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ORIGINAL ARTICLE

Diet and exercise during growth have site-specific skeletal effects: a co-twin control study

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Abstract Exercise and improved nutrition offer safe, low-cost and widely applicable approaches to potentially reduce the burden of fractures. We conducted a cross-sectional study of 30 monozygotic and 26 dizygotic male twin pairs, aged 7–20 years to test the following hypotheses: (1) Associations between bone mass and dimensions and exercise are greater than between bone mass and dimensions and protein or calcium intakes; (2) exercise or nutrient intake are associated with appendicular bone mass before puberty and axial bone mass during and after puberty. Total body and posteroanterior (PA) lumbar spine bone mineral content (BMC) and mid-femoral shaft dimensions were measured using dual energy X-ray absorptometry (DEXA). Relationships between within-pair differences in nutrient intake (determined by weighed-food diaries) or exercise duration (determined by questionnaire) and within-pair differences in BMC and bone dimensions were tested using linear regression analysis. In multivariate analyses, within-pair differences in exercise duration were associated with within-pair differences in total body, leg and spine BMC, and cortical thickness. Every-hour-per-week difference in exercise was associated with a 31-g (1.2%) difference in total body BMC, a 10-g (1.4%) difference in leg BMC, a 0.5-g difference in spine BMC and a 0.1-mm difference in cortical thickness ($p < 0.01$-$p < 0.1$). A 1-g difference in protein intake was associated with a 0.8-g (0.4%) difference in arm BMC ($p < 0.05$). These relationships were present in peri-pubertal and post-pubertal pairs but not in pre-pubertal pairs. Exercise during growth appears to have greater skeletal benefits than variations in protein or calcium intakes, with the site-specific effects evident in more mature twins.

Keywords Bone mineral content · Calcium · Exercise Growth · Protein · Twins

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Introduction

Fragility fractures are a public health problem affecting up to 50% of women and 20% of men [1]. Of all the fractures in the community, at least 40–50% occur in women without osteoporosis [2]. Drug therapy to prevent fractures is not proven to be effective in this moderate risk group. It is also not a feasible option [3]. Population-based approaches such as modifying exercise and nutrition may offer potentially safe, low-cost and widely applicable alternatives to reduce the burden of fractures, provided these interventions are efficacious.

The optimal time to intervene appears to be during growth, as the magnitude of the osteogenic effects of exercise or calcium interventions are generally greater in children than in adults, with the effect size of exercise being larger than that for calcium [4–7]. For example, mechanical loading during growth appears to produce far greater benefits to skeletal size and mass than exercise during adulthood, with the potential for these benefits to be maintained into adulthood [8, 9]. Exercise of even modest intensity introduced into school physical education curricula increases bone mass during growth [4, 10].

Protein malnutrition, and perhaps a low dietary calcium intake, reduces peak bone mass during growth, while correction of protein malnutrition reduces bone

loss in adults and may reduce fracture risk [11–15]. Matkovic et al. reported lower fracture rates in people living in a community with high calcium intakes, and the benefits appeared to be confined to a higher peak bone size and mass achieved during growth rather than to less bone loss during ageing [16]. Few studies have reported the effect of protein or calcium on bone dimensions in children, even though these two nutrients may be important determinants of bone's material and structural strength [17–19]. Furthermore, whether variations in protein intake within the "normal" range affect bone mass accrual is uncertain [20].

Growth velocity is higher in the limbs than spine before puberty and decelerates during the peri-pubertal years when axial growth accelerates. Therefore, region-specific effects of disease or lifestyle factors may be produced according to the age of exposure. For example, anorexia nervosa of early onset reduces bone mass and size in the axial and appendicular skeleton, while later onset of the disease affects only the axial skeleton [21].

Given the responsiveness of the skeleton to mechanical loading and nutritional factors during growth, and the potential for long-term benefits from this childhood exposure, modifying lifestyle factors such as weight-bearing exercise and nutritional intake during this time may offer safe, accessible, inexpensive and potentially effective ways of reducing the burden of fractures in the community.

