**Placental size is associated differentially with postnatal bone size and density**

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**Abstract**

We investigated relationships between placental size and offspring adolescent bone indices using a population-based mother-offspring cohort. The Avon Longitudinal Study of Parents and Children (ALSPAC) recruited pregnant women from the South West of England between 1991 and 1993. 12,942 singleton babies were born at term and survived at least the first 12-months and from these, 8933 placentas were preserved in formaldehyde, with maternal permission for their use in research studies. At approximately 15.5 years the children underwent a DXA [Dual energy X-ray Absorptiometry) scan (measurements taken of whole body minus head bone area (BA), bone mineral content (BMC), and areal bone mineral density (aBMD)]. A pQCT (peripheral quantitative computed tomography) scan (Stratec XCT2000L) at the 50% tibial site was performed at this visit and at approximately 17.7 years. In 2010 a sample of 1,680 placentas were measured and photographed. To enable comparison of effect size across different variables, predictor and outcome variables were standardised to z-scores and therefore results may be interpreted as partial correlation coefficients. Complete placental, DXA and pQCT data were available for 518 children at 15.5 years. After adjustment for sex, gestational age at birth and age at time of pQCT, placental area was positively associated with endosteal circumference [β (95% CI): 0.21 (0.13, 0.30), p<0.001], periosteal circumference [β (95% CI): 0.19 (0.10, 0.27), p<0.001] and cortical area [β (95%CI): 0.10 (0.01, 0.18), p=0.03], and was negatively associated with cortical density [β (95%CI): -0.11 (-0.20, -0.03), p=0.01] at age 15.5 years. Similar relationships were observed for placental volume, and after adjustment for additional maternal and offspring covariates. These results suggest that previously observed associations between placental size and offspring bone development persist into older childhood, even during puberty, and that placental size is differentially related to bone size and volumetric density.

**Keywords:** Osteoporosis; Epidemiology; Placenta; DXA; pQCT; ASLPAC**Introduction**

The size of the placenta reflects its ability to transfer nutrients ([1](#_ENREF_1)) to the developing fetus, and indices of placental morphology are subject to wide variations ([2](#_ENREF_2)). There is increasing evidence that attributes such as placental area and volume may predict the risk of common chronic non-communicable diseases (NCDs) in later life. For example, low placental weight at birth has been associated with increased risk of hypertension and coronary heart disease in adulthood ([3](#_ENREF_3),[4](#_ENREF_4)). Osteoporosis constitutes a further important NCD and is a major public health concern due its association with age-related fragility fractures. We have previously shown that birthweight is associated with bone mineral content (BMC) in adulthood, and that poor early postnatal growth predicts adverse proximal femoral morphology ([5](#_ENREF_5),[6](#_ENREF_6)) and increased risk of hip fracture risk in older age ([7](#_ENREF_7),[8](#_ENREF_8)). Although placental transfer of nutrition from mother to fetus is critical in the determination of birthweight ([9](#_ENREF_9)), there is a paucity of evidence relating to associations between placental morphology and offspring bone mass. Recently, using data from a large prospective mother-offspring cohort, The Southampton Women’s Survey, we observed that placental volume, measured by high-resolution ultrasound in mid-pregnancy, was positively associated with neonatal bone size and content measured by DXA ([10](#_ENREF_10)). It remains unclear however, whether these associations might persist into later childhood and whether placental size may have differential relationships with bone size and density. The aim of this study, therefore, was to investigate whether placental size is associated with indices of bone size, geometry and density in the offspring, assessed using pQCT during adolescence, in a UK population-based mother-offspring cohort.

**Methods**

*The Avon Longitudinal Study of Parents and Children (ALSPAC)*

ALSPAC is a large prospective birth cohort study, the aim of which is the investigation of genetic and environmental influences on childhood health and development. Details of ALSPAC have been published previously ([11](#_ENREF_11),[12](#_ENREF_12)), but in brief, all pregnant women living in the former county of Avon, UK with an expected delivery date between the 1st April 1991 and 31st December 1992 were eligible to take part, of whom 14,541 were recruited during early pregnancy (80-90% of the target population). Information from early pregnancy onwards was collected from a variety of sources including self-completed questionnaires, medical records and annual examination and assessment of the children at dedicated research clinics. The study website contains details of all the data that are available through a fully searchable data dictionary (<http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/>).

*Placental and birth measurements*

12,942 singleton infants were born at term (≥37 completed weeks). Premature infants were excluded as prematurity is known to effect skeletal development ([13](#_ENREF_13)). Length of gestation was estimated from the date of the mother’s last menstrual period. Birthweights were extracted from hospital records and birth length (crown to heel) measured using a Harpenden neonatometer (Holtain Ltd, Crymlych Wales) by ALSPAC staff who visited all study participants within a day after birth. At delivery, the placenta was collected and stored in 10% formaldehyde for later assessment. In 2010 a sample of 1,680 placentas, all from one maternity hospital and taken in the order in which they were stored, were removed from their containers, trimmed as per a standard protocol, and measured ([14](#_ENREF_14)). Direct measurements were made of placental thickness, volume and weight. Both sides of the placenta (maternal and fetal) were then photographed using a digital camera. Each photograph included a ruler to measure the length and breadth of the surface (Figure 1). Length was defined as the maximal diameter, and breadth was measured at 90 degrees to the midpoint of the length. To calculate area, the placenta was assumed to be elliptical in shape, and area was defined as the product of length and breadth, multiplied by π/4. Maximum thickness was measured using a calibrated needle and volume was estimated as the product of area and maximum thickness.

