The influence of diet and physical activity on bone density of children aged 5-7 years: The Belfast HAPO family study

The influence of diet and physical activity on bone density of children aged 5-7 years: The Belfast HAPO family study

Claire Casey [a], Bridie J. Kemp [b, c], Laura Cassidy [b], Chris C. Patterson [a], Mark A. Tully [d], Alyson J. Hill [e], David R. McCance [a, b]

[a] Centre for Public Health, Queen's University Belfast, Belfast, BT12 6BA, Northern Ireland, UK

[b] Regional Centre for Endocrinology and Diabetes, Royal Victoria Hospital, Belfast, BT12 6BA, Northern Ireland, UK

[c] School of Nursing and Midwifery, Queen's University Belfast, Belfast, BT12 6BA, Northern Ireland, UK

[d] Institute of Mental Health Sciences, School of Health Sciences, Ulster University, Newtownabbey, BT37 0QB, Northern Ireland, UK

[e] Nutrition Innovation Centre for Food and Health, Ulster University, Coleraine, BT52 1SA, Northern Ireland, UK

Corresponding author: David R McCance, Regional Centre for Endocrinology and Diabetes, Royal Victoria Hospital, Belfast, BT12 6BA, Northern Ireland, U.K.

Email: david.mccance@belfasttrust.hscni.net

Abstract

Objective: Osteoporosis is a global health issue, and modifiable behavioural factors need to be identified in childhood to reduce the risk of osteoporosis in later life. The aim of this study was to investigate the influence of diet and physical activity on bone density of children aged 5-7 years participating in the Belfast Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) Family study.

Design and methods: Pregnant women were recruited to the Belfast centre of the HAPO study at 24-32 weeks gestation. Offspring were followed up at 5-7 years as part of the Belfast HAPO Family Study. Heel bone mineral density (BMD) and bone mineral apparent density (BMAD) were measured and calculated, respectively. Physical activity in the offspring was measured by accelerometery and dietary intakes were measured using a 4-day food diary.

Results: Results from 793 offspring were analysed. Mean age of the offspring ± standard deviation was 6.4 ± 0.5 years. A mean of 48.3 ± 22.4 minutes each day was spent in moderate to vigorous physical activity (MVPA). Median (interquartile range) dietary calcium and vitamin D intakes were 844 (662-1073) mg/day and 1.7 (1.1-2.5) µg/day, respectively. Neither dietary vitamin D nor calcium intakes were significantly associated with offspring heel BMD or BMAD in multiple regression. However, controlling for confounders, a 30-minute greater MVPA was associated with significantly larger heel BMD (0.018 g/cm^2 in boys and 0.010 g/cm^2 in girls) and BMAD (0.005 g/cm^3 in boys and 0.003 g/cm^3 in girls).

Conclusion: Physical activity was associated with better BMD and BMAD in 5–7-year-old children. Dietary calcium and vitamin D were not predictive of BMD and BMAD.

Keywords: children, nutrition, physical activity, bone health, osteoporosis, public health

1. Introduction

The development of peak bone mass during childhood and adolescence to achieve optimum adult bone health and reduce the risk of osteoporosis in future life is of the utmost importance (1, 2). It is thought that attaining maximal bone mineral content during childhood and adolescence may compensate for age-associated bone loss and therefore reduce the risk of fractures and bone fragility associated with osteoporosis (3). Determinants of bone mass include genetic and behavioural factors such as diet and physical activity (4).

A substantial proportion of the variance in bone mass is explained by genetics, and therefore cannot be modified. There are, however, several environmental factors which can be positively influenced to increase bone mass during childhood. These include ensuring children and adolescents have adequate vitamin D and calcium intake. The classical actions of vitamin D in the maintenance of calcium-phosphate homeostasis, and the subsequent achievement of normal bone mineralisation are well known (5). However, suboptimal vitamin D concentrations are commonplace globally during childhood (6). This is due to insufficient dietary vitamin D intake, and reduced vitamin D production due to a lack of sunlight exposure (7). The consumption of calcium-containing foods such as milk, yogurt and cheese is essential to bone health (8), as calcium is an essential bone-forming mineral for optimal growth and development, where it can affect the acquisition of bone mass (9). It is also widely recognised that the intrauterine environment has an important long-term influence on adult health via fetal programming (10). It is therefore plausible that maternal vitamin D deficiency may have a negative influence on offspring bone development in utero thus affecting peak bone mass attained by offspring at skeletal maturity.

Physical activity has been suggested as a major determinant of bone health in people of all ages and appears to be an important factor for bone mineral accrual in the early stages of puberty

(11, 12). High levels of physical activity during growth is correlated with favourable bone mineral density and high peak bone mass, alongside superior neuromuscular function and greater muscle strength, all of which decrease the risk of fractures (13). In a 15-year prospective controlled study conducted in Sweden, 131 children took part in an intervention involving 40 minutes of physical activity per school day (200 minutes per week) from age 6 to 9 years to age 14 to 16 years. The intervention group were compared to 78 children of the same ages, who carried out 60 minutes of physical education per week, as per national recommended physical activity guidance in schools (14). There were higher musculoskeletal gains (bone mineral content, bone mineral density (BMD) and knee flexion peak torque muscle strength) across the 7 year study in the intervention group compared to the normal physical education group, with gains remaining beneficial after a further 7 year follow-up (14). Within the United Kingdom, children and adolescents are advised to do vigorous intensity activities that strengthen muscles and bones at least 3-times a week (15). These exercises for children include simple and inexpensive activities such as running, skipping and ball games.

