Muscle Density and Bone Quality of the Distal Lower Extremity Among Individuals with Chronic Spinal Cord Injury

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Background: Understanding the related fates of muscle density and bone quality after chronic spinal cord injury (SCI) is an important initial step in determining endocrine-metabolic risk. **Objective:** To examine the associations between muscle density and indices of bone quality at the distal lower extremity of adults with chronic SCI. **Methods:** A secondary data analysis was conducted in 70 adults with chronic SCI (C2-T12; American Spinal Injury Association Impairment Scale [AIS] A-D; ≥2 years post injury). Muscle density and cross-sectional area (CSA) and bone quality indices (trabecular bone mineral density [TbBMD] at the distal tibia [4% site] and cortical thickness [CtTh], cortical area [CtAr], cortical BMD [CtBMD], and polar moment of inertia [PMI] at the tibial shaft [66% site]) were measured using peripheral quantitative computed tomography. Calf lower extremity motor score (cLEMS) was used as a clinical measure of muscle function. Multivariable linear regression analyses were performed to determine the strength of the muscle-bone associations after adjusting for confounding variables (sex, impairment severity [AIS A/B vs AIS C/D], duration of injury, and wheelchair use). **Results:** Muscle density was positively associated with TbBMD ($b = 0.85$ [0.04, 1.66]), CtTh ($b = 0.02$ [0.001, 0.034]), and CtBMD ($b = 1.70$ [0.71, 2.69]) ($P < .05$). Muscle CSA was most strongly associated with CtAr ($b = 2.50$ [0.12, 4.88]) and PMI ($b = 731.8$ [161.7, 1301.9]) ($P < .05$), whereas cLEMS was most strongly associated with TbBMD ($b = 7.69$ [4.63, 10.76]) ($P < .001$). **Conclusion:** Muscle density and function were most strongly associated with TbBMD at the distal tibia in adults with chronic SCI, whereas muscle size was most strongly associated with bone size and geometry at the tibial shaft. **Key words:** *bone mineral density, bone quality, muscle density, muscle size, osteoporosis, peripheral quantitative computed tomography, spinal cord injury*

Spinal cord injury (SCI) is associated with sublesional muscle atrophy,[1-3] changes in muscle fiber type,[4,5] reductions in hip and knee region bone mineral density (BMD),[6-8] and increased central and regional adiposity after injury.[9,10] Adverse changes in muscle and bone health in individuals with SCI contribute to an increased risk of osteoporosis,[11-13] fragility fractures,[14] and endocrine-metabolic disease (eg, diabetes, dyslipidemia, heart disease).[15-17] Cross-sectional studies have shown a higher prevalence of lower extremity fragility fractures among individuals with SCI ranging from 1% to 34%.[18-20] Fragility fractures are associated with negative health and functional outcomes, including an increased risk of morbidity and hospitalization,[21,22] mobility limitations,[23] and a reduced quality of life.[24] Notably, individuals with SCI have a normal life expectancy, yet fracture rates increase annually from 1% per year in the first year to 4.6% per year in individuals greater than 20 years post injury.[25,26]

Muscle and bone are thought to function as a muscle-bone unit, wherein muscle contractions impose loading forces on bone that produce changes in bone geometry and structure.[27,28] A growing body of evidence has shown that individuals with SCI (predominantly those with motor complete injury) exhibit similar patterns of decline in muscle cross-sectional area (CSA) and BMD in the acute and subacute stages following injury.[4,11,29] Prospective studies have exhibited a decrease in BMD of 1.1% to 47% per year[6,7,30] and up to 73% in the 2 to 7 years following SCI.[8,14,31,32] Decreases in muscle CSA

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have been well-documented following SCI, with greater disuse atrophy observed after complete SCI versus incomplete SCI, presumably due to the absence of voluntary muscle contractions and associated mobility limitations.[1,2,16] Muscle quality is also compromised early after SCI, resulting in sublesional accumulation of adipose tissue in the chronic stage of injury[3,33,34]; the exact time course of this event has been poorly elucidated to date. Adipose tissue deposition within and between skeletal muscle is linked to an increase in noncontractile muscle tissue and a reduction in muscle force-generating capacity on bone.[35,36] Skeletal muscle fat infiltration is up to 4 times more likely to occur in individuals with SCI,[1,16,37] contributing to metabolic complications (eg, glucose intolerance),[16] reduced muscle strength and function,[38] and mobility limitations[3] – all factors that may be associated with a deterioration in bone quality after SCI.

