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1 **The independent and interactive associations of physical activity intensity and vitamin D status with bone**  
2 **mineral density in prepubertal children: The PANIC Study**

3

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16

17 *Abstract*

18

19 **Purpose:** The sex-specific independent and interactive associations of physical activity (PA) intensity and

20 serum 25-hydroxyvitamin D (25(OH)D) levels with areal bone mineral density (aBMD) were investigated in

21 prepubertal children.

22 **Methods:** The participants were 366 prepubertal Finnish children (190 boys, 176 girls) aged 6-8 years. Linear

23 regression analysed the associations of sedentary time (ST), light PA (LPA), moderate PA (MPA), moderate-to-

24 vigorous PA (MVPA) and vigorous PA (VPA) measured by accelerometry, and serum 25(OH)D with total

25 body less head (TBLH) and lower-limb aBMD, measured by dual-energy X-ray absorptiometry.

26 **Results:** There was no interaction between PA intensity or serum 25(OH)D and sex with aBMD. MPA and

27 MVPA were positively associated with TBLH and lower-limb aBMD ( $\beta = 0.11$ , 95% CI 0.02-0.20,  $p = 0.01$ ).

28 Serum 25(OH)D was positively associated with TBLH and lower-limb aBMD ( $\beta = 0.09$ , 95% CI 0.01-0.18,  $p =$

29 0.03). There were no interactions between PA intensity and serum 25(OH)D with aBMD.

30 **Conclusion:** Vitamin D status, MPA and MVPA levels in active prepubertal children were positively associated

31 with aBMD. The influence of MVPA is due to the MPA component, though our findings regarding the role of

1 VPA should be interpreted with caution, as shorter accelerometer epochs are needed to more accurately assess  
2 VPA. This study adds evidence to the promotion of MPA and behaviours to encourage optimal vitamin D status  
3 in supporting skeletal health in childhood, though these need not be used in conjunction to be beneficial, and a  
4 sex-specific approach is not necessary in prepubertal children.

5  
6 Trial registration number: NCT01803776

7 Date of registration: 4/03/2013

8 **Key words:** Childhood • Bone mass • Accelerometry • DXA • Growth

9  
10 Declarations

11

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22

23 *Conflicts of interest/competing interests*

24 The authors declare that there are no conflicts of interest.

25

26 *Ethics approval*

27 The study was conducted according to the ethical guidelines of the Declaration of Helsinki. The study protocol  
28 was approved by the Research Ethics Committee of the Hospital District of Northern Savo.

29

30 *Consent to participate*

1 The parents or caregivers of the children gave their written informed consent, and the children provided their  
2 assent to participation.

3

4 *Consent for publication*

5 Not applicable.

6

7 *Availability of data and material*

8 The datasets analysed during the current study are available from the corresponding author on reasonable  
9 request.

10

11 *Code availability*

12 Not applicable.

13

14 *Mini Abstract*

15 It is unclear how physical activity intensity and vitamin D status are related to bone health in prepubertal  
16 children. We found positive associations between vitamin D status and moderate-to-vigorous physical activity  
17 with bone in boys and girls. This highlights the importance of lifestyle factors for skeletal health prepuberty.

18

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24 Cambridge, for her assistance in processing the physical activity data.

25

## 1 Introduction

2

3 Bone mineral content (BMC) and areal bone mineral density (aBMD) increase substantially during childhood  
4 and adolescence [1]. In the two years surrounding the pubertal growth spurt, more than one quarter of adult bone  
5 is accrued [1]. The timing of the pubertal growth spurt is sex-specific, with peak BMC velocity occurring  
6 around 8 months after peak height velocity, on average at age 12.5 years in females, and at age 14.1 years in  
7 males, though there is considerable variation between individuals and populations [2]. The pubertal period is a  
8 time of elevated fracture risk that is partly due to increases in linear length of long bones outpacing BMC and  
9 aBMD accrual over this period [3]. Children who accrue more bone during prepuberty tend to have higher BMC  
10 and aBMD and a lower fracture risk in adolescence [4]. Further, the bone accrued during growth may track into  
11 adulthood, increasing peak bone mass and therefore reducing the risk of osteoporosis in later life [3].

12

13 Physical activity (PA) measured by accelerometry has been positively associated with aBMD in boys and girls  
14 aged 4 to 6 [5] and 11 years [6] as well as in male and female adolescents aged 12.5-17.5 years [7]. Vigorous  
15 PA (VPA), in addition to moderate-to-vigorous PA (MVPA), has been positively associated with aBMD in  
16 adolescents [7]. However, in children aged 11, moderate PA (MPA) had a stronger influence on aBMD than  
17 light PA (LPA) and VPA [6]. As such, the individual associations of LPA, MPA, MVPA and VPA with aBMD  
18 are currently unclear. VPA was positively associated with hip aBMD but not with total body aBMD in boys and  
19 girls aged 4-6 [5], suggesting that the associations of PA with aBMD may be more pronounced in weight-  
20 bearing bones. This is supported by athlete studies, which have observed greater aBMD in athletes who  
21 participate in impact loading sports compared to those who participate in active loading sports or healthy  
22 controls, suggesting mechanical loading is important for bone adaptation [8]. Furthermore, previous studies  
23 have used samples of children at various pubertal stages [6,7]. However, the associations of PA with aBMD  
24 may vary depending on age and pubertal status [9], and it remains unclear whether the associations between PA  
25 with aBMD are sex-specific [10]. It is therefore important to investigate the associations of PA intensity with  
26 total body and site-specific aBMD in prepubertal boys and girls.

27

28 Serum 25-hydroxyvitamin D (25(OH)D) has been associated positively with aBMD in cross-sectional studies  
29 among boys and girls aged 1.8-6 years [11] and 7-19 years [12]. However, these studies did not address possible  
30 sex differences in these associations, so it is unknown whether the relationships of serum 25(OH)D with aBMD

1 differ between boys and girls. This requires further exploration, as serum 25(OH)D has been shown to be  
2 inversely associated with the rate of BMC accrual in prepubertal girls [13]. Therefore, the relationships of serum  
3 25(OH)D with aBMD in prepubertal boys and girls remain unclear.

