Intrinsic and Modifiable Contributors to Distal Radius Microstructure, Macrostructure and Strength in Premenopausal Women

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May 31, 2017

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Abstract

Peak bone mass is predictive of lifetime fracture risk, yet young adult women are not screened or counseled on strategies for improving their bone health. Thus, the purpose of this cross-sectional analysis was to identify measurable factors describing genetic and lifestyle characteristics predictive of bone macro- and microstructure and mechanical behavior. We hypothesized that serum vitamin D, current daily calcium intake, site-specific loading, and grip strength would be associated with favorable bone structure and mechanical properties. Seventy-two women aged 21-40 were included in this cross-sectional analysis. High resolution peripheral quantitative computed tomography was used to measure total bone mineral density (BMD), trabecular BMD, mean cross-sectional area (CSA), trabecular number, cortical BMD, cortical thickness, and cortical porosity (%) in the ultradistal radius. Quantitative analysis of clinical computed tomography (CT) scans of the distal forearm were used to calculate integral BMD, bone volume (BV), and bone mineral content (BMC) in the ultradistal and total distal radius, and mean energy equivalent strain in the ultradistal radius was calculated from continuum finite element models generated from CT images. Hierarchical regression models were used to assess the predictive capability of intrinsic (age, height) and modifiable (body mass, grip strength, physical activity) predictors. Vitamin D and calcium intake were not correlated with any bone parameter. Age and height explained 32% of the variance in variables related to bone size, with grip strength and adult loading due to physical activity each explaining an additional 5 to 19% in these and other measures. Body mass explained 10% of the variance in bone strain under a given force, with higher body mass being associated with lower strain ($r=-0.210$, $p<0.05$). Overall, results suggest that meaningful differences in bone structure and strength can be predicted by measurable subject characteristics. This highlights the contribution of modifiable site-specific mechanical loading on bone structure and strength.

Highlights:

- Potential predictors of radius micro- and macrostructure and strength are presented.
- Grip strength positively predicts cortical porosity, bone area, and mineral content.
- Adult loading predicts greater porosity, trabecular density, and mineral content.
- Greater body mass predicts lower finite-element predicted radial bone strain.

Key Words: HRpQCT; Bone QCT; Finite Element Model; Physical Activity; Bone Adaptation;

1. Introduction

Peak bone mass, achieved during late adolescence[1–4], is a critical determinant of lifetime bone health. Increased peak bone mass in females has been linked to decreased fracture risk [5–8] and delayed onset of osteoporosis [9]. Therefore, understanding the factors affecting bone at the onset of adulthood is an important step in identifying women at higher risk for fracture later in life. Additionally, since young adult bone is responsive to weight-bearing exercise [10], detecting low bone mass or diminished bone quality in this age group may allow for targeted mechanical loading interventions to reduce fracture risk later in life. Such exercised-based interventions may offer an effective means of preventing fractures at a lower cost compared to bisphosphonates, which limit bone loss but fail to rebuild bone in osteoporotic women. However, due to their overall low risk of fracture, young adults are not routinely screened for low peak bone mass or counseled on methods to improve their bone health [11]. Furthermore, routine screening tools such as DXA and fracture risk calculators are expensive and invalid for young adults, respectively [12].

The forearm is a particularly appropriate site for developing screening tools and preventative strategies for premenopausal adult women. The distal radius is the most common fracture site in adults, accounting for 17.5% of all fractures [13]. Among older women, distal

forearm fracture is associated with a 1.95-fold increased risk of any subsequent fracture and a 5.18-fold increased risk of vertebral fracture [14]. Additionally, the forearm is a suitable site for studying the interaction between skeletal loading and bone structure, which is thought to adapt to its habitual mechanical environment [15]. While the lower extremities are subjected to high volumes of ambulatory loading, the upper extremities experience less frequent loading and may be more sensitive to prescribed exercise. This may make it easier to detect skeletal changes in response to loading, which is an important step in correlating tissue strain with adaptation to better design osteogenic loading regimes at other sites. Additionally, differences in forearm loading between individuals can be determined from retrospective physical activity surveys, while ambulatory loading is difficult to recall without pedometer data. Measuring variability between individuals is important, as exercise history may affect sensitivity to loading and help identify patients who benefit most from novel loading.

