved by the local ethical committee (# VF 95/300 MC) and was conducted in accordance with the Second Helsinki Declaration. All participants or their parents gave written informed consent.

Results

General characteristics of the twins are shown in Table I. GA was negatively ($r = -0.152$, $P < 0.001$) and BW-SDS positively ($r = 0.126$, $P < 0.001$) associated with year of birth in all twin girls. No significant effect of birth year was found for BW or BL. The median within-pair difference in BW-SDS was 0.65 [95% confidence interval (CI): 0.0–2.59] in all twin pairs. The within-pair differences in BW-SDS were significantly smaller in MZ twins (0.60; 95% CI: 0.0–2.39) compared with both DZ female–female (0.66; 95% CI: 0.0–3.04) and DZ male–female (0.70; 95% CI: 0.08–2.60) twin pairs (both $P < 0.001$). The within-pair correlation for BW was significantly higher in MZ ($r = 0.75$; 95% CI: 0.71–0.78) than in DZ twin girls ($r = 0.64$; 95% CI: 0.59–0.69, $P < 0.001$) (Fig. 1). The results of the ACE model on BW are shown in Table II. Both the additive genetic and the shared and non-shared environmental variance components were significant for BW (both $P < 0.0001$).

The age at menarche was not significantly different between MZ, SSDZ and OSDZ twin girls, respectively (Table I). There was a significant

Table I Descriptive characteristics on 2505 twin pairs.

	SSMZ twins Girls	SSDZ twins Girls	OSDZ twins Girls/boys
Twin-pairs (*n*)	733	625	1147
Twins (*n*)	1466	1250	1147/1147
Post-menarche (*n*)	1242	1014	957/—
Pre-menarche (*n*)	176	191	137/—
Missing data (*n*)	48	45	53/—
Age at examination (years)	17.4 (12.4; 21.8)	16.9 (12.3; 21.7)	17.4 (12.3; 21.8)
Menarche <12 years (%)	9.4	10.5	11.5/—
Age at menarche (years)	13.2 (11.0; 15.9)	13.0 (11.0; 16.0)	13.2 (11.0; 15.8)/—
BW (g)	2400 (1375; 3375)[a,b]	2625 (1375; 3600)	2625 (1625/3625)/2750 (1625/3625)
BL (cm)	48.0 (40.0; 52.0)[a,b]	48.0 (41.0; 53.0)	48.0 (41.0; 53.0)/49.0 (42.0; 54.0)
GA (weeks)	38.0 (32.0; 40.0)[a,b]	38.0 (32.0; 40.0)[b]	39.0 (33.0; 40.0)
BW (SD)	−0.73 (−2.71; 1.48)[a,b]	−0.40 (−2.71; 1.50)	−0.44 (−2.65; 1.48)/−0.44 (−2.48; 1.54)

Data represent medians (2.5th percentile; 97.5th percentile). SSMZ, same sex monozygotic twin. SSDZ, same sex dizygotic twins. OSDZ, opposite-sex dizygotic twins. GA, gestational age. BW-SDS, birthweight standard deviation score according to the study by Glinianaia *et al.* (2000).
[a]$P < 0.05$ versus SSDZ girls.
[b]$P < 0.05$ versus OSDZ girls.

