



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

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1 **The influence of diet and physical activity on bone density of children aged 5-7 years:**

2 **The Belfast HAPO family study**

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18 **Abstract**

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

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

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

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

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

40 **1. Introduction**

41 The development of peak bone mass during childhood and adolescence to achieve optimum
42 adult bone health and reduce the risk of osteoporosis in future life is of the utmost importance
43 (1, 2). It is thought that attaining maximal bone mineral content during childhood and
44 adolescence may compensate for age-associated bone loss and therefore reduce the risk of
45 fractures and bone fragility associated with osteoporosis (3). Determinants of bone mass
46 include genetic and behavioural factors such as diet and physical activity (4).

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

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

64 (11, 12). High levels of physical activity during growth is correlated with favourable bone
65 mineral density and high peak bone mass, alongside superior neuromuscular function and
66 greater muscle strength, all of which decrease the risk of fractures (13). In a 15-year
67 prospective controlled study conducted in Sweden, 131 children took part in an intervention
68 involving 40 minutes of physical activity per school day (200 minutes per week) from age 6 to
69 9 years to age 14 to 16 years. The intervention group were compared to 78 children of the same
70 ages, who carried out 60 minutes of physical education per week, as per national recommended
71 physical activity guidance in schools (14). There were higher musculoskeletal gains (bone
72 mineral content, bone mineral density (BMD) and knee flexion peak torque muscle strength)
73 across the 7 year study in the intervention group compared to the normal physical education
74 group, with gains remaining beneficial after a further 7 year follow-up (14). Within the United
75 Kingdom, children and adolescents are advised to do vigorous intensity activities that
76 strengthen muscles and bones at least 3-times a week (15). These exercises for children include
77 simple and inexpensive activities such as running, skipping and ball games.

78 There is currently limited literature regarding the relative influence of dietary vitamin D and
79 calcium intake and objectively measured moderate to vigorous physical activity (MVPA) on
80 BMD in children, taking into consideration maternal vitamin D blood concentrations during
81 pregnancy. Therefore, the aim of this study was to describe the dietary and exercise habits of
82 5–7-year-old offspring of mothers who participated in the Belfast Hyperglycaemia and Adverse
83 Pregnancy Outcome (HAPO) Family study and investigate the influence of diet and objectively
84 measured physical activity on bone health markers, whilst controlling for several relevant
85 confounders including intrauterine influences.

86 **2. Materials and Methods**

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

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

105 Measurements obtained in the 5-7 year follow-up offspring included: weight (to the nearest 0.1
106 kg; scale model 708; Seca, Birmingham, UK), height (to the nearest 0.1 cm using a calibrated
107 stadiometer) and head circumference (to the nearest 0.1cm, standard plastic measuring tape).
108 Offspring weight, height, head circumference and body mass index (BMI) (kg/m²) were once
109 again converted to SDS using the 1990 British Growth Standard (18).

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

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

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

135 studies have investigated Calcaneal DXL measurements as a marker of BMD for fracture risk
136 (24, 25). Muschitz and colleagues showed similar sensitivity and specificity of DXA (measured
137 at the hip, femoral neck, and lumbar spine) compared with DXL calcaneal measurement
138 techniques for vertebral fractures (25). The device was modified and approved for use in the
139 pediatric population by lowering the tube current and modifying the software to include a
140 function for measuring calcaneal height, making it possible to calculate volumetric bone
141 mineral apparent density (BMAD) (26). $BMAD (g/cm^3)$ was calculated using the areal BMD
142 (g/cm^2) value divided by the height (cm) of the calcaneal bone ($BMAD (g/cm^3) = BMD$
143 $(g/cm^2)/calcaneal\ height (cm)$).

