Published in final edited form as:

Placenta. 2020 July 22; 99: 101–107. doi:10.1016/j.placenta.2020.07.017.

Placental volume at 11 weeks is associated with offspring bone mass at birth and in later childhood: Findings from the Southampton Women's Survey

SJ Woolford^{*,a}, EM Curtis^{*,a}, S D'Angelo^{*,a}, P Mahon^a, L Cooke^c, JK Cleal^c, SR Crozier^a, KM Godfrey^{a,b}, HM Inskip^{a,b}, C Cooper^{a,b,d,+}, NC Harvey^{a,b,+}

^aMRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, UK

^bNIHR Southampton Nutrition Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton, UK

^cInstitute of Developmental Sciences, University of Southampton, Southampton, UK

^dNIHR Oxford Biomedical Research Centre, University of Oxford, Oxford, UK

Abstract

Objectives—To investigate associations between placental volume (PV) at 11 weeks gestation and offspring bone outcomes at birth, 6 years and 8 years.

Methods—3D ultrasound scanning was used to assess 11 week PV in a subset (n=236) of the Southampton Women's Survey (a prospective mother-offspring cohort). Maternal anthropometric measures and lifestyle information were obtained pre-pregnancy and at 11 weeks' gestation. Offspring dual-energy x-ray absorptiometry scanning was performed within 2 weeks postnatally and at 6 and 8 years. Linear regression was used to assess associations between PV and bone outcomes, adjusting for offspring age at DXA and sex, and maternal age, height, smoking status, walking speed and triceps skinfold thickness. β are SD change in bone outcome per SD change in PV.

Results—In adjusted models, 11 week PV was positively associated with bone area (BA) at all time points, with evidence of persisting associations with increasing childhood age [birth: n=80, $\beta=0.23$ (95% CI=0.03, 0.42), 6 years: n=110, $\beta=0.17$ (-0.01, 0.36), 8 years: n=85, $\beta=0.13$ (-0.09, 0.36)]. Similar associations between 11 week PV and bone mineral content (BMC) were observed. Associations with size-corrected bone mineral content were weaker at birth but strengthened in later childhood [birth: n=78, $\beta=0.07$ (-0.21, 0.35), 6 years: n=107, $\beta=0.13$ (-0.08, 0.34), 8 years: n=71, $\beta=0.19$ (-0.05, 0.43)].

Conclusions—11 week PV is associated with DXA bone measures at birth, with evidence of persisting associations into later childhood. Further work is required to elucidate the contributions of placental morphology and function to gestational influences on skeletal development.

Corresponding author Prof Nicholas Harvey, nch@mrc.soton.ac.uk, Full postal address: MRC Lifecourse Epidemiology Unit, Tremona Road, Southampton, SO16 6YD, UK.

^{*}Joint first author

⁺joint senior author

Keywords

osteoporosis; epidemiology; placenta; childhood; bone; DXA

Introduction

Placental morphology is variable and can greatly affect the efficiency of nutrient transfer to the developing fetus [1]. Furthermore, the role of the placenta within the developmental origins of health and disease (DOHaD) paradigm is well recognised, with placental size and function being associated with a number of adulthood non-communicable diseases, including hypertension [2], ischaemic heart disease [3] and diabetes [4]. Osteoporosis is another such chronic non-communicable disease and represents a significant public health burden due to its association with age-related fractures and the resulting morbidity and mortality [5,6]. Greater peak bone mass (PBM) can significantly delay the onset of osteoporosis [7], with adult PBM likely to be influenced by bone development in utero and during early childhood [8,9]. Therefore, much importance has been placed on elucidating the early determinants of bone mineral accrual within the human lifecourse.

There is growing evidence of associations between placental morphology and offspring bone health. We have previously shown in the Southampton Women's Survey (SWS) cohort that estimated placental volume (PV) in mid-pregnancy (at 19 weeks' gestation) is associated with neonatal bone size and mineral content [10]. We have also shown in the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort that placental size measured at birth is associated with offspring bone size and density at 9.9 years and in later adolescence [11]. However, it is not known whether relationships between placental size and offspring bone mass are evident when placental size is assessed in the first trimester. The Southampton Women's Survey (SWS) cohort offers an ideal opportunity to investigate this relationship as PV was measured in a subset of participants at 11 weeks' gestation using 3D ultrasound scanning (3DUS) methods (which is superior to 2D ultrasound PV estimates used in many older studies) and bone measures in the offspring were subsequently characterised in detail using dual-energy X-ray absorptiometry (DXA) scanning.

Therefore, the aim of this study was to investigate whether PV measured at 11 weeks gestation using 3DUS is associated with offspring bone composition at birth within the SWS, a longitudinal prospective cohort study of women and their children, and whether these relationships persist into later childhood.