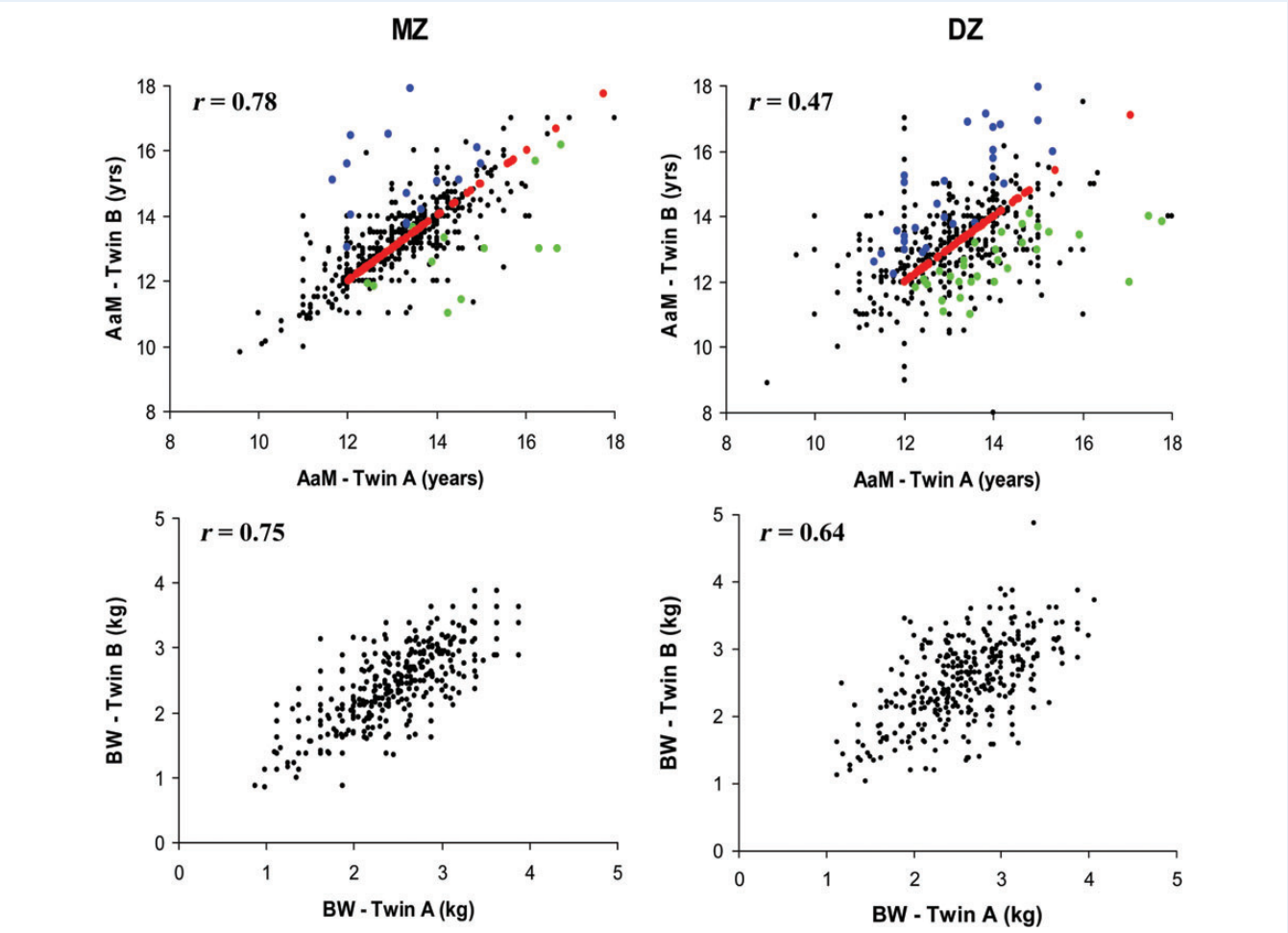

Figure 1 Within twin-pair (interclass) correlations for BW and menarcheal age in MZ and DZ female twin pairs. Interclass correlation coefficients (*r*) adjusted for digit preference and right censorings (pairs in which none or only one twin have had menarche at the time of examination) are presented. Solid black circles represent twin pairs with no right censorings; green circles, right censorings for twin A; blue circles, right censorings for twin B; red circles, right censorings for both twins. BW, birthweight; MZ, monozygotic; DZ, dizygotic.

Table II Results from the two-stage frailty model and the derived sources of variation.

