Peripheral quantitative computed tomography (pQCT) in 12- and 24-month-old children – Practical aspects and descriptive data

Saara Valkama, MD[1,2], Elisa Holmlund-Suila, MD, PhD[1,2], Alex Ireland, PhD[3], Helena Hauta-alus, PhD[1,2,4], Maria Enlund-Cerullo, MD, MSc[1,2,5], Jenni Rosendahl, MD, PhD[1,2], Sture Andersson, MD, PhD[1], and Outi Mäkitie, MD, PhD[1,2,5,6]

[1] Children's Hospital, Pediatric Research Center, University of Helsinki and Helsinki University Hospital, Helsinki, Finland

[2] Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of Helsinki, Helsinki, Finland

[3] Musculoskeletal Science and Sports Medicine Research Centre, Department of Life Sciences, Manchester Metropolitan University, Manchester, United Kingdom

[4]National Institute for Health and Welfare, Helsinki, Finland

[5] Folkhälsan Research Center, Helsinki, Finland

[6] Department of Molecular Medicine and Surgery, Karolinska Institutet, and Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden

Corresponding author:

Dr. Saara Valkama

Children's Hospital, Pediatric Research Center, University of Helsinki and Helsinki University Hospital, P.O.Box 281, FIN-00029 HUS, Helsinki, Finland

Email: saara.valkama@hus.fi

Telephone: +358 50 3138565

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ABSTRACT

Background

Peripheral quantitative computed tomography (pQCT) is a useful tool to assess detailed bone characteristics. Its utility in infants is however limited due to lack of reference data and technical challenges. The purpose of this study was to provide data on length- and weight-adjusted pQCT values and to present a quality grading system for healthy children aged 12 and 24 months.

Material and methods

As a part of the Vitamin D intervention in Infants (VIDI) trial, we collected pQCT and anthropometric data from 855 children at 12 months and from 784 children at 24 months. Bone mineral content (BMC; mg/mm), volumetric bone mineral density (vBMD; mg/cm^3), cross-sectional area (CSA; mm^2), polar-moment of inertia (PMI; mm^4), and periosteal circumference (PsC; mm) were assessed for total bone at 20% distal site of the left tibia using pQCT (Stratec XCT2000L). We evaluated the impact of scan quality on bone measures. Total bone parameters were assessed for boys and girls separately. The means of the bone parameters were also compared in relation to age. The associations between bone parameters and weight, length, sex and scan quality were analyzed.

Results

We included scans with sufficient quality (Grade 1-5) in the final analyses: 679/855 (79%) at 12 months and 709/784 (90%) at 24 months. Altogether 39% of the scans at 12 months and 51% at 24 months were of good or excellent quality (Grade 1-2). Scan quality had an impact on BMCs at 12 and 24 months (p=0.001 and p=0.017, respectively) but not on other bone parameters. Boys presented greater total bone BMC, CSA, PMI and PsC values at 12 and 24 months but vBMDs were similar. All bone parameters showed a significant increase between 12 and 24 months for both sexes. When adjusting bone parameters for weight, length and scan quality, differences between

sexes disappeared. Weight was the strongest modifier of BMC, CSA, PMI and PsS at 12 and 24 months.

Conclusions

This study increases our understanding on bone parameters in young children and demonstrates the suitability of pQCT in bone research in infants. The described pQCT data and scan quality grading system should prove useful in evaluating data reliability in research settings.

1. Introduction

Interest towards childhood bone health has grown in recent years. Bone mineral accrual during childhood affects skeletal health in adulthood, and high peak bone mass attained during early life may be protective against osteoporosis and fractures at older age [1-3]. Chronic diseases and pharmaceutical agents in childhood may have a negative effect on bone strength and contribute to increased fracture risk [4-7]. In order to evaluate the impact of various factors that may affect bone health, adequate tools are needed to distinguish between normal and impaired bone strength.

Several different bone densitometry methods can be applied in children [8]. Dual-energy X-ray absorptiometry (DXA) remains the most common in clinical practice for its wide availability, precision, low radiation exposure and for being relatively easy and inexpensive to use [9]. The current recommendations, updated by the International Society for Clinical Densitometry Official Positions in 2013, support the use of DXA for clinical densitometry in infants and young children [10, 11]. However, especially for children younger than 3 years, normative data for any measurement technique are scarce. DXA-derived lumbar spine BMD is the only assessment method for which sufficient reference data in this age group exist [10].

Peripheral quantitative computed tomography (pQCT) has been successfully used in neonates, infants and small children [12-15], as well as in preschool- and school-aged children [16]. The advantage of pQCT compared with DXA is that it allows three-dimensional examination of bone geometry as well as separation between different cortical and trabecular bone compartments and muscle. It also allows volumetric bone mineral density and other bone measures to be examined without being influenced by the size of the child. While DXA provides data mostly for central bone parameters and body composition, pQCT allows examination of bone parameters at peripheral sites. pQCT is also relatively inexpensive and involves low radiation exposure (<1μSv per scan). Some pQCT reference data exist for children older than 5 years of age [17, 18] but, to the best of our

knowledge, no such large pQCT data have been reported for younger children and especially for infants.

We carried out a large prospective vitamin D intervention study (VIDI) among 975 healthy term-born infants during 2013-2016 [19]. As a part of the trial, we collected data on tibial total bone measures at 12 and 24 months of age using pQCT as a densitometric method. Here, we describe the practical approach how to conduct pQCT assessments in young children, present a grading system to evaluate the impact of scan quality on measurements, demonstrate how weight, length, scan quality and sex affect bone parameters, and provide length- and weight-adjusted tibial pQCT data for 12 and 24 months old healthy girls and boys.

2. Materials and Methods

2.1 Participants

The study cohort consisted of 975 children who were included in our randomized, double-blind Vitamin D Intervention in Infants (VIDI) trial between January 2013 and June 2016. The VIDI study compared the effects of vitamin D_3 supplementation on bone strength and infections during the first 24 months of life in children who received either 10 µg (Group10) or 30 µg (Group30) daily supplemental dose from age 2 weeks to 24 months [19]. All children were born full-term and with normal weight and were of Northern European ethnicity. Study protocol, inclusion and exclusion criteria and primary outcome measures have previously been described in more detail [19, 20].