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

147 **2.1 Statistical Analysis**

148 Statistical analysis was performed using SPSS version 24 (IBM Corp, Armonk, NY, USA).
149 Continuous variables were examined using normal scores plots and were reported as mean and
150 standard deviation (SD) or median and interquartile (IQR) range for heavily skewed variables.
151 Categorical variables were reported as frequency and percentage. Differences between male
152 and female offspring were investigated with independent samples t-tests for continuous
153 variables and chi-square tests for categorical variables. Maternal total 25OHD, offspring
154 dietary calcium and vitamin D were logarithmically transformed as their distributions were
155 positively skewed. Maternal total 25OHD is composed of 25OHD₂ and 25OHD₃, of which
156 25OHD₃ was the main constituent. Vitamin D deficiency was defined as 25OHD ≤ 50 nmol/L
157 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

172 **3. Results**

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

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

188 **Table 1** provides a descriptive analysis of the Belfast HAPO family study offspring at the 5-7-
189 year follow-up. Statistical significance was found between the 404 (50.9%) male and the 389
190 (49.1%) female offspring for BMD and BMAD, with females having slightly greater BMD and
191 BMAD compared with their male counterparts (0.29 vs. 0.28 g/cm^2 and 0.10 vs. 0.09 g/cm^3 ,
192 respectively, both $p < 0.001$). Independent samples t-tests showed male offspring spent
193 significantly more time doing MVPA per day than female offspring (53.0 vs 43.5 minutes/day,
194 $p < 0.001$) and had greater median dietary calcium intakes (871 vs. 822 mg/day; $p = 0.05$). Chi-
195 squared tests revealed a higher proportion of male offspring also achieved the recommendation

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

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

204 The influence of dietary calcium, dietary vitamin D and MVPA on bone health in the offspring
205 was assessed by multiple regression controlled for several confounders including maternal age,
206 maternal education, parity, smoking, offspring sex, birthweight SDS, gestational age at
207 delivery, offspring age and height at follow up. In both males and females, MVPA was the
208 only significant ($p < 0.05$) predictor associated with offspring heel BMD and BMAD (**Table**
209 **2**).

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

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219 4. Discussion

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

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

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

243 (36-40). Several of these observational studies reported that maternal vitamin D was positively
244 associated with offspring BMD/BMC; however, the numbers in these studies were small. By
245 contrast, the largest of these observational studies among 3,960 maternal-offspring pairs found
246 no association with maternal vitamin D in the third trimester and offspring BMC at 9-10 years,
247 but only 6% of mothers were vitamin D deficient (< 27.5 nmol/l) (39). In the present study,
248 61% of mothers were vitamin D deficient, though this did not appear to influence offspring
249 BMD/BMAD. Garcia and colleagues observed an inverse association between fetal 25OHD
250 and BMC and bone area during childhood at a similar time point to our study (6 years old) (36).
251 However, after controlling for childhood 25OHD, the association was lost. This might suggest
252 that vitamin D deficiency present in early life, can be compensated for in childhood, and will
253 not have a lasting effect on BMC (36). It would be of interest to explore whether dietary vitamin
254 D or sunlight vitamin D are more pertinent, as results from this present study suggest dietary
255 vitamin D may not play as significant a role in BMD and BMAD in childhood as previously
256 thought.

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

268 serum 25OHD was significantly associated with BMD at several sites (although not including
269 the heel) (42). This would suggest that dietary vitamin D intake is a less relevant measure than
270 serum 25OHD. This may be due to limitations of the food diary which did not consider the use
271 of vitamin D supplements and excludes the production of vitamin D₃ from sunlight and could
272 explain the subpar vitamin D intake in our cohort. In another study, vitamin D supplementation
273 in healthy children and adolescents did not improve BMD over a 1–2-year period (43),
274 however, further research was suggested to investigate the role of vitamin D supplementation
275 on BMD in subjects with a low serum 25OHD (<35 nmol/L) compared to those with higher
276 baseline serum 25OHD (≥30 nmol/L) (43).

