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Original Article

Muscle indices do not fully account for enhanced upper extremity bone mass and strength in gymnasts

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Abstract

Objectives: Muscular forces are an important determinant of bone strength, but bone may also adapt to non-muscular loading. We tested the hypothesis that loads associated with childhood gymnastics yield high arm bone mass (BMC), bone size and bone strength, independent of arm lean mass (FFM) and muscle cross-sectional area (CSA). **Methods:** Total body DXA and distal radius pQCT scans were performed on 33 post-menarcheal girls (19 ex/gymnasts, 14 non-gymnasts). Physical activity and calcium intake were assessed by questionnaire. For the non-dominant arm, pQCT measured bone strength indices and bone CSA (total, cortical) (4%, 33% sites); DXA measured arm FFM, arm BMC and skull BMC. Multiple regression analyses assessed gymnastic exposure, arm FFM, gynecological age and stature as predictors of bone parameters. **Results:** Bone outcomes at loaded upper extremity sites were 10-42% greater in ex/gymnasts than non-gymnasts. Gymnastic exposure remained a consistent, significant predictor of upper extremity skeletal parameters after accounting for the effects of muscle parameters, gynecological age and height. **Conclusions:** Considering the effects of either arm FFM or muscle CSA, indices of bone mass, geometry and theoretical strength are disproportionately elevated after gymnastic exposure. Thus, non-muscular loading may be a distinct and important determinant of human skeletal structure.

Keywords: Radius, pQCT, Mechanical Loading, Bone Strength, Muscle

Introduction

It is well established that muscle is a major correlate and determinant of skeletal properties[1-9]. Systemic anabolic agents induce growth of both muscle and bone[6,10-11], and genetic factors may affect muscle and skeletal properties simultaneously, directly and indirectly[12-13]. Furthermore, muscle contraction determines skeletal properties by direct mechanical stimulation[9-10,13-14]. New evidence indicates that muscle tissue also exerts biochemical effects upon bone[9].

Accordingly, many researchers have emphasized muscle tissue and resultant influences on bone as the primary determinants of bone mass, structure and strength during growth, explaining the variation in skeletal phenotype throughout the lifespan[21,3,5,15]. Sole reliance upon this view of skeletal formation and maintenance may underestimate the importance of direct (non-muscular) mechanical loading, which may play an independent or additive role in bone growth and structure.

Researchers have used animal models to apply isolated, non-muscular mechanical loads to mammalian femora, yielding improvements in bone mineral content, structural properties and fatigue resistance at cortical, cortico-cancellous and cancellous sites that cannot be explained by muscular factors[16-18]. In humans, many studies indicate that "weight-bearing" and impact-loading are highly osteogenic[19-28], more osteogenic than non-impact and non-"weight-bearing" activities[19,25-28]. In particular, gymnastics provides a useful model of combined impact and "weight-bearing", generating ground reaction forces of up to 10-15 times body weight[29]. Accordingly, exposure to gymnastics activity during growth has been associated with elevated bone mass and enlarged bone size[30-33].

In females, artistic gymnastics training includes floor exercises, balance beam, uneven parallel bars and vaulting, thereby loading the distal radii and arms in axial compression, bending and torsion. Training on the uneven parallel bars

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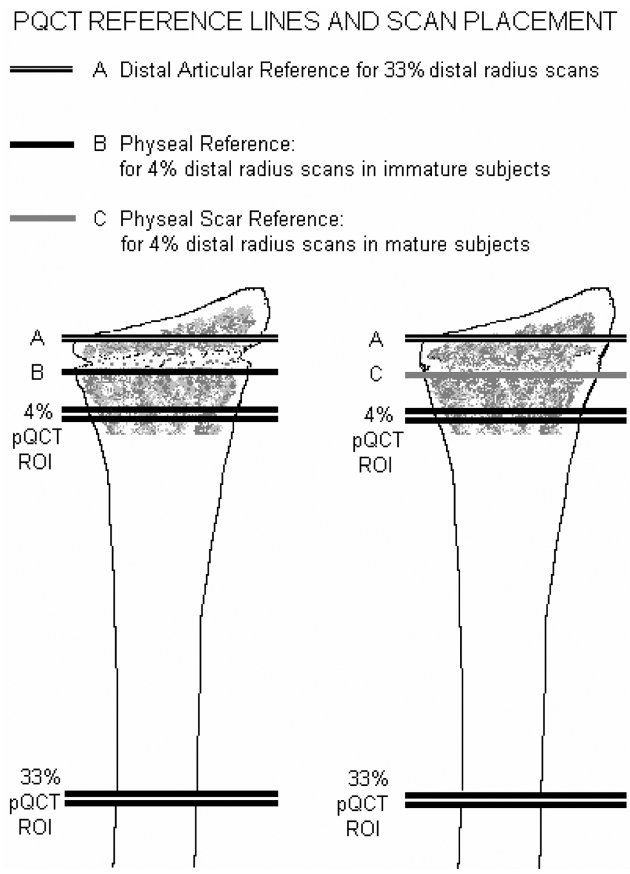


Figure 1. Reference Lines and Regions of Interest for Distal Radius pQCT Scans. Relative positions of pQCT regions of interest for the distal radius are depicted in adjacent figures; 33% scans are positioned relative to an articular reference, whereas 4% pQCT scans are positioned relative to the physis or physeal scar.

also applies tensile loads to the same anatomical sites. The applied forces are greater than body weight alone, due to the effect of inertia as the gymnast accelerates through complex sets of maneuvers. The resultant forces produce high skeletal loads, which are further increased by the muscular system as it generates propulsive and stabilizing forces. Thus, gymnastics generates extreme forces in both the dominant and non-dominant upper extremities. In contrast, daily life and recreational or sporting activities preferentially load the dominant arm. Therefore, non-dominant arm and radius sites may be assessed as barometers of unique loading via gymnastics activity[33]. The present study exploits the gymnastic model to investigate differences in human skeletal mass, geometry and strength indices attributed to gymnastic loading at the non-dominant distal radius, hypothesizing that these adaptations are not explained by muscular indices alone.