*Skeletal assessment at 9.9, 15.5 and 17.7 years*

Children were invited to attend the Focus@9 research clinic at age 9 years, in which whole body DXA assessment of bone mass by DXA (Lunar Prodigy, GE Corporation, Wisconsin, USA) was undertaken. At approximately 15.5 years of age, all children within the ALSPAC cohort were invited to attend a research clinic as part of a study investigating the effects of physical activity on cortical bone ([15](#_ENREF_15)). Height (to the nearest 0.1cm) and weight (to the nearest 50g) were measured using a Harpenden Stadiometer (Holtain Ltd, Crymlych Wales) and Tanita Body Fat Analyser (Tanita UK Ltd, Uxbridge, UK) respectively. Measurements were then made of whole body bone area (BA), bone mineral content (BMC) and areal bone mineral density (BMD) using a DXA scanner with specific paediatric software (Lunar Prodigy, GE Corporation, Wisconsin, USA). The children also underwent pQCT assessment of their mid (50% from the distal endplate) right tibia using a Stratec XCT2000L instrument (Stratec, Pforzheim, Germany). Cortical BMD (BMDC) and cortical BMC (BMCC) were obtained. Periosteal circumference (PC), endosteal circumference (EC) and cortical thickness (CT) were derived using a circular ring model. Cortical bone was defined using a threshold above 650 mg/cm3, as previously described ([15](#_ENREF_15)). The within subject coefficients of variation (CV) for pQCT measurements are displayed in parentheses: tibial length (4.04%), BMCC (2.71%), BMDC (1.29%), PC (1.58%), EC (4.03%). All scans were reviewed and those with artefact were excluded from analysis. Whole body DXA and tibial pQCT assessments were repeated in the cohort at approximately 17.7 years of age using identical methods.

*Pubertal assessment*

Questionnaires that addressed maturation were mailed to participants at age 13.5 years, permitting ranking of participants according to age of pubertal onset. The puberty questionnaire, known to participants as the Growing and Changing Questionnaire, could be answered by the child, either parent, a guardian, or any combination of these individuals; the participants recorded who completed the questionnaire. The respondent was asked to examine line drawings representing the five Tanner stages for pubic hair and to record which drawing most closely represented the child’s current stage of development.

Ethical approval was obtained from the ALSPAC Law and Ethics committee, and the Local Research Ethics Committees. Parental written informed consent and child's assent were obtained for all measurements made.

*Statistical analysis*

All variables were checked for normality. Sex differences between baseline characteristics were compared using unpaired t-tests and chi-squared tests. Pubertal stage information was missing for

 42.6% of individuals and in these cases data were imputed: Individuals who did not have pubertal stage information were assigned a value of 4.5, which was close to the mean value (4.46) and stands midway between the two most commonly observed tanner stages- 4 and 5. A sensitivity analysis was undertaken using the complete case data. Univariate and multivariate linear regression were used to relate placental measurements to offspring DXA and pQCT measurements. Although not necessarily true confounders, gestational age at birth is strongly associated with placental size, and offspring age and sex are strongly associated with the bone outcomes: We therefore included these as covariates in all models in order to increase the precision of our estimates. We hypothesised that maternal factors, which have previously been associated with offspring bone mass and body composition, might act through placental size and thus incorporated these into a second model. Puberty has a marked impact on bone development and in a third model we included the child’s pubertal stage at 13.5 years to investigate whether relationships might be mediated via altered timing of pubertal transition. Finally, in order to assess the contribution of the child’s current body size, in a further model we additionally included child’s height and weight at the relevant assessment. The covariates included in the 4 models are summarised below:

Model 1: Child’s gestational age at delivery, age at pQCT and sex

Model 2: As model 1 and maternal age at delivery, height, weight and parity

Model 3: As model 2 and child’s pubertal stage at 13.5 years

Model 4: As model 3 and child’s height and weight at 15.5 years

In order to assess the contribution of placental size to change in bone outcomes, we used a conditional multiple regression analysis, in which the 4 models were used as before, but for each pQCT or DXA measure, we additionally included the corresponding measure at the previous time point. This effectively yields the association between placental size and the bone measure at a later time point, over and above that explained by the association between placental size and the bone measure at the earlier time point. We undertook separate analyses to assess whether associations might be mediated via birthweight and by including birthweight in model 1. Placental measurements and sex interactions were examined, however these provided little evidence of sex differences and we therefore analysed boys and girls together. In line with convention, DXA-derived whole body bone variables were analysed minus head. To enable comparison of effect sizes across relationships, all predictor and outcome variables were standardised to z-scores with a mean of 0 and an SD of 1. Regression coefficients are therefore representative of SD change in outcome per unit SD change in predictor, and may be interpreted as partial correlation coefficients. Analysis was performed using SPSS Version 21 (IBM UK Ltd, Portsmouth, UK).

