Preterm birth and adolescent bone mineral content

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Running Title: Preterm Birth and Bone Mineral Content

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Abstract

Objective: The purpose of this paper was to determine the influence of preterm low birth weight on bone mineral content in adolescence.

Study Design: In 2007-2008 adolescent data was obtained for 25 males and 16 females who were born preterm (<37 weeks gestation) between October 1st 1989 and December 31st 1995 with a birth weight of less than 1850 grams. Preterm low birth weight individuals were age and sex matched to full term (>37 weeks) normal birth weight (>2500g) controls. Total body, hip and spine bone mineral content (BMC) was assessed using dual energy x-ray absorptiometry (DXA).

Results: Male preterm individuals had less BMC at the proximal femur in adolescence compared to controls ($p<0.05$). However, once adjusted for age, maturity, height, weight, physical activity and diet there were no differences between groups ($p<0.05$) in any bone parameters.

Conclusion: These finding suggest that preterm birth and low birth weight did not influence bone accrual in these individuals at adolescence.

Keywords: bone mineral content, preterm, low birth weight, adolescent

Abbreviations: BMC – bone mineral content, BMD – Bone mineral density, SGA – small for gestational age, AGA – appropriate for gestational age, PBMAS – Pediatric Bone Mineral Accrual Study, APHV – age at peak height velocity, PAQ-A – Physical Activity Questionnaire for Adolescents, DXA – dual X-ray absorptiometry
Introduction:

Osteoporosis is the most common bone disorder in the world and a major cause of loss of independence in the elderly; with approximately 60% of women and 30% of men over the age of 50 suffering from an osteoporotic fracture in their remaining lifetime. Osteoporosis, through its association with age-related fractures, is one of the most common causes of longstanding pain, functional impairment, disability and death in elderly populations, and a major contributor to medical care costs worldwide. Although the consequences of poor skeletal health are mainly observed later in life and fracture prevention has been directed at delaying the rate of age-related bone loss, the most effective time to influence bone health appears to be at the opposite end of the lifecycle. The amount of bone gained during childhood and adolescence impacts greatly on lifetime skeletal health. Therefore, understanding the determinants of childhood and adolescent bone mineral accrual are imperative. Many factors influence bone mineral accumulation during childhood and adolescence, including genetics, gender, maturation, diet and physical activity. However, a proportion of the variance in bone mass in the general population cannot be explained by genetic or environmental factors in childhood and adolescence. Recently, it has been suggested that this residual variance may be explained by genetic programming of the mother and fetus, and pattern of growth in infancy. This is supported by the finding that adult bone mineral content, density and estimated bone strength is positively correlated with birth weight and weight at one year of age, suggesting that fetal and early development may be important in determining lifetime skeletal health.

The positive correlation between birth weight and bone mass that has been demonstrated in the general population suggests that having a birth weight within the lower range of normal may be a marker of future low bone mass and increased fracture risk. In population studies the
average birth weight is between 3040-3400 grams; therefore, the question arises as to the effect of low birth weight (<2500g) on bone development and skeletal health\textsuperscript{3,8}. It has been suggested that low birth weight individuals have lower bone mineral content (BMC) and bone mineral density (BMD) in adulthood compared to term-born, normal birth weight peers\textsuperscript{11,12}.

Confounding the effect of low birth weight and bone accrual is the fact that low birth weight individuals can also be preterm, gestation less than or equal to 37 weeks. Approximately 80% of fetal bone is accrued in the last trimester, with peak accretion rates starting at about 35 weeks of gestation\textsuperscript{13}. Consequently, preterm infants may be born with a bone accrual deficit regardless of birth weight. Preterm infants are also postnatally exposed to several other factors which may further adversely affect bone health, including the use of medications (such as furosemide), immobilization, compromised respiration, nutritional problems and infections\textsuperscript{11}. Evaluations of preterm infants at the time of birth suggest that not only are they born with lower bone strength but that demineralization of bone may occur during the first postnatal weeks\textsuperscript{13,14}, potentially further compromising bone strength.

The purpose of this paper was to determine the influence of being born preterm (\leq 37 weeks gestation) low birth weight (<2500g) on bone mineral content in adolescence. We hypothesized that being born preterm will result in lower BMC in adolescence.