Materials and methods

Subjects from an ongoing longitudinal study of gymnastics-related bone accrual were enrolled in the present study. At initial recruitment, gymnasts and non-gymnasts were matched for age and body size. The Institutional Review Board of SUNY Upstate Medical University approved the introduction of supplemental peripheral quantitative computed tomography (pQCT) scans to the existing dual energy X-ray absorptiometry (DXA) protocol. Consenting subjects underwent a pQCT scan of the non-dominant forearm at the time of their annual assessment. Annual assessments included densitometry, anthropometry and questionnaires assessing calcium intake, physical activity and physical maturity[30].

Total body DXA scans were obtained using a Hologic QDR 4500W scanner (coefficient of variation=1%). Skull BMC was derived from the total body scan, and analyzed as

pQCT ROI	Analysis	Contour Mode	Peel Mode	Inner Threshold	Threshold	Separation Mode	Peel By
33% radius	calcbd	1	2	540 mg/cm^3	711 mg/ cm^3	-	-
	cortbd	-	-	-	711 mg/ cm^3	2	-
	SSI	-	-	-	711 mg/ cm^3	2	-
4% radius	calcbd	3	4	450 mg/cm^3	169 mg/cm^3	-	5%
	cortbd	-	-	450 mg/cm^3	169 mg/cm^3	4	-
	SSI	-	-	-	169 mg/cm^3	2	-
65% MUSCSA	calcbd	3	2	711 mg/ cm^3	40 mg/ cm^3	-	-
	cortbd	-	-	51 mg/ cm^3	711 mg/ cm^3	4	-

For each analysis type, pQCT analysis settings are presented by region of interest.
Calcbd and cortbd are standard pQCT analyses that provide compartmental output.
The SSI analysis provides an index of total bone theoretical strength.
MusCSA evaluates anatomical muscle cross-sectional area.

Table 1. pQCT analysis settings by region of interest.

Activity	Ex-gymnasts (n=9) Count (%)	Non-gymnasts (n=14) Count (%)
Light tumbling/cheerleading	2 (22%)	8 (57%)
Circuits/Weight-training	3 (33%)	3 (21%)
Rowing	0 (0%)	1 (7%)
Basketball	1 (11%)	9 (64%)
Volleyball	0 (0%)	4 (29%)
Lacrosse	3 (33%)	8 (57%)
Tennis	0 (0%)	4 (29%)
Softball	1 (11%)	3 (21%)
Field Hockey	1 (11%)	1 (7%)
Golf	0 (0%)	1 (7%)
Color Guard/Marching Band	2 (22%)	0 (0%)
Track & Field (running, jumping, etc.)	4 (44%)	4 (29%)
Soccer	2 (22%)	7 (50%)
Long distance running (>1 mi/interval)	1 (11%)	5 (36%)
Dance	2 (22%)	9 (64%)
Ice skating	0 (0%)	1 (7%)
Equestrian Events	0 (0%)	1 (7%)
Downhill Skiing	1 (11%)	1 (7%)
"Cardio" (elliptical machine, aerobics, etc.)	2 (22%)	8 (57%)
Diving	1 (11%)	0 (0%)
Swimming (lessons/team)	0 (0%)	7 (50%)

For non-gymnasts, participation is listed for all years of study participation (late childhood through menarche and beyond, over 5-10 years).
For ex-gymnasts, participation is listed for all years since discontinuing gymnastics training; therefore, all participation is post-menarcheal (pre-menarche was dominated by gymnastics).
Clearly, many subjects have been/are active in several organized physical activities; some vary activity over a span of years, while others overlap training in multiple activities.
Most non-gymnasts participate in multiple sports, rotating by season.
Many subjects cross-train in the off-season or year-round for performance enhancement.

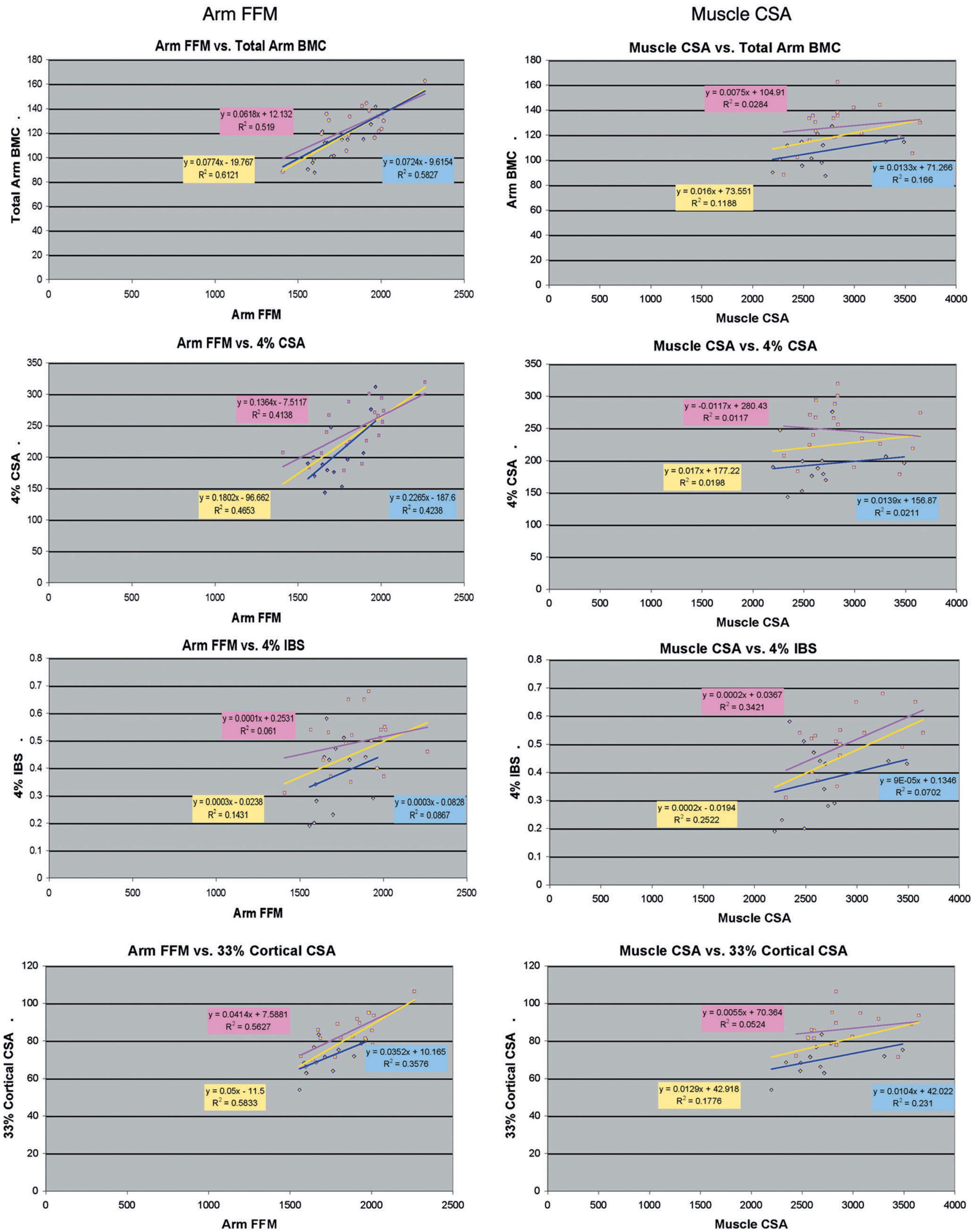
Table 2. Non-gymnastic activity participation by activity group.

