Vitamin D status and parathyroid hormone relationship in adolescents and its association with bone health parameters:analysis of the Northern Ireland Young Hearts Project

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SHORT COMMUNICATION

Vitamin D status and parathyroid hormone relationship in adolescents and its association with bone health parameters: analysis of the Northern Ireland Young Heart's Project

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Abstract
Summary In girls, a plateau in parathyroid hormone (PTH) was observed at a 25-hydroxyvitamin D (25(OH)D) concentration of approximately 60 nmol/l. In boys, there was no plateau in PTH concentrations as 25(OH)D concentration increased. A 25(OH)D threshold of 60 nmol/l appears to have implications for bone health outcomes in both girls and boys.

Introduction Our objective was to investigate if there is a threshold 25(OH)D concentration where a plateau in PTH concentration is evident and to examine the impact of this relationship on bone mineral density (BMD) and bone turnover in a representative sample of adolescents.
Methods We conducted a cross-sectional analysis among 1,015 Northern Irish adolescents aged 12 and 15 years. Serum 25(OH)D, PTH, osteocalcin, type I collagen cross-linked C-telopeptide (CTx), and BMD of the nondominant forearm and heel were measured. Nonlinear regression analysis was used to model the association between 25(OH)D and PTH.
Results In girls, a plateau in PTH was observed at a 25 (OH)D concentration of approximately 60 nmol/l (PTH= $47.146+370.314\times\exp^{(-0.092\times25(OH)D)}$) while no plateau in PTH was observed in boys (PTH=$42.144+56.366\times\exp^{(-0.022\times25(OH)D)}$). Subjects with 25(OH)D levels <60 nmol/l had significantly higher osteocalcin concentrations ($P<0.05$) compared with those who had ≥60 nmol/l, while no significant ($P>0.05$) differences were noted for CTx concentrations. In girls only, nondominant forearm BMD but not heel BMD was significantly higher ($P=0.046$) in those with 25(OH)D concentrations≥60 nmol/l.
Conclusions Serum 25(OH)D levels above 60 nmol/l in Northern Irish adolescent girls prevent an increase in serum PTH levels and maintaining 25(OH)D >60 nmol/l in both girls and boys may lead to improved bone health outcomes.

Keywords Adolescents · Bone turnover · PTH · Vitamin D

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Introduction and hypothesis

It is recognized that severe hypovitaminosis D (defined by a serum 25-hydroxyvitamin D (25(OH)D) concentration

<25 nmol/l) causes rickets in children and osteomalacia in adults [1]. In contrast, the effects of less severe forms of hypovitaminosis D (serum 25(OH)D concentrations between 25 and 50 nmol/l) on the skeleton are less well understood [2–4]. It is well established that there is an inverse relationship between low 25(OH)D concentrations and parathyroid hormone (PTH), which can increase the rate of bone turnover and promote loss of bone mineral [5]. Indeed, while a number of criteria can be considered, the serum 25(OH)D value at which PTH plateaus (referred to as the point of inflection) is considered by many as the most appropriate criterion for defining adequate vitamin D status in adults [5–7]. Elevations in PTH concentrations may not be driven by the same mechanism in adolescents as in adults and may not necessarily be detrimental to bone health. For example, serum PTH concentrations are normally raised during adolescence [8,9] when the rate of bone remodeling and consolidation is at a peak. Thus, the appropriateness of defining vitamin D adequacy on the basis of suppression of PTH in children and adolescents is not established. Notwithstanding this issue, an inverse relationship between serum 25(OH)D and PTH during adolescence had been shown in a number of studies (for review, see [10]) but with variable points of inflection between 25 and 93 nmol/l. Furthermore, three of these studies (in adolescent girls) provide evidence for a possible adverse effect on bone mineral acquisition and bone remodeling in adolescents with serum 25(OH)D levels below such cutoffs [10].

