2017

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Kun Zhu

Wendy Oddy

Patrick Holt

Wendy Chan She Ping-Delfos
The University of Notre Dame Australia, wendy.chan.she.ping-delfos@nd.edu.au

Jennifer Mountain

See next page for additional authors

This article was originally published as:
Zhu, K., Oddy, W., Holt, P., Chan She Ping-Delfos, W., Mountain, J., Lye, S., Pennell, C., Hart, P., & Walsh, J. (2017). Tracking of vitamin D status from childhood to early adulthood and its association with peak bone mass. *American Journal of Clinical Nutrition, 106* (1), 276-283.
http://doi.org/10.3945/ajcn.116.150524

This article has been accepted for publication in American Journal of Clinical Nutrition
Published by Oxford University Press.

Zhu, K., Oddy, W., Holt, P., Chan She Ping-Delfos, W., Mountain, J., Lye, S. … Walsh, J. (2017). Tracking of vitamin D status from childhood to early adulthood and its association with peak bone mass. *American Journal of Clinical Nutrition, 106*(1), 276-283.
doi: 10.3945/ajcn.116.150524

Tracking of vitamin D status from childhood to early adulthood and its association with peak bone mass

Kun Zhu[1,2], Wendy H Oddy[3], Patrick Holt[4], Wendy Chan She Ping-Delfos[5], Jenny Mountain[6], Stephen Lye[7], Craig Pennell[8], Prue H Hart[4], John P Walsh[1,2]

[1]Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Perth, Australia;

[2]School of Medicine and Pharmacology, University of Western Australia, Perth, Australia;

[3] Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia;

[4] Telethon Kids Institute, Perth, Australia;

[5] University of Notre Dame, Perth, Australia;

[6] School of Population Health, University of Western Australia, Perth, Australia;

[7] Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada;

[8] School of Women's and Infants' Health, University of Western Australia, Perth, Australia

Authors' last names: Zhu, Oddy, Holt, Ping-Delfos, Mountain, Lye, Pennell, Hart, Walsh

Corresponding author: Kun Zhu, PhD, Department of Endocrinology and Diabetes, Sir Charles Gardiner Hospital, Nedlands, WA 6009, Australia; Email: kun.zhu@uwa.edu.au; Phone: +61 8 6457 4969; Fax: +61 8 6457 4109

Sources of Support: The University of Western Australia, the Raine Medical Research Foundation, Telethon Kids Institute, Women and Infants Research Foundation, Curtin University, Edith Cowan University, the Australian National Health and Medical Research Council, Lions Eye Institute WA, and the Canadian Institutes of Health Research.

Running title: Vitamin D status during growth and peak bone mass

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BMC, bone mineral content; BMD, bone mineral density; BMI, body mass index; CV, coefficient of variation; DXA, dual-energy x-ray absorptiometry; ICC, intraclass correlation coefficient; IPAQ, International Physical Activity Questionnaire

Abstract

Background: There are few longitudinal studies of vitamin D status from childhood to early adulthood, and it is uncertain whether vitamin D predicts peak bone mass in young adults.

Objective: The purpose of this longitudinal study was to evaluate long-term stability of vitamin D status from age 6 to 20 in healthy individuals and to study associations between serum 25-hydroxyvitamin D (25(OH)D) at different developmental stages and bone mass measured at age 20.

Design: Participants are offspring of the Western Australian Pregnancy Cohort (Raine) Study. Serum 25(OH)D was assessed at age 6, 14, 17 and 20, and whole body bone mineral content (BMC) and density (BMD) measured at age 20 using dual-energy x-ray absorptiometry (DXA). This analysis included 821 participants (385 females) who had ≥3 serum 25(OH)D measures and DXA data. Using latent class growth analysis, four vitamin D status trajectories were identified: consistently lower (n=259), decreasing (n=125), increasing (n=138), and consistently higher (n=299).

Results: There were significant correlations between serum 25(OH)D concentrations at different time points in both sexes (r=0.346-0.560, P<0.001), with stronger correlations at adjacent time points. In males, but not females, serum 25(OH)D at 6, 17 and 20 years was positively associated with total body BMC and BMD at 20 years (covariate-adjusted increments of 40.7-53.9 g and 14.7-18.6 mg/cm^2, respectively, per 25 nmol/L 25(OH)D); when 25(OH)D at all four ages were included in the same model, the level at age 6 remained significant. Males in the "consistently higher" trajectory had 3.2-3.4% higher total body BMC and BMD than those who were "consistently lower", accounting for age, anthropometric and lifestyle factors.

Conclusion: Within both sexes, there are moderate associations between vitamin D status measured in pre-puberty, adolescence and early adulthood. Vitamin D status in childhood is a significant predictor of peak bone mass in males but not females.

Key words: vitamin D status, tracking, early adulthood, peak bone mass, Raine Study

Introduction

The physiological importance of vitamin D in calcium homeostasis and bone mineralization is well-established (1), but vitamin D deficiency in children and adolescents is common. In cross-sectional studies, the prevalence of vitamin D deficiency in adolescents ranges from 17 to 47% in different countries (2). Even in Australia, a country of low latitude, the 2011-2012 National Health Measures Survey showed that 15% of children aged 12-17 years and 31% of adults aged 18-34 years were vitamin D-deficient (defined as serum 25-hydroxy vitamin D (25(OH)D) < 50 nmol/L) (3).

