ORIGINAL ARTICLE

JBMR®

Birth Weight and Adult Bone Metabolism Are Unrelated: Results From Birth Weight–Discordant Monozygotic Twins

Morten Frost,[1,2] Inge Petersen,[1] Thomas L Andersen,[3] Bente L Langdahl,[4] Thora Buhl,[5] Lene Christiansen,[1] Kim Brixen,[2] and Kaare Christensen[1,6,7]

[1]The Danish Twin Registry, Danish Ageing Research Centre, University of Southern Denmark, Odense, Denmark
[2]Department of Endocrinology and Metabolism, Odense University Hospital, Odense, Denmark
[3]Department of Clinical Cell Biology, Vejle Hospital, Vejle, Denmark
[4]Department of Endocrinology and Internal Medicine, Tage-Hansens Gade (THG), Aarhus University Hospital, Aarhus, Denmark
[5]Department of Nuclear Medicine, Gentofte Hospital, Copenhagen, Denmark
[6]Department of Clinical Biochemistry and Pharmacology, Odense University Hospital, Odense, Denmark
[7]Department of Clinical Genetics, Odense University Hospital, Odense, Denmark

ABSTRACT

Low birth weight (BW) has been associated with poor bone health in adulthood. The aim of this study was to investigate the association between BW and bone mass and metabolism in adult BW-discordant monozygotic (MZ) twins. A total of 153 BW–extremely discordant MZ twin pairs were recruited from the Danish Twin Registry. Serum vitamin D (25-hydroxyvitamin D [25OHD]) and bone turnover markers (BTMs) amino-terminal propeptide of type I procollagen (P1NP), pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (1CTP), and cross-linked C-telopeptide (CTX) were quantified. Femoral neck (FN), total hip (TH), lumbar spine (LS), and whole-body (WB) bone mineral density (BMD) (ie, FN-BMD, TH-BMD, LS-BMD, and WB-BMD, respectively) were measured using dual-energy X-ray absorptiometry (DXA). Twins were studied as single individuals using regression analyses with or without adjustment for height, weight, age, sex, and intrapair correlation. Within-pair differences were assessed using Student's *t* test and fixed-regression models. BW was not associated with BTMs, LS-BMD, TH-BMD, FN-BMD, or WB-BMD, but BW was associated with WB-BMC, and WB-Area after adjustments. Compared to the co-twin, twins with the highest BW were heavier and taller in adulthood (mean differences ± SD): 3.0 ± 10.5 kg; 1.6 ± 2.6 cm; both $p < 0.001$). Within-pair analyses showed that LS-BMD, TH-BMD, and FN-BMD tended to be higher in twins with highest BW (for all: mean difference 0.01 ± 0.1 g/cm^2; $p = 0.08$, 0.05, and 0.10, respectively). No difference was observed after adjustment for adult body size. Intrapair differences in BW were not associated with differences in any of the biochemical parameters or BMD. Small differences between twins in BMD were explained by dissimilarities in body size. These results suggest that BW and adult bone metabolism are unrelated. © 2013 American Society for Bone and Mineral Research.

KEY WORDS: EPIDEMIOLOGY; METABOLIC BONE DISEASE; GROWTH AND DEVELOPMENT; MONOZYGOTIC TWINS

Introduction

The etiology of osteoporosis is complex, and numerous modifiable and permanent factors change the risk of osteoporosis, including age, body weight, and genetics.[1] Furthermore, epidemiological studies suggest that factors in early life influence bone health in adulthood. Birth weight (BW), a proxy for intrauterine environment and fetal growth, has been associated with whole-body (WB) bone mineral density (BMD) at 6 months and 9 years of age.[2,3] Similar associations have been reported with WB, femoral neck (FN), and lumbar spine (LS) area, as well as LS bone mineral content (BMC),[4,5] femoral,[6] cortical, and trabecular bone sizes,[7] and bone strength assessed using peripheral quantitative computed tomography in adults.[8] It has been suggested that these associations may originate from a detrimental intrauterine environment that through fetal programming lead to an impairment of adult bone metabolism.[9] Nevertheless, shared risk factors, eg, socioeconomics and

Received in original form November 27, 2013; revised form April 21, 2013; accepted May 9, 2013. Accepted manuscript online May 23, 2013.
Address correspondence to: Morten Frost, MD, PhD, Department of Epidemiology, J.B.Winsløwsvej 9B, 5000 Odense, Denmark. E-mail: frostnielsen@yahoo.com
Journal of Bone and Mineral Research, Vol. 28, No. 12, December 2013, pp 2561–2569
DOI: 10.1002/jbmr.1995

lifestyle, or genetics, may influence intrauterine growth as well as the risk of osteoporosis.

Little is known about the pathophysiological processes that may couple intrauterine growth and adult bone metabolism. It has been suggested that nutrition and vitamin D affect the interaction. Thus, low levels of maternal 25-hydroxyvitamin D (25OHD) during pregnancy were associated with lower WB-BMC and LS-BMC when their child was 9 years of age.[10] Furthermore, intrauterine growth restriction due to undernutrition causes shorter bones with lower BMC in rats.[11] Steer and Tobias[3] reported that the association between BW and BMD in 9-year-old children was attenuated after adjustment for parental height and that this association either disappeared or changed direction when adjusted for body size. BMD was lower in adults born prematurely; however, BMD was appropriate for stature,[12] indicating that adult body size needs to be taken into consideration when these associations are investigated. In addition, Jensen and colleagues[4] reported that correction for adult body size and not infant body size eliminated the association between BW and bone mass in adulthood, indicating that postnatal growth rather than intrauterine programming mediates the association.