Methods

Participants

The SWS is a prospective mother-offspring cohort study, investigating the effects of pre-pregnancy and pre-natal characteristics on offspring health. Details of the SWS have been described elsewhere [12]. In brief, between 1998 and 2002, 12,583 non-pregnant women aged 20-34 years were recruited via their GP from the general population of Southampton, England. Anthropometry and lifestyle assessments were carried out at entry into the study

and again in early (11 weeks' gestation) and late pregnancy (34 weeks' gestation) in those women who became pregnant. Maternal height was measured using a stadiometer (Seca, Birmingham, UK), weight with calibrated digital scales (Seca, Birmingham, UK) and skin fold thickness (biceps, triceps, subscapular and supra-iliac) with Holtain callipers (CMS Instruments, London, UK). Research nurses carrying out these measurements underwent training and regular re-assessment to ensure consistency. Information on parity, smoking habit and socioeconomic status was gathered via questionnaire. Women were also asked to characterise their walking speed into one of five groups (very slow, stroll at an easy pace, normal speed, fairly brisk or fast). A total of 3,158 singleton pregnancies were followed. Only singleton pregnancies and the first child born during the study time frame were included in the analysis. Child sex, gestational age, birthweight (measured using calibrated digital scales [Seca, Birmingham, UK]) and crown-heel length (measured using a neonatometer [CMS Ltd, UK]) were recorded at birth.

Prenatal 3D ultrasound

A subset of pregnancies underwent 3DUS at the 11 weeks' gestation follow-up appointment on an opportunistic basis as part of a feasibility trial for future 3DUS assessment. A high resolution 3DUS system (Kretz Voluson 730, Zipf, Austria) fitted with a 3D curvilinear multi-frequency transducer was used to collect PV data. In order to accommodate the exposure being derived from ultrasound, gestational age at 11 week 3DUS was calculated using an algorithm accounting for last menstrual period, cycle length and measurements taken from the 11 week 3DUS [13]. An experienced sonographer (PM) located the longest longitudinal section of the placenta on screen. From this location, a mechanical sweep within the transducer was activated to collect a 3D acquisition of the placenta in voxels. Using the 4D VIEW application (GE Medical Systems, Kretztechnik, Zipf, Austria) sequential parallel measurements of placental area in the longitudinal plane were acquired using the 3D multiplane function of the ultrasound system software. Each longitudinal section of placenta then had its border traced on-screen by the sonographer. This circumference excluded the placental vascular bed. The distance between the parallel slices was set to 5 mm increments at the periphery of the placenta and in 10mm increments towards the centre of the placenta. The software calculated the volume of tissue between slices using the area traced and the distance between the slices, to obtain the full PV in mm³. This measurement process was repeated three times and the mean volume was used in the final analysis. The coefficient of variation between the three PV measurements ranged between 0.1% and 13%, with an average of 1.2%. Any large or anteriorly sited placentae were omitted from the analysis if the entirety of the placental tissue was not captured in scans, as were any scans where increased maternal body habitus or bowel gas content obscured placental boundaries.

Neonatal DXA assessment

A random subset of mothers recruited to the SWS from specific GP practices were approached to participate in a bone assessment substudy. Specific GP practices were chosen in order to avoid participants being recruited into multiple substudies. This subset was representative of the population of Southampton as a whole. Neonates underwent DXA assessment within two weeks of birth. The child's crown-heel length and weight were

obtained from birth records, measured as described above. The child's age at the time of DXA assessment was also recorded. A Lunar DPX-L scanner (GE Corporation, Madison, WA, USA) with specific paediatric software (paediatric small scan mode v4.7c) was used. Neonates were completely undressed, pacified and fed if necessary and swaddled in a standard towel. The neonate was then placed on a waterproof mat in a standardised position for DXA assessment. Rice bags were placed at the bottom of the towel to minimise movement. Whole body scans were obtained, generating data on bone indices. Short-term and long-term coefficients of variation for this instrument for adult whole body BMD were 0.8% and 1.4% respectively. DXA scans were reviewed and those with excessive movement or clothing artefacts were omitted from the analysis.

6-year and 8-year DXA assessment

At 6 and 8 years of age, children were invited for a repeat DXA assessment. The child's height, using a Leicester height measurer (Seca, Birmingham, UK), and weight, using calibrated digital scales (Seca, Birmingham, UK) were measured. The child's age at the time of DXA assessment was also recorded. A Hologic Discovery scanner (Hologic Inc., Bedford, MA, USA) was used in paediatric scan mode. To encourage compliance children were shown an age-appropriate DVD and a bright sheet with appropriate pictures was laid on the couch. Whole body scans were obtained, generating data on bone indices. Coefficients of variation for this instrument for whole body BMD were 0.75%. DXA scans were reviewed and those with excessive movement or clothing artefacts were omitted from the analysis.