4  
5 In addition to their independent relationships with aBMD, it is also possible that PA and vitamin D interact to  
6 influence aBMD. One cross-sectional study found that adolescents meeting the PA guidelines and having serum  
7 25(OH)D  $\geq$  75 nmol/L had greater BMC than adolescents meeting the PA guidelines and having serum  
8 25(OH)D  $<$  75 nmol/L [14]. As male and female adolescents were not analysed separately in the previous cross-  
9 sectional study [14], it remains unknown whether these associations differ depending on sex. This is of interest  
10 due to the sex-specific patterns of BMC and aBMD accrual during growth [2]. It also remains unknown whether  
11 PA and serum 25(OH)D interact to influence aBMD in prepubertal children, as maturation independently affects  
12 aBMD [1].

13  
14 Thus, the aim of this study was to investigate the sex-specific independent and interactive associations of PA  
15 intensity and serum 25(OH)D concentration on total body less head (TBLH) and lower-limb aBMD in  
16 prepubertal children.

17  
18 Methods

19  
20 Study Design and Participants

21  
22 This study utilized baseline data (2007 to 2009) of the Physical Activity and Nutrition in Children (PANIC)  
23 Study, which is an ongoing longitudinal study in a population sample of Finnish children (ClinicalTrials.gov  
24 registration number NCT01803776). Children aged 6-8 years who were registered for the first grade in one of  
25 the 16 public schools in Kuopio, Finland were invited to participate. Children were eligible to participate in the  
26 study if they had no disability that could prevent their participation in the assessments or the intervention and  
27 had a custodian who was able to communicate in Finnish to fill out the questionnaires and participate in the  
28 intervention. Of the 736 children invited to participate, 512 (70%) attended the baseline examinations. We  
29 excluded two children at baseline due physical disabilities, and six children withdrew from the study. The total  
30 sample from baseline was therefore 504 children. The participants did not differ in sex, age, height standard

1 deviation score (SDS) and body mass index (BMI) SDS from all children who started the first grade in Kuopio  
2 between 2007 and 2009. The study was conducted according to the ethical guidelines of the Declaration of  
3 Helsinki. The study protocol was approved by the Research Ethics Committee of the Hospital District of  
4 Northern Savo. The parents or caregivers of the children provided their written informed consent, and the  
5 children provided their assent to participation.

6

7 The children were included in these analyses if they had complete data for general health and pubertal status,  
8 anthropometric measures, dual-energy X-ray absorptiometry (DXA) measurements, serum 25(OH)D, and PA  
9 measured by combined heart rate and movement sensor, and if they were prepubertal based on stages described  
10 by Tanner [15]. The children were excluded from the analyses if they currently or previously used oral  
11 corticosteroids, as this can independently affect aBMD [3]. Altogether 366 prepubertal children (190 boys, 176  
12 girls) were included in the analysis. The reduction in sample size from baseline examinations was largely  
13 accounted for by missing serum 25(OH)D data and invalid or missing PA data. Exclusion and inclusion criteria  
14 are displayed in Figure 1.

15

#### 16 Assessment of General Health and Pubertal Status

17

18 General health was assessed by questionnaires completed by parents. The questionnaires included items on  
19 children's chronic diseases and allergies diagnosed by a physician and detailed information on children's  
20 medication use. A research physician performed a clinical examination and classified the girls as entered clinical  
21 puberty if their breast development had started and boys if their testicular volume assessed by palpation and by  
22 an orchidometer was  $\geq 4$  mL, according to criteria described by Tanner [15].

23

#### 24 Anthropometry

25

26 Stature was measured using the Frankfurt plane with a wall-mounted stadiometer, three times to an accuracy of  
27 0.1 cm. Body weight was measured twice (InBody 720 bioelectrical impedance device, Biospace, Seoul, South  
28 Korea) to an accuracy of 0.1 kg. For stature and body weight, the mean of the values was used in analyses. BMI  
29 ( $\text{kg}/\text{m}^2$ ) was calculated, and the BMI cut-offs of the International Obesity Task Force were applied to classify  
30 children as normal weight, overweight, or obese [16,17].

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## Assessment of Bone Mineral Density

TBLH and lower-limb aBMD ( $\text{g}/\text{cm}^2$ ), and TBLH and lower-limb BMC (g) were measured using the Lunar Prodigy Advance DXA device (GE Medical Systems, Madison, WI, USA) and the Encore software, Version 10.51.006 (GE Company, Madison, WI, USA). DXA provides valid and reliable data on aBMD in children (coefficient of variation 0.01 % – 4.37%) [18,19]. The primary outcome variables was TBLH aBMD, as recommended by the International Society for Clinical Densitometry [19]. Lower-limb aBMD derived from the whole body DXA scan were also measured, as evidence indicates that the influence of PA on aBMD may be site-specific [5]. The lower-limb is automatically defined using Encore software (GE Company, Madison, WI, USA), and an average of both sides was used. We included measurements of BMC in order to check whether the associations were broadly similar between PA and serum 25(OH)D with aBMD and BMC. To allow comparisons with other populations, we used paediatric cross-calibration equations to account for differences in DXA outcomes between manufacturers [20].

## Assessment of Physical Activity and Sedentary Time

PA was assessed using an individually calibrated combined heart rate and movement sensor (Actiheart, CamNtech Ltd, Papworth, UK) [21,22]. The device was attached to the chest with standard electrocardiogram electrodes (Bio Protech Inc, Wonju, South Korea) and set to record heart rate and body movement in 60-second epochs. Participants were instructed to wear the monitor continuously for a minimum of four consecutive days, although some children wore the monitor for up to nine days. In order to capture different PA patterns between weekends and weekdays, the wear period was scheduled to include an entire weekend [23]. Heart rate data were pre-processed using robust Gaussian Process regression [24] and individually calibrated to PA energy expenditure using a maximal exercise test on a cycle ergometer [25]. Intensity was modelled from the combined sensing signal using a branched equation framework [26] as described in previous publications from this cohort [27]. However, we primarily used the uniaxial acceleration signal as the PA exposure in present analyses, as it has been suggested that mechanical loading has a stronger influence on bone than metabolic loading [28,10].