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

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

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

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

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

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

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

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335 **References**

- 336 1. Bonjour JP, Chevalley T, Ferrari S, Rizzoli R. The importance and relevance of peak
337 bone mass in the prevalence of osteoporosis. *Salud Publica Mex* 2009;51 Suppl 1:S5-17.
- 338 2. Wren TA, Kalkwarf HJ, Zemel BS, Lappe JM, Oberfield S, Shepherd JA, et al.
339 Longitudinal tracking of dual-energy X-ray absorptiometry bone measures over 6 years
340 in children and adolescents: persistence of low bone mass to maturity. *J Pediatr*
341 2014;164(6):1280-5.e2.
- 342 3. Behringer M, Gruetzner S, McCourt M, Mester J. Effects of weight-bearing activities on
343 bone mineral content and density in children and adolescents: a meta-analysis. *J Bone*
344 *Miner Res* 2014;29(2):467-78.
- 345 4. Golden NH, Abrams SA. Optimizing bone health in children and adolescents. *Pediatrics*
346 2014;134(4):e1229-43.
- 347 5. Anderson PH, Turner AG, Morris HA. Vitamin D actions to regulate calcium and
348 skeletal homeostasis. *Clin Biochem* 2012;45(12):880-6.
- 349 6. Voortman T, van den Hooven EH, Heijboer AC, Hofman A, Jaddoe VW, Franco OH.
350 Vitamin D deficiency in school-age children is associated with sociodemographic and
351 lifestyle factors. *J Nutr* 2015;145(4):791-8.
- 352 7. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health
353 consequences. *Am J Clin Nutr* 2008;87(4):1080s-6s.
- 354 8. Arundel P, Shaw N. Vitamin D and bone health: A practical clinical guideline for
355 management in children and young people. Bath, United Kingdom 2015.
- 356 9. Pekkinen M, Viljakainen H, Saarnio E, Lamberg-Allardt C, Mäkitie O. Vitamin D is a
357 major determinant of bone mineral density at school age. *PLoS ONE* 2012;7(7):e40090.
- 358 10. Karras SN, Fakhoury H, Muscogiuri G, Grant WB, van den Ouweland JM, Colao AM, et
359 al. Maternal vitamin D levels during pregnancy and neonatal health: evidence to date and
360 clinical implications. *Ther Adv Musculoskelet Dis* 2016;8(4):124-35.
- 361 11. Hind K, Burrows M. Weight-bearing exercise and bone mineral accrual in children and
362 adolescents: a review of controlled trials. *Bone* 2007;40(1):14-27.
- 363 12. Proia P, Amato A, Drid P, Korovljevic D, Vasto S, Baldassano S. The Impact of Diet and
364 Physical Activity on Bone Health in Children and Adolescents. *Front Endocrinol*
365 *(Lausanne)* 2021;12:704647.
- 366 13. Karlsson MK, Rosengren BE. Exercise and Peak Bone Mass. *Current Osteoporosis*
367 *Reports* 2020;18(3):285-90.
- 368 14. Rosengren BE, Rempe J, Jehpsson L, Dencker M, Karlsson MK. Physical activity at
369 growth induces bone mass benefits into adulthood – A fifteen-year prospective
370 controlled study. *JBMR Plus* 2022;6(1):e10566.
- 371 15. Department of Health. Start active, stay active. A report on physical activity for health
372 from the four home countries' Chief Medical Officers. London, United Kingdom 2011.
- 373 16. The HAPO Study Cooperative Research Group. The Hyperglycemia and Adverse
374 Pregnancy Outcome (HAPO) Study. *Int J Gynaecol Obstet* 2002;78(1):69-77.
- 375 17. The HAPO Study Cooperative Research Group. Hyperglycemia and adverse pregnancy
376 outcomes. *N Engl J Med* 2008;358(19):1991-2002.
- 377 18. Cole TJ, Freeman JV, Preece MA. British 1990 growth reference centiles for weight,
378 height, body mass index and head circumference fitted by maximum penalized
379 likelihood. *Stat Med* 1998;17(4):407-29.
- 380 19. Migueles JH, Cadenas-Sanchez C, Ekelund U, Delisle Nyström C, Mora-Gonzalez J, Löff
381 M, et al. Accelerometer Data Collection and Processing Criteria to Assess Physical
382 Activity and Other Outcomes: A Systematic Review and Practical Considerations. *Sports*
383 *Med* 2017;47(9):1821-45.