There is currently limited literature regarding the relative influence of dietary vitamin D and calcium intake and objectively measured moderate to vigorous physical activity (MVPA) on BMD in children, taking into consideration maternal vitamin D blood concentrations during pregnancy. Therefore, the aim of this study was to describe the dietary and exercise habits of 5–7-year-old offspring of mothers who participated in the Belfast Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) Family study and investigate the influence of diet and objectively measured physical activity on bone health markers, whilst controlling for several relevant confounders including intrauterine influences.

2. Materials and Methods

The Belfast HAPO Family Study was an observational study in which women who participated in the HAPO study at the Belfast centre were invited with their offspring to attend for a further follow-up examination 5-7 years later. Details of the HAPO study have been published elsewhere (16, 17). Briefly, the HAPO study was a 15-centre multicultural and multinational study designed to examine the association between maternal hyperglycaemia and adverse pregnancy outcomes in singleton pregnancies whose results on oral glucose tolerance testing (OGTT) were below the traditional thresholds for overt diabetes. Each participant underwent a standard 75g OGTT between 24-32 weeks gestation (average 28 weeks), with sampling of plasma glucose fasting and at one hour and two hours. At the time of the OGTT maternal height, weight and blood pressure were measured, and information on education, smoking status and alcohol use collected by a standardised questionnaire. All neonatal anthropometric measurements were obtained within 72 hours of birth by trained HAPO personnel, and a detailed description has been published elsewhere (17). Neonatal birth weight, height and head circumference were converted to standard deviation scores (SDS) using the 1990 British Growth Standard, which takes account of the child's gestational age and sex (18).

Maternal 25-hydroxy vitamin D (25OHD) was measured at an average of 28 weeks gestation during pregnancy using a liquid chromatography tandem-mass spectrometry (LC-MS/MS) method [Xevo TQ-S® & ACQUITY UPLC (Waters Corporation, Milford, MA, USA)].

Measurements obtained in the 5-7 year follow-up offspring included: weight (to the nearest 0.1 kg; scale model 708; Seca, Birmingham, UK), height (to the nearest 0.1 cm using a calibrated stadiometer) and head circumference (to the nearest 0.1cm, standard plastic measuring tape). Offspring weight, height, head circumference and body mass index (BMI) (kg/m^2) were once again converted to SDS using the 1990 British Growth Standard (18).

Offspring physical activity was measured for seven days using an Actigraph GT1M accelerometer and analysed using Actilife Software v6 13.3 (Actigraph Inc., Pensacola, FL, USA). An accelerometery record was considered valid if it contained at least four valid days of data and a day was considered valid if the device was worn for at least 10 hours in the day (19). Freedson cut-off points were used to calculate time spent in moderate or vigorous intensity physical activity each day (20) and the average steps/day was also extracted as a measure of overall physical activity. Accelerometery data were used to calculate if a child was physically active/ inactive. Individuals were classified as active if they accumulated ≥60 minutes/day of moderate to vigorous physical activity (MVPA) per day and inactive as <60 minutes of MVPA/day as per recommended guidelines (15).

Offspring dietary intakes were calculated from a 4-day weighed food diary using the nutritional software package Q-Builder (Questionnaire Design System), version 2.0 (Tinuviel Software, Anglesey, UK) which uses UK food composition tables to quantify nutrient intakes. The Recommended Nutrient Intake (RNI) for dietary vitamin D and calcium in this study was defined as 10 µg/day and 550 mg/day respectively, as per UK guidelines (21, 22).

Heel BMD (g/cm^2) was measured using dual-energy X-ray absorptiometry and laser (DXL) Calscan technique (Rothband, Haslingden, UK), carried out by a trained member of staff in the Belfast Health and Social Care Trust. This method of assessing bone mass is quick, easy, and well tolerated by children and provides a low dose of absorbed radiation. Its clinical utility as a relevant marker of bone health compared with traditional DXA has been confirmed in various studies over the last 15 years. For example, in a group of healthy adults and those with suspected osteoporosis, Kullenberg showed that DXL heel measurement showed a similar pattern of T-scores to axial and forearm measures, with equally high levels of sensitivity and specificity, concluding that DXL heel measurements represented a valid and convenient alternative to axial measurements for the diagnosis of osteoporosis (23). In addition, various

studies have investigated Calcaneal DXL measurements as a marker of BMD for fracture risk (24, 25). Muschitz and colleagues showed similar sensitivity and specificity of DXA (measured at the hip, femoral neck, and lumbar spine) compared with DXL calcaneal measurement techniques for vertebral fractures (25). The device was modified and approved for use in the pediatric population by lowering the tube current and modifying the software to include a function for measuring calcaneal height, making it possible to calculate volumetric bone mineral apparent density (BMAD) (26). BMAD (g/cm^3) was calculated using the areal BMD (g/cm^2) value divided by the height (cm) of the calcaneal bone (BMAD (g/cm^3) = BMD (g/cm^2)/calcaneal height (cm).