	Variance	Kendall's τ	Pearson's correlation
MZ twins	1.880 (0.169)	0.484 (0.016)	0.776 (0.053)[a]
DZ twins	0.670 (0.110)	0.251 (0.024)	0.472 (0.052)[a]
	Age at menarche	**BW**	
Additive genetic (A)	0.61 (0.38–0.84)[a]	0.27 (0.18–0.38)	
Common environmental (C)	0.17 (0.00–0.36)[a]	0.48 (0.40–0.58)	
Non-shared factors (E)	0.22 (0.12–0.33)[a]	0.23 (0.21–0.27)	

Estimated directly from Pearson's correlation for menarche age and from a standard variance component model for BW. BW-SDS, birthweight standard deviation score.
[a]Bootstrap-based standard errors.

trend towards a lower menarcheal age in the most recent birth years (Table III). The within-pair correlation for the age at menarche was significantly higher in MZ (0.78 (95% CI: 0.67–0.88) than in SSDZ twin girls (0.47; 95% CI: 0.37–0.57, $P < 0.001$) (Fig. 1). The results of the ACE model on the age at menarche are shown in Table II. The additive genetic variance component for the age at menarche was strongly significant ($P < 0.0001$), while the shared environmental component was only borderline significant ($P = 0.055$).

Table III Hazard regression model on age at menarche in relation to BW-SDS, birth cohort (1973–1982) and zygosity (SSDZ, same sex dizygotic; OSDZ, opposite-sex dizygotic) in 3466 twin girls.

Variables	HR (95% CI)	*P*-value
BW-SDS	0.962 (0.928; 0.998)	0.04
Birth cohort	1.032 (1.014; 1.050)	<0.001
SSDZ[a]	1.027 (0.923; 1.144)	0.62
OSDZ[a]	1.049 (0.950; 1.159)	0.34

[a]SSDZ and OSDZ twins are presented with reference to monozygotic twins (MZ). Data are presented as HRs and 95% CIs.

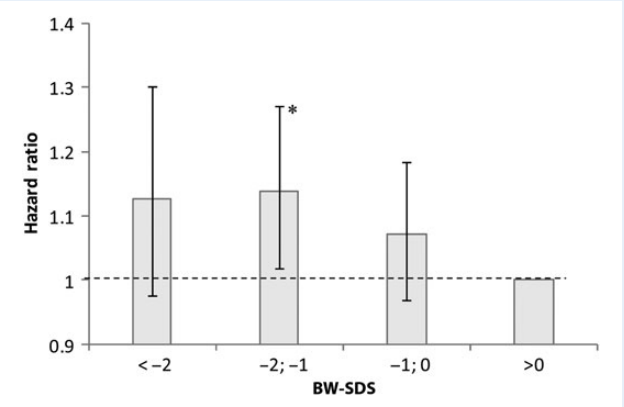

Figure 2 BW and the risk of early age at menarche in 3466 twin girls. Data are presented as HRs and 95% CIs with reference to the group with a GA-adjusted BW above the mean (BW-SDS >0). *$P = 0.01$. BW, birthweight; GA, gestational age; BW-SDS, birthweight-standard deviation score.

BW-SDS, but not BW, BL or GA *per se* ($P \geq 0.15$) was positively associated with the age at menarche in all twin girls after adjustment for zygosity and year of birth (Table III). Categorical dose–response analysis demonstrated that the assumption of linearity was not strongly violated, although the risk of early menarche appeared to level off, or even slightly declined, in the lowest BW-SDS group (Fig. 2). No interaction ($P = 0.74$) between BW-SDS and zygosity was found on age at menarche.

In paired analysis, BW-SDS was not significantly associated with menarcheal age within all twin pairs [hazard ratio (HR): 1.01; 95% CI: 0.91–1.12, $P = 0.88$]. Divided by zygosity, BW-SDS was not associated with age at menarche within either MZ twin pairs (HR: 0.94; 95% CI: 0.81–1.10, $P = 0.45$) or DZ twin pairs (HR: 1.07; 95% CI: 0.93–1.24, $P = 0.35$). Comparing the within-pair differences in the age at menarche between twin girls discordant by more than 1 BW-SDS (HR 1.05; 95% CI: 0.93–1.19, $P = 0.4$) and 2 BW-SDS (HR: 1.04; 95% CI: 0.87–1.23, $P = 0.7$) revealed similar non-significant results.

Discussion

In the present study including 2505 healthy twin pairs, we found a modest, but significant, association between lower BW for GA and earlier age at menarche in all twin girls. However, being the smaller twin at birth was not associated with earlier menarcheal age compared with being the larger co-twin in paired analysis. Furthermore, the age at menarche or birth size did not differ between twin girls from same-sex and opposite-sex pregnancies. The heritability of age at menarche and BW was estimated to be 61 and 27%, respectively.