The association between lean tissue mass and bone size (eg, BMD and bone mineral content) in individuals with SCI has been well-established using dual energy x-ray absorptiometry (DXA).[9,10,29,34] However, DXA is unable to measure true volumetric BMD (vBMD), bone geometry, and bone structure. Peripheral quantitative computed tomography (pQCT) is an imaging technique that improves our capacity to measure indices of bone quality and muscle density and CSA at fracture-prone sites (eg, tibia).[3,39] Recent evidence from cross-sectional pQCT studies has shown that muscle CSA and calf lower extremity motor score (cLEMS) were associated with indices of bone quality at the tibia in individuals with SCI.[13,40] However, neither study measured muscle density (a surrogate of fatty infiltration when evaluating the functional muscle-bone unit). Fatty infiltration of muscle is common after SCI[1,16,37] and may affect muscle function or the muscle-bone unit, but the association between muscle density and bone quality indices at the tibia in individuals with chronic SCI is unclear. Muscle density measured using pQCT may be an acceptable surrogate of muscle quality when it is difficult to assess muscle strength due to paralysis.[3,39] Additionally, investigating which muscle outcome (muscle density, CSA, cLEMS) is most strongly associated with vBMD and bone structure may inform modifiable targets for improving bone quality and reducing fracture risk after chronic SCI.

The primary objective of this secondary analysis was to examine the associations between pQCT-derived calf muscle density and trabecular vBMD at the tibia among adults with chronic SCI. The secondary objective was to examine the associations between calf muscle density, CSA, and function and tibial vBMD, cortical CSA and thickness, and polar moment of inertia (PMI). First, we hypothesize that calf muscle density will be a positive correlate of trabecular and cortical vBMD, cortical CSA and thickness, and PMI at the tibia in individuals with chronic SCI. Second, we hypothesize that of the key muscle variables (cLEMS, CSA and density), calf muscle density and cLEMS will be most strongly associated with trabecular vBMD, whereas calf muscle CSA will be most strongly associated with cortical CSA and PMI.

Materials and Methods

Study design and data collection

A secondary analysis of baseline data from a prospective, observational cohort study was conducted.[41] The prospective, observational cohort study was designed to examine a number of primary and secondary research questions related to musculoskeletal health in individuals with chronic SCI. Seventy participants (50 men and 20 women) with SCI (C2-T12, American Spinal Injury Association Impairment Scale [AIS] A-D) were included. Indices of bone quality and muscle density and CSA were determined using pQCT scans of the distal lower extremity (4% and 66% tibia length, respectively). Past and current medical history, demographic characteristics, lifestyle, and impairment data were obtained via participant interview and chart abstraction. Neurological level of injury and AIS classification of participants were determined by a physiatrist using the International Standards for Neurologic Classification of SCI. Duration of injury (years) was calculated as the date of injury minus the date of the baseline assessment. To isolate the effect of voluntary muscle activation on muscle status, the cLEMS of the scanned limb (ankle dorsiflexors, long toe extensors, and plantar

flexors) was used in the analyses. Supine height (cm), body mass (kg), and waist circumference (cm) were measured according to SCI-specific anthropometric measurement protocols previously published.[41,42] The proportion of participants who met the body mass index (BMI) (≥22 kg/m^2) and waist circumference (≥94 cm) criteria for SCI-specific definitions of obesity were determined.[43,44] Mobility status was dichotomously classified as using or not using a wheelchair for community mobility. History of tobacco use and co-morbidities were assessed via a subset of questions from the Canadian Multicentre Osteoporosis Study medical history questionnaire.[45] The CAGE questionnaire was used to determine alcohol use.[46]

Participants

Eligible participants were 18 years of age or older, had a traumatic SCI (C2-T12, AIS A-D), were able to provide informed consent, and were able to understand instructions in English. Participants were at least 2 years post injury prior to enrollment. Exclusion criteria were (a) current or prior conditions other than paralysis known to adversely influence bone metabolism, including metabolic disorders, chronic alcoholism, oral glucocorticoids use ≥7.5 mg/day for 3 months or longer, malignancy, and known liver, kidney, or intestinal disease; (b) contraindications to pQCT testing, including bilateral lower extremity metal implants or severe hip and knee flexion contractures; and (c) women who were pregnant or planning to become pregnant. Bisphosphonate exposure was recorded but was not an exclusion criterion. Recruitment strategies were described in a previous publication.[41] Written informed consent was obtained from all participants prior to the conduct of formal assessments to determine study eligibility. The current study was approved by the University Health Network and University of Waterloo research ethics boards.