Written informed consent was obtained from all study participants. Ethical approval was obtained from the Northern Ireland Regional Ethics Committee and the research adhered to the tenets of the Declaration of Helsinki.

2.1 Statistical Analysis

Statistical analysis was performed using SPSS version 24 (IBM Corp, Armonk, NY, USA). Continuous variables were examined using normal scores plots and were reported as mean and standard deviation (SD) or median and interquartile (IQR) range for heavily skewed variables. Categorical variables were reported as frequency and percentage. Differences between male and female offspring were investigated with independent samples t-tests for continuous variables and chi-square tests for categorical variables. Maternal total 25OHD, offspring dietary calcium and vitamin D were logarithmically transformed as their distributions were positively skewed. Maternal total 25OHD is composed of $25OHD_2$ and $25OHD_3$, of which $25OHD_3$ was the main constituent. Vitamin D deficiency was defined as 25OHD $\leq$50 nmol/L as per guidelines (27).

158 Simple linear regression was initially used to explore the relationships of four predictor
159 variables (maternal vitamin D during pregnancy, offspring dietary vitamin D, offspring dietary
160 calcium and offspring MVPA) with the offspring bone density measurements (BMD and
161 BMAD) at 5-7 years as dependent variables. Each of the four predictor variables was then
162 entered separately into a multiple regression model together with confounding variables chosen
163 based on the literature and previous bivariate analysis results. These confounder variables were
164 maternal age at OGTT, maternal education, parity, cigarette smoking during pregnancy,
165 offspring birth weight SDS, gestational age at delivery, age, and height of offspring at 5–7-
166 year follow-up. Finally, prediction models for BMD and BMAD were derived using only the
167 statistically significant predictor and confounding variables. A p-value <0.05 was considered
168 statistically significant.

169 **2.2 Role of the funding source**

170 The funders had no role in the study design; the collection, analysis, and interpretation of data;
171 in the writing of the report; and in the decision to submit the paper for publication

3. Results

Figure 1 shows the recruitment and flow chart of participants to the Belfast HAPO Family Study. In total, of the n = 1677 original HAPO participants, complete serum 25OHD, accelerometer data, BMD measurements and dietary results were available for n = 793 offspring. Compared to the 884 excluded participants, they were older, more likely to be employed, less often smokers, had more years of education and lower BMIs and fasting glucose levels.

In the Belfast centre of the HAPO study, of the 793 mothers included in this study, mean ± standard deviation age of the mothers was 30.3 ± 5.3 years at time of the index pregnancy, 18% (n = 143) of participants smoked and 26% (n = 206) consumed alcohol during pregnancy. At the time of OGTT, 37.3% (n = 296) of mothers had a BMI >33kg/m^2, and 15.1% (n = 120) were diagnosed with GDM (following IADPSG criteria). The median (inter-quartile range) maternal 25OHD concentration at 28 weeks gestation was 41.4 (26.3 - 65.1) nmol/L with 61% (n = 484) of women having deficient 25OHD concentrations (≤ 50 nmol/L). Offspring were born at an average gestation of 39.9 ± 1.4 weeks, had a mean birthweight of 3413 ± 482 g and measured 50.8 ± 2.3 cm in length.

Table 1 provides a descriptive analysis of the Belfast HAPO family study offspring at the 5-7-year follow-up. Statistical significance was found between the 404 (50.9%) male and the 389 (49.1%) female offspring for BMD and BMAD, with females having slightly greater BMD and BMAD compared with their male counterparts (0.29 vs. 0.28 g/cm^2 and 0.10 vs. 0.09 g/cm^3, respectively, both p <0.001). Independent samples t-tests showed male offspring spent significantly more time doing MVPA per day than female offspring (53.0 vs 43.5 minutes/day, p <0.001) and had greater median dietary calcium intakes (871 vs. 822 mg/day; p = 0.05). Chi-squared tests revealed a higher proportion of male offspring also achieved the recommendation

of ≥60 minutes of MVPA per day (130 (32.2%) vs 72 (18.5%), p <0.001). Less than 1% (n = 2) of both male and female participants met the RNI for dietary vitamin D.

To determine the relationship between BMD/BMAD and MVPA, dietary vitamin D and calcium, scatter plots and simple regression analyses were used, stratified by gender. These showed modest but statistically significant (p <0.001) associations for both BMD and BMAD with MVPA in both males and females (**supplementary material**). However, no significant relationships were observed between, maternal 25OHD, dietary vitamin D or calcium and BMD or BMAD (Data not shown).

