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ORIGINAL ARTICLE

Calcium and vitamin-D supplementation on bone structural properties in peripubertal female identical twins: a randomised controlled trial

D. A. Greene · G. A. Naughton

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Abstract
Summary A randomised controlled trial was used in assessing the impact of 6 months of daily calcium and vitamin-D supplementation on trabecular and cortical bone acquisition at distal tibial and radial sites using peripheral quantitative computed tomography (pQCT). Daily supplementation was associated with increased bone density and bone strength at the distal tibia and radius.
Introduction pQCT has not been used to assess bone responses to calcium and vitamin-D supplementation on peripubertal children. This randomised controlled trial aimed to assess the impact of a 6-month daily calcium and vitamin-D supplementation on trabecular and cortical bone acquisition at distal tibial and radial sites using pQCT.
Methods Twenty pairs of peripubertal female identical twins, aged 9 to 13 years, were randomly assigned to receive either 800 mg of calcium and 400 IU of vitamin D3, or a matched placebo. Bone structural properties at the distal tibia and distal radius were acquired at baseline and 6 months.
Results The calcium-supplemented group showed greater gains in trabecular density, trabecular area and strength strain index at the 4% of distal tibial and radial sites compared with the placebo group (p=0.001). Greater gains in cortical area at the 38% and 66% of tibial sites were also found in twins receiving the calcium supplement (p=0.001).
Conclusions Daily supplementation for a period of 6 months was associated with increased trabecular area, trabecular density and strength strain index at the ultra-distal tibia and radius and increased cortical area at tibial mid-shaft.

Keywords Bone strength · Calcium · pQCT · Supplementation · Trabecular

D. A. Greene (✉) · G. A. Naughton
Centre of Physical Activity Across the Lifespan (CoPAAL),
School of Exercise Science, Australian Catholic University,
Locked Bag 2002,
Strathfield, NSW 2763, Australia
e-mail: david.greene@acu.edu.au

Introduction

Peripubertal development may or may not be the ideal period to potentially and permanently advance bone mass given that gains in bone mass peak towards the end of puberty [1–3]. Bone loss in later life is related to the quality of peak bone mass established over the first two decades of life [4]. Strategies to permanently maximise peak bone mass during childhood remain under-researched.

Modifiable lifestyle factors such as physical activity and nutrition are important for optimising the genetic potential for peak bone mass during skeletal consolidation. Calcium is a primary bone-forming mineral that must be supplied to the diet and is the most important during childhood when approximately 200 mg/d is accreted into the skeleton [5]. Vitamin D appears to be essential for calcium uptake, bone development and remodelling. Even mild vitamin-D insufficiency can have detrimental effects on bone mineral acquisition [6, 7]. Therefore, increasing calcium and vitamin D intake during childhood and early adolescence is proposed as an effective way to maximise peak bone mass.

A significant, positive effect of calcium supplementation on bone mineral density has been demonstrated in pre- and peripubertal children [5, 8–14]. Milk products with the addition of protein and phosphorus are also associated with bone density gains in this age group [15]. However, positive effects on bone are not consistently reported [16–18]. Specifically, a meta-analysis [18] of calcium supple-

mentation on bone density in healthy children showed some trials observing significant bone effects at different pubertal stages and at some bone sites, but not others. Furthermore, a number of studies challenge the permanency of calcium-related gains in bone mass in childhood [19].

Few studies [9, 12] have adequately controlled genetic and environmental factors in ascertaining the effects of calcium and vitamin D supplementation on bone density. Most studies have compared unrelated individuals. Co-twin study designs confer substantial advantages by controlling the additive genetic effects on bone mineral measurements during a period of strong genetic variance [20]. In paediatric twin studies, the likelihood of similar phenotypical lifestyle factors such as nutrition and physical activity is also high.

Traditionally, bone mass measurements were quantified using two-dimensional scanning technology such as Dual X-ray Absorptiometry (DXA). In the past decade however, technological advances in non-invasive three-dimensional methods of bone assessment have developed considerably. The combination of bone mass and biomechanical properties can determine bone strength and predict fracture risk. Peripheral quantitative computed tomography (pQCT) provides a three-dimensional assessment of bone properties which includes, but is not limited to, quantification of bone architecture at the trabecular level and the calculation of bone strength known as strength strain index (SSI). SSI is calculated as the product of section modulus (which is directly proportional to the maximum stress in bone) and volumetric cortical bone mineral density (BMD) normalised to the maximum physiological cortical BMD of human bones. Few studies [8, 12, 16, 21, 22] have used pQCT to assess the effect of calcium supplementation on bone density in children. No study has combined pQCT technology to assess changes to bone properties, in particular trabecular bone at radial and tibial sites, in related individuals, randomly assigned to receive either a calcium and vitamin-D supplement or a matched placebo in a double-blind manner.