We conducted a co-twin control study involving young, healthy male twins to test two hypotheses. First, the within-pair differences in weight-bearing exercise are a better predictor of the within-pair differences in bone mass and dimensions at loaded sites than are protein or calcium intakes. Second, the within-pair differences in weight-bearing exercise or nutrient intake will predict within-pair differences in appendicular bone mass and dimensions before puberty and axial bone mass and dimensions during and after puberty.

Methods

Male twin pairs registered on the National Health and Medical Research Council (NHMRC) twin registry were invited to participate in this study conducted at the Austin Hospital from 1997 to 2001. Data was obtained from 30 monozygotic (MZ) and 26 dizygotic (DZ) male-male twin pairs aged 7–20 years. Zygosity was ascertained via questionnaire and confirmed from blood samples. All pairs were tested on the same day. Written informed consent was obtained from participants (and their parents if the twins were under the age of 18 years). The study was approved by the Human Research Ethics Committee and the Radiation Safety Committee of the Austin and Repatriation Medical Center, and the Australian Twin Registry.

Total body and posteroanterior (PA) lumbar spine BMC and mid-femoral shaft dimensions were measured using dual energy X-ray absorptiometry (DPX-L, version 1.3z, Lunar, Madison, WI, USA). The coefficient of variation (CV) determined on five adults, each scanned three times by the same technician, was 2–4%. Adults are used to calculate the CV for bone density, as successive repeated scans on children are not permitted by the Ethics Committee. The pediatric PA spine program and ruler function were used to obtain periosteal and endocortical widths and cortical thickness at the mid-femoral shaft. The interobserver CV for re-analysis of the mid-femoral shaft scans by the same technician was 1.5%. Height and sitting height were determined using a Holtain stadiometer. Body mass was measured using a seca electric scale (Seca, Hamburg, Germany). Pubertal staging was determined using a physician-assisted report based on Tanner staging for pubic hair and genital development [10]. Hours per week of organized weight-bearing activity (exercise duration) were determined using a modified parental-assisted physical activity questionnaire [22]. Dietary intakes were assessed using 3-day weighed-food diaries, under parental supervision. Twin pairs were randomly allocated 2 weekdays and 1 day on the weekend to complete the food diaries, with all days of the week at various intervals throughout the year covered within the study population. This method of assessment is the most appropriate to obtain information about the frequency and consistency of eating and to calculate nutrient data, especially when investigating more than one nutrient [23]. Furthermore, agreement between 3-day diet diaries and the reference method of 14×24 h diet records was greater than for food frequency questionnaires [24]. All diet diaries were cross-checked for completeness, then analyzed by a nutritionist (S.I.B.) using FoodWorks nutrition program (Xyris software, Australia, version 2.1).

Baseline characteristics are expressed as mean ± standard error (SE). Differences between MZ and DZ twin pairs for absolute and percentage values for within-pair differences were performed using unpaired *t*-tests. Let Y_i represent the response variable of twin*i* where $i = 1$ or 2 and X_{1i}, ..., X_{qi} represent the covariates. The difference in each dependent variable between members of a pair was calculated as $D = Y_1 - Y_2 = a_1 D_1 + ... + a_q D_q + E$, where $D_j = X_{ji} - X_{j2}$ and E = measurement error = $E_1 - E_2$. To adjust for age-related and genetic factors that contribute to the variation in the mean response, the pair differences were expressed as a percentage of the pair mean, i.e., $\%D = 100 \times (D/((Y_1 + Y_2)/2))$. Both D and $\%D$ were regressed through the origin against D_1, ..., D_q by linear regression analysis. Subsequent analyses were performed using multiple regression. Covariates examined were within-pair differences in lean mass, fat mass, and height [25]. Regression coefficients (± 95% confidence intervals [CI]) are reported as an indication of the weight or importance of each of the predictor variables [26]. A significance level of $p < 0.05$ was used. However, values of $p < 0.1$ are reported to indicate trends in relationships. Data was analyzed using StatView (Version 5.0, SAS Institute, USA).