**Results**

*Baseline characteristics*

5515 children underwent assessment at the 15.5 year assessment. Of these, 518 (10%; 230 boys and 288 girls) had complete placental, DXA and pQCT measurements. Table 1 shows the offspring, placental and maternal characteristics. Offspring DXA indices at 9.9, 15.5 and 17.7 years are shown in Online Supplementary Table 1, and participant flow through the study is documented in Online Supplementary Figure 1. Mean (SD) age for boys and girls was 15.3 (0.2) and 15.4 (0.2) years respectively. Mean (SD) maternal age at delivery was 29.3 (4.4) years; 50.8% of women were primiparous. At birth, boys were heavier and longer than girls, however by 9.9 years of age there was no difference in height, weight nor any of the DXA variables between the sexes. At age 15.5 years boys were taller and heavier, and had higher whole body (less head) BA, BMC and BMD (all p<0.001) than the girls. Similarly boys had higher cortical area, cortical thickness, cortical content, periosteal circumference and endosteal circumference at the tibial 50% site (p all <0.001); conversely boys had lower cortical density than girls (p<0.001). Placental measurements did not differ by offspring sex, but girls were on average at a greater stage of puberty than boys when assessed at 13.5 years.

*Placental size and offspring pQCT indices at age 15.5 years*

Table 2 summarises the relationships observed between placental measurements and offspring bone mass. We observed strong positive relationships between placental area and child’s cortical area, periosteal circumference and endosteal circumference at age 15.5 years, which remained robust after adjusting for gestational age, age at pQCT and sex (all p<0.05). Conversely there was a negative association between placental area and cortical BMD [β= -0.11 (95%CI: -0.20, -0.03); p=0.01]. These relationships remained, but were attenuated, after additional adjustments for maternal age at delivery, maternal parity, height and weight, and also after inclusion of child’s pubertal stage at 13.5 years, (except for placental area and cortical area, p=0.06; Table 2, Figure 2). Placental area, adjusted for gestational age, sex and age at pQCT, was weakly correlated with height at 15.5 years (rpartial=0.07, p=0.048), as was placental volume (rpartial: 0.1, p=0.03), but adjustment for child’s height and weight at 15.5 years did not materially alter the associations observed. Inclusion of birthweight modestly attenuated the beta coefficients, but the overall pattern of associations remained similar (Online Supplementary Table 2).

The strongest observed relationships were between placental area and measurements of endosteal and periosteal circumference [EC:β= 0.21 (95%CI: 0.13, 0.30); PC: β= 0.19 (95%CI: 0.10, 0.27), both p<0.001]. Similar relationships were observed between placental volume and child pQCT measurements. There was no association between placental size and cortical thickness; a weak association was observed between placental volume and cortical content, however this relationship was no longer present after maternal and pubertal covariates were incorporated into the regression model. When examined separately by offspring sex, relationships appeared similar in boys and girls, with the p-value for the interaction placental size\*sex on pQCT outcomes >0.05. In a sensitivity analysis using the complete case data, results were not materially different from those using imputed values where pubertal status was missing.

Online Supplementary Table 3 demonstrates that mean placental area and volume did not differ by pubertal stage at 13.5 years. Online supplementary Table 4 similarly summarises the mean pQCT indices (represented as SD scores) by pubertal status at 13.5 years. Here, there was a trend for greater cortical area, thickness, content and density with later pubertal stage, both in boys and girls (p≤0.01).

*Placental size and offspring pQCT indices at age 17.7 years*

Table 3 summarises the relationships observed between placental measurements and offspring bone mass at age 17.7 years. Although the previously observed associations were attenuated, in the fully adjusted models including child height and weight, positive relationships remained between placental size (area and volume) and endosteal circumference and periosteal circumference. The negative association between placental volume and cortical density remained, and that between placental area and cortical density was attenuated (p=0.18). Relationships were similar in boys and girls. Again, inclusion of birthweight modestly attenuated the beta coefficients, but the overall pattern of associations remained similar (Online Supplementary Table 5). In a further analysis, in which pQCT indices at age 17.7 years were conditioned on those at 15.5 years, relationships were further attenuated (Online Supplementary Table 6).

*Placental size and offspring DXA measurements of bone mass*

At age 9.9 years, positive relationships were observed between each of placental area and volume, and offspring WB (minus head) BA and WB (minus head) BMC (Table 4), similar in boys and girls. No associations between placental measures and child WB (minus head) BMD were seen. At 15.5 years, there were similar positive associations between placental area or volume and DXA BA, but those with BMC were partially attenuated (p=0.07 and 0.09 respectively). At 17.7 years, the associations were attenuated further with only that between placental area and BA remaining statistically significant (Table 4), albeit with a beta coefficient of similar magnitude to that at earlier timepoints. Associations remained similar in models 2 and 3, but inclusion of child’s height and weight in model 4 removed all relationships. Indeed both placental area and placental volume, adjusted for gestational age at birth, sex and age at the 9 year visit, were weakly correlated with height at 9.9 years (rpartial for placental area: 0.08, p=0.02; rpartial placental volume: 0.13, p=0.0001). Inclusion of birthweight in the regression analysis also attenuated associations with bone area to below statistical significance (Online Supplementary Table 7). Finally, in conditional analyses in which DXA indices at 17.7 years were controlled for those at 15.5 years (Online Supplementary Table 8), and those at 15.5 years were controlled for those that 9.9 years (Online Supplementary Table 9), in a similar pattern to those with pQCT, all associations were attenuated.

**Discussion**

To our knowledge, this is the first study to investigate relationships between placental size and offspring bone indices using pQCT. We found that our previously documented positive associations between placental size and neonatal bone mass by DXA persisted into later childhood but appeared attenuated across puberty. However, placental area and volume were positively associated with offspring cortical area, periosteal circumference and endosteal circumference at both age 15.5 and 17.7 years, even after adjustment for a range of covariates, including pubertal status and current body size. Conversely, placental size was negatively related to cortical density.