Evidence supports that restricted fetal growth may advance onset and progression of puberty in girls (van Weissenbruch and Delemarre-van de Waal, 2006). In the present study, the risk of early menarche increased by ~4% for each unit decrease in BW-SDS in healthy twin girls. In comparison with girls with a BW-SDS above the mean, the girls with BWs one to two standard deviations below the mean had a 14% increased risk of early age at menarche. However, this effect seems to plateau or even decrease slightly in the infants with BWs two standard deviations below the mean. Our results are in accordance with studies in non-twin populations showing an association between small size at birth within the normal BW range and earlier age at menarche (Adair, 2001; Tam *et al.*, 2006; Sloboda *et al.*, 2007). In our study menarcheal age was not associated with BW, BL or GA, respectively. Thus, factors related to restricted intrauterine growth rather than prematurity seem to govern this relationship.

Determination of the genetic and environmental contributions to the association between menarcheal age and BW has to our knowledge not previously been studied. Despite the significant within-cohort association, lower BW was not associated with earlier menarche within twin-pairs. Neither in MZ nor in DZ twin-pairs, did the smaller twin have a higher risk of earlier menarcheal age than the larger co-twin. This supports that shared factors within twin-pairs are the main contributors to the association between BW and age of menarche. However, conclusive evidence in support for shared genetics as opposed to shared environmental factors could not be determined due to lack of associations in either MZ or DZ twin-pairs. Although the strong concordance in BW within twin-pairs complicated the interpretation of the paired analyses, even markedly discordant intrauterine growth did not influence menarcheal age within pairs in the present cohort. Thus, the assumption that discordant intrauterine growth influences pubertal timing through non-shared biological effects is not supported by the present study.

Rapid infant statural growth and weight gain (Tam *et al.*, 2006; Ong *et al.*, 2009), and increased mid-childhood adiposity (Adair, 2001; Lee *et al.*, 2007), all strongly influenced by the post-natal nutritional environment, have been associated with earlier menarcheal age independent of BW. Thus, the shared nutritional environment within twin-pairs during childhood may influence pubertal timing through early growth and body composition. These potential confounders could not be taken into account due to lack of information on early growth and adiposity during childhood in the present study. Although, early growth patterns, body composition during childhood and pubertal timing are influenced by post-natal nutritional factors, these are all strong heritable traits (Kaprio *et al.*, 1995; Kirk *et al.*, 2001; Beardsall *et al.*, 2009). Intriguingly *el al.* (Ong *et al.*, 2007) have shown that young maternal age at menarche was predictive of increased early post-natal weight gain, high mid-childhood adiposity and early menarcheal age, but not low BW, in their daughters. However, early age at menarche has previously been associated with an increased risk of giving birth to low BW infants (Scholl *et al.*, 1989). Thus, pre- and post-natal growth, body composition and pubertal timing may be partly influenced by common genes shared within twin-pairs.

Early childhood weight gain and high mid-childhood adiposity have in most studies been found to be more important determinants of

menarcheal age than low BW (Adair, 2001; Tam *et al.*, 2006; Ong *et al.*, 2009). Hypothetically, this may be due to stronger biological than genetic effects on intrauterine growth (Murphy *et al.*, 2006; Beardsall *et al.*, 2009) compared with post-natal growth (Beardsall *et al.*, 2009) and maturational timing (Kaprio *et al.*, 1995; Kirk *et al.*, 2001). In accordance with previous twin studies (Kaprio *et al.*, 1995; Kirk *et al.*, 2001; Gielen *et al.*, 2008; Beardsall *et al.*, 2009), we estimated the heritability of age at menarche and BW to 61 and 27%, respectively. BW was more strongly influenced by environment than by genetic factors, while the age at menarche was predominantly influenced by genetic factors. Thus, the strong association between childhood adiposity and age at menarche may be genetically, rather than environmentally, determined. However, the genes involved are still largely unknown.