There is evidence that vitamin D status during childhood and adolescence is associated with bone mineral accretion (4-6), but the results have been inconsistent (7, 8). A 3-year, longitudinal study of Finnish girls aged 9-15 years showed that vitamin D status at baseline had significant correlation with change in bone mineral density (BMD) at the lumbar spine and femoral neck (4). In the Avon Longitudinal Study of Parents and Children, $25(OH)D_3$ measured during childhood (9.9 or 11.8 or 7.6 years) positively associated with cortical bone mineral content, cortical thickness and bone strength measured at 50% mid-tibia using peripheral quantitative computed tomography (pQCT) at 15.5 years (9). However, in a cross-sectional study from Northern Ireland of adolescents aged 12 and 15, higher serum 25(OH)D was associated with higher forearm (but not heel) BMD in girls, but not in boys (7), and in a cross-sectional study of American adolescents, there were no significant associations between vitamin D status and bone mass in males or females (8). It is uncertain whether vitamin D status is stable in individuals during childhood and adolescence, and whether a single measurement (as is typical in cross-sectional and longitudinal studies) is a valid long term measure of vitamin D status. In a longitudinal study of 99 South African adolescents followed from age 11 to 20, there was no significant correlation between 25(OH)D in the

earlier and later years of adolescence, and measurement of 25(OH)D at a single time point did not reflect long term vitamin D status (10). By contrast, a large study of Norwegian adults showed significant correlations between serum 25(OH)D concentrations measured 14 years apart (r = 0.42 to 0.52) (11).

To our knowledge, tracking of vitamin D status from childhood to early adulthood and its associations with peak bone mass have not been evaluated in a long-term prospective study. In the Western Australian Pregnancy Cohort (Raine) Study, serum 25(OH)D was assessed in the offspring at age 6, 14, 17 and 20 years, and whole body dual-energy x-ray absorptiometry (DXA) scanning was performed at age 20. A longitudinal study of Canadian children showed that whole body peak bone mass was generally achieved by 18.5 years of age in girls and 20 years in boys (12). The aims of our study were firstly to determine whether tracking of vitamin D status occurs from childhood to young adulthood, and secondly to examine whether vitamin D status at key developmental stages (childhood, adolescence and skeletal maturity) and vitamin D trajectories are predictors of peak bone mass.

Subjects and methods

Participants

This longitudinal, prospective study included data from 821 offspring (436 males and 385 females) from the Western Australian Pregnancy Cohort (Raine) Study. The original study recruited 2900 pregnant women from the antenatal clinic at King Edward Memorial Hospital and nearby private clinics in Perth, Western Australia between May 1989 and November 1991. Inclusion criteria were a gestational age between 16 and 20 weeks, English language skills sufficient to understand the study demands, an expectation to deliver at King Edward Memorial Hospital, and an intention to remain in Western Australia to enable future follow-up of their child (13). All offspring were invited to attend periodic follow-ups. Compared with the general Western Australian population, the Raine cohort at birth was characterized by higher proportions of high-risk births and fathers employed in managerial and professional positions, but comparison of participants remaining in the study at the 14-year follow-up suggested that attrition resulted in a cohort comparable with the general population (14). A total of 1306 offspring participated in the clinical component of the 20 year follow-up, and 1183 had a valid whole body DXA scan. The current study is restricted to participants who had a whole body DXA at 20 years and measurements of serum 25(OH)D at three or more of the study time points at age 6, 14, 17 and 20 years. The study at each follow-up was approved by the Human Research Ethics Committee of Princess Margaret Hospital (year 6, 14 and 17) and University of Western Australia (year 20). At each study visit, written informed consent was obtained from parents and/or offspring, as appropriate.

Vitamin D status at 6, 14, 17 and 20 years

Fasting venous blood was collected at age 6, 14, 17 and 20, and serum was then securely stored at -80°C. Serum 25(OH)D at ages 6 and 14 was measured using an enzyme

immunoassay (EIA) (Immunodiagnostic Systems (IDS) Ltd, Scottsdale, AZ, USA), whereas at ages 17 and 20, isotope-dilution liquid chromatography/tandem mass spectrometry (LC-MS/MS) was performed by RMIT Drug Discovery Technologies (Melbourne, Victoria, Australia) according to published methodology (15). In 12 participants aged 14 years both methods were used, with a strong correlation ($r^2 = 0.933$) (16). Analysis of 50 samples from participants aged 6 years revealed that EIA overestimated 25(OH)D compared with LC-MS/MS, particularly for 25(OH)D values over 100 nmol/L. Therefore an equation developed using Weighted Deming Regression (17) was used to calculate standardized 25(OH)D values at year 6: standardized 25(OH)D = 22.3 + 0.58 * EIA. Since the enzyme immunoassay at the 6 and 14-year follow-ups did not differentiate between serum 25(OH)D_2 and 25(OH)D_3, analyses at all four time points were performed on total serum 25(OH)D concentrations (18). The inter-assay coefficients of variations (CVs) for the EIA were low standard (40.3 nmol/L) 4.6%, medium standard (72.0 nmol/L) 6.4%, and high standard (132.0 nmol/L) 8.7%. For the LC-MS/MS, the CVs for 25(OH)D_3 were low standard (27.1 nmol/L) 7.1%, medium standard (75.4 nmol/L) 5.0%, and high standard (163.8 nmol/L) 5.3%; the CVs for 25(OH)D_2 were low standard (23.4 nmol/L) 8.8%, medium standard (66.0 nmol/L) 6.7%, and high standard (150.1 nmol/L) 6.7%.

