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Vitamin K status is associated with childhood bone mineral content

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In adult bone, vitamin K contributes to bone health, probably through its role as co-factor in the carboxylation of osteocalcin. In children, the significance of vitamin K in bone-mass acquisition is less well known. The objective of this longitudinal study was to determine whether biochemical indicators of vitamin K status are related to (gains in) bone mineral content (BMC) and markers of bone metabolism in peripubertal children. In 307 healthy children (mean age 11·2 years), BMC of the total body, lumbar spine and femoral neck was determined at baseline and 2 years later. Vitamin K status (ratio of undercarboxylated (ucOC) to carboxylated (cOC) fractions of osteocalcin; UCR) was also measured at both time points. Markers of bone metabolism, sex steroids, vitamin D status and growth hormones were measured at baseline only. Large variations in the levels of the UCR were found at both time-points, indicating a substantial interindividual difference in vitamin K status. Improvement of vitamin K status over 2 years (n 281 children) was associated with a marked increase in total body BMC (r −49·1, $P<0·001$). The UCR was associated with pubertal stage, markers of bone metabolism, sex hormones and vitamin D status. A better vitamin K status was associated with more pronounced increase in bone mass in healthy peripubertal children. In order to determine the significance of these findings for childhood bone health, additional paediatric studies are needed.

Children: Puberty: Vitamin K: Osteocalcin: Undercarboxylated osteocalcin: Bone mineral content: Bone turnover

Several studies in adults suggest a beneficial role for vitamin K in bone mineral metabolism and bone fracture prevention, although precise mechanisms have not been entirely elucidated[1,2]. A well-recognized concept is the vital role of vitamin K as a co-factor in the posttranslational carboxylation of osteocalcin, a protein synthesized by osteoblasts[3]. In this carboxylation process, glutamate (Glu) residues are converted into γ-carboxyglutamate (Gla)[4]. The common property of all Gla-proteins is their high affinity for Ca which is essential for their function. Osteocalcin, the most abundant non-collagenous protein found in human bone, consists of forty-nine amino-acids three of which are Gla[5,6]. Here, we will designate the 3-Gla molecule as carboxylated osteocalcin (cOC). In order to adequately carboxylate osteocalcin, the osteoblast requires sufficient vitamin K[7]. In the case of vitamin K deficiency, undercarboxylated osteocalcin (ucOC) will be produced. In the healthy adult population, osteocalcin is carboxylated to a variable extent, suggesting that the dietary vitamin K intake is insufficient for full osteocalcin carboxylation[8]. Markedly higher osteocalcin carboxylation is obtained by increased vitamin K intake[9,10]. Previous studies in postmenopausal women found a clear association between elevated ucOC levels and increased fracture risk[11,12]. Bioavailable vitamin K is mainly derived from nutritional sources such as green leafy vegetables and cheese[13].

Research in the elderly population has revealed that serum ucOC and the ratio of ucOC to cOC (UCR) are reliable and stable markers for vitamin K status of bone, and that a high vitamin K intake may improve bone mineral content (BMC) and strength and diminish fracture risk[2,10,11,14,15]. Also in children, the amount of ucOC relative to the total (or carboxylated) osteocalcin is used to study the relationship between vitamin K status and bone health[16–18]. Using the UCR, we have recently shown that the majority of healthy children have a suboptimal vitamin K status of bone[18]. Additionally, a marked correlation between the bone metabolism markers and the fractions of osteocalcin was found in these children[18]. Recently, another study in a large cohort of healthy girls aged 11–12 years showed that better vitamin K status was associated with increased BMC[16].

Abbreviations: BMC, bone mineral content; cOC, carboxylated osteocalcin; ucOC, undercarboxylated osteocalcin; DEXA, dual-energy X-ray absorptiometry; UCR, ratio of ucOC to cOC; FN, femoral neck; IGF, insulin-like growth factor; LS, lumbar spine; TB, total body; Δ, difference.

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The objective of the present study was to assess the vitamin K status in healthy children during puberty and to study its association with bone mass and changes in bone mass. In addition, the associations between vitamin K status and biochemical markers of bone metabolism, sex steroids and growth hormones were analyzed.