The influence of dietary calcium, dietary vitamin D and MVPA on bone health in the offspring was assessed by multiple regression controlled for several confounders including maternal age, maternal education, parity, smoking, offspring sex, birthweight SDS, gestational age at delivery, offspring age and height at follow up. In both males and females, MVPA was the only significant (p <0.05) predictor associated with offspring heel BMD and BMAD (**Table 2**).

Table 3 displays a prediction model for BMD and BMAD derived using only the statistically significant predictor (offspring MVPA) and confounding variables (offspring height for BMD; and offspring height and age for BMAD). The prediction models explained 16% and 13% of the variation in BMD and 9% and 4% of the variation in BMAD in boys and girls, respectively.

4. Discussion

This study aimed to investigate the influence of mother's serum 25OHD concentrations during pregnancy and offspring diet (dietary calcium and vitamin D) on objectively measured physical activity on bone health markers of 5–7-year-old offspring of mothers who participated in the Belfast HAPO Family study, adjusting for several confounding factors (detailed in **Table 2**). Overall, no association was observed between mother's serum 25OHD concentration, offspring dietary calcium and vitamin D intake and heel BMD in 5–7-year-old offspring. By contrast, offspring gender, height and MVPA were associated with heel BMD and offspring gender, age, height and MVPA were associated with heel BMAD.

Gender, age and height have all been associated with BMD/BMAD during childhood throughout the existing literature (28-30). Physical activity has also been positively associated with bone mass, during all periods of life (31). Weight bearing activities and high impact activities in particular (common activities promoted and performed in childhood), stimulate bone metabolism and formation, ultimately leading to increased bone mass and BMD (32). It has been noted that physical activity is of particular relevance to BMD, as MVPA has been positively associated with BMD during childhood (33, 34), has been associated with higher BMD in adolescents (35), and it appears to have a direct influence on BMD in later adult-life (31). However, unlike this present study, there has previously been limited controlling for confounding variables. Thus, results of this study and associated literature, suggest that early intervention involving MVPA could significantly contribute to optimum bone health during childhood and beyond, more so than dietary vitamin D and calcium intakes.

In the current study, we did not find an association between maternal serum 25OHD during pregnancy and offspring BMD. Observational studies have reported conflicting results regarding maternal vitamin D and subsequent offspring BMD/ bone mineral content (BMC)

(36-40). Several of these observational studies reported that maternal vitamin D was positively associated with offspring BMD/BMC; however, the numbers in these studies were small. By contrast, the largest of these observational studies among 3,960 maternal-offspring pairs found no association with maternal vitamin D in the third trimester and offspring BMC at 9-10 years, but only 6% of mothers were vitamin D deficient (< 27.5 nmol/l) (39). In the present study, 61% of mothers were vitamin D deficient, though this did not appear to influence offspring BMD/BMAD. Garcia and colleagues observed an inverse association between fetal 25OHD and BMC and bone area during childhood at a similar time point to our study (6 years old) (36). However, after controlling for childhood 25OHD, the association was lost. This might suggest that vitamin D deficiency present in early life, can be compensated for in childhood, and will not have a lasting effect on BMC (36). It would be of interest to explore whether dietary vitamin D or sunlight vitamin D are more pertinent, as results from this present study suggest dietary vitamin D may not play as significant a role in BMD and BMAD in childhood as previously thought.

The classical role of vitamin D in bone metabolism is well established. The most accurate marker of vitamin D is serum or plasma 25OHD. As this was not available for the offspring in the present study, we used dietary vitamin D as an alternative marker. Dietary vitamin D levels were low compared to the RNI of 10µg, and we found no association between dietary vitamin D and BMD. This is in concordance with a Canadian study by Hazell and colleagues, where forearm BMD was measured in children aged 1.8-6.6 years (41). Dietary vitamin D was not associated with the BMD, however, plasma 25OHD >75 nmol/L was associated with forearm BMD, as was a marker of sun exposure (41). The Healthy Lifestyle in Europe by Nutrition in Adolescence study (HELENA) was a cross-sectional study of 227 adolescents aged 12-17 years (42), measuring dietary vitamin D by 24-hour dietary recall, found no associations observed between dietary vitamin D and BMD. However, in a subsample of 55 female participants,

serum 25OHD was significantly associated with BMD at several sites (although not including the heel) (42). This would suggest that dietary vitamin D intake is a less relevant measure than serum 25OHD. This may be due to limitations of the food diary which did not consider the use of vitamin D supplements and excludes the production of vitamin D_3 from sunlight and could explain the subpar vitamin D intake in our cohort. In another study, vitamin D supplementation in healthy children and adolescents did not improve BMD over a 1–2-year period (43), however, further research was suggested to investigate the role of vitamin D supplementation on BMD in subjects with a low serum 25OHD (<35 nmol/L) compared to those with higher baseline serum 25OHD (≥30 nmol/L) (43).