a non-loaded control site. Total Arm Bone Mineral Content (Arm BMC) was derived from the arm region of interest of the total body scan, including the humerus, radius, ulna, carpals, metacarpals and phalanges; all are loaded by gymnastic activity. The arm region of interest was also used to derive total arm non-bone, lean or fat-free mass (arm FFM).

For analysis of bone geometry and theoretical strength, pQCT scans were performed using a Norland-Stratec XCT 2000 scanner at three distal radius regions of interest (ROI): for the metaphysis, at 4% of ulnar length; for the diaphysis, at 33% of ulnar length and 65% of ulnar length (evaluated for muscle CSA, not bone output). A scout view was used to place two different scan reference lines; the diaphyseal scans were performed relative to a distal articular reference, whereas the metaphyseal scan was performed relative to a physeal reference (Figure 1). Scan slices were approximately 2 mm thick. The conditions for automated pQCT scan analyses are shown in Table 1. At the 4% pQCT site, we evaluated total bone cross-sectional area (CSA) and index of structural strength in axial compression (IBS)[34]. At the 33% site, we evaluated cortical shell cross-sectional area (cCSA) and polar strength-strain index (SSI, index of theoretical bone strength). IBS and SSI were used to calculate "fall strength ratios", analogous to ratios used by Ruff and Rauch et al. to indicate theoretical resistance to a low trauma fall on an outstretched arm (Fall strength=IBS or SSI/(total body mass * forearm length)[33,35,36].

As an alternative muscle index, pQCT also measured anatomical muscle cross-sectional area (MUSCSA) relative to an articular reference at 65% of ulnar length. MUSCSA analyses were performed as follows (Table 1): 1) Muscle ROI was circumscribed by hand to include the entire soft tissue envelope; 2) CALCBD analyses isolated muscle and bone from other tissues; 3) CORTBD analyses isolated 65% bone area from muscle area; and 4) Bone area was subtracted from lean tissue area to yield MUSCSA[37].

GROUP-SPECIFIC REGRESSION PLOTS FOR MUSCLE INDICES VS. BONE OUTCOMES



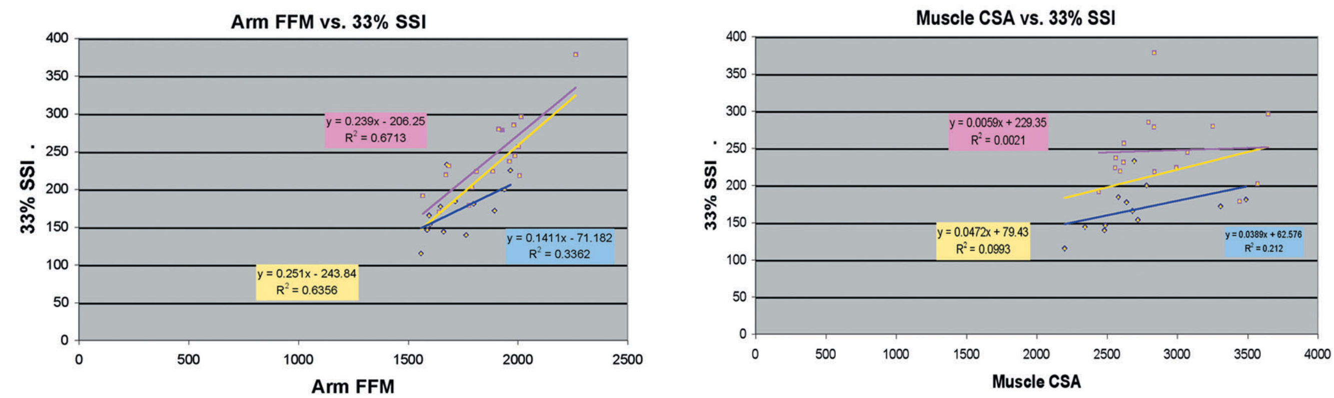


Figure 2. Group-specific Regression Plots for Muscle Indices vs. Bone Outcomes. Values for upper extremity bone outcomes are plotted against muscle indices; regressions based on Arm FFM (left) are contrasted against analogous plots based on 65% Muscle CSA (right). Gymnasts and ex-gymnasts are plotted in pink, and non-gymnasts are depicted in blue. Regression lines and equations are depicted for the total sample (yellow), the "gymnast" group (pink) and the "non-gymnast" group (blue). In no case was a significant interaction detected for gymnastic exposure vs. muscle indices after accounting for gynecological age and body size (by multiple regression).