Results

Absolute and percentage within-pair differences for anthropometry, body composition, BMC and periosteal width were greater for DZ than MZ twin pairs ($p < 0.05$) (Table 1). DZ and MZ pairs did not differ in within-pair differences in energy, macronutrient and calcium intakes or hours per week of exercise.

Associations were detected by univariate analysis (Table 2, panel a). In the multivariate analyses, height, lean mass and fat mass adjusted within-pair differences in exercise predicted differences in total body, leg and spine BMC, and cortical thickness, accounting for 13%, 15%, 9% and 4% of the variances, respectively (Table 2, panel b). When calcium and protein intakes were added to the analysis, only differences in exercise predicted total body, leg and lumbar spine BMC (Fig. 1a–c).

For every hour difference in exercise there was a 30.5-g (15.1 g to 45.8 g, $p < 0.01$) or 1.2% (0.1% to 2.4%, $p < 0.05$) difference in total body BMC, a 10.1-g (2.4 g to 17.8 g, $p < 0.05$) or 1.4% (−0.02% to 2.9%, $p < 0.1$) difference in leg BMC and a 0.5-g (0.0 g to 1.0 g, $p < 0.05$) difference in spine BMC (Table 2, panel c). The relationship between exercise and BMC was observed for the peri-pubertal and post-pubertal twins but not for pre-pubertal twins ($p < 0.05$).

Differences in exercise duration were greater in more mature twins than they were in less mature twins (4.1 ± 0.5 h/week vs 2.3 ± 0.3 h/week, $p < 0.01$). In more mature twins, every hour difference in exercise was associated with a 38.1-g (18.1 g to 58.1 g, $p < 0.01$) or 1.2% (0.5% to 1.9%, $p < 0.01$) difference in total body BMC, 15.0-g (4.6 g to 25.4 g, $p < 0.01$) or 1.4% (0.3% to 2.5%, $p < 0.05$) difference in leg BMC, and a 0.6-g (0.2 g to 1.0 g, $p < 0.01$) difference in spine BMC (data not shown). Within-pair differences in exercise duration were also associated with differences in cortical thickness ($r = 0.2$, $p < 0.1$); for every hour difference in exercise there was a 0.1-mm (0.0 mm to 0.2 mm, $p < 0.1$) difference in cortical thickness (Table 2, panel c, Fig. 2).

In multivariate analysis, height, lean-mass and fat-mass-adjusted, within-pair differences in protein intake were associated with differences in arm BMC, accounting for 4% of the variance (Table 2, panel b). With the inclusion of exercise duration and calcium intake in the regression analysis, within-pair differences in protein intake remained predictors of differences in arm BMC; for every 1-g difference in protein intake there was a 0.8-g (0.1 g to 1.5 g, $p < 0.05$) or 0.4% (0.1% to 0.7%, $p < 0.05$) difference in arm BMC (Fig. 3, Table 2, panel c). The relationship between protein intake and arm BMC was present in peri-pubertal and post-pubertal twins but not in pre-pubertal twins. In more mature

Table 1 Baseline characteristics (mean ± standard error [SE]), and within-pair differences (%) for male twins aged 7–20 years

