

# Chapter 5 Extremes in vitamin K status of bone are related to bone ultrasound properties in children with juvenile idiopathic arthritis

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**Accepted for publication in Clinical and Experimental Rheumatology**

## 5.1 Abstract

Osteopenia is a common complication of juvenile idiopathic arthritis (JIA). In adults, low bone density and increased fracture risk are associated with low vitamin K status of bone. The vitamin K-dependent protein osteocalcin plays an important role in bone metabolism. Its activity depends upon post-translational carboxylation in which vitamin K is an essential co-factor. Hence, vitamin K deficiency leads to undercarboxylated (i.e. inactive) osteocalcin (ucOC). Little is known about the vitamin K status and bone health in children with juvenile idiopathic arthritis (JIA). We studied the vitamin K status of bone and its association with bone mass properties in children with JIA compared to healthy children. We performed a cross sectional study in 55 children with JIA and 54 healthy controls between 6-18 years of age. Bone markers, ultrasound bone mass properties and vitamin K status of bone were determined. Overall, no differences in vitamin K status of bone were found between the study groups. Among children with JIA, a high ratio of ucOC/cOC indicating low vitamin K status was associated with low bone ultrasound parameters, whereas children with a high vitamin K status had markedly higher bone properties. This association was independent of physical activity, age, gender and BMI. These results suggest that vitamin K may be one of multiple risk factors for low bone mass in children with JIA, in addition to other recognized determinants of bone mass. The question remains whether JIA patients would benefit from increased dietary vitamin K intake.

## 5.2 Introduction

Juvenile idiopathic arthritis (JIA) is the most prevalent rheumatic disease in childhood (1). In these patients, reduced generalized bone mass resulting in osteopenia or osteoporosis is frequently observed (2;3). In children with JIA, multiple risk factors for the development of systemic osteopenia are present. The main contributors are glucocorticoid treatment, systemic inflammation and physical inactivity resulting from disease severity (4-7). Osteopenia in JIA patients may lead to increased fracture risk in childhood (8). Low bone mass and increased fractures are noted in later (adult) life as well probably due to an inadequate build up of peak bone mass in adolescence (8;9).

Several studies in adults suggest a beneficial role for vitamin K in bone mineral acquisition and bone fracture prevention, although precise mechanisms have not been entirely elucidated (10-12). A recognized concept is the vital role of vitamin K as a co-factor in the posttranslational carboxylation of osteocalcin, a protein synthesized by osteoblasts (13;14). In this carboxylation process, glutamate acid (Glu) residues are converted into  $\gamma$ -carboxyglutamate acid (Gla) (15). The common property of all Gla-proteins is their high affinity for calcium which is essential for the function of these proteins. Osteocalcin is the most abundant non-collagenous protein found in human bone and consists of 49 amino-acids of which three are Gla (16;17). The Gla residues in osteocalcin are positioned in such a way that they point directly to the calcium ions in the crystal structure of hydroxyapatite, the mineral matrix in bone. In order to adequately carboxylate osteocalcin, the osteoblast requires sufficient vitamin K (18). In 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 (19). Markedly higher osteocalcin carboxylation is obtained by increasing vitamin K intake (20). Bioavailable vitamin K is mainly derived from nutritional sources such as green leafy vegetables and cheese (21;22).

Research in the elderly population has revealed that a high vitamin K intake may improve bone quality and diminish fracture risk (23-25). The amount of ucOC relative to the total (or carboxylated) osteocalcin as well as absolute levels of circulating ucOC are used as indicators for the vitamin K status of bone (14;26). Serum vitamin K concentrations fluctuate with recent dietary vitamin K intake and are no reliable markers for tissue vitamin K status (22).

Also in children, several studies have confirmed the positive relationship between vitamin K and bone health (27-30). Kalkwarf et al. reported that in healthy girls better vitamin K status was associated with decreased bone turnover as indicated by the level of bone markers (29). A study by O'Connor et al. in healthy girls (11-12 years) showed that optimal vitamin K status was related to increased bone mineral content (27). There are no data on vitamin K status in children with juvenile idiopathic arthritis and its possible contribution to bone health in this group. Children in general seem to be at risk of a reduced dietary intake of vitamin K, as was recently reported in British children (31). Furthermore, children with JIA are at risk of malnutrition due to reduced food intake and increased energy expenditure (32). All this may lead to subclinical vitamin K deficiency of bone in children with JIA. This shortage may affect bone mineralization in a population already susceptible for osteopenia. The present study examines vitamin K status in children with JIA compared to vitamin K status in healthy children. Furthermore, we studied the association between vitamin K status of bone, bone markers and quantitative ultrasound properties of calcaneal bone in these children.