Twin studies are of particular importance when the outcome in adulthood of early life factors are investigated, as a result of the possibility to control for several potential confounders, including maternal lifestyle, gestational age, and genetics. Among 1411 unselected female twin-pairs, self-reported BW was a positive predictor of both BMC and BMD; however, adjustment for adult height decreased the associations between intrapair differences in BW and BMC, indicating that the association was explained predominantly by skeletal dimension.[13]

Thus, BW appears to be highly important for adult bone status; however, the relative importance of BW and shared risk factors remain uncertain. Therefore, the aim of this study was to investigate the association between BW and bone mass as well as turnover in adulthood. In order to control for the effects of genetics as well as maternal and social confounding, adult monozygotic (MZ), BW-discordant twins (twins with large difference in birth weight) were recruited from the Danish Twin Registry. If BW has an independent impact on bone in later life once important confounders are controlled for, significant differences in bone mass and turnover markers would be anticipated within MZ BW-discordant twin pairs.

Subjects and Methods

Study population

The participants in the present study were recruited from the Danish Twin Registry, which comprises information on more than 77,855 unselected Danish twins.[14] First, MZ twin-pairs were identified. Second, the 379 most–BW-discordant MZ twin-pairs were selected as potential participants of the present study, which aimed to include at least 150 MZ twin-pairs. Third, a total of 336 twin-pairs were successively invited by mail to participate in the clinical study that was conducted at three different medical centers in 2010 and which included a structured interview on comorbidity, including fracture history after the age of 50 years and concomitant medication.

In all, 178 twin-pairs declined participation or were ineligible, mainly because one or both of the twins were unwilling to participate ($n = 74$ and 25 pairs, respectively), or one or both of the twins were too ill to participate ($n = 16$ and 4 pairs, respectively). Fifty-nine pairs refused participation or the twin-pair was ineligible due to other causes, including low mobility, travel distance to the medical center, and present or recent pregnancy (<6 months). Thus, a total of 158 twin-pairs were included in the study.

The study was approved by the Local Ethics Committee (S-20090033) and conducted in accordance with the Helsinki II declaration, and all participants consented in writing to participate in the study.

BW

Information on BW was retrieved from midwife records and the Danish Birth Record Registry in those born before and after 1973, respectively. Twin-pairs that disagreed on BW were excluded from further analyses.

Twin status

Zygosity status was assigned on the basis of physical resemblance, which is known to provide >95% accuracy of classification.[15] Zygosity was confirmed in the study population by use of 12 highly polymorphic microsatellite markers, and identity in all of these indicated >99.8% likelihood of monozygosity.

Biochemical tests

Blood samples were drawn between 7:00 a.m. and 9:00 a.m. after an overnight fast of at least 8 hours and were immediately separated into plasma and serum, aliquotted, and stored at –80°C until analyses.

The following bone turnover markers (BTMs) were assessed: serum total amino-terminal propeptide of type I procollagen (P1NP) and cross-linked C-telopeptide (CTX) were determined using an electrochemiluminescent method (Roche Diagnostic, Mannheim, Germany), and serum type-1 collagen C-terminal telopeptide (ICTP) was measured by a competitive enzyme immunoassay (Orion Diagnostica, Espoo, Finland).

Plasma 25-hydroxyvitamin D3 (25OHD) was determined by high-performance liquid chromatography followed by tandem mass spectrometry (LC-MS/MS). Deuterated 25-hydroxy-vitamin D3 (d6-25-OH-D3) was used as internal standard and the analysis was calibrated by using serum-based calibrators (Chromsystems, Munich, Germany). Serum was purified before LC-MS/MS by protein precipitation and solid-phase extraction of the supernatant. The interassay precision was 8% at 80 nmol/L and 15% at 20 nmol/L. The laboratory participated with satisfactory results in the external quality control scheme from DEQAS Vitamin D External Quality Assessment Scheme, London, UK

Body composition, BMC, and BMD

Body weight was measured to the nearest 0.1 kg (SECA, Hamburg, Germany), with the participants dressed in light clothing without shoes, and height was measured by use of a stadiometer to the nearest 0.1 cm (Harpenden; Holtain Ltd.,

Crymmych, Wales). Body mass index (BMI, kg/m^2) was calculated as weight divided by height[2].

Dual-energy X-ray absorptiometry (DXA) (Discovery A; Hologic Inc., Waltham, MA, USA, and Lunar Prodigy; GE Medical Systems, Madison, WI, USA) was used to measure LS (L_1–L_4) BMC, bone area, and BMD (LS-BMC, LS-AREA, LS-BMD) as well as similar measures in TH (TH-BMC, TH-AREA, and TH-BMD), FN (FN-BMC, FN-AREA, and FN-BMD), and WB (WB-BMC, WB-AREA, and WB-BMD). The coefficients of variation were 1.5% for LS and TH measurements and 0.7% for WB.

Statistical analyses

Data are reported as mean ± SD or median and interquartile range [IQR] according to distribution.

Data was analyzed in two different ways. In the first set of analyses, study participants were considered as individuals in order to investigate if BW and 25OHD, BTMs, or bone parameters were associated. In the second set of analyses, the study population was evaluated as twin-pairs with the intention to find out if there were any intrapair differences in these variables.

Subgroup analyses were performed in twin-pairs with a BW difference of at least 0.5 kg.

All calculations were performed using STATA version 11 (STATA Corp, College Station, TX). The level of significance was set at $p < 0.05$. Data were not corrected for multiple comparisons.

Analyses of twins considered as single individuals

The associations between BW and bone parameters and biochemical markers were assessed in the first set of analyses using regression analyses. The univariate model (A) included BW, whereas the multivariate model (B) also comprised height, weight, age, and sex. Adult body height was included in the regression models in order to adjust for body size, which was considered a potential confounder. Also, body weight was included, because it may be an intermediary factor in the pathway between BW and adult bone metabolism. STATA's cluster option was used to take account for possible within-pair correlations that might underestimate the standard errors in both analyses.