To evaluate bone parameters in relation to gymnastic loading, girls exposed to artistic gymnastics during growth (both current and ex-gymnasts) were grouped together as "GYM" and compared to non-gymnasts, "NON". Any year for which the annual mean for gymnastic participation reached a threshold of at least 5 hours per week was defined as one year of gymnastic activity. Although many NON participated in low level gymnastics, tumbling and cheerleading over the years of longitudinal study, no annual mean gymnastic exposure exceeded the threshold level. Ex-gymnasts were defined as girls for whom more than 10 weeks had passed since discontinuing gymnastic exposure.

Data were screened for normality of distribution, and for variables where the distribution deviated significantly from normal, natural log transformation (ln) was performed (MUSCSA only). Linear regressions were plotted to indicate relationships between muscle indices and bone outcomes (Figure 2). Correlations were used to assess relationships between total body mass (weight) and muscle indices versus bone outcomes. For body weight, Pearson's correlations were performed, reporting r and significance at the 0.05 level for the total sample and GYM and NON by group; for arm FFM and MUSCSA, Spearman correlations and rho are reported due to non-normality of MUSCSA distribution. GYM and NON subject characteristics and unadjusted bone outcomes were compared using t-tests, providing 95% confidence intervals and significance levels (α=0.05).

To test the focal hypothesis, multiple regression analyses assessed the predictive value of gymnastic exposure and arm muscle parameters (arm FFM or 65% MUSCSA), simultaneously accounting for the effects of gynecological age and height (physical maturity and body size). Fall strength regression models (4% Fall IBS, 33% Fall SSI) did not include height, as these ratios include forearm length and body weight in the denominator (routinely generating a significant negative correlation with height). For all other bone outcomes, all independent variables (gymnastic exposure, arm FFM or 65% MUSCSA, gynecological age and stature) were entered simultaneously. In addition, all bone outcomes were tested for an interaction between gymnastic exposure and muscle parameters (gymnastic exposure * arm FFM/65% MUSCSA), secondarily entering the interaction term to assess the significance of F change. For each model, r^2 and significance is reported; for each independent variable, beta coefficients, squared semipartial correlation coefficients and significance are reported. Squared semipartial correlation coefficients indicate the predictive value of each independent variable, after accounting for the effects of all other entered predictors. Adjusted percentage differences in bone outcomes are presented graphically, with 95% confidence intervals, to illustrate gymnast advantages in bone parameters over non-gymnast values after adjustment for muscle parameters, gynecological age and height (as appropriate) (Figures 3-4).

Results

Thirty-three post-menarcheal ex/gymnasts and non-gymnasts underwent pQCT and DXA scans (mean chronological age=16.6 years). All GYM had participated in at least 5.8 hours per week of gymnastic activity for at least 2 of the preceding 10 years (range=2 to 9 years). GYM included 9 current gymnasts and 10 ex/gymnasts. All GYM were exposed to gymnastic loading for at least 2 years during late childhood; 79% of GYM continued gymnastics through early puberty and peri-menarcheal development. The grand mean annual gymnastic participation (mean of all GYM annual means) was equal to 12.2 hours per week (6.5 to 17.3 hours per week). Gymnastic participation intensity/duration ranged from a minimum of 6.5

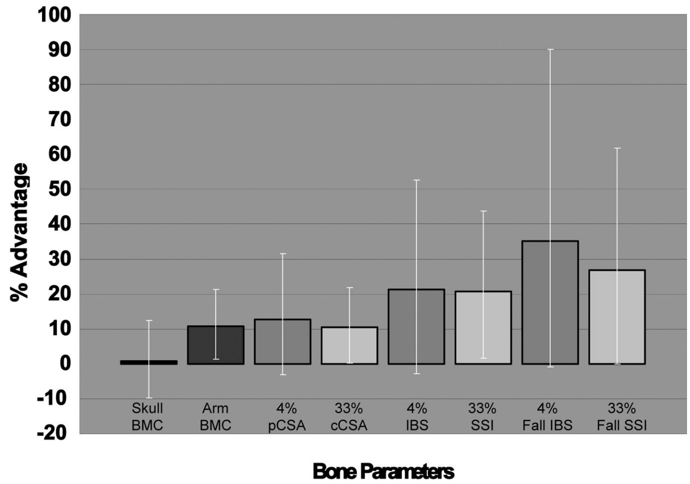


Figure 3. Bone Parameters Adjusted for Arm FFM: Gymnast Percentage Advantage vs. Non-gymnasts. Gymnastic exposure differences are presented after adjustment for arm fat-free mass, gynecological age and height (as appropriate). Columns represent gymnast percentage advantages, or the difference between the gymnast adjusted mean and the non-gymnast adjusted mean (zero reference), with 95% confidence intervals. In our comparisons, the term "advantage" was used for lack of a better term, as it describes the higher values for gymnast parameters relative to non-gymnast parameters concisely. These "advantages" are clearly theoretical, as we cannot provide experimental failure results, and the results should be interpreted accordingly. Results are color-coded: Skull and Total Arm BMC (black, dark gray), 4% Metaphysis (medium gray) and 33% Diaphysis (light gray).