Statistical analysis

All baseline characteristics were checked for normality of distribution. Unpaired *t*-tests and Mann-Whitney *U* tests were used to compare normally distributed and non-normally distributed maternal baseline characteristics respectively against the larger SWS cohort. The same tests were used to compare offspring baseline characteristics by sex. Bone outcomes of interest were whole body bone area (BA), whole body bone mineral content (BMC) and whole body areal bone mineral density (aBMD). To allow for body size, whole body size-corrected bone mineral content (scBMC) was also assessed (i.e. BMC adjusted for BA and the offspring's length or height, and weight using linear regression). For 6- and 8-year DXA data outcomes were whole body measurement, excluding heads. All 11 week PVs were pre-adjusted for gestational age at 11 week 3DUS scan before further analysis using linear regression. Outcomes of interest at neonatal, 6-year and 8-year DXA assessment were initially related to 11 week PV using univariable linear regression analyses.

A series of multivariable linear regression analyses were then carried out to adjust for confounding variables. A directed acyclic graph (DAG) was first constructed using the DAGitty web application (www.dagitty.net) incorporating maternal and offspring factors informed by literature review and biological plausibility (see Supplementary Figure 1). A minimal set was generated by DAGitty using these covariates which included: maternal age, smoking status, triceps skinfold thickness, walking speed (all measured at 11 weeks' gestation), maternal height and parity (both measured at study recruitment) and offspring sex and gestational age at 11 week 3DUS scan. Offspring age at DXA scan was included as an

additional covariate to increase precision of the associations. Furthermore, gestational age at delivery was included as an additional covariate for neonatal data only, due to positive associations being evident in preliminary analyses between gestational age at delivery and bone outcomes at this time point, but not at later ages. A further model including birthweight in addition to the previous covariates was also used to investigate whether PV-bone associations might be explained in part by birthweight. Finally, in order to investigate potential relationships over time, we additionally performed a multilevel model looking at the effect of placental volume on scBMC over time, taking into account the within-subject correlation.

Analyses at each timepoint used all available participants with data for the dependent variable of interest. 11 week PV and outcome variables were transformed to normality using a Fisher-Yates transformation (z-scores with a mean of 0 and a SD of 1) [14]. Regression coefficients represent an SD change in the normalised outcome variable per unit SD change in the normalised 11 week PV. Stata V15.1 (StataCorp LP, College Station, TX, USA) was used for all analyses.

The SWS was approved by the Southampton and South West Hampshire Local Research Ethics Committee. Written consent was obtained from the parent or carer of all participants at each study stage.

Results

Characteristics of mothers and offspring

242 mothers underwent 3DUS at 11 weeks gestation. Of those, 236 mothers had scan images which captured the entirety of placental tissue, allowing calculation of placental volume, and forming the sample for this study. Figure 1 shows a flowchart, demonstrating how our sample was reached in relation to the wider SWS cohort. The mothers' baseline characteristics are summarised in Table 1A. The mean (SD) age of mothers at 11 weeks gestation was 30.7 (3.8). Their mean (SD) height was 164.2 (6.4) and their median (IQR) BMI pre-pregnancy was 23.6 kg/m² (21.7, 26.4). Compared with mothers of singleton livebirths from the larger SWS cohort, mothers in this sub-study were slightly older (30.7 years vs 29.9 years). Mothers in this sub-study also had a lower Index of Multiple Deprivation (IMD) 2004 (15.7 vs 17.7), indicating that they were less deprived than the cohort as a whole, and the proportion of nulliparous mothers was greater (59.3% vs 50.4%). Fewer mothers in this study smoked before pregnancy (25.4% versus 30.9%) and fewer mothers were smoking at 11 weeks gestation (12.6% versus 16.2%). No clear differences between this study sample and the larger SWS cohort were evident for maternal height, pre-pregnancy BMI, triceps skinfold thickness at 11 weeks' gestation or walking speed at 11 weeks gestation.

As demonstrated in Table 1B, when compared to the larger SWS cohort, offspring at birth in this sub-study were more likely to be older at DXA scanning (9 days vs 4 days) and have lower BA (107.6 cm² vs 117.7 cm²) and lower BMC (56.4 g vs 62.7 g). The full characteristics of the 83 offspring (45 boys) assessed at birth; 111 offspring (51 boys) assessed at 6 years; and 87 offspring (38 boys) at 8 years are presented in Online

Supplementary Tables 1A, 1B and 1C respectively. Their dates of birth ranged between September 2003 and January 2006, while the difference between recruitment and dating scan ranged between 9 months to almost 7 years.

When observing complications of pregnancy within our sample, only 2 mothers (0.9%) developed gestational diabetes, 7 (3.0%) had pre-eclampsia and 11 (4.7%) had a premature delivery (before the 37th week of pregnancy).