Latent class growth analysis which can discern classes defined by different developmental trajectories was used to estimate trajectories of vitamin D status, in which serum 25(OH)D concentrations at each time point were categorized into four groups according to quartile. Sex was used as an active covariate in the models and a series of models with between 1 and 8 trajectories were estimated. Four trajectories were chosen based on a combination of statistical criteria, parsimony and interpretability (19). Participants were assigned to the trajectory class for which they had the highest posterior probability of membership. The four

vitamin D status trajectories identified were: consistently lower (most values in the two bottom quartiles, n = 259), decreasing (moving from the two top quartiles to the two bottom quartiles over time, n = 125), increasing (moving from the two bottom quartiles to the two top quartiles over time, n =138), and consistently higher (most values in the two top quartiles, n = 299).

Whole body DXA at 20 years

Whole body scanning was performed at the 20 year follow-up visit using DXA on a Norland XR-36 densitometer (Norland Medical Systems, Inc., Fort Atkinson, WI, USA), according to manufacturer-recommended procedures. Analysis of scans was performed using built-in machine software (version 4·3·0) which provided estimates of whole body BMC (g), bone area (cm^2) and areal BMD (g/cm^2). Daily calibration was performed prior to each scanning session, and the inter-scan coefficient of variation was less than 2%.

Other assessments

At age 6, 14, 17 and 20 years, body weight was measured to the nearest 0.1 kg with subjects dressed in light clothes, and height measured with a hypsometer to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$, and data on organized sports participation and TV watching collected using a questionnaire. Month and year of menarche in girls were recorded at 14 years. A validated semi-quantitative food frequency questionnaire from the Cancer Council Victoria (20) was used to assess dietary intake including calcium and alcohol intake at 20 years. Physical activity level at 20 years was assessed using the International Physical Activity Questionnaire (IPAQ), and categorized as low, medium and high according to the IPAQ scoring protocol (21). Information on smoking habit and oral contraceptive using in females at 20 years was collected using a questionnaire.

144

145 *Statistical analysis*

146 Variables are presented as mean (SD) unless otherwise stated. The normality of continuous
147 variables was checked through the construction of histograms. The characteristics of
148 participants included in the present study were compared with those of the whole Raine Study
149 cohort to determine whether participants were representative of the broader cohort. These
150 comparisons as well as those between male and female participants were made using
151 Student's t test and chi-square test. The tracking of vitamin D status was assessed using
152 Pearson's correlation analysis to calculate the correlation coefficients between serum
153 25(OH)D measured at different time points. In addition, intraclass correlation coefficient
154 (ICC) was obtained from a linear mixed model with 25(OH)D measures at all time points as
155 the dependent variable, age as timeline, subject effects as random and season as covariate (for
156 models with raw values), and the square root of the ICC is given as an estimate of the
157 correlation between any two 25(OH)D measures for the same individual. Associations
158 between serum 25OHD at different ages (6, 14, 17 and 20 years) and total body BMC and
159 BMD at age 20 were evaluated using linear regression analyses in males and females
160 separately, adjusting for covariates including season of blood collection, age, height, body
161 weight, TV watching (22), organized sports participation or physical activity level at the time
162 of 25(OH)D assessment and at age 20, and calcium intake, smoking, alcohol consumption
163 and bone area (for the models for BMC only) at 20 years. To account for seasonal variation
164 of vitamin D status, deseasonalized vitamin D concentrations were calculated using a
165 published formula (23), and the above analyses were repeated using the deseasonalized
166 values (without inclusion of season as a covariate).

167

Comparisons between those with serum 25(OH)D concentrations below or above the sufficiency level of 50 nmol/L (24) at age 17 and 20 and between the four vitamin D status trajectories (consistently lower, decreasing, increasing, consistently higher) on bone outcomes were made by analysis of covariance (ANCOVA) with Bonferroni post hoc test adjusted for age, height, body weight, physical activity, TV watching, calcium intake, smoking, alcohol consumption and bone area (for BMC only) at 20 years (the models for age 17 additionally adjusted for covariates at 17 years). In females, further analyses were made including age of menarche and oral contraceptive use as covariates. The homogeneity of variance of each model was checked by Levene's Test. Statistical significance level was set at $P < 0.05$ (two-tailed). All analyses were performed using IBM SPSS (version 22, IBM, Chicago, IL, USA) and R (version 3.3.3, R Foundation for Statistical Computing, Vienna, Austria).

Results

Characteristics of participants

In total 821 participants (436 males and 385 females) who underwent whole body DXA scanning at age 20 years, and who had measurements of serum 25(OH)D from three or more study visits at age 6, 14, 17 and 20 were included in this analysis (**Supplemental Figure 1**). Compared with Raine participants who had whole body DXA at 20 years but were not included in the analysis (n = 362), the subsample included in our study (n = 821) did not differ in age, body weight, BMI and total body BMD. The characteristics of participants at 6, 14, 17 and 20 are presented in **Table 1**. Serum 25(OH)D concentrations did not differ significantly between males and females at 6 and 17 years, but at age 14, mean 25(OH)D was significantly higher in males, whereas at age 20 it was higher in females. In girls, the mean age of menarche was 12.8 ± 1.1 years, and at age 20, 56.5% of females were using an oral contraceptive.