The purpose of this 12-month randomised, double-blind, placebo-controlled trial was to assess the effect of daily calcium and vitamin-D supplementation on bone material properties using pQCT in peripubertal female identical twins. Data presented are limited to the baseline to 6-month comparison only.

Materials and methods

Participants

Forty-nine female twin pairs, aged 9 to 13 years, enrolled with the Australian Twin Registry were invited to participate between March 2007 and July 2007 to participate in a 12-month randomised, double-blind, placebo-controlled trial. Twenty-two pairs of peripubertal female identical twins initially agreed to participate in the clinical trial (Fig. 1). Twenty pairs of twins commenced the study at baseline. The study was approved by both the Human Research Ethics Committee of Australian Catholic University and the Australian Twin Registry (20060739); however, clinical trial registration number was not sought. For each twin pair, written informed consent was obtained from each participant and from at least one parent or legal guardian. Participants were healthy, used no medications known to affect calcium metabolism, and were of white ethnicity.

Study design

One member in each twin pair was randomly assigned using computer-generated numbers to receive 800 mg of elemental Calcium from citrate and carbonate, 400 IU of vitamin D3 (as Cholecaliferol), 400 mg of Magnesium from citrate, and amino acid chelate and oxide in four orange-flavoured chewable tablets (Active Calcium™ Chewable); the other twin was given a matched placebo in a double-blinded manner. The placebo tablet was identical in appearance, taste and composition but contained no active ingredient. All tablets were supplied by USANA Health Sciences, Inc., Sydney, Australia. Allocation concealment

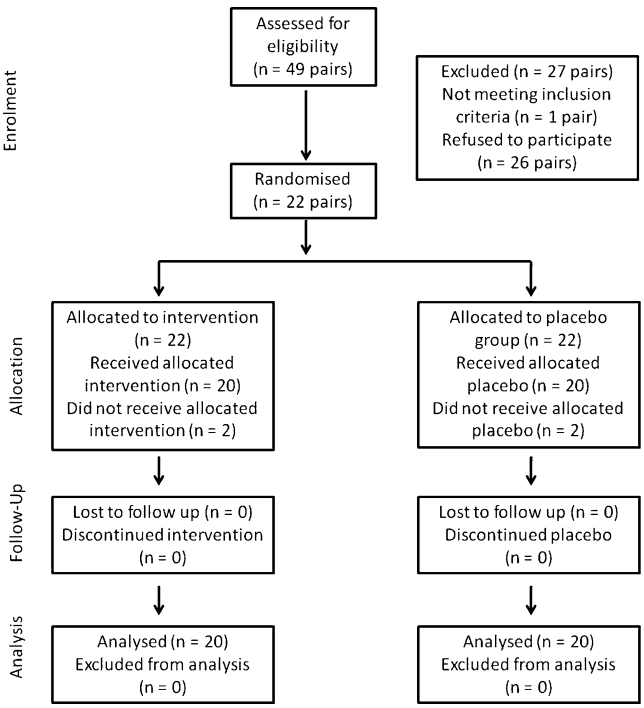

Fig. 1 Box-flow of number of participants recruited and withdrawn from trial

occurred via sealed envelopes provided to parents by a central administrator not involved in the study. Twins were instructed to take two tablets with their morning and evening meals for a total of four tablets per day. Tablet compliance was assessed at 6 months by tablet count and parental responses to key questions including a request to report if tablets were incorrectly consumed. Participants visited the Australian Catholic University, Strathfield campus, at baseline and 6 months.

Anthropometric measures and pubertal assessment

Body weight was measured using an electronic scale accurate to 500 g (Wedderburn UW150, Sydney, Australia) with participants dressed in light clothing and without shoes. Standing height was measured to 0.1 cm using a standard stadiometer (Wedderburn UW150). Pubertal status was determined using proxy-reported assessments of Tanner stage for pubic hair and breast development [23, 24]. Menstrual history was determined by questionnaire and included age at menarche, and number of menses in the previous 12 months. Menses was coded into three categories; eumenorrheic (once per month), oligomenorrehic (once every 2 months) and amenorrehic (more than 2 months apart).