Acquisition and analysis of indices of bone quality using pQCT

Bone quality and muscle density and CSA variables were measured using a pQCT scanner (XCT-2000; Stratec Medizintechnik, Germany), which acquired a transaxial image from 145 projection scans by a narrow fan beam emitted from an x-ray tube. The right tibia was scanned, except in participants with severe lower spasticity or contractures or those with a calf girth that exceeded the gantry opening or those with hardware or prior trauma in the region of interest. Where feasible, the left tibia was scanned as an alternate. Our protocol for pQCT acquisition and analysis has been described in a previous publication.[41] Single 2.5-mm slices were completed at distal tibia (4% tibia length) and proximal one-third tibia (66% tibia length). A voxel size of 0.2 mm was used at the 4% site and 0.5 mm was used at the 66% site. pQCT scans from 5 participants were not obtained because of the following reasons: missed appointment ($n = 2$), died after study enrollment but before pQCT scan acquisition ($n = 1$), or had severe lower extremity spasticity or a calf circumference that exceeded the size of the gantry opening ($n = 2$).

Trabecular vBMD (mg/cm^3) was determined at the 4% tibia site. Cortical vBMD (mg/cm^3), cortical thickness (mm), cortical CSA (mm^2), and PMI (mm^4) were determined at the 66% tibia sites. These measurement sites are consistent with standard protocols for measuring vBMD and bone structure using pQCT. Trabecular vBMD at the distal tibia was chosen as the primary outcome because accelerated bone loss and fractures often occur at skeletal sites with a higher proportion of trabecular bone.[14] Precision for this technique has been reported in individuals with and without SCI.[47] The precision (in root mean square coefficient of variation) was 2% or less for all BMD and geometric variables.[47] Analyses of the pQCT scans were performed using the manufacturer's software (Stratec XCT-2000, version 6.00) that applied an iterative contour detection algorithm.[48]

Muscle density (mg/cm^3) and CSA (cm^2) were calculated from pQCT scans at the 66% site of the tibial shaft.[49,50] This site was chosen because it is the region of the calf with the largest muscle CSA and circumference.[50] Images have a slice width of 2.5 mm and voxel size of 0.5 mm. Tissue segmentation and the calculation of muscle density and CSA were performed using SliceOmatic software version 4.3 for PC (SliceOMatic; Tomovision, Montreal, Canada).

Manual watershed segmentation of muscle from bone was performed using SliceOmatic V4.3 (Tomovision, Montreal, Canada).[3,39] The analysis protocol to determine muscle density and CSA and precision for this technique was previously reported.[39] Muscle CSA was adjusted for tibia length in meters (cm^2m) in all analyses to provide a more relevant surrogate measure of muscle force-generating potential on bone and correct for differences in muscle length among participants.[13,50]

Statistical analyses

Descriptive analyses of demographic and clinical characteristics, indices of bone quality, and muscle outcomes were expressed as mean ± standard deviation (*SD*) for continuous variables and number (%) for categorical variables. Secondary analyses using baseline data from this cohort study have been previously published.[3,13,41,51] Independent samples *t* tests were performed to compare bone quality and muscle outcomes in participants with chronic SCI grouped by sex (men vs women) and AIS classification (AIS A/B vs AIS C/D). Pearson's correlations were performed to determine the strength of the associations between muscle density and indices of bone quality. Bivariate and multivariable linear regression analyses were performed to determine potential correlates of bone quality outcomes. We examined the following potential confounding variables known to affect BMD and bone microarchitecture in individuals with SCI: AIS classification (motor complete or incomplete; AIS A/B vs AIS C/D), duration of injury (years), sex (male/female), and community wheelchair use (yes/no). We previously demonstrated that history of bisphosphonate use was not associated with bone quality indices in this cohort of 70 individuals with chronic SCI.[41] Therefore, we did not control for history of bisphosphonate use in our models. Separate models examining the associations between muscle density, CSA, and function and bone quality indices (trabecular vBMD, cortical vBMD, CSA, and thickness, and PMI) were created for the entire sample. Muscle outcomes (muscle density, CSA, and cLEMS) and confounding variables found to be significant at $P < .20$ in bivariate regression analyses were entered into a multivariable linear regression model. Sex and duration of injury were controlled for in all models. AIS classification was not entered in the same models as cLEMS due to risk of collinearity. A minimum of 10 observations for each independent variable were included to avoid over-fitting the models.[52] The estimate of the coefficients, 95% confidence intervals, *P* values, and R^2 values were reported for the bivariate and multivariable analyses. Regression diagnostic statistics were conducted to assess bias in the models and check for influential cases using standardized residuals and DFBeta values.[53] Multicollinearity was assessed using variance inflation factor. The *P* values were reported to 3 decimal places. Analyses were performed using IBM SPSS version 22.0 (IBM, Armonk, NY).