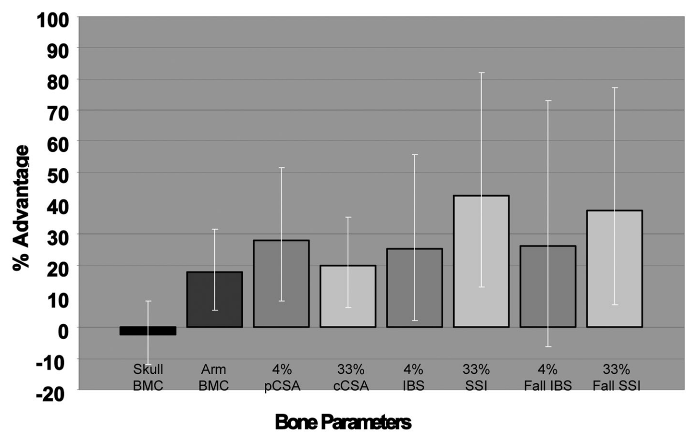


Figure 4. Bone Parameters Adjusted for Muscle CSA: Gymnast Percentage Advantage vs. Non-gymnasts. Gymnastic exposure differences are presented after adjustment for 65% forearm muscle CSA, gynecological age and height (as appropriate). Columns represent gymnast percentage advantages, or the difference between the gymnast adjusted mean and the non-gymnast adjusted mean (zero reference), with 95% confidence intervals. In our comparisons, the term "advantage" was used for lack of a better term, as it describes the higher values for gymnast parameters relative to non-gymnast parameters concisely. These "advantages" are clearly theoretical, as we cannot provide experimental failure results, and the results should be interpreted accordingly. Results are color-coded: Skull and Total Arm BMC (black, dark gray), 4% Metaphysis (medium gray) and 33% Diaphysis (light gray).

hours per week for two of the preceding 10 years, to a maximum of 16 hours per week for 7 of the preceding 10 years. Ex-gymnasts had discontinued gymnastic exposure an average of 4.8 years prior to the densitometric scans (range 0.7 yrs to 7.6 yrs), with an average gynecological age at gymnastic cessation of -0.5±1.7 years (-4.7 to +1.3 yrs). After discontinuing gymnastics, ex-gymnasts engaged in a range of physical activities similar to those of non-gymnasts in type, intensity and duration, yielding similar annual mean physical activity levels for the year prior to the focal scans (Table 2, Table 3).

The 19 GYM and 14 NON were well-matched, such that only arm FFM and gymnastic activity differed significantly by

Variable	GYM (n=19)		NON (n=14)	
	Mean	95% CI	Mean	95% CI
Chronological Age (years)	**16.7**	(15.7–17.7)	**16.2**	(15.0–17.3)
Gynecological Age (years)	**3.4**	(2.4–4.4)	**3.5**	(2.3–4.7)
Height (m)	**1.60**	(156.8–163.3)	**1.62**	(158.1–165.7)
Weight (kg)	**54.6**	(51.7–57.3)	**55.3**	(52.1–58.6)
BMI (kg/m^2)	**21.3**	(20.5–22.1)	**21.1**	(20.1–22.0)
DXA Total Body FFM (kg)	**39.2**	(37.6–40.8)	**38.8**	(36.9–40.7)
DXA % Body Fat (%)	**23.2**	(21.1–25.2)	**25.3**	(22.9–27.7)
DXA Arm FFM* (kg)	**18.5***	(17.7–19.3)	**17.2**	(16.3–18.2)
pQCT Muscle CSA (cm^2)	**28.8**	(26.9–30.6)	**26.7**	(24.5–28.8)
Calcium Intake (mg/day)	**663.3**	(602.2–935.0)	**713.3**	(572.2–960.0)
Weight-bearing Activity (h/wk) (ex-gymnasts vs. non-gymnasts)	**5.9**	(2.8–9.0)	**6.3**	(3.1–9.5)
Gymnastic Activity* (h/wk) (current gymnasts only)	**5.8***	(3.1–8.5)	**N/A**	(N/A)
Skull BMC (g)	**430.3**	(403.5–457.2)	**439.6**	(408.3–470.9)
Total Arm BMC** (g)	**126.2****	(118.7–133.7)	**109.3**	(100.5–118.0)
4% CSA** (mm^2)	**244.5****	(224.0–265.0)	**202.4**	(178.5–226.2)
4% IBS** (g^2/cm^4)	**0.494****	(0.442–0.547)	**0.374**	(0.313–0.435)
4% Fall Strength Ratio**	**0.369****	(0.321–0.418)	**0.278**	(0.222–0.335)
33% cCSA** (mm^2)	**85.3****	(80.8–89.8)	**70.9**	(65.8–76.0)
33% SSI** (mm^3)	**242.1****	(220.0–264.2)	**172.0**	(146.7–197.2)
33% Fall Strength Ratio**	**0.175****	(0.159–0.191)	**0.128**	(0.109–0.146)

Subject characteristics and bone outcomes (unadjusted data) are presented by gymnastic exposure group. For 33% bone outcome analyses, GYM n=17, NON n=13.

BMI=Body Mass Index; FFM= non-bone fat-free or lean mass; h/wk= hours per week;
BMC=bone mineral content; CSA= total bone cross-sectional area;
SSI=polar strength-strain index; Fall Strength Ratio= SSI/(body mass*forearm length)

* greater mean for GYM (ex-gymnasts) than NON (non-gymnasts) by t-test ($p<0.05$)
**For all unadjusted upper extremity bone outcomes (loaded sites), GYM>NON (t-test $p<0.02$)

Table 3. Subject characteristics and bone outcomes by gymnastic exposure group.

group (Table 3). No difference was detected between GYM and NON for skull BMC (t-test $p>0.60$) (Table 3). In contrast, for bone outcomes, t-tests indicated larger unadjusted values for other GYM at all loaded sites (t-test $p<0.02$). Racial composition was not a factor in GYM and NON group differences (1 mixed-race non-gymnast (African-American/white); 1 Asian non-gymnast; all other subjects were white). For 33% site analyses, one NON and two GYM (1 ex-gymnast, 1 current gymnast) were excluded due to pQCT scan motion artifacts. Similarly, two 65% scans were excluded from muscle CSA analyses (1 NON, 1 GYM (ex-gymnast)).