Physical activity and dietary assessment

Intensity and type of physical activity was estimated using Bouchard's Physical Activity record [25]. Activities were ranked on a scale from 1 to 9 according to energy expenditure with the least vigorous activity, scoring 1, and the most vigorous activity, scoring 9. Activity intensity was estimated for every 15 min over three 24-h periods. Results were analysed by the same investigator and expressed as mean kilojoules of energy expenditure per three days. Participants completed a medical and fracture history questionnaire at the time of baseline testing. Previous medical treatment and medication usage in the past 12 months were included. A 3-day-food diary was completed by participants for two weekdays and one weekend day. Total caloric (kJ), calcium intake (mg) and macronutrient data were estimated using Foodworks™ software program (Xyris Software 2007, Version 5.0, Brisbane, Australia).

Bone mineral measurement

The non-dominant tibia and radius were measured by pQCT (XCT 2000, Stratec Medizintechnik, Pforzheim, Germany) using software version 5.50d at baseline and 6 months for all participants. The scanner was positioned at the site of the tibia whose distance to the distal anatomic reference line (cortical end plate) corresponded to 4%, 14%, 38% and 66% of tibial length. Similarly, the scanner was positioned at the radius at a distance to the distal cortical end plate that corresponded to 4% and 66% of radial length. Variables measured and scan sites are shown in Fig. 2. Medullary cross-sectional area (mm^2) was calculated by subtracting cortical cross-sectional area (mm^2) from total bone area (mm^2).Tibial length (cm) was determined externally using the mid-point of the distal medial malleolus and the proximal medial tibial plateau as landmarks. Radial length was determined externally using the mid-point of the styloid and olecranon processes. Software from the pQCT permitted a planar scout scan to determine an anatomic reference line. To analyse trabecular bone, a contour mode with a threshold of 180 mg/cm^3 was used to separate soft tissue and bone. A constant default threshold of 711 mg/cm^3 was used to identify and remove cortical bone. A region of interest (ROI) identified the tibia or radius automatically or was manually adjusted to ensure the entire tibial or radial ROI was completely enclosed. To manually demarcate the ROI, a trace function was selected. While measurements during growth are problematic, standard practices at least partially address the issue of locating sites in growing bones.The precision of repeat measurements in our department is 0.7% to 1.4% of the radius and 0.8% to 2.9% of the tibia after repositioning in eight adults. Precision was not determined using children due to ethical concerns of repeated exposure to radiation. Scans were analysed by the same investigator (PW) independent from this study and blinded to group allocation.

Statistical methods

Statistical analyses were performed using SPSS version 16.0 for Windows (SPSS, Chicago, IL, USA). Means and standard deviations are reported for descriptive statistics. Paired *t* tests were used to assess within-pair differences in volumetric mineral bone density (vBMD), bone area, muscle cross-sectional area and strength strain index. Within-pair differences were calculated as the (calcium twin at 6 months–calcium twin at baseline)–(placebo twin at 6 months–placebo twin at baseline). To comply with common practice in growth-related reports of bone responses, analyses included adjustments via covariance for limb length. However, given the absence of difference in results, unadjusted data are presented.

A p value of <0.05 was considered significant for statistical tests.

Results

All 20 twin pairs enrolled in the study completed 6 months of intervention. Baseline descriptive characteristics are