Participants attended follow-up visits at 12 and 24 months of age. Length (cm) and weight (kg) were measured and corresponding standard deviation scores (SDS) determined using Finnish pediatric growth references [21]. Length adjusted weight percentage was calculated and standardized into sex-specific standard deviation score (SDS). Blood 25-hydroxyvitamin D

concentrations were analyzed at birth (umbilical cord blood), and at 12 and 24 months of age, as previously reported [19].

The VIDI study protocol was approved by the Research Ethics Committee of the Hospital District of Helsinki and Uusimaa (ID 107/13/03/03/2012) and was registered in ClinicalTrials.com (NCT01723852). All families who agreed to participate gave a written informed consent after having received information about the study.

2.2 pQCT data collection

Bone outcomes were measured using pQCT technique (XCT2000L Research+, Stratec Medizintechnik GmbH, Pforzheim, Germany) at 12 and 24 months of age. Because dominant leg cannot be determined in this age group, we used the left tibia for all participants. The length of tibia was measured from the medial malleolus to the medial condyle, and the scanning site at 20% distal length was marked with a color line. We measured a single slice with a 2.0 mm thickness, 0.40 mm voxel size and 25 mm/s scan speed. We used a leg stabilizer to fix the position of the leg during measurement, and the leg was also supported by the researcher (Fig. 1).

All children were measured in a sitting position on the parent's lap. The region of interest (ROI) around tibia was manually set for each scan. All pQCT measurements were performed by experienced and trained research personnel.

2.3 pQCT quality assessment and analysis

Since no scan quality grading for this age group has previously been reported, we developed our own grading protocol, based on an earlier study in pubertal children [22]. We agreed on the grading criteria prior to scan analysis and in uncertain cases, reviewed the scans together for consensus. Grading criteria were based on the amount of movement artefacts and whether the cortex of the tibia was intact or not. If the bone shape could not be recognized or the scanning site was incorrect,

the scan was defined as failed. All scans were individually reviewed and graded from Grade 1 to 6 as follows: 1=excellent, 2=good, 3=moderate, 4=sufficient, 5=poor, 6=failed (Fig. 2). Grade 6 measurements were omitted from further analyses.

We used the loop function of the manufacturer's software version 6.20 C to analyze the Grade 1-5 scans. A threshold of 180 mg/cm3 with peeling mode 1 was used to filter out soft tissue in order to determine total bone, and a threshold of 400 mg/cm3 to define the cortical compartment. These thresholds were based on a previous study in infants [13]. The precision of the thresholds was pretested with a smaller subset of 100 scans before running the final analyses.

We determinated bone mineral content (BMC; mg/mm), volumetric bone mineral density (vBMD; mg/cm^3), cross-sectional area of the bone (CSA; mm^2), polar moment of inertia (PMI; mm^4) and periosteal circumference (PsC; mm) for total bone at 20% distal length of the left tibia. We found that in our cohort 99% of the scans at 12 months and 89% at 24 months had a mean cortical thickness less than 2 mm. As previous studies have shown, there are significant problems measuring cortical bone outcomes if cortical thickness is less than 2 mm [23]. Therefore we decided to exclude cortical bone results from our analysis.

2.4 Statistical analysis

Results are presented as means and 95% confidence intervals (95% CI) or medians and 25% and 75% IQRs. The normality of the variables was assessed using visual inspection and Kolmogorov-Smirnov test. Logarithmic transformation was used for non-normally distributed variables where appropriate. We used an independent samples T-test in unadjusted analyses when comparing anthropometric, biochemical and bone characteristics between two groups. Analysis of covariance (ANCOVA) was used for adjusted analyses between sexes. Comparisons between groups of three or more were performed using analysis of variance (ANOVA) for normally distributed variables or Kruskall-Wallis test for variables lacking equal variance. Tukey test was used as a post hoc test,

with Bonferroni correction for multiple comparison. Pearson Chi-Square was used for comparison of categorical variables. Pearson correlation was used to explore the association between bone parameters and other variables. Covariates for linear regression were chosen based on these correlations. A multiple regression analysis was assessed to observe linear associations between bone parameters and independent variables. In linear regression, residuals were plotted to assess their normal distribution and evaluate homoscedasticity.

Statistical significance for all tests was set at $p < 0.05$. Statistical analyses were performed using IBM SPSS version 24 (IBM) for Windows.

3. Results

3.1 Cohort characteristics

A total of 975 participants (50% girls) were included in the study. Of them, 865/975 (89%) children attended a follow-up visit at 12 months and 823/975 (84%) at 24 months. Cohort characteristics are presented in Table 1.

Length (cm) and weight (kg) were greater in boys than in girls at birth and during entire follow-up ($p<0.001$). Length SDS at birth and length-adjusted-weight SDS at 12 months were also greater in boys than girls ($p=0.039$ and $p=0.012$, respectively).

Majority of the children were vitamin D sufficient (25-OHD > 50 nmol/L) throughout the study: 96% at birth, 99% at 12 months, and 99% at 24 months. As previously reported [19], the vitamin D intervention did not have an impact on the pQCT outcomes at 12 or 24 months. Therefore we pooled the data from both intervention groups in further analyses.

3.2 pQCT grading

Altogether 855/865 (99%) children were scanned with pQCT at 12 months and 784/823 (95%) at 24 months (Fig. 3).

We included Grade 1-5 scans in the final analyses: 679/855 (79%) at 12 months and 709/784 (90%) at 24 months. The number of excellent or good quality scans (Grade 1-2) was 264/679 (39%) at 12 months and 360/709 (51%) at 24 months. Distribution into different Grade groups did not differ between girls and boys at 12 or 24 months ($p=0.382$ and $p=0.281$, respectively). When comparing the means of bone parameters according to grading, no difference was observed between each Grade (1 to 5) (ANOVA $p > 0.1$ for all) or between good-quality (Grade 1-2) and all (Grade 1-5) scans.

3.3 Sex-specific total bone pQCT outcomes

Table 2 illustrates the sex-specific total bone results at 12 and 24 months. In unadjusted analyses, boys had significantly greater BMC, CSA, PMI and PsC values ($p<0.01$ for all) than girls at 12 and 24 months, whereas no difference between the sexes was observed for vBMD. After adjusting data for length (cm), weight (kg) and Grade, all differences between sexes disappeared.