Tracking of vitamin D status

There were significant correlations between serum 25(OH)D concentrations measured at different time points in both males (raw values r = 0.360−0.560; deseasonalized values r = 0.440−0.673, all P<0.001) and females (raw values r = 0.346−0.537; deseasonalized values r = 0.399−0.629, P<0.001), and the associations were stronger at adjacent time points (**Table 2**). The square root of ICC range was 0.667-0.697 for these values (Table 2), and were very similar (0.684 for males and 0.667 for females) when using the unstandardized (measured) values at year 6 in the models. When analyzed according to whether serum 25(OH)D was below or above the median at age 6, it was found that the majority of participants (64.4-69.7% of males and 58.9-66.5% of females) remained in the same group (i.e. below or above the median) at age 14, 17 and 20 (**Supplemental Table 1)**.

205

206 *Relationship between vitamin D status and peak bone mass*

207 In males, serum 25(OH)D concentration using the raw values at age 6, 17 and 20 years was
208 positively associated with total body BMC and BMD at age 20, with regression coefficients
209 of 40.7-53.9 g for BMC and 14.7-18.6 mg/cm^2 for BMD per 25 nmol/L increase in 25(OH)D
210 (all $P<0.05$) after adjustment for season of blood collection, age, height, weight, TV watching,
211 organized sports participation or physical activity level at the time of 25(OH)D assessment
212 and at age 20, and calcium intake, smoking, alcohol consumption and bone area (for BMC
213 only) at age 20 (**Table 3**). When measured (unstandardized) 25(OH)D values at year 6 were
214 used instead for the year 6 models, the regression coefficient (95% CI) was slightly lower as
215 31.3 (3.6, 59.0) g for BMC, and 10.8 (1.1, 20.5) mg/cm^2 for BMD. Serum 25(OH)D
216 concentrations at age 14 in males and at age 6, 14, 17 and 20 in females were not
217 significantly correlated with total body bone measures at 20 years (Table 3). In females, the
218 results remained similar after further adjustment for age of menarche and oral contraceptive
219 use (data not shown). Using deseasonalized 25(OH)D values yielded similar results (Table 3).

220

221 In a further regression model in males where 25(OH)D at the four different ages were
222 simultaneously included, only 25(OH)D at year 6 remained significant, with regression
223 coefficient (95% CI) of 50.6 (6.7, 94.4) g for BMC, and 18.1 (2.6, 33.6) mg/cm^2 for BMD per
224 25 nmol/L increase in serum 25(OH)D. When measured (unstandardized) 25(OH)D values at
225 year 6 were used instead, the regression coefficient (95% CI) was slightly lower at 29.3 (3.9,
226 54.8) g for BMC, and 10.5 (1.5, 19.5) mg/cm^2 for BMD. Using deseasonalized 25(OH)D
227 values yielded similar results (data not shown).

228

There were 1.0%, 3.9%, 15.4% and 16.7% participants with serum 25(OHD) below 50 nmol/L at age 6, 14, 17 and 20, respectively. Comparing to those with serum 25(OHD) below 50 nmol/L, those with serum 25(OHD) ≥ 50 nmol/L had 3.8-4.1% higher total body BMC and BMD at age 17 and 20 (**Table 4**).

Vitamin D trajectories and peak bone mass

The mean serum 25(OH)D values at each time point for the four vitamin D status trajectories identified (consistently lower, decreasing, increasing and consistently higher) are presented in **Figure 1**. At age 20, males in the "consistently higher" vitamin D trajectory had 3.2% and 3.4% higher total body BMC and BMD, respectively, compared with those in the "consistently lower" category, after accounting for age, height, body weight, physical activity, TV watching, calcium intake, smoking, alcohol consumption and bone area (for BMC only) at 20 years (BMC 3222 ± 21 vs 3123 ± 24 g, P = 0.013; BMD 1135 ± 7 vs 1098 ± 8 mg/cm^2, P = 0.008) (**Figure 2**). In females, there were no significant differences between the four trajectory classes in total body bone measures at 20 years after adjustment for these covariates (Figure 2), or after further adjustment for age of menarche and oral contraceptive use (data not shown).

Discussion

In this longitudinal study of 821 boys and girls examined at age 6, 14, 17 and 20, we found evidence of tracking of vitamin D status, with significant associations between vitamin D in individuals assessed at pre-puberty, adolescence and early adulthood in both genders. In males, but not females, serum 25(OH)D at age 6, 17 and 20 was a significant predictor of total body BMC and BMD at 20 years, with the level at age 6 remaining significant when 25(OH)D at four different ages were included in the same model. Males in the "consistently higher" vitamin D trajectory had significantly higher total body BMC and BMD than those in the "consistently lower" category, after accounting for age, anthropometric and lifestyle factors. Childhood and adolescence are critical periods for bone mineral accretion, and achieving optimal peak bone mass at skeletal maturity is considered an effective strategy against osteoporosis in later life. An increase in peak bone mass by one standard deviation is estimated to reduce the osteoporotic fracture risk in later life by 50% (25). Therefore the magnitude of the difference observed in total body BMD in our study (~0.35 SD) may be clinically relevant, with implications for reducing the fracture risk in later life.