As expected, when evaluated across the total sample, arm FFM was a strong correlate of most upper extremity bone mass, strength and geometry outcomes (Figure 2, Table 4). Surprisingly, 65% MUSCSA correlated less strongly with bone outcomes (Figure 2, Table 4). When broken down by activity group, the consistency of the correlations was particularly reduced for MUSCSA. In contrast, arm FFM correlations indicated strong positive linear relationships with most bone outcomes that were similar for GYM and NON (Figure 2, Table 4). Total body weight was not a consistent, significant correlate of bone parameters when assessed across the total sample, yet it exhibited higher, more positive correlations with GYM bone parameters than with NON outcomes (Table 5).

For all skeletal parameters, multiple regression models yielded significant predictive value, explaining 34% to 75% of variation based on gymnastic exposure, arm muscle parameters, gynecological age and height (as appropriate) (Table 6). There were no significant interactions between gymnastic exposure and either arm muscle parameter, so

Variable	Arm FFM			Ln Muscle CSA		
	TOTAL	GYM	NON	TOTAL	GYM	NON
Arm FFM vs. Muscle CSA	**0.62****	0.44	0.52	**0.62****	0.44	0.52
Skull BMC	0.06	0.13	0.17	0.13	0.28	0.23
Total Arm BMC	**0.72****	**0.50***	**0.81*****	**0.47***	0.27	0.42
4% CSA	**0.66****	**0.66****	0.39	0.25	0.00	0.28
4% IBS	**0.37***	0.22	0.40	**0.43***	**0.57***	0.12
4% Fall IBS	0.16	-0.11	0.33	0.38	0.43	0.21
33% cCSA	**0.72****	**0.62****	**0.66***	**0.49****	0.35	0.54
33% SSI	**0.76****	**0.75****	**0.62***	**0.42***	0.16	**0.68***
33% Fall SSI	**0.52****	0.44	0.41	**0.39***	0.20	0.52

Spearman correlation coefficients (rho) are listed for Arm FFM and the natural logarithm of Muscle CSA with bone outcomes and each other.
FFM=fat free mass; CSA=cross-sectional area; BMC=bone mineral content;
SSI=polar strength-strain index
* $p<0.05$; ** $p<0.01$; *** $p<0.001$

Table 4. Arm FFM and muscle CSA correlations, for total sample and each group, by outcome.

Variable	Total Sample	EX-GYM	NON
Skull BMC	**+0.43 ***	**+0.69 ****	+0.26 (NS)
Total Arm BMC	**+0.46 ****	**+0.67 ****	+0.35 (NS)
4% pCSA	+0.25 (p=0.16)	**+0.47 ***	+0.09 (NS)
4% IBS	-0.05 (NS)	-0.09 (NS)	+0.07 (NS)
33% cCSA	+0.04 (NS)	+0.22 (NS)	-0.10 (NS)
33% SSI	+0.10 (NS)	+0.36 (p=0.16)	-0.20 (NS)

Pearson correlation coefficients (r) are listed for Total Body Weight vs. bone outcomes.
Fall strength ratios are not included; total body weight is a factor in their denominators.
FFM=fat free mass; BMC=bone mineral content; pCSA=periosteal cross-sectional area; cCSA=cortical cross-sectional area;
IBS=index of structural strength in axial compression; SSI=polar strength-strain index
* $p<0.05$; ** $p<0.01$, (NS) $p>0.20$

Table 5. Correlations of total body weight with outcomes, by group.

models did not include interaction terms. For the skull, a non-loaded control site, BMC was not predicted by either muscle parameter after accounting for the significant predictive effects of gynecological age and height. In contrast, after accounting for muscle indices, gynecological age and height, gymnastic activity exposure was a significant predictor of all upper extremity skeletal parameters. Total arm FFM exhibited significant predictive value for all upper extremity bone parameters except 4% Fall IBS. In contrast, 65% MUSCSA was a poor predictor of upper extremity skeletal parameters, only exhibiting significant explanatory value for 4% IBS. Figures 3 and 4 present adjusted mean percentage differences in GYM compared to NON, for all bone parameters, accounting for arm muscle indices, gynecological age and height (as appropriate).

Discussion

In this cohort of post-menarcheal female gymnasts, ex-gymnasts and non-gymnasts, our results demonstrate the predictive value of gymnastic exposure for indices of bone mass, size and strength in the upper extremity (arm, radial metaphysis and radial diaphysis). This predictive value persists after accounting for physical maturity, height and either arm muscle mass or forearm muscle cross-sectional area. These findings suggest that skeletal adaptations to gymnastic

A	GYM/NON			Arm FFM			Gynecological Age			Height			Model	
	β	SS CC	*p*	β	SS CC	*p*	β	SS CC	*p*	β	SS CC	*p*	r^2	*p*
SKULL BMC	2.8	0.02	ns	-0.4	-0.09	ns	**14.6**	**0.54**	***	**3.2**	**0.32**	*	0.48	***
ARM BMC	**12.1**	**0.30**	**	**0.1**	**0.41**	***	0.9	0.11	ns	**0.8**	**0.26**	*	**0.75**	***
4% CSA	**26.9**	**0.25**	*	**0.1**	**0.39**	**	**-7.6**	**-0.34**	**	1.1	0.13	ns	**0.63**	***
4% IBS	**0.1**	**0.29**	*	**0.0**	**0.29**	*	**0.0**	**0.52**	***	-0.0	-0.18	0.17	**0.56**	***
4% FALL IBS	**0.1**	**0.41**	*	-0.0	-0.05	ns	**0.0**	**0.41**	**	------	------	------	0.34	**
33% CCSA	**7.8**	**0.29**	*	**0.0**	**0.50**	***	-0.6	-0.12	ns	-0.1	-0.08	ns	**0.72**	***
33% SSI	**39.1**	**0.30**	**	**0.2**	**0.49**	***	-2.7	-0.10	ns	0.2	0.02	ns	**0.74**	***
33% FALL SSI	**0.0**	**0.40**	**	**0.0**	**0.30**	*	-0.0	-0.11	ns	------	------	------	**0.46**	***