Sex-specific pQCT data for total BMC, vBMD, CSA, PMI and PsC for children at 12 and 24 months are presented in Suppl. fig. 1. As seen in these figures, all values increased both in girls and boys from 12 to 24 months, the changes being of similar magnitude in both sexes.

3.4 Associations of weight, length, sex and scan quality with total bone pQCT parameters

We examined the associations between total bone parameters and independent variables (weight, length, sex and Grade) with a multiple linear regression (Tables 3 and 4). Weight associated with all bone parameters except vBMD at 12 and 24 months ($p<0.001$ for all) and was the strongest modifier of BMC, CSA, PMI and PsC. Length was significantly associated with BMC, CSA, PMI and PsC at 24 months ($p<0.005$ for all) but not at 12 months whereas Grade was significantly associated with BMC at 12 and 24 months ($p=0.001$ and 0.017, respectively). Sex was not a significant modifier of any of the bone variables. The model could not explain the variance in vBMDs.

When all scans taken at 12 and 24 months were pooled and divided into five subgroups according to weight, all bone parameters differed among the subgroups (Fig. 4, Suppl. table 1). Similar differences were seen when the scans were compared in subgroups according to length (Fig. 5, Suppl. table 2). Suppl. fig. 2 illustrates the distribution of Grade 1-2 and Grade 3-5 scans for BMC and vBMD according to weight and length and sex.

Discussion

As part of the Vitamin D Intervention in Infants (VIDI) trial, we here report detailed pQCT data on tibial total bone measurements in 679 children at 12 months and in 709 children at 24 months of age. All children were born at term, healthy and with normal birth measurements. In addition, we present a grading protocol and a practical approach to be considered when implementing pQCT measurements in bone health studies involving infants and toddlers. To our knowledge, this is the largest study to report bone parameters measured with pQCT in small children.

Despite the fact that the pQCT method has served as a useful tool for bone assessments in the research field since the early 1970s [24], pQCT studies among pediatric populations are rather limited. Some studies have established pQCT reference data on radius or tibia in older children and adolescents [16-18, 25], but no reference data for smaller children and especially infants exist. This was also noted in the 2013 ISCD Pediatric Official Positions guidelines on bone densitometry where the lack of pQCT reference data for infants and young children was highlighted [10]. Measurement techniques differ greatly between the studies [25] and there is no consensus on how measurements should be assessed, which scan locations to use, or which variables to report. One European study reported age- and height-related reference data for tibial trabecular and cortical vBMD, BMC, and CSA in healthy 5-19-year-old participants using 4%, 14% and 38% distal tibial length of the dominant leg as scanning sites [26]. Another pQCT study established height-specific

normal ranges and sex-specific centile curves for trabecular BMD, total and cortical CSA, cortical BMC, BMD, and cortical thickness in 416 subjects aged 5 to 18 years, using the 4% and 66% tibial sites of non-dominant tibia [27]. A third study reported reference curves for cortical BMD in relation to age and race in 665 children and adults from 5 to 35 years of age, using a scanning site at 38% of left tibia [28]. Our aim was to measure tubular bone that is not too close to the growth plate, in order to avoid potential sources of positioning and measurement error. Therefore, we chose to use the 20% distal length of the left tibia as a measurement site. This was in line with the study by Binkley et al. which reported reference centile curves for 231 subjects between 5 and 22 years using a single pQCT slice at 20 % distal tibial length [18].

Studies on sex-related differences in bone parameters are limited in this age group. One previous study using DXA measurements in infants and toddlers aged 1-36 months showed boys to have greater lumbar spine BMC than girls without any difference in areal BMD [29]. In our analyses, boys presented greater total bone BMC, CSA, PMI and PsC than girls but no sex-related difference was seen in vBMD. However, when we adjusted the bone variables for length, weight and quality grading, differences between sexes disappeared. In a multiple regression analysis, bone results were strongly influenced by body size and sex was not as significant predictor for bone parameters as were weight or length. This should be noted when interpreting pQCT data in infants. In line with this, we present the data for various bone parameters by weight and length and not by age or sex.

Obtaining pQCT scans in this age group is technically challenging in order to avoid movement during scanning: we excluded altogether 21% of the scans at 12 months and 10% at 24 months due to movement artifacts. However, in contrast to the pQCT study by Blew et al. that showed a correlation between greater amount of movement artifacts and total bone CSA [22], grading only impacted BMC in our study. We performed a single scan and did not use a scout view (a 2D coronal projection used to position scans) in order to keep the scanning and radiation exposure as low as

possible. It is possible that repeated measurements or the use of a scout view could have improved the precision of the measurements [22, 30]. Despite these limitations we regard our unique data as valuable and the provided data important for improving and expanding bone health research in early childhood.

In conclusion, we performed pQCT measurements at the 20% distal tibial site in 855 children at 12 months and in 784 children at 24 months of age and included 679 (79%) of the scans taken at 12 months and 709 (90%) scans taken at 24 months in the final analyses. We examined a large number of healthy children with good adherence to the study protocol, permitting assessment in the same child at 12 and 24 months. Newborns with a growth restriction were excluded and the vast majority of the participants were vitamin D sufficient. Our study demonstrates that pQCT is a feasible tool when evaluating bone health in infants and toddlers. We provide length- and weight-specific pQCT values for 12 and 24 months old children and present a novel grading system for scan quality assessment. We describe how anthropometric factors, scan quality or sex associate with bone parameters. However, future studies are needed to evaluate how other factors, such as dietary patterns or age at walking, influence pQCT outcomes, and to see how the presented data can be utilized in various bone health and intervention studies in this age group.

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Author contributions

Study design: SV, EHS, SA and OM, Study conduct: SV, EHS, SA and OM, Data collection: SV, EHS, HH, MEC, JR, SA and OM, Data analysis: SV, EHS, AI, OM, Data interpretation: SV, EHS, SA and OM, Drafting manuscript: SV and OM, Revising manuscript content: EHS, AI, HH, MEC, JR, and SA, Approving final version of manuscript: SV, EHS, AI, HH, MEC, JR, SA and OM. SV takes responsibility for the integrity of the data analysis.