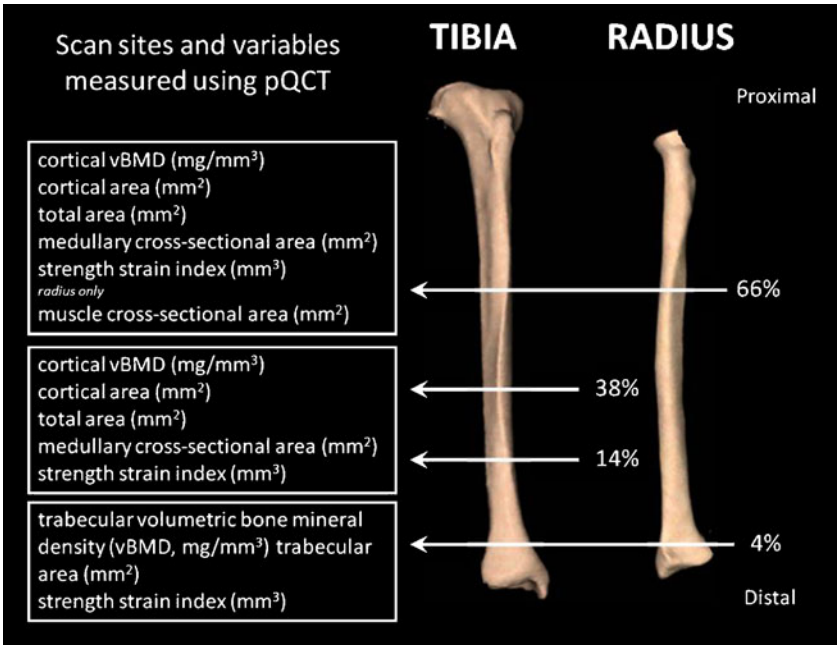

Fig. 2 Tibial and radial scan sites and bone variables measured using pQCT

compared with 6-month characteristics in Table 1. No differences in descriptive data were observed between the calcium and placebo groups for any baseline or 6-month comparisons. All twin pairs were premenarcheal at baseline and only two twin pairs commenced menstruation at 6 months. Frequency distribution of pubertal development from baseline to 6 months is shown in Table 2. Tablet compliance did not differ between the calcium and placebo groups. The calcium group consumed 98.3% of their tablets (95%–100% compliance range) and the placebo group consumed 98% of their tablets (94.7%–100% compliance range). The calcium-supplemented group received an

Table 1 Baseline and 6-month descriptive characteristics of calcium and placebo groups

	Treatment n=20	Placebo n=20	95% CI		p value
	Mean (SD)	Mean (SD)	Lower	Upper	
Age (years)	11.08 (1.1)	11.08 (1.1)	−0.71	0.71	1.000
Weight (kg)					
Baseline	39.4 (9.0)	39.7 (8.8)	−6.05	5.41	0.911
6 months	40.7 (8.4)	41.4 (8.7)	−6.17	4.81	0.803
Height (cm)					
Baseline	149.0 (9.6)	149.2 (10.2)	−6.45	6.21	0.968
6 months	151.3 (9.7)	150.9 (9.9)	−5.91	6.64	0.906
Tanner stage (I–V)					
Baseline					
Breast development	1.31 (0.47)	1.35 (0.49)	−0.35	0.25	0.744
Pubic hair	1.45 (0.68)	1.5 (0.68)	−0.37	0.37	1.000
6 months					
Breast development	1.85 (0.58)	1.85 (0.58)	−0.48	0.39	0.819
Pubic hair	2.05 (0.68)	2.1 (0.71)	−0.49	0.41	0.823
Physical activity (mean kJ per day)	131.1 (16.5)	132.8 (16.7)	−12.31	8.91	0.747
3-day-food intake (per day)					
Energy (kJ)	7,874.1 (687.1)	7,966.5 (496.2)	−476.05	291.22	0.629
Calcium (mg)	763.1 (158.4)	786.4 (156.6)	−124.07	77.58	0.643

Table 2 Frequency distribution of pubertal development from baseline to 6 months for combined groups

	Baseline (n=40)		6months (n=40)	
	Supplemented twin	Placebo twin	Supplemented twin	Placebo twin
Breast development				
Tanner stage 1	13	13	5	5
Tanner stage 2	7	7	13	13
Tanner stage 3	0	0	2	2
Pubic hair				
Tanner stage 1	12	12	4	4
Tanner stage 2	6	6	10	10
Tanner stage 3	2	2	6	6

average of 1,563.1 mg of calcium per day (diet plus supplement) compared with the placebo group which received an average of 786.4 mg of calcium per day.

Group means (±SD) at baseline and 6 months are shown in Tables 3 (tibia) and 4 (radius), and results from paired t tests for within co-twin differences from baseline expressed as percent change are shown in Tables 5 (tibia) and 6 (radius). No within-pair differences were found at baseline. The calcium-supplemented group showed greater gains in trabecular vBMD (13.89±0.48 mg/mm^3, or 5.2%, p=0.001), trabecular area (24.7±0.14 mm^2, or 5.4%, p=0.001) and strength strain index (65.4±8.9 mm^3, or 6.6%, p=0.001) at the 4% distal tibial site compared with the placebo group. Greater gains in cortical area at the 38% (12.15±1.2 mm^2, or