- 384 20. Freedson PS, Melanson E, Sirard J. Calibration of the computer science and applications,
385 inc. Accelerometer. *Med Sci Sports Exerc* 1998;30(5):777-81.
- 386 21. Department of Health. 1991. London, United Kingdom; Dietary Reference Values for
387 Food Energy and Nutrients for the United Kingdom. Report on Health and Social
388 Subjects.
- 389 22. Scientific Advisory Committee on Nutrition. Vitamin D and Health. London, United
390 Kingdom; 2016.
- 391 23. Kullenberg R, Falch JA. Prevalence of osteoporosis using bone mineral measurements at
392 the calcaneus by dual X-ray and laser (DXL). *Osteoporos Int* 2003;14(10):823-7.
- 393 24. Ceroni D, Martin XE, Delhumeau C, Farpour-Lambert NJ, De Coulon G, Dubois-
394 Ferrière V, et al. Recovery of decreased bone mineral mass after lower-limb fractures in
395 adolescents. *J Bone Joint Surg Am* 2013;95(11):1037-43.
- 396 25. Muschitz C, Dimai HP, Kocijan R, Kaider A, Zendeli A, Kühne F, et al. The
397 discriminatory capacity of BMD measurements by DXA and dual X-ray and laser (DXL)
398 at the calcaneus including clinical risk factors for detecting patients with vertebral
399 fractures. *Osteoporos Int* 2013;24(8):2181-90.
- 400 26. Söderpalm AC, Kullenberg R, Wikland KA, Swolin-Eide D. Pediatric Reference Data
401 for Bone Mineral Density in the Calcaneus for Healthy Children 2, 4, and 7 Years of Age
402 by Dual-Energy X-Ray Absorptiometry and Laser. *Journal of Clinical Densitometry*
403 2005;8(3):305-13.
- 404 27. Institute of Medicine (US) Committee. Dietary Reference Intakes for Calcium and
405 Vitamin D. Washington, D.C. 2011.
- 406 28. Zemel BS, Leonard MB, Kelly A, Lappe JM, Gilsanz V, Oberfield S, et al. Height
407 adjustment in assessing dual energy x-ray absorptiometry measurements of bone mass
408 and density in children. *J Clin Endocrinol Metab* 2010;95(3):1265-73.
- 409 29. Boot AM, de Ridder MAJ, Pols HAP, Krenning EP, de Muinck Keizer-Schrama SMPF.
410 Bone Mineral Density in Children and Adolescents: Relation to Puberty, Calcium Intake,
411 and Physical Activity*. *The Journal of Clinical Endocrinology & Metabolism*
412 1997;82(1):57-62.
- 413 30. Kalkwarf HJ, Gilsanz V, Lappe JM, Oberfield S, Shepherd JA, Hangartner TN, et al.
414 Tracking of bone mass and density during childhood and adolescence. *J Clin Endocrinol*
415 *Metab* 2010;95(4):1690-8.
- 416 31. Bielemann RM, Martinez-Mesa J, Gigante DP. Physical activity during life course and
417 bone mass: a systematic review of methods and findings from cohort studies with young
418 adults. *BMC Musculoskelet Disord* 2013;14:77.
- 419 32. Lombardi G, Ziemann E, Banfi G. Physical Activity and Bone Health: What Is the Role
420 of Immune System? A Narrative Review of the Third Way. *Frontiers in Endocrinology*
421 2019;10.
- 422 33. Harvey NC, Cole ZA, Crozier SR, Kim M, Ntani G, Goodfellow L, et al. Physical
423 activity, calcium intake and childhood bone mineral: a population-based cross-sectional
424 study. *Osteoporos Int* 2012;23(1):121-30.
- 425 34. Heidemann M, Mølgaard C, Husby S, Schou AJ, Klakk H, Møller NC, et al. The
426 intensity of physical activity influences bone mineral accrual in childhood: the childhood
427 health, activity and motor performance school (the CHAMPS) study, Denmark. *BMC*
428 *Pediatr* 2013;13:32.
- 429 35. Janz KF, Letuchy EM, Burns TL, Francis SL, Levy SM. Muscle power predicts
430 adolescent bone strength: Iowa bone development study. *Med Sci Sports Exerc*
431 2015;47(10):2201-6.
- 432 36. Garcia AH, Erler NS, Jaddoe VWV, Tiemeier H, van den Hooven EH, Franco OH, et al.
433 25-hydroxyvitamin D concentrations during fetal life and bone health in children aged 6