## 5.3 Patients and Methods

### Study subjects

From October 2003 to January 2004, 55 JIA patients between 6-18 years of age, classified according to International League Against Rheumatism (ILAR) criteria were enrolled into this study (33). Patients were consecutively recruited from the outpatient clinic from the department of Pediatric Immunology (Utrecht, the Netherlands) at routine follow up visit.

Fifty-four healthy controls, between 6-18 years of age as well, were recruited from a population of children undergoing minor surgery (e.g. strabismus correction, phimosis correction), and friends or relatives of above mentioned patients. Exclusion criteria for controls were JIA or other inflammatory diseases and the use of systemic corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs) or anti-tumor necrosis factor (TNF)  $\alpha$  medication.

This study was approved by the Central Committee on Research Involving Human Subjects (CCMO, the Netherlands) and by the Medical Ethical Committee of the University Medical Centre Utrecht (METC-UMCU, the Netherlands).

Written informed consent was obtained from the parents of all children and also from children being  $\geq 12$  years of age.

## Data collection procedure

Body height and weight of all subjects were measured in a standardized manner without shoes and heavy clothing, to the nearest centimeter and 100 g, respectively. From these values the BMI ( $\text{weight}/\text{height}^2$ ) was calculated. The values of height, weight and BMI were compared with the reference values for healthy subjects matched for age and sex, and Z-scores were calculated (34). Pubertal stage was determined according to Tanner's sexual maturity scale and divided into three categories: prepuberty (prepubertal stage), puberty (pubertal stages II-IV) and end of puberty (pubertal stage V). Leisure-time participation in sport activities (yes/no) and the amount of hours spend on sport activities per week were assessed with a short self-report questionnaire. No distinctions were made in the quantity of weight bearing and intensity of sport activities. Seven JIA patients had missing data for sport-activities and mean number of hours of sport. Duration of disease and current use of medication in JIA patients were derived from medical records. Disease-activity (active disease versus clinical (partial) remission) in JIA patients was based upon the preliminary criteria for clinical remission for different categories of JIA (35). These criteria define inactive disease as the absence of active arthritis; no fever, rash, serositis, splenomegaly, or generalized lymphadenopathy attributable to JIA; no active uveitis; normal erythrocyte sedimentation rate (ESR); and a physician's global assessment of disease activity rated at the best score possible for the instrument used. According to these criteria, partial clinical remission was defined as the absence of disease activity during 6 continuous months while using medication. Clinical remission off medication was defined as the absence of disease activity during 12 months.

From the Child Health Questionnaire (CHQ), the physical functioning (PF) and psychosocial summary scores (PSS) were obtained for JIA patients and controls (36). Four JIA patients did not fill out the complete questionnaire to compute the CHQ-PF; nine JIA patients and four controls did fill out the complete questionnaire to compute the CHQ-PSS. In the control group, information about medical history and current use of any medication was ascertained by a short interview.

## Assessment of vitamin K status, bone turnover and inflammation markers

After blood sampling and serum preparation, all samples were frozen and kept at -80 °C until use. The inflammation marker high sensitivity C-reactive protein (hsCRP) was measured by nephelometry; ESR was measured using the Westergren technique. Five JIA patients did not have values for hsCRP; one JIA patient and one control did not have values for ESR. In serum, two biochemical markers of bone turnover were measured. Bone-specific alkaline phosphatase (BAP, Hybritech, Belgium) was measured as a marker for bone formation by radio immunoassay. As a marker for bone resorption, N-telopeptide cross-links of collagen breakdown (NTX, Ostex, Washington) were determined. Undercarboxylated (ucOC) and carboxylated (cOC) fractions of osteocalcin (Takara, Japan) were measured by enzyme-linked immunosorbent assay (ELISA). We used the ucOC/cOC ratio (UCR) because it is a sensitive indicator for the vitamin K status of bone and relatively independent of bone turnover (14;26;28). Elevated levels of UCR are indicative of an inferior vitamin K status of bone and relate to a suboptimal nutritional vitamin K intake (19).

