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Adiponectin is associated with bone strength and fracture history in paralyzed men with spinal cord injury

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Abstract

Summary—We explored the association between adiponectin levels and bone strength in paralyzed men with spinal cord injury. We found that bone strength was inversely associated with circulating adiponectin levels. Thus, strength estimates and adiponectin levels may improve fracture risk prediction and detection of response to osteogenic therapies following spinal cord injury.

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Purpose—Previous research has demonstrated an inverse relationship between circulating adiponectin and bone mineral density, suggesting that adiponectin may be used as a biomarker for bone health. However, this relationship may reflect indirect effects on bone metabolism via adipose-mediated mechanical pathways rather than the direct effects of adipokines on bone metabolism. Thus, we explored the association between circulating adiponectin levels and bone strength in 27 men with spinal cord injury.

Methods—Plasma adiponectin levels were quantified by ELISA assay. Axial stiffness and maximal load to fracture of the distal femur were quantified via finite element analysis using reconstructed 3D models of volumetric CT scans. We also collected information on timing, location, and cause of previous fractures.

Results—Axial stiffness and maximal load were inversely associated with circulating adiponectin levels ($R^2=0.44$, $p=0.01$; $R^2=0.58$, $p=0.05$) after adjusting for injury duration and lower extremity lean mass. In individuals with post-SCI osteoporotic fractures, distal femur stiffness ($p=0.01$) and maximal load ($p=0.005$) were lower, and adiponectin was higher ($p=0.04$) than those with no fracture history.

Conclusions—Based on these findings, strength estimates may improve fracture risk prediction and detection of response to osteogenic therapies following spinal cord injury. Furthermore, our findings suggest that circulating adiponectin may indeed be a feasible biomarker for bone health and osteoporotic fracture risk in paralyzed individuals with spinal cord injury.

Keywords

Adiponectin; Biomarker; Finite element analysis; Fracture; Rehabilitation medicine; Spinal cord injury

Introduction

A reciprocal cross-regulation links bone and adipose metabolism [1]. Bone participates in adipose metabolism by releasing osteocalcin into the circulation. Osteocalcin reduces fat mass by increasing the release of the adipokine and adiponectin [2, 3]. At the same time, adipose tissue serves a dual role in bone metabolism: as a contributor to total body weight, thus a source of mechanical loading, and as an endocrine organ that produces adipokines, aromatase, and potentially other factors that affect bone cell activities.

Animal models have shown that limb unloading results in immediate osteocyte and osteoblast apoptosis [4] and increased osteoclastic bone resorption with reduced bone formation [5]. Thus, mechanical loading facilitates bone formation and suppresses bone resorption. In addition, body adiposity has a direct hormonal impact on bone metabolism. Increased adiposity is negatively associated with circulating levels of adiponectin [6], a polypeptide hormone expressed specifically and abundantly in adipose tissue in both visceral and marrow fat depots [7, 8]. Adiponectin facilitates osteoblast differentiation and bone formation directly via mitogen-activated protein kinase pathways [9], while it also enhances osteoclast formation and bone resorption directly via the RANKL pathway and indirectly by inhibiting osteoprotegerin production in osteoblasts [10]. Though the net outcome of these hormonal actions of adiponectin on human bone metabolism is not always clear, inferential

data suggest that adiponectin-mediated bone resorption may predominate. For example, circulating adiponectin levels appear to be inversely related to bone mineral density (BMD) in premenopausal [11, 12] and postmenopausal [13] women as well as young and elderly men [14, 15], and the risk of fracture increases with increasing serum adiponectin levels at least in men [6]. However, this reciprocal relation between bone strength and serum adiponectin level may also involve factors associated with indirect results of age-related increase in adipose tissue mass (thus, mechanical loading). Thus, this relationship may not represent direct signaling between fat and bone tissue [16].