Although considered the gold-standard measure of fracture risk, DXA areal densitometry measurements are inherently limited by their two-dimensional nature. Most distal radius fractures occur in women who are osteopenic rather than osteoporotic as measured using DXA [16], indicating that factors besides bone mineral density (BMD) affect bone strength and fracture risk. Indeed, a combination of density, volumetric structure, and other factors comprising bone quality can explain differences in the biomechanical behavior of bone better than bone mass or density alone [17,18]. High-resolution peripheral quantitative computed tomography (HRpQCT) and quantitative analysis of clinical computed tomography (CT) scans allow for the measurement of volumetric density and micro- and macrostructure of bone in the forearm. Additionally, 3D finite element models explicitly modeling volumetric structure can be

constructed directly from CT images and used to estimate tissue-level bone strain under physiologic loading conditions [17].

Our approach to developing screening tools and fracture prevention strategies for young adult women is to identify measurable factors reflecting genetic and lifestyle characteristics affecting bone strength. Previous studies have highlighted weight, height, and physical activity as potential determinants of peak bone mass, but have generally focused on whole-body loading and bone mass measured by DXA [19–22]. Attempts to relate site-specific loading and radius bone structure have compared athletes and non-active controls [23–25], but have not considered the effects of loading due to recreational exercise habits among average women. Thus, the purpose of this study was to identify intrinsic and modifiable factors that affect radius macro- and microstructure and mechanical behavior under simulated loads in average adult women. We hypothesized that individuals with high levels of serum vitamin D, calcium intake, site-specific physical activity and grip strength would have stronger bone structure and experience lower strain for a given external force, independent of intrinsic factors such as age and height.

2. Materials and Methods

2.1 Participants

Healthy females age 21-40 were recruited for this cross-sectional study as part of a larger, institutionally approved longitudinal experiment. Women were recruited from the greater Worcester area, and as of November 2016, 1343 subjects had responded to online surveys expressing initial interest in participation. Of those, 374 were screened using a telephone survey, while 969 were no longer interested when contacted for screening. Based on telephone survey responses, women with irregular menstrual cycles or whose body mass index fell outside the normal range (18-25 kg/m^2) were excluded. Additionally, individuals were excluded if they had

no regular dietary or supplemental calcium intake or were taking medications known to affect bone metabolism. Because subjects were being screened for a prospective loading intervention study, individuals with a history of radius fracture or injury of the non-dominant shoulder or elbow, and those regularly participating (> 2 time per month) in sports that apply high-impact loads to the forearm (e.g. gymnastics, volleyball) were also excluded. Those satisfying the initial inclusion criteria and still interested were screened for 25-hydroxyvitamin D serum levels and forearm DXA T-score (n=120). Qualified subjects had 25-hydroxyvitamin D serum above 20 ng/ml and a DXA T-score between -2.5 and 1. Overall, 258 individuals were excluded based on screening criteria, and 34 were no longer interested in participating after screening. Data for qualified subjects (n=82) were collected either during the screening or a single visit within approximately two weeks of screening. All participants provided written, informed consent between January 2014 and November 2016.

2.2 Anthropometrics and Loading Assessments

Height was measured using a wall-mounted stadiometer, and body mass was measured using an analog scale. Non-dominant grip strength was measured using a hydraulic hand-grip dynamometer [Baseline; White Plains, NJ] three times and averaged. Grip strength measurements were taken in a seated position with the elbow bent ninety degrees in flexion. Average daily calcium intake (mg/day) was estimated using a 10-item questionnaire that tallied weekly consumption of calcium-containing foods and beverages [26].