## Assessment of bone ultrasound parameters

To assess bone mineral density and bone structure, quantitative ultrasound measurements were performed using an ultrasonic device (Hologic QDR 4500, Hologic Inc, Waltham, MA). This equipment is an easy and rapid means of evaluating bone density without ionising radiation and it provides information about bone mass and architecture (37). It has been shown that bone density measured by bone ultrasound and DEXA are highly correlated (38;39). However, it is recommended that the definitive diagnosis of osteoporosis or osteopenia is established using DEXA (40).

For every bone ultrasound measurement, two parameters are determined automatically: broadband ultrasound attenuation (BUA, dB/MHz) and speed of sound (SOS, m/s). BUA is an indicator of bone quantity whereas SOS is used as parameter for bone stiffness. Two measurements at the right and left os calcis were performed for each participant. These four values were averaged to one outcome for BUA and SOS respectively. Comparison of these data to the mean of BUA and SOS of the left and right os calcis separately revealed no differences. For that reason, the overall means for BUA and SOS were used in data-analyses.

Acoustic phantoms provided by the manufacturer were scanned monthly and showed no drift over the time period of the study.

## Statistical analysis

Normality of distribution for all subjects was checked for all study parameters. Z-scores for height, weight and BMI were calculated with the equation  $Z\text{-score} = (\text{observed value} - \text{mean value})/\text{SD}$ . Characteristics of children with JIA and healthy children were compared using independent t-tests. A Chi-square test was performed to compare the distribution of gender across groups. ANOVA was used to compare the distribution of pubertal stages across groups.

Differences in hsCRP and ESR were assessed using the Mann-Whitney test. Outcome parameters (bone turnover markers, bone parameters and vitamin K status) between groups were compared using multivariate regression analyses adjusting for the potential confounders pubertal stage, gender and BMI. In the analyses for the bone ultrasound parameters, we also added variables like sport activities, CHQ physical functioning and the inflammation marker hsCRP in order to investigate whether differences between groups could be explained by these factors.

We examined the association of vitamin K status and the bone turnover markers BAP and NTX in both groups using multivariate linear regression analyses, adjusting for potential confounders pubertal stage, gender and BMI. Secondly, we studied the relationship of vitamin K status and bone density in both groups with use of univariate regression analyses.

The plotting of vitamin K status against bone properties showed a non-linear relationship between these variables. Therefore, vitamin K status was categorized into three equal groups based on the UCR (low-median-high). The UCR groups were used as independent (dummy) variables in multivariate regression analyses studying the association of vitamin K status with the bone ultrasound properties BUA and SOS in JIA patients and healthy children separately. The analyses were adjusted for age, gender and BMI. We also introduced variables like sport activities, CHQ physical functioning and hsCRP in these models in order to investigate whether these factors could explain the association between BUA/SOS and low versus high UCR. For JIA patients only, we also introduced disease-variables into these models.

Finally, we studied potential associations between vitamin K status of bone and disease-variables in JIA patients only using linear regression analyses. All statistical tests were executed using a two-sided significance level of 5%.

SPSS Base 12.0.2 for Windows (SPSS Inc, Chicago, Illinois, USA) was used for all analysis.

## 5.4 Results

### Subjects

The characteristics of the study subjects are shown in table 1. Age, height, weight, BMI and pubertal stages were comparable in patients and controls. There was a trend toward more girls in the JIA sample. As expected, the inflammation markers ESR and hsCRP were elevated in patients with JIA. The physical functioning (PF) score was significantly lower in the JIA group and a trend towards lower psychosocial summary score (PSS) was observed. These results, both derived from the CHQ, indicate that JIA patients are impaired in their daily physical functioning compared to controls. Participation in sports was similar across both groups, but patients with JIA spent fewer hours on sport activities than healthy controls. The clinical characteristics of JIA patients are shown in table 2. Most patients were classified as having oligoarthritis (n = 29) or poly-arthritis (n = 20) and only a minority with systemic disease (n = 6). Disease activity was absent in 40 patients, of whom 32 were in partial remission. Partial remission was defined as the absence of disease activity during 6 months while using medication. Active disease was present in 15 patients (table 2). Mean duration (sd) of disease for all subtypes was 59.1 (41.6) months.