Results

Description of study participants

The study sample included 70 adults with chronic SCI (50 men and 20 women). The mean ± *SD* age of participants was 48.8 ± 11.5 years with a mean ± *SD* duration of injury of 15.5 ± 10 years and LEMS of 11.0 ± 15.7. Participants had a mean ± *SD* height of 174.5 ± 10.3 cm and weight of 80.1 ± 18.4 kg with a BMI of 26.3 ± 5.6 kg/m^2 and waist circumference of 97.4 ± 14.8 cm. Thirty-two (49.2%) and 35 (out of 63; 55.6%) participants met the criteria for BMI and waist circumference SCI-specific definitions of obesity, respectively. Forty-five (64.3%) and 25 (35.7%) participants met impairment criteria for AIS A/B and AIS C/D, respectively. Fifty-one (72.9%) participants had been exposed to bisphosphonate therapy, defined as current or past bisphosphonate use for at least 6 months. Fifty-seven (81.4%) and 61 (87.1%) participants reported a history of calcium and vitamin D supplementation, respectively. Forty (57.0%) and 43 (61.4%) participants had a history of smoking and alcohol use, respectively. Nineteen (27.1%) participants had a history of fragility or low-trauma fracture. Descriptive characteristics of the study participants are shown in **Table 1.** **Table 2** summarizes the mean ± *SD* for indices of bone quality and muscle density, CSA, and function in participants with SCI grouped by sex (men vs women) and impairment severity (AIS A/B vs AIS C/D).

Table 1. Descriptive characteristics of study participants with chronic SCI ($n = 70$)

Characteristics	All participants	Range
Demographic characteristics		
Sex, *n* (%)		
Male	50 (71.4)	—
Female	20 (28.6)	—
Age, mean (*SD*), years	48.8 (11.5)	24-77
Height, mean (*SD*), cm	174.5 (10.3)	140-193
Weight, mean (*SD*), kg	80.1 (18.4)	48.1-137.4
BMI, mean (*SD*), kg/m^2	26.3 (5.6)	16.6-41.6
Waist circumference[a], mean (*SD*), cm	97.4 (14.8)	69.9-148.0
Impairment and mobility characteristics		
Time post injury, mean (*SD*), years	15.5 (10.0)	2-41
Age at injury, mean (*SD*), years	33.7 (14.7)	14-66
Impairment group, *n* (%)		
Paraplegia AIS A/B	23 (32.9%)	—
Paraplegia AIS C/D	11 (15.7%)	—
Tetraplegia AIS A/B	22 (31.4%)	—
Tetraplegia AIS C/D	14 (20.0%)	—
LEMS, mean (*SD*)	11.0 (15.7)	0-48
Wheelchair use, *n* (%)	54 (83.1)	—
Bone health and lifestyle characteristics		
History of fragility fracture, *n* (%)	19 (27.1)	—
Bisphosphonate exposure[b], *n* (%)	51 (72.9)	—
Past calcium supplement use[c], *n* (%)	57 (81.4)	—
Past vitamin D supplement use, *n* (%)	61 (87.1)	—
History of smoking, *n* (%)	40 (57.0)	—
History of alcohol use[c], *n* (%)	43 (61.4)	—

Note: AIS = American Spinal Injury Association Impairment Scale; BMI = body mass index; LEMS = lower extremity motor score; SCI = spinal cord injury; *SD* = standard deviation.

[a]Waist circumference ($n = 68$).

[b]Bisphosphonate exposure is defined as current use or past history of bisphosphonate medications (including alendronate [Fosamax/Fosavance], risedronate [Actonel/Actonel Delayed Release], zoledronate [Aclasta], etidronate [Didrocal], and pamidronate [Aredia]).

[c]Past calcium supplement use was unknown for one participant. Three participants did not respond regarding history of alcohol use.

Bivariate and multivariable regression analyses of muscle-bone associations

Moderately strong positive associations were observed between muscle density and trabecular vBMD ($r = 0.473$, $P < .001$), cortical vBMD ($r = 0.415$, $P = .001$), and cortical thickness ($r = 0.421$, $P < .001$) (**Figure 1**), whereas weak-to-moderate positive associations were observed between muscle density and cortical CSA ($r = 0.367$, $P = .003$) and PMI ($r = 0.245$, $P = .049$).