Shared intrauterine environment between opposite-sex twins may adversely influence the female twin due to increased androgenic milieu during intrauterine development (Padmanabhan *et al.*, 2006). Although these effects have been reported in many different species from rodents to sheep (Ryan and Vandenbergh, 2002; Korsten *et al.*, 2009), significant effects in human are non-consistent (Christensen *et al.*, 1998; Lummaa *et al.*, 2007; Medland *et al.*, 2008). We found no differences in the age at menarche in twin girls with a co-twin boy compared with a co-twin girl. This is in contrast to the findings from Kaprio *et al.* (1995) showing later age at menarche in 434 OSDZ twin girls compared with 378 same sex DZ twin girls. Apart from being a smaller study than our present study, we have no obvious explanation for this discrepancy.

Despite the fact that twins provide the potential to separate the effects of genetic and environmental factors on phenotypic traits, twins have a different *in utero* environment than singletons, and estimates of environmental versus genetic contributions to these traits should therefore be extrapolated to the non-twin population with caution. Multiple pregnancy has a negative influence on offspring birth size largely due to increased nutritional demand, although limited uterine stretch may also participate (Gluckman and Hanson, 2004). Although such maternal constraint may not be entirely benign, the post-natal effects of growth restriction seem less severe in twins compared with a corresponding low BW in singletons (Christensen *et al.*, 2006). In our twin population the age at menarche was similar to contemporary normative Danish references (Juul *et al.*, 2006) and the smaller MZ twin girls did not have earlier menarcheal age than the larger DZ twins girls. Thus, the adverse effects of low BW seem to have the same influence on twins and singletons, but presumably at a higher threshold level in twins than in singletons possibly due to differences in the mechanisms leading to growth restriction.

The strength of the present study relies on the large number of participants and the good response rate limiting ascertainment bias. In addition, birth size and GA at birth was collected from the Danish Birth Register, which is considered to be highly accurate. The use of self-reported menarcheal age will lead to some degree of recall bias (Bergsten-Brucefors, 1976), even with the narrow time window between the age at menarche and the recall by questionnaire in the present study. However, this recall is not likely to be associated with BW and systematic errors are therefore unlikely to have influenced our results.

In conclusion, we found a significant, although modest association between lower BW for GA and earlier age at menarche in a large cohort of healthy twins. However, being the smaller twin at birth within a pair was not associated with earlier menarcheal age compared with the larger co-twin, even in genetically identical twins. This indicates that the association is governed by shared genetics and/or shared environmental factors. Our findings confirm that age at menarche is a strongly heritable trait, but does not support that low BW leads to prenatal programming of earlier menarche by non-shared environmental effects in twins. To what extent the effect of BW on menarcheal age is governed by shared genetics or shared environment could not be determined. The age at menarche or birth size did not differ between DZ twin girls with a different or opposite-sex twin, questioning the previously proposed androgenic influence on timing of pubertal development in female twins sharing intrauterine milieu with a male co-twin.

Authors' roles

K.S. contributed to participation in study design, analysis, manuscript drafting and critical discussion. A.J. contributed to participation in study design and critical discussion. K.C. contributed to participation in study design, execution and critical discussion. A.S. contributed to participation in the study execution and data-preparation T.S. contributed to statistical analysis. T.K.J. contributed to participation in study design and critical discussion.

Funding

Departmental funds were used to support all authors during the study period and preparation.

Conflict of interest

The authors have nothing to declare.

References

Adair LS. Size at birth predicts age at menarche. *Pediatrics* 2001;**107**:E59.

Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet* 1989;**2**:577–580.

Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* 1993;**36**:62–67.

Beardsall K, Ong KK, Murphy N, Ahmed ML, Zhao JH, Peeters MW, Dunger DB. Heritability of childhood weight gain from birth and risk markers for adult metabolic disease in prepubertal twins. *J Clin Endocrinol Metab* 2009;**94**:3708–3713.

Bergsten-Brucefors A. A note on the accuracy of recalled age at menarche. *Ann Hum Biol* 1976;**3**:71–73.

Bhargava SK, Ramji S, Srivastava U, Sachdev HP, Kapani V, Datta V, Satyanarayana L. Growth and sexual maturation of low birth weight children: a 14 year follow up. *Indian Pediatr* 1995;**32**:963–970.