1 The acceleration signal was summarised as a fraction of time spent in 25 acceleration thresholds ( $\text{m/s}^2$ ) across  
2 the movement intensity continuum. Thresholds for sedentary time (ST) ( $\leq 0.06 \text{ m/s}^2$ ), LPA ( $> 0.06 \text{ m/s}^2$  and  $\leq$   
3  $0.75 \text{ m/s}^2$ ), MPA ( $> 0.75 \text{ m/s}^2$  and  $\leq 4.00 \text{ m/s}^2$ ) and VPA ( $> 4.00 \text{ m/s}^2$ ) were used to categorise the data into the  
4 fractions of time spent in each intensity. These thresholds are based on previous research which assessed  
5 uniaxial acceleration during treadmill walking and running at different speeds [28,29]. The combined sensing  
6 data were used to characterise the proportion of children meeting the physical activity guidelines of 60 minutes  
7 MVPA per day, based on time spent  $> 4$  metabolic equivalents (METs), with  $3.5 \text{ ml O}_2/\text{min}/\text{kg}$  used to define  
8 resting metabolic rate (1 MET) [30]. Average sleep duration was subtracted from sedentary time to give  
9 sedentary time excluding sleep, as described in previous publications from this cohort [27].

10

11 Non-wear time was classified as zero-acceleration lasting  $> 90$  minutes combined with non-physiological heart  
12 rate and diurnal imbalance in non-wear was minimised when summarising the data to reduce bias and error as  
13 previously described [27,31]. Criteria for a valid PA measurement were  $\geq 48$  hours of good-quality data with  $\geq$   
14  $32$  hours of weekday data and  $\geq 16$  hours of weekend data as well as  $\geq 12$  hours of morning, noon, afternoon  
15 and evening wear time to protect against bias from over-representation from specific times of day and to  
16 optimise the diurnal bias minimisation procedure [31,27].

17

#### 18 Serum 25(OH)D Assessment

19

20 Serum 25(OH)D concentration was assessed from venous blood samples. Children were required to fast for 12  
21 hours prior to blood sampling. Blood was immediately centrifuged and stored at  $-75^\circ\text{C}$  until biochemical  
22 analyses. Serum 25(OH)D concentration was analysed by LIAISON 25(OH)D TOTAL Assay (DiaSorin Inc.,  
23 Stillwater, USA), a chemical luminescence immunoassay, using an automatic immunoanalyser (DiaSorin  
24 S.p.A.). Total variation, including intra-assay and inter-assay variation, for this method is 8.2–11.0% in the  
25 concentration range of 21–123 nmol/L. The 25(OH)D analyses were performed in Eastern Finland Laboratory  
26 Centre Joint Authority Enterprise (ISLAB). ISLAB has been participating in the Vitamin D External Quality  
27 Assessment Scheme (DEQAS) since 2008 with DiaSorin LIAISON 25(OH)D assay meeting the performance  
28 targets. The closest DEQAS survey was carried out in January 2011, soon after analysing the study samples, and  
29 it showed a 6.2% positive bias from the mean value of all methods, 7.1% positive bias from the mean of the  
30 LIAISON method and a 1.1% negative bias from the LC-MS/MS method. We applied cut-offs to the data based

1 on thresholds for deficiency ( $< 25$  nmol/L), insufficiency ( $25$  nmol/L –  $49.9$  nmol/L) and sufficiency ( $> 50$   
2 nmol/L) described by the British Paediatric and Adolescent Bone Group and the Institute of Medicine [32,33].  
3 As the threshold for sufficiency varies from  $> 50$  nmol/L to  $> 75$  nmol/L, we applied thresholds for  $50$  nmol/L –  
4  $74.9$  nmol/L, and  $\geq 75$  nmol/L [33,34].

5

6

## 7 Statistical Analyses

8

9 The IBM SPSS Statistics for Mac software, Version 26.0 (IBM Corp.), was used for the statistical analyses.

10 Differences in age, stature, body weight, BMI, TBLH aBMD, lower-limb aBMD, ST, LPA, MPA, MVPA, VPA  
11 and serum 25(OH)D between the included and excluded children were tested. Body weight, BMI, VPA and  
12 serum 25(OH)D had skewed distributions, whereas other continuous variables were normally distributed.

13 For normally distributed variables, the mean and standard deviation (SD) were calculated for the total sample,  
14 and for boys and girls separately. Independent samples t-tests were used to test for sex differences. For skewed  
15 variables, the median and interquartile range (IQR) were calculated for the total sample, and for girls and boys  
16 separately. Mann-Whitney U tests were used to test for sex differences. The Fisher's exact test was used to test  
17 for differences in categorical variables between sexes.

18

19 Linear regression was used to assess the associations of ST, LPA, MPA, MVPA, VPA and serum 25(OH)D with  
20 TBLH aBMD and lower-limb aBMD. Interaction terms for sex with each PA intensity and serum 25(OH)D  
21 were computed from standardised values (Z-scores) in order to assess whether the association between PA,  
22 serum 25(OH)D and bone outcomes differed based on sex. These interaction terms were not significant, so all  
23 further analysis was carried out in the total sample. We have also presented the results split by sex, due to the  
24 sex differences in aBMD in our sample, and the sex-specific patterns of bone accrual during growth [1]. The  
25 linear interaction of PA and serum 25(OH)D on TBLH aBMD and lower-limb aBMD was explored. For the  
26 total sample analyses, in Model 1, the predictor variable was entered unadjusted, and in Model 2, the data were  
27 adjusted for age, stature and sex. For testing the interactions, the main effects and the interaction term were  
28 entered in Model 1, with adjustment for age, stature and sex in Model 2. For the analyses stratified by sex, the  
29 same process was followed, though sex was not included as a covariate. We repeated all analyses with TBLH  
30 and lower-limb BMC as the outcome variables (results not presented). In all models, the variance inflation

1 factor (VIF) was  $< 5$ , and the average VIF was not considerably greater than 1, indicating that the model was  
2 robust [35]. Independence of residuals was confirmed with the Durbin-Watson statistic, with all values between  
3 1 and 3. Standardised regression coefficients ( $\beta$ ) with 95% confidence intervals (CI) were reported and  
4 statistical significance was set at alpha level 0.05.

5

6

7 Results

8

### 9 *Characteristics of Children*

10 Descriptive characteristics of the 366 prepubertal children included in this study are provided in Table 1. These  
11 did not differ significantly from the children excluded from this study for age, stature, body weight, BMI status,  
12 TBLH aBMD, lower-limb aBMD, TBLH BMC, lower-limb BMC, ST, LPA, MPA, MVPA, VPA and serum  
13 25(OH)D. For the participants included in the study, stature, body weight, TBLH aBMD, TBLH BMC, lower-  
14 limb BMC, MPA and MVPA were greater in boys than girls, and LPA was lower in boys than girls. The  
15 proportion of children meeting the PA guidelines and the proportion of children in categories for serum  
16 25(OH)D levels differed between boys and girls, though the mean levels of serum 25(OH)D did not differ  
17 between boys and girls (see Table 1).