To estimate forearm loading due to physical activity, a site-specific arm bone loading index (*armBLI*) algorithm [23] was used to score activity histories collected using the validated Bone Loading History Questionnaire (BLHQ) [27]. The *armBLI* algorithm scores activities based on the magnitude, rate, and frequency of loads applied to the non-dominant arm as:

$$armBLI=\Sigma[(Magnitude + Velocity) \times Frequency \times Non\text{-}Dominance]$$

where the non-dominance multiplier corrects for activities loading the dominant arm preferentially. The multiplier is 0.33 for predominantly unilateral activities (e.g., tennis), 0.66 for somewhat unilateral activities (e.g. softball), and 1.0 for bilateral activities (e.g. gymnastics). For each individual, an overall score is calculated as the products of activity-specific training volumes and *armBLI* indices summed over all activities performed. For the present study, physical activity training volumes were generated using the retrospective BLHQ, which has been used to collect activity histories in premenopausal adult women [27]. Briefly, training volume is calculated as the product of years of participation, the seasons participated per year (fraction out of four), and a frequency score ranging from 1 to 4 reflecting training sessions per week (1=1-3 times per month, 2=1-2 times per week, 3=3-5 times per week, and 4=>5 times per week). To assess the relative importance of upper-extremity physical activity during different stages of development, separate mean annual scores (armBLI/year) were calculated for adolescent (age 10-18) and adult (age 19-current age) loading.

2.3 High-Resolution Peripheral Quantitative Computed Tomography

High-resolution peripheral quantitative computed tomography (HRpQCT; XtremeCT, Scanco Medical) scans of the distal radius in the non-dominant arm were performed according to the manufacturer's standard *in vivo* scanning protocol. The scans consisted of 110 slices with an isotropic voxel size of 82 µm, encompassing a 9.02 mm axial region beginning 9.5 mm proximal to a reference line placed at the distal endplate. All scans were performed by trained technicians, and daily and weekly quality control scans were performed. Each scan was graded for motion on a scale from 1 (no motion) to 5 (severe motion artifact) [28], and only scans scoring 3 or better were included in the analysis.

HRpQCT scans were analyzed using the manufacturer's semi-automatic standard morphological [29] and cortical [30–33] analyses. Total bone mineral density (BMD) (mgHa/cm^3), trabecular BMD (mgHa/cm^3), mean cross-sectional area (CSA; mm^2), and trabecular number (mm^{-1}) were calculated using the standard manufacturer's analysis, and cortical BMD (mgHa/cm^3), cortical thickness (mm), and cortical porosity (%) were calculated using the dual-threshold method [30–33].

2.4 Quantitative CT Analysis and Continuum FE Modeling

Clinical CT scans of the distal-most 12 cm of the non-dominant forearm were acquired using established methods [34]. Axial CT scans of this continuous volume of interest allow macrostructure to be assessed in a larger region than possible with HRpQCT *in vivo*, and facilitate construction of continuum finite element models including the distal articulating surface to simulate physiologic loading through the carpals. A calibration phantom with known calcium hydroxyapatite equivalent concentrations was included for quantitative analysis (QCT), and calculations of integral parameters were made using established protocols [35]. Bone volume (BV; cm^3), bone mineral content (BMC; g), and bone mineral density (BMD; g/cm^3) were calculated for the ultradistal region in the clinical CT images matching the HRpQCT scanned region, as well as a total region comprising everything distal to and 45 mm proximal to the subchondral plate (the transverse slice with maximal cross-sectional area). The clinical CT region corresponding to the smaller HRpQCT-scanned region was identified using a custom Matlab script utilizing a mutual information image registration algorithm considering pixel intensities. A laboratory precision study yielded mean rotation errors of 0.47±0.38°, 0.46±0.41°, and 0.32±0.24° in the x, y, and z directions, respectively, for a similar data set [36]. Mechanical behavior of the entire distal radius under physiologic loading was estimated using continuum

finite element (FE) models [17] to simulate distal compressive forearm loading of 300 N (approximately one half body-weight) through the palm of the hand. Energy equivalent strain ($\bar{\varepsilon}$) was selected as the primary outcome because it has been previously related to bone adaptation [34]. This scalar quantity represents the total work done on the bone tissue, provided by the multi-axial stress-strain state:

$$\bar{\varepsilon} = \sqrt{\frac{2U}{E}},$$

where E is the elastic modulus, and U is the strain energy density calculated as:

$$U = \frac{1}{2}[\sigma_1\varepsilon_1 + \sigma_2\varepsilon_2 + \sigma_3\varepsilon_3],$$

where σ_n and ε_n are the principal stress and strain components, respectively. Mean energy equivalent strain within the matched ultradistal region of the continuum model was used for further analysis.