This was a large prospective cohort study, with detailed characterisation of mothers and children, using two validated methods of bone mineral assessment, however there are some limitations that should be considered when interpreting the results. Firstly, the placentas were not measured immediately after collection, but were stored for several years in formaldehyde. The effect of this on placental size and shape is uncertain, however as all placentas were stored identically, this is unlikely to have affected the relationship observed between placental size and offspring bone mass. Secondly, we have reported on a subset of the ALSPAC cohort, and thus there is the possibility that our participants may not be representative of the entire cohort, thereby reducing the generalizability of our findings. Again, as all analyses were within the present cohort, there is no reason to suppose that this would have influenced the associations observed. Thirdly, only mid-tibial pQCT scans were performed so it is not possible to examine the placental influences on trabecular bone. Finally, although prospective, this is an observational study and so causality in any observed associations cannot be definitively established.

These results complement our previous findings from another UK mother-offspring cohort, the Southampton Women’s Survey ([10](#_ENREF_10)). Here, placental volume at 19 weeks’ gestation, estimated from high-resolution ultrasound measurements, was positively associated with whole body BA, BMC and more weakly with BMD from DXA at birth. Similarly, in the current study we observed positive associations between placental area or volume and whole body DXA BA and BMC at 9 years, but much weaker associations with BMD. Interestingly, although the direction of associations was maintained, the magnitude of placenta-bone associations was much attenuated by the age of 17.7 years, suggesting that pubertal transition might modify these relationships. However, in the present study, we also assessed children at 15.5 and 17.7 years using pQCT, which allowed detailed measurements of bone indices without the effect of overall size that confounds DXA measures: Periosteal and endosteal circumference were positively associated with placental size, but we observed an inverse association between placental size and volumetric cortical BMD at the tibia, suggesting a disparity between influences on bone size and volumetric density, as has been observed with other aspects of intrauterine growth ([16](#_ENREF_16)).

The mechanisms which might underlie associations between placental size and offspring bone development are poorly characterised, but may comprise direct effects of the placenta on long-term postnatal growth trajectories, shared determinants of placental size and bone indices, or mediation through factors such as age at pubertal onset. There is scant evidence to inform the first two hypotheses, although we have previously demonstrated associations between patterns of intrauterine growth and postnatal skeletal development ([5](#_ENREF_5),[16](#_ENREF_16),[17](#_ENREF_17)); early growth, adult hip morphology ([6](#_ENREF_6),[18](#_ENREF_18)) and risk of hip fracture ([7](#_ENREF_7),[8](#_ENREF_8)); and positive relationships between expression of placental calcium transporters and offspring BMC at birth ([19](#_ENREF_19)).

In relation to the third hypothesis, there may be two components to a potential maturational explanation for our findings. These children were assessed towards the end of the pubertal period, during which substantial linear growth has occurred. The concept of “cortical consolidation” describes the way in which mineralisation may lag behind growth in bone size during modelling, with mineralisation and volumetric density catching up with skeletal size by the time of peak bone mass ([20](#_ENREF_20)). Indeed late puberty is a time of rapid bone remodelling, with increased cortical porosity and active periosteal apposition, both characteristics which would be consistent with our findings.

One hypothesis therefore, is that greater placental size leads to earlier onset of puberty, resulting in larger bones at 15.5 years, but with cortical density lagging behind proportionate to bone size (with larger bones at having lower cortical density compared with smaller bones). Indeed, such a mechanism was proposed in a recent study from ALSPAC cohort ([21](#_ENREF_21)), based on all children who underwent pQCT at 15.5 and 17.7 years, linking birthweight to bone outcomes. Here, relationships between birthweight and pQCT measures were somewhat attenuated by adjustment for puberty, and those with cortical density were not apparent at 17.7 years. We found that associations between placental size and pQCT measures at 15.5 years were not appreciably changed by adjustment for puberty; however relationships between placental size and pQCT measures at 17.7 years, whilst robust for periosteal and endosteal circumference, were much weaker for cortical density, consistent with a maturational etiology, and further supported by the conditional models, demonstrating that the strongest placental associations with the earlier time points of follow-up. Conversely, whilst increasing pubertal stage at 13.5 years was associated with larger bones by pQCT, there was no evidence of placental size having been greater in children who were at a later stage of puberty at 13.5 years. Additionally, increasing pubertal stage at 13.5 years was associated with increasing, rather than decreasing cortical density. It must be noted though that the 2 year interval between pubertal staging and pQCT measures somewhat limits the inferences that can be made. Furthermore, the correlation between birthweight and placental area was 0.4, suggesting much scope for relationships between placental size and outcomes independent of birthweight, consistent with previous documentation of the role of placental size versus function ([22](#_ENREF_22),[23](#_ENREF_23)). Inclusion of birthweight in the base model removed associations between placental size and DXA bone area, most likely due to the strong association between birthweight and overall size, thus potentially on the causal pathway. In contrast, associations between placental size and pQCT measures of periosteal and endosteal circumference, and cortical density, although attenuated, remained similar to those without the inclusion of birthweight, suggesting relationships over and above those mediated through size at birth. Consistent with these findings, although placental size was weakly correlated with height in childhood, and whilst the DXA associations were removed by addition of height in the models, those with the pQCT indices remained statistically significant, further supporting the notion that the placenta pQCT relationships were not purely mediated via linear growth.