18

### 19 *Associations of PA Variables and Serum 25(OH)D with aBMD in Total Sample*

20 Results from the regression analyses in the total sample are provided in Table 2. MPA and MVPA were  
21 positively associated with TBLH aBMD and lower-limb aBMD, adjusted for age, sex and stature. A 10-minute  
22 difference in either MPA or MVPA was associated with a 0.001 g/cm<sup>2</sup> higher TBLH aBMD and 0.002 g/cm<sup>2</sup>  
23 higher lower-limb aBMD. Serum 25(OH)D was positively associated with TBLH and lower-limb aBMD,  
24 adjusted for age, sex and stature. A 10 nmol/L difference in serum 25(OH)D was associated with 0.002 g/cm<sup>2</sup>  
25 higher TBLH aBMD and 0.003 g/cm<sup>2</sup> higher lower-limb aBMD. There were no interactions between PA  
26 variables and serum 25(OH)D with TBLH aBMD and lower-limb aBMD.

27

### 28 *Associations of PA Variables and Serum 25(OH)D with aBMD in Boys*

29 Results from the regression analysis in boys are provided in Table 3. Serum 25(OH)D was positively associated  
30 with lower-limb aBMD, adjusted for age and stature. A 10 nmol/L difference in serum 25(OH)D was associated

1 with 0.003 g/cm<sup>2</sup> higher lower-limb aBMD. There were no associations between any PA variable and with  
2 TBLH and lower-limb aBMD. There were no interactions between PA variables and serum 25(OH)D with  
3 TBLH and lower-limb aBMD.

4  
5  
6 *Associations of PA Variables and Serum 25(OH)D with aBMD in Girls*

7 Results from the regression analysis in girls are provided in Table 4. MPA and MVPA were positively  
8 associated with TBLH and lower-limb aBMD, adjusted for age and stature. A 10-minute difference in either  
9 MPA or MVPA was associated with a 0.002 g/cm<sup>2</sup> higher TBLH and lower-limb aBMD. There were no  
10 associations between serum 25(OH)D with TBLH aBMD and lower-limb aBMD. There were no interactions  
11 between PA variables and serum 25(OH)D with TBLH and lower-limb aBMD.

12  
13 We repeated all analyses with TBLH and lower-limb BMC as the outcome variables, and found the associations  
14 were broadly similar to those with TBLH and lower-limb aBMD as the outcome variable, with the exception of  
15 serum 25(OH)D, which was not associated with any measures of BMC.

16  
17 Discussion

18  
19 This study is the first to address the sex-specific independent and interactive associations of objectively  
20 measured PA intensity and serum 25(OH)D with aBMD in prepubertal children. Positive associations of serum  
21 25(OH)D, MPA and MVPA with TBLH aBMD and lower-limb aBMD were observed in the total sample, and  
22 these associations did not differ between sexes. The magnitude of the association was similar for serum  
23 25(OH)D, MPA and MVPA, though serum 25(OH)D did not interact with PA intensity to determine aBMD.  
24 Our findings imply that these are independent determinants of aBMD in prepubertal children.

25  
26 Reference values from the Bone Mineral Density in Childhood Study indicate that TBLH aBMD in the 50<sup>th</sup>  
27 percentile of non-black children aged seven were 0.58 g/cm<sup>2</sup> for boys, and 0.61 g/cm<sup>2</sup> for girls [36]. After  
28 applying cross-calibration equations to allow comparison between DXA manufactures, the median levels of  
29 TBLH aBMD in our study were similar to the reference values, with 0.60 g/cm<sup>2</sup> for boys, and 0.59 g/cm<sup>2</sup> for  
30 girls [20]. Altogether 79.5% of our sample met the recommended 60 minutes of MVPA per day, based on the  
31 combined sensing data [30]. The International Study of Childhood Obesity, Lifestyle and the Environment

1 (ISCOLE) found that the average percentage of children aged 9-11 years from 12 countries meeting the PA  
2 guidelines was considerably lower than this (44%) [37]. It is difficult to draw comparisons between studies, as  
3 the prevalence of children meeting the PA guidelines varies dependent on the PA intensity thresholds used [38].  
4 Even so, it is likely that our sample were more active than the ISCOLE sample in Finland, with 61% of children  
5 assessed meeting the PA guidelines compared to the 79.5% in our sample [37].

6

7 The threshold for vitamin D deficiency varies from 25 to 50 nmol/L, and a review of evidence from the Institute  
8 of Medicine suggests that there are risks to bone health in children at serum 25(OH)D concentration < 30  
9 nmol/L [33,34,39]. Although living in a northern country is considered a risk factor for vitamin D deficiency  
10 [39], only < 1% of our sample had serum 25(OH)D below 25 nmol/L, with 21% below 50 nmol/L. Previous  
11 research in this sample showed that vitamin D intake was higher than in other European countries, which may  
12 explain the relatively high levels of 25(OH)D in this study [40]. The majority of milk products as well as  
13 margarines available in Finland are fortified with vitamin D, and consumption of milk products was the main  
14 determinant of serum 25(OH)D in our study population [40]. At the time of our data collection, vitamin D  
15 supplement use was recommended for this age group from October to March for children who did not use  
16 fortified milk products [40]. Vitamin D intake from supplements was low: about 40% of the children did not use  
17 supplements at all, and many of those who used supplements did not use them regularly [40]. The  
18 recommendations for both vitamin D intake from food and supplements and the level of vitamin D fortification  
19 in milk products and margarines in Finland has now been increased, and a wider range of other fortified  
20 products are now available [41,42].