Number of pairs	MZ twin pairs		DZ twin pairs	
	30		26	
Age (years)	11.5 ± 0.4		11.1 ± 0.4	
Maturity				
Pre–pre	18		15	
Peri–peri / post–post	12		11	
Anthropometry and body composition				
-	Mean ± SE	% difference	Mean ± SE	% difference
Height (cm)	146.4 ± 2.3	1.5 ± 0.2	148.0 ± 2.5	3.6 ± 0.5*
Sitting height (cm)	77.4 ± 1.1	1.9 ± 0.4	77.7 ± 1.2	3.3 ± 0.6*
Leg length (cm)	69.2 ± 1.2	2.2 ± 0.4	70.3 ± 1.4	4.7 ± 0.7*
Weight (kg)	39.0 ± 1.8	6.0 ± 1.2	42.1 ± 2.2	14.6 ± 3.0*
Lean mass (kg)	29.8 ± 1.5	3.5 ± 0.6	31.9 ± 1.6	10.8 ± 1.7*
Fat mass (kg)	7.0 ± 0.7	20.9 ± 3.3	7.8 ± 0.9	45.8 ± 7.3*
Bone mineral content (g)				
Total body	1,576 ± 93	4.9 ± 0.7	1,641 ± 95	13.5 ± 2.2*
Arms	167 ± 12	9.7 ± 1.2	182 ± 12	18.5 ± 3.0*
Legs	566 ± 40	6.9 ± 1.2	607 ± 44	17.6 ± 3.2*
Lumbar spine	25.9 ± 1.9	9.0 ± 1.8	26.7 ± 1.8	15.8 ± 2.3*
Bone dimensions (mm)				
Cortical thickness	4.3 ± 0.1	14.7 ± 2.0	4.3 ± 0.1	13.2 ± 1.9
Periosteal diameter	16.4 ± 0.4	5.2 ± 0.9	16.8 ± 0.4	11.6 ± 2.1*
Endosteal diameter	7.9 ± 0.2	14.7 ± 2.0	8.2 ± 0.3	19.1 ± 2.1
Lifestyle				
Energy (kj)	8,651 ± 215	9.9 ± 1.5	8,727 ± 283	9.8 ± 1.4
Protein (g)	75 ± 3	13.6 ± 1.6	77 ± 3	14.9 ± 2.4
Carbohydrate (g)	271 ± 7	8.7 ± 1.7	267 ± 8	13.1 ± 2.3
Fat (g)	77 ± 3	15.6 ± 2.5	82 ± 4	13.3 ± 1.7
Calcium (mg)	925 ± 53	21.3 ± 2.5	941 ± 64	25.9 ± 4.0
WB exercise (h/week)	2.7 ± 0.3	19.5 ± 10.0	3.3 ± 0.5	26.0 ± 10.9

* $p < 0.05$, DZ pairs differ from MZ pairs

Table 2 Beta coefficients (±95% confidence interval [CI]) for within-pair differences in protein, calcium and exercise duration vs (a) within-pair differences in bone mineral content (BMC) and bone dimensions; (b) within-pair differences in size-adjusted BMC and bone dimensions; and (c) when all within-pair differences in protein, calcium, exercise duration and size are included in the regression equation. The regression coefficient represents the relationship between the independent and dependant variables. For each unit change in the independent variable, the regression coefficient indicates the direction (+ or -) and magnitude of the relationship. $N = 56$ set of MZ and DZ male twins aged 7–20 years (*Cal* calcium, *Prot* protein, *Exer*exercise, *Ht* height)