Secondly, it is notable from pQCT studies that bone size, for example, periosteal circumference, tends to be inversely related to cortical density ([24](#_ENREF_24)). The bending strength of a bone is proportional to the fourth power of the radius ([25](#_ENREF_25)) and thus greater diameter bones require lower cortical density to achieve the same strength as narrower bones ([26](#_ENREF_26)). Since the skeleton adapts its structure to the prevailing loads imposed on it, and cortical density encompasses cortical porosity as well as tissue mineralisation, this then provides a second possible mechanism. Indeed, when both periosteal circumference and cortical density were regressed simultaneously on placental volume or area, the predominant association was with periosteal circumference, suggesting that the primary effect is on bone size, an observation which could support either of these two maturational hypotheses.

Both of these potential explanations would be compatible with the observed increased incidence of childhood fractures during the transition into puberty, where increase in bone size appears to outstrip mineralisation ([27](#_ENREF_27)); reassuringly however, the relative catch-up in mineralisation by young adulthood ([20](#_ENREF_20)), suggests that by the time peak bone mass has been achieved, a larger placenta is likely to be associated with greater adult bone strength.

In conclusion, we have demonstrated that previously observed associations between placental size and offspring bone size persist into late childhood, and that placental size is differentially related to bone size and volumetric density over the pubertal period. Whilst these results further our understanding of relationships between early development and postnatal skeletal growth, the magnitudes of association are modest by late adolescence and the elucidation of underlying mechanisms must remain a subject for further investigation.

**Conflict of interest**

All authors report no conflict of interest

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**Figure legends**

Figure 1: Image of the fetal side of a placenta and umbilical cord. Lines illustrate measurements of length (dark) and width (light)

Figure 2: Associations between placental characteristics and childhood pQCT measurements at 15.5 yrs. Data are adjusted for child’s gestational age at delivery, age at pQCT, sex and pubertal stage at 13.5 years; maternal age at delivery, maternal height, maternal weight, parity.

Table 1: Baseline characteristics of mothers, placentas and children

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Women**  |  |  |  |  |
|  | n | Mean (%) | (SD) |  |  |  |  |
| **Mothers** |  |  |  |  |  |  |  |
| Age (years) | 518 | 29.3 | (4.40) |  |  |  |  |
| Height (cm) | 504 | 164.9 | (6.6) |  |  |  |  |
| Weight (kg) | 492 | 61.5 | (9.9) |  |  |  |  |
| Body mass index (BMI; kg/m2) | 490 | 22.6 | (3.4) |  |  |  |  |
| Parity |  |  |  |  |  |  |  |
| Primiparous (Parity=0) | 257  | (50.8) |  |  |  |  |  |
| Multiparous (Parity≥1) | 249 | (49.2) |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
|  | **Boys** | **Girls** |  |
|  | n | Mean/ (%) | (SD) | n | Mean | (SD) | p-value |
| **Child** |  |  |  |  |  |  |  |
| Birth weight (g) | 228 | 3540.5 | 553.3 | 287 | 3414.4 | 446.1 | 0.004 |
| Age at 15.5 year visit (years)  | 230 | 15.3 | (0.2) | 288 | 15.4 | (0.3) | 0.2 |
| Height at 15.5 year visit (cm) | 230 | 175.0 | (8.2) | 288 | 165.0 | (5.9) | <0.001 |
| Weight at 15.5 year visit (kg)\* | 230 | 64.1 | (12.0) | 288 | 59.1 | (9.9) | <0.001 |
|  Gestational age at birth (weeks) | 230 | 39.6 | 1.6 | 288 | 39.7 | 1.4 | 0.4 |
|  Tanner stage at 13.5 years Stage 1 Stage 2 | 1732 | (11/8)(22.4) |  | 1121 | (5.2)(9.9) |  | p<0.001 |
|  Stage 3 | 42 | (29.4) |  | 50 | (23.6) |  |  |
|  Stage 4 | 41 | (28.7) |  | 87 | (41.0) |  |  |
|  Stage 5 | 11 | (7.7) |  | 43 | (20.3) |  |  |
| **Placental measurements** |  |  |  |  |  |  |  |
|  Area (cm2) | 230 | 286.1 | (59.2) | 288 | 284.8 | (53.2) | 0.8 |
|  Volume (cm3) | 230 | 793.8 | (192.9) | 288 | 797.1 | (176.5) | 0.8 |
|  No. of cotyledons/ cm3  | 195 | 1.7 | (0.6) | 266 | 1.8 | (0.7) | 0.04 |
| **Tibial pQCT scan at 15.5 years** |  |  |  |  |  |  |  |
|  Cortical area (cm2) | 230 | 331.2 | (47.7) | 288 | 276.5 | (35.8) | <0.001 |
|  Cortical BMD (mg/cm2) | 230 | 1076.2 | (36.3) | 288 | 1126.2 | (24.5) | <0.001 |
|  Cortical thickness (mm) | 230 | 5.7 | (0.7) | 288 | 5.3 | (0.6) | <0.001 |
|  Cortical content (mg) | 230 | 356.8 | (54.6) | 288 | 311.4 | (40.4) | <0.001 |
|  Periosteal circumference (mm) | 230 | 76.0 | (5.2) | 288 | 69.2 | (4.4) | <0.001 |
|  Endosteal circumference (mm) | 230 | 40.2 | (5.0) | 288 | 36.2 | (4.8) | <0.001 |
|  |  |  |  |  |  |  |  |
| **Tibial pQCT scan at 17.7 years** |  |  |  |  |  |  |  |
|  Cortical area (cm2) | 228 | 336.6 | (51.5) | 311 | 270.5 | (34.6) | <0.001 |
|  Cortical BMD (mg/cm2) | 228 | 1105.6 | (36.6) | 311 | 1133.7 | (24.6) | <0.001 |
|  Cortical thickness (mm) | 228 | 5.9 | (0.7) | 311 | 5.2 | (0.5) | <0.001 |
|  Cortical content (mg) | 228 | 372.5 | (59.0) | 311 | 306.7 | (39.7) | <0.001 |
|  Periosteal circumference (mm) | 228 | 77.6 | (5.2) | 311 | 68.9 | (4.5) | <0.001 |
|  Endosteal circumference (mm) | 228 | 40.8 | (5.1) | 311 | 36.0 | (4.6) | <0.001 |