21

22

### 23 *Physical Activity Intensity and aBMD*

24 The positive associations of PA with aBMD we found in the total sample and in girls are in agreement with the  
25 results of previous population studies on children and adolescents [5,6,10,43]. MPA and MVPA were positively  
26 associated with TBLH and lower-limb aBMD in the total sample and in girls. The Avon Longitudinal Study of  
27 Parents and Children (ALSPAC) reported that MVPA was positively associated with TBLH aBMD and lower-  
28 limb aBMD in children aged 11 (40% boys and 10% girls prepubertal), and MPA was shown to have a stronger  
29 influence on TBLH aBMD and lower-limb aBMD than LPA and VPA [6]. Similarly, the positive association  
30 we observed between MVPA with aBMD was due to the MPA component, highlighting the importance of MPA

1 compared to VPA for aBMD. Findings from the Southampton Women's Survey (SWS) study showed no  
2 associations between MVPA and total body bone indices in children aged four, though MVPA was positively  
3 related to hip BMC and aBMD in boys and girls [10]. These differences could be due to heterogeneity in the age  
4 and pubertal status of the participants in these studies. In younger children, such as those in the SWS study, the  
5 smaller body size and the lower BMC may limit the ability to detect a relationship between MVPA and total  
6 body bone indices [10]. The importance of MVPA has also been demonstrated longitudinally from age 12 to 25  
7 years by the ALSPAC study [43]. Greater levels of MVPA in early- or mid-adolescence associated with greater  
8 femoral neck aBMD in early adulthood, and there were no associations between LPA trajectory and femoral  
9 neck aBMD at age 25 years, though as the independent roles of MPA and VPA were not examined and TBLH  
10 measures were not included direct comparisons cannot be drawn [43]. Even so, our findings support those of  
11 the ALSPAC study, highlighting the importance of MVPA, but not LPA, for bone health.

12  
13 As it is mainly mechanical loading which is thought to increase aBMD [3], we expected that VPA, resulting in  
14 increased mechanical loads on the bone, would have a stronger positive association with aBMD than MPA. The  
15 method of capturing PA may have influenced our findings [6]. Although all but 15 children recorded some VPA  
16 (data not presented), as we used a 60-second epoch the ability to detect short bursts of VPA is reduced [44],  
17 leading to a potential misclassification of VPA as MPA. This could affect our findings, by overestimating the  
18 association between MPA with aBMD and underestimating the association between VPA with aBMD. There  
19 are also differences in the methods used to process accelerometer data between studies. Whereas we justified  
20 our PA intensity thresholds on the basis of acceleration, as did the SWS, the ALSPAC study justified PA  
21 intensity thresholds on the basis of METs [6,10]. Theoretically, using the acceleration data may strengthen the  
22 relationships of PA measures with aBMD, as mechanical loading may be more important for bone health than  
23 metabolic energy expenditure [28]. However, as bouts of VPA typically last less than 10 seconds in children  
24 [44], the 60-second epoch may limit our ability to capture the high-intensity mechanical loading which is likely  
25 important for aBMD [28]. Furthermore, ground reaction forces generated during jumping, which are thought to  
26 have an osteogenic effect, were not associated with accelerometer counts in children [28]. This may further  
27 weaken the observed association between VPA and aBMD. This may also explain why the associations between  
28 lower-limb aBMD and TBLH aBMD with MPA and MVPA were of equal magnitude. Although we expected to  
29 observe stronger associations between PA intensity and aBMD in weight-bearing bones, we were likely limited

1 in our ability to capture the high-intensity mechanical loading which may have site-specific effects [28]. This  
2 may have resulted in the associations between PA and lower-limb aBMD being underestimated.

3

4 Although we did not find significant associations between MPA and MVPA with aBMD in boys only, the  
5 associations followed the same direction and a similar magnitude to those in the total sample and in girls only.

6 Our ability to detect these associations in boys is likely limited by our reduced statistical power when we  
7 stratified our analyses by sex. Previous studies have found the associations between PA intensity and aBMD to  
8 be similar between boys and girls [5,6]. In children aged 11 years the associations between PA variables and  
9 TBLH and lower-limb aBMD were similarly positive between boys and girls, with no interaction between PA  
10 intensity and sex with aBMD [6]. Further, although a formal sex-by-activity interaction was not tested, the Iowa  
11 Bone Development Study reported positive associations between total PA and VPA with total body and site-  
12 specific BMC and aBMD in boys and girls [5]. It is therefore unlikely that our findings reflect sex differences in  
13 the associations between PA intensity and aBMD. Our findings indicate that PA intensity, specifically MPA, is  
14 important to bone health in prepubertal children. However, given the aforementioned methodological  
15 limitations, it is possible that influence of VPA was underestimated. Further research with assessment of PA  
16 using shorter epochs and with comparison of acceleration-only thresholds with thresholds based on METs is  
17 needed to quantify the associations of VPA with aBMD.

18

19

#### 20 *Vitamin D Status and aBMD*

21 We found a positive association between serum 25(OH)D and TBLH and lower-limb aBMD in the total sample,  
22 and between serum 25(OH)D and lower-limb aBMD in boys only. Previous research has also reported positive  
23 associations between serum 25(OH)D and whole body aBMD in boys and girls aged 7-19 years [12], though  
24 these analyses were not stratified by sex, so direct comparisons cannot be drawn. Even so, as the associations  
25 between serum 25(OH)D with TBLH aBMD and lower-limb aBMD are a similar direction and magnitude in  
26 boys and girls, it is likely that the smaller sample size when we stratified by sex may have affected our ability to  
27 detect an association in girls only. This is supported by experimental data, with meta-analyses indicating that  
28 sex did not modify the effect of vitamin D supplementation on bone health in children and adolescents age 10 –  
29 17 years [45].

30

1 Our findings should be considered in context of the relatively low prevalence of vitamin D deficiency in our  
2 sample. In the aforementioned meta-analysis, the authors suggested that the positive influence of vitamin D on  
3 bone is less likely to occur in children with sufficient serum 25(OH)D [3,45]. Therefore, it is possible that  
4 stronger positive associations would be observed in a sample with greater prevalence of vitamin D deficiency.

5

#### 6 *Physical Activity Intensity, Vitamin D Status and aBMD*

7 We did not observe an interaction between PA intensity and serum 25(OH)D with TBLH and lower-limb aBMD  
8 in our prepubertal sample, in the total sample or in boys and girls separately. However, in adolescents, MVPA  
9 and serum 25(OH)D interact to influence BMC [14]. In physically active adolescents, high levels of serum  
10 25(OH)D were positively associated with BMC. This association was not present in physically inactive  
11 adolescents in the same study. The differences in observed interactions may be due to the differences in pubertal  
12 status of the samples, differences in the methods of processing the accelerometry data and differences in  
13 baseline PA levels between the studies. Further longitudinal research would be valuable in assessing how these  
14 associations change throughout puberty.