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TABLE 1. Anthropometric and biochemical characteristics as mean (95% CI) at birth and during follow-up according to sex.

ANTHROPOMETRIC CHARACTERISTICS			
BIRTH	**Boys (N=490)**	**Girls (N=485)**	**p**
Length (cm)	50.7 (50.5; 50.8)	50.0 (49.9; 50.2)	<0.001
Length SDS	-0.3 (-0.3; -0.2)	-0.1 (-0.2; -0.1)	0.039
Weight (kg)	3.6 (3.5; 3.6)	3.5 (3.5; 3.5)	<0.001
Length adjusted weight SDS	0.1 (0.0; 0.2)	0.1 (0.0; 0.2)	0.851
12 MONTHS	**Boys (N=427)**	**Girls (N=438)**	**p**
Length (cm)	76.5 (76.2; 76.7)	74.5 (74.3; 74.8)	<0.001
Length SDS	-0.5 (-0.6; -0.4)	-0.6 (-0.7; -0.5)	0.051
Weight (kg)	10.2 (10.1; 10.3)	9.4 (9.3; 9.5)	<0.001
Length adjusted weight SDS	0.1 (0.0; 0.2)	-0.1 (-0.2; -0.0)	0.012
Tibial length	137.6 (136.4; 138.8)	136.0 (134.8; 137.2)	0.068
24 MONTHS	**Boys (N=408)**	**Girls (N=414)**	**p**
Length (cm)	88.7 (88.4; 89.0)	87.0 (86.7; 87.3)	<0.001
Length SDS	-0.2 (-0.3; -0.1)	-0.3 (-0.4; -0.2)	0.350
Weight (kg)	13.0 (12.8; 13.1)	12.2 (12.0; 12.3)	<0.001
Length adjusted weight SDS	0.0 (-0.1; 0.1)	-0.1 (-0.2; 0.0)	0.116
Tibial length	168.3 (167.4; 169.2)	165.9 (165.1; 166.8)	<0.001
BIOCHEMICAL CHARACTERISTICS			
25-OHD (nmol/L)	**Boys**	**Girls**	**p**
Birth[a]	83.1 (80.8; 85.4)	79.9 (77.6; 82.3)	0.059
12 months[b]	98.1 (95.2; 101.0)	99.7 (96,9; 102.5)	0.434
24 months[c]	101.1 (98.4; 103.9)	103.4 (100.7; 106.1)	0.255

Number of participants are given if less than 95% from total n: [a] n=483/472, [b] n=393/411, [c] n=402/412

Analyzed with independent samples t-test

TABLE 2. Sex-specific bone characteristics at 12 and 24 months as mean and 95% CI

	Unadjusted model			Adjusted model^		
	Boys	**Girls**	**p[a]**	**Boys**	**Girls**	**p[b]**
12 MONTHS						
N (%)	328 (48)	351 (52)	N/A	328 (48)	350 (52)	N/A
BMC (mg/mm)	37.0 (36.2; 37.9)	33.9 (33.0; 34.7)	<0.00	35.9 (35.1; 36.8)	34.9 (34.1; 35.7)	0.078
vBMD (mg/cm^3)	301.2 (293.3; 309.2)	293.6 (285.8; 301.3)	0.175	299.7 (291.4; 308.0)	295.0 (287.0; 303.1)	0.449
CSA (mm^2)	127.2 (123.6; 130.8)	120.6 (117.1; 124.1)	0.009	124.2 (120.5; 127.8)	123.5 (119.9; 127.0)	0.793
PMI (mm^4)	2822.2 (2651.8; 2992.6)	2550.5 (2385.8; 2715.3)	0.002	2691.9 (2518.0; 2865.8)	2672.3 (2504.6;	0.651^^
PsC (mm)	39.7 (39.1; 40.2)	38.6 (38.1; 39.1)	0.005	39.2 (38.6; 39.7)	39.0 (38.0; 39.6)	0.723
24 MONTHS						
N (%)	335 (47)	374 (53)	N/A	335 (47)	374 (53)	N/A
BMC (mg/mm)	56.1 (55.3; 56.9)	52.9 (52.1; 53.7)	<0.00	54.8 (54.0; 55.5)	54.1 (53.4; 54.8)	0.212
vBMD (mg/cm^3)	381.6 (373.4; 389.8)	374.6 (366.8; 382.3)	0.221	380.2 (371.8; 388.5)	375.8 (367.9; 383.7)	0.472
CSA (mm^2)	150.8 (147.8; 153.8)	145.1 (142.3; 147.9)	0.006	147.8 (145.0; 150.7)	147.8 (145.1; 150.5)	0.995
PMI (mm^4)	3860.1 (3709.5; 4010.6)	3577.4 (3434.9; 3719.8)	0.003	3711.5 (3565.6; 3857.3)	3710.5 (3572.8;	0.950^^
PsC (mm)	43.4 (42.9; 43.8)	42.5 (42.1; 42.9)	0.005	42.9 (42.5; 43.3)	42.9 (42.5; 43.3)	0.988

^Covariates appearing in the adjusted model are: Grade, length (cm) and weight (kg).

^^logarithmic transformation

[a] t; test, [b] analysis of covariance

BMC= bone mineral content; vBMD= volumetric bone mineral density; CSA= cross; sectional area, PMI= polar moment of inertia, PsC=periosteal circumference (a circular ring model)

Table 3. Standardized coefficient, partial correlation and adjusted R^2 of multiple linear regression on bone parameters at 12 months of age.