In the present study, there was no association between dietary calcium and BMD. In a small study (n=195) of children and adolescents with a wide age range (7-19 years), no association was observed between dietary calcium intake and BMD in regression analysis (9), despite calcium intake in the above study reportedly being substantially higher than in the current study (median 1,506 mg/day in boys and 1,407 mg/day in girls), though this was possibly reflective of an older age-group. Despite the relatively high intake of dietary calcium in both studies, no association was observed with BMD. The HELENA study measured dietary calcium by 24-hour dietary recall, and again, despite dietary calcium intakes being more comparable to the current study (792 mg/day vs 844 mg/day, respectively), no associations were observed between dietary calcium and BMD (42). A blood measure of calcium status was not reported in the current study or the above studies. Perhaps, like vitamin D, more research is required regarding blood calcium levels and BMD/BMAD, compared with recalled dietary intakes.

In this study, BMD was measured at the heel using DXL-measured BMD. Results from this current study are similar to those of other studies using the same method to measure BMD. Previous authors have concluded that both DXA and DXL techniques effectively identify the same individuals with low BMD (44). However, there are limited reference range data for

calcaneus BMD measured by DXL in young children with which to compare the results of this study. Thus, data from this study adds to the current reference data for heel BMD in a paediatric cohort.

Other observational studies which have examined the association between maternal vitamin D levels and offspring BMD, these frequently differ in the age of the offspring at follow up (new-born to age 20 years), few use the gold standard method of 25OHD measurement (LC-MS/MS) and in many there is inadequate controlling for relevant confounding variables (33, 34, 36). Against that background, in the present study, the association between physical activity and bone health observed in the present study was observed using an objective measurement of physical activity (accelerometery) and persisted after controlling for a detailed series of confounding variables including dietary vitamin D and calcium. This highlights the novelty of this present study, as we are unaware of any other study that has similarly controlled for these important confounding variables.

There are several strengths of this present study. Firstly, the large number of participants and their representativeness of the local population; secondly, the rigorous nature of the research methodology including controlling for confounding variables; and thirdly, the detailed offspring endpoints and objective accelerometery measurements of physical activity in the offspring. However, there were also limitations. Firstly, the observational nature of the study, limiting the study's ability to infer causality of MVPA with greater association with BMD or BMAD compared with dietary vitamin D and calcium; secondly, the lack of blood vitamin D and calcium measurements in the offspring, which could explain the very low numbers of children meeting dietary vitamin D RNI; thirdly, the lack of information on vitamin D and calcium supplement use, although it is doubtful whether these would have been prevalent in the population under study; and fourthly that 385 offspring were excluded due to invalid accelerometer results, reducing the overall numbers included in the study and causing a

potential lack of representativeness of the included 793 participants, as there was some under-representation of mothers who were unemployed, smokers, younger, less well educated, more overweight or obese and with higher fasting glucose levels.

In conclusion, the results of this large observational study suggest that offspring MVPA, but not maternal 25OHD, dietary calcium and vitamin D (adjusted for several confounding factors), are associated with higher offspring heel BMD and BMAD. Our findings suggest dietary intakes of calcium and vitamin may not have as major a role in bone health as is often quoted and would make the case for a greater focus on physical activity during childhood and adolescence to optimise adult bone health. However, to substantiate these findings, future research should include measurements such as blood concentrations of vitamin D and calcium, as well as details of supplement use, to determine the most significant contributions to BMD in early childhood.

Funding: The HAPO study was funded by grants from the National Institute of Child Health and Human Development and the National Institute of Diabetes and Digestive and Kidney Diseases (RO1-HD34242 and RO1- HD34243) and Diabetes UK (RD04/ 0002756), which supported the enrolment and collection of data on participants.

Disclosure statement: The authors have nothing to disclose.