**Methods:**

*Participants*

All preterm (<37 weeks gestation) infants who were born at the Royal University Hospital in Saskatoon, Saskatchewan between October 1\textsuperscript{st} 1989 and December 31\textsuperscript{st} 1995 with a birth weight of less than 1850 grams (n= 359) who were relatively healthy were invited to take
part in this study. Individuals were excluded if they had major congenital malformations, gastrointestinal diseases or neurodevelopmental abnormalities (e.g. seizures, moderate and severe cerebral palsy). Ventilated infants with fluid restrictions who could not meet their caloric requirements in the Neonatal Intensive Care Unit, as well as infants who received total parenteral nutrition for more than 30 days were also excluded. In 2007-2008, seventy six eligible individuals (21%) consented to take part. All multiple births (n = 16) were removed prior to analysis as their births are not considered independent events. Multiples also often have different reasons than singleton births for being born earlier or of a low birth weight which may influence bone development. Complete data were available for 28 males and 22 females. Small for gestational age was defined as a birth weight less than the 10th percentile; participants were classified as either AGA (n= 41) or SGA (n= 9). SGA infants were excluded from the present analysis due to low participant number. Therefore, 41 AGA preterm low birth weight individuals were included in the present analysis. Forty of the preterm infants (98%) were Caucasian and one was Métis. The control group were Caucasian subjects recruited from the University of Saskatchewan’s Pediatric Bone Mineral Accrual Study (PBMAS) (n=127) who were born full term (>37 weeks gestation) and with a weight appropriate for gestational age (10th-90th percentile) between 1979 - 1985. The PBMAS has been described in detail elsewhere\textsuperscript{15} in short; the study utilized a mixed-longitudinal design to examine bone development throughout childhood, adolescence and young adulthood. Children were eight to 15 years at study entry and have been followed from 1991-present. This study received ethical approval from the University of Saskatchewan’s Biomedical Ethics Board. Informed consent was obtained from all parents or guardians and assent was provided by all participants.
**Chronological age:** The chronological age (years) of each participant was recorded to the nearest 0.01 year by subtracting the decimal year of the participant’s date of birth from the decimal year of the day of testing. One-year intervals were used to construct chronological age groups. Age groups were set up such that the 15-year age group included individuals between 14.50 and 15.49 years. These age groupings were then used to match the preterm individuals to a PBMAS control.

**Anthropometry:** Anthropometric measurements of adolescent participants included standing height, sitting height and weight. Heights were recorded to the nearest millimeter using a wall mounted stadiometer (Holtain Limited, Britain) and body mass to the nearest 0.5 kilogram using a calibrated physician’s scale (Toledo Scale Company, Model 2830, Canada). All measures were performed twice and if the difference was greater than 0.4 a third measure was recorded. The mean or median was then reported depending on whether two or three measures were recorded, respectively. Participants wore t-shirts and loose fitting shorts, with shoes and jewelry removed during all measures.

**Adolescent Maturation:** The range of variability between individuals of the same chronological age in maturity may be large especially as individuals approach their adolescent growth spurt; therefore, it is essential that maturity is considered when examining physiological parameters in adolescence. Age at peak height velocity (APHV) reflects the maximum growth in stature during a one year time interval in adolescence and also acts as an indicator of somatic maturation. It provides a benchmark of the maximum growth during adolescence within and between individuals. APHV is a maturational landmark which is easily assessed, does not require invasive procedures and occurs in both males and females; therefore, it provides the best means of assessing maturity in the present cohort. APHV was estimated for the preterm
individuals using Mirwald’s et al (2002) maturity offset equation and measured directly as part of the PBMAS\(^5\). The coefficient of determination ($R^2$) for the estimation is 0.92 for males and 0.91 for females.

*Physical activity and dietary questionnaires:* The Physical Activity Questionnaire for Adolescents (PAQ-A) was developed for the PBMAS to assess general levels of physical activity during the school year for students beyond grade three. It is a self administered seven-day recall questionnaire, which asks students to recall their physical activity for the last seven days. The PAQ-A was designed for large sample studies and can be completed in approximately 10 to 15 minutes. Activity is scored on a five point scale with 1 representing low activity and 5 representing high activity. The PAQ-A has been shown to be reliable and valid in children and adolescents\(^{19-21}\). Calcium and vitamin D intake were assessed through the use of two 24-hour recall questionnaires, administered approximately 6 months apart to capture seasonal intake fluctuations. Dietary data were analyzed using the Food Processor and Nutritional Software version 8.5 (ESHA research software, Salem, Oregon).