In individuals with spinal cord injury (SCI) and subsequent paralysis, the long bones of the lower extremity adapt to mechanical unloading. Therefore, SCI represents a unique population to assess the direct relation between adiponectin levels and bone metabolism, independent of mechanical factors. In a recent study of people with SCI who use a wheelchair or walk as their primary means of mobility, we found an inverse association between serum adiponectin and BMD in wheelchair users, but not in walkers, independent of body composition [17]. This suggested that walking (thus, mechanical loading) might mitigate the effect of adiponectin on bone loss. However, bone mineral density is only one of the determinants of the bone strength (thus, fracture risk) [18]. In fact, higher bone mineral density in long bones does not always lead to stronger bones [19]. Therefore, we sought to explore the association between circulating adiponectin levels and long bone strength in individuals with chronic spinal cord injury who do not walk due to lower extremity paralysis (minimal mechanical loading).

Methods

Subjects

We studied participants with SCI who were enrolled in an exercise-based clinical trial to improve bone health. Subjects were recruited from individuals who receive care at our outpatient rehabilitation clinic or Veterans Affairs (VA) Medical Center. Participants were eligible for the parent study if they were 18 years or older, had a C4 or lower SCI [American Spinal Injury Association (ASIA) A, B, or C] with 3/5 greater biceps strength and were nonambulatory due to their injury. Subjects were excluded in the following: if they were actively being treated for epilepsy, actively using medications potentially affecting bone metabolism, including parathyroid hormone (PTH) and PTH analogs, bisphosphonates, androgenic steroids, estrogenic steroids, anti-epileptics, lithium, or oral glucocorticoid (use for more than 3 months); if they had a history of peripheral nerve compression or rotator cuff injury that limited the ability to exercise, uncontrolled diabetes, active renal disease, implanted defibrillator or pacemaker, an active grade 2 or greater pressure ulcer in a location that could be worsened with exercise; if they had an active bone fracture or lower extremity contractures; or if they were pregnant or lactating. For this study, we excluded women and those who reported bisphosphonate use within the year prior to enrollment for a total of 27 men who completed baseline testing between October 2010 and October 2013. The Institutional Review Boards approved all protocols, and all participants gave their written informed consent to participate.

Motor score

Motor level and completeness of injury were confirmed by physical exam at study entry by the study physician according to the American Spinal Injury Association Impairment Scale (AIS). Participants were classified as AIS A (sensory and motor complete, no sensory or motor function below the neurological level of injury, 18 individuals); AIS B (motor complete, preservation of sensory but no motor function below the neurological level of injury, four individuals); or AIS C (motor incomplete, sensory and motor function preserved below the neurological level, and more than half the key muscles below the neurological level are not strong enough to overcome gravity, five individuals).

Dual X-ray absorptiometry (DXA) for bone mineral density

We used a 5th generation GE Healthcare iDXA dual X-ray absorptiometry (DXA) scanner with enCore configuration version 12.3 to determine bone mineral density. Fractures are most common at the knee (distal femur or proximal tibia) after SCI. Therefore, bone density was determined at both SCI-specific (proximal tibia, distal femur) and standard (hip, radius) skeletal sites as described previously [20].

Volumetric computed tomography (CT) and finite element analysis for bone strength

The distal femur was scanned using one of two 128-slice multi-detector CT scanners (Siemens Definition Flash with imaging conducted at 120 kV, 80 mA s, $n=20$ or GE Lightspeed pro with imaging conducted at 16 120 kV, 50 mAs, $n=7$). CT slices of 180, each with a slice thickness of 1 mm and reconstructed to 0.5 mm (360 slices), were obtained, delivering a three-dimensional (3D) representation of approximately 18 cm of anatomy. All scans included a calibration phantom in the imaging field to ensure quality serial data within a participant. Reconstructed images were then transferred to a workstation for strength analysis.

A 10-mm section of the distal femur (measured from just beneath femoral condyle) was segmented from the surrounding tissue by adaptive thresholding based on expectation-maximization algorithm, followed by a region growing algorithm [21]. Segmented CT images were then reconstructed into a three-dimensional volumetric model of cortical bone with tetrahedral elements (one voxel = one element) [22]. The finite element model assumed a Young's modulus of elasticity of 17 GPa, and a Poisson ratio of 0.3 for initial material properties of cortical bone, and used the Grey-scale information from the CT scans to scale material properties for each individual element to account for differences in bone mineral content. Subsequently, femoral axial stiffness was calculated by simulating a 1 % (i.e., 0.1 mm) compression, recording the sum of the reaction forces of all elements and dividing the force, F , by the displacement, x (i.e., stiffness = F/x). Maximum axial (compressive) load was also determined from axial stiffness and cortical bone cross-sectional area. This approach of estimating bone strength is a well-established engineering method and has been used in orthopedic biomechanics research for decades [23–25].