### Bone markers, bone mass and vitamin K status of bone

The outcome parameters for the JIA patients and the control group are shown in table 3. Patients with JIA showed an increased level of BAP (marker for bone formation) and a lower level of NTX (marker for bone resorption) compared to the control group. In addition, the patients had lower bone ultrasound variables, both for BUA as well as for SOS, even when adjusting for potential confounders. The differences in the bone ultrasound parameter SOS between the groups were not explained by sport activities, CHQ physical functioning or inflammation markers. CHQ physical functioning and inflammation markers did also not explain the difference in the bone ultrasound parameter BUA between groups. However, after adjusting for sport activities in the model for BUA, the difference between groups was not longer statistically significant.

**Table 1: Characteristics of the study groups**

	<b>Control group (n = 54)</b>	<b>JIA group (n = 55)</b>	<b>p</b>
<b>Demographics</b>			
Gender, male <sup>  </sup>	21 (39)	12 (22)	0.052
Age, years	11.9 (3.0)	11.3 (3.2)	0.368
Height, cm	153.2 (17.2)	148.8 (18.2)	0.201
Height sds	0.05 (0.9)	-0.06 (0.9)	0.526
Weight, kg	46.6 (15.4)	42.3 (14.4)	0.135
Weight-height sds	0.46 (0.98)	0.23 (0.97)	0.231
BMI, kg/m <sup>2</sup>	19.3 (3.1)	18.5 (3.0)	0.180
BMI sds	0.46 (0.97)	0.22 (0.94)	0.190
Pubertal stage *			0.626
	Prepuberty	27 (50)	26 (47)
	Puberty	15 (28)	14 (25)
	End of puberty	12 (22)	15 (28)
<b>Inflammation markers</b>			
hsCRP, mg/l * <sup>§</sup>	0.43 (0.2-13.3)	1.3 (0.2 - 97.6)	0.001
ESR, mm/hour * <sup>§</sup>	4.3 (1-25)	6.5 (1-40)	0.014
<b>Child Health questionnaire (CHQ)</b>			
CHQ Physical Functioning <sup>† † §</sup>	100 (33.3-100)	83.3 (33.3-100)	<0.0001
CHQ Psychosocial Summary Score <sup>† † §</sup>	53.7 (37.9-61.2)	51.3 (24.9-59.1)	0.055
<b>Sport</b>			
Sport activities, yes <sup>  </sup>	44 (81.5 %)	36 (75%)	0.427
Sport activities per week/year, hours <sup>† §</sup>	1.7 (0-13.7)	1.0 (0-17.5)	0.047

Values are presented as mean (sd). Pubertal stages, male gender and sport activities are presented as n (%). p values based on t tests, except for \*, based on ANOVA; <sup>||</sup>, based on Chi square test; <sup>§</sup>, based on Mann-Whitney test.

<sup>†</sup> Variable presented as median (minimum-maximum).

\* Variable presented as geometric mean (minimum-maximum).

<sup>§</sup> Lower values indicate greater functional impairment, maximal score is 100.

**Table 2: Characteristics of JIA patients**

	<b>Total group JIA</b>	<b>Oligoarticular JIA</b>	<b>Polyarticular JIA</b>	<b>Systemic JIA</b>
<b>Demographics</b>				
No. of subjects	55	29	20	6
Extended form		10 (34.5)		
Gender, male	12 (21.8)	8 (27.6)	4 (20.0)	0 (0)
Age, years <sup>†</sup>	11.3 (3.2)	11.0 (3.0)	11.1 (2.9)	13.4 (3.9)
<b>Disease variables</b>				
Duration of disease, months <sup>†</sup>	59.1 (41.6)	58.3 (38.7)	54.4 (38.9)	64.2 (48.3)
RF positive	5 (9.1)	2 (6.9)	3 (15.0)	0
ANA positive	22 (40.0)	12 (41.4)	9 (45.0)	1 (16.7)
<b>Medication</b>				
NSAID use	41 (74.5)	22 (75.9)	17 (85.0)	3 (50.0)
Methotrexate use	29 (52.7)	13 (44.8)	13 (65.0)	4 (66.7)
Corticosteroid use	1 (1.8)	0	0	1 (16.7)
<b>Disease activity</b>				
Complete remission	8 (14.5)	6 (20.7)	1 (5.0)	1 (16.7)
Partial remission	32 (58.2)	17 (58.6)	11 (55.0)	4 (66.7)
Active disease	15 (27.3)	6 (20.7)	8 (40.0)	1 (16.6)