Table 3 Measurements at baseline and 6 months

Site	Tibial variable	Baseline				p value	6months				p value
		Treatment n=20		Placebo n=20			Treatment n=20		Placebo n=20		
		Mean	(SD)	Mean	(SD)		Mean	(SD)	Mean	(SD)	
4%	Trabecular vBMD (mg/mm^3)	262.66	25.53	265.77	26.08	0.58	278.45	25.95	267.67	26.04	0.05
	Trabecular area (mm^2)	470.35	52.02	463.54	38.60	0.45	499.72	52.27	468.15	39.12	0.01
	Subcortical density (mg/mm^3)	307.27	48.03	308.89	47.05	0.16	321.77	43.11	310.94	47.77	0.39
	Subcortical area (mm^2)	577.16	64.98	568.11	45.18	0.41	586.15	63.90	576.65	44.90	0.38
	Stress/Strain index (mm^3)	1,104.77	330.74	1,104.33	325.28	0.98	1,177.00	321.63	1,111.09	324.48	0.04
14%	Cortical BMD (mg/mm^3)	975.44	28.36	972.66	28.96	0.42	981.66	29.37	979.91	29.93	0.66
	Cortical area (mm^2)	118.07	17.27	117.36	20.27	0.61	121.77	17.62	119.52	19.53	0.19
	Total bone area (mm^2)	320.12	40.89	319.36	43.56	0.72	321.67	44.19	320.05	42.22	0.59
	Medullary cavity CSA (mm^2)	202.05	33.13	201.80	35.08	0.89	199.90	33.67	200.53	37.49	0.41
	Stress/Strain index (mm^3)	1,025.37	182.45	1,022.58	200.77	0.84	1,050.48	182.99	1,031.10	201.57	0.23
38%	Cortical BMD (mg/mm^3)	1,062.98	44.27	1,066.02	47.59	0.78	1,091.90	44.12	1,085.93	43.55	0.57
	Cortical area (mm^2)	209.55	28.66	208.83	25.57	0.72	224.53	29.80	211.66	26.90	0.01
	Total bone area (mm^2)	331.35	43.33	330.44	45.45	0.66	336.12	46.89	330.61	45.33	0.77
	Medullary cavity CSA (mm^2)	121.8	22.56	121.61	26.81	0.73	111.59	24.50	118.95	25.15	0.05
	Stress/Strain index (mm^3)	1,077.03	199.89	1,077.28	204.98	0.98	1,111.97	202.97	1,105.91	216.79	0.58
66%	Cortical BMD (mg/mm^3)	1,022.56	29.27	1,023.77	39.24	0.84	1,029.94	29.02	1,029.47	39.75	0.94
	Cortical area (mm^2)	252.91	34.09	256.95	37.86	0.16	276.87	35.84	267.05	42.32	0.03
	Total bone area (mm^2)	371.12	69.44	375.82	68.22	0.92	376.02	62.34	376.56	61.19	0.37
	Medullary cavity CSA (mm^2)	118.21	26.72	118.87	27.07	0.74	99.15	28.36	109.51	26.66	0.01
	Muscle cross-sectional area (mm^2)	4,382.68	572.28	4,605.20	312.24	0.11	4,519.42	511.65	4,711.53	343.67	0.11

Table 4 Measurements at baseline and 6 months

Site	Radial variable	Baseline				*p* value	6months				*p* value
		Treatment *n*=20		Placebo *n*=20			Treatment *n*=20		Placebo *n*=20		
		Mean	(SD)	Mean	(SD)		Mean	(SD)	Mean	(SD)	
4%	Trabecular vBMD(mg/mm^3)	295.15	56.58	285.83	52.43	0.11	311.98	58.80	293.03	51.57	0.01
	Trabecular area (mm^2)	125.02	18.31	122.42	17.74	0.39	133.87	17.12	127.86	17.44	0.03
	Subcortical density (mg/mm^3)	269.57	2.99	270.42	4.15	0.38	271.73	2.97	272.23	4.07	0.62
	Subcortical area (mm^2)	180.34	4.77	179.15	5.02	0.13	184.19	4.89	182.55	6.38	0.11
	Stress/Strain Index (mm^3)	142.00	6.19	142.88	6.83	0.55	155.87	8.88	148.58	10.35	0.01
66%	Cortical BMD (mg/mm^3)	1,076.90	6.50	1,078.23	6.44	0.45	1,082.83	8.06	1,082.09	7.82	0.59
	Cortical area (mm^2)	126.12	8.53	124.65	6.30	0.35	129.98	7.46	127.93	5.80	0.88
	Total bone area (mm^2)	179.91	23.89	179.22	22.67	0.55	181.39	27.72	180.18	26.17	0.66
	Medullary cavity CSA (mm^2)	53.79	8.11	54.57	9.13	0.61	51.41	9.75	52.25	10.38	0.57
	Stress/Strain Index (mm^3)	1,030.95	42.14	1,031.26	41.40	0.84	1,043.34	42.10	1,040.64	42.70	0.33
	Muscle cross-sectional area (mm^2)	2,047.34	50.78	2,036.48	53.97	0.35	2,062.62	52.88	2,045.71	53.11	0.17