To our knowledge, no study exists that has investigated the association between 25(OH)D and PTH in a large representative sample of young adolescent boys and girls. Therefore, the objective of the present study was to determine whether a point of inflection exists for adolescent girls and boys from a large representative sample of young adolescents and furthermore to investigate whether this threshold in serum 25(OH)D concentrations might differentiate between measures associated with beneficial or adverse effects on bone health.

Methods

Design

This is a cross-sectional investigation of the association between serum 25(OH)D and PTH in 1,015 Northern European Caucasian adolescents and its impact on bone health indices, using data from the Northern Ireland Young Heart's Project [11]. The Northern Ireland Young Heart's Project is the second in a series of cross-sectional studies examining a representative sample of adolescents from Northern Ireland. Details of subject selection, inclusion and exclusion criteria, and venepuncture as well as physical, lifestyle, and dietary information have been described in detail elsewhere [12]. In brief, all subjects in the current analysis were either 12 or 15 years of age at the time of investigation. In total, there were 505 boys (266 and 239 aged 12 and 15 years, respectively) and 510 girls (260 and 250 aged 12 and 15 years, respectively). Eleven percent, 16%, 16%, 6%, 2%, 7%, 10%, 11%, 11%, and 11% of the group were sampled during January, February, March, April, May, June, September, October, November, and December, respectively. None of the subjects were sampled during July or August because of the summer vacation. Complete records were available for 1,015 adolescents who had provided a blood sample and for whom data on pubertal status, anthropometry, bone mineral density (BMD), habitual physical activity, and food intakes were also available. Nonfasting serum samples, which were stored at −80°C since collection, were sent to the University College Cork for analysis of serum 25(OH)D, PTH, C-terminal telopeptide of type I collagen (CTx), and osteocalcin.

Ethics

Ethical approval was obtained from the Research Ethics Committee of the Queen's University of Belfast and written informed consent was obtained from each subject and from each subject's parent or guardian before participation.

Experimental techniques

Assessment of bone mineral density

BMD of the nondominant forearm (distal radius) and dominant heel (os calcis) was measured by dual-energy X-ray absorptiometry with a Norland Lunar peripheral instantaneous X-ray imager bone densitometer (Lunar Corporation, Madison, WI, USA), which has a precision of 0.5%. Before each scan, the densitometer was calibrated by using quasianthropomorphic phantoms according to the manufacturer's recommendations. The results of the scan were expressed as BMD (gram calcium hydroxyapatite per square centimeter).

Biochemical analysis

Concentrations of 25(OH)D were measured in serum samples by using an enzyme-linked immunosorbent assay (ELISA; OCTEIA 25-hydroxy vitamin D; Immuno Diagnostic Systems, Ltd., Boldon, UK). The intra-assay and interassay coefficients of variation (CVs) for the ELISA method were 5.9% and 6.6%, respectively. The quality and accuracy of serum 25(OH)D analysis in our laboratory are ensured on an ongoing basis by participation in the Vitamin D External Quality Assessment Scheme (DEQAS) from Charing Cross

Hospital (London, UK). This ELISA is used for the quantitative determination of 25(OH)D. It has 100% cross-reactivity with 25(OH)D3, and, whereas 75% cross-reactivity with 25(OH)D2 was also reported, Carter et al. [13] reported that the assay did not underestimate 25(OH)D2 in the DEQAS samples. A comparison of the performance of our ELISA with that of a commonly used radioimmunoassay in relation to the DEQAS samples shows very good correlation (ELISA=1.2238×radioimmunoassay−5.5514; r=0.96). Intact PTH concentrations were measured in serum by using an ELISA (MD Biosciences Inc., St. Paul, MN, USA). The intra-assay and interassay CVs were 3.4% and 3.8%, respectively. β-Isomerized CTx was measured in the serum samples by using an ELISA (Nordic Bioscience Diagnostics A/S, Herlev, Denmark). The intra-assay and interassay CVs were 6% and 5%, respectively. Osteocalcin concentrations were measured in serum samples by using an ELISA (Metra Osteocalcin EIA Kit; Quidel Corporation, San Diego, CA, USA). The intra-assay and interassay CVs were 6.0% and 7.6%, respectively.