11 week placental volume and DXA bone outcomes at birth, 6 years and 8 years

Table 2 summarises the relationships between 11 week PV and DXA bone outcomes. 11 week PV was positively associated with all bone outcomes at all time points, with associations remaining similar after adjustment. At birth, the most strongly associated outcomes were BA ($\beta=0.23$ [95%CI=0.03, 0.42]) and BMC ($\beta=0.26$ [0.06, 0.46]). Similar associations were observed for aBMD ($\beta=0.21$ [-0.04, 0.47]). The magnitude of the associations for these three bone outcomes tended to decrease with increasing age (see Figure 2A, B and C). 11 week PV was only weakly associated with scBMC at birth ($\beta=0.07$ [-0.21, 0.35]). This association tended to strengthen, albeit with modest magnitude, with increasing age (see Figure 2D).

The correlation between 11 week PV and birthweight was 0.2. Adjusting for birthweight either led to attenuation or minimal material alteration of the relationships between 11 week PV and all bone outcomes at all time points, with the exception of scBMC whose associations were somewhat strengthened after adjustment (see Online Supplementary Table 2).

Multilevel model

The multilevel model examining the effect of placental volume on scBMC over time showed that time was not significant if it was either entered as a continuous variable ($\beta=-0.01$ [-0.04, 0.02]), or as a categorical variable when comparing 6 year and 8 year with birth (6 years vs birth: $\beta=-0.06$ [-0.31, 0.19], 8 years vs birth: $\beta=-0.07$ [-0.35, 0.20]).

Discussion

To our knowledge, this is the first study to examine the relationship between early pregnancy placental volume and offspring bone measures. We have shown that 11 week PV is positively associated with neonatal BA and BMC and that these associations are robust when adjusted for maternal factors previously associated with offspring bone mass. In addition, we observed that associations for BA and BMC at birth persist into later childhood at 6 years and 8 years, albeit with attenuated relationships with increasing age (see Figure 2A and 2B). In contrast, we found evidence that size-corrected BMC may be more strongly associated with 11 week PV in older childhood than at birth. Whilst the observed associations are relatively modest, regression coefficients for all bone outcomes at all time points (excluding aBMD at 8 years) were >0 , suggesting a persistent signal for the associations between early pregnancy placental volume and offspring bone metrics in early childhood.

This study used a well-characterised prospective mother-offspring cohort study, with “gold-standard” measures of placental volume and bone mass. However, several limitations should be considered in the interpretation of our results. Firstly, our sample was a subset of the larger SWS cohort, and both mothers and offspring differed in several ways from the overall study population, especially in regards to neonatal BA and BMC. Although this may limit the generalisability of our findings, all described observations are in relation to internal comparisons. Secondly, DXA scanning of neonates and children is subject to several limitations, due to their lower BMD when compared with adults and the propensity of younger participants to move during scanning. To allow for this, specific paediatric software was used at all three DXA scanning time points which minimises the loss of edge detection and images with excessive movement artefacts were excluded from the analysis. Furthermore, previous studies have demonstrated that DXA measurements of bone mass correlate well with whole body bone mineral content in small mammals such as piglets [15]. It should also be noted that intrauterine ultrasound measurements are prone to variation due to operator dependent factors. However, all measurements were carried out by a single experienced sonographer, using a standard protocol. Finally, placentas which were not captured in their entirety in 3DUS scans were omitted from the analysis and placental position (which can impact measurement of placental volume) was not recorded during 3DUS scanning, due to difficulties regarding commenting on placental position at this very early stage of pregnancy. If anything, this may have conservatively biased our results, with the associations between very large placentae and offspring bone outcomes not being represented.

The data we present here complement our previous findings in the SWS cohort [10]. In this earlier analysis, 19 week PV was positively associated with neonatal BA and BMC. Similarly, in the present study we have shown that 11 week PV is also associated with neonatal BA and BMC, but that the associations persist, albeit with reduced magnitude, in relation to outcomes at 6 and 8 years. We have also previously demonstrated in the ALSPAC cohort that postpartum PV is associated with several offspring bone metrics at 9.9 years of age, which also included BA and BMC [11]. We found similar positive associations in the present study between 11 week PV and BMC at a roughly comparable time point (8 years). The general pattern of a waning magnitude of association for DXA bone measures with increasing offspring age is consistent with the findings in the ALSPAC cohort, which showed attenuation from 9.9 years, though 15.5 years to 17.7 years. In contrast, in this earlier study we found evidence that associations with measures of bone from peripheral quantitative computed tomography (pQCT), such as tibial cortical density and periosteal circumference, persisted during adolescence from 15.5 years to 17.7 years, suggesting that placental size is likely to be relevant to aspects of later bone health.