Christensen K, Basso O, Kyvik KO, Juul S, Boldsen J, Vaupel JW, Olsen J. Fecundability of female twins. *Epidemiology* 1998;**9**:189–192.

Christensen K, Wienke A, Skytthe A, Holm NV, Vaupel JW, Yashin AI. Cardiovascular mortality in twins and the fetal origins hypothesis. *Twin Res* 2001;**4**:344–349.

Christensen K, Petersen I, Skytthe A, Herskind AM, McGue M, Bingley P. Comparison of academic performance of twins and singletons in adolescence: follow-up study. *BMJ* 2006;**333**:1095.

Demerath EW, Choh AC, Czerwinski SA, Lee M, Sun SS, Chumlea WC, Duren D, Sherwood RJ, Blangero J, Towne B *et al.* Genetic and

environmental influences on infant weight and weight change: the Fels Longitudinal Study. *Am J Hum Biol* 2007;**19**:692–702.

Elks CE, Perry JR, Sulem P, Chasman DI, Franceschini N, He C, Lunetta KL, Visser JA, Byrne EM, Cousminer DL *et al*. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. *Nat Genet* 2010;**42**:1077–1085.

Frost M, Petersen I, Brixen K, Beck-Nielsen H, Holst JJ, Christiansen L, Hojlund K, Christensen K. Adult glucose metabolism in extremely birthweight-discordant monozygotic twins. *Diabetologia* 2012;**55**:3204–3212.

Gielen M, Lindsey PJ, Derom C, Smeets HJ, Souren NY, Paulussen AD, Derom R, Nijhuis JG. Modeling genetic and environmental factors to increase heritability and ease the identification of candidate genes for birth weight: a twin study. *Behav Genet* 2008;**38**:44–54.

Glidden DV. A two-stage estimator of the dependence parameter for the Clayton-Oakes model. *Lifetime Data Anal* 2000;**6**:141–156.

Glinianaia SV, Skjaerven R, Magnus P. Birthweight percentiles by gestational age in multiple births. A population-based study of Norwegian twins and triplets. *Acta Obstet Gynecol Scand* 2000;**79**:450–458.

Gluckman PD, Hanson MA. Maternal constraint of fetal growth and its consequences. *Semin Fetal Neonatal Med* 2004;**9**:419–425.

Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. *N Engl J Med* 2008;**359**:61–73.

Holt JD, Prentice RL. Survival analysis in twin studies and matched-pair experiments. *Biometrika* 1974;**61**:17–30.

Ibanez L, Ferrer A, Marcos MV, Hierro FR, de ZF. Early puberty: rapid progression and reduced final height in girls with low birth weight. *Pediatrics* 2000;**106**:E72.

Jacobsen BK, Oda K, Knutsen SF, Fraser GE. Age at menarche, total mortality and mortality from ischaemic heart disease and stroke: the Adventist Health Study, 1976–88. *Int J Epidemiol* 2009;**38**:245–252.

Juul A, Teilmann G, Scheike T, Hertel NT, Holm K, Laursen EM, Main KM, Skakkebaek NE. Pubertal development in Danish children: comparison of recent European and US data. *Int J Androl* 2006;**29**:247–255.

Kaprio J, Rimpela A, Winter T, Viken RJ, Rimpela M, Rose RJ. Common genetic influences on BMI and age at menarche. *Hum Biol* 1995; **67**:739–753.

Kirk KM, Blomberg SP, Duffy DL, Heath AC, Owens IP, Martin NG. Natural selection and quantitative genetics of life-history traits in Western women: a twin study. *Evolution Int J Org Evolution* 2001;**55**:423–435.

Korsten P, Clutton-Brock T, Pilkington JG, Pemberton JM, Kruuk LE. Sexual conflict in twins: male co-twins reduce fitness of female Soay sheep. *Biol Lett* 2009;**5**:663–666.

Kyvik KO, Green A, Beck-Nielsen H. The new Danish Twin Register: establishment and analysis of twinning rates. *Int J Epidemiol* 1995; **24**:589–596.