Subjects and methods

Subjects and design

Originally, this study was designed to evaluate associations between bone markers, sex steroids and (changes in) bone mass throughout puberty in healthy children. The results of this study and a detailed description of the study design were previously published[19]. The present study consists of a cross-sectional study at baseline in which the associations between vitamin K status and biochemical markers of bone metabolism, sex steroids and growth hormones are investigated. It also contains a prospective part which assesses the vitamin K status in healthy children during puberty and its association with bone mass and changes in bone mass over 2 years.

A total of 307 children aged between 8 and 14 years, recruited from a number of primary and secondary schools in the villages around Amsterdam, participated in this study. The children were white, reported to be healthy and did not take any medication. At the first visit, height, weight, pubertal development and bone densitometry were measured and serum samples were collected. Height and weight were measured using a stadiometer and a calibrated scale, respectively, subjects wearing underwear only. Pubertal development was assessed in boys on genital stages (G1–5) and in girls on breast stages (B1–5) according to Tanner. After 2·0 (SD 0·10) years, bone densitometry measurement was repeated in 281 children. At this study visit, height, weight and pubertal development were determined for the second time and collection of serum samples took place. Dietary intakes or vitamin K intake were not recorded since validated FFQ that include vitamin K_1 (phylloquinone) and all forms of vitamin K_2 (menaquinones-*n*) are not available at present. The study protocol was approved by the Committee of Ethics on Human Research of the VU University Medical Centre.

Collection and preparation of samples

Blood samples were drawn in the morning after overnight fasting. After blood sampling and serum preparation, samples were frozen and kept at −70°C until use.

Experimental techniques

Serum carboxylated and undercarboxylated osteocalcin. The ucOC and cOC fractions of osteocalcin were measured by ELISA (Takara, Japan). The UCR was used as an indicator of vitamin K status. Elevated levels of the UCR are indicative of an inferior vitamin K status and are related to suboptimal nutritional vitamin K intake[8,20]. Δ-UCR is defined as the difference of the natural log-transformed UCR at follow-up minus baseline. This means that a negative figure for Δ-UCR indicates a decrease in UCR over time which means an improved vitamin K status. Vice versa, a positive figure for Δ-UCR represents an increase in UCR over time, suggesting a deteriorated vitamin K status.

Bone markers. Bone-specific alkaline phosphatase, marker of bone formation, was measured with an assay by wheat germ agglutinin[21]. Procollagen type-I amino terminal propeptides, marker of bone formation, and type I carboxy terminal telopeptides, marker of bone resorption, were estimated by radioimmunoassay of Orion Diagnostica (Espoo, Finland).

Vitamin D. The level of 25(OH)-vitamin D_3 was measured using a competitive binding assay (Nichols) after alcohol extraction. The level of $1,25(OH)_2$-vitamin D was measured using the IDS-assay (Tyne and Wear, UK).

Hormones. Oestradiol (in girls only) was determined by radioimmunoassay (Sorin Biomedica, Saluggia, Italy) as well as testosterone (in boys only; Coat-A-Count, DPC, Los Angeles, CA, USA). Insulin-like growth factor (IGF)-1 and IGF-BP3 were determined by immunoradiometric assays (DSL, Webster, TX, USA).

All samples were analyzed using the same assay lot run to reduce interassay variation. Levels of ucOC and cOC were determined at baseline and after 2 years; all other biochemical markers of bone metabolism and vitamin D status, sex steroids, IGF-1 and IGF-BP3 measurements were determined at baseline only.

Bone mineral content

BMC (g) and bone size (anterior–posterior projected bone area (cm^2)) of the L1–L4 region of the lumbar spine (LS), the femoral neck (FN) and the total body (TB) were measured at baseline and at follow-up with dual-energy X-ray absorptiometry (DEXA) using the Hologic QDR-2000 (Hologic Inc., Waltham, MA, USA). All scans were carried out in the array mode and analyzed by the same investigator. The reproducibility of the different scans has been described previously[22]. Δ-BMC is defined as the difference in BMC at follow-up minus BMC at baseline.

Statistical methods

Normality of distributions was checked for all study parameters. The distributions of ucOC and UCR, testosterone and oestradiol were skewed to the right, and their absolute values are given as median and range. The data of ucOC, UCR, testosterone and oestradiol were converted to natural logarithms, prior to use in regression- and correlation analyses. Paired samples *t* tests were used for comparison of continuous parameters (age, height, weight, BMI, bone-DEXA-parameters) between baseline and follow-up. Comparisons of ucOC and UCR between baseline and follow-up were performed using Wilcoxon signed rank-tests. ANOVA was used to compare the distribution of pubertal stages at baseline and after 2 years. Possible associations of anthropometric data with the UCR were investigated using bivariate correlation tests.