We cannot derive specific mechanistic conclusions from the present analyses and as this was an observational study causality cannot be inferred from these data. Placental size has a well-documented relationship with birthweight, which is perhaps unsurprising as the size of the placenta partly reflects its ability to transfer nutrients to the fetus [16]. Several previous studies have documented associations between birthweight and placental volume measured in early pregnancy [17,18], in later pregnancy [19–22] and at delivery [23]. As bone contributes to birthweight, it is possible that our observed findings simply reflect a larger

placenta facilitating greater birthweight and corresponding greater skeletal size. Indeed, as with PV measured at 19 weeks' gestation in our previous SWS study, inclusion of birthweight in the present DXA models led to attenuation of the relationships with neonatal bone mass (other than for scBMC), suggesting this may be at least partly the case. However, this adjustment had no material effect on associations with DXA measures at 6 and 8 years, consistent with findings in the ALSPAC cohort [11], suggesting that, particularly in the longer term, associations are not fully explained by birthweight.

Whilst it is logical to assume the causal direction of placenta – birthweight – bone, the determinants of birthweight are complex. These include contributions from genotype, maternal pelvis size and the functional capacity per unit volume of the placenta itself. As a result, it may be that placental volume is in part dictated by an opposite sequence of causality, with the genetic and physiological potential for bone density and birthweight contributing to the demand for maternal-fetal nutrient transfer, and subsequently placental development [24,25]. Further possible mechanisms might involve the capacity of the placenta to transport calcium and amino acids to the skeleton of the developing fetus. Indeed we have previously demonstrated links between activity of placental calcium [26–28] and amino acid transporters [29–31] and offspring bone mass and body composition. In this regard, a larger placenta is simply one dimension of potential variation, with transporter density and activity also contributing to foetal growth. Therefore, both the overall size of the placenta, as well as its ability to transport nutrients per unit surface area, are likely to be key variables in determining offspring bone outcomes.

Early growth is an important contributor to peak bone mass achieved in young adulthood [8,9] and has implications for the onset of osteoporosis [7] and hip fracture risk [32] in later life. In this study, we found that for every 1 SD increase in 11 week PV there is an increase of 0.26SD in neonatal BMC, when fully adjusting for a range of offspring and maternal factors. Whilst these size-related associations were somewhat attenuated in relation to bone measures at 6 and 8 years, there was, consistent with previous findings [11], evidence to support associations between 11 week PV and scBMC with increasing childhood age. These data suggest a persistent relationship between placental volume, when measured in even the earliest stages of pregnancy, and offspring skeletal development throughout early childhood.

Conclusion

We have shown that 11 week placental volume is associated with measures of bone size and mineral content at birth, remaining robust after adjusting for a wide range of offspring and maternal factors previously associated with bone outcomes. These associations were somewhat attenuated in older childhood, with the exception of size-corrected bone mineral content, consistent with the pattern for more size-independent bone measures observed in our ALSPAC cohort analysis. When this study is taken together with our previous work, greater placental size, measured at several different time points (early-pregnancy [11 weeks' gestation], mid-pregnancy [19 weeks' gestation] and at birth) has now been shown to be associated with improved offspring bone outcomes. These findings provide further support for the importance of the placenta in setting the trajectory of bone accrual in childhood and inform approaches to future mechanistic investigations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank the administrative staff, research nurses and participants of the SWS for their contributions. SJW, EMC and SD are joint first authors. CC and NCH are joint senior authors.

Funding

This work was supported by grants from Medical Research Council (MRC) [4050502589 (MRC LEU)], Bupa Foundation, British Heart Foundation, National Institute for Health Research (NIHR) Southampton Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, and NIHR Oxford Biomedical Research Centre, University of Oxford. EC has been supported by the Wellcome Trust (201268/Z/16/Z). The work leading to these results was supported by the European Union's Seventh Framework Programme (FP7/2007–2013), projects EarlyNutrition, ODIN and LifeCycle under grant agreements numbers 289346, 613977 and 733206, and by the BBSRC (HDHL-Biomarkers, BB/P028179/1), as part of the ALPHABET project, supported by an award made through the ERA-Net on Biomarkers for Nutrition and Health (ERA HDHL), Horizon 2020 grant agreement number 696295.