Vitamin D has well-established physiological roles in calcium absorption and bone mineralization, and an association between vitamin D status in childhood/adolescence and peak bone mass is biologically plausible. The basis for the sex difference observed in the present study is uncertain, but may arise from differences in sex hormone effects in bone between males and females. Estrogens and androgens influence the growth and maintenance of bones and muscles and are responsible for sexual dimorphism. Estrogen is needed for closure of epiphyseal growth plates in both sexes (26), and the more rapid epiphyseal maturation in girls compared with boys is mostly due to higher circulating estradiol levels (27). Estrogen stimulates renal 1-α hydroxylase activity, converting 25(OH)D to the more

biologically active hormone 1,25$(OH)_2$D (28), stimulates vitamin D receptor expression via activation of the ERK 1/2 signaling pathway (29), and increases intestinal calcium absorption through vitamin D-dependent, and possibly vitamin D-independent mechanisms (30). Estrogen also reduces circulating levels of sclerostin, an inhibitor of Wnt signaling, which has anti-anabolic effects on bone (31). It is thus possible that in females, estrogenic effects may counteract the effects of lower 25(OH)D levels, whereas in males this compensatory effect is absent, explaining the sex difference in our results. In the same cohort, associations between vitamin D status, atopy and asthma at age 6 and 14 years were seen mainly in boys (16). Another possible explanation is that males experience a longer period of bone mineral accretion and require a greater amount of calcium than females (12), and therefore may be more at risk from suboptimal intestinal calcium absorption associated with lower vitamin D levels. Consistent with our findings, in a Korean study of 1926 men and 2350 women aged 10-40 years, significant positive associations between serum 25(OH)D and BMD of spine and hip were observed in men but not women (32).

The significant, moderate correlations observed between serum 25(OH)D concentrations at age 6, 14, 17 and 20 in our West Australian cohort differ from a study of South African adolescents, in which no association was found between 25(OH)D values measured in early and late adolescence (10). Our results are consistent with data from Norwegian adults, in whom, after seasonal adjustment, the correlation coefficients of serum 25(OH)D concentrations measured 14 years apart ranged between 0.42 to 0.52, similar to those seen for cardiovascular risk factors, blood pressure and lipids (11), and close to the coefficients observed in our study. In the majority of our participants, vitamin D status at 6 years with respect to the quartile they were in predicted their vitamin D status at subsequent visits. Such tracking in vitamin D status from childhood to young adulthood may be due to multiple

296 factors known to affect vitamin D status, including genetic factors, body fatness, and lifestyle
297 factors such as dietary intake and sunlight exposure (18, 33). In a meta-analysis of six
298 randomized controlled trials of vitamin D supplementation in healthy children, there was no
299 significant effect of vitamin D on BMC or BMD overall, but there was a significant positive
300 effect on total body BMC in children with low baseline 25(OH)D levels (<35 nmol/L) (34).
301 Therefore, optimizing vitamin D status in children and adolescents could play an important
302 role in promoting the attainment of optimal peak bone mass.
303
304 A strength of this study is the measurement of serum 25(OH)D at multiple time points,
305 allowing the longitudinal analysis of vitamin D at different developmental stages and use of
306 trajectories as predictors of peak bone mass. Other strengths include the large sample size,
307 prospective, detailed data collection and assessment of bone mass at the age of accrual of
308 peak bone mass (12). Our study also has limitations. Firstly, its observational nature means
309 we cannot assume that the relationships between vitamin D status and bone are causal.
310 Although we adjusted for important confounding variables including anthropometric
311 measures and lifestyle factors, the significant associations observed may be due to potential
312 residual or uncontrolled confounders. Secondly, the majority of participants were Caucasian,
313 with median serum 25(OH)D concentrations between 70 and 80 nmol/L at different time
314 points; the study findings may not be applicable to other ethnic groups or communities with
315 substantially different vitamin D status. Thirdly, two different methods were used to measure
316 serum 25(OH)D. This may affect measured 25(OH)D values, but should have minimal or no
317 effect on ranking and trajectory. In addition, we used standardized values at age 6 to correct
318 for between-method differences and also presented analyses results using unstandardized
319 values, whereas at age 14 there was good agreement between the methods. Finally, we did
320 not measure BMD at fracture-relevant sites such as spine and hip, but previous studies have

shown the value of total body BMD in predicting hip fracture (35) and a close relationship between BMD measures of total body, lumbar spine and hip (36).

In conclusion, we found moderate associations between serum 25(OH)D measured at pre-puberty, adolescence and early adulthood in both genders, and evidence of tracking of vitamin D status across key developmental stages. In males, but not females, vitamin D status in childhood and adolescence was a significant, independent predictor of peak bone mass at age 20. Optimizing vitamin D status during childhood may play a role in achievement of optimal peak bone mass, particularly in males, which may in turn reduce the risk of fracture in adult life.

Acknowledgments

We gratefully acknowledge the efforts of the Raine Study participants, the Raine Study team for study co-ordination and data collection, and Denise Anderson for developing the Weighted Deming Regression equation for calculating standardized 25(OH)D values at 6 years.

Authors' contributions: KZ, CP, and JPW designed research; WO, PH, WCSP-D, JM, SL, CP, PHH conducted research; KZ analyzed data; KZ and JPW wrote the paper and had primary responsibility for final content. All authors read and approved the final manuscript.

Conflict of Interest Statement: KZ, WO, PH, WCSP-D, JM, SL, CP, PHH, and JPW declare that they have no conflict of interest.