	(a) Univariate regression			(b) Size-adjusted (Δ Ht, Δ lean mass, Δ fat mass)			(c) All lifestyle + size-adjusted		
	Δ Cal	Δ Prot	Δ Exer	Δ Cal	Δ Pro	Δ Exer	Δ Cal	Δ Pro	Δ Exer
Δ Bone mineral content (g)									
Total body	0.0(−0.2 to 0.2)	3.5ˆ(−0.7 to 7.6)	30.9ˆ(−4.0 to 65.8)	0.0(−0.1 to 0.1)	1.3(−0.6 to 3.2)	32.0#(17.1 to 47.0)	0.0(−0.1 to 0.1)	1.3(−0.9 to 3.5)	30.5#(15.1 to 45.8)
Arms	0.0(−0.0 to 0.1)	0.8*(0.0 to 1.6)	6.5ˆ(−0.1 to 13.1)	0.0(−0.0 to 0.0)	0.7*(0.2 to 1.2)	3.4(−1.5 to 8.3)	0.0(−0.0 to 0.0)	0.8*(0.1 to 1.5)	2.5(−2.3 to 7.3)
Legs	0.0(−0.1 to 0.1)	1.6(−0.5 to 3.7)	6.9(−10.8 to 24.6)	−0.0(−0.1 to −0.0)	0.3(−0.6 to 1.2)	10.5#(3.1 to 17.9)	0.0(−0.1 to 0.1)	0.3(−0.8 to 1.4)	10.1*(2.4 to 17.8)
Lumbar spine	−0.0(−0.0 to 0.0)	0.0(−0.1 to 0.1)	0.9#(0.3 to 1.5)	−0.0(−0.0 to 0.0)	0.0(−0.1 to 0.1)	0.6*(0.1 to 1.1)	−0.0(0.1 to 1.5)	0.0(−0.0 to 0.1)	0.5*(0.0 to 1.0)
Δ Bone mineral content (%)									
Total body	0.0(−0.0 to 0.0)	0.3*(0.0 to 0.5)	1.1(−1.0 to 3.1)	0.0(−0.0 to 0.0)	0.2*(0.0 to 0.3)	1.4*(0.2 to 2.5)	0.0(−0.0 to 0.0)	0.1ˆ(−0.0 to 0.3)	1.2*(0.1 to 2.4)
Arms	0.0(−0.0 to 0.0)	0.4*(0.1 to 0.8)	2.0(−1.0 to 4.9)	0.0(−0.0 to 0.0)	0.4#(0.1 to 0.6)	1.1(−1.2 to 3.3)	−0.0(−0.0 to 0.0)	0.4*(0.1 to 0.7)	0.7(−1.5 to 2.8)
Legs	0.0(−0.0 to 0.0)	0.3ˆ(−0.0 to 0.7)	0.7(−2.2 to 3.5)	0.0(−0.0 to 0.0)	0.1(−0.0 to 0.3)	1.5ˆ(−0.1 to 3.0)	0.0(−0.0 to 0.0)	0.1(−0.1 to 0.3)	1.4ˆ(−0.2 to 2.9)
Lumbar spine	−0.0(−0.0 to 0.0)	0.1(−0.2 to 0.4)	1.9(−0.7 to 4.5)	−0.0(−0.0 to 0.0)	0.1(−0.2 to 0.4)	1.0(−1.7 to 3.6)	−0.0(−0.0 to 0.0)	0.2(−0.2 to 0.6)	0.7(−2.1 to 3.5)
Δ Bone dimensions and widths (mm)									
Cortical thickness	−0.0(−0.0 to 0.0)	0.0(−0.0 to 0.0)	0.1(−0.0 to 0.2)	−0.0(−0.0 to 0.0)	0.0(−0.0 to 0.0)	0.1*(0.0 to 0.3)	−0.0(−0.0 to 0.0)	0.0(−0.0 to 0.0)	0.1ˆ(−0.0 to 0.2)
Periosteal	−0.0(−0.0 to 0.0)	0.0(−0.0 to 0.1)	0.2(−0.2 to 0.5)	−0.0(−0.0 to 0.0)	0.0(−0.0 to 0.0)	0.1(−0.2 to 0.5)	−0.0(−0.0 to 0.0)	0.0(−0.0 to 0.1)	0.1(−0.2 to 0.5)
Endocortical	0.0(−0.0 to 0.0)	0.0(−0.0 to 0.0)	−0.0(−0.3 to 0.3)	0.0(−0.0 to 0.0)	0.0(−0.0 to 0.0)	−0.1(−0.4 to 0.2)	0.0(−0.0 to 0.0)	−0.0(−0.1 to 0.0)	−0.1(−0.3 to 0.2)
Δ Bone dimensions and widths (%)									
Cortical thickness	−0.0(−0.0 to 0.0)	0.2(−0.1 to 0.5)	1.2(−1.5 to 4.0)	−0.0(−0.0 to 0.0)	0.1(−0.2 to 0.5)	2.4(−0.5 to 5.3)	−0.0(−0.0 to 0.0)	0.4(−0.1 to 0.8)	1.8(−1.2 to 4.8)
Periosteal	−0.0(−0.0 to 0.0)	0.1(−0.2 to 0.3)	0.7(−1.3 to 2.6)	−0.0(−0.0 to 0.0)	0.0(−0.2 to 0.2)	0.7(−1.2 to 2.5)	−0.0(−0.0 to 0.0)	0.0(−0.3 to 0.3)	0.6(−1.4 to 2.5)
Endocortical	0.0(−0.0 to 0.0)	−0.0(−0.4 to 0.4)	−0.8(−4.0 to 2.5)	0.0(−0.0 to 0.0)	−0.1(−0.5 to 0.3)	−1.8(−5.0 to 1.4)	0.0(−0.0 to 0.0)	−0.3(−0.8 to 0.2)	−1.2(−4.5 to 2.1)