5.8%, p=0.001) and 66% (13.86±1.5 mm^2, or 5.7%, p=0.001) tibial sites were found in the twins receiving the calcium supplement compared with placebo twins Supplemented twins also showed a concomitant decrease in tibial medullary cavity cross-sectional area at the 38% (−7.55±0.28 mm^2, or−6.2%, p=0.001) and 66% (−9.7±0.58 mm^2, or −8.1%, p=0.001) sites compared with the placebo group (Tables 3 and 5).The calcium-supplemented group showed greater gains in trabecular vBMD (9.6±0.8 mg/mm^3, or 3.3%, p=0.001), trabecular area (3.4±0.5 mm^2, or 2.8%, p=0.02), and strength strain index (8.2±0.8 mm^3, or 5.7%, p=0.001) at the 4% distal radial site compared with the placebo

Table 5 Within co-twin differences from baseline values in tibial variables at 6 months, expressed as percent change

Site	Tibial variable	Baseline to 6months (*n*=20 pairs)		
		Percentage gain (%)		*p* value
		Mean	SD	
4%	Trabecular vBMD(mg/mm^3)	5.2	1.96	0.001
	Trabecular area (mm^2)	5.4	1.33	0.001
	Subcortical density (mg/mm^3)	3.4	0.82	0.32
	Subcortical area (mm^2)	0.8	0.07	0.63
	Stress/Strain index (mm^3)	6.6	1.26	0.001
14%	Cortical BMD (mg/mm^3)	0.1	0.04	0.33
	Cortical area (mm^2)	1.3	0.35	0.12
	Total bone area (mm^2)	0.3	0.02	0.38
	Medullary cavity CSA (mm^2)	−0.4	0.02	0.44
	Stress/Strain index (mm^3)	1.7	0.22	0.09
38%	Cortical BMD (mg/mm^3)	0.8	0.03	0.28
	Cortical area (mm^2)	5.8	0.8	0.001
	Total bone area (mm^2)	0.5	0.03	0.29
	Medullary cavity CSA (mm^2)	−6.2	1.6	0.001
	Stress/Strain index (mm^3)	0.7	0.06	0.44
66%	Cortical BMD (mg/mm^3)	0.1	0.02	0.54
	Cortical area (mm^2)	5.7	0.39	0.001
	Total bone area (mm^2)	−0.7	0.03	0.59
	Medullary cavity CSA (mm^2)	−8.1	1.82	0.001
	Muscle cross-sectional area (mm^2)	0.6	0.02	0.61

Table 6 Within co-twin differences from baseline values in radial variables at 6 months, expressed as percent change

Site	Radial variable	Baseline to 6months (n=20 pairs)		
		Percentage gain (%)		p value
		Mean	SD	
4%	Trabecular vBMD(mg/mm^3)	3.3	0.28	0.001
	Trabecular area (mm^2)	2.8	0.36	0.02
	Subcortical density (mg/mm^3)	0.1	0.03	0.36
	Subcortical area (mm^2)	0.3	0.01	0.38
	Stress/Strain index (mm^3)	5.7	0.51	0.001
66%	Cortical BMD (mg/mm^3)	0.2	0.03	0.21
	Cortical area (mm^2)	0.5	0.02	0.48
	Total bone area (mm^2)	−0.4	0.02	0.33
	Medullary cavity CSA (mm^2)	−0.1	0.01	0.22
	Stress/Strain index (mm^3)	0.3	0.01	0.28
	Muscle cross-sectional area (mm^2)	0.3	0.03	0.21

group (Tables 4 and 6). No differences were found between the calcium-supplemented and placebo groups at the 66% distal radial site.