B	GYM/NON			ln MusCSA			Gynecological Age			Height			Model	
	β	SS CC	*p*	β	SS CC	*p*	β	SS CC	*p*	β	SS CC	*p*	r^2	*p*
SKULL BMC	-10.5	-0.09	ns	18.9	0.04	ns	**15.4**	**0.57**	***	**3.3**	**0.38**	**	0.55	***
ARM BMC	**19.0**	**0.50**	***	26.7	0.19	0.12	0.4	0.04	ns	**1.4**	**0.51**	***	0.63	***
4% CSA	**54.0**	**0.55**	***	-2.7	-0.01	ns	**-9.2**	**-0.42**	**	**2.2**	**0.32**	*	0.57	***
4% IBS	**0.1**	**0.37**	**	**0.4**	**0.38**	**	**0.0**	**0.51**	***	-0.0	-0.06	ns	**0.65**	***
4% FALL IBS	**0.1**	**0.32**	*	0.3	0.29	0.06	**0.0**	**0.43**	**	------	------	------	0.44	***
33% CCSA	**14.2**	**0.58**	***	23.5	0.25	0.08	-0.8	-0.16	ns	0.2	0.12	ns	0.58	***
33% SSI	**72.7**	**0.61**	***	65.2	0.14	ns	-3.8	-0.14	ns	2.0	0.23	0.11	0.57	***
33% FALL SSI	**0.0**	**0.56**	***	0.1	0.19	ns	-0.00	-0.10	ns	------	------	------	0.46	**

6A: Regression results for models that include Arm FFM.
6B: Regression results for models that include Muscle CSA.

Bold variable names indicate models for which both gymnastic group and the focal muscle index are significant predictors.
SSCC= Squared semi-partial correlation coefficients; these indicate percentage of variance explained by the focal variable after accounting for the effects of the other independent variables.
β=Slope indicates the factor by which the relevant independent variable is multiplied in the regression equation.
* $p<0.05$; ** $p\leq0.01$; *** $p\leq0.001$; ns, $p>0.20$

Table 6. Multiple regression results, by muscle index.

loading are at least partially independent of muscular indices. This evidence contrasts with the perspective of authors who ascribe the influence of "weight-bearing" and impact loading to muscular forces alone[2,15].

Evidence from the current study, other human studies[19,25-28] and animal models[16-18] lends support to a more direct, yet more complex, model: 1) gymnastic loading stimulates bone directly through non-muscular means; 2) gymnastic loading generates muscle tissue adaptations, thereby increasing muscular stimulation of bone (mechanically and biochemically); and 3) training improves muscular capacity to generate and withstand greater impact forces. In other words, positive feedback between non-muscular loading and muscular stimuli may act together to enhance bone structure, resulting in adaptation to higher peak loads during training (suggested by high arm FFM vs. bone correlations). This model acknowledges direct loading of the skeletal system, as well as the vital role played by the muscular system in the generation and transmission of loads and in the support of the skeleton during loading. As noted earlier, gymnastic-specific osteogenic loads are a composite of forces that exert axial compression, bending, torsion and tension, including muscular and non-muscular stimuli.

Research evaluating non-gymnasts supports the contention that non-muscular aspects of impact loading generate greater bone benefits than loading attributed to muscular forces alone[19,25-28]. In particular, Ducher et al. aimed to directly address the muscle-bone relationship using MRI and DXA to compare dominant vs. non-dominant distal radii of tennis players, thereby controlling for inter-individual genetic, hormonal and nutritional differences[19]. They identified side-to-side BMC differences of 13.5%, similar to our unadjusted gymnast vs. non-gymnast differences (15.5%). Muscle volume and grip strength were strongly, positively correlated with bone parameters within each arm. However, differences between dominant and non-dominant arms were not explained by differences in muscle volume or grip strength; there was no correlation between muscle volume asymmetry and bone volume asymmetry. Their results strongly implicated non-muscular forces as factors in bone adaptation via impact loading[19].

Few studies have assessed the relative importance of muscular vs. non-muscular factors using the gymnastic model of bone accrual. Taaffe and Marcus identified significantly greater arm muscle strength and aBMD in a group of female gymnasts and non-gymnasts[25]. At local and uninvolved sites, both muscle strength and body weight were strongly, positively correlated with aBMD in non-gymnasts, but not in gymnasts. Paralleling our findings, Taaffe and Marcus concluded that impact loading dominated development of gymnast aBMD, whereas in non-gymnasts, the effects of muscular loading are discernible, yet largely accounted for by body weight (body size, habitual weight-bearing)[25].

Similarly, in a pediatric comparison of pre-pubertal non-athletes, swimmers and gymnasts, Cassell et al. identified a higher correlation between body weight and total body BMD in gymnasts compared to swimmers, concluding that impact-loading was superior to non-impact muscular stimuli for improvement of bone quality[39]. In the current study, compared to NON, our GYM also generally exhibited higher, more positive correlations for body weight vs. bone outcomes. These findings suggest that, in GYM, elevated arm bone mass and radius size/strength may be partly attributable to the role of the forearm as an impact/"weight-bearing" limb. However, compared to NON, GYM also exhibited a stronger positive correlation for body weight versus skull BMC. The absence of a relationship between gymnastic loading and skull BMC suggests that gymnastic loading is not responsible for the body weight/skull BMC correlation. The strength of the link between body weight and bone mineral content in GYM may be due to some other, unknown factor.