In order to explore the association between vitamin K status (UCR), bone markers and hormones, we used multivariate linear regression analysis. The (log-transformed) UCR at baseline was used as dependent variable and bone markers and hormones at baseline as independent variables. Analyses

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were adjusted for sex, age, pubertal stage, weight and height, but only when these variables were associated with the UCR outcome at $P<0{\cdot}05$. In these analyses, pubertal stage was dichotomized into a prepubertal/early stage (Tanner stage 1–2) *v.* late/end of puberty-stages (Tanner stage 3–5). Because data on bone markers and hormones after 2 years were lacking, we could not perform their association with vitamin K status at 2 years.

Multiple linear regression analyses were also used to examine the association of (increase in) bone density and (changes in) vitamin K status. Dependent variables were the TB-BMC, LS-BMC and FN-BMC at baseline and at follow-up. In addition, differences (Δ) in TB-BMC, LS-BMC and FN-BMC, indicating gains in BMC over time, were also used as dependent variables. In these analyses, we adjusted BMC for (site-specific) bone size in order to minimize size-related effects on (longitudinal) estimates of bone mass by DEXA, according to the recommendation for children by Prentice *et al.* [23]. The independent variables of interest in the regression analyses were the UCR at baseline and follow-up, and the changes in UCR (Δ-UCR) over time. Furthermore, besides adjustment for bone size, other potential confounders (sex, age, pubertal stage (early *v.* late)) were included into the model, but only when these variables were associated with the BMC outcome at $P<0{\cdot}05$. Weight and height were also considered in the models but because of multicollinearity with bone area, these variables were omitted from the definitive models. In the regression analyses for Δ-BMC, besides sex, other potential confounders included in the model were the differences in bone size, weight, height and pubertal stage.

The statistical tests were executed using a two-sided significance level of 5 %. A value of $P<0{\cdot}05$ was considered to be statistically significant. SPSS Base 12.0.2 for Windows (SPSS Inc., Chicago, IL, USA) was used for all analyses.

Results

Anthropometric, dual-energy X-ray absorptiometry- and vitamin K-parameters at baseline and follow-up

In Table 1, the anthropometric variables and DEXA-parameters at baseline and follow-up are shown. Over 2 years time, significant increases in weight, height and BMI were noted. At baseline, most children were prepubertal or in early puberty. Expectedly, more children were found in later pubertal stages after 2 years. As expected, BMC increased significantly in the course of time in all children.

Table 1 also shows the levels of ucOC, cOC and UCR at the start of the study and after 2 years. Large ranges in the level of the UCR were found at both time-points, indicating a substantially interindividual difference in vitamin K status. The median ucOC increased significantly from baseline to follow-up whereas the cOC showed a marginal, borderline-significant, increase. However, the median UCR did not change over time.

The UCR at baseline was significantly associated with pubertal stage (r 0·165, $P=0{\cdot}004$), baseline-weight (r 0·183, $P=0{\cdot}001$) and baseline-BMI (r 0·190, $P=0{\cdot}001$). The UCR at follow-up was associated with gains in height (r 0·342, $P<0{\cdot}01$) and weight (r 0·204, $P=0{\cdot}001$) over 2 years.

Table 1. Characteristics of the study subjects at baseline and follow up (2 years)

(Values are means with standard deviation or number and percentage*)

	Baseline			Follow-up			
	Mean	SD	%	Mean	SD	%	*P*†
Subjects (*n*)	307			281			
Anthropometry							
Male sex (*n*)	156		50·8	139		49·5	
Age (years)	11·2	1·3		13·2	1·3		<0·001
Height (cm)	150·6	10·5		162·5	11·0		<0·001
Weight (kg)	39·9	9·1		50·7	11·1		<0·001
BMI (kg/m^2)	17·4	2·4		19·0	2·7		<0·001
Pubertal stage (*n*)							
1	112		36·5	28		9·9	<0·001
2	96		31·3	46		16·4	
3	67		21·8	60		21·4	
4	23		7·5	48		17·4	
5	9		2·9	99		35·2	
Bone DEXA parameters							
BMC-LS (g)	30·9	8·2		42·9	13·0		<0·001
BMC-FN (g)	3·3	0·6		3·9	0·8		<0·001
BMC-TB (g)	1236·5	310·1		1619·9	420·9		<0·001
Vitamin K status							
ucOC (ng/ml)*	35·1		5·4–64·2	43·1		0·2–10·8	0·004
cOC (ng/ml)	27·2	8·0		28·5	9·2		0·052
UCR*	1·4		0·2–8·3	1·5		0·2–10·8	0·137