Abbreviations

DOHaD	developmental origins of health and disease
PBM	peak bone mass
PV	placental volume
SWS	Southampton Women,s Survey Education
ALSPAC	Avon Longitudinal Study of Parents and Children
3DUS	3D ultrasound scanning
DXA	dual-energy X-ray absorptiometry
BA	bone area
BMC	bone mineral content
aBMD	areal bone mineral density
scBMC	size-corrected bone mineral density
DAG	directed acyclic graph
IMD	index of multiple deprivation
PMCA	plasma membrane calcium-dependent ATPases

References

1. Huppertz B, Burton G, Cross JC, Kingdom JCP. Placental Morphology: From Molecule to Mother – A Dedication to Peter Kaufmann – A Review. *Placenta*. 2006; 27:3–8. DOI: 10.1016/j.placenta.2006.01.007

2. Barker DJP, Thornburg KL, Osmond C, Kajantie E, Eriksson JG. The surface area of the placenta and hypertension in the offspring in later life. *Int J Dev Biol.* 2010; 54(2-3):2–3. DOI: 10.1387/ijdb.082760db
3. Thornburg K, O'tierney P, Louey S. The placenta is a programming agent for cardiovascular disease. *Placenta.* 2010; 31:S54–S59. [PubMed: 20149453]
4. Eriksson J, Forsen T, Tuomilehto J, Osmond C, Barker D. Fetal and childhood growth and hypertension in adult life. *Hypertension.* 2000; 36(5):790–794. [PubMed: 11082144]
5. Kim SM, Moon YW, Lim SJ, Yoon BK, Min YK, Lee DY, Park YS. Prediction of survival, second fracture, and functional recovery following the first hip fracture surgery in elderly patients. *Bone.* 2012; 50(6):1343–1350. [PubMed: 22421579]
6. Abrahamsen B, Van Staa T, Ariely R, Olson M, Cooper C. Excess mortality following hip fracture: a systematic epidemiological review. *Osteoporosis international.* 2009; 20(10):1633–1650. [PubMed: 19421703]
7. Hernandez C, Beaupre G, Carter D. A theoretical analysis of the relative influences of peak BMD, age-related bone loss and menopause on the development of osteoporosis. *Osteoporosis international.* 2003; 14(10):843–847. [PubMed: 12904837]
8. Cooper C, Cawley M, Bhalla A, Egger P, Ring F, Morton L, Barker D. Childhood growth, physical activity, and peak bone mass in women. *Journal of Bone and Mineral Research.* 1995; 10(6):940–947. [PubMed: 7572318]
9. Dennison EM, Syddall HE, Sayer AA, Gilbody HJ, Cooper C. Birth weight and weight at 1 year are independent determinants of bone mass in the seventh decade: the Hertfordshire cohort study. *Pediatric research.* 2005; 57(4):582. [PubMed: 15695596]
10. Holroyd CR, Harvey NC, Crozier SR, Winder NR, Mahon PA, Ntani G, Godfrey KM, Inskip HM, Cooper C. Placental size at 19 weeks predicts offspring bone mass at birth: Findings from the Southampton Women's Survey. *Placenta.* 2012; 33(8):623–629. DOI: 10.1016/j.placenta.2012.04.007 [PubMed: 22640438]
11. Holroyd CR, Osmond C, Barker DJ, Ring SM, Lawlor DA, Tobias JH, Smith GD, Cooper C, Harvey NC. Placental Size Is Associated Differentially With Postnatal Bone Size and Density. *Placenta.* 2016; 31(10):1855–1864. DOI: 10.1002/jbmr.2840
12. Inskip HM Group atSS, Godfrey KM Group atSS, Robinson SM Group atSS, Law CM Group atSS, Barker DJ Group atSS, Cooper C Group atSS. Cohort profile: The Southampton Women's Survey. *International Journal of Epidemiology.* 2005; 35(1):42–48. DOI: 10.1093/ije/dyi202 [PubMed: 16195252]
13. Pike KC, Crozier SR, Lucas JSA, Inskip HM, Robinson S, Southampton Women's Survey Study G, Roberts G, Godfrey KM. Patterns of fetal and infant growth are related to atopy and wheezing disorders at age 3 years. *Thorax.* 2010; 65(12):1099–1106. DOI: 10.1136/thx.2010.134742 [PubMed: 20956394]
14. Armitage P, Berry G, Matthews JNS. *Statistical methods in medical research.* John Wiley & Sons. 2008
15. Koo WW, Walters J, Bush AJ. Technical considerations of dual-energy X-ray absorptiometry-based bone mineral measurements for pediatric studies. *Journal of Bone and Mineral Research.* 1995; 10(12):1998–2004. [PubMed: 8619381]
16. Fowden A, Sferruzzi-Perri A, Coan P, Constancia M, Burton G. Placental efficiency and adaptation: endocrine regulation. *The Journal of physiology.* 2009; 587(14):3459–3472. [PubMed: 19451204]
17. Antsaklis A, Anastasakis E, Komita O, Theodora M, Hiridis P, Daskalakis G. First trimester 3D volumetry. Association of the gestational volumes with the birth weight. *The Journal of Maternal-Fetal & Neonatal Medicine.* 2011; 24(8):1055–1059. [PubMed: 21247238]
18. Effendi M, Demers S, Giguère y, Forest JC, Brassard N, Girard M, Gouin K, Bujold E. Association between first-trimester placental volume and birth weight. *Placenta.* 2014; 35(2):99–102. DOI: 10.1016/j.placenta.2013.11.015 [PubMed: 24345759]
19. Kinare AS, Natekar AS, Chinchwadkar MC, Yajnik CS, Coyaji KJ, Fall CH, Howe DT. Low midpregnancy placental volume in rural Indian women: A cause for low birth weight? *American journal of obstetrics and gynecology.* 2000; 182(2):443–448. [PubMed: 10694350]