Table 2: Associations between placental characteristics and childhood pQCT measurements at 15.5 yrs



|  |  |
| --- | --- |
| pQCT at 15.5 years (n=518) |  Placental measurement |
| **Area (SD)** | **Volume (SD)** |
|  | B1 (95% CI) | p | B2(95% CI) | p | B3(95% CI) | p | B4 | p | B1(95% CI) | p | B2 (95% CI) | p | B3(95% CI) | p | B4 | p |
| Cortical area (SD) | 0.10(0.01, 0.18) | 0.03 | 0.08 (-0.01, 0.17) | 0.07 | 0.08(-0.01, 0.17) | 0.06 | 0.01(-0.06, 0.08) | 0.79 | 0.14(0.05, 0.23) | 0.003 | 0.09(-0.02, 0.19) | 0.06 | 0.10(0.01, 0.19) | 0.04 | 0.06(-0.02, 0.13) | 0.12 |
| Cortical BMD (SD) | -0.11(-0.20, -0.03) | 0.01 | -0.14 (-0.22, -0.05) | 0.003 | -0.13(-0.22, -0.05) | 0.002 | -0.16(-0.24, -0.07) | <0.001 | -0.09(-0.18, 0.004) | 0.06 | -0.10 (-0.20, -0.01) | 0.04 | -0.09(-0.18, 0.003) | 0.06 | -0.10(-0.19, -0.01) | 0.03 |
| Cortical thickness (SD) | -0.04(-0.13, 0.04) | 0.36 | -0.07 (-0.16, 0.02) | 0.14 | -0.07(-0.15, 0.02) | 0.14 | -0.11(-0.19, -0.03) | 0.01 | -0.01(-0.10, 0.09) | 0.90 | -0.04 (-0.13, 0.06) | 0.46 | -0.03(-0.12, 0.07) | 0.55 | -0.05(-0.14, 0.03) | 0.22 |
| Cortical content (SD) | 0.07(-0.02, 0.15) | 0.13 | 0.05 (-0.04, 0.13) | 0.30 | 0.05(-0.04, 0.13) | 0.27 | -0.03(-0.09, 0.04) | 0.43 | 0.11(0.02, 0.20) | 0.02 | 0.06 (-0.03, 0.16) | 0.18 | 0.07(-0.02, 0.16) | 0.12 | 0.03(-0.04, 0.10) | 0.39 |
| Periosteal circum (SD) | 0.19(0.10, 0.27) | <0.001 | 0.18 (0.10, 0.27) | <0.001 | 0.18(0.10, 0.27) | <0.001 | 0.11(0.04, 0.18) | 0.002 | 0.22(0.13, 0.31) | <0.001 | 0.17(0.08, 0.27) | <0.001 | 0.18(0.08, 0.27) | <0.001 | 0.13(0.06, 0.21) | <0.001 |
| Endosteal circum (SD) | 0.21(0.13, 0.30) | <0.001 | 0.24 (0.15, 0.32) | <0.001 | 0.24(0.15, 0.32) | <0.001 | 0.20(0.12, 0.29) | <0.001 | 0.21(0.12, 0.30) | <0.001 | 0.19(0.10, 0.29) | <0.001 | 0.19(0.10, 0.28) | <0.001 | 0.17(0.08, 0.26) | <0.001 |

1Model 1: Adjusted for child’s gestational at delivery, age at pQCT and sex; 2Model 2: As model 1 and maternal age at delivery, height, weight and parity;

3Model 3: As model 2 and child’s pubertal stage at 13.5 years; 4Model 4: As model 3 and child’s height and weight at 15.5 yearsTable 3: Associations between placental characteristics and childhood pQCT measurements at 17.7 yrs