DEXA, dual-energy X-ray absorptiometry; BMC, bone mineral content; LS, lumbar spine; FN, femur neck; TB, total body; ucOC, undercarboxylated osteocalcin; cOC, carboxylated osteocalcin; UCR, ratio of ucOC and cOC.

* ucOC and UCR are presented as median and range.

† *P* values are presented for differences in values at baseline and follow-up; *P* values are based on paired *t* tests except for pubertal stage (based on ANOVA) and ucOC and UCR (based on Wilcoxon signed-rank test).

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Associations of vitamin K status with bone markers and hormones (cross-sectional)

In Table 2, the associations of vitamin K status (UCR) with bone markers (resorption and formation), vitamin D status and hormones (growth, sex steroids) at baseline are shown. The UCR was found to have a positive correlation with markers of bone formation (bone-specific alkaline phosphatase and procollagen type-I amino terminal propeptides) and the marker for bone resorption (type I carboxy terminal telopeptides). The UCR was not related to the level of IGF-1 and IGF-BP3. No significant association was found for 25(OH) vitamin D_3 and UCR. However, an evident correlation between 1,25-$(OH)_2$ vitamin D and the UCR was found. In girls, oestradiol was associated with the UCR, whereas in boys, testosterone was correlated with the UCR.

Associations of bone mineral content and vitamin K status (longitudinal)

Table 3 shows the associations between bone mass and vitamin K status (UCR) at baseline and follow-up. In addition, this table depicts the associations between the increase in BMC and changes in UCR over time. The association of bone mass and vitamin K status was more evident for TB-BMC than for the other sites (FN and LS). At baseline, the UCR was inversely associated with TB-BMC, but this association merely showed a statistical trend. At follow-up, the UCR was inversely associated with TB-BMC after 2 years, even when adjusted for covariates. Considering changes over time, improvement of the UCR was inversely associated with more pronounced increase in whole-body bone mass (Table 3). The association of FN-BMC with vitamin K status was not consistent at the different time-points. At baseline, the UCR was related to FN-BMC whereas at follow-up, no significant association was found. In addition, improvements in UCR were inversely associated with more pronounced increases in FN-BMC. No statistically significant associations were found between LS-BMC and vitamin K status.

Discussion

In the present study in healthy peripubertal children, we have found that gains in whole-body bone mass over 2 years are associated with changes in vitamin K status of bone, even after adjusting for the potential confounders bone size, sex, body-height and -weight, and pubertal stage. Furthermore, vitamin K status was related to BMC, most evident at follow-up. In addition, vitamin K status was associated with markers of bone formation, vitamin D status and sex steroids at baseline.

The relationship between vitamin K status and bone health has already been studied extensively in the adult population[14]. It is recognized that circulating ucOC levels may be useful in predicting fracture rate and relate to bone mass in the elderly[11,24]. Also in healthy children, evidence for the usefulness of the carboxylation of osteocalcin as biochemical marker for vitamin K status is available[16–18]. In these studies, vitamin K status is expressed as the amount of ucOC relative to the amount of total or cOC. In the present study, better vitamin K status was associated with higher bone mass at baseline and after 2 years of follow-up, although this relation was more evident at follow-up. The associations found were more pronounced for bone mass of the whole body than for site-specific bone mass at FN or LS. It has been suggested that in growing children, the TB-BMC is a preferable outcome measure to monitor changes in overall bone mass over time because it takes bone size and shape of all skeletal regions into account[25]. The association of current vitamin K status and bone mass in healthy children has also been described in other observational studies[16,17]. O'Connor and co-workers also found that better vitamin K status, expressed as % cOC, was positively related to current

Table 2. Bone markers and hormones at baseline and their associations with the ratio of undercarboxylated osteocalcin to carboxylated osteocalcin (UCR)*