*Dual X-ray Absorptiometry:* Body composition measurements were performed using a Hologic dual energy X-ray absorptiometry (DXA) 2000 or 4500 scanner. Previously developed conversion factors were applied to scans so that all outcomes reflected the Hologic 2000 equivalent values\(^{22}\). Three different scans were performed; whole body, lumbar spine, and proximal femur (hip). Bone mineral content (BMC, grams), lean mass and fat mass were derived from the scans. All scans were administered and analyzed by a certified radiology technologist. Quality control phantom scans were performed daily. The coefficients of variation (CV\%) for these measures from our laboratory, based on duplicate measures in young healthy female university students (20-30 yrs), were 0.5\% for whole body BMC, 0.7\% for lumbar spine BMC
and 1.0% for the proximal femur BMC. Fat and lean tissue mass were assessed from the whole-body scans and our laboratory has determined coefficients of variation for these measures to be 3.0% and 0.5%, respectively.

**Statistics:** All variables are presented as means ± standard deviation. Group differences in age, height, weight, APHV, lean mass, fat mass, physical activity, diet and bone measures were assessed using ANOVA. Analysis of covariance (ANCOVA) were used to assess absolute bone mineral content differences between groups while controlling for age, height, weight, adolescent maturity (APHV), physical activity, calcium and vitamin D, which have all been found to influence bone development. Total body, lumbar spine and proximal femur BMC Z-score were calculated using sex, age, size and ethnicity specific pediatric reference standards\(^{22}\). All analyses were performed using SPSS version 18.0. Alpha was set as \(p<0.05\).

**Results:**

Adolescent anthropometric, body composition and lifestyle characteristics for male and female preterm low birth weight and control groups are presented in Table 1. As expected both male and female controls had a significantly greater birth weight than the preterm low birth weight individuals \((p<0.05)\). Controls were also heavier and consumed more vitamin D in adolescence than male and female preterm individuals \((p<0.05)\). Female preterm low birth weight individuals had less fat mass and a lower percent body fat compared to the control group \((p<0.05)\). Male preterm low birth weight individuals were significantly shorter and had a significantly older APHV compared to controls \((p<0.05)\), suggesting that the preterm individuals may be later maturers. However, their APHV is within the normal range.
Unadjusted bone parameters for preterm low birth weight individuals and controls are presented in Table 2. Male preterm low birth weight individuals had significantly less BMC at the proximal femur in adolescence compared to controls \( (p<0.05) \). However, once adjusted for age, maturity (APHV), height, weight, physical activity and diet there were no differences between groups \( (p<0.05) \) (Figures 1-2). Z-scores for total body, lumbar spine and proximal femur BMC are presented in Table 3. Z-scores ranged from -4.90-4.22 for total body BMC, -4.69-2.83 at the lumbar spine and -4.62-2.82 at the proximal femur in males. Female Z-scores ranged from -2.25-4.33 for total body BMC, -2.2-2.91 at the lumbar spine and -1.67-2.24 at the proximal femur.

**Discussion:**

The aim was to investigate the effect of preterm low birth weight on bone mineral content in adolescence. The main finding was that male preterm low birth weight infants had decreased absolute proximal femur bone mineral content in adolescence compared to controls. However, when age, maturity, height, weight, physical activity and diet were considered there were no differences in BMC between the groups at any site; suggesting that preterm birth had no influence on bone accrual in these adolescents.

Although osteoporosis is often thought of as a disease of the elderly, intrauterine programming has been suggested to contribute to the risk of osteoporosis later in life\(^{10}\). Population studies suggest that a lower birth weight significantly predicts future low BMC, BMD and estimated bone strength, independent of adult body weight and height, and should be considered a risk factor for osteoporosis\(^{3,8,9}\). Since individuals with birth weights within the lower range of normal have been found to have compromised bone we hypothesized that preterm
low birth weight infants would have lower BMC than their full term peers. Congruent with these findings we found that preterm low birth weight male adolescents had lower BMC at the hip than term born controls; however, once differences in body size, maturity, physical activity and diet were adjusted for there were no differences in bone parameters between groups. In contrast to previous findings in the adult general population once differences in adolescent body size were considered we found no influence of preterm low birth weight on bone mineral content in this group. Weiler et al (2002) also found absolute total body, lumbar spine, and total hip BMC was reduced in a group preterm young adults. However, once adult weight, height or lean mass were considered all differences in BMC between birth weight cohorts disappeared, suggesting that preterm adolescents have an appropriate amount of BMC for their body size.