# $p < 0.01$
* $p < 0.05$
ˆ $p < 0.1$

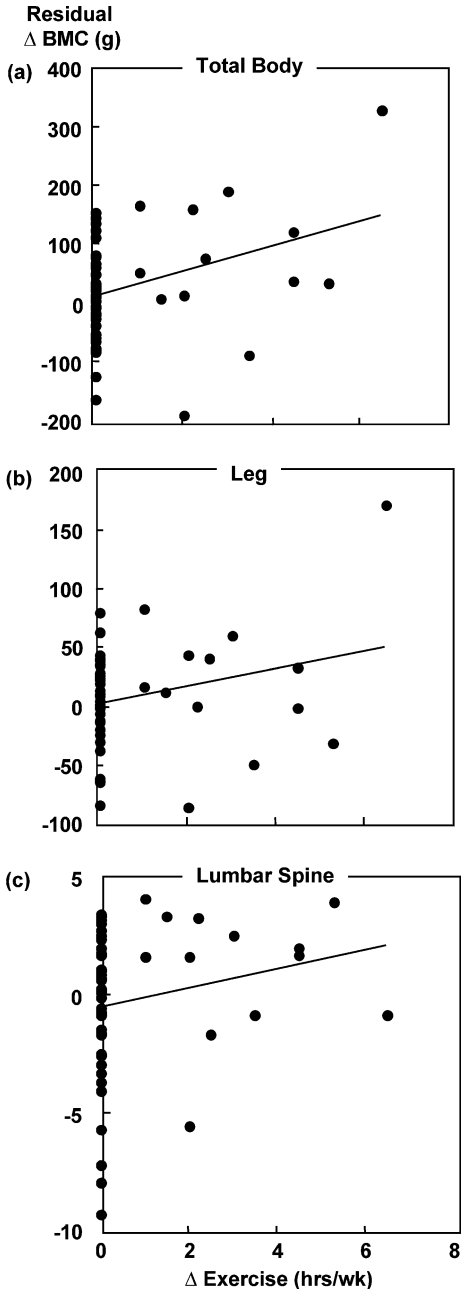

Fig. 1 Within-pair differences in exercise duration plotted against within-pair differences in bone mineral content (*BMC*), adjusted for differences in size (lean, fat, height) and nutrient intake (calcium, protein) for 56 male twin pairs aged 7–20 years. $p < 0.05$

twins, every 10-g difference in protein intake was associated with a 14.2-g (0.0 g to 29.2 g, $p < 0.1$) or 5.8% (0.9% to −10.7%, $p < 0.05$) difference in arm BMC (data not shown). No relationships were detected between calcium intake and bone mass or dimensions in univariate or multivariate analyses (Table 2, panels a–c).