Two other studies evaluate muscle strength and bone properties in gymnasts vs. non-gymnasts. Liang et al. compared ulnar and tibial bending stiffness in non-athletes, world-class synchronized swimmers and world-class gymnasts, correlating bone outcomes with average extension/flexion power in gymnasts and synchronized swimmers (elbow, knee)[40]. Unfortunately, activity group comparisons were not adjusted for body size differences, confounding interpretation of results. Helge et al., compared muscular strength and axial aBMD in 15-17 year old female gymnasts (n=6), rhythmic gymnasts (n=5) and non-gymnasts (n=6)[41]. However, this study was limited by an extremely small sample size and evaluated only axial sites, which provide a more ambiguous model than our appendicular site. Thus, neither study effectively distinguishes the osteogenic potency of non-muscular vs. muscular stimuli.

Although our results suggest an independent osteogenic influence for non-muscular components of gymnastic loading, our findings also support the importance of the functional muscle-bone unit. Arm muscle mass exhibited significant predictive value for most upper extremity skeletal parameters (not 4% Fall IBS); higher explanatory value was attributed to arm FFM than gymnastic exposure for all but 4% Fall IBS and 33% Fall SSI. In contrast, MUSCSA only exhibited significant predictive value for 4% IBS, and greater explanatory value was attributed to gymnastic exposure than MUSCSA for all upper extremity bone parameters except 4% IBS (explanatory value and significance of MUSCSA for 4% IBS were comparable to those of gymnastic exposure).

MUSCSA would have been expected to act as a more specific index of proximal forearm muscular function than arm FFM, as both anatomical and physiological muscle cross-sectional areas have been shown to demonstrate strong positive correlations with muscle contractile force[42,43]. In contrast, total arm FFM is merely a muscle mass index, providing only a crude summary of global upper extremity muscular factors. The predictive value of arm FFM may stem primarily from common underlying growth and development factors and common responses to training exposures, serving more as correlate than cause. Alternatively, in the context of gymnastic activity, arm FFM may indicate capacity of the upper extremity muscular system to generate and support gymnastic maneuvers, deriving predictive value from a positive correlation with the total of muscle volumes (reflecting joint torques) for the upper extremity[43]. Regardless, our results support a view that the osteogenic potency of gymnastic loading is not solely attributable to local muscular action; other stimuli appear to contribute an additive effect.

Limitations

As a pediatric observational study, this work relies upon statistical methods within a quasi-experimental design. As such, this cross-sectional analysis is limited in its capacity to characterize differences as "adaptations". Similarly, due to its observational nature, this study is subject to a gymnastic participation bias; however, it is neither feasible nor ethical to perform a randomized, controlled gymnastic activity intervention. Although our design cannot rule out the potential influence of genetic factors, analyses of skull BMC (skeletal index for a non-loaded site) do not indicate an underlying bias among gymnasts for globally enhanced bone parameters. In fact, the observed site-specific enhancement of skeletal parameters suggests regional adaptation to mechanical loading exposure. In addition, other work has indicated that mechanical loading induces skeletal adaptations that do not result from pre-existing genetic differences, including prospective studies of gymnastic exposure, arm to arm racquet sport adaptations and targeted loading within animal models[19-23,31,44]. Finally, the relationship between muscle and bone may be modulated by other important factors, including underlying hormonal milieu and nutritional adequacy[4,45]. In order to limit potential confounding influences, our analyses included only post-menarcheal females and accounted statistically for the potential influence of gynecological age and body size (height). Calcium intake did not differ between groups or correlate with upper extremity outcomes, reducing the likelihood of dietary influence.

Assessment of bone strength indices was limited to densitometric methods. As a result, our work provides a theoretical estimate of bone strength, rather than a measure of experimental failure, and the results should be interpreted accordingly. Furthermore, densitometric output may be affected by soft tissue interference, and partial volume effects may reduce pQCT accuracy. However, the arm and forearm (distal radius) consist of small, minimally variable soft tissue envelopes, reducing potential error from this source, and the magnitudes of the activity group differences observed in this study (10-42%) are unlikely to result from measurement error alone.

Our observational study did not directly measure the muscular forces or biological factors that are applied to the bone; arm FFM and MUSCSA were used as surrogate indices of muscular forces. Unfortunately, neither arm FFM nor MUSCSA is an ideal index, as muscles with equal CSA and mass may yield different contraction speeds and force generation[42]. We have limited potential differences in this regard by comparing only females and accounting for gynecological age. Nonetheless, it is possible that "gymnast muscle" generates greater stimulation per kg (or cm^2) than "non-gymnast muscle" (GYM may have higher specific tension than NON) via different fiber composition and/or efficiency of neural activation[43], potentially underestimating the influence of muscular forces in statistical analyses. Finally, total arm FFM includes muscles that do not act directly upon the distal radius. However, model inclusion of proximal upper extremity muscle mass (total arm FFM) is appropriate, as these muscles provide the stability required for force transmission across the radius and may indirectly generate bending loads. Regardless of the aforementioned limitations, our results are supported by animal studies that generated experimental bone strength improvements via highly specific mechanical loading protocols[16-18]. These animal studies are strengthened by their capacity to disentangle muscle-bone interactions *in vivo*; they corroborate our hypothesis by demonstrating that mechanical loading is osteogenically potent when isolated from muscular involvement[16-18].

Conclusion

This work, and other research, provides strong evidence that bone is enhanced by gymnastic activity during growth. As expected, muscle mass and bone properties are closely related, and arm FFM is a useful indicator of upper extremity bone characteristics. However, as a more specific index of muscular contraction force, muscle CSA is a comparatively weak predictor of gymnasts' bone properties. Our regression results indicate that gymnastic exposure acts as an independent factor in arm skeletal structure, linked to elevated bone mass, size and theoretical strength, including higher theoretical resistance to fracture in a low trauma fall. Therefore, direct mechanical loading via non-muscular means may act as a distinct and important determinant of human skeletal structure.

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