Lakshman R, Forouhi NG, Sharp SJ, Luben R, Bingham SA, Khaw KT, Wareham NJ, Ong KK. Early Age at Menarche Associated with Cardiovascular Disease and Mortality. *J Clin Endocrinol Metab* 2009; **94**:4953–4960.

Lee JM, Appugliese D, Kaciroti N, Corwyn RF, Bradley RH, Lumeng JC. Weight status in young girls and the onset of puberty. *Pediatrics* 2007; **119**:e624–e630.

Luke B, Hediger M, Min SJ, Brown MB, Misiunas RB, Gonzalez-Quintero VH, Nugent C, Witter FR, Newman RB, Hankins GD *et al*. Gender mix in twins and fetal growth, length of gestation and adult cancer risk. *Paediatr Perinat Epidemiol* 2005;**19**(Suppl. 1):41–47.

Lummaa V, Pettay JE, Russell AF. Male twins reduce fitness of female co-twins in humans. *Proc Natl Acad Sci USA* 2007;**104**: 10915–10920.

Maisonet M, Christensen KY, Rubin C, Holmes A, Flanders WD, Heron J, Ong KK, Golding J, McGeehin MA, Marcus M. Role of prenatal characteristics and early growth on pubertal attainment of British girls. *Pediatrics* 2010;**126**:e591–e600.

Martinussen T, Scheike TH. *Dynamic Regression Models for Survival Data*. New York: Springer-Verlag, 2006.

Medland SE, Loehlin JC, Willemsen G, Hatemi PK, Keller MC, Boomsma DI, Eaves LJ, Martin NG. Males do not reduce the fitness of their female co-twins in contemporary samples. *Twin Res Hum Genet* 2008; **11**:481–487.

Murphy VE, Smith R, Giles WB, Clifton VL. Endocrine regulation of human fetal growth: the role of the mother, placenta, and fetus. *Endocr Rev* 2006;**27**:141–169.

Ong KK, Northstone K, Wells JC, Rubin C, Ness AR, Golding J, Dunger DB. Earlier mother's age at menarche predicts rapid infancy growth and childhood obesity. *PLoS Med* 2007;**4**:e132.

Ong KK, Emmett P, Northstone K, Golding J, Rogers I, Ness AR, Wells JC, Dunger DB. Infancy weight gain predicts childhood body fat and age at menarche in girls. *J Clin Endocrinol Metab* 2009; **94**:1527–1532.

Padmanabhan V, Manikkam M, Recabarren S, Foster D. Prenatal testosterone excess programs reproductive and metabolic dysfunction in the female. *Mol Cell Endocrinol* 2006;**246**:165–174.

Poulsen P, Grunnet LG, Pilgaard K, Storgaard H, Alibegovic A, Sonne MP, Carstensen B, Beck-Nielsen H, Vaag A. Increased risk of type 2 diabetes in elderly twins. *Diabetes* 2009;**58**:1350–1355.

Ryan BC, Vandenbergh JG. Intrauterine position effects. *Neurosci Biobehav Rev* 2002;**26**:665–678.

Scholl TO, Hediger ML, Vasilenko P III, Ances IG, Smith W, Salmon RW. Effects of early maturation on fetal growth. *Ann Hum Biol* 1989; **16**:335–345.

Sloboda DM, Hart R, Doherty DA, Pennell CE, Hickey M. Age at menarche: influences of prenatal and postnatal growth. *J Clin Endocrinol Metab* 2007; **92**:46–50.

Tam CS, de ZF, Garnett SP, Baur LA, Cowell CT. Opposing influences of prenatal and postnatal growth on the timing of menarche. *J Clin Endocrinol Metab* 2006;**91**:4369–4373.

Vagero D, Leon D. Ischaemic heart disease and low birth weight: a test of the fetal-origins hypothesis from the Swedish Twin Registry. *Lancet* 1994; **343**:260–263.

van Weissenbruch MM, Delemarre-van de Waal HA. Early influences on the tempo of puberty. *Horm Res* 2006;**65**(Suppl. 3): 105–111.

Veening MA, van Weissenbruch MM, Roord JJ, de Delemarre-van Waal HA. Pubertal development in children born small for gestational age. *J Pediatr Endocrinol Metab* 2004;**17**:1497–1505.