The lack of a difference between low birth weight individuals and controls in the current and Weiler et al cohorts compared to the findings in the large population studies may be related to advances in general medical and neonatal practices. The individuals in population studies\(^3\) were born early in the 20\(^{th}\) century between 1931-1939 whereas the individuals in the Weiler et al (2002) study were born between 1978-1982 and the current cohort between 1989-1995. It could be argued that there have been many advances in neonatal care and feeding regimes in the preceding 40 years between studies which may help explain the differences in findings. Perhaps the preterm low birth weight individuals in the late twentieth century were more closely monitored to ensure adequate growth compared to individuals born within the lower range of normal in the 1930’s. This suggests that neonatologists and clinicians caring for preterm infants should feel encouraged that the strategies adopted in those preceding years may be assisting to negate the previously suggested negative impact of preterm birth. Conversely, it may also be possible that differences in bone values between preterm and term born individuals do not
become apparent until older adulthood. The large population cohort studies that have found differences in bone parameters independent of body size have been in older adults in their 6th and 7th decades of life. It may be that disparities in BMC between preterm low birth weight individuals and full term peers are most evident at birth and in later adulthood when bone loss accelerates, the process of accelerated bone mineral accrual during adolescence may be masking the effect of being born preterm low birth weight.

Although there were no differences in bone parameters between preterm low birth weight adolescents and full term controls in the present study, it is important to note that the preterm males had negative Z-Scores for lumbar spine and proximal femur BMC and females had a negative proximal femur Z-score. This finding suggests that while there were no differences between groups, the preterm adolescents may have lower BMC than average. Hovi et al. (2009) found young adults who were born with a very low birth weight had lower total body, lumbar spine and femoral neck BMD Z-Scores. The range of Z-Scores for the very low birth weight individuals in the Hovi et al. (2009) cohort were all negative. In contrast the Z-Scores for the present preterm infants range from -4.9 to +4.3 suggesting that while some preterm adolescents have lower BMC than average, others actually have higher than average BMC.

Along with body size and composition, environmental factors such as physical activity and diet have been found to independently impact bone development. Preterm infants consumed significantly less vitamin D than their normal birth weight peers in adolescence. This could potentially have a negative impact on bone development; however, despite this disparity there were no differences in the adjusted bone mineral content between groups. Physical activity during adolescence is known to positively impact skeletal health; with the most active children and adolescents accruing greater bone. There was no difference in physical activity between
the groups. It is possible that physical activity during adolescence is attenuating the effect of preterm low birth weight on bone mass. Early physical activity interventions in very low birth infants have been found to attenuate the decrease in bone strength often observed during the first few weeks of life in this population\textsuperscript{24}. Likewise physical activity in adolescence may help to attenuate the effect of preterm low birth weight on bone health and decrease the risk of low BMC.

The current study has some limitations. While DXA is considered the gold standard for measuring body composition it does not allow for the assessment of bone structure or the separate analysis of cortical and trabecular bone which may be influenced differently by preterm birth. Future studies should examine bone structure as well as bone mass through the use of imaging tools such as peripheral quantitative computed tomography which allows for the assessment of bone structure. We found that preterm adolescents had lower intakes of vitamin D; however, caution should be taken when interpreting dietary values as they were obtained from two 24 hour food recalls which may not be representative of normal intake. Nevertheless, the 24-hour recall has been found to be a suitable method to assess individual nutrient intakes of children and adolescents\textsuperscript{25} and we found good agreement between the two evaluations. We removed multiple births from the current; however, it may be that multiple birth low birth weight individuals are at an increased risk of low BMC. SGA individuals were also removed due to low participant numbers. Studies are required which examine the impact on bone mass of being a multiple low birth weight individual as well as being born SGA.

In summary we found that preterm birth and low birth weight were not associated with negative bone health in adolescence. Preterm individuals had lower absolute BMC; however, once adjusted for age, height, weight, physical activity and diet, there was no influence of
preterm birth and low birth weight on bone parameters. This suggests that adolescent individuals born preterm low birth weight have an appropriate amount of BMC for their size. However, prospective longitudinal studies are required to confirm the influence of preterm birth on attainment of adolescent and adult BMC and to determine if the risk of developing osteoporosis later in life is increased.

Acknowledgements
The authors wish to acknowledge the Royal University Hospital Foundation for providing funding for this project. PBMAS was in part funded by the Canadian Institute of Health Research (CIHR) and the Saskatchewan Health Research Foundation (SHRF). We wish to thank Stephanie Kendel for completing the nutritional analysis of the 24-hour dietary recalls. We would also like to thank all the participants and their families who gave generously of their time, without their support this study would not have been possible.
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Figure Captions

Figure 1 – Total body BMC (± SE) for preterm low birth weight individuals and controls. BMC was adjusted for age, height, weight, physical activity and diet.