Values are presented as n (%), except for <sup>†</sup> age and duration of disease, presented as mean (sd). Disease-activity (active disease versus clinical (partial) remission) in JIA patients was based upon the preliminary criteria for clinical remission for different categories of JIA (35).

Markers representing the vitamin K status of bone (UCR, ucOC and cOC) were similar across groups. In the group of JIA patients, no associations were found between the vitamin K status of bone and disease-variables like duration of disease, disease activity, subtype of JIA, inflammation markers and use of medication (data not shown). A remarkably large variation of the level of ucOC and the UCR was found, suggesting a substantial interindividual difference in vitamin K status of bone in both JIA patients and controls. To further investigate the significance of these differences, we studied its association with (markers of) bone metabolism in both groups.

**Table 3: Outcome variables of bone markers, bone ultrasound parameters and vitamin K status in JIA patients and controls**

	<b>Control group (n = 54)</b>	<b>JIA group (n = 55)</b>	<b>p *</b>
<b>Bone markers</b>			
BAP, ng/ml	189.0 (155.7)	288.4 (147.2)	<0.001
NTX, ng/ml	89.8 (43.3)	58.6 (30.2)	<0.001
<b>Bone ultrasound parameters</b>			
BUA, dB/MHz	53.4 (12.9)	48.6 (10.0)	0.041
SOS, m/sec	1555.9 (22.9)	1545.0 (20.7)	0.017
<b>Vitamin K status</b>			
ucOC, ng/ml	34.8 (22.1)	28.2 (19.8)	0.167
cOC, ng/ml	17.1 (7.9)	15.3 (6.8)	0.267
UCR	2.3 (1.4)	2.1 (1.4)	0.490

BAP is used as indicator of bone formation; NTX is used as indicator of bone resorption.

Elevated levels of UCR are indicative of an inferior vitamin K status of bone.

Values are presented as mean (sd). \* p values are based on multivariate analyses with adjustment for gender, BMI and pubertal stage.

## Association of bone markers and vitamin K status of bone

Although no actual differences in vitamin K status were found between the study groups, we examined whether the adequacy of vitamin K in bone, indicated by the UCR, was associated with markers of bone turnover (BAP and NTX). In the group of healthy children, no significant associations between the UCR and bone markers were found. In the JIA-sample, multivariate analysis showed a trend for the positive association of the bone formation marker BAP and UCR ( $p = 0.068$ ), independent of the effects of pubertal stage, gender and BMI. A non-significant association was found for the bone resorption marker NTX and UCR in the JIA-group ( $p = 0.116$ ).

## Bone mass and vitamin K status of bone

Next, we studied the association of the bone ultrasound properties (SOS and BUA) with the UCR as indicator of vitamin K status of bone. Figure 1 shows the bone mass variables per subgroup of vitamin K status (low-median-high) in healthy children and patients with JIA. Using multivariate regression analyses, we investigated whether these extremes in vitamin K status (expressed as low versus high or median UCR) were associated with bone mass parameters in the JIA group. No significant differences for bone ultrasound parameters between the different UCR-groups were found in the healthy children.

However, patients in the low UCR group had higher bone mass properties compared to the patients in the high UCR group (figure 1B/D).

In patients with JIA, those with a high UCR, indicating suboptimal vitamin K status, had a 3 % lower SOS and a 7 % lower BUA, independent of age, gender and BMI. The difference in SOS between the low and high UCR group was not explained by sport activities, CHQ-physical functioning or the inflammatory marker hsCRP. CHQ physical functioning and inflammation markers did also not explain the difference in the bone ultrasound parameter BUA between the low and high UCR group.

However, after adjusting for sport activities in the model for BUA, the difference between groups only showed a clear trend. Also, disease-variables like duration of disease, subdiagnosis, current disease activity, current medication or cumulative dosages of methotrexate or prednisone did not explain the difference in bone mass in JIA patients between the low and high UCR group.