Analyses of within-pair differences

Paired *t* test and the chi-square test were used to compare within MZ twin-pair differences (always twin with highest BW compared to twin with lowest BW) of continuous and categorical variables in the second set of analyses. Subgroup analyses were carried out in twin-pairs with a difference in BW of >0.5 kg. Furthermore, both univariate and multivariate fixed-effect regression models including BW, height, weight, age, and sex were used to assess the association between BW and 25OHD, BTM, or bone parameters within twin-pairs.

These analyses were repeated in twin pairs with a small difference in BW (<0.5 kg) as well as in those twin pairs that both provided information on BW and agreed on BW. Also, analyses were repeated with adjustment for scanner site.

Results

Basic description of the study population

A total of 158 twin-pairs were included in the study, but 9 twin-pairs were excluded prior to any analysis due to one of the following reasons: because the twins either disagreed on BW (one pair), were not MZ (one pair), were pregnant (one pair), were treated with specific osteoporosis medication (three pairs; four individuals within the three pairs), or DXA scans were not performed (three pairs). Thus, the study population comprised 149 twin-pairs.

Basic anthropometrics at birth and at inclusion in the study are presented in Table 1. The study population consisted of 79 male and 70 female twin-pairs with a median age of 57 (interquartile ranges, 33–63) years. BW differed by at least 0.5 kg in 45 twin-pairs. Seven participants had a BW of <1.5 kg. Furthermore, 67 individuals (22.5%) reported use of tobacco products on a daily basis, and 31 of these were the heavier twin at birth ($p > 0.1$). Ten participants (3.4%) consumed more than 21 units of alcohol per week, and BW was highest in 7 of these individuals ($p > 0.1$). Twenty-eight participants (9.4%) reported present use of vitamin D supplements, and 15 of these twins were the heavier twin at birth ($p > 0.1$). None of the twin pairs were discordant for menopause.

Participants investigated as single individuals

Results of these analyses are presented in Table 2. BW was associated with P1NP ($\beta = 4.90$, $p = 0.04$) before but not after adjustments. Neither BTMs nor 25OHD was significantly associated with BW before or after adjustment for potential confounding factors (Table 2).

BW was not associated with FN-BMD. Associations between BW and FN-BMC ($\beta = 0.47$, $p < 0.001$) as well as FN-AREA ($\beta = 0.39$, $p < 0.001$) were present in univariate but not multivariate analyses (Table 2). BW was not associated with TH-BMD, whereas BW was associated with TH-BMC ($\beta = 5.36$, $p < 0.001$) and TH-AREA ($\beta = 4.48$, $p < 0.001$) in univariate but not multivariate analyses. LS-BMD, LS-BMC and LS-Area were not associated with BW (Table 2). WB-BMD, WB-BMC, and WB-AREA were all associated with BW ($\beta = 0.05$, $p = 0.001$, $\beta = 349.1$, $p < 0.001$, and $\beta = 200.2$, $p < 0.001$, respectively) in univariate analyses, and WB-BMC and WB-AREA but not WM-BMD were associated with BW in multiple regression analyses ($\beta = 99.8$, $p = 0.02$ and $\beta = 53.2$, $p < 0.001$) (Table 2).

Participants investigated as twin-pairs

Within twin-pair comparisons are presented in Table 3. Body weight and height were greater in twins with highest BW (mean difference ± SD: 3.0 ± 10.5 kg and 1.64 ± 2.6 cm, both $p < 0.001$). Body weight and height but not BMI were all higher in twins with largest BW in subgroup analyses comprising twin-pairs with either double-verification of BW or large difference in BW (Table 3).

Levels of 25OHD and BTMs did not differ between BW-discordant twins, and similar results were retrieved from subgroup analyses (Table 3). FN-BMD was not significantly different between groups, but FN-BMC and FN-AREA were higher

Table 1. Anthropometrics of the Study Population

	Men ($n = 158$)	Women ($n = 140$)	Twins with highest BW ($n = 149$)	Twins with lowest BW ($n = 149$)
Age (years)	47.9 (15)	48.6 (16)	–	–
Birth length (cm)	49 (3)	48 (3)	48.7 (2.6)	48.1 (3.0)
BW (kg)	2.7 (0.6)	2.4 (0.5)	2.9 (0.5)	2.3 (0.5)
Height (cm)	179.0 (7.0)	165.4 (6.1)	173.4 (9.5)	171.7 (9.5)
Weight (kg)	85.7 (13.7)	65.5 (10.7)	77.6 (17.0)	74.6 (14.7)
BMI (kg/m^2)	26.7 (3.9)	24.1 (4.2)	25.7 (4.6)	25.3 (4.0)
Vitamin D				
25OHD (mmol/L)	61.3 (31.3)	73.3 (31.0)	67.3 (29.3)	66.6 (34.0)
BTMs				
P1NP (μg/L)	52.0 (19.2)	48.3 (21.1)	50.1 (20.6)	50.5 (19.8)
CTX (μg/L)	3.5 (1.6)	3.3 (1.1)	0.43 (0.20)	0.45 (0.20)
1CTP (μg/L)	0.47 (0.21)	0.41 (0.19)	3.34 (1.25)	3.46 (1.56)
Bone parameters				
FN-BMD (g/cm^2)	0.90 (0.18)	0.84 (0.16)	0.88 (0.18)	0.87 (0.17)
TH-BMD (g/cm^2)	1.03 (0.13)	0.93 (0.13)	0.99 (0.15)	0.98 (0.14)
LS-BMD (L_1–L_4) (g/cm^2)	1.09 (0.15)	0.98 (0.15)	1.04 (0.16)	1.03 (0.16)
WB-BMD (g/cm^2)	1.19 (0.11)	1.07 (0.11)	1.14 (0.13)	1.13 (0.13)

Data are presented as mean (SD). The twins with the highest BW and those with the lowest BW were compared by use of Student's *t* test. Neither of the variables differed between groups.