20. Thame M, Osmond C, Wilks R, Bennett FI, Forrester TE. Second-trimester placental volume and infant size at birth. *Obstetrics & Gynecology*. 2001; 2(10):279–283. DOI: 10.1016/S0029-7844(01)01414-4
21. Wolf H, Oosting H, Treffers PE. Second-trimester placental volume measurement by ultrasound: Prediction of fetal outcome. *American Journal of Obstetrics and Gynecology*. 1989; 160(1):121–126. DOI: 10.1016/0002-9378(89)90102-6 [PubMed: 2643317]
22. Clapp JF, Rizk KH, Appleby-Wineberg SK, Crass JR. Second-Trimester Placental Volumes Predict Birth Weight at Term. *Journal of the Society for Gynecologic Investigation*. 1995; 2(1):19–22. DOI: 10.1177/107155769500200104 [PubMed: 9420843]
23. Kabir N, Kawser CA, Rahman F, Kabir ML, Rahman A. The relationship of placental weight with birth weight. *Mymensingh Med*. 2007; 16(2):177–180.
24. Clynes, MA, Harvey, NC, Dennison, EM. Early growth, bone development and risk of adult fracture. *Osteoporosis: A lifecourse epidemiology approach to skeletal health*. Harvey, N, Cooper, C, editors. CRC Press; Boca Raton: 2018. 33–42.
25. Harvey N, Dennison E, Cooper C. Osteoporosis: a lifecourse approach. *J Bone Miner Res*. 2014; 29(9):1917–1925. DOI: 10.1002/jbmr.2286 [PubMed: 24861883]
26. Hosking D. Calcium homeostasis in pregnancy. *Clinical endocrinology*. 1996; 45(1):1–6. [PubMed: 8796131]
27. Belkacemi L, Bédard I, Simoneau L, Lafond J. Calcium channels, transporters and exchangers in placenta: a review. *Cell calcium*. 2005; 37(1):1–8. [PubMed: 15541458]
28. Martin R, Harvey N, Crozier S, Poole J, Javaid M, Dennison E, Inskip H, Hanson M, Godfrey K, Cooper C. Placental calcium transporter (PMCA3) gene expression predicts intrauterine bone mineral accrual. *Bone*. 2007; 40(5):1203–1208. [PubMed: 17336174]
29. Cetin I. Placental transport of amino acids in normal and growth-restricted pregnancies. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. 2003; 110:S50–S54. [PubMed: 12965090]
30. Cleal JK, Day PE, Simner CL, Barton SJ, Mahon PA, Inskip HM, Godfrey KM, Hanson MA, Cooper C, Lewis RM, Harvey NC. Placental amino acid transport may be regulated by maternal vitamin D and vitamin D-binding protein: results from the Southampton Women's Survey. *Br J Nutr*. 2015; 113(12):1903–1910. DOI: 10.1017/s0007114515001178 [PubMed: 25940599]
31. Day PE, Ntani G, Crozier SR, Mahon PA, Inskip HM, Cooper C, Harvey NC, Godfrey KM, Hanson MA, Lewis RM, Cleal JK. Maternal Factors Are Associated with the Expression of Placental Genes Involved in Amino Acid Metabolism and Transport. *PloS one*. 2015; 10(12):e0143653.doi: 10.1371/journal.pone.0143653 [PubMed: 26657885]
32. Cooper C, Eriksson JG, Forsen T, Osmond C, Tuomilehto J, Barker DJ. Maternal height, childhood growth and risk of hip fracture in later life: a longitudinal study. *Osteoporosis International*. 2001; 12(8):623–629. [PubMed: 11580075]

Figure 1.

Flowchart detailing how study sample was derived from the SWS cohort.

¹Numbers of offspring with BMC measurements.

Figure 2.

11 week placental volume (SD, pre-adjusted for gestational age at 11 week 3DUS scan) and bone outcomes at birth, 6 years and 8 years (adjusted models).

Data are regression coefficients (95% confidence interval) adjusted for maternal age, smoking status, triceps skinfold thickness, walking speed (all measured at 11 weeks gestation), maternal height and parity (measured at study recruitment) and offspring sex and age at DXA assessment. Neonatal outcomes are additional adjusted for gestational age at delivery.