Results of the bivariate and multivariable regression analyses of muscle-bone associations are presented in **Table 3**. Muscle density was positively associated with trabecular vBMD ($b = 1.94$ [1.03, 2.85]), cortical vBMD ($b = 1.62$ [0.72, 2.51]), cortical thickness ($b = 0.03$ [0.01, 0.05]), cortical CSA ($b = 2.12$ [0.77, 3.47]), and PMI ($b = 342.6$ [1.30, 683.9]) ($P < .05$). Muscle CSA and cLEMS were positively associated with all indices of bone quality (trabecular vBMD, cortical

Table 2. Indices of bone quality and muscle density, CSA, and function in participants with chronic SCI grouped by sex and impairment severity ($n = 65$[a])

Anatomic site	Variable (units)	All participants with chronic SCI ($n = 65$[a])	Male participants with chronic SCI ($n = 45$)	Female participants with chronic SCI ($n = 20$)	Participants with AIS A/B ($n = 40$)	Participants with AIS C/D ($n = 25$)
4% tibia	Trabecular vBMD (mg/cm^3)	130.1 (54.5)	137.2 (55.3)	114.1 (50.3)	101.0 (38.7)	176.7 (42.6)[d]
66% tibia	Cortical vBMD (mg/cm^3)	1,082.1 (51.8)	1,179.8 (53.7)	1,137.6 (66.4)[c]	1,139.1 (73.0)	1,168.8 (46.7)
	Cortical thickness (mm)	3.21 (0.94)	3.37 (0.95)	2.84 (0.83)[c]	2.84 (0.92)	3.80 (0.65)[d]
	Cortical CSA (mm^2)	256.0 (76.7)	278.8 (74.9)	204.7 (53.4)[c]	228.5 (67.5)	300.0 (70.8)[d]
	PMI (mm^4)	45,304 (18,602)	52,188 (17,505)	29,816 (9,551)[c]	40,867 (15,750)	52,404 (20,840)[d]
	Muscle density (mg/cm^3)	53.5 (13.3)	54.2 (13.6)	51.9 (12.7)	49.4 (13.8)	60.2 (9.3)[d]
	Muscle CSA[b] (cm^{2}m)	19.9 (8.7)	22.1 (8.7)	14.9 (6.5)[c]	16.3 (6.8)	25.7 (8.3)[d]
	cLEMS	2.8 (4.7)	3.09 (4.97)	2.05 (4.20)	0 (0)	7.2 (5.2)[d]

Note: Values given as mean (*SD*). SCI = spinal cord injury; AIS = American Spinal Injury Association Impairment Scale; cLEMS = calf lower extremity motor score; CSA = cross-sectional area; pQCT = peripheral quantitative computed tomography; *SD* = standard deviation; vBMD = volumetric bone mineral density.

[a]Five participants did not complete pQCT scans.

[b]Muscle CSA (cm^2) was multiplied by tibia length (m).

[c]Significant difference between male and female participants ($P < .05$).

[d]Significant difference between AIS A/B and AIS C/D groups ($P < .05$).

thickness, cortical CSA, and PMI) ($P \leq .001$), except for cortical vBMD ($P > .05$). After adjusting for sex, duration of injury, AIS classification, and wheelchair use, muscle density remained a significant positive correlate of trabecular vBMD ($b = 0.85$ [0.04, 1.66]), cortical thickness ($b = 0.02$ [0.001, 0.034]), and cortical vBMD ($b = 1.70$ [0.71, 2.69]) ($P < .05$), whereas muscle CSA was a significant positive correlate of cortical CSA ($b = 2.50$ [0.12, 4.88]) and PMI ($b = 731.8$ [161.7, 1301.9]) ($P < .05$). Muscle CSA was not significantly associated with trabecular vBMD and cortical thickness after adjusting for confounding variables ($P > .05$). After adjusting for sex, duration of injury, and wheelchair use, cLEMS remained a significant positive correlate of trabecular vBMD ($b = 7.69$ [4.63, 10.76]), cortical CSA ($b = 5.85$ [0.27, 11.43]), and cortical thickness ($b = 0.10$ [0.03, 0.16]) ($P < .05$).