Bone parameter and modifying variables	β (95% CI)	r	p	Adjusted R^2
BMC (mg/mm)				0.169*
Weight	0.353 (0.275; 0.435)	0.317	<0.001	
Length	0.054 (-0.020; 0.123)	0.054	0.160	
Grade	-0.117 (-0.145; -0.037)	-0.127	0.001	
Sex	-0.067 (-0.281; 0.015)	-0.068	0.078	
vBMD (mg/cm^3)				0.004**
Weight	0.053 (-0.035; 0.141)	0.046	0.237	
Length	0.025 (-0.055; 0.102)	0.023	0.553	
Grade	-0.063 (-0.108; 0.010)	-0.063	0.104	
Sex	-0.031 (-0.225; 0.100)	-0.029	0.449	
CSA (mm^2)				0.058*)
Weight	0.235 (0.151; 0.322)	0.205	<0.001	
Length	0.027 (-0.050; 0.102)	0.026	0.506	
Grade	-0.035 (-0.085; 0.030)	-0.036	0.347	
Sex	-0.011 (-0.179; 0.137)	-0.010	0.793	
PMI (mm^4)^				0.072*
Weight	0.257 (0.174; 0.343)	0.225	<0.001	
Length	0.030 (-0.048; 0.104)	0.028	0.470	
Grade	-0.026 (-0.078; 0.037)	-0.027	0.482	
Sex	-0.018 (-0.193; 0.120)	-0.017	0.651	
PsC (mm)				0.065*
Weight	0.246 (0.162; 0.333)	0.215	<0.001	
Length	0.028 (-0.049; 0.103)	0.026	0.492	
Grade	-0.035 (-0.085; 0.030)	-0.037	0.343	
Sex	-0.014 (-0.186; 0.129)	-0.014	0.723	

*= p<0.001 (significant), **=p>0.05 (insignificant) ^=logarithmic transformation
Grade is a categorical variable (1-5)
Sex is a dichotomous variable: boys value of 1, girls value of 2

Table 4. Standardized coefficient, partial correlation and adjusted R^2 of multiple linear regression on bone parameters at 24 months of age.

Bone parameter and modifying variables	β (95% CI)	r	p	Adjusted R^2
BMC (mg/mm)				0.334*
Weight	0.426 (0.342; 0.521)	0.336	<0.001	
Length	0.176 (0.126; 0.376)	0.147	<0.001	
Grade	-0.073 (-0.097; -0.010)	-0.090	0.017	
Sex	-0.040 (-0.206; 0.046)	-0.047	0.212	
vBMD (mg/cm^3)				0.002**
Weight	0.083 (-0.026; 0.194)	0.057	0.133	
Length	-0.014 (-0.173; 0.133)	-0.010	0.794	
Grade	-0.023 (-0.070; 0.037)	-0.023	0.549	
Sex	-0.028 (-0.211; 0.098)	-0.027	0.472	
CSA (mm^2)				0.121*
Weight	0.233 (0.133; 0.339)	0.168	<0.001	
Length	0.148 (0.066; 0.353)	0.108	0.004	
Grade	-0.018 (-0.063; 0.038)	-0.019	0.618	
Sex	-0.0002 (-0.145; 0.144)	-0.0002	0.995	
PMI (mm^4)^				0.136*
Weight	0.239 (0.140; 0.344)	0.173	<0.001	
Length	0.163 (0.090; 0.374)	0.120	0.001	
Grade	-0.012 (-0.059; 0.041)	-0.013	0.725	
Sex	-0.002 (-0.148; 0.139)	-0.002	0.950	
PsC (mm)				0.126*
Weight	0.232 (0.133; 0.338)	0.168	<0.001	
Length	0.156 (0.078; 0.364)	0.114	0.003	
Grade	-0.022 (-0.066; 0.034)	-0.024	0.532	
Sex	-0.001 (-0.146; 0.143)	-0.001	0.988	

*= p<0.001 (significant), **=p>0.05 (insignificant) ^=logarithmic transformation
Grade is a categorical variable (1-5)
Sex is a dichotomous variable: boys value of 1, girls value of 2

2

Figure 1. The pQCT scanner used in the study (Stratec XCT2000 L Research +) and a study subject positioning during pQCT imaging at age 24 months.

Figure 2. Images illustrating scan quality at 20% distal site of the left tibia in 24-month-old children. A) Grade 1: Cortex intact. B) Grade 2: Minimal movement artifacts visible. C) Grade 3: Some movement artifacts visible. D) Grade 4: Several movement artifacts visible. E) Grade 5: Cortex broken but bone shape definable. F) Grade 6: Unanalyzable scan, bone shape cannot be defined (excluded from analyses).

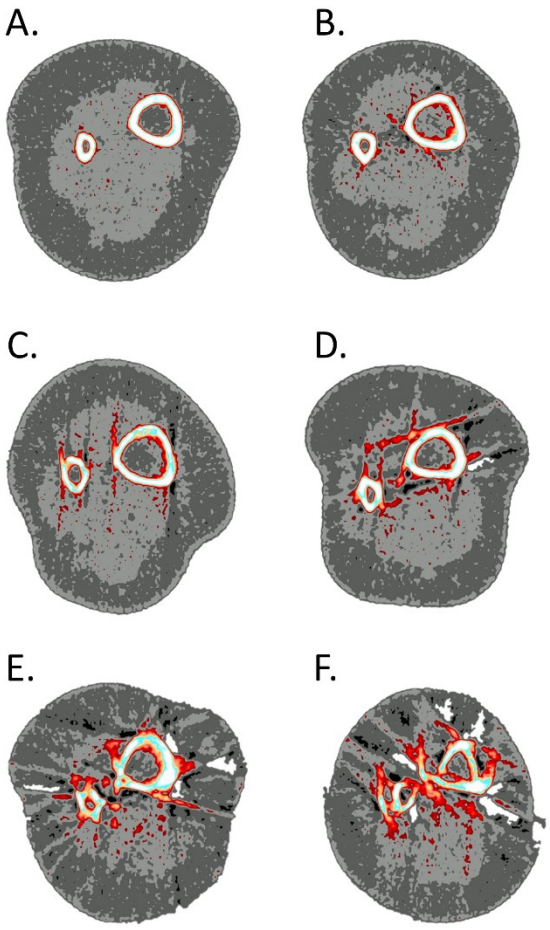


Figure 3. Participant flow chart.