References

1. Bonjour JP, Chevalley T, Ferrari S, Rizzoli R. The importance and relevance of peak bone mass in the prevalence of osteoporosis. *Salud Publica Mex* 2009;51 Suppl 1:S5-17.
2. Wren TA, Kalkwarf HJ, Zemel BS, Lappe JM, Oberfield S, Shepherd JA, et al. Longitudinal tracking of dual-energy X-ray absorptiometry bone measures over 6 years in children and adolescents: persistence of low bone mass to maturity. *J Pediatr* 2014;164(6):1280-5.e2.
3. Behringer M, Gruetzner S, McCourt M, Mester J. Effects of weight-bearing activities on bone mineral content and density in children and adolescents: a meta-analysis. *J Bone Miner Res* 2014;29(2):467-78.
4. Golden NH, Abrams SA. Optimizing bone health in children and adolescents. *Pediatrics* 2014;134(4):e1229-43.
5. Anderson PH, Turner AG, Morris HA. Vitamin D actions to regulate calcium and skeletal homeostasis. *Clin Biochem* 2012;45(12):880-6.
6. Voortman T, van den Hooven EH, Heijboer AC, Hofman A, Jaddoe VW, Franco OH. Vitamin D deficiency in school-age children is associated with sociodemographic and lifestyle factors. *J Nutr* 2015;145(4):791-8.
7. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr* 2008;87(4):1080s-6s.
8. Arundel P, Shaw N. Vitamin D and bone health: A practical clinical guideline for management in children and young people. Bath, United Kingdom 2015.
9. Pekkinen M, Viljakainen H, Saarnio E, Lamberg-Allardt C, Mäkitie O. Vitamin D is a major determinant of bone mineral density at school age. *PLoS ONE* 2012;7(7):e40090.
10. Karras SN, Fakhoury H, Muscogiuri G, Grant WB, van den Ouweland JM, Colao AM, et al. Maternal vitamin D levels during pregnancy and neonatal health: evidence to date and clinical implications. *Ther Adv Musculoskelet Dis* 2016;8(4):124-35.
11. Hind K, Burrows M. Weight-bearing exercise and bone mineral accrual in children and adolescents: a review of controlled trials. *Bone* 2007;40(1):14-27.
12. Proia P, Amato A, Drid P, Korovljev D, Vasto S, Baldassano S. The Impact of Diet and Physical Activity on Bone Health in Children and Adolescents. *Front Endocrinol (Lausanne)* 2021;12:704647.
13. Karlsson MK, Rosengren BE. Exercise and Peak Bone Mass. *Current Osteoporosis Reports* 2020;18(3):285-90.
14. Rosengren BE, Rempe J, Jehpsson L, Dencker M, Karlsson MK. Physical activity at growth induces bone mass benefits into adulthood – A fifteen-year prospective controlled study. *JBMR Plus* 2022;6(1):e10566.
15. Department of Health. Start active, stay active. A report on physical activity for health from the four home countries' Chief Medical Officers. London, United Kingdom2011.
16. The HAPO Study Cooperative Research Group. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. *Int J Gynaecol Obstet* 2002;78(1):69-77.
17. The HAPO Study Cooperative Research Group. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med* 2008;358(19):1991-2002.
18. Cole TJ, Freeman JV, Preece MA. British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. *Stat Med* 1998;17(4):407-29.
19. Migueles JH, Cadenas-Sanchez C, Ekelund U, Delisle Nyström C, Mora-Gonzalez J, Löf M, et al. Accelerometer Data Collection and Processing Criteria to Assess Physical Activity and Other Outcomes: A Systematic Review and Practical Considerations. *Sports Med* 2017;47(9):1821-45.

20. Freedson PS, Melanson E, Sirard J. Calibration of the computer science and applications, inc. Accelerometer. *Med Sci Sports Exerc* 1998;30(5):777-81.
21. Department of Health. 1991. London, United Kingdom; Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects.
22. Scientific Advisory Committee on Nutrition. Vitamin D and Health. London, United Kingdom; 2016.
23. Kullenberg R, Falch JA. Prevalence of osteoporosis using bone mineral measurements at the calcaneus by dual X-ray and laser (DXL). *Osteoporos Int* 2003;14(10):823-7.
24. Ceroni D, Martin XE, Delhumeau C, Farpour-Lambert NJ, De Coulon G, Dubois-Ferrière V, et al. Recovery of decreased bone mineral mass after lower-limb fractures in adolescents. *J Bone Joint Surg Am* 2013;95(11):1037-43.
25. Muschitz C, Dimai HP, Kocijan R, Kaider A, Zendeli A, Kühne F, et al. The discriminatory capacity of BMD measurements by DXA and dual X-ray and laser (DXL) at the calcaneus including clinical risk factors for detecting patients with vertebral fractures. *Osteoporos Int* 2013;24(8):2181-90.
26. Söderpalm AC, Kullenberg R, Wikland KA, Swolin-Eide D. Pediatric Reference Data for Bone Mineral Density in the Calcaneus for Healthy Children 2, 4, and 7 Years of Age by Dual-Energy X-Ray Absorptiometry and Laser. *Journal of Clinical Densitometry* 2005;8(3):305-13.
27. Institute of Medicine (US) Committee. Dietary Reference Intakes for Calcium and Vitamin D. Washington, D.C. 2011.
28. Zemel BS, Leonard MB, Kelly A, Lappe JM, Gilsanz V, Oberfield S, et al. Height adjustment in assessing dual energy x-ray absorptiometry measurements of bone mass and density in children. *J Clin Endocrinol Metab* 2010;95(3):1265-73.
29. Boot AM, de Ridder MAJ, Pols HAP, Krenning EP, de Muinck Keizer-Schrama SMPF. Bone Mineral Density in Children and Adolescents: Relation to Puberty, Calcium Intake, and Physical Activity*. *The Journal of Clinical Endocrinology & Metabolism* 1997;82(1):57-62.
30. Kalkwarf HJ, Gilsanz V, Lappe JM, Oberfield S, Shepherd JA, Hangartner TN, et al. Tracking of bone mass and density during childhood and adolescence. *J Clin Endocrinol Metab* 2010;95(4):1690-8.
31. Bielemann RM, Martinez-Mesa J, Gigante DP. Physical activity during life course and bone mass: a systematic review of methods and findings from cohort studies with young adults. *BMC Musculoskelet Disord* 2013;14:77.
32. Lombardi G, Ziemann E, Banfi G. Physical Activity and Bone Health: What Is the Role of Immune System? A Narrative Review of the Third Way. *Frontiers in Endocrinology* 2019;10.
33. Harvey NC, Cole ZA, Crozier SR, Kim M, Ntani G, Goodfellow L, et al. Physical activity, calcium intake and childhood bone mineral: a population-based cross-sectional study. *Osteoporos Int* 2012;23(1):121-30.
34. Heidemann M, Mølgaard C, Husby S, Schou AJ, Klakk H, Møller NC, et al. The intensity of physical activity influences bone mineral accrual in childhood: the childhood health, activity and motor performance school (the CHAMPS) study, Denmark. *BMC Pediatr* 2013;13:32.
35. Janz KF, Letuchy EM, Burns TL, Francis SL, Levy SM. Muscle power predicts adolescent bone strength: Iowa bone development study. *Med Sci Sports Exerc* 2015;47(10):2201-6.
36. Garcia AH, Erler NS, Jaddoe VWV, Tiemeier H, van den Hooven EH, Franco OH, et al. 25-hydroxyvitamin D concentrations during fetal life and bone health in children aged 6