Role of the Sponsor: None of the funding agencies had any role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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Table 1 Characteristics of male and female participants at each time point

	Male	Female	P [1]
6 years	***n = 271***	***n = 228***	
Age, year	5.9 ± 0.2	5.9 ± 0.2	0.822
Weight, kg	21.4 ± 3.6	20.9 ± 2.8	0.031
Height, cm	116.1 ± 5.1	115.5 ± 4.5	0.186
25(OH)D measured, nmol/L	105.4 ± 34.3	101.2 ± 28.8	0.138
25(OH)D standardized, nmol/L	83.4 ± 19.9	81.0 ± 16.7	0.138
25(OH)D deseasonalized, nmol/L	83.2 ± 18.9	81.3 ± 15.7	0.226
Watch TV ≥ 2 hours/day, %	25.1	19.2	0.057
Participated in organized sports, %	35.9	32.6	0.363
14 years	***n = 412***	***n = 366***	
Age, year	14.1 ± 0.2	14.1 ± 0.2	0.285
Weight, kg	57.4 ± 12.6	56.0 ± 10.4	0.091
Height, cm	165.9 ± 8.5	162.8 ± 6.3	<0.001
25(OH)D, nmol/L	89.6 ± 31.0	83.7 ± 28.6	0.005
25(OH)D deseasonalized, nmol/L	89.9 ± 26.6	83.3 ± 25.4	<0.001
Watch TV ≥ 2 hours/day, %	47.7	44.5	0.391
Participated in organized sports, %	94.0	87.6	0.002
17 years	***n = 409***	***n = 366***	
Age, year	17.0 ± 0.2	17.1 ± 0.3	0.037
Weight, kg	70.7 ± 13.2	62.8 ± 10.9	<0.001
Height, cm	177.5 ± 7.0	166.7 ± 6.6	<0.001
25(OH)D, nmol/L	74.7 ± 28.6	75.1 ± 26.2	0.857
25(OH)D deseasonalized, nmol/L	75.4 ± 25.2	75.1 ± 23.9	0.871

Watch TV ≥ 2 hours/day, %	31.3	28.8	0.497
Participated in organized sports, %	85.5	72.3	<0.001
20 years	***n = 436***	***n = 385***	
Age, year	20.0 ± 0.4	20.0 ± 0.4	0.312
Weight, kg	76.8 ± 14.1	65.5 ± 12.7	<0.001
Height, cm	178.3 ± 7.1	166.3 ± 6.4	<0.001
BMI, kg/m^2	24.1 ± 3.9	23.7 ± 4.6	0.198
25(OH)D, nmol/L	70.6 ± 24.3	75.2 ± 26.2	0.009
25(OH)D deseasonalized, nmol/L	71.3 ± 22.2	75.6 ± 24.8	0.010
Watch TV ≥ 2 hours/day, %	26.5	26.1	0.931
Physical activity, %			
Low	8.0	13.1	<0.001
Moderate	33.6	52.6	
High	58.4	34.4	
Current smoker, %	15.6	12.5	0.274
Alcohol intake ≥3 units/day, %	16.6	4.6	<0.001
Calcium intake, mg/day	1029.6 ± 435.8	803.4 ± 334.9	<0.001
Total body BMC, g	3182 ± 429	2719 ± 325	<0.001
Total body bone area, cm^2	2830 ± 192	2649 ± 181	<0.001
Total body BMD, mg/cm^2	1122 ± 107	1025 ± 83	<0.001

Values are mean ± SD unless otherwise stated. 25(OH)D, 25 hydroxyvitamin D; BMC, bone mineral content; BMD, bone mineral density.

[1] Student's t-test or chi-square test.

Table 2 Correlations between individual participants' 25(OH)D concentrations measured at 6, 14, 17 and 20 years

	Raw values			Deseasonalized values		
	Year 14	**Year 17**	**Year 20**	**Year 14**	**Year 17**	**Year 20**
Male	*n = 412*	*n = 409*	*n = 422*	*n = 412*	*n = 409*	*n = 422*
Year 6 (n = 271)	0.441[1]	0.455 [1]	0.399 [1]	0.483 [1]	0.473 [1]	0.440 [1]
Year 14 (n = 412)		0.560 [1]	0.360 [1]		0.577 [1]	0.483 [1]
Year 17 (n = 409)			0.501 [1]			0.673 [1]
Square root of ICC [2]		0.667			0.691	
Female	*n = 366*	*n = 366*	*n = 371*	*n = 366*	*n = 366*	*n = 371*
Year 6 (n = 228)	0.473 [1]	0.394 [1]	0.358 [1]	0.493 [1]	0.430 [1]	0.399 [1]
Year 14 (n = 366)		0.412 [1]	0.346 [1]		0.463 [1]	0.487 [1]
Year 17 (n = 368)			0.537 [1]			0.629 [1]
Square root of ICC [2]		0.667			0.697	

[1] $P < 0.001$, Pearson correlation coefficients.

[2] Square root of ICC (intraclass correlation coefficient) obtained from linear mixed model with subject effects as random, age as timeline and adjusted for season for raw values.

Table 3 Associations between serum 25-hydroxyvitamin D at each time point and 20 year total body bone measures