BW = birth weight; BMI = body mass index; 25OHD = 25-hydroxyvitamin D; BTM = bone turnover marker; P1NP = amino-terminal propeptide of type I procollagen; CTX = cross-linked C-telopeptide; 1CTP = pyridinoline cross-linked carboxyterminal telopeptide of type I collagen; FN = femoral neck; BMD = bone mineral density; TH = total hip; LS = lumbar spine; WB = whole body.

in twins with the largest BW (mean difference ± SD: 0.14 ± 0.5 g and 0.09 ± 0.3 cm^2, both $p < 0.01$). TH-BMD tended to be higher in twins with largest BW (0.01 ± 0.1 g/cm^2, $p = 0.05$) in the complete study population but not in subgroup analyses (Table 3). TH-BMC and TH-AREA were higher in twins with largest BW in the complete study population (1.04 ± 3.3 g and 0.57 ± 2.3 cm^2, respectively, both $p < 0.01$) (Table 3). TH-AREA was higher in those with largest BW when analyses were restricted to twin-pairs with a difference in BW of at least 0.5 kg (0.89 ± 2.6 g, $p = 0.03$). LS-BMD tended to be larger in twins with the largest BW (0.01 ± 0.1 g/cm^2, $p = 0.08$), but the difference was not present in the subgroup comprising twins with large difference in BW (Table 3). Neither LS-BMC nor LS-AREA differed between groups. WB-BMD was similar but WB-BMC and WB-AREA were higher in twins with highest BW (76.8 ± 181 g and 56.9 ± 97 cm^2, both $p < 0.001$). These differences were also present in those with a large difference in BW (Table 3). Stratifying analyses according to sex provided similar results (data not shown).

Within-pair differences in BW and BTMs or 25OHD were not associated in univariate or multivariate analyses (Table 4). Intrapair differences in BW tended to be associated with FN-BMD ($\beta = 0.024$, $p = 0.07$), but no association was present in multivariate analyses. Intrapair differences in BW were associated with FN-BMC in univariate but not multivariate analyses ($\beta = 0.24$, $p = 0.001$), whereas differences in BW and FN-AREA were associated before adjustments ($\beta = 0.15$, $p = 0.002$), but not after. Intrapair differences in BW tended to be associated with differences in TH-BMD before ($\beta = 0.021$, $p = 0.07$), but not after adjustments. TH-BMC and TH-AREA were associated with BW in univariate ($\beta = 1.79$ and $\beta = 1.10$, $p < 0.001$ and $p = 0.001$, respectively), but not multivariate analyses. Differences in BW were not associated with any of the LS parameters. Intrapair differences in BW were not associated with WB-BMD in any analyses, but intrapair differences in BW were associated with WB-BMC and WB-AREA before ($\beta = 133.77$ and $\beta = 103.96$, both $p < 0.001$) adjustments, and WB-Area and intrapair differences in BW were associated after adjustments ($\beta = 46.06$, $p = 0.004$) (Table 4).

Similar results were found in twin pairs with a difference in BW of <0.5 kg. Restricting the analyses to those twin pairs (128 twin pairs; 86% of the twin pairs) in which both twins had provided information on BW and agreed on BW provided identical results (data not shown). Also, adjustment for scanner site did not influence the results (data not shown).

Fracture history

A total of 20 (6.7%) individuals reported a fracture after the age of 50 years, and BW was highest in 10 of these ($p > 0.1$). Three (1.0%) individuals had had a hip fracture after the age of 50 years (2 with highest BW). Two participants reported a vertebral (0.7%) and another 8 (2.7%) individuals reported a forearm fracture (1 and 4 with highest BW, respectively). In all, 30 (10.1%) individuals out of the 295 reporting information on height loss had lost at least 4 cm since youth, and 18 of these individuals were among those with the largest BW ($p > 0.1$).

Discussion

BMD tended to be higher in the twin with the largest BW; however, bone mass was appropriate when dissimilarities in body size were taken into consideration, suggesting that

Table 2. Regression Analyses of Birth Weight and 25OHD, BTMs, and Bone Parameters

	Birth weight Model A β	*p*	Birth weight Model B β	*p*
Height and weight				
Height (cm)	7.1	<0.001		
Weight (kg)	6.7	0.003		
Vitamin D				
25OHD (mmol/L)	−0.94	>0.1	0.55	>0.1
BTMs				
P1NP (μg/L)	4.77	0.04	3.72	>0.1
CTX (μg/L)	0.03	>0.1	0.011	>0.1
1CTP (μg/L)	−0.12	>0.1	−0.10	>0.1
Bone parameters				
FN-BMD (g/cm^2)	0.03	>0.1	0.02	>0.1
FN-BMC (g)	0.47	<0.001	0.14	>0.1
FN-Area (cm^2)	0.39	<0.001	0.07	>0.1
TH-BMD (g/cm^2)	0.03	0.08	−0.01	>0.1
TH-BMC (g)	5.36	<0.001	0.69	>0.1
TH-Area (cm^2)	4.48	<0.001	1.04	0.05
LS-BMD (L_1–L_4) (g/cm^2)	0.02	>0.1	−0.01	>0.1
LS-BMC (L_1–L_4) (g)	1.49	>0.1	−5.32	>0.1
LS-Area (L_1–L_4) (cm^2)	−2.43	>0.1	−4.45	>0.1
WB-BMD (g/cm^2)	0.05	0.001	0.02	>0.1
WB-BMC (g)	349.13	<0.001	99.8	0.02
WB-Area (cm^2)	200.21	<0.001	53.2	<0.001

Model A: birth weight. Model B: Model A + height, weight, age, sex.