Statistical analysis

Statistical analysis of the data was carried out using SPSS© for Windows™ version 15 (SPSS Inc., Chicago, IL, USA). The association between serum 25(OH)D and PTH was modeled using nonlinear regression analysis as used previously [14,15]. Models were preformed separately for boys and girls as we had previously reported a significant sex effect on serum 25(OH)D concentration [16]. We also examined the association between serum 25(OH)D and PTH during winter and summer within both genders. We defined winter according to the time of year when there is no skin synthesis of vitamin D at our northerly latitude, i.e., from October to March [17]. Summer was defined from April to September. To account for the direct effect of dietary calcium intake on serum PTH concentrations, nonlinear regression was also preformed on subjects with dietary calcium intakes above and below the UK reference nutrient intake (RNI; boys=1,000 mg/day and girls=800 mg/day) [18]. To investigate differences in BMD and bone turnover markers between subjects with a serum 25(OH)D concentration less than or greater than a given threshold based on the point of inflection of serum PTH (girls) or mean PTH concentration (boys, as no point of inflection was evident), Mann–Whitney rank sum tests were used. P values <0.05 were considered statistically significant.

Results

Association between 25(OH)D and PTH

Nonlinear regression models were used to investigate the point of inflection for serum PTH in the boys and girls separately. The exponential decay function showed the lowest residual sum of squares and thus was selected for the graphical representation (Fig. 1). For these models, the equation was given by: $PTH=a+b\times\exp^{(-c\times 25(OH)D)}$. In girls, a plateau in PTH was observed at a serum 25(OH)D concentration of approximately 60 nmol/l ($PTH=47.146+370.314\times\exp^{(-0.092\times 25(OH)D)}$). Season did not affect the observed 25 (OH)D threshold of 60 nmol/l in girls, i.e., during winter ($PTH=46.271+330.787\times\exp^{(-0.086\times 25(OH)D)}$; n=407) and during summer ($PTH=38.836+18.955*\exp^{(-0.007*25(OH)D)}$; n=103). In boys, there was no plateau in serum PTH as 25 (OH)D concentration increased ($PTH=42.144+56.366\times\exp^{(-0.022\times 25(OH)D)}$). For any given serum 25(OH)D concentration, serum PTH concentrations were consistently higher in boys than in girls (data not shown).

To investigate whether the relationship between serum 25(OH)D and PTH was influenced by dietary calcium intake, nonlinear regression was preformed with dietary calcium as a covariate in sex-specific analysis. In each model, the subjects were divided into those with calcium intakes above and below the UK RNI (800 and 1,000 mg/

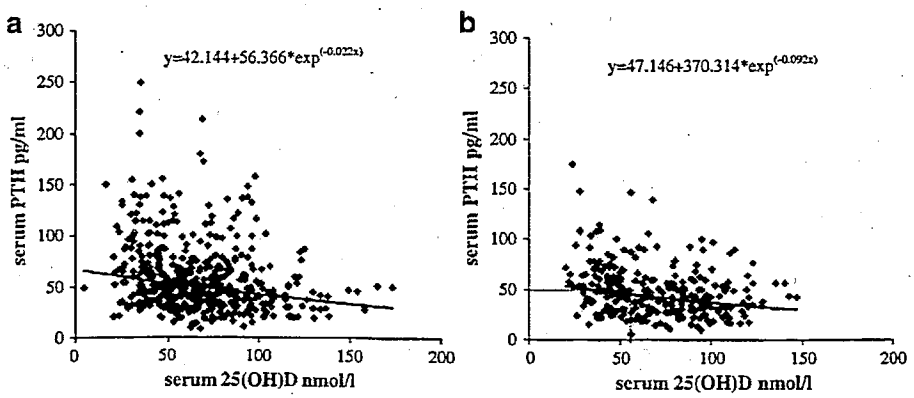

Fig. 1 Association between serum 25(OH)D and PTH concentrations in adolescent a males n=505 and b females n=510, using nonlinear regression analysis

day, for girls and boys, respectively). The results show that dietary calcium did not affect the nonlinear relationship between serum 25(OH)D and PTH (data not shown).