	Regression coefficients (95% CI) per 25 nmol/L increase in serum 25(OH)D concentration			
	Year 6 (M = 271, F = 228)	Year 14 (M = 412, F = 366)	Year 17 (M = 409, F = 366)	Year 20 (M = 422, F = 371)
Male (raw values)				
Total body BMC, g	**53.9 (6.2, 101.6)** [1]	12.8 (-10.6, 36.2)	**40.7 (13.5, 68.0)**	**43.5 (12.0, 74.9)**
Total body BMD, mg/cm^2	**18.6 (1.9, 35.3)**	4.7 (-3.5, 13.0)	**14.7 (5.1, 24.3)**	**15.7 (4.7, 26.7)**
Male (deseasonalized values)				
Total body BMC, g	**48.4 (0.01, 96.8)**	6.0 (-19.8, 31.9)	**42.6 (13.4, 71.7)**	**38.8 (6.1, 71.6)**
Total body BMD, mg/cm^2	16.6 (-0.3, 33.5)	2.2 (-6.9, 11.3)	**15.2 (5.0, 25.5)**	**14.0 (2.6, 25.5)**
Female (raw values)				
Total body BMC, g	2.4 (-39.8, 44.6)	1.6 (-16.4, 19.7)	-8.1 (-31.1, 14.8)	7.9 (-13.0, 28.8)
Total body BMD, mg/cm^2	1.1 (-15.1, 17.2)	0.02 (-6.9, 6.9)	-2.2 (-11.0, 6.6)	2.7 (-5.2, 10.7)
Female (deseasonalized values)				
Total body BMC, g	-5.2 (-50.6, 40.2)	5.5 (-14.0, 25.0)	-4.3 (-27.5, 19.0)	6.1 (-14.9, 27.2)
Total body BMD, mg/cm^2	-2.4 (-19.7, 14.9)	1.2 (-6.3, 8.7)	-0.8 (-9.6, 8.1)	2.1(-5.9, 10.1)

BMC, bone mineral content; BMD, bone mineral density.

[1] Multiple linear regression models adjusted for age, height, weight, TV watching, organised sports participation or physical activity level at time of 25(OH)D measurement and 20 year, calcium intake, smoking, and alcohol consumption at 20 years, and additionally adjusted for bone area for the models for BMC and season of blood collection for models using raw values.

Table 4 Comparisons between those with 25-hydroxyvitamin D below or above 50 nmol/L at 17 and 20 years and total body bone measures at age 20

	25(OH)D at year 17		25(OH)D at year 20	
	<50 nmol/L	**≥50 nmol/L**	**<50 nmol/L**	**≥50 nmol/L**
Male, n	65	344	76	346
Total body BMC, g	3106 ± 37	3226 ± 16 [1]	3096 ± 32	3215 ± 15 [2]
Total body BMD, mg/cm^2	1094 ± 13	1136 ± 6 [1]	1088 ± 11	1133 ± 5 [2]
Female, n	54	312	56	315
Total body BMC, g	2672± 30	2718 ± 12	2701 ± 29	2707 ± 11
Total body BMD, mg/cm^2	1006 ± 11	1025 ± 5	1018 ± 11	1023 ± 4

Values are estimated mean ± SEM. BMC, bone mineral content; BMD, bone mineral density.

[1] P = 0.004, [2] P = 0.001 compared with 25(OH)D < 50 nmol/L at the same age, analysis of covariance (ANCOVA) adjusted for season of blood collection, age, height, weight, TV watching, organised sports participation or physical activity level at time of 25(OH)D measurement and 20 year, calcium intake, smoking, and alcohol consumption at 20 years, and additionally adjusted for bone area for the models for BMC.

Figure legends

Figure 1 Mean serum 25-hydroxy vitamin D (25(OH)D) both sexes combined at each time point by trajectory classes, error bar represents standard deviation.

Figure 2 Comparison of estimated means of total body bone mineral content (BMC) and density (BMD) by vitamin D status trajectory classes. N = 136, 62, 64 and 174 for males and 123, 63, 74 and 125 for females in the consistently lower, decreasing, increasing and consistently higher groups, respectively. Error bar represents standard error, analysis of co-variance with Bonferroni post hoc test, adjusted for age, height, body weight, bone area (for the models for BMC only), physical activity, TV watching, calcium intake, smoking and alcohol consumption at 20 years.