¹ Outcomes are whole body measurements for neonatal data and whole body measurements, without heads, for 6 year data and 8 year data.

Table 1
Characteristics of mothers and neonates compared with SWS singleton livebirths not included in this study

A) Maternal characteristics	
	SWS participants not in sample n=2,920
Maternal age at 11 weeks gestation (years)	29.9 (3.7)
Height (cm)	163.1 (6.5)
BMI pre-pregnancy (kg/m ²)	24.2 (21.9, 27.5)
Triceps skinfold at 11 weeks gestation (mm)	20.3 (6.0)
Non-white ethnicity	2 (0.9)
Nulliparous	140 (59.3)
Smoking before pregnancy	60 (25.4)
Smoking at 11 weeks gestation	32 (13.6)
Walking speed at 11 weeks gestation	
Very slow	1 (0.4)
Stroll at an easy pace	39 (17.0)
Normal speed	112 (48.9)
Fairly brisk	73 (31.9)
Fast	4 (1.8)
Serum 25(OH) Vitamin D at 11 weeks gestation (nmol/l)	65.8 (25.9)
Index of Multiple Deprivation 2004	15.7 (9.2, 23.4)
Placental volume at 11 weeks gestation, corrected for gestational age (cm ³)	40.2 (31.2, 50.2)
B) Neonatal DXA characteristics	
	SWS participants not in sample n=917
Gestational age at 11 week 3DUS scan (weeks)	11.8 (0.6)
Gestational age at birth (weeks)	39.9 (38.9, 40.9)
Birth weight (g)	3427.9 (471.9)
Birth crown-heel length (cm)	49.6 (2.0)
Age (days)	9.0 (2.0, 12.0)
BA (cm ²) ²	107.6 (24.9)

BMC (g) ²	62.7 (16.0)	56.4 (14.8)
aBMD (g/cm ²) ²	0.5 (0.0)	0.5 (0.0)
scBMC (g) ²	62.1 (2.9)	62.2 (2.5)

Data are mean (SD), median (IQR) or number (%). Bold text denotes *p* value <0.05.

¹ Characteristics are based on offspring with data on neonatal BMC. ² Outcomes are whole body measurement.

² Outcomes are whole body measurement

Table 2

11 week placental volume (SD, pre-adjusted for gestational age at 11 week 3DUS scan) and bone outcomes at birth, 6 years and 8 years.

A) BA (SD)					
	Unadjusted		Adjusted ³		
	n	β (95% CI)	n	β (95% CI)	
Birth ¹	83	0.20 (-0.05, 0.45)	80	0.23 (0.03, 0.42)*	
6 years ²	111	0.18 (-0.01, 0.38)	110	0.17 (-0.01, 0.36)	
8 years ²	87	0.10 (-0.10, 0.31)	85	0.13 (-0.09, 0.36)	
B) BMC (SD)					
	Unadjusted		Adjusted ³		
	n	β (95% CI)	n	β (95% CI)	
Birth ¹	83	0.23 (-0.02, 0.48)	80	0.26 (0.06, 0.46)*	
6 years ²	111	0.24 (0.05, 0.43)*	110	0.20 (0.02, 0.39)*	
8 years ²	87	0.17 (-0.04, 0.38)	85	0.14 (-0.08, 0.35)	
C) aBMD (SD)					
	Unadjusted		Adjusted ³		
	n	β (95% CI)	n	β (95% CI)	
Birth ¹	83	0.16 (-0.09, 0.41)	80	0.21 (-0.04, 0.47)	
6 years ²	111	0.23 (0.04, 0.42)*	110	0.19 (-0.01, 0.38)	
8 years ²	87	0.10 (-0.10, 0.31)	85	-0.00 (-0.22, 0.22)	
D) scBMC (SD)					
	Unadjusted		Adjusted ³		
	n	β (95% CI)	n	β (95% CI)	
Birth ¹	81	0.05 (-0.20, 0.31)	78	0.07 (-0.21, 0.35)	
6 years ²	108	0.14 (-0.05, 0.34)	107	0.13 (-0.08, 0.34)	
8 years ²	72	0.18 (-0.04, 0.41)	71	0.19 (-0.05, 0.43)	

Table shows regression coefficient (95% confidence interval) from univariable and multivariable linear regression analyses. Asterisk denotes 95%CI which do not include zero.

¹ Outcomes are whole body measurements.

²Outcomes are whole body measurements, without heads.

³Adjusted for maternal age, smoking status, triceps skinfold thickness, walking speed (all measured at 11 weeks gestation), maternal height and parity (measured at study recruitment) and offspring sex and age at DXA assessment. Neonatal outcomes are additional adjusted for gestational age at delivery.