Differences in bone health parameters between subjects with 25(OH)D levels less than a serum 25(OH)D threshold

Girls with serum 25(OH)D concentrations <60 nmol/l (the point of inflection) had significantly higher PTH ($P<0.001$) and osteocalcin ($P=0.014$) concentrations and lower nondominant forearm BMD ($P=0.046$) than girls with serum 25(OH)D concentrations ≥60 nmol/l (Table 1). Similar statistical trends remained for PTH, osteocalcin, and nondominant forearm BMD in girls during winter and summer separately (data not shown). As a plateau for PTH was not observed in boys, we divided them into two groups depending on the mean PTH concentration of 57.3 pg/ml (as described previously by Outila et al. [15]). By using ($y=42.144+56.366\times\exp^{(-0.022x)}$; Fig. 1), a mean serum PTH concentration of 57.3 pg/ml can be reached at a serum 25(OH)D concentration of 60 nmol/l ($y=42.144+56.366\times\exp^{(-0.022x)}$). This value was used to investigate differences in bone health indices in boys. Boys with a 25(OH)D concentration <60 nmol/l had significantly higher serum PTH ($P<0.001$) and osteocalcin ($P=0.001$) than boys with 25(OH)D concentrations ≥60 nmol/l (Table 1). There was no relationship between vitamin D cutoffs chosen and CTx in either girls or boys nor was there a relationship between vitamin D cutoffs chosen and BMD in boys.

Difference in bone turnover markers and BMD between subjects with PTH levels less than a given threshold (data not shown)

The PTH concentration which was achieved at a serum 25(OH)D level of 60 nmol/l in girls was 48.6 pg/ml (from equation). Girls with serum PTH concentrations ≥48.6 pg/ml had significantly higher ($P<0.001$) concentrations of osteocalcin and CTx and lower nondominant forearm BMD ($P<0.001$) than girls with PTH concentrations <48.6 pg/ml. The serum PTH threshold of 48.6 pg/ml had no significant effect on heel BMD in girls. In boys, while a plateau in serum PTH was not evident, a serum PTH concentration of 57.3 pg/ml was reached at a serum 25(OH)D concentration of approximately 60 nmol/l (from equation). Boys with serum PTH concentrations ≥57.3 pg/ml had significantly higher ($P<0.001$) concentrations of osteocalcin and CTx than boys with PTH concentrations <57.3 pg/ml while there were nonsignificant ($P>0.05$) differences in BMD at either site in boys with serum PTH concentrations < or ≥57.3 pg/ml.

Conclusions

The findings of the present study in a large representative sample of healthy adolescents from Northern Ireland show that a point of inflection (i.e., the serum 25(OH)D value at which PTH plateaus) exists for girls (~60 nmol/l) but not boys. The reasons for the sex-specific difference in the

Table 1 Mean serum level of bone turnover markers and BMD in males and females, stratified by serum 25(OH)D concentration<and ≥60 nmol/l

	Boys		Girls	
	<60nmol/l (n=240)	≥ 60nmol/l (n=265)	< 60nmol/l (n=266)	≥ 60nmol/l (n=244)
Serum 25(OH)D (nmol/l)				
Mean	43.2a	86.3b	41.4a	86.1b
SD	10.5	21.5	10.5	18.9
Serum PTH (pg/ml)				
Mean	63.6a	51.6b	61.6a	45.9b
SD	36.1	30.8	32.2	41.8
Serum osteocalcin (ng/ml)				
Mean	37.6a	34.1b	26.1a	23.1b
SD	14.9	16.9	12.9	10.5
Serum CTX (nmol BCE/l)				
Mean	1.10	1.08	0.79	0.74
SD	0.43	0.59	0.47	0.41
BMD forearm (g/cm^3)				
Mean	0.357	0.362	0.362a	0.375b
SD	0.063	0.059	0.066	0.069
BMD heel (g/cm^3)				
Mean	0.511	0.513	0.466	0.474
SD	0.090	0.095	0.078	0.078