Discussion

Summary of findings

Consistent with our hypothesis, we observed moderately strong positive associations between calf muscle density and trabecular and cortical vBMD and cortical thickness at the tibia in adults with chronic SCI. However, we found weak to nonexistent positive associations between calf muscle density and cortical CSA and PMI at the tibial shaft after adjusting for relevant confounding variables. Alternatively, we demonstrated that calf muscle CSA was a stronger positive correlate of cortical CSA and PMI at the tibial shaft in individuals with chronic SCI than calf muscle density. As such, our findings suggest calf muscle density may exert a greater influence on tibial vBMD and cortical thickness in individuals with chronic SCI than calf muscle CSA. In addition, cLEMS was most strongly associated with trabecular vBMD at the distal tibia consistent with previous findings in individuals with chronic SCI.[13] The current study contributes to a growing body of research on the muscle-bone unit theory under conditions of disuse and neurological impairment and provides data on the contributions of muscle density to bone quality indices at the tibia.[13,40] Thus, changes in tibial vBMD and cortical thickness over time following SCI may be more closely linked to calf muscle density and function, whereas changes in tibial bone size and geometry following SCI may be more closely linked to calf muscle size.

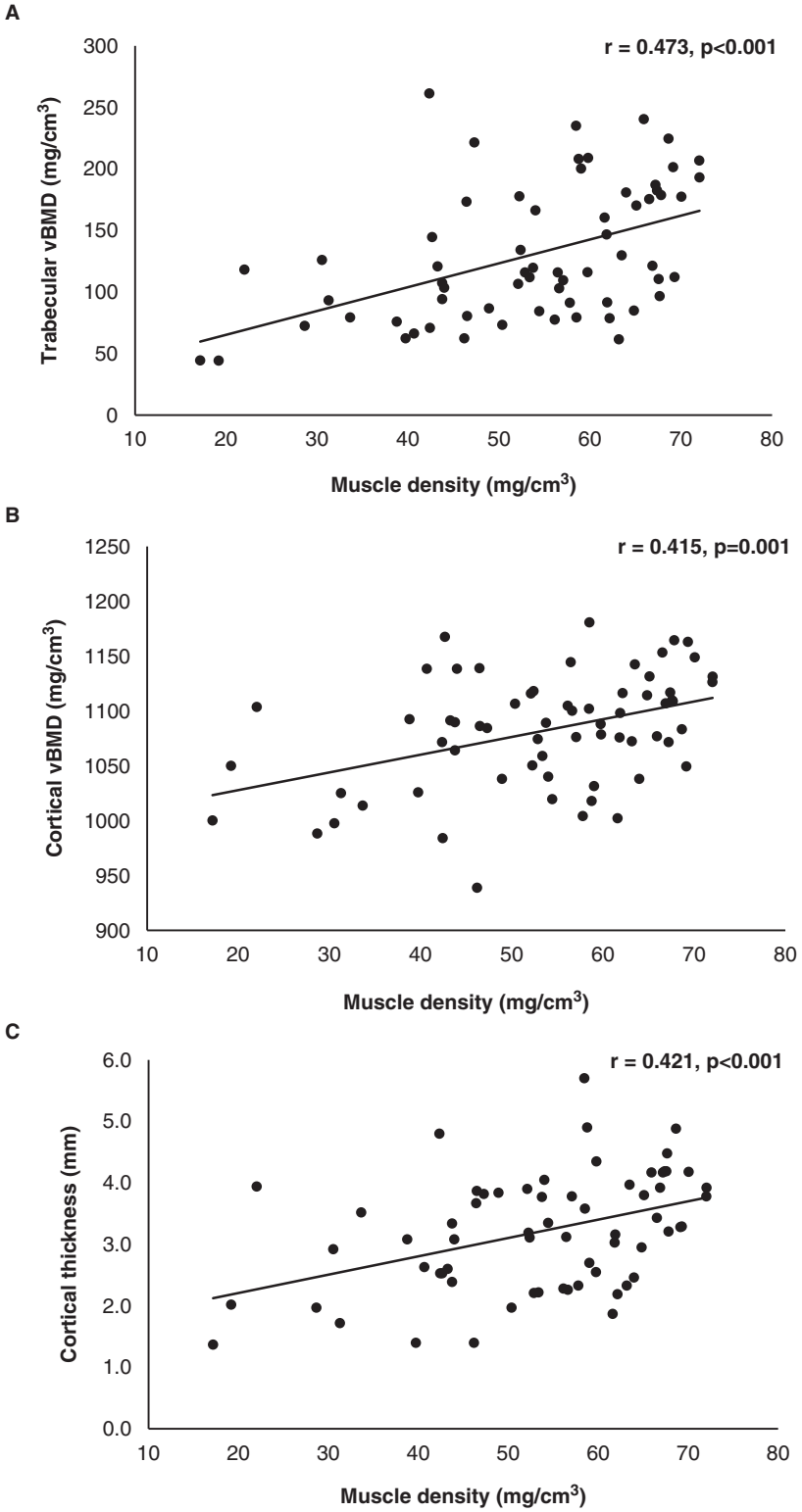

Figure 1. Pearson's correlations were performed to determine the strength of the associations between muscle density and indices of bone quality in our participants with chronic spinal cord injury. Moderately strong associations were observed between muscle density and trabecular volumetric bone mineral density (vBMD) ($r = 0.473$, $P < .001$), cortical vBMD ($r = 0.415$, $P < .001$), and cortical thickness ($r = 0.421$, $P < .001$).

Table 3. Bivariate and multivariable regression analyses of muscle-bone associations in participants with chronic SCI ($n = 65$[a])