years: a population-based prospective cohort study. *Lancet Diabetes Endocrinol* 2017;5(5):367-76.
37. Ioannou C, Javaid MK, Mahon P, Yaqub MK, Harvey NC, Godfrey KM, et al. The effect of maternal vitamin D concentration on fetal bone. *J Clin Endocrinol Metab* 2012;97(11):E2070-7.
38. Javaid MK, Crozier SR, Harvey NC, Gale CR, Dennison EM, Boucher BJ, et al. Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. *Lancet* 2006;367(9504):36-43.
39. Lawlor DA, Wills AK, Fraser A, Sayers A, Fraser WD, Tobias JH. Association of maternal vitamin D status during pregnancy with bone-mineral content in offspring: a prospective cohort study. *Lancet* 2013;381(9884):2176-83.
40. Zhu K, Whitehouse AJ, Hart PH, Kusel M, Mountain J, Lye S, et al. Maternal vitamin D status during pregnancy and bone mass in offspring at 20 years of age: a prospective cohort study. *J Bone Miner Res* 2014;29(5):1088-95.
41. Hazell TJ, Pham TT, Jean-Philippe S, Finch SL, El Hayek J, Vanstone CA, et al. Vitamin D status is associated with bone mineral density and bone mineral content in preschool-aged children. *J Clin Densitom* 2015;18(1):60-7.
42. Mouratidou T, Vicente-Rodriguez G, Gracia-Marco L, Huybrechts I, Sioen I, Widhalm K, et al. Associations of dietary calcium, vitamin D, milk intakes, and 25-hydroxyvitamin D with bone mass in Spanish adolescents: the HELENA study. *J Clin Densitom* 2013;16(1):110-7.
43. Winzenberg T, Powell S, Shaw KA, Jones G. Effects of vitamin D supplementation on bone density in healthy children: systematic review and meta-analysis. *Bmj* 2011;342:c7254.
44. Söderpalm A-C, Kullenberg R, Swolin-Eide D. The relationship between dual energy x-ray absorptiometry (DXA) and dxa with laser (DXL) measurements in children. *J Clin Densitom* 2008;11(4):555-60.

Table 1 Characteristics of offspring at the 5–7-year follow-up from the Belfast HAPO Family Study

	All (N=793)	Male (N=404)	Female (N=389)	p value
Age (year)[§]	6.4 ± 0.5	6.4 ± 0.5	6.4 ± 0.6	0.97
White European[#]	793 (100%)	404 (100%)	389 (100%)	1.00
Height (cm)[§]	118.3 ± 5.6	118.6 ± 5.6	117.9 ± 5.7	0.07
Height SDS[§]	0.10 ± 0.97	0.11 ± 0.95	0.09 ± 0.99	0.77
Weight (kg)[§]	23.1 ± 4.0	23.0 ± 3.7	23.2 ± 4.4	0.60
Weight SDS[§]	0.37 ± 1.05	0.36 ± 1.04	0.38 ± 1.07	0.73
Head circumference (cm)[§]	52.3 ± 1.5	52.7 ± 1.4	51.9 ± 1.4	<0.001
Head circumference SDS[§]	-0.38 ± 1.05	-0.38 ± 0.91	-0.37 ± 1.18	0.87
BMI (kg/m^2)[§]	16.4 ± 1.9	16.3 ± 1.7	16.6 ± 2.1	0.04
BMI SDS[§]	0.42 ± 1.02	0.41 ± 1.03	0.44 ± 1.01	0.61
Heel BMD (g/cm^2)[§]	0.29 ± 0.04	0.28 ± 0.05	0.29 ± 0.04	<0.001
Heel BMAD (g/cm^3)[§]	0.10 ± 0.02	0.09 ± 0.02	0.10 ± 0.02	<0.001
Dietary calcium (mg/day)[§]	844 (662-1073)	871 (670-1100)	822 (657-1051)	0.05
Met calcium RNI[†];n(%)[#]	697 (87.9%)	356 (88.1%)	341 (87.7%)	0.93
Dietary vitamin D (µg/day)[§]	1.7 (1.1-2.5)	1.7 (1.2-2.6)	1.7 (1.1-2.4)	0.12
Met vitamin D RNI[‡]; n(%)[#]	2 (0.3%)	1 (0.2%)	1 (0.3%)	1.00
MVPA (minutes/day)[§]	48.3 ± 22.4	53.0 ± 23.8	43.5 ± 19.6	<0.001
Met daily MVPA recommendations[‡]; n(%)[#]	202 (25.5%)	130 (32.2%)	72 (18.5%)	<0.001
Sedentary activity (minutes/day)[§]	326 ± 105	326 ± 109	327 ± 99	0.97
Steps per day[§]	9833 ± 2685	10168 ± 2813	9486± 2502	<0.001