15

#### 16 *Clinical Relevance*

17 Despite positive associations between serum 25(OH)D, MPA and MVPA with TBLH and lower-limb aBMD,  
18 the clinical relevance of these findings requires consideration. To place our findings into a clinical context, an  
19 increase of MPA or MVPA by 10 minutes per day, as indicated by our study, equates to 2-3% of the annual  
20 change in TBLH aBMD in children aged seven [46]. Likewise, our study indicates a 10 nmol/L increase in  
21 serum 25(OH)D equates to 5-6% of the annual change in TBLH aBMD in children aged seven [46]. Even so,  
22 there are clearly other factors of more importance in determining aBMD in prepubertal children, such as lean  
23 mass and fat mass [47], and longitudinal research is needed to examine how these associations track into  
24 adulthood.

25

#### 26 *Strengths and Limitations*

27 Strengths of this study include the population-based sample of children, the analysis of boys and girls  
28 separately, the objective measurement of vitamin D status from blood samples, the objective measurement of  
29 PA and the measurement of bone outcomes, including the lower-limbs, by DXA.

30



1 There are several limitations that should be taken into account when interpreting the results. The assessment and  
2 interpretation of aBMD measurements are not simple in growing children. The determinants of volumetric bone  
3 mineral density (vBMD) remain unclear, as it is not possible to obtain true measures for it from DXA. Although  
4 aBMD is a partly body size-corrected measure for vBMD, it may underestimate the vBMD of short children and  
5 overestimate the vBMD of tall children. Therefore, the International Society for Clinical Densitometry  
6 recommends adjusting TBLH aBMD using height z-score [19]. We used TBLH aBMD measured by DXA, as  
7 recommended by the International Society for Clinical Densitometry, and additionally lower-limb values which  
8 are less commonly used in children [19]. To account for body size, we adjusted the data for age, sex and for  
9 stature, which are all components of height z-score.

10

11 As previously discussed, we used the 60-second epoch to capture PA. As children's MPA and VPA commonly  
12 occurs in short bouts (< 10 seconds), both of them may have been underestimated, possibly resulting in the  
13 attenuation of the associations of MPA and VPA with aBMD [44]. Although stature and age were controlled  
14 for, as in previous studies [5,6,14], residual confounding remains a potential limitation in all observational  
15 studies. Future research should use shorter epochs for investigating the relationships of the whole PA intensity  
16 spectrum with aBMD. The cross-sectional study design means that evidence for causal relationships cannot be  
17 provided and there is also a possibility of bidirectional relationships.

18

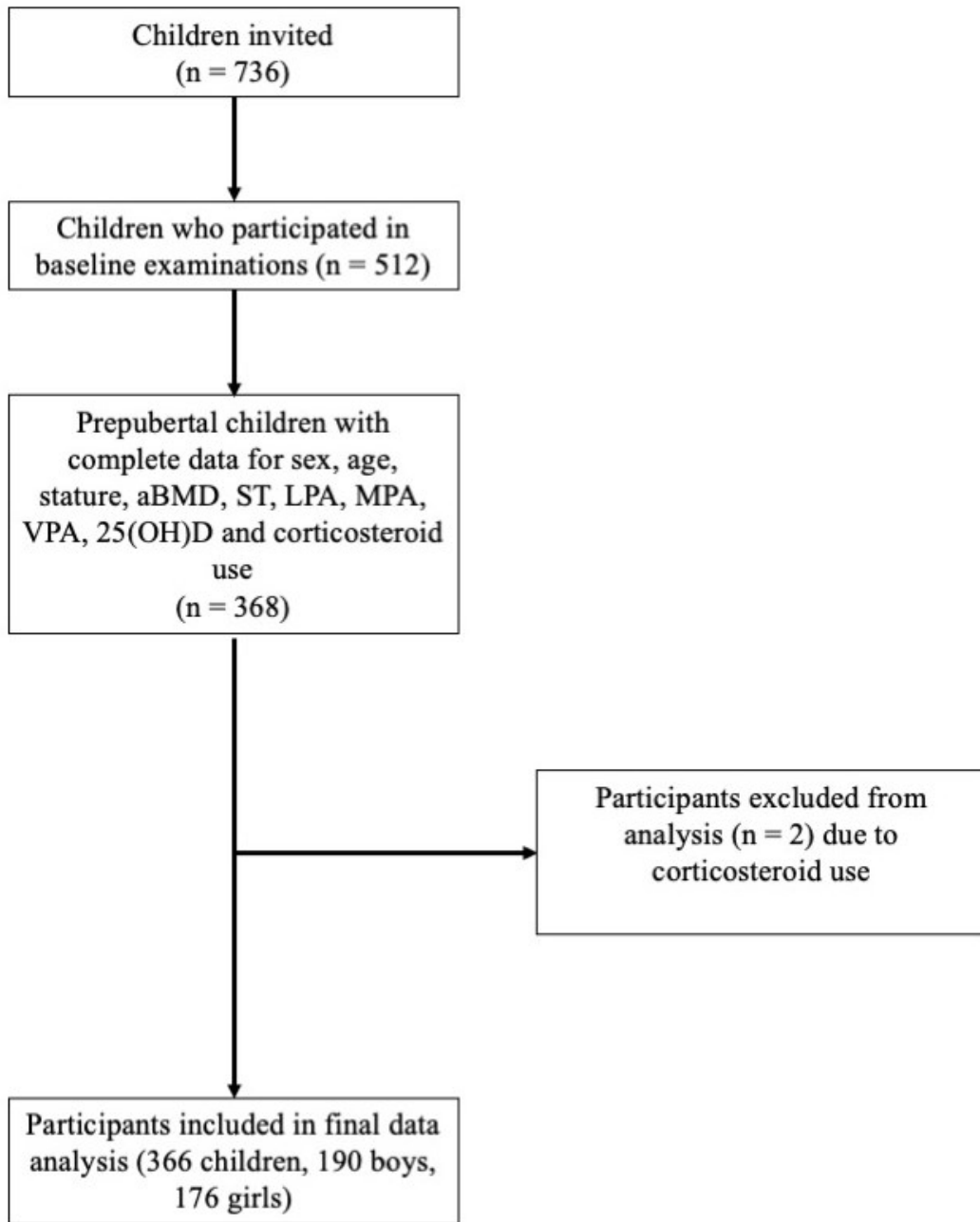
19 Although children had not participated in the intervention before collecting the baseline data, the reasons for not  
20 participating in the study were not asked, and a possibility of selection bias exists whereby more motivated  
21 families interested in health issues may be more eager to participate in this kind of study. This may be one of the  
22 reasons why the children in our study sample were more active than other cohorts in Finland and in other  
23 European countries [37]. However, our sample did not differ in age, sex distribution, or BMI-SDS from all  
24 children who started the first grade in the city of Kuopio in 2007–2009 based on data from the standard school  
25 health examinations, indicating our sample were representative of children of the same age in Kuopio, Finland.  
26 Even so, as our sample were more active and had lower levels of vitamin D deficiency than other cohorts both  
27 in Finland and in other European countries, it is unknown how these observations extend to children with low  
28 levels of PA and deficient vitamin D status [37,40]. This offers a potential direction for future research. As there  
29 are clearly other factors of more importance in determining aBMD in this sample, further research considering  
30 the mediating role of hormonal factors and body composition may extend the understanding of determinants of

1 aBMD in prepubertal children [47]. Investigating these associations longitudinally would be valuable in  
2 understanding the role of maturation in these relationships and exploring whether these associations track  
3 beyond prepuberty.