Discussion

In this cross-sectional study of young male twins, we report that exercise was a better predictor of BMC than

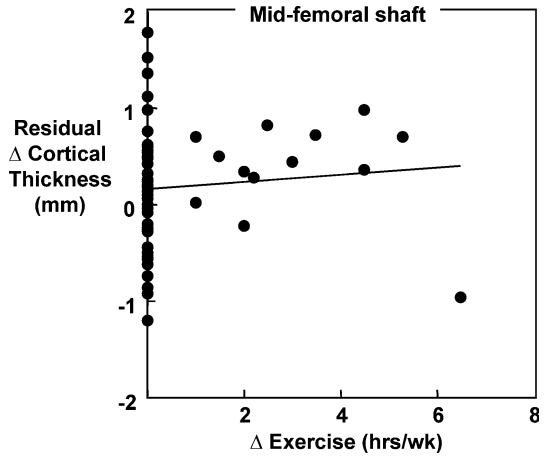

Fig. 2 Within-pair differences in exercise duration plotted against cortical thickness adjusted for differences in size (lean, fat, height), and nutrient intake (calcium, protein) for 56 male twin pairs aged 7–20 years. $p < 0.1$

are protein or calcium intakes at weight-bearing sites; legs and lumbar spine. Greater exercise duration was associated with greater cortical thickness at the mid-femoral shaft. Protein intake predicted differences in BMC at the arms. These relationships were observed in the peri-pubertal and post-pubertal twins but not in the pre-pubertal male twins.

The results support the view that exercise is likely to be a more important determinant of BMC in healthy children than are calcium or protein intakes. Several studies suggest benefits of exercise (up to 12% higher BMD) are greater than calcium supplementation (up to 5%) [4, 5, 10, 27–30]. The effect of exercise was limited

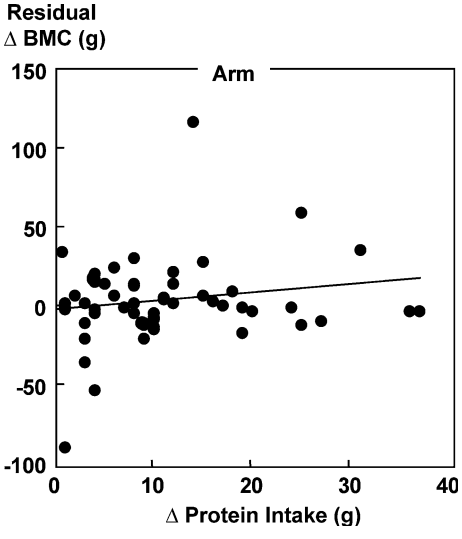

Fig. 3 Within-pair differences in protein intake plotted against differences in arm bone mineral content (*BMC*) adjusted for differences in size (lean, fat, height), calcium intake and exercise duration for 56 male twin pairs aged 7–20 years. $p < 0.05$

to weight-bearing sites and was dose-dependant, with larger differences in BMC observed with greater differences in exercise duration. A similar dose-dependent relationship between exercise and BMD was reported in pre-pubertal female gymnasts, where higher values for BMD were observed with increasing number of training hours [31].

The exercises were organized activities that fulfilled the criteria for impact loading (weight bearing) and of diverse strains, as the reported activities were varied, e.g., football, soccer, basketball, tennis, marital arts, etc. However, loading magnitude was not quantified. These results, however, support the view that participating in weight-bearing activities at a level that is obtainable by normal children has skeletal benefits [32].

There was a trend towards greater cortical thickness, with increasing exercise duration, at the mid-femoral shaft. Bradney et al. reported enhanced cortical thickening at the mid-femoral shaft in exercising pre-pubertal boys due to endocortical contraction [10]. Using peripheral quantitative computed tomography (pQCT), Haapasalo et al. reported similar cortical thickness, but greater medullary area at the proximal humerus and radial shaft in the playing arm compared with the non-playing arm of male tennis players. Greater cortical thickness and similar medullary area at the mid-humerus and distal humerus was observed [8]. Bass et al. also reported site-specific differences in the skeletal response to loading, with greater side-to-side differences in medullary and periosteal areas at the mid-humerus, but only periosteal expansion at the distal humerus in female tennis players [33].