2.5 Statistical Analysis

The normality of each measured variable was assessed by visual inspection of histogram distributions. Correlation coefficients were calculated between subject characteristics, HRpQCT values, QCT parameters, and FE-strain to identify potential predictors of bone structure and strength. Pearson and spearman coefficients were used for variables with normal and non-normal distributions, respectively. As a result of this initial analysis, subsequent regression models included age and height as intrinsic covariates and body mass, grip strength, and loading scores were included as extrinsic predictors. A series of hierarchical linear regression models were fitted for each dependent structure and strength variable. Non-modifiable intrinsic factors were added as a first block of independent variables, and then a single modifiable factor was added in a second block. This analysis allowed the total variance explained by the intrinsic factors as a

group and the predictive capability of each individual modifiable factor to be determined. The overall model residuals were visually inspected for normality and homoscedasticity using a plot of residuals versus predicted values. An alpha level of 0.05 was used to detect significance. All statistical analyses were performed using SPSS v22.0.

3. Results

3.1 Subject Characteristics

Descriptive statistics, presented as means and standard deviations, are summarized in Table 1. Ten enrolled subjects were excluded from analysis due to incomplete physical activity data (n=3) or HRpQCT motion artifact (n=7). Thus, all results are reported for the seventy-two subjects for which data were complete. Estimated daily calcium intake was below the recommended daily value (1000 mg/day) and the average intake reported for women ages 19-50 in the United States [37]. Average grip strength was similar to previously reported values for young adult women [38,39]. Correlation coefficients between potential predictors and bone structure and strength parameters are provided in Table 2. Neither serum 25-hydroxyvitamin D levels nor daily calcium intake were significantly correlated with any bone structure or strength parameter and were thus excluded from regression analysis. Age and height, which were significantly correlated with one or more structural parameters, were included as intrinsic model covariates. Body mass, grip strength, adolescent upper-extremity loading and adult loading were included as potential extrinsic predictors.

3.2 Predictors of HRpQCT Microstructure

Mean and standard deviations for all HRpQCT parameters, as well as the hierarchical regression results, are presented in Table 3. Mean values for HRpQCT-measured parameters agree well with those previously reported for young adult women [40]. Age and height

accounted for 9.6% of the variance in trabecular BMD (p=0.031), and adding adult loading score to the model accounted for an additional 7.1% of the variance (p=0.019). Intrinsic factors alone explained 11.9% of the variance in cortical BMD (p=0.013), and adding grip strength to the model explained an additional 17.0% of the variance (p<0.001). Total cross sectional area was strongly predicted by age and height, which explained 31.6% of the variance (p<0.001). Adding grip strength to the model significantly improved the prediction of total area, explaining an additional 17.9% of the variance (p<0.001). Intrinsic factors alone explained 17.4% of the variance in trabecular number (p=0.001), and body mass accounted for an additional 7.6% percent of the variance (p=0.011). Cortical porosity was not significantly predicted by intrinsic factors alone, but adding either grip strength or adult loading score improved model predictions by 5.6% (p=0.043) and 8.3% (p=0.013), respectively. None of the models predicting total BMD or cortical thickness were significant.

3.3 Predictors of QCT Macrostructure and Strain

Mean and standard deviations for all clinical QCT parameters and FE-derived mean energy equivalent strain, as well as the hierarchical regression results, are presented in Table 4. Age and height alone explained 32.0% of the variance in ultradistal bone volume (p<0.001), and grip strength explained an additional 19.5% (p<0.001). Ultradistal bone mineral content was significantly predicted by intrinsic factors, with age and height predicting 9.7% of the variance in BMC (p=0.029). Adding grip strength or adult loading score explained an additional 12.3% (p=0.002) and 5.0% (p=0.049) of variance in ultradistal BMC, respectively. Total bone volume was strongly predicted by age and height, with 39.7% of the variance (p<0.001) accounted for by intrinsic factors alone. Adding grip strength explained an additional 14.1% of the variance in total bone volume (p<0.001). Age and height explained 20.9% of the variance in total region

BMC ($p<0.001$). Adding body mass to the model explained an additional 5.3% ($p=0.030$) of the variance in total BMC, adding grip strength explained 16.3% ($p<0.001$), and adding adult loading score explained an additional 6.1% of variance ($p=0.020$). Mean energy equivalent strain, calculated for the ultradistal region using continuum finite element models, was not significantly predicted by age or height. Adding body mass to the model significantly improved the prediction of strain, explaining an additional 10.0% of the variance ($p=0.008$). Neither ultradistal nor total BMD were significantly predicted by any intrinsic or extrinsic predictors.