Discussion

This is the first randomised controlled trial to assess bone responses to calcium and vitamin-D supplementation in female peripubertal identical twins using pQCT. The treatment group were found to have greater gains in BMD and strength strain index relative to the placebo group after 6-month intervention. Results indicate that compared with placebo conditions, an average calcium supplementation of 1,563 mg per day during early adolescence is effective in enhancing bone accrual at distal tibial and radial sites. Specifically, gains following supplementation were observed for trabecular area, trabecular volumetric density and strength strain index at the 4% site for both the radius and tibia. However, gains in cortical area and a concomitant reduction in medullary cavity cross-sectional area was observed only at 38% and 66% of the tibial region, were not apparent at the radius.

When DXA was used to assess calcium supplementation in children equivocal results were reported [9, 11, 12, 17]. On average, positive skeletal changes between 2% to 3% in bone mineral content or areal bone mineral density (aBMD) were reported at whole body and regional sites using DXA. However, the planar nature of DXA precludes true volumetric and geometric assessment of bone. Studies using DXA also report ultra-distal or lumbar spine as sites representative of trabecular bone. The present study used three-dimensional scanning technology (pQCT) to assess changes to bone properties, and in particular trabecular bone, at the distal tibia and distal radius. The distal tibia is an ideal site for trabecular bone assessment due to the larger trabecular surface area [8]. Furthermore, isolation of trabecular bone using pQCT avoids partial volume effects associated with cortical bone growth in children.

Sufficient calcium intake is necessary to offset daily losses of calcium and low calcium absorption efficiency. Calcium must be absorbed in sufficient quantities to meet the demands of growth during childhood and adolescence. When absorbed calcium is insufficient to offset daily losses, bone mineral is scavenged [26]. Calcium retention is dose-responsive to calcium intake until a biological threshold is obtained. No further increase in intake will alter calcium retention once threshold has been reached. The threshold is predominately determined by skeletal needs and is estimated to be approximately 1,400–1,600 mg/d for optimally nourished children [26]. Previous work by Johnston et al. (1992) found increased lumbar spine BMD in supplemented prepubertal twins with daily calcium intakes at the upper threshold limit. The supplemented group had an average daily calcium intake of 1,612 mg/d (1,000 mg/d calcium citrate) compared with 908 mg/d in the placebo group after a 3-year-supplementation period. A shorter trial of 18 months also found significant changes in lumbar spine aBMD in supplemented peripubertal girls with an average daily calcium intake of 1,300 mg/d compared with 960 mg/d in the control group [27]. In the present study, the supplemented group achieved positive skeletal responses with an average calcium intake of 1,563 mg/d compared with the placebo group of 786 mg/d. The results support the hypothesis that sub-threshold dietary calcium intake in the control group was insufficient to illicit positive skeletal responses, particularly at the ultra-distal tibial and radial sites.

An established relationship exists between calcium and vitamin D. Specifically, calcium and vitamin D decrease

excessive bone remodelling by reducing parathyroid hormone secretion. Vitamin D also acts by facilitating active calcium transport, thereby increasing absorption efficiency [26]. It is important when evaluating evidence relating to the efficacy of calcium and vitamin D that both nutrients are considered together. Meta-analyses [16] and other systematic reviews [28] that analyse studies using either calcium or vitamin D alone as the primary supplement are likely to produce misleading results. Only one study [8] has combined calcium and vitamin-D supplementation to asses bone material responses in a group of unrelated preadolescent females. Previous twin studies [9, 12, 14] have used calcium alone to examine changes in bone material responses to supplementation. Moderate skeletal responses were found at whole body and regional sites using DXA. It is plausible that positive skeletal responses to supplementation at trabecular sites were achieved in these studies but escaped detection due to imaging limitations inherently found with DXA. Therefore, results in the present study are strengthened by the use of pQCT.

Previous longitudinal calcium supplementation resulted in greater cortical thickness at the proximal radius in females during late adolescence [29]. In the present study, differences in cortical thickness were observed at the tibia but not the radius. However, the absence of baseline pQCT data and variations of sites selected for proximal radius measurements impair comparisons of pQCT findings.