Biochemical analyses

Plasma samples were drawn into an EDTA tube and immediately delivered to the core blood research laboratory at our facility. The samples were centrifuged for 15 min at 2,600 rpm

(1,459×g) at 4 °C and stored at −80 °C until batch analysis. All biochemical analyses were performed at the Clinical and Epidemiologic Research Laboratory, Department of Laboratory Medicine at Children's Hospital in Boston. Assays were performed in duplicate and any duplicate with >10 % CV was repeated. Total adiponectin was quantified by enzyme-linked immunosorbent assay (ELISA) assay (Alpco Diagnostics, Salem, NH) with a detection limit of 0.075 ng/ml. Total osteocalcin was measured as an indicator of bone formation by electrochemiluminescence immunoassay on a 2010 Elecsys autoanalyzer (Roche Diagnostics, Indianapolis, IN) with a detection limit of 0.50 ng/ml. C-telopeptide was measured as an indicator of bone resorption by electrochemiluminescence immunoassay on a 2010 Elecsys autoanalyzer (Roche Diagnostics) with a detection limit of 0.01 ng/ml. 25 OH vitamin D was quantified by enzyme immunoassay (Immunodiagnostic Systems Inc., Fountain Hills, AZ) with a detection limit of 2.0 ng/ml.

Variable definition

Information regarding SCI, medical history, medication use, and fracture history was obtained by a questionnaire at the time of enrollment. Age and body mass index (BMI) were considered as continuous variables. For fracture history, information was collected on timing (before SCI, at time of SCI, or after SCI), location, and cause of fracture (traumatic or osteoporotic). Digit and rib fractures were excluded. Osteoporotic fractures that occurred after SCI were considered in the analysis. Bone mineral density (g/cm²) was assessed as a continuous variable. For subjects age 50 or older, T-score was used to classify hip bone density (total hip and femoral neck) according to the World Health Organization (WHO) definitions of normal (T-score ≥ 1), osteopenia (T-score −1 and −2.5) and osteoporosis (T-score ≤ −2.5). For subjects under the age of 50, Z-score was used to classify bone density at the hip as normal (Z-score ≥ 2) or as lower than expected for age and sex (Z-score < 2). 25 OH vitamin D levels were categorized as sufficient (≥ 30 ng/ml) or deficient (<30 ng/ml).

Statistical analysis

We used univariate regression models to assess relationships between clinical variables and distal femur stiffness and maximum load and multivariate models to determine significant clinical predictors of stiffness and maximum load. For multivariate models, we used backward stepwise elimination, wherein stiffness and maximum load were used separately as the dependent variable, and all independent variables are initially included in the model. Subsequently, independent variables that do not reach statistical significance are eliminated. One might instead use a forward stepwise model, wherein independent variables are added to the model in random order until the overall predictive power cannot be further improved. It is possible that regression models constructed via both approaches may contain different predictor variables [26], especially if the sample size is small [27]. Therefore, as a sensitivity analysis for our main result, we also constructed forward stepwise models for femoral stiffness and maximal load and evaluated the agreement between this model selection procedure and the backward stepwise approach used in primary analysis, in terms of the selection of final independent variables. To compare subject characteristics, *t* tests or χ^2 tests were used as appropriate. All analyses were performed using SAS 9.2 (SAS Institute, Inc., Cary, NC).