4. Discussion

The purpose of this study was to identify measureable factors affecting bone structure and mechanical behavior in healthy adult women. As intrinsic factors, age and height were significant predictors of trabecular number, trabecular and cortical BMD, integral BMC and bone size but not integral BMD, cortical thickness, cortical porosity or energy equivalent strain. Age was negatively correlated with trabecular density and number and higher cortical density. Height was positively correlated with measures of bone size and mineral content and negatively correlated with measures of density. Regression results showed that higher values for grip strength was associated with lower cortical density and higher cortical porosity, cross-sectional area, BV and BMC. In other words, individuals with higher grip strength tended to have more porous cortices but larger bones with higher mineral content. Similar morphological trends were seen in individuals with higher levels of site-specific adult loading, who tended to have more porous cortices, higher density in the trabecular region and higher mineral content. Greater body mass predicted higher trabecular number, bone mineral content in the total region, and lower ultradistal strain. This suggests that within the normal BMI range, greater body mass is associated with improved mechanical behavior (i.e. lower strains under a given load), which may

be attributed to more interconnected trabeculae supporting the ultradistal region. Taken together, these results suggest that meaningful differences in bone morphology and mechanical behavior can be predicted by measurable subject characteristics.

In the current study, upper extremity mechanical loading was considered through the inclusion of body mass, grip strength, and questionnaire-based physical activity scores. These measures are to some extent related, as more active individuals may have more muscle mass, which affects both grip strength and body mass. However, each contributes to a different aspect or mode of loading. For example, individuals with greater body mass experience larger compressive loads during weight-bearing exercises, to which bone adapts. This is consistent with the observation that heavier individuals experienced lower-magnitude strains for a given compressive force, indicating stronger bone. This also supports the previously reported effects of body mass on lower-extremity mechanical loading [41], areal BMD [42], and fracture risk [43].

Grip strength is a functionally useful measure of muscle mass and strength, and has been associated with bone density, macrostructure, and strength using peripheral QCT [21,44,45]. The relationship between muscle mass and bone mass is complex. While body size is somewhat genetically predetermined, bones may also adapt to larger muscle forces over time. Biomechanically, grip strength is related to muscle forces applied at the distal articular surface, which leads to bending at the ultradistal region [46]. If radius structure were adapted to resist higher bending forces in individuals with higher grip strength, then cross-sectional area would be larger and bone mineral would be concentrated near the peripheral cortical compartment to maximize mass moment of inertia. Our results partially support this logic; higher grip strength was related to increased bone size and total BMC, but decreased cortical BMD. The importance of loading mode may also explain the inability of grip strength to predict FE bone strain under

compression; if grip strength is mostly related to the resistance to bending loads, its effect may be minimal during axial compressive loading. This highlights the potential importance of different loading modes on bone structural adaptation.

Physical activity during growth and adulthood has been associated with improvements to bone structure [47]. Implied, is that the mechanical forces transmitted through the skeleton during physical activity elicit tissue-level strains that stimulate remodeling and bone formation. However, there is a lack of consensus whether loading during adolescence or early adulthood are more significant in determining peak bone mass [48–50]. We found that adolescent loading did not significantly contribute to the prediction of any bone structural or strength parameter, while adult loading was associated with favorable ultradistal and total BMC as well as trabecular BMD. Variations between previous and the current results may be related to differences in questionnaires or anatomic sites. As opposed to other skeletal loading questionnaires, the *armBLI* scores activities based on the magnitude and frequency of forearm loading rather than using ground reaction forces [51] or estimations of loading at the hip and spine [27]. The relationship between loading and structure may also be site-specific, especially considering the differences in habitual loading between the upper and lower extremities.