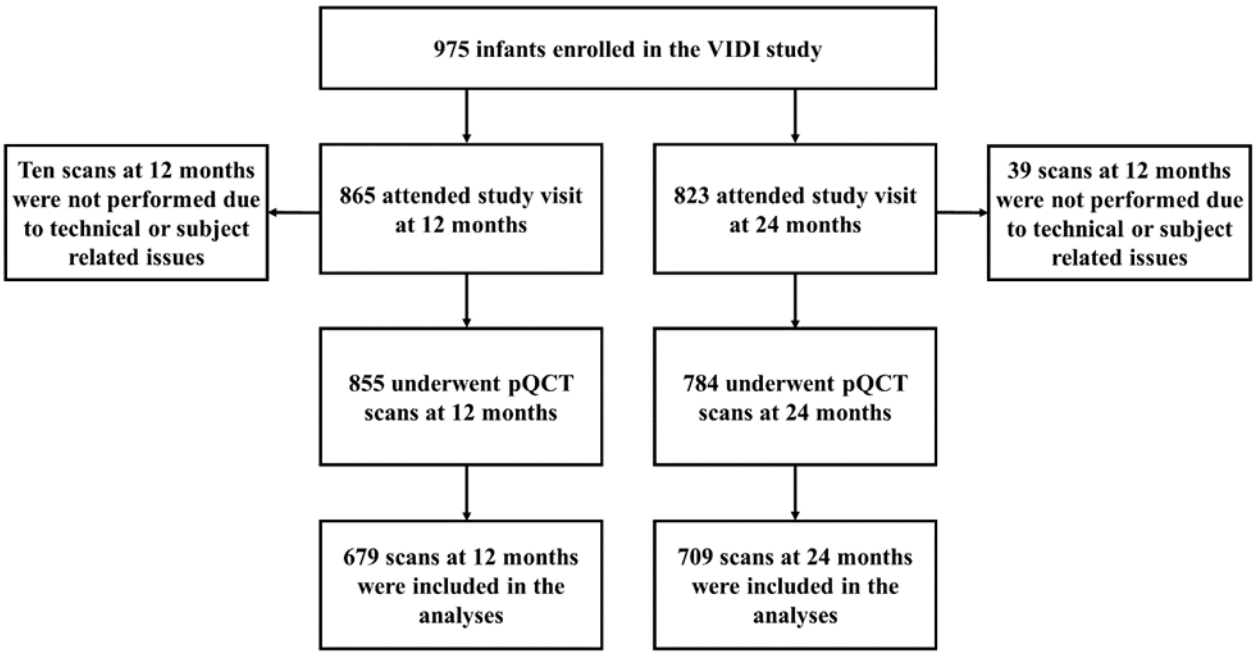
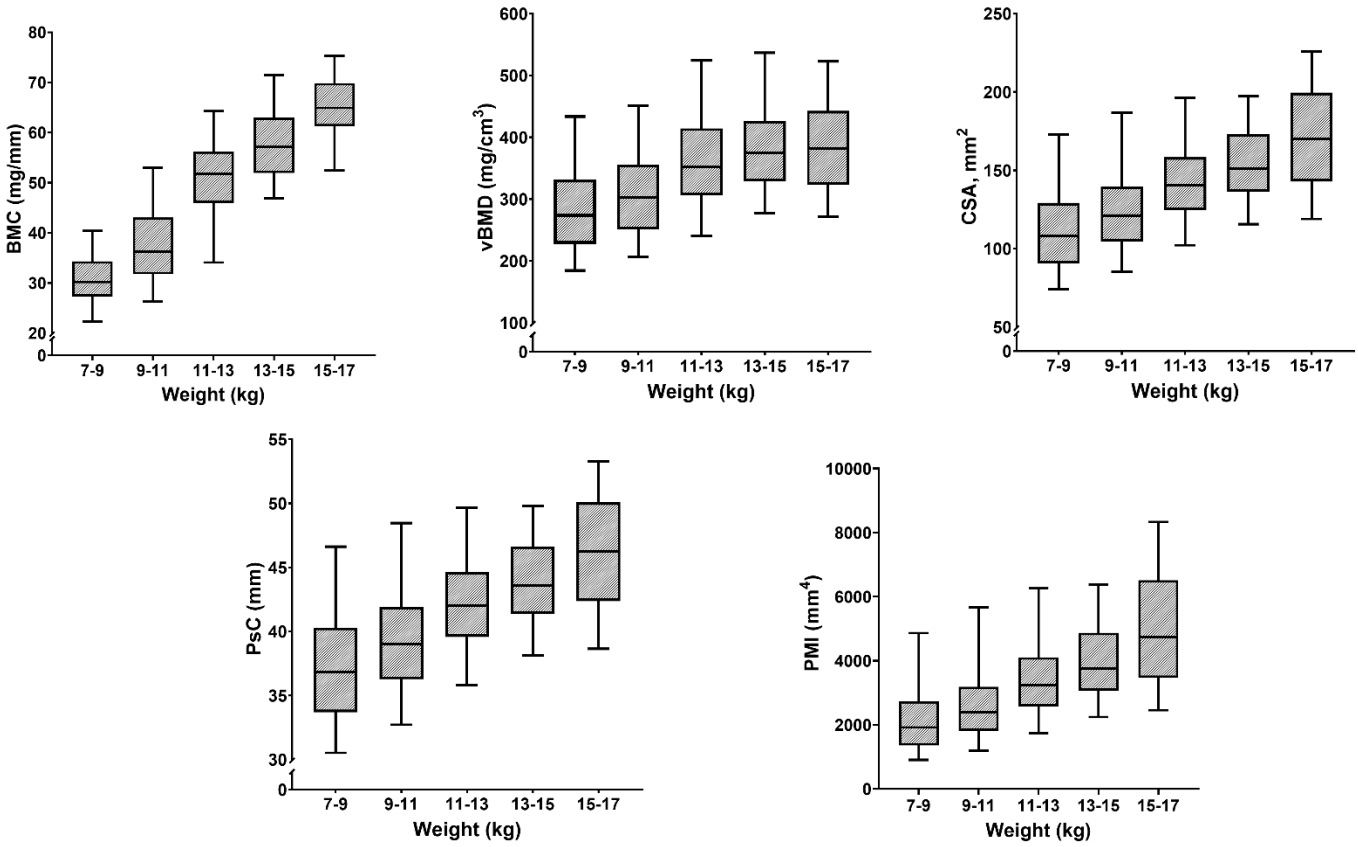
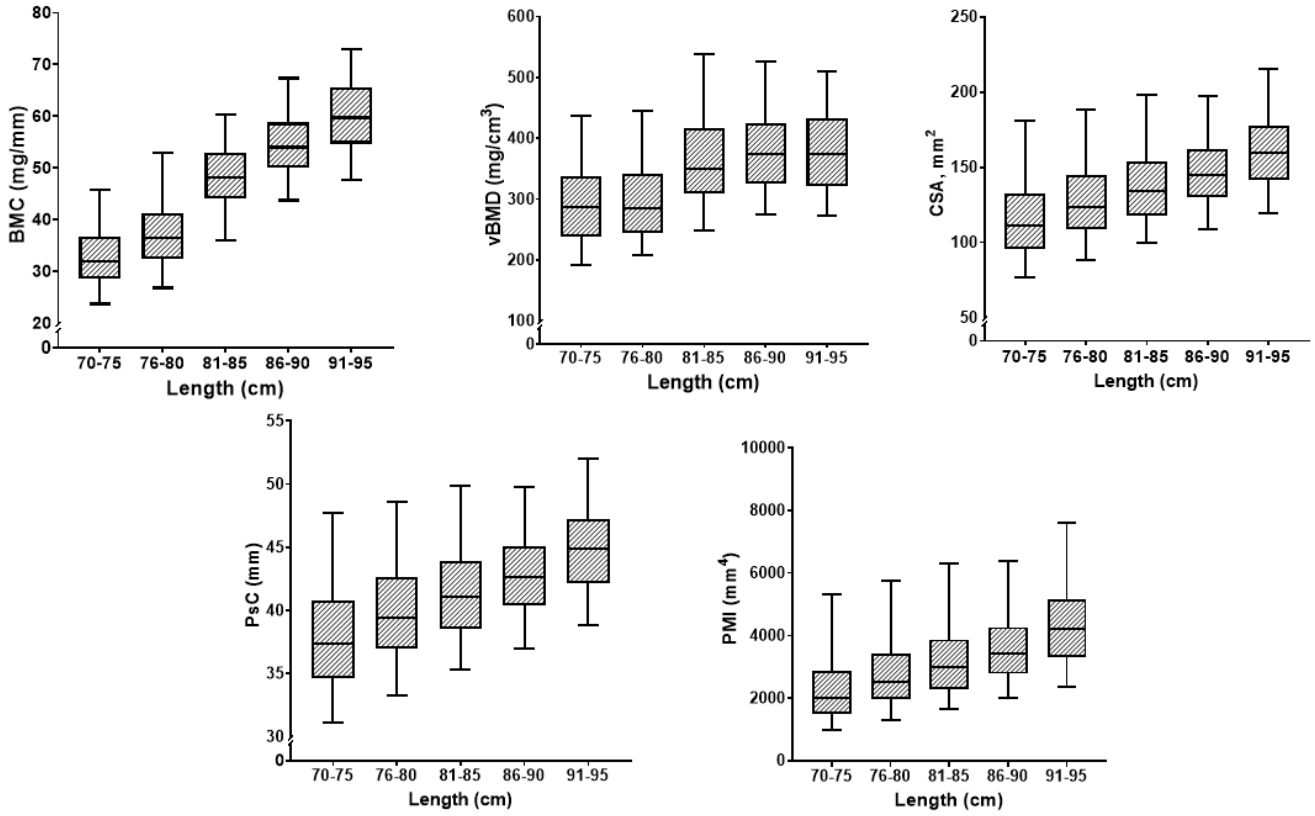