**Figure 1: The bone ultrasound parameters SOS and BUA per group of vitamin K status in healthy children and JIA patients**

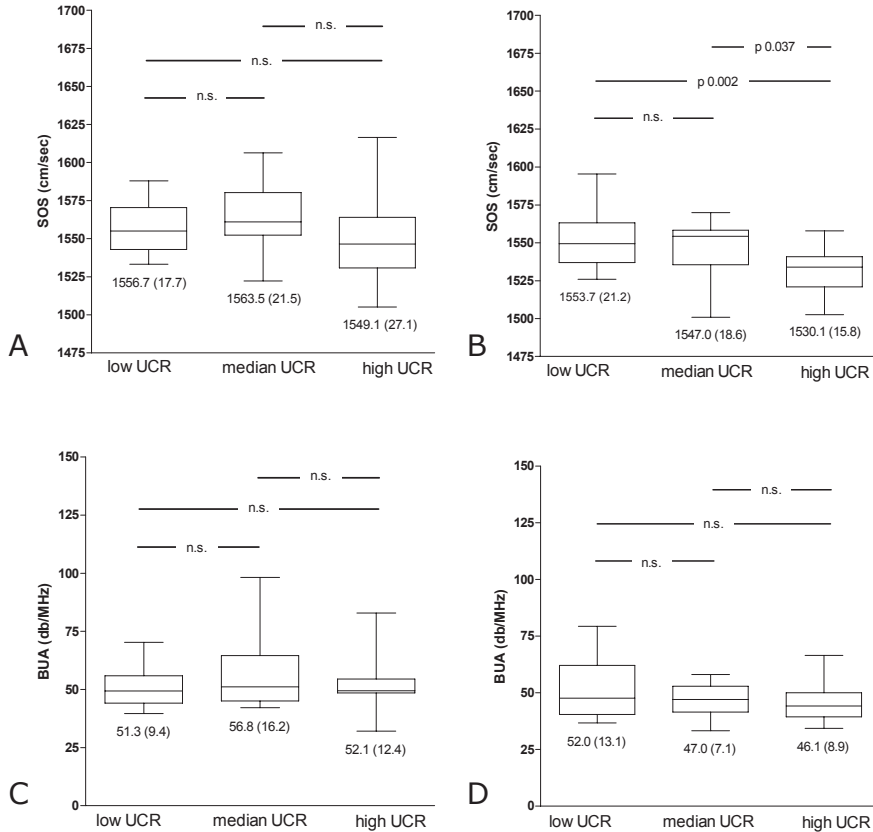


Figure 1A depicts box diagrams for SOS parameters in low, middle and high UCR-groups in healthy children. Figure 1B depicts box diagrams for SOS parameters in low, middle and high UCR-groups in JIA-patients. Figure 1C depicts box diagrams for BUA parameters in low, middle and high UCR-groups in healthy children. Figure 1D depicts box diagrams for BUA parameters in low, middle and high UCR-groups in JIA-patients. p values are based on multivariate analyses with the UCR as the independent variable of interest, comparing subjects with high UCR or median UCR to those with low UCR (reference category). Analyses were adjusted for age, gender and BMI. Box diagrams: the upper limit of the box is the 75th percentile; the lower limit of the box is the 25th percentile; the horizontal line within box represents the median; the whiskers show the range of values. Values per box are presented as mean (sd). Elevated levels of UCR are indicative of an inferior vitamin K status of bone.

## 5.5 Discussion

In this cross-sectional study, we describe the finding of low bone mineral density in children with JIA in comparison to healthy controls. No differences in vitamin K parameters between the study groups were found, although large interindividual variability in vitamin K status of bone was observed. Within the group of JIA patients, children with a high UCR indicating suboptimal vitamin K status had lower bone ultrasound parameters compared to children with a low UCR, independent of confounding factors.