Our findings are supported by Moyer-Mileur et al. [8] who reported a significant difference in trabecular density at the distal tibia after 6-month calcium (carbonate) and vitamin-D supplementation between two groups of non-related preadolescent females aged 11.9 years. Significant gains in trabecular bone content and density at the distal tibia were found in the supplemented group (800 mg/d calcium carbonate and 400 IU/d vitamin D) compared with controls. Despite the use of an identical supplement in quantity during the present study, and ingredient and dosage from the same supplier (USANA Health Sciences, Inc., Sydney), no baseline calcium intakes were reported for the treatment group in the study by Moyer-Mileur et al. [8]. The present study used the manufacturer-recommended 4% distal site to assess trabecular bone at the tibia and radius, as opposed to the single, 10% distal tibial site used by Moyer-Mileur et al. [8]. Similar gains in trabecular bone have been observed in clinical trials of children in whom higher calcium intake caused a decrease in bone resorption with a concomitant increase in trabecular bone formation [26]. Unfortunately it is beyond the scope of the present study to determine if the remodelling transient that has resulted in increased trabecular density was due to greater trabecular thickness, greater trabecular number or a combination of both factors. Potential confounding factors in the present study such as daily physical activity, pubertal stage and maturational status did not differ between twin pairs and therefore have limited use in explaining the variation observed in trabecular bone acquisition between supplemented and placebo groups.

We further explored differences between twins and between sites for patterns in bone structural changes. Specifically, we subtracted gains at the tibia from gains at the radius and compared the differences between the supplemented and placebo twin. The gain was approximately 20-fold higher in the supplemented twin. However, speculation about causal mechanisms remains difficult despite identical genetics in monozygotic twins and similar practices in nutrition and physical activity. One possible explanation may be that a greater uptake of calcium occurred at the tibia and radius triggered by the greater availability of calcium and vitamin D. Although hypothetical, a further explanation may lie in a higher responsiveness to mechanical loading triggered by the presence of these micronutrients.

Studies comparing the osteogenic benefits of combining physical activity and calcium supplementation in prepubertal children have found evidence of increased cortical areas at the distal tibia [16, 22]. Intervention groups assigned to receive calcium supplements (carbonate or phosphate) were exposed to moderate impact loads from gross motor activities such as skipping, hopping and jumping. No increase in cortical area was found in supplemented groups at unloaded sites such as the radius–ulna [16] or due to fine motor tasks [22]. The present study found evidence of increased cortical areas at 38% and 66% distal tibial sites in the supplemented group compared with controls despite the absence of difference in daily physical activity within pairs. Furthermore, cortical areas did not differ between groups at 66% distal radial site. It is possible or postulated that increased calcium intake may have a potentiating effect in facilitating daily physical activity to exert a positive influence on bone development at the distal tibia [30]. Daily weight-bearing exposure to loading at the distal tibia and not the distal radius may explain the observation of greater gains in lower body compared with upper body bone development.

The present study has significant strengths and some potential limitations. First, the use of three-dimensional imaging (pQCT) to assess bone avoids inherent imaging problems associated with two-dimensional DXA technology. Unlike DXA, pQCT analyses cross-sectional images of long bones and provides evaluation of bone size, shape and density. Second, our co-twin study design controls genetic and environmental confounders in contrast to most studies in which unrelated individuals are used. The homogeneity of participants in the study is a strength given that up to 80% of variability in bone mass is genetically determined and the remaining 20% of variability explained by

environmental factors such as nutrition and physical activity [31]. Furthermore, no between-pair differences in daily physical activity or dietary habits were found at baseline or 6-month follow-up. We acknowledge however, that six monthly changes in biological measures of maturation, bone formation and resorption markers via blood and urine sampling would have more definitively described mechanistic evidence of bone and dietary outcomes. Furthermore, the dietary analysis software used in the present study did not calculate vitamin D intake. Most of the vitamin D used by our bodies each day comes from cutaneous synthesis, although it is reasonable to assume baseline values were not different between twin pairs. Due to these limitations, it was outside the scope of the study to determine if positive skeletal gains at the distal tibia and radius were due to greater calcium intake only, or enhanced calcium resorption due to vitamin D, or a direct effect of vitamin D alone.

To our knowledge, this is the first randomised controlled trial in peripubertal female monozygotic twins to assess the effects of calcium and vitamin-D supplementation on bone structural properties using pQCT. Our findings indicate that supplementation with 800 mg calcium and 400 IU vitamin D3 per day for a period of 6 months was associated with increased trabecular area, trabecular density and strength strain index at the ultra-distal tibia and radius and increased cortical area at tibial mid-shaft. All participants are being followed from 6- to 12-month-supplementation period to determine the sustainability of gains in bone mineral measures.

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Conflicts of interest None.

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