Figure 4. Box plots showing distal tibial pQCT values for total bone measured at 12 and 24 months of age according to weight (kg)*. The middle line represents the median value, the top of the box is the 75th percentile and the bottom of the box is the 25th percentile. The whiskers represent the 5% and 95% percentiles.



*Weight groups are: 7-8.99 kg, 9-10.99 kg, 11-12.99 kg, 13-14.99 kg, 15-16.99 kg

Figure 5. Box plots showing distal tibial pQCT values for total bone measured at 12 and 24 months of age according to length (cm). The middle line represents the median value, the top of the box is the 75th percentile and the bottom of the box is the 25th percentile. The whiskers represent the 5% and 95% percentiles.



SUPPLEMENTAL TABLE 1. Bone parameters for total bone at 12 and 24 months stratified by weight.

Variable	Group 1 (7.00-8.99 kg)	Group 2 (9.00-10.99 kg)	Group 3 (11.00-12.99 kg)	Group 4 (13.00-14.99 kg)	Group 5 (15.00-16.99 kg)	*p* value[a], ANOVA
N	169	496	483	202	36	N/A
BMC (mg/mm)	30.2 (27.3 to 34.3)	36.2 (31.7 to 43.1)	51.8 (46.0 to 56.2)	57.2 (51.9 to 63.0)	64.9 (61.2 to 69.8)	<0.001[b]
vBMD (mg/cm^3)	273.7 (227.6 to 331.8)	303.1 (251.4 to 355.9)	352.3 (306.9 to 414.5)	374.6 (328.9 to 426.6)	381.7 (323.3 to 442.8)	<0.001[c]
CSA (mm^2)	108.0 (90.4 to 129.2)	121.1 (104.7 to 139.7)	140.5 (124.6 to 158.4)	151.2 (136.3 to 173.0)	170.2 (142.9 to 199.6)	<0.001[d]
PsC (mm)	36.8 (33.7 to 40.3)	39.0 (36.3 to 41.9)	42.0 (39.6 to 44.6)	43.6 (41.4 to 46.6)	46.2 (42.4 to 50.1)	<0.001[e]
PMI* (mm^4)	1918.2 (1356.0 to 2735.8)	2388.7 (1808.3 to 3183.9)	3243.0 (2578.6 to 4104.2)	3757.9 (3061.4 to 4868.6)	4737.1 (3463.8 to 6508.7)	<0.001[f]

Data presented as median (IQR; 25% to 75%)

Abbreviations: ANOVA, analysis of variance; BMC, bone mineral content; vBMD, volumetric bone mineral density; CSA, cross-sectional area; PsC, periosteal circumference; PMI, polar moment of inertia

a. Subgroups compared with Tukey post hoc test with statistically significant difference at $p < 0.010$
b. Tukey post hoc test insignificant between Group 4 vs Group 5
c. Tukey post hoc test insignificant between Group 3 vs Group 4 and Group 5, Group 4 vs Group 5

d. Tukey post hoc test insignificant between Group 4 vs Group 5

e. Tukey post hoc test insignificant between Group 4 vs Group 5

f. Tukey post hoc test insignificant between Group 4 vs Group 5

* ANOVA was performed after logarithmic transformation

SUPPLEMENTAL TABLE 2. Bone parameters for total bone at 12 and 24 months stratified by length.