4  
5 In conclusion, the results of our study indicated that serum 25(OH)D, MPA and MVPA were positively  
6 associated with aBMD in active prepubertal children with sufficient vitamin D status, though these factors did  
7 not interact with each other, and these associations were not dependent on sex. The positive association between  
8 MVPA with aBMD was due to the MPA component, though as we were limited in our ability to capture short  
9 duration PA, it is possible that the importance of VPA was underestimated. Further research with shorter  
10 accelerometer epochs is needed to explore the associations of VPA with aBMD in prepubertal children. This  
11 study adds evidence to the promotion of MPA and behaviours to encourage optimal vitamin D status in  
12 supporting skeletal health in childhood, though our findings indicate that these strategies do not need to be used  
13 in conjunction to be beneficial, and a sex-specific approach is not needed in prepubertal children.

14

1 Tables and Figures



2

3 Figure 1. Participant flow chart.

4 Bone mineral density, aBMD; sedentary time, ST; light physical activity, LPA; moderate physical activity,

5 MPA; vigorous physical activity, VPA; serum 25-hydroxyvitamin D, 25(OH)D).

6

1 Table 1. Descriptive characteristics of children  
2

	Total		Boys		Girls		P value for sex difference
	n = 366		n = 190		n = 176		
	Mean / Median	SD / IQR	Mean / Median	SD / IQR	Mean / Median	SD / IQR	
Age (years)	7.7	0.4	7.7	0.4	7.6	0.4	0.083
Stature (cm)	128.8	5.5	129.9	5.4	127.7	5.4	< 0.001
Weight (kg)	26.0	23.6 – 29.0	26.9	24.1 – 29.9	25.2	23.1 – 28.7	0.005
BMI (kg/m <sup>2</sup> )	15.6	14.7 – 16.0	15.8	14.7 – 17.1	15.6	14.6 – 16.8	0.251
<b>IOTF Definition</b>							
% (cases) normal weight	88.8 (325)		88.9 (169)		88.6 (156)		0.965
% (cases) overweight	8.2 (30)		7.9 (15)		8.5 (15)		
% (cases) obese	3.0 (11)		3.2 (6)		2.9 (5)		
TBLH BMC (g)	675.2	130.2	699.0	130.6	649.5	125.2	< 0.001
TBLH aBMD (g/cm <sup>2</sup> )	0.721	0.046	0.726	0.046	0.715	0.045	0.019
Lower-limb BMC (g)	318.9	65.3	326.6	65.5	310.7	64.2	0.020
Lower-limb aBMD (g/cm <sup>2</sup> )	0.809	0.063	0.813	0.063	0.804	0.062	0.203
<b>Proportion of children meeting MVPA guidelines (&gt; 4 METs)</b>							
% (cases) < 60 mins/day	20.5 (75)		15.8 (30)		25.6 (45)		0.027
% (cases) ≥ 60 mins/day	79.5 (291)		84.2 (160)		74.4 (131)		
ST (minutes per day spent ≤ 0.06 m/s <sup>2</sup> )	177	67	177	73	177	61	0.913
LPA (minutes per day spent > 0.06 m/s <sup>2</sup> and ≤ 0.75 m/s <sup>2</sup> )	553	47	545	51	561	42	0.002
MPA (minutes per day spent > 0.75 m/s <sup>2</sup> and ≤ 4.00 m/s <sup>2</sup> )	128	42	136	45	118	36	< 0.001
VPA (minutes per day spent > 4.00 m/s <sup>2</sup> )	2	1 – 5	2	1 – 4	2	1 – 5	0.059
MVPA (minutes per day spent > 0.75 m/s <sup>2</sup> )	131	43	139	47	122	37	< 0.001
25(OH)D (nmol/L)	65.7	52.3 – 79.1	67.4	52.2 – 83.4	63.7	52.3 – 74.9	0.081
<b>25(OH)D thresholds</b>							
% (cases) < 25 nmol/L	0.8 (3)		0.5 (1)		1.1 (2)		0.013
% (cases) 25 nmol/L – 49.9 nmol/L	19.1 (70)		19.5 (37)		18.8 (33)		
% (cases) 50 nmol/L – 74.9 nmol/L	48.4 (177)		41.6 (79)		55.7 (98)		
% (cases) ≥ 75 nmol/L	31.7 (116)		38.4 (73)		24.4 (43)		

3  
4 Body mass index, BMI; International Obesity Task Force, IOTF; Bone mineral content, BMC; areal bone  
5 mineral density, aBMD; metabolic equivalents, METs; moderate-to-vigorous physical activity, MVPA;  
6 sedentary time, ST; light physical activity, LPA; moderate physical activity, MPA; vigorous physical activity,  
7 VPA; serum 25-hydroxyvitamin D, 25(OH)D.  
8