Figure 1

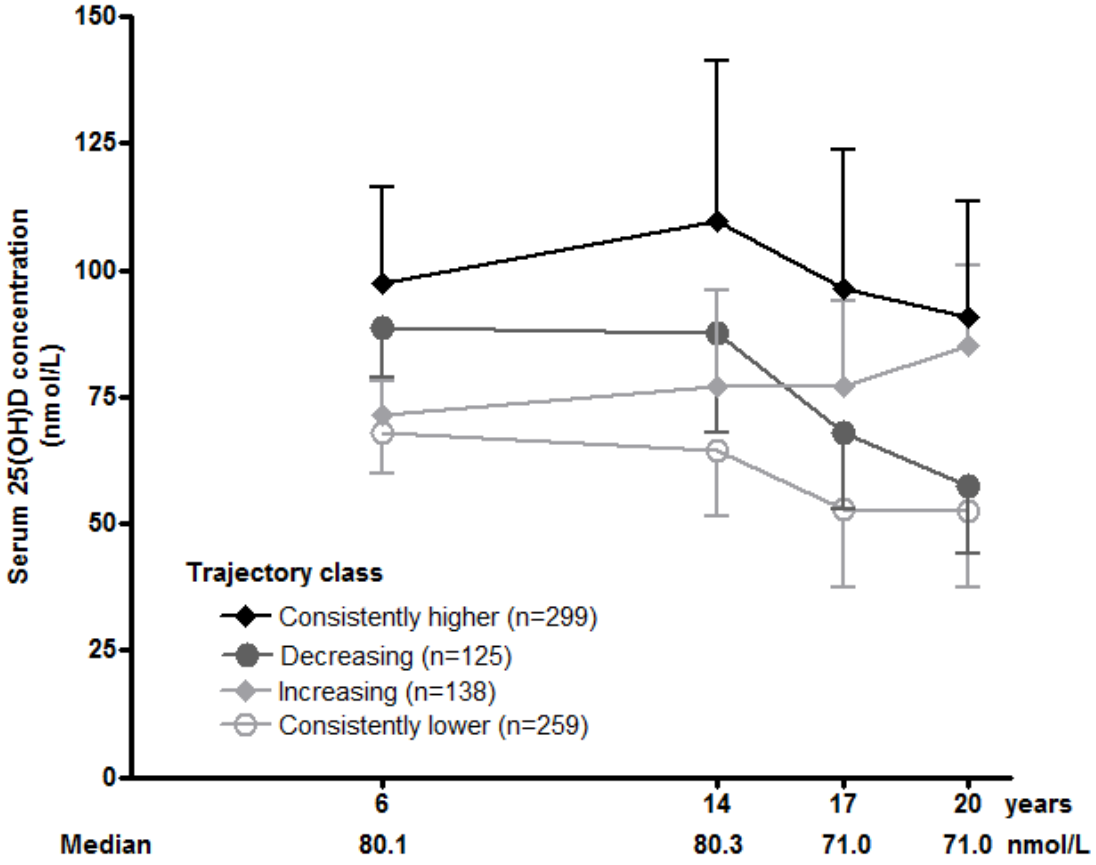
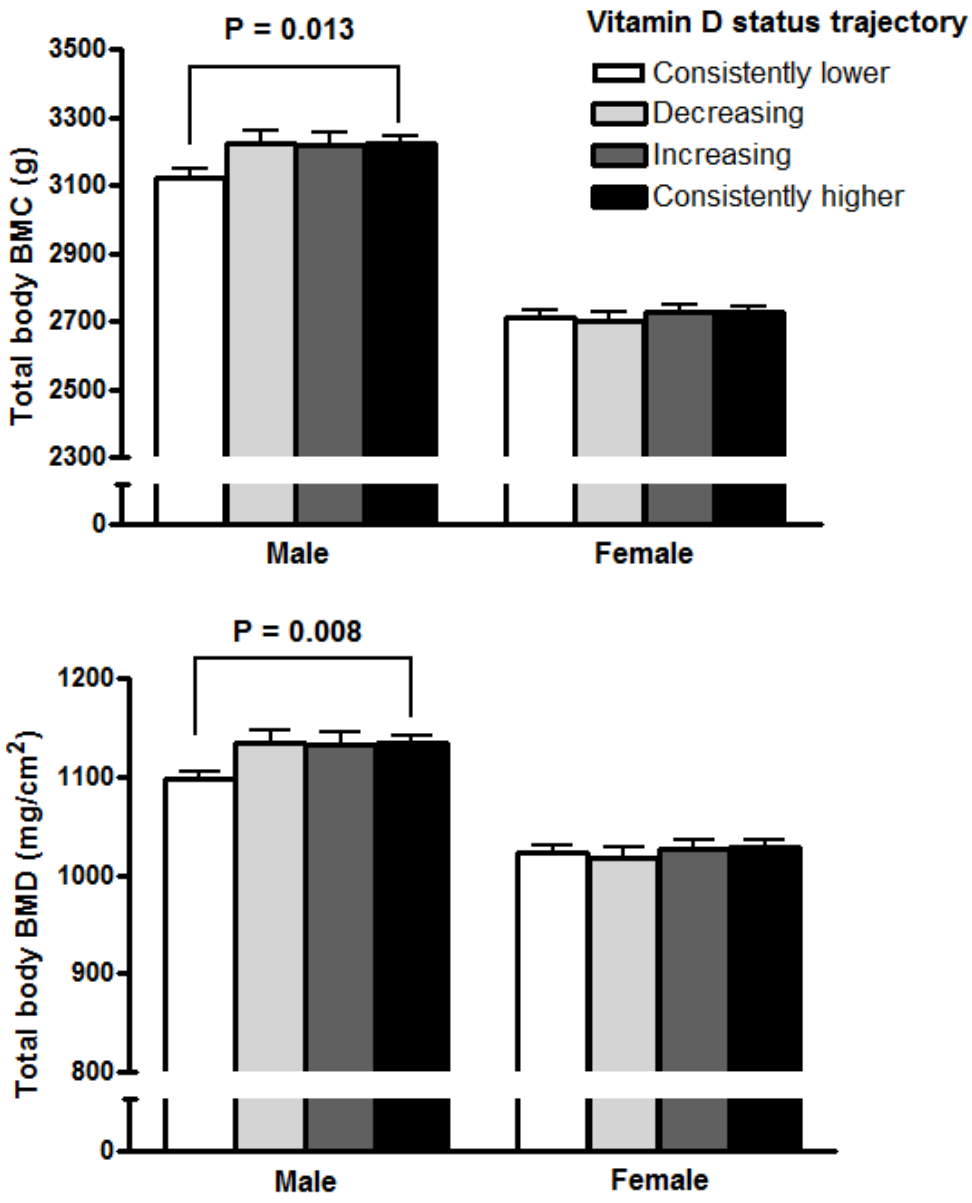

Serum 25(OH)D concentration (nmol/L)
150
125
100
75
50
25
0
Trajectory class
Consistently higher (n=299)
Decreasing (n=125)
Increasing (n=138)
Consistently lower (n=259)
6
14
17
20
years
Median
80.1
80.3
71.0
71.0
nmol/L

Figure 2


P = 0.013
Vitamin D status trajectory
Consistently lower
Decreasing
Increasing
Consistently higher
Total body BMC (g)
3500
3300
3100
2900
2700
2500
2300
0
Male
Female
P = 0.008
Total body BMD (mg/cm2)
1200
1100
1000
900
800
0
Male
Female