Results

Subject characteristics

Subject characteristics are presented in Table 1. Participants were aged 40.7 ± 11.5 (SD) years (ranged from 21.1 to 63.6 years) and were 13.2 ± 11.7 (0.12 to 37.5) years post-injury. All participants used a wheelchair as their primary mode of mobility. The majority was paraplegic (70.4 %) and had motor complete SCI (81.5 %). The mean BMI was 25.5 ± 6.2 (13.8–38.9), mean total mass was 82.7 ± 21.0 kg, and mean total lean mass was 52.6 ± 10.7 kg. 56 % of participants were vitamin D-deficient (<30 ng/ml). A majority of subjects (70.4 %) had not consumed anything for at least 8 h prior to testing. Adiponectin levels did not vary significantly based on time since last meal or snack ($p=0.48$).

Relationship between bone mineral density, axial stiffness, and maximal load

Left and right femoral axial stiffness and maximal load were closely correlated ($r=0.70$ for axial stiffness and $r=0.83$ for maximal load, $p<0.0001$ for both). Therefore, we used average stiffness and maximal load values within each participant across sides for subsequent analyses. Distal femur axial stiffness was modestly but significantly correlated with baseline bone mineral density measured at the distal femur ($r=0.58$, $p=0.002$), proximal tibia ($r=0.52$, $p=0.007$), and femur neck ($r=0.40$, $p=0.04$) and tended to correlate with total hip BMD ($r=0.35$, $p=0.07$). Distal femur maximal load was also significantly correlated with baseline BMD measured at all four sites (distal femur, $r=0.83$, $p<0.0001$; proximal tibia, $r=0.76$, $p<0.0001$; femur neck, $r=0.57$, $p=0.001$; and total hip, $r=0.59$, $p=0.001$; Table 2).

Clinical factors associated with axial stiffness or maximal load at the distal femur

Univariate analysis showed that axial stiffness was negatively associated with years post-injury ($R^2=0.27$, $p=0.005$) and adiponectin ($R^2=0.32$, $p=0.002$) and positively associated with lower extremity lean mass ($R^2=0.20$, $p=0.02$) (Table 3). Similarly, maximal load was negatively associated with years post-injury ($R^2=0.29$, $p=0.004$) and adiponectin ($R^2=0.33$, $p=0.002$), while it was positively associated with lower extremity lean mass ($R^2=0.40$, $p=0.0005$) and modestly associated with BMI ($R^2=0.17$, $p=0.03$), total lean mass (kg; $R^2=0.23$, $p=0.01$), and total mass (kg; $R^2=0.12$, $p=0.08$) (Table 3). A multivariate analysis showed that adiponectin remained significantly associated with femoral axial stiffness and maximal load after adjusting for years post-injury and lower extremity lean mass in the maximal load model (Table 4). The backward stepwise and the forward stepwise procedures used in the sensitivity analysis procedures yielded exactly the same models for femoral stiffness and maximal load. We performed a sensitivity analysis using an indicator variable for those who were scanned on the Siemens Definition Flash scanner ($n=20$) and those who were scanned on the GE Lightspeed pro scanner ($n=7$). We found no significant variation in femoral stiffness or maximal load based on the CT scanner used. Similarly, the effect estimates of adiponectin for both femoral stiffness and maximal load were unchanged when adjusting for CT scanner.

Factors associated with history of post-SCI osteoporotic fracture

Six participants reported post-SCI osteoporotic fractures. There was no difference in mean age based on fracture history (44 years in the fracture group versus 40 years in the no fracture group, $p=0.41$). Those who reported an osteoporotic fracture had significantly lower bone density at all tested skeletal sites (Table 5), compared to those with no fracture history. Similarly, distal femur axial stiffness (160.59 ± 49.0 vs. 99.51 ± 56.3 MPa, $p=0.01$) and maximal load (91.11 ± 40.8 vs. 38.39 ± 14.6 kg, $p=0.005$) were lower in the fracture group compared to those with no fracture (Fig. 1). Participants with osteoporotic fractures had significantly higher adiponectin levels compared to those who did not report an osteoporotic fracture (5656.7 ± 3003.3 vs. 3802.2 ± 1380.4 ng/ml, $p=0.04$).