25OHD = 25-hydroxyvitamin D; BTM = bone turnover marker; P1NP = amino-terminal propeptide of type I procollagen; CTX = cross-linked C-telopeptide; 1CTP = pyridinoline cross-linked carboxyterminal telopeptide of type I collagen; FN = femoral neck; BMD = bone mineral density; BMC = bone mineral content; TH = total hip; LS = lumbar spine; WB = whole body.

differences in BW and adult bone mass and metabolism are unrelated. BW-discordant MZ twins provide an excellent opportunity of investigating the impact of BW on aspects of bone health in adulthood due to the possibility of controlling for genetics and prenatal as well as postnatal, shared family, social, and cultural environments. To the best of our knowledge, this is the first study to use the discordant-twin approach to assess the association between BW and bone parameters, BTMs, and 25OHD. Based on these results obtained from discordant twins, low BW is unlikely to be an independent and clinically important risk factor for osteoporosis later in life. Thus, although different with regard to height and weight, BW-discordant adult MZ twins reveal no differences in BMI or bone status after adjustment for body size.

In agreement with our study, BW and intrapair differences in BW were associated with adult height in singletons[5,16] and Dutch twins,[17] respectively. Furthermore, as previously reported,[4,5,18] BW and several bone parameters were associated in our study when the participants were investigated as single individuals. With the exception of WB-Area, neither of these associations remained after adjustments for adult body size. The residual association between BW and adult bone area could be explained by the fact that an imperfect proxy for adult size was used in the regression models. Although we performed a large number of statistical tests, adjustment for multiple testing was not executed. Therefore, any positive finding should be interpreted with caution.

The BW-discordant twins, including those that were extremely BW-discordant, differed with regard to adult height and weight. Although the growth of an individual is likely to be reflected in adult bone size, factors with direct effect on changes in bone size during growth may also influence adult height. The present study allows for evaluation of neither causality nor direction. Nevertheless, although the twins with the largest BW were slightly taller in adulthood compared to those with the lowest BW, the difference in adult height is unlikely to reflect adult bone metabolism.

Antoniades and colleagues[13] showed that the intrapair difference in BW in female, unselected twins was associated with bone mass, primarily mediated through skeletal size. Although different methods were used for the recruitment of the study populations, results from these studies appear similar. Since size-adjusted bone mass was comparable in BW discordant MZ twins, we found no evidence of any fetal programming of adult bone metabolism. Rather, it would appear that bone mass was appropriate for body size. Furthermore, the absence of differences in 25OHD or BTMs suggests that BW discordance conveys no information on the metabolism of these important bone-related issues in adulthood.

Nevertheless, it has been suggested that inappropriate intrauterine environment alters fetal development and causes lasting changes in the physiology, predisposing to development of several adverse health outcomes in adulthood, including osteoporosis.[19,20] According to animal studies, maternal protein

Table 3. Bone Markers, 25-OHD, BTMs, and Bone Parameters in BW-Discordant MZ Twins

	MZ pairs ($n = 149$)		Pairs with BW difference of >0.5 kg ($n = 45$)	
Within-pair differences	Mean (SD)	p	Mean (SD)	p
BW status				
BMI (kg/m^2)	0.5 (3.5)	0.09	0.8 (3.4)	>0.1
Height (cm)	1.64 (2.6)	<0.001	2.51 (3.1)	<0.001
Weight (kg)	3.0 (10.5)	<0.001	4.5 (9.8)	0.004
Vitamin D				
25OHD (nmol/L)	0.7 (30.4)	>0.1	−0.7 (36.0)	>0.1
BTMs				
P1NP (μg/L)	−0.5 (21.0)	>0.1	−0.28 (27.3)	>0.1
CTX (μg/L)	−0.02 (0.2)	>0.1	−0.04 (0.2)	>0.1
1CTP (μg/L)	−0.11 (1.8)	>0.1	−0.05 (1.4)	>0.1
Bone parameters				
FN-BMD (g/cm^2)	0.01 (0.1)	0.10	0.00 (0.1)	>0.1
FN-BMC (g)	0.14 (0.5)	<0.01	0.07 (0.6)	>0.1
FN-Area (cm^2)	0.09 (0.3)	<0.01	0.07 (0.4)	>0.1
TH-BMD (g/cm^2)	0.01 (0.1)	0.05	<0.01 (0.1)	>0.1
TH-BMC (g)	1.04 (3.3)	<0.001	0.65 (3.3)	>0.1
TH-Area (cm^2)	0.57 (2.3)	<0.01	0.89 (2.6)	0.03
LS-BMD (L_1–L_4) (g/cm^2)	0.01 (0.1)	0.08	0.01 (0.1)	>0.1
LS-BMC (L_1–L_4) (g)	1.71 (41.9)	>0.1	0.23 (40.2)	>0.1
LS-Area (L_1–L_4) (cm^2)	0.50 (33.2)	>0.1	−1.69 (33.7)	>0.1
WB-BMD (g/cm^2)	0.005 (0.06)	>0.1	<0.01	>0.1
WB-BMC (g)	76.8 (180.9)	<0.001	85.7 (185.2)	0.004
WB-Area (cm^2)	56.9 (96.8)	<0.001	72.9 (122.5)	<0.001

Twin with the highest BW was compared to the co-twin by use of Student's t test (paired).