|  |  |
| --- | --- |
| pQCT at 17.7 years (n=539) | Placental measurement |
| **Area (SD)** | **Volume (SD)** |
|  | B1 (95% CI) | p | B2(95% CI) | p | B3(95% CI) | p | B4 | p | B1(95% CI) | p | B2 (95% CI) | p | B3(95% CI) | p | B4 | p |
| Cortical area (SD) | 0.05(-0.04, 0.13) | 0.27 | 0.05(-0.04, 0.13) | 0.30 | 0.05(-0.04, 0.13) | 0.30 | -0.01(-0.09, 0.07) | 0.82 | 0.07(-0.02, 0.16) | 0.11 | 0.05(-0.04, 0.14) | 0.27 | 0.05(-0.04, 0.14) | 0.26 | -0.003(-0.08, 0.08) | 0.94 |
| Cortical BMD (SD) | -0.06(-0.14, 0.03) | 0.18 | -0.06(-0.15, 0.03) | 0.18 | -0.06(-0.14, 0.03) | 0.17 | -0.05(-0.13, 0.04) | 0.26 | -0.10(-0.19, -0.02) | 0.02 | -0.09(-0.18, 0.003) | 0.06 | -0.08(-0.17, 0.01) | 0.08 | -0.07(-0.16, 0.02) | 0.14 |
| Cortical thickness (SD) | -0.04(-0.12, 0.05) | 0.39 | -0.05(-0.14, 0.04) | 0.27 | -0.05(-0.14, 0.04) | 0.27 | -0.09(-0.17,-0.01) | 0.04 | 0.003(-0.08, 0.09) | 0.94 | -0.02(0.11, 0.07) | 0.69 | -0.02(-0.11, 0.08) | 0.75 | -0.05(-0.14, 0.03) | 0.22 |
| Cortical content (SD) | 0.04(-0.05, 0.12) | 0.37 | 0.04(-0.05, 0.12) | 0.43 | 0.04(-0.05, 0.12) | 0.43 | -0.02(-0.10, 0.06) | 0.66 | 0.05(-0.03, 0.14) | 0.23 | 0.04(-0.06, 0.13) | 0.44 | 0.04(-0.05, 0.13) | 0.41 | -0.02(-0.10, 0.07) | 0.72 |
| Periosteal circum (SD) | 0.14(0.05, 0.22) | 0.002 | 0.15(0.06, 0.23) | <0.001 | 0.14(0.06, 0.23) | <0.001 | 0.08(0.01, 0.15) | 0.02 | 0.15(0.07, 0.24) | <0.001 | 0.13(0.04, 0.22) | 0.004 | 0.13(0.04, 0.22) | 0.004 | 0.07(-0.01, 0.14) | 0.08 |
| Endosteal circum (SD) | 0.17(0.08, 0.25) | <0.001 | 0.19(0.10, 0.27) | <0.001 | 0.19(0.10, 0.27) | <0.001 | 0.15(0.07, 0.24) | <0.001 | 0.15(0.06, 0.23) | 0.001 | 0.14(0.05, 0.23) | 0.003 | 0.13(0.04, 0.23) | 0.004 | 0.10(0.01, 0.19) | 0.03 |

1Model 1: Adjusted for child’s gestational age at delivery, age at pQCT and sex; 2Model 2: As model 1 and maternal age at delivery, height, weight and parity; 3Model 3: As model 2 and child’s pubertal stage at 13.5 years; 4Model 4: As model 3 and child’s height and weight at 17.7 years

Table 4: Associations between placental characteristics and childhood bone DXA measurements

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **9.9 years** | **15.5 years** |  | **17.7 yrs** |  |  |
| **Placental measure** | **WB BA (SD)** | **WB BMC (SD)** | **WB BMD (SD)** | **WB BA (SD)** | **WB BMC (SD)** | **WB BMD (SD)** | **WB BA****(SD)** | **WB BMC** **(SD)** | **WB BMD** **(SD)** |  |
| B (95%CI) | B (95%CI) | B (95%CI) | B (95%CI) | B (95%CI) | B (95%CI) | B (95%CI) | B (95%CI) | B (95%CI) |  |
| **Area (SD)** | 0.12\*\*(0.03, 0.2) | 0.10\*(0.01, 0.18) | 0.05(-0.04, 0.14) | 0.09\*(0.01, 0.18) | 0.07(-0.01, 0.16) | 0.03(-0.06, 0.11) | 0.10(0.02, 0.18)\* | 0.08(-0.001, 0.16) | 0.04(-0.04, 0.12) |  |
| **Volume (SD)** | 0.14\*\*(0.05, 0.23) | 0.12\*\*(0.03, 0.22) | 0.08(-0.01, 0.17) | 0.12\*\*(0.04, 0.21) | 0.09(-0.001, 0.18) | 0.02(-0.07, 0.11) | 0.04(-0.01, 0.08) | 0.02(-0.03, 0.07) | -0.02(-0.09, 0.05) |  |

WB = Whole body minus head; BA= Bone Area; BMC = Bone Mineral Content; BMD = Bone Mineral Density; All associations adjusted for child’s gestational age at delivery, age at DXA and sex; \*p<0.05; \*\*p<0.01; \*\*\*p<0.001

**References**

1. C.P. S 1994. In: Case RM, Waterhouse JM (eds.) Human Physiology: Age, Stress, and the Environment. Oxford University Press, Oxford, pp 3-27.

2. Hamilton WJ BJ, Mossman HW 1945 Human Embryology. W. Heffer & Sons.

3. Eriksson J, Forsen T, Tuomilehto J, Osmond C, Barker D 2000 Fetal and childhood growth and hypertension in adult life. Hypertension **36**(5)**:**790-794.

4. Forsen T, Eriksson JG, Tuomilehto J, Teramo K, Osmond C, Barker DJ 1997 Mother's weight in pregnancy and coronary heart disease in a cohort of Finnish men: follow up study. BMJ **315**(7112)**:**837-840.

5. Harvey NC, Cole ZA, Crozier SR, Ntani G, Mahon PA, Robinson SM, Inskip HM, Godfrey KM, Dennison EM, Cooper C 2013 Fetal and infant growth predict hip geometry at six years old: findings from the Southampton Women's Survey. Pediatr Res.