Periosteal apposition before puberty continues in boys during puberty, while endocortical contraction predominates in girls [34]. The surface of bone undergoing the greatest apposition may be more responsive to exercise. However, we were not able to detect a greater difference in cortical thickness with increasing difference in exercise duration in pre-pubertal males. A greater biomechanical advantage would be achieved with periosteal apposition, as this confers greater benefits to bone strength than the same amount of bone on the endocortical surface [35]. Previous exercise interventions in children have reported similar effects on BMC accrual in both males and females. However, limited data exists defining whether the observations resulted from greater endocortical contraction in girls, but greater periosteal apposition in boys.

Greater cortical thickness with increasing exercise duration was confined to the mediolateral mid-femoral shaft. Jones et al. observed that the relative contribution of the periosteal and endocortical surfaces to cortical thickness at the humerus in tennis players was 60:40 in the anteroposterior direction and 80:20 in the mediolateral direction [36]. Thus, loading appears to affect both the periosteal and endocortical surfaces. However, the response varies along the length of bone. The inability to identify surface-specific effects of exercise at the mid-femoral shaft, and detection of only a trend between exercise duration and cortical thickness, may be due to the small number of twin pairs that varied sufficiently in exercise duration.

The relationship between leg and spine BMC and exercise duration was evident in the peri-pubertal and post-pubertal twins, but not in pre-pubertal twins. Haapasalo et al. [37] noted greater side-to-side differences in forearm BMD in tennis players of Tanner stages III to V than those in Tanner Stages I and II, relative to controls. We report similar observations. However, it is possible that the greater duration of loading and the greater within-pair differences in exercise duration in more mature individuals contributed to this finding. Moreover, the exercise questionnaire only provided details of the type and duration of exercise, but not intensity. Therefore, we were unable to determine if the pre-pubertal and more mature twins experienced similar load magnitudes.

Protein intakes at or above 1 g/kg body weight were associated with differences in arm BMC. This relationship remained when differences in energy intake were included in the regression model. The deleterious effects of protein malnutrition on bone mass during growth are documented, with protein deficiency being more detrimental than a low calcium intake [11–14, 19]. Many studies report associations between protein intake and bone mass or fracture risk [38–41], and between calcium intake and bone mass, in children [42–44]. No associations were detected between calcium intake and BMC or bone dimensions, perhaps because participants had adequate calcium intakes. For example 22 of the twin pairs (39%) had calcium intakes above 800 mg/day. Fewer pairs (25%) had one twin above and one twin below this level. Only one pair had calcium intakes that varied by >800 mg/day; however, both twins had intakes above recommended levels. The effect of calcium supplementation on bone mass accrual is reported, with the benefits most obvious in children with lower calcium intakes [5, 45]. However, no association between within-pair differences in calcium intake and BMC was found, even after twins were divided into those with larger (<250 mg/day) or smaller (>250 mg/day) within-pair differences in calcium intake.

We were able to detect differences in BMC despite the small number of twins with large differences in exercise activity. However, despite the large number of twins with larger differences in calcium, we were still unable to detect difference in BMC. These data suggest that exercise has potent effects on the skeleton during growth while the effects of calcium remain unclear.

In summary, these data support the view that in healthy children with adequate dietary intakes, exercise has a greater osteogenic effect than calcium or protein. Studies are needed to further understand the role of protein and calcium intake in skeletal growth. Targeted exercise interventions or encouraging weight-bearing physical activity in normal healthy children should be promoted, but the optimal type, duration and frequency

of activity needed to benefit bone should be more accurately defined.

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