25OHD = 25-hydroxyvitamin D; BTM = bone turnover marker; BW = birth weight; MZ = monozygotic; P1NP = amino-terminal propeptide of type I procollagen; CTX = cross-linked C-telopeptide; 1CTP = pyridinoline cross-linked carboxyterminal telopeptide of type I collagen; FN = femoral neck; BMD = bone mineral density; BMC = bone mineral content; TH = total hip; LS = lumbar spine; WB = whole body.

restriction or feeding with high-fat diet during pregnancy affect skeletal structures and strength in the offspring.[21,22] Furthermore, a high-fat diet during pregnancy reduced development of osteoblasts due to epigenetic regulation[23] and changed the expression of a number of genes that may influence bone metabolism, including the leptin receptor and pro-opiomelanocortin in the offspring.[24] Whether the epigenetic profile varies with different BW after control for genetics and environment remains unanswered. Furthermore, studies in humans have linked maternal magnesium and folate intake[3] and vitamin D levels in late pregnancy[10] with bone health in childhood. On the other hand, infants with 1-alpha-hydroxylase deficiency, vitamin D receptor mutations, or inability to synthesize vitamin D present normal skeletal phenotype at birth, indicating that intrauterine bone development is normal even in the absence of vitamin D.[25]

Our results do not exclude a causative link between prenatal life and skeletal attainment and preservation in adulthood. We solitarily investigated BW as a proxy for intrauterine life conditions. However, accelerated weight gain and growth in infancy have also been associated with several diseases, including obesity[26] and hip fractures,[27] and abnormal growth in early life may reflect aspects of fetal programming that is not captured by BW. Accordingly, it has been shown that low BW is followed by metabolic changes in cases of rapid catch-up growth,[28] possibly due to leptin resistance.[29] Interestingly, leptin may influence postnatal bone development directly through osteoblasts or indirectly via r-adrenergic tone in bone, as reviewed by Devlin and Bouxsein.[30] Moreover, an unfavorable intrauterine environment could lead to epigenetic modulations, changing gene expression, and subsequently the risk of development of disease in adulthood. BW and expression of genes related to a number of metabolic functions were associated in newborn MZ twins.[31] Furthermore, prenatal stress has been associated with shorter telomere lengths in adulthood,[32] but it has also been suggested that early-life factors may cause mitochondrial dysfunction and by that increase the risk of disease in later life.[33] Further studies on the interaction between the effects of early-life factors on epigenetic modulations, mitochondrial function, and telomere length and bone health throughout are needed.

Causes of low BW may differ between twins and singletons.[34] Nevertheless, when we investigated twins as single individuals, we found associations between BW and bone parameters as previously shown in studies based on singletons. Furthermore, Andrew and colleagues[35] reported that height and BMD were

Table 4. Fixed-Effect Regression Analyses of Intrapair Differences in Birth Weight and 25OHD, BTMs, and Bone Parameters

	Birth weight Model A β	*p*	Birth weight Model B β	*p*
Height and weight				
Height (cm)	3.23	<0.001		
Weight (kg)	5.49	<0.001		
Vitamin D				
25OHD (mmol/L)	1.84	>0.1	0.97	>0.1
BTMs				
P1NP (μg/L)	−0.01	>0.1	3.80	>0.1
CTX (μg/L)	−0.04	>0.1	−0.01	>0.1
1CTP (μg/L)	−0.25	>0.1	−0.31	>0.1
Bone parameters				
FN-BMD (g/cm^2)	0.024	0.07	0.019	>0.1
FN-BMC (g)	0.24	0.001	0.18	0.06
FN-Area (cm^2)	0.15	0.002	0.11	0.08
TH-BMD (g/cm^2)	0.021	0.07	0.01	>0.1
TH-BMC (g)	1.79	<0.001	0.78	>0.1
TH-Area (cm^2)	1.10	0.001	0.56	>0.1
LS-BMD (L_1–L_4) (g/cm^2)	0.021	>0.1	0.010	>0.1
LS-BMC (L_1–L_4) (g)	2.47	>0.1	1.72	>0.1
LS-Area (L_1–L_4) (cm^2)	0.56	>0.1	1.67	>0.1
WB-BMD (g/cm^2)	0.01	>0.1	0.01	>0.1
WB-BMC (g)	133.77	<0.001	53.33	0.08
WB-Area (cm^2)	103.96	<0.001	46.06	0.004

Model A: birth weight; Model B: Model A + height, weight, age, sex.

25OHD = 25-hydroxyvitamin D; BTM = bone turnover marker; P1NP = amino-terminal propeptide of type I procollagen; CTX = cross-linked C-telopeptide; 1CTP = pyridinoline cross-linked carboxyterminal telopeptide of type I collagen; FN = femoral neck; BMD = bone mineral density; BMC = bone mineral content; TH = total hip; LS = lumbar spine; WB = whole body.

similar in twins from the St. Thomas' UK Twin Register and age-matched singletons, showing that twin status and height are unrelated. Fracture rates in twins have not been compared to single-born individuals. Although the limited number of participants in the present study clearly prevents us from any interpretation of fracture risk, the present study and earlier reports provide no support of any major difference in bone health in terms of BMD and BTMs in twins and singletons, suggesting that the conclusions drawn from this study are valid in both groups. Conversely, whereas bone health of twins most likely mirrors that of non-twins, the same may not apply to individuals born prematurely at very low or extremely low BW. Compared to controls, BMD at age 22 years was lower after correction for height in individuals born prematurely with a very low BW (<1.5 kg).[36] Similarly, Smith and colleagues[37] reported substantially lower height-adjusted BMD in individuals born at very low BW compared to controls at time of peak bone mass. These results indicate that very low BW may have a detrimental and clinically important impact on bone status in adulthood. Due to the low number of individuals with a BW of less than 1.5 kg in our study, we were unable to investigate whether extremely low BW is particularly unfavorable to bone.