Cortical porosity was significantly predicted by grip strength and adult loading score. However, correlation coefficients in both cases were negative, indicating that more active individuals with greater muscle mass have more porous cortices. This is somewhat surprising, as increased cortical porosity is associated with diminished structural integrity and increased fracture risk [33]. However, increased cortical porosity in this population may reflect more active remodeling units rather than degradation, driven by adaptation to increased applied loading.

We found that neither serum vitamin D levels nor estimated daily calcium intake were correlated with any structural or strength parameters. While another study also found no significant association between serum vitamin D and peak bone mass [52], this result may also be related to limitations of the study design. Vitamin D level and calcium intake were considered during screening, and only individuals with levels above 20 ng/ml and who had some regular dietary or supplemental calcium intake were included. Thus, by excluding individuals with lower levels, variability may have been limited such that statistically significant correlations were not detectable. Additionally, both serum vitamin D level and the calcium survey are short-term measurements that do not necessarily reflect lifelong dietary patterns. This is important considering that the effects of vitamin D and calcium are dependent on long-term intake [53,54].

This study has several strengths. By obtaining both HRpQCT and clinical CT scans in each subject, we were able to measure microstructure in the clinically important, fracture-prone ultradistal region while assessing macrostructure and volumetric density for the entire distal radius. Validated continuum finite element models allowed for the estimation of mechanical behavior under simulated physiologic loading. While fracture is a common outcome in studies of post-menopausal women, the overall low fracture rate in younger adults inhibits the use of fracture as a primary outcome. Additionally, non-invasive predictions of mechanical behavior under simulated loading allows for the subject-specific estimation of bone strain. Such models are useful in determining patient fracture risk and developing prescribed loading interventions tuned to individual variation in bone strain.

The current study is not without limitations. Our sample size was relatively small, and subjects were recruited as part of a longitudinal study with inclusion criteria developed for the evaluation of a loading intervention. To target individuals who would most likely benefit from

new loading, anyone already regularly participating in activities involving frequent, high impact loading of the upper extremities was excluded. Additionally, only women with a DXA radius T-score falling within the range -2.5 to 1 were included. Therefore, the current results cannot be generalized to women with extreme levels of upper-extremity loading, those with clinical osteoporosis, or those with T-score more than 1 SD above the population mean. Additionally, there may have been limitations in applying the *armBLI* algorithm to adult women with retrospective rather than prospective, calendar-based training histories. The accuracy with which adolescent activity was recalled may have been limited and introduced additional variability, contributing to the lack of significant predictions by adolescent loading. Further, the *armBLI* was validated against DXA areal density measurements [23] rather than volumetric structure or FE-derived strain. Considering these differences, a more rigorous validation of the *armBLI* may be required in adult women using CT-based measurements.

In summary, we have identified several intrinsic and modifiable factors predictive of radius micro- and macrostructure and tissue-level strain. Vitamin D and calcium were not significantly associated with any bone parameter, indicating that maintaining normal serum vitamin D levels and regular calcium intake may be sufficient for sustaining normal bone structure in early adulthood. Additionally, we have shown that individuals with higher levels of adult physical activity, grip strength, and body mass generally tend to have favorable bone structure. Women with higher body mass within a normal BMI range also had lower levels of strain under a given force, suggestive of adaptation to increased loads during functional activities. Overall, these results suggest the importance of engaging in bone-building behaviors in early adulthood and contribute to the systematic design of prescribed loading interventions to better address the growing incidence of osteoporotic fracture.

Acknowledgements

This work was supported by the National Institutes of Health [grant number R01AR063691]; and the National Science Foundation [grant numbers DGE1106756 (MM), DGE1144804 (MM)]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

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Table 1: Descriptive statistics for all subjects (n=72)

Subject Characteristics	Mean	SD
Age (years)	28.3	5.3
Body Mass (kg)	63.6	8.6
Height (cm)	164.6	6.9
ND Grip Strength (kg)	26.4	5.2
Vit D (ng/mL)	32	9
Daily calcium intake (mg/day)	682	400
Adolescent Loading Score (armBLI/year)	50	48
Adult Loading Score (armBLI/year)	54	47

Vit D Serum Vitamin D level, *ArmBLI* Arm Bone Loading Index

Table 2*:* Correlation coefficients between subject characteristics and bone structure and strength parameters. *p<0.05, **p<0.01