Figure 2 - Lumbar Spine and Proximal Femur BMC (± SE) in preterm low birth weight and control individuals. BMC was adjusted to account of possible differences in age, height, weight, physical activity and diet.
Tables:

Table 1 – Anthropometric, body composition and lifestyle data for adolescent male and female preterm low birth weight and control individuals (mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>♀ Preterm (n=16)</th>
<th>♂ Preterm (n=25)</th>
<th>♀ Controls (n=56)</th>
<th>♂ Controls (n=71)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth Weight (g)</td>
<td>1278.2±378.7</td>
<td>1411.2±323.5</td>
<td>3306.6±582.2*</td>
<td>3682.7±671.5*</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>14.9±1.8</td>
<td>15.0±1.8</td>
<td>15.0±1.7</td>
<td>15.1±1.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.9±11.5</td>
<td>165.0±11.6</td>
<td>163.4±8.1</td>
<td>173.4±9.8*</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>44.3±19.0</td>
<td>56.1±21.9</td>
<td>58.7±13.0*</td>
<td>66.5±14.3*</td>
</tr>
<tr>
<td>Lean Mass (kg)</td>
<td>37.0±9.1</td>
<td>45.2±12.3</td>
<td>37.2±5.9</td>
<td>47.4±10.7</td>
</tr>
<tr>
<td>Fat Mass (g)</td>
<td>13.4±5.9</td>
<td>13.0±8.4</td>
<td>18.6±9.0*</td>
<td>12.3±7.2</td>
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<tr>
<td>% BF</td>
<td>24.9±6.0</td>
<td>20.5±8.2</td>
<td>30.9±8.5*</td>
<td>19.3±8.7</td>
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<tr>
<td>APHV</td>
<td>12.8±0.7</td>
<td>14.2±0.5</td>
<td>12.5±0.8</td>
<td>13.6±0.6*</td>
</tr>
<tr>
<td>PA Score</td>
<td>2.6±0.6</td>
<td>2.5±0.7</td>
<td>2.5±0.7</td>
<td>2.7±0.7</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>921.4±442.9</td>
<td>1390.9±718.2</td>
<td>1058.5±483.2</td>
<td>1356.4±666.0</td>
</tr>
<tr>
<td>Vitamin D (IU)</td>
<td>145.9±155.5</td>
<td>255.6±176.8</td>
<td>260.3±199.6*</td>
<td>364.2±235.1*</td>
</tr>
</tbody>
</table>

♀ = female, ♂ = male, %BF = percent body fat, APHV = age at peak height velocity, PA = physical activity

* control group significant different than preterm low birth weight
Table 2 – Absolute bone parameters for preterm low birth weight and control individuals (means±SD)

<table>
<thead>
<tr>
<th></th>
<th>♀ Preterm</th>
<th>♂ Preterm</th>
<th>♀ Control</th>
<th>♂ Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB BMC (g)</td>
<td>1960.5±622.5</td>
<td>1786.7±461.8</td>
<td>2195.1±620.6</td>
<td>1918.2±414.9</td>
</tr>
<tr>
<td>LS BMC (g)</td>
<td>50.5±15.9</td>
<td>49.2±18.1</td>
<td>49.7±15.1</td>
<td>49.3±18.1</td>
</tr>
<tr>
<td>PF BMC (g)</td>
<td>26.9±7.9</td>
<td>31.5±11.1</td>
<td>27.9±5.7</td>
<td>38.5±10.4*</td>
</tr>
</tbody>
</table>

♀=female, ♂=male, TB BMC=total body bone mineral content, LS BMC=lumbar spine bone mineral content, PF BMC=proximal femur bone mineral content

* control group significant different than preterm low birth weight

Table 3 – Bone mineral content Z-scores (mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>♀ Preterm</th>
<th>♂ Preterm</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB BMC Z-score</td>
<td>0.67±2.0</td>
<td>0.12±2.2</td>
</tr>
<tr>
<td>LS BMC Z-score</td>
<td>0.29±1.0</td>
<td>-0.92±1.8</td>
</tr>
<tr>
<td>PF BMC Z-score</td>
<td>-0.38±1.0</td>
<td>-0.26±1.5</td>
</tr>
</tbody>
</table>

♀=female, ♂=male, TB BMC=total body bone mineral content, LS BMC=lumbar spine bone mineral content, PF BMC=proximal femur bone mineral content