A number of concerns need to be addressed in our study. First, biochemical tests were only performed once in each individual and minor changes in bone metabolism may not have been detected. Second, information on bone structure and strength was not available because only DXA was performed. Third, because the study population consisted of BW-discordant MZ twins, one would anticipate higher levels of underlining comorbidity which would contribute bias toward a significant difference in bone mass. Twenty twin-pairs were not included due to health-related problems, indicating that twins in the present study were healthier than those not included in the study, possibly limiting the likelihood of detection of differences in health status. Fourth, the present study was particularly sensitive to erroneous classification of BW discordance that would deter us from assessing within-pair difference in bone mass in adulthood. In order to reduce the risk of misclassification, information on BW was based on midwives records or a public register. However, the majority of the participants were in agreement with regard to who was born with highest BW, and analyses repeated in twin-pairs who agreed on BW were similar to those obtained in the complete study population. Finally, the power of fixed-effects models is limited and, although a large number of comparisons were performed, neither of the analyses was adjusted for multiple testing. Therefore, the results should be interpreted with caution.

The major strength of the study was the inclusion of a large number of highly informative MZ twins and the ability of the discordant-twin design to control for both genetics and potential effects of maternal and paternal behavior as well as gestational age on the association between BW and bone mass.

In conclusion, BTMs and most bone parameters were similar in adult, BW-discordant MZ twins. Thus, based on our study, BW and

bone metabolism after controlling for genetics, shared environment, and adult body size are unrelated. Low BW does not appear to confer abnormalities on bone metabolism in later life and is unlikely to contribute substantial risk to the development of osteoporosis.

Disclosures

All authors state that they have no conflicts of interest.

Acknowledgments

This study was supported by the European Union's Seventh Framework Programme (FP7/2007-2011) under grant agreement #259679. Grants were received from the Novo Nordisk Foundation and the Region of South Denmark. The Danish Twin Registry is supported by a grant from The National Program for Research Infrastructure 2007 from the Danish Agency for Science Technology and Innovation. We are most grateful to Marianne Snorgaard and Frans Boedker at the Danish Twin Registry, University of Southern Denmark, for their assistance in the recruitment of study participants and study management.

Authors' roles: Study design: MF, KB, and KC. Study conduct: MF, BL, TB, TLA, LC, and KC. Data collection: MF, TLA, BL, LC, and BS. Data analysis: MF, TLA, IP, and KC. Data interpretation: MF, IP, KB, and KC. Drafting manuscript: MF. Revising manuscript content: MF, BL, KB, LC, and KC. Approving final version of manuscript: MF, IP, BL, TB, TLA, LC, KB, and KC. MF takes responsibility for the integrity of the data analysis.