Discussion

We examined circulating adiponectin, an adipokine released by adipose tissue, and elements of bone strength (axial stiffness and maximal load) in 27 healthy, community-dwelling men with chronic motor complete SCI enrolled in an exercise-based clinical trial. In this study, all participants used a wheelchair due to lower extremity paralysis and the inability to ambulate. We found a significant inverse relationship between adiponectin and bone strength at the distal femur, a frequently fractured skeletal site following SCI. This relationship remained significant after accounting for injury duration and total lean mass. Moreover, we found that participants with post-SCI osteoporotic fractures had significantly higher adiponectin levels. Therefore, our results suggest that, in the absence of mechanical loading due to paralysis following SCI, adiponectin-mediated bone resorption dominates bone metabolism promoting bone loss.

Recent studies highlighted an inverse relation between circulating adiponectin level and bone mineral density, suggesting that circulating adiponectin may be used as a biomarker for bone health. However, the evidence for this relation has been equivocal. On one hand, both cross-sectional and longitudinal studies reported a negative association in healthy adults [13, 14, 28–30], adolescents [30], and individuals with metabolic syndrome [31]. On the other hand, this relationship did not always remain significant after adjusting for body mass, fat mass, lean mass, or BMI [13, 14, 29, 30, 32, 33]. Thus, one or more of these factors may mediate the observed relationship between adiponectin and bone health. Indeed, bone–fat interactions are mediated by both direct effects such as adipokine signaling in bone cells, as well as indirect effects via body weight (thus, mechanical loading). It is also possible that fat tissue aromatase activity may also have an effect on bone mass by increasing circulating estrogen [34]. The interplay between these mediators may obscure the interpretation of observed relationships and may lead to contradictory observations. For example, anorexia nervosa and obesity, two conditions at the extreme ends of the nutritional spectrum, are both associated with a marked increase in risk for fractures [35]. Thus, the prior observations between bone strength and serum adiponectin level may involve a multitude of pathways and may not represent direct signaling between adipose and bone tissue.

To dissociate the direct role of adiponectin, we previously explored the association between bone biomarkers (including serum adiponectin levels) and bone mineral density in the long bones of lower extremity in individuals with SCI. SCI-related paralysis results in pure

mechanical unloading. In the previous study, we found a negative association between serum adiponectin levels and bone mineral density, independent of body composition in a cohort of 149 men with chronic SCI [17]. Importantly, this association was present in wheelchair users (similar to the population in this study), but not in individuals who used assisted walking as the primary means of mobility, confirming that the adiponectin-mediated bone loss may dominate adipokine–bone interaction in the absence of mechanical loading.

However, bone mineral density in long bones does not always relate to actual bone strength [18, 19]. The latter depends not only on the mineralization, but also on the composition (e.g., porosity) and organization (e.g., micro- and macro-architecture) of the bone [18, 36]. Consistent with this, in our study, only ~35 % of the axial stiffness and ~70 % of the maximal load of the distal femur was explained by the bone mineral density at the same site. Nonetheless, our current results are in agreement with our previous result and suggest that in the absence of mechanical loading, not only bone mineral density [17] but also overall bone strength and maximal load are inversely related to circulating adiponectin level.

It has also been suggested that circulating adiponectin level may be a biomarker of fracture risk [6, 37]. Indeed, the risk of fracture appears to increase with increasing serum adiponectin levels in men [6], and higher adiponectin levels are associated with greater bone loss at the lumbar spine over the course of a year in physically active older women [38]. Similarly, adiponectin was associated with fracture risk in elderly men participating in the MrOS study (Sweden) and in the Health ABC study (USA) [6, 37]. Consistent with these earlier studies, we also found an association between adiponectin levels and history of osteoporotic fracture occurring after SCI. Specifically, among participants in our study, individuals with osteoporotic fractures had significantly lower axial stiffness and maximal load and significantly higher serum adiponectin levels compared to those without a history of post-SCI fractures. Thus, while our sample size was relatively small (6 with fractures versus 21 with no history of fractures), this finding, combined with our primary result (an inverse association between serum adiponectin and bone strength), does suggest that serum adiponectin levels may be predictive of incident osteoporotic fractures. Markers of bone turnover have been explored in the general population [39] and in SCI [40], but do not show promise in fracture risk prediction. In this study, we found no association between markers of bone turnover and bone strength or fracture history. To our knowledge, adiponectin is the first identified biomarker of fracture history in SCI.