	Unadjusted muscle-bone associations			Muscle-bone associations adjusted for confounding variables		
	***b* (95% CI)**	***P* value**	**R^2**	***b* (95% CI)**	***P* value**	**R^2**
Trabecular vBMD, mg/cm³						
Muscle density	1.94 (1.03, 2.85)	**<.001**	0.223	0.85 (0.04, 1.66)[c]	**.040**	0.544
Muscle CSA[b]	3.46 (2.14, 4.78)	**<.001**	0.304	1.29 (-0.28, 2.86)[c]	.105	0.532
cLEMS	7.80 (5.67, 9.93)	**<.001**	0.459	7.69 (4.63, 10.76)[d]	**<.001**	0.503
Cortical vBMD, mg/cm³						
Muscle density	1.62 (0.72, 2.51)	**.001**	0.172	1.70 (0.71, 2.69)[c]	**.001**	0.248
Muscle CSA[b]	0.79 (-0.70, 2.28)	.294	0.017	0.88 (-1.17, 2.94)[c]	.394	0.109
cLEMS	2.40 (-0.29, 5.08)	.079	0.048	2.77 (-1.14, 6.68)[d]	.161	0.104
Cortical thickness, mm						
Muscle density	0.03 (0.01, 0.05)	**<.001**	0.177	0.02 (0.001, 0.034)[c]	**.042**	0.370
Muscle CSA[b]	0.05 (0.03, 0.07)	**<.001**	0.210	0.02 (-0.01, 0.05)[c]	.195	0.343
cLEMS	0.10 (0.05, 0.14)	**<.001**	0.233	0.10 (0.03, 0.16)[d]	**.003**	0.313
Cortical CSA, mm²						
Muscle density	2.12 (0.77, 3.47)	**.003**	0.135	0.98 (-0.29, 2.25)[c]	.127	0.438
Muscle CSA[b]	4.94 (3.10, 6.78)	**<.001**	0.313	2.50 (0.12, 4.88)[c]	**.040**	0.456
cLEMS	8.12 (4.59, 11.65)	**<.001**	0.251	5.85 (0.27, 11.43)[d]	**.040**	0.456
PMI, mm⁴						
Muscle density	342.6 (1.30, 683.9)	**.049**	0.060	107.7 (-206.1, 421.47)[c]	.495	0.414
Muscle CSA[b]	1205.8 (760.2, 1651.3)	**<.001**	0.317	731.8 (161.7, 1301.9)[c]	**.013**	0.469
cLEMS	1611.6 (709.8, 2513.4)	**.001**	0.168	1412.0 (311.3, 2512.6)[d]	**.013**	0.449

Note: AIS = American Spinal Injury Association Impairment Scale (AIS); cLEMS = calf lower extremity motor score; CSA = cross-sectional area; SCI = spinal cord injury; vBMD = volumetric bone mineral density. Boldface *P* values indicate statistical significance ($P < .05$).

[a]Five participants did not complete pQCT scans.

[b]Muscle CSA (cm^2) was multiplied by tibia length (m).

[c]Adjusted model for sex, duration of injury, AIS classification (AIS A/B vs AIS C/D), and wheelchair use.

[d]Adjusted model for sex, duration of injury, and wheelchair use.