- 434 years: a population-based prospective cohort study. *Lancet Diabetes Endocrinol*
435 2017;5(5):367-76.
- 436 37. Ioannou C, Javaid MK, Mahon P, Yaqub MK, Harvey NC, Godfrey KM, et al. The effect
437 of maternal vitamin D concentration on fetal bone. *J Clin Endocrinol Metab*
438 2012;97(11):E2070-7.
- 439 38. Javaid MK, Crozier SR, Harvey NC, Gale CR, Dennison EM, Boucher BJ, et al.
440 Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a
441 longitudinal study. *Lancet* 2006;367(9504):36-43.
- 442 39. Lawlor DA, Wills AK, Fraser A, Sayers A, Fraser WD, Tobias JH. Association of
443 maternal vitamin D status during pregnancy with bone-mineral content in offspring: a
444 prospective cohort study. *Lancet* 2013;381(9884):2176-83.
- 445 40. Zhu K, Whitehouse AJ, Hart PH, Kusel M, Mountain J, Lye S, et al. Maternal vitamin D
446 status during pregnancy and bone mass in offspring at 20 years of age: a prospective
447 cohort study. *J Bone Miner Res* 2014;29(5):1088-95.
- 448 41. Hazell TJ, Pham TT, Jean-Philippe S, Finch SL, El Hayek J, Vanstone CA, et al. Vitamin
449 D status is associated with bone mineral density and bone mineral content in preschool-
450 aged children. *J Clin Densitom* 2015;18(1):60-7.
- 451 42. Mouratidou T, Vicente-Rodriguez G, Gracia-Marco L, Huybrechts I, Sioen I, Widhalm
452 K, et al. Associations of dietary calcium, vitamin D, milk intakes, and 25-
453 hydroxyvitamin D with bone mass in Spanish adolescents: the HELENA study. *J Clin*
454 *Densitom* 2013;16(1):110-7.
- 455 43. Winzenberg T, Powell S, Shaw KA, Jones G. Effects of vitamin D supplementation on
456 bone density in healthy children: systematic review and meta-analysis. *Bmj*
457 2011;342:c7254.
- 458 44. Söderpalm A-C, Kullenberg R, Swolin-Eide D. The relationship between dual energy x-
459 ray absorptiometry (DXA) and dxa with laser (DXL) measurements in children. *J Clin*
460 *Densitom* 2008;11(4):555-60.

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

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	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

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

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

479

480 [†] Calcium RNI ≥ 550mg/day

481 [‡] Dietary Vitamin D RNI ≥ 10 µg/day

482 [‡] MVPA recommendations ≥ 60 minutes/day

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

484 [#] Chi squared test used to determine statistical significance

485 **Table 2 Multiple regression models to assess the association of maternal and offspring influences on offspring BMD and BMAD in 403**
 486 **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

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

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

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

491 25OHD, 25-hydroxyvitamin D; MVPA, moderate to vigorous physical activity; BMD, bone mineral density; BMAD, bone mineral apparent density, CI, confidence interval

492

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

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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

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

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

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499 **Figure 1 Participant flow chart**