Variable	Group 1 (70-75 cm)	Group 2 (76-80 cm)	Group 3 (81-85 cm)	Group 4 (86-90 cm)	Group 5 (91-95 cm)	*p* value[a], ANOVA
N	345	320	169	427	117	N/A
BMC (mg/mm)	31.9 (28.5 to 36.7)	36.5 (32.4 to 41.3)	48.2 (44.1 to 53.0)	54.0 (50.0 to 58.8)	59.7 (54.6 to 65.6)	<0.001
vBMD (mg/cm^3)	287.4 (237.6 to 338.0)	284.9 (244.3 to 341.6)	348.6 (309.7 to 417.2)	374.7 (326.0 to 424.7)	374.8 (320.7 to 433.1)	<0.001[b]
CSA (mm^2)	110.9 (95.5 to 132.2)	123.8 (109.0 to 144.8)	134.6 (117.9 to 153.7)	144.8 (130.1 to 161.8)	160.0 (141.3 to 177.6)	<0.001[c]
PsC (mm)	37.3 (34.6 to 40.8)	39.4 (37.0 to 42.7)	41.1 (38.5 to 43.9)	42.7 (40.4 to 45.1)	44.8 (42.1 to 47.2)	<0.001[d]
PMI* (mm^4)	2018.1 (1508.0 to 2869.7)	2501.7 (1950.6 to 3422.7)	2976.8 (2282.4 to 3874.7)	3430.9 (2778.0 to 4269.2)	4218.4 (3309.4 to 5148.5)	<0.001

Data presented as median (IQR; 25% to 75%)

Abbreviations: ANOVA, analysis of variance; BMC, bone mineral content; vBMD, volumetric bone mineral density; CSA, cross-sectional area; PsC, periosteal circumference; PMI, polar moment of inertia

a. Subgroups compared with Tukey post hoc test with statistically significant difference at $p < 0.010$

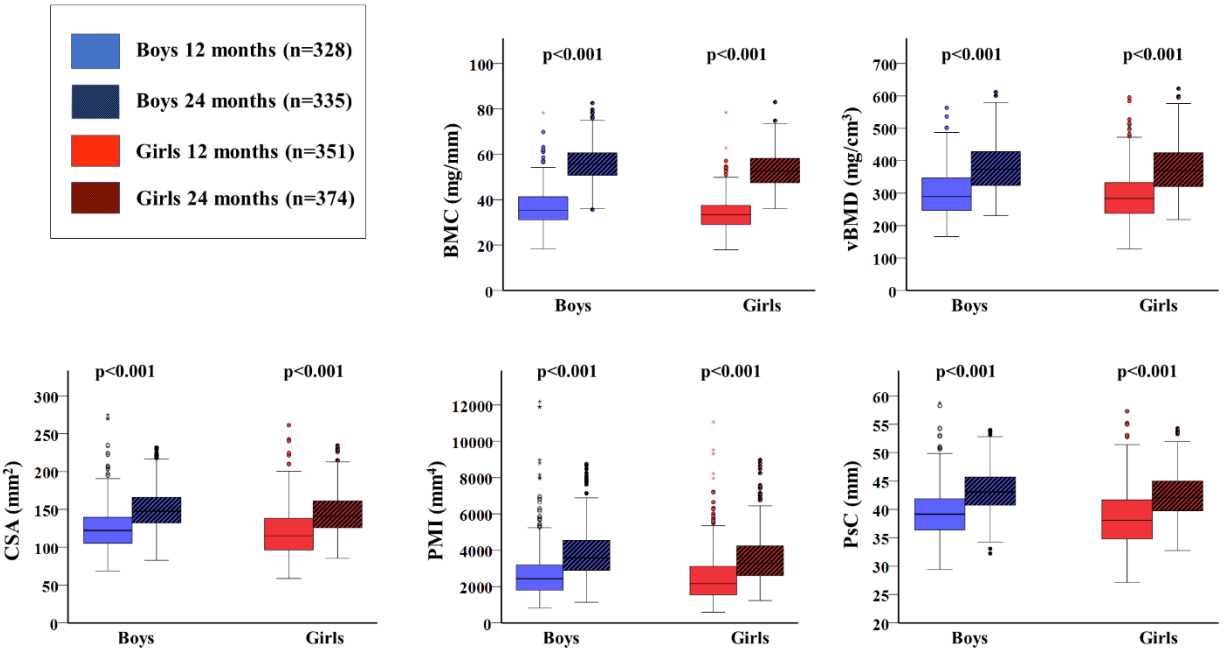
b. Tukey post hoc test insignificant between Group 1 vs Group 2, Group 3 vs. Group 4 and Group 5, Group 4 vs. Group 5

c. Tukey post hoc test insignificant between Group 2 vs Group 3

d. Tukey post hoc test insignificant between Group 2 vs Group 3

* ANOVA was performed after logarithmic transformation

Supplemental figure 1. Box plots showing comparison of the unadjusted distal tibial pQCT values for total bone between 12 and 24 months of age according to sex. The middle line represents the median value, the top of the box the 75th percentile and the bottom of the box the 25th percentile. The whiskers represent the 95% confidence interval.



Supplemental figure 2. A scatterplot of total bone BMC vs length, vBMD vs length, BMC vs weight, and vBMD vs weight. Blue dots represents Grade 1-2 scans and red dots Grade 3-5 scans. Girls are marked with triangles and boys with circles. Blue solid line represents a trendline for girls with Grade 1-2 scans and a red solid line boys with Grade 1-2 scans. Blue dashed line represents a trendline for girls with Grade 3-5 scans and a red dashed line boys with Grade 3-5 scans.