Several other studies have reported the presence of low bone mineral density in children with JIA (3;9;41;42). Besides conventional DEXA measurements, quantitative ultrasound methods have also been used to detect low bone density in these patients (43-45). To our best knowledge, this is the first study in which vitamin K status in relation to bone health in JIA patients is examined. Although we did not find a linear relationship between bone properties and vitamin K status in JIA patients, it was demonstrated that in the extremes of vitamin K status, bone ultrasound properties were significantly different. We choose to divide the group into three equal groups based on the UCR because a clear definition of optimal vitamin K status based on osteocalcin carboxylation is lacking presently. The relationship between suboptimal vitamin K status and bone health was shown in another study in pediatric patients with long-standing vitamin K deficiency caused by the anti-coagulation drug warfarin; these children showed a reduced bone density compared to healthy children (46).

In addition to its role in bone mass accrual, vitamin K may also be important for bone structure and geometry as was recently shown by Knapen et al. (47). In the present study, we used calcaneal ultrasound to measure bone mass. Both ultrasound parameters reflect bone density as well as bone micro-architecture, whereas SOS is more indicative for bone quantity and BUA for bone strength (48). In the present study, the difference in bone ultrasound parameters between the low and high UCR-group in JIA patients was more evident for the SOS than for the BUA, suggesting that vitamin K might be more important in bone density than bone strength. In a small group of healthy children, Sugiyama et al. also found that carboxylation of osteocalcin was related to the bone ultrasound property SOS (30).

In contrast to a limited number of previous studies in healthy children, we did not find a relation between vitamin K status and bone density in

our healthy control group. A study in a large cohort of healthy girls aged 11-12 years showed that better vitamin K status, expressed as the % of undercarboxylated osteocalcin, was associated with increased bone mineral content (27). In comparison to the latter study, our control group had a broader range of age, which might explain the absent relation between vitamin K status and bone mass variables. This was also the case in a study by Kalkwarf et al. who did not find consistent associations between markers of vitamin K status and bone mass variables in a large cohort of healthy girls aged 3-16 years (29). Additionally, in this study, suboptimal vitamin K status was associated with increased levels of bone markers indicating bone metabolic activity (29). Again in our control group, we did not observe any association of bone turnover markers and vitamin K status. We merely observed a trend towards higher bone turnover markers in children with JIA with lower vitamin K status.

When considering the levels of the bone turnover markers, it was quite remarkable that the bone formation marker BAP was significantly higher in the patients than in the healthy subjects, whereas the bone resorption marker NTX was lower. This is in contrast to findings of other studies in children with JIA, reporting reduced markers of bone formation and increased markers of bone resorption (3;49). However, the majority of our patients are in partial or complete remission whereas these studies mainly described children with active JIA early in the course of disease. Most likely, a catch-up phenomenon of bone growth and bone mineral acquisition occurs in patients who are adequately treated. This hypothesis is supported by a study of Reed et al. who noted that improvement of disease activity leads to increased bone formation markers (50).

In order to appreciate the findings of the present study, some aspects of the cross sectional study design need to be discussed. Nowadays, most patients will achieve clinical remission due to prompt and adequate treatment. As a result, the majority of the JIA-patients in this study are in a relatively well clinical condition, resulting in few active disease-cases. This might explain the absent relationship of current clinical disease characteristics, bone ultrasound parameters and vitamin K status in the present study. In addition, we would like to make some additional remarks about the bone measurements using calcaneal ultrasound. At present, no reference ultrasound data are available to allow comparisons between our study groups and large age and sex matched cohorts. However, the groups in our study were quite comparable in

anthropomorphic data. Furthermore, the question may rise whether calcaneal ultrasound is representative for total body BMD (51). When considering the clinical relevance of calcaneal ultrasound measurements, other studies have shown that this method is a surrogate measure for fractures (52).

In conclusion, children with JIA with suboptimal vitamin K status of bone have lower bone ultrasound variables as compared to those with a better vitamin K status. These results suggest a possible contributory role for vitamin K to bone health in children with JIA, besides other recognized determinants of bone mass. The question remains whether JIA patients would benefit from increased dietary vitamin K intake. This will need to be confirmed in a prospective intervention study in JIA patients, preferably in a selected group of patients with severe active disease who are at highest risk of developing reduced bone health.

## Acknowledgements

The authors thank Danielle de Vries for her indispensable and excellent work in data acquisition and Kirsten Teunissen for her excellent laboratory work.

This study was supported by funding of the Wilhelmina Children's Hospital, University Medical Centre Utrecht, the Netherlands.

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