Bone health parameter with different letters within a gender were significantly different ($P<0.05$)

nonlinear relationship between 25(OH)D and PTH are unclear and require further investigation. One possible explanation for the sex-specific difference, among others, may relate to differing sex hormone influences on skeletal metabolism between the genders [19]. For example, estrogen may play a more important role than androgen in skeletal mineralization during puberty [20].

Outila et al. [15] in a study of Finnish adolescents (n= 178) reported a lack of a plateau in PTH concentrations with increasing 25(OH)D concentrations among 14–16-year-old adolescent girls during wintertime. The majority of females in their study (95%) were postmenarcheal which is far higher than what is reported in our study (50%). Thus, these differences in pubertal development may partly explain the lack (or presence) of a plateau in PTH between the studies. Several estimates of the serum 25(OH)D value at which PTH plateaus all employing nonlinear models have been reported separately for adolescent girls at 90 nmol/l [21] and 40 nmol/l [15] and for boys at 83 nmol/l [14]. While the reason why there is such variability in the point of inflection in these studies is unclear, it may relate to the fact that serum PTH is also affected by age and sex [22], pubertal status [8], and dietary calcium [23] and phosphorus [24], as well as by serum 25 (OH)D levels.

An important question is whether having serum 25(OH) D concentrations below the point of inflection has any ramifications for bone health. This could be of public health importance in light of the fact that approximately 52% of adolescent females (n=510) in the present study had year-round serum 25(OH)D concentrations less than the point of inflection. While a plateau in serum PTH with increasing serum 25(OH)D concentrations was not evident in adolescent boys, we split the group on the serum 25(OH)D level at the mean PTH concentration; this alternative approach was used by Outila et al. who did not find a plateau in their study of adolescent girls [15]. Approximately 50% of adolescent males (n=510) in the present study had year-round serum 25(OH)D concentrations less than this cutoff value (<60 nmol/l). It is evident from the current study that maintaining a serum 25(OH)D concentration ≥60 nmol/l is associated with lower bone turnover in both adolescent boys and girls and higher nondominant forearm BMD in adolescent girls.

We have previously reported in the same group of adolescents, using a multivariate statistical approach in which the group were stratified by tertile of 25(OH)D concentration, that, during winter, low to moderate vitamin D status (serum 25(OH)D <57 nmol/l) was associated with lower nondominant forearm BMD in girls compared to that seen with high vitamin D status (serum 25(OH) D >57 nmol/l) [16]. The results of the current study adds considerable strength to the notion that this effect is mediated by serum PTH as a plateau in PTH was observed with 25(OH)D concentrations ≥60 nmol/l. Furthermore, marked clear differences existed in nondominant forearm BMD and bone turnover markers between girls with serum PTH concentrations < and ≥48.6 pg/ml (the point of inflection) which further supports the role of PTH.

The main strength of the present study is that it was conducted in a large representative sample of Northern Ireland adolescents, both male and female. However, its cross-sectional design limits the possibility of drawing definitive conclusions on the relationship between 25(OH) D and PTH. A prospective longitudinal study design should be undertaken to verify the cross-sectional associations between 25(OH)D and PTH and associated changes in BMD and bone turnover observed in adolescents in the current study .

In conclusion, a point of inflection of serum PTH exists in adolescent girls but not boys, which may be determined by serum 25(OH)D levels. This finding appears to have implications for bone turnover and BMD of nondominant forearm in the girls.

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Conflicts of interest None.

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