			Age (years)	Height (cm)	Body Mass (kg)	ND Grip Strength (kg)	Vitamin D (ng/mL)	Calcium Intake (mg/day)	Adolescent Loading (armBLI/year)	Adult Loadin (armBLI/yea
HRpQCT		Total BMD (mg Ha/cm^3)	0.022	**-0.253***	-0.099	**-0.254***	-0.169	-0.096	-0.182	0.005
		Trabecular BMD (mg Ha/cm^3)	**-0.334****	-0.076	-0.005	0.089	-0.062	-0.035	0.075	**0.237***
		Cortical BMD (mg Ha/cm^3)	**0.301***	**- 0.262***	0.029	**-0.472****	-0.194	-0.148	-0.085	-0.220
		Total Area (mm^2)	-0.181	**0.550****	**0.343****	**0.620****	0.105	0.058	0.145	0.190
		Trabecular Number (1/mm)	**-0.457****	0.080	0.190	0.069	0.079	0.083	0.162	0.153
		Cortical Thickness (mm)	0.157	-0.160	-0.031	-0.165	-0.180	-0.081	**-0.232***	-0.024
		Cortical Porosity (%)	-0.184	0.159	-0.027	**0.285***	0.096	0.069	0.034	0.228
Clinical QCT	UD	Bone Volume (cm^3)	-0.161	**0.558****	**0.365****	**0.642****	0.084	0.074	0.121	0.212
		Bone Mineral Content (g HA)	-0.059	**0.309****	**0.307****	**0.451****	-0.033	-0.029	-0.002	**0.236***
		BMD (g HA/cm^3)	0.020	**-0.249***	-0.087	-0.199	-0.182	-0.090	-0.173	0.005
	Total	Bone Volume (cm^3)	-0.041	**0.630****	**0.445****	**0.619****	0.134	0.057	0.114	0.201
		Bone Mineral Content (g HA)	-0.094	**0.449****	**0.422****	**0.558****	0.111	0.049	0.087	**0.282***
		BMD (g HA/cm^3)	-0.140	-0.231	-0.039	-0.061	-0.050	-0.071	-0.061	0.098
	FE	Mean Energy Eqiv. Strain (με)	-0.004	-0.014	**-0.261***	-0.092	0.031	0.006	-0.029	-0.177

Table 3: HRpQCT parameter values (mean±SD) and hierarchical linear regression results

Parameter	Mean	SD	Predictors	R^2	ΔR^2	p	Beta
Total BMD (mg Ha/cm^3)	298.23	51.53	Age, Height	0.064		0.101	
			+Body Mass	0.067	0.003	0.640	0.068
			+Grip Strength	0.088	0.024	0.184	-0.175
			+Adolescent Loading	0.068	0.004	0.584	-0.065
			+Adult Loading	0.076	0.012	0.360	0.110
Trabecular BMD (mg Ha/cm^3)	162.86	30.03	**Age, Height**	**0.096**		**0.031**	
			+ Body Mass	0.110	0.014	0.309	0.146
			+Grip Strength	0.122	0.026	0.164	0.181
			+Adolescent Loading	0.101	0.005	0.525	0.074
			+Adult Loading	**0.167**	**0.071**	**0.019**	**0.272**
Cortical BMD (mg Ha/cm^3)	969.24	44.06	**Age, Height**	**0.119**		**0.013**	
			+ Body Mass	0.147	0.028	0.142	0.207
			+Grip Strength	**0.289**	**0.170**	**<0.001**	**-0.464**
			+Adolescent Loading	0.120	0.001	0.749	-0.037
			+Adult Loading	0.139	0.020	0.215	-0.144
Total Area (mm^2)	274.23	49.23	**Age, Height**	**0.316**		**<0.001**	
			+ Body Mass	0.321	0.005	0.493	0.086
			+Grip Strength	**0.494**	**0.179**	**<0.001**	**0.477**
			+Adolescent Loading	0.316	<0.001	0.945	-0.007
			+Adult Loading	0.325	0.009	0.354	0.095
Trabecular Number (1/mm)	2.00	0.26	**Age, Height**	**0.174**		**0.001**	
			+ Body Mass	**0.249**	**0.076**	**0.011**	**0.342**
			+Grip Strength	0.178	0.004	0.564	0.072
			+Adolescent Loading	0.195	0.022	0.179	0.148
			+Adult Loading	0.198	0.024	0.158	0.158
Cortical Thickness (mm)	0.77	0.15	Age, Height	0.047		0.189	
			+ Body Mass	0.049	0.002	0.738	0.049
			+Grip Strength	0.060	0.013	0.337	-0.128
			+Adolescent Loading	0.072	0.025	0.178	-0.159
			+Adult Loading	0.048	0.001	0.846	0.024
Cortical Porosity (%)	1.20	0.67	Age, Height	0.050		0.169	
			+ Body Mass	0.091	0.041	0.084	-0.252
			+Grip Strength	**0.106**	**0.056**	**0.043**	**0.267**
			+Adolescent Loading	0.050	<0.001	0.946	0.008
			+Adult Loading	**0.133**	**0.083**	**0.013**	**0.294**