Values are mean ± SD or median (IQR) for continuous variables and number (%) for categorical variables.

SDS, standard deviation score; BMI, body mass index; BMD, bone mineral density; BMAD, bone mineral apparent density; RNI, Recommended nutrient intake; MVPA, Moderate to vigorous physical activity; IQR, interquartile range.

[†] Calcium RNI ≥ 550mg/day

[‡] Dietary Vitamin D RNI ≥ 10 µg/day

[‡] MVPA recommendations ≥ 60 minutes/day

[§] Independent samples t-test used to determine statistical significance

[#] Chi squared test used to determine statistical significance

Table 2 Multiple regression models to assess the association of maternal and offspring influences on offspring BMD and BMAD in 403 male and 387 female offspring aged 5-7 years

Predictor Variable	BMD‡				BMAD‡			
	Male		Female		Male		Female	
	Coefficient (95% CI)	p value	Coefficient (95% CI)	p value	Coefficient (95% CI)	p value	Coefficient (95% CI)	p value
Maternal 25OHD during pregnancy*	-0.004 (-0.008, 0.001)	0.13	-0.001 (-0.005, 0.004)	0.81	-0.001 (-0.003, 0.000)	0.17	0.000 (-0.002, 0.002)	0.91
Offspring dietary calcium*	0.001 (-0.007, 0.009)	0.87	0.007 (-0.001, 0.016)	0.08	-0.001 (-0.003, 0.002)	0.71	0.003 (0.000, 0.006)	0.07
Offspring dietary vitamin D*	-0.002 (-0.006, 0.003)	0.51	0.002 (-0.003, 0.007)	0.41	-0.001 (-0.002, 0.001)	0.49	0.001 (-0.001, 0.002)	0.40
Offspring MVPA†	0.018 (0.013, 0.023)	<0.001	0.010 (0.004, 0.016)	0.002	0.005 (0.003, 0.007)	<0.001	0.003 (0.000, 0.005)	0.03

* Regression coefficients represent the difference in BMD/BMAD associated with a doubling in the predictor variable.

† Regression coefficient represent the difference in BMD/BMAD associated with a 30-minute increase in MVPA per day.

‡ Adjusted also for maternal age, education, parity, smoking, birthweight SDS, gestational age at delivery, offspring age, height at follow-up.

25OHD, 25-hydroxyvitamin D; MVPA, moderate to vigorous physical activity; BMD, bone mineral density; BMAD, bone mineral apparent density, CI, confidence interval

Table 3. Multiple regression analysis of significant predictors of BMD and BMAD in 403 male and 387 female offspring

Predictor Variable	BMD				BMAD			
	Male		Female		Male		Female	
	Coefficient (95%CI)	p value	Coefficient (95%CI)	p value	Coefficient (95%CI)	p value	Coefficient (95%CI)	p value
Offspring MVPA[†]	0.017 (0.012, 0.022)	<0.001	0.010 (0.004, 0.016)	<0.001	0.005 (0.003, 0.007)	<0.001	0.003 (0.000, 0.005)	0.02
Age (year)	–	–	–	–	0.005 (0.002, 0.009)	0.003	0.003 (0.000, 0.006)	0.05
Height at follow-up (cm)	0.0020 (0.0012, 0.0027)	<0.001	0.0025 (0.0018, 0.0032)	<0.001	-0.0005 (-0.0009,-0.0002)	<0.001	-0.0005 (-0.0008,-0.0002)	0.003
Constant	0.020 (-0.067, 0.107)	0.64	-0.013 (-0.097, 0.071)	0.76	0.117 (0.085, 0.148)	<0.001	0.131 (0.099, 0.162)	<0.001

[†] Regression coefficient represents the difference in BMD/BMAD associated with a 30-minute increase in MVPA per day.

BMD, bone mineral density; BMAD, bone mineral apparent density; CI, confidence interval; MVPA, moderate to vigorous physical activity

Figure 1 Participant flow chart