Table 2. Determinants of aBMD in boys and girls age 6-8 years

	TBLH aBMD				Lower-limb aBMD			
	$\beta$	95% CI		<i>p</i>	$\beta$	95% CI		<i>p</i>
ST <sup>a</sup>	-0.05	-0.14	0.03	0.22	-0.07	-0.15	0.02	0.13
LPA <sup>a</sup>	-0.04	-0.13	0.04	0.34	-0.02	-0.10	0.07	0.68
MPA <sup>a</sup>	<b>0.11</b>	<b>0.03</b>	<b>0.20</b>	<b>0.01</b>	<b>0.11</b>	<b>0.02</b>	<b>0.20</b>	<b>0.01</b>
VPA <sup>a</sup>	0.07	-0.01	0.16	0.09	0.07	-0.01	0.16	0.09
MVPA <sup>a</sup>	<b>0.11</b>	<b>0.03</b>	<b>0.20</b>	<b>0.01</b>	<b>0.11</b>	<b>0.03</b>	<b>0.20</b>	<b>0.01</b>
25(OH)D <sup>a</sup>	<b>0.09</b>	<b>0.01</b>	<b>0.18</b>	<b>0.03</b>	<b>0.09</b>	<b>0.01</b>	<b>0.18</b>	<b>0.03</b>
ST*25(OH)D <sup>b</sup>	0.03	-0.06	0.12	0.50	0.03	-0.05	0.12	0.45
LPA*25(OH)D <sup>b</sup>	-0.01	-0.09	0.08	0.88	-0.02	-0.10	0.07	0.71
MPA*25(OH)D <sup>b</sup>	0.00	-0.09	0.08	0.94	0.00	-0.08	0.09	0.92
VPA*25(OH)D <sup>b</sup>	-0.07	-0.16	0.01	0.09	-0.06	-0.15	0.02	0.14
MVPA*25(OH)D <sup>b</sup>	-0.01	-0.09	0.08	0.85	0.00	-0.09	0.09	0.99

The values are standardised regression coefficients ( $\beta$ ), 95% confidence intervals (CI) of standardised regression coefficients, and *p* values from linear regression models.

<sup>a</sup> adjustment for age, sex and stature.

<sup>b</sup> adjustment for age, sex, stature and the main effects of PA intensity and 25(OH)D.

Bone mineral density, aBMD; sedentary time, ST; light physical activity, LPA; moderate physical activity, MPA; vigorous physical activity, VPA; moderate-to-vigorous physical activity, MVPA; serum 25-hydroxyvitamin D, 25(OH)D.

Table 3. Determinants of aBMD in boys age 6-8 years

	TBLH aBMD				Lower-limb aBMD			
	$\beta$	95% CI		<i>p</i>	$\beta$	95% CI		<i>p</i>
ST <sup>a</sup>	-0.01	-0.14	0.11	0.81	-0.03	-0.16	0.09	0.58
LPA <sup>a</sup>	-0.10	-0.22	0.02	0.12	-0.07	-0.19	0.06	0.29
MPA <sup>a</sup>	0.09	-0.03	0.21	0.15	0.09	-0.03	0.21	0.15
VPA <sup>a</sup>	0.08	-0.04	0.20	0.21	0.08	-0.04	0.20	0.18
MVPA <sup>a</sup>	0.09	-0.03	0.21	0.13	0.09	-0.03	0.21	0.14
25(OH)D <sup>a</sup>	0.12	0.00	0.24	0.05	<b>0.14</b>	<b>0.02</b>	<b>0.26</b>	<b>0.02</b>
ST*25(OH)D <sup>b</sup>	-0.02	-0.14	0.11	0.80	0.00	-0.12	0.12	0.99
LPA*25(OH)D <sup>b</sup>	0.07	-0.06	0.19	0.29	0.04	-0.08	0.17	0.50
MPA*25(OH)D <sup>b</sup>	0.04	-0.08	0.17	0.48	0.05	-0.07	0.17	0.44
VPA*25(OH)D <sup>b</sup>	-0.05	-0.18	0.08	0.42	-0.04	-0.17	0.08	0.51
MVPA*25(OH)D <sup>b</sup>	0.04	-0.08	0.16	0.53	0.04	-0.08	0.17	0.47

The values are standardised regression coefficients ( $\beta$ ), 95% confidence intervals (CI) of standardised regression coefficients, and *p* values from linear regression models.

<sup>a</sup> adjustment for age and stature.

<sup>b</sup> adjustment for age, stature and the main effects of PA intensity and 25(OH)D.

Bone mineral density, aBMD; sedentary time, ST; light physical activity, LPA; moderate physical activity, MPA; vigorous physical activity, VPA; moderate-to-vigorous physical activity, MVPA; serum 25-hydroxyvitamin D, 25(OH)D.

Table 4. Determinants of aBMD in girls age 6-8 years

	TBLH aBMD			Lower-limb aBMD		
	$\beta$	95% CI	<i>p</i>	$\beta$	95% CI	<i>p</i>
ST <sup>a</sup>	-0.10	-0.22 0.02	0.09	-0.11	-0.22 0.01	0.08
LPA <sup>a</sup>	0.03	-0.09 0.15	0.60	0.04	-0.08 0.17	0.48
MPA <sup>a</sup>	<b>0.14</b>	<b>0.02 0.26</b>	<b>0.02</b>	<b>0.13</b>	<b>0.01 0.25</b>	<b>0.03</b>
VPA <sup>a</sup>	0.07	-0.06 0.19	0.28	0.06	-0.06 0.18	0.33
MVPA <sup>a</sup>	<b>0.14</b>	<b>0.02 0.26</b>	<b>0.02</b>	<b>0.13</b>	<b>0.01 0.25</b>	<b>0.03</b>
25(OH)D <sup>a</sup>	0.05	-0.07 0.18	0.39	0.03	-0.09 0.15	0.63
ST*25(OH)D <sup>b</sup>	0.09	-0.03 0.21	0.15	0.08	-0.04 0.20	0.19
LPA*25(OH)D <sup>b</sup>	-0.08	-0.20 0.05	0.24	-0.08	-0.20 0.05	0.24
MPA*25(OH)D <sup>b</sup>	-0.05	-0.17 0.07	0.42	-0.03	-0.16 0.09	0.58
VPA*25(OH)D <sup>b</sup>	-0.09	-0.22 0.03	0.14	-0.08	-0.20 0.05	0.23
MVPA*25(OH)D <sup>b</sup>	-0.06	-0.18 0.07	0.37	-0.04	-0.16 0.08	0.53

The values are standardised regression coefficients ( $\beta$ ), 95% confidence intervals (CI) of standardised regression coefficients, and *p* values from linear regression models.

<sup>a</sup> adjustment for age and stature.

<sup>b</sup> adjustment for age, stature and the main effects of PA intensity and 25(OH)D.

Bone mineral density, aBMD; sedentary time, ST; light physical activity, LPA; moderate physical activity, MPA; vigorous physical activity, VPA; moderate-to-vigorous physical activity, MVPA; serum 25-hydroxyvitamin D, 25(OH)D.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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