R^2 Total variance explained by the model, ΔR^2 Additional variance explained by predictor, *p* significance of F-value change, *Beta* Standardized coefficient

Table 4: Clinical QCT and FE-derived strain values (mean±SD) and hierarchical linear regression results

Parameter	Mean	SD	Predictors	R^2	ΔR^2	p	Beta
Ultradistal Bone Volume (cm^3)	2.85	0.44	**Age, Height**	**0.320**		**<0.001**	
			+ Body Mass	0.327	0.007	0.399	0.105
			+Grip Strength	**0.514**	**0.195**	**<0.001**	**0.498**
			+Adolescent Loading	0.320	0.000	0.842	-0.020
			+Adult Loading	0.333	0.014	0.244	0.119
Ultradistal Bone Mineral Content (g)	0.83	0.12	**Age, Height**	**0.097**		**0.029**	
			+ Body Mass	0.128	0.030	0.128	0.217
			+Grip Strength	**0.220**	**0.123**	**0.002**	**0.396**
			+Adolescent Loading	0.115	0.018	0.240	-0.135
			+Adult Loading	**0.148**	**0.050**	**0.049**	**0.229**
Ultradistal BMD (g HA/cm3)	0.29	0.05	Age, Height	0.063		0.106	
			+ Body Mass	0.066	0.003	0.620	0.072
			+Grip Strength	0.073	0.010	0.404	-0.111
			+Adolescent Loading	0.070	0.007	0.471	-0.085
			+Adult Loading	0.076	0.013	0.334	0.116
Total Bone Volume (cm3)	12.63	1.52	**Age, Height**	**0.397**		**<0.001**	
			+ Body Mass	0.412	0.014	0.200	0.149
			+Grip Strength	**0.538**	**0.141**	**<0.001**	**0.423**
			+Adolescent Loading	0.397	0.000	0.953	-0.006
			+Adult Loading	0.415	0.018	0.158	0.135
Total Bone Mineral Content (g)	5.03	0.62	**Age, Height**	**0.209**		**<0.001**	
			+ Body Mass	**0.263**	**0.053**	**0.030**	**0.287**
			+Grip Strength	**0.372**	**0.163**	**<0.001**	**0.455**
			+Adolescent Loading	0.211	0.002	0.673	0.673
			+Adult Loading	**0.270**	**0.061**	**0.020**	**0.252**
Total BMD (g HA/cm3)	0.40	0.03	Age, Height	0.062		0.111	
			+ Body Mass	0.080	0.018	0.253	0.167
			+Grip Strength	0.065	0.003	0.630	0.064
			+Adolescent Loading	0.063	0.002	0.724	-0.042
			+Adult Loading	0.095	0.033	0.120	0.185
Mean Energy Eqiv. Strain (με)	534.69	151.27	Age, Height	<0.001		0.987	
			+ Body Mass	**0.101**	**0.100**	**0.008**	**-0.394**
			+Grip Strength	0.010	0.010	0.421	-0.110
			+Adolescent Loading	0.006	0.006	0.523	0.078
			+Adult Loading	0.033	0.033	0.134	-0.184

R^2 Total variance explained by the model, ΔR^2 Additional variance explained by predictor, *p* significance of F-value change, *Beta* Standardized coefficient