6. Javaid MK, Lekamwasam S, Clark J, Dennison EM, Syddall HE, Loveridge N, Reeve J, Beck TJ, Cooper C 2006 Infant growth influences proximal femoral geometry in adulthood. J Bone Miner Res **21**(4)**:**508-512.

7. Cooper C, Eriksson JG, Forsen T, Osmond C, Tuomilehto J, Barker DJ 2001 Maternal height, childhood growth and risk of hip fracture in later life: a longitudinal study. Osteoporos Int **12**(8)**:**623-629.

8. Javaid MK, Eriksson JG, Kajantie E, Forsen T, Osmond C, Barker DJ, Cooper C 2011 Growth in childhood predicts hip fracture risk in later life. Osteoporos Int **22**(1)**:**69-73.

9. Harvey N, Dennison E, Cooper C 2014 Osteoporosis: a lifecourse approach. J Bone Miner Res **29**(9)**:**1917-25.

10. Holroyd CR, Harvey NC, Crozier SR, Winder NR, Mahon PA, Ntani G, Godfrey KM, Inskip HM, Cooper C 2012 Placental size at 19 weeks predicts offspring bone mass at birth: findings from the Southampton Women's Survey. Placenta **33**(8)**:**623-629.

11. Boyd A, Golding J, Macleod J, Lawlor DA, Fraser A, Henderson J, Molloy L, Ness A, Ring S, Davey Smith G 2013 Cohort Profile: the 'children of the 90s'--the index offspring of the Avon Longitudinal Study of Parents and Children. Int J Epidemiol **42**(1)**:**111-27.

12. Fraser A, Macdonald-Wallis C, Tilling K, Boyd A, Golding J, Davey Smith G, Henderson J, Macleod J, Molloy L, Ness A, Ring S, Nelson SM, Lawlor DA 2013 Cohort Profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. Int J Epidemiol **42**(1)**:**97-110.

13. Longhi S, Mercolini F, Carloni L, Nguyen L, Fanolla A, Radetti G 2015 Prematurity and low birth weight lead to altered bone geometry, strength, and quality in children. J Endocrinol Invest **38**(5)**:**563-8.

14. Barker D, Osmond C, Grant S, Thornburg KL, Cooper C, Ring S, Davey-Smith G 2013 Maternal cotyledons at birth predict blood pressure in childhood. Placenta **34**(8)**:**672-5.

15. Sayers A, Tobias JH 2010 Fat mass exerts a greater effect on cortical bone mass in girls than boys. J Clin Endocrinol Metab **95**(2)**:**699-706.

16. Harvey NC, Mahon PA, Robinson SM, Nisbet CE, Javaid MK, Crozier SR, Inskip HM, Godfrey KM, Arden NK, Dennison EM, Cooper C 2010 Different indices of fetal growth predict bone size and volumetric density at 4 years of age. J.Bone Miner.Res. **25**(4)**:**920-927.

17. Harvey NC, Mahon PA, Kim M, Cole ZA, Robinson SM, Javaid K, Inskip HM, Godfrey KM, Dennison EM, Cooper C 2012 Intrauterine growth and postnatal skeletal development: findings from the Southampton Women's Survey. Paediatr.Perinat.Epidemiol. **26**(1)**:**34-44.

18. 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 2011 Self-reported weight at birth predicts measures of femoral size but not volumetric BMD in eldery men: MrOS. J Bone Miner Res **26**(8)**:**1802-7.

19. Martin R, Harvey NC, Crozier SR, Poole JR, Javaid MK, Dennison EM, Inskip HM, Hanson M, Godfrey KM, Cooper C, Lewis R 2007 Placental calcium transporter (PMCA3) gene expression predicts intrauterine bone mineral accrual. Bone **40**(5)**:**1203-1208.

20. Walsh JS, Paggiosi MA, Eastell R 2012 Cortical consolidation of the radius and tibia in young men and women. J Clin Endocrinol Metab **97**(9)**:**3342-8.

21. Steer CD, Sayers A, Kemp J, Fraser WD, Tobias JH 2014 Birth weight is positively related to bone size in adolescents but inversely related to cortical bone mineral density: findings from a large prospective cohort study. Bone **65:**77-82.

22. Hosking DJ 1996 Calcium homeostasis in pregnancy. Clin Endocrinol (Oxf) **45**(1)**:**1-6.

23. Barker DJP, Eriksson JG, Kajantie E, Alwasel SH, Fall CHD, Roseboom TJ, Osmond C 2011 The maternal and placental origins of chronic disease. In: Burton GJ, Barker DJP, Moffett A, Thornburg K (eds.) The Placenta And Human Developmental Programming, 1 ed. Cambridge University Press, Cambridge, pp 5-16.

24. Pandey N, Bhola S, Goldstone A, Chen F, Chrzanowski J, Terranova CJ, Ghillani R, Jepsen KJ 2009 Interindividual variation in functionally adapted trait sets is established during postnatal growth and predictable based on bone robustness. J Bone Miner Res **24**(12)**:**1969-80.

25. Ruff CB, Hayes WC 1988 Sex differences in age-related remodeling of the femur and tibia. J Orthop Res **6**(6)**:**886-96.

26. Seeman E 2008 Structural basis of growth-related gain and age-related loss of bone strength. Rheumatology (Oxford) **47 Suppl 4:**iv2-8.

27. Cooper C, Dennison EM, Leufkens HG, Bishop N, van Staa TP 2004 Epidemiology of childhood fractures in Britain: a study using the general practice research database. J Bone Miner Res **19**(12)**:**1976-81.