(Values are means and standard deviations (medians and ranges for sex steroids) with regression coefficient (B) and 95 % CI)

	Mean	SD	Median	Range	B	95 % CI	P†
Bone formation							
BAP (U/l)	194·6	61·0			0·001	0·000, 0·003	0·040
PINP (μg/l)	754·0	308·5			0·001	0·000, 0·001	<0·001
Bone resorption							
ICTP (U/l)	15·8	6·9			0·014	0·002, 0·026	0·018
Growth factor							
IGF-1 (U/l)	29·5	14·1			0·005	−0·012, 0·012	0·177
IGF-BP3 (mg/l)	4·5	0·8			0·054	−0·057, 0·165	0·377
Vitamin D							
25-(OH) vitamin D_3 (nmol/l)	69·6	18·8			0·000	−0·004, 0·005	0·872
1,25-$(OH)_2$ vitamin D (pmol/l)	136·4	39·0			0·007	0·005, 0·009	<0·001
Sex steroids							
Testosterone in boys (nmol/l)			0·7	0·3–22·0	0·257	0·127, 0·388	<0·001
Oestradiol in girls (pmol/l)			37·0	16·0–318·0	0·178	0·018, 0·337	0·029

BAP, bone-specific alkaline phosphatase; PINP, procollagen type-I amino terminal propeptides; ICTP, type I carboxyterminal telopeptide; IGF-1, insulin-like growth factor-1; IGF-BP3, IGF-1 binding protein 3.

* For details of subjects and procedures Subjects and methods and Table 1.

† *P* values, B and 95 % CI for B are based on multivariate linear regression analyses with natural log-transformed UCR as dependent variable, adjusted for sex, pubertal stage (early *v.* late) and weight. Log-transformed values of testosterone and oestradiol were used in regression analyses. Direct interpretation of the coefficients requires back transformation to original units.

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Table 3. Associations between (changes in, Δ) bone mineral content (BMC) and (changes in) the ratio of undercarboxylated osteocalcin to carboxylated osteocalcin (UCR). Values (regression coefficient (B), 95 % CI and *P*) are based on linear regression analyses with BMC parameters as dependent variables and the natural log-transformed UCR as the variable of interest. Direct interpretation of the coefficients requires back transformation to original units*

	UCR baseline†		
BMC parameters at baseline	B	95 % CI	*P*
BMC-TB	−10·9	−22·3, 0·44	0·060
BMC-LS	0·42	−0·13, 0·96	0·132
BMC-FN	0·07	0·010, 0·13	0·022
	UCR follow-up‡		
BMC parameters at follow-up	B	95 % CI	*P*
BMC-TB	−19·1	−36·2, −2·0	0·029
BMC-LS	−0·02	−0·81, 0·78	0·969
BMC-FN	0·03	−0·06, 0·12	0·560
	Δ-UCR§‖		
Δ-BMC	B	95 % CI	*P*
Δ-BMC-TB	−49·1	−63·0, −35·2	<0·001
Δ-BMC-LS	0·19	−0·25, 0·64	0·392
Δ-BMC-FN	−0·10	−0·15, −0·05	<0·001

TB, total body; LS, lumbar spine; FN, femoral neck.
* For details of subjects and procedures see Subjects and methods.
† Adjusted for bone size, age, sex and pubertal stage (early *v.* late) at baseline.
‡ Adjusted for bone size, age, sex and pubertal stage (early *v.* late) at follow-up.
§ Adjusted for difference in bone size (follow-up to baseline), difference in body-weight, difference in body height, sex and differences in pubertal stage.
‖ Δ-UCR is the difference in natural log-transformed UCR at follow up *v.* baseline. A negative figure for Δ-UCR indicates a decrease in UCR over time. Vice versa, a positive figure for Δ-UCR represents an increase in UCR over time. Higher UCR values indicate an inferior vitamin K status.

BMC of TB and LS[16]. Kalkwarf and colleagues found that % ucOC was related to markers of bone turnover in a group of healthy girls[17]. In addition, indicators of vitamin K status were not consistently associated with 4-year changes in BMC[17]. In our study, we have found similar associations of the UCR and markers of bone turnover. However, in contrast to the findings from the study by Kalkwarf, we have found that improvement of the vitamin K status over time, indicated by a decrease in UCR, is related to an additional increase in BMC. A possible explanation for this divergent finding is that the broad age range of the healthy girls (3–16 years) in the study cohort of Kalkwarf have obscured the relation between vitamin K status and bone mass variables, despite the large number of participants (*n* 245).