Association between muscle density and bone quality

Substantial evidence links elevated muscle adiposity in the leg and impaired metabolic status (eg, decreased insulin sensitivity, muscle mitochondrial dysfunction) in individuals with SCI.[1,16] However, the associations between calf muscle density (a surrogate of fatty infiltration when evaluating the muscle-bone unit) and lower extremity bone quality under conditions of disuse and neurological impairment are not as well understood. In a cross-sectional study of stroke survivors with subacute lower limb hemiparesis, MacIntyre et al[54] observed a moderately strong association between muscle density and cortical vBMD at the tibia. Similar to stroke, sublesional adiposity following SCI is attributed to persistent mobility limitation and neuromuscular impairment in the paralyzed limbs. We demonstrated that muscle density was a moderately strong positive correlate of indices of bone quality at the tibia, including trabecular and cortical vBMD, and cortical thickness, after adjusting for confounding variables known to influence vBMD and bone microarchitecture. Our findings suggest that lower muscle density or fatty infiltration at the calf is linked to lower vBMD at the distal lower extremity among individuals with chronic SCI. However, changes in muscle density in larger muscle groups (eg, quadriceps) may assert different or more potent effects on lower extremity bone quality (eg, femur) following SCI.[55]

Associations between muscle CSA and function and bone quality

A unique finding of the current study is that muscle CSA was a stronger correlate of cortical CSA and PMI than muscle density. Conflicting evidence was recently published[40] suggesting that the association between muscle and bone outcomes was weak in adults with complete SCI paraplegia due to the absence of voluntary muscle contraction. Alternatively, Totosy de Zepetnek et al[13] demonstrated that associations between muscle CSA and function and indices of bone quality were significant among individuals with chronic SCI and remained significant after adjusting for duration of injury. Further, Totosy de Zepetnek et al[13] observed a stronger association between cLEMS and trabecular vBMD at the distal tibia compared to muscle CSA, supporting the importance of muscle function as a possible target for intervention after injury. Similarly, we found moderately strong positive associations between cLEMS and trabecular vBMD, cortical thickness, and cortical CSA. Our findings suggest that muscle size and function may represent therapeutic targets for preventing bone loss and attenuating fracture risk following SCI, in addition to being related to glucose metabolism and muscle strength.[2,11,13,55]

Study strengths and limitations

Strengths of this study include its diverse, large cohort of adult men and women with chronic motor complete and incomplete SCI and the multivariable regression analysis of muscle-bone associations controlled for relevant confounders. However, the cohort with chronic SCI included a large proportion of individuals who were 15 years or more post injury. A broader distribution of duration of injury might elucidate different muscle-bone relationships of varying strength and significance over time in individuals with SCI. Further, the findings regarding muscle-bone associations may be influenced by the active or remote exposure to bisphosphonate therapy in our participants with chronic SCI. A large proportion of participants (>70%) were current or past bisphosphonate users, primarily to treat low BMD by preventing declines in bone mass or attenuating bone resorption. Also, the nature of the cross-sectional design limits our ability to make causal inferences from the data regarding the muscle-bone unit theory in response to SCI.

Resolution limitations with pQCT analysis affected our ability to differentiate between soft and adipose or noncontractile tissues contained within the fascia border of the calf muscle group

in participants with extreme muscle atrophy. Our protocol may have overestimated calf muscle CSA by failing to account for the contributions of intra- and intermuscular adipose stores.[1] Magnetic resonance imaging is a more sensitive technique for measuring fatty infiltration of skeletal muscle across the entire length of the leg,[33] yet recent evidence demonstrates that pQCT-derived muscle density of the leg may represent a valid proxy for intra- and intermuscular fat.[39,56] There were several technical challenges related to pQCT acquisition in the cohort of individuals with SCI that precluded accurate analysis, including lower leg edema, spasticity, and calf girths that exceeded the size of the pQCT gantry. Also, we did not measure and were unable to control for metabolic biomarkers that may explain greater variability in the association between muscle density and bone quality outcomes, particularly in individuals with SCI.

Conclusions and future directions

Our findings demonstrate that calf muscle density and cLEMS were more strongly associated with trabecular vBMD at the tibia in adults with chronic SCI than calf muscle size, evolving our understanding of the muscle-bone unit theory as it relates to SCI. Alternatively, muscle size represented an important determinant of tibial bone size and geometry in individuals with chronic SCI. Because changes in muscle precede changes in bone in the subacute phases of injury, muscle density, size, and function may represent modifiable targets for rehabilitation interventions with the goal of improving bone quality and reducing fracture risk.[57] Future research should examine the effect of therapeutic interventions (eg, electrical stimulation, vibration, resistance training) targeting muscle density, size, and function on longitudinal changes in tibial vBMD and bone microarchitecture and fracture risk after chronic SCI.

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