References

1. Ralston SH, Uitterlinden AG. Genetics of osteoporosis. Endocr Rev. 2010;31(5):629–62.
2. Ay L, Jaddoe VW, Hofman A, Moll HA, Raat H, Steegers EA, Hokken-Koelega AC. Foetal and postnatal growth and bone mass at 6 months: the Generation R Study. Clin Endocrinol (Oxf). 2011;74 (2):181–90.
3. Steer CD, Tobias JH. Insights into the programming of bone development from the Avon Longitudinal Study of Parents and Children (ALSPAC). Am J Clin Nutr. 2011;94(6):1861S–4S.
4. Jensen RB, Vielwerth S, Frystyk J, Veldhuis J, Larsen T, Mølgaard C, Greisen G, Juul A. Fetal growth velocity, size in early life and adolescence, and prediction of bone mass: association to the GH-IGF axis. J Bone Miner Res. 2008;23(3):439–6.
5. Gale CR, Martyn CN, Kellingray S, Eastell R, Cooper C. Intrauterine programming of adult body composition. J Clin Endocrinol Metab. 2001;86(1):267–72.
6. Javaid MK, Prieto-Alhambra D, Lui LY, Cawthon P, Arden NK, Lang T, Lane NE, Orwoll E, Barrett-Conner E, Nevitt MC, Cooper C. Cummings SR; Osteoporotic Fractures in Men (MrOS) Research Group. Self-reported weight at birth predicts measures of femoral size but not volumetric BMD in elderly men: MrOS. J Bone Miner Res. 2011;26(8):1802–7.
7. de Bono S, Schoenmakers I, Ceesay M, Mendy M, Laskey MA, Cole TJ, Prentice A. Birth weight predicts bone size in young adulthood at cortical sites in men and trabecular sites in women from The Gambia. Bone. 2010;46(5):1316–21.
8. Oliver H, Jameson KA, Sayer AA, Cooper C, Dennison EM. Growth in early life predicts bone strength in late adulthood: the Hertfordshire Cohort Study. Bone. 2007;41(3):400–5.
9. Cooper C, Westlake S, Harvey N, Javaid K, Dennison E, Hanson M. Review: developmental origins of osteoporotic fracture. Osteoporos Int. 2006;17(3):337–47.
10. Javaid MK, Crozier SR, Harvey NC, Gale CR, Dennison EM, Boucher BJ, Arden NK, Godfrey KM, Cooper C. Princess Anne Hospital Study Group. Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. Lancet. 2006;367 (9504):36–43.
11. Romano T, Wark JD, Wlodek ME. Calcium supplementation does not rescue the programmed adult bone deficits associated with perinatal growth restriction. Bone. 2010;47(6):1054–63.
12. Dalziel SR, Fenwick S, Cundy T, Parag V, Beck TJ, Rodgers A, Harding JE. Peak bone mass after exposure to antenatal betamethasone and prematurity: follow-up of a randomized controlled trial. J Bone Miner Res. 2006;21(8):1175–86.
13. Antoniades L, MacGregor AJ, Andrew T, Spector TD. Association of birth weight with osteoporosis and osteoarthritis in adult twins. Rheumatology (Oxford). 2003;42(6):791–6.
14. Skytthe A, Christiansen L, Kyvik KO, Bødker FL, Hvidberg L, Petersen I, Nielsen MM, Bingley P, Hjelmborg J, Tan Q, Holm NV, Vaupel JW, McGue M, Christensen K. The Danish twin registry: linking surveys, national registers, and biological information. Twin Res Hum Genet. 2013;16(1):104–11.
15. Christiansen L, Frederiksen H, Schousboe K, Skytthe A, von Wurmb-Schwark N, Christensen K, Kyvik K. Age- and sex-differences in the validity of questionnaire-based zygosity in twins. Twin Res. 2003;6 (4):275–8.
16. Nilsen ST, Finne PH, Bergsjo P, Stamnes O. Males with low birthweight examined at 18 years of age. Acta Paediatr Scand. 1984;73(2):168–75.
17. Ijzerman RG, Stehouwer CD, van Weissenbruch MM, de Geus EJ, Boomsma DI. Intra-uterine and genetic influences on the relationship between size at birth and height in later life: analysis in twins. Twin Res. 2001;4(5):337–43.
18. Cooper C, Fall C, Egger P, Hobbs R, Eastell R, Barker D. Growth in infancy and bone mass in later life. Ann Rheum Dis. 1997;56(1):17–21.
19. Barker DJP. Mothers, babies, and health in later life 2nd ed. Edinburgh/New York: Churchill Livingstone; 1998.
20. Fall C, Hindmarsh P, Dennison E, Kellingray S, Barker D, Cooper C. Programming of growth hormone secretion and bone mineral density in elderly men: a hypothesis. J Clin Endocrinol Metab. 1998;83(1):135–9.
21. Lanham SA, Bertram C, Cooper C, Oreffo RO. Animal models of maternal nutrition and altered offspring bone structure. Bone development across the lifecourse. Eur Cell Mater. 2011;22:321–32.
22. Mehta G, Roach HI, Langley-Evans S, Taylor P, Reading I, Oreffo RO, Aihie-Sayer A, Clarke NM, Cooper C. Intrauterine exposure to a maternal low protein diet reduces adult bone mass and alters growth plate morphology in rats. Calcif Tissue Int. 2002;71(6):493–8.
23. Chen JR, Zhang J, Lazarenko OP, Kang P, Blackburn ML, Ronis MJ, Badger TM, Shankar K. Inhibition of fetal bone development through epigenetic down-regulation of HoxA10 in obese rats fed high-fat diet. FASEB J. 2012 Mar;26(3):1131–41.
24. Page KC, Malik RE, Ripple JA, Anday EK. Maternal and postweaning diet interaction alters hypothalamic gene expression and modulates response to a high-fat diet in male offspring. Am J Physiol Regul Integr Comp Physiol. 2009;297(4):R1049–57.
25. Malloy PJ, Feldman D. Genetic disorders and defects in vitamin d action. Endocrinol Metab Clin North Am. 2010;39(2):333–46.
26. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ. 2000;320(7240):967–71.
27. Javaid MK, Eriksson JG, Kajantie E, Forsén T, Osmond C, Barker DJ, Cooper C. Growth in childhood predicts hip fracture risk in later life. Osteoporos Int. 2011;22(1):69–73.

28. Coupe B, Grit I, Darmaun D, Parnet P. The timing of "catch-up growth" affects metabolism and appetite regulation in male rats born with intrauterine growth restriction. Am J Physiol Regul Integr Comp Physiol. 2009;297(3):R813–24.
29. Coupe B, Grit I, Hulin P, Randuineau G, Parnet P. Postnatal growth after intrauterine growth restriction alters central leptin signal and energy homeostasis. PLoS One. 2012;7(1):e30616.
30. Devlin MJ, Bouxsein ML. Influence of pre- and peri-natal nutrition on skeletal acquisition and maintenance. Bone. 2012;50(2):444–51.
31. Gordon L, Joo JH, Andronikos R, Ollikainen M, Wallace EM, Umstad MP, Permezel M, Oshlack A, Morley R, Carlin JB, Saffery R, Smyth GK, Craig JM. Expression discordance of monozygotic twins at birth: effect of intrauterine environment and a possible mechanism for fetal programming. Epigenetics. 2011;6(5):579–92.
32. Entringer S, Epel ES, Kumsta R, Lin J, Hellhammer DH, Blackburn EH, Wüst S, Wadhwa PD. Stress exposure in intrauterine life is associated with shorter telomere length in young adulthood. Proc Natl Acad Sci U S A. 2011;108(33):E513–8.
33. Barut A, Barut F, Kandemir NO, Aktunc E, Arikan I, Harma M, Harma MI, Gun BD. Placental chorangiosis: the association with oxidative stress and angiogenesis. Gynecol Obstet Invest. 2012;73(2):141–51.
34. Phillips DI, Davies MJ, Robinson JS. Fetal growth and the fetal origins hypothesis in twins—problems and perspectives. Twin Res. 2001;4(5):327–31.
35. Andrew T, Hart DJ, Snieder H, de Lange M, Spector TD, MacGregor AJ. Are twins and singletons comparable? A study of disease-related and lifestyle characteristics in adult women. Twin Res. 2001;4(6):464–77.
36. Hovi P, Andersson S, Järvenpää AL, Eriksson JG, Strang-Karlsson S, Kajantie E, Mäkitie O. Decreased bone mineral density in adults born with very low birth weight: a cohort study. PLoS Med. 2009;6(8):e1000135.
37. Smith CM, Wright NP, Wales JK, Mackenzie C, Primhak RA, Eastell R, Walsh JS. Very low birth weight survivors have reduced peak bone mass and reduced insulin sensitivity. Clin Endocrinol (Oxf). 2011;75(4):443–9.