A previous study conducted by our group has shown that the majority of healthy children have a suboptimal vitamin K status, based on the extent of osteocalcin carboxylation. In the latter study, we found high circulating levels of ucOC and high UCR levels in children in comparison with the adult population[18]. Also in the present study, we have found high levels of UCR in children, suggesting a relative vitamin K shortage in bone. Furthermore, the UCR was correlated to pubertal stages, indicating that in advanced pubertal stages, coinciding with highest growth velocities, higher UCR levels are found. Comparable associations of UCR and pubertal development were also observed in our previous study[18]. It may be reasoned that the high levels of UCR result from an imbalance between dietary vitamin K intake and the metabolic requirement for vitamin K during growth. A gradual decline in vitamin K intake in children in recent years is described by Prynne *et al.*[26]. In the USA, the RDA for vitamin K in children aged 4–18 years is 55–75 μg vitamin K[27]. Bounds and co-workers found an average daily intake of 51 (SD 30) μg vitamin K in American children aged 2–8 years whereas others found a median intake of 45 μg vitamin K per day in American girls aged 3–16 years[17,28]. In the latter study, wide ranges of the percentage of ucOC (% ucOC) were found, indicating that intakes of vitamin K were not sufficient, despite dietary intakes approximating the RDA. It could also be that the high levels of UCR and ucOC are only a reflection of a physiological situation during normal growth and bone mass acquisition. However, from an evolutionary point of view, it seems unlikely that large amounts of non-functional ucOC are meant to be synthesized. It could also be that a relative vitamin K shortage in this period has no adverse effects as long as levels of cOC (the functional form of osteocalcin) are sufficiently high. In the present study, the average cOC concentrations at baseline and after 2 years did remain constant.

In the present study, we found that vitamin K status was only related to the active form of vitamin D which is $1,25(OH)_2$ vitamin D levels, and not to 25(OH) vitamin D_3. In the literature, the relationship between vitamin D and vitamin K remains subject to debate. Several studies suggest a contribution of vitamin D in osteocalcin expression[29]. However, vitamin D is not involved in the carboxylation of osteocalcin. The synergistic effect of the combined supplementation of vitamin D and vitamin K on bone mass has been shown in some intervention trials in adults[1,30].

Our report describes the associations of vitamin K status and bone mass in peripubertal healthy children. One of the limitations of this study may be the lack of longitudinal data on physical activity. Weight-bearing physical activity is another important determinant of bone mass[31]. Nevertheless, it is not likely that physical activity is also a determinant of vitamin K status of bone and therefore not a true confounder. Also, other nutritional factors like Ca metabolism (e.g. urinary Ca excretion or milk intake) or carbonated beverage consumption were not taken into account[32].

Taking together the findings from the present and other observational studies on vitamin K and bone in children, one may conclude that a suboptimal vitamin K status in children probably has a negative impact on childhood bone health. Whilst (bone) growth continues, the relative lack of vitamin K may lead to inferior bone quality/strength or suboptimal bone mineralization resulting in increased fracture risk. Indeed, an increased fracture risk is observed in healthy children in the peripubertal years because the increase of bone mass fails to keep up with the increase in height[33,34]. Furthermore, recent data in adults suggest that vitamin K supplementation results in improved bone geometry besides its effect on bone density[35]. Hence, the question remains what happens to bone mass if vitamin K is supplemented in this period of life. We speculate that vitamin K supplementation in healthy adolescents will lead to improved bone health and decreased fracture risk, in analogy to the situation in another population characterized by high bone metabolism, i.e. postmenopausal women[15,24,36]. An adequate vitamin K status during puberty may lead contribute to a higher peak bone mass, which is the

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maximal amount of bone mineral accrued during life. Achievement of optimal peak bone mass in adolescence may be a possible strategy in the prevention of osteoporosis in later life[37,38]. The effect of vitamin K supplementation on bone mass will need to be studied in intervention studies in the adolescent population.

Conclusion

We found that an improved vitamin K status over time is associated with a more pronounced increase in bone mass over 2 years in healthy peripubertal children. In order to determine the significance of these findings, both longitudinal observational studies and placebo-controlled randomized intervention trials in children are needed.

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