By providing mechanistic insight, this observation has substantial implications for development of clinical care paradigms for SCI as well as for other conditions characterized by prolonged immobility and/or paralysis. For example, all individuals with motor complete SCI develop osteoporosis below the level of the injury [41, 42]. As a result, individuals with complete SCI are twice as likely to experience fractures compared to healthy controls [43]. Fracture risk prediction is limited by the lack of SCI-specific guidelines. Moreover, DXA may lack the sensitivity to detect small gains in response to osteogenic therapies that might translate to improvements in bone strength and reduced fracture risk. Thus, strength estimates alone or used in combination with biomarkers predictive of osteoporotic fractures, if demonstrated in future studies to be sufficiently sensitive and specific, might be indispensable tools to develop therapeutic approaches to effectively minimize post-injury

fractures and medical complications due to osteoporosis secondary to lower extremity paralysis.

Our study was limited by the relatively small sample size ($n=27$) and the cross-sectional design. There were few subjects with a history of osteoporotic fractures in this cohort, and therefore, these findings must be confirmed in larger studies of incident fracture. Nonetheless, the results of our study provide evidence that, in the absence of mechanical loading, adiponectin-mediated bone resorption dominates bone metabolism and promotes loss of bone strength and that serum adiponectin level may indeed be a feasible biomarker for bone health and osteoporotic fracture risk. Given the relatively small sample size and the cross-sectional design of our study, further work is needed to confirm these observations in a longitudinal study and to assess adiponectin as a biomarker of bone strength and incident fracture risk in a larger population.

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Fig. 1. Three-dimensional (3D) reconstruction of the bones of lower extremity and von Mises stress distribution. **a** 3D reconstruction of the tibia and femur. **b–c** von Mises stress distribution in response to the same axial force (10 kN) in subjects with high axial stiffness with no fracture history (**b**) and low axial stiffness with a history of post-SCI osteoporotic fracture (**c**)

Table 1

Participant characteristics

Variable	n=27
<i>Demographics</i>	
Age (years) [mean±SD]	40.7±11.5
Years since injury [mean±SD]	13.2±11.7
Age at injury [mean±SD]	27.4±10.9
White [n (%)]	24 (88.9 %)
Injury level [n (%)]	
Paraplegia	19 (70.4 %)
Tetraplegia	8 (29.6 %)
Motor complete injury [n (%)]	22 (81.5 %)
<i>Body composition [mean±SD]</i>	
Weight (kg)	82.67±21.0
Total fat mass (kg)	27.28±11.9
Total lean mass (kg)	52.60±10.7
BMI (kg/m ²) [mean±SD]	25.5±6.2
25 OH vitamin D (ng/ml) [mean±SD]	30.9±9.8
<i>Vitamin D status [n (%)]</i>	
Deficient (<30 ng/ml)	15 (55.6 %)
Sufficient (≥ 30 ng/ml)	12 (44.4 %)
<i>Bone mineral density (BMD) (g/cm²) [mean±SD]</i>	
SCI-specific skeletal sites	
Distal femur	0.729±0.262
Proximal tibia	0.738±0.287
<i>Traditional skeletal sites</i>	
Total hip	0.788±0.232
Femur neck	0.809±0.236
<i>Hip bone density classification^a [n (%)]</i>	
Normal BMD	10 (37.0 %)
Osteopenia	2 (7.4 %)
Osteoporosis/BMD lower than expected for age	15 (55.6 %)
<i>Distal femur strength [mean±SD]</i>	
Average distal femur stiffness (MPa ^b)	147.02±55.9
Average distal femur maximal load (kg)	79.40±42.7
<i>Post-SCI fracture osteoporotic fracture [n (%)]</i>	
No	21 (84.8 %)
Yes	6 (23.1 %)
<i>Bone biomarkers [mean±SD]</i>	
Adiponectin (ng/ml)	4,214.35±1953.88
Osteocalcin (ng/ml)	22.02±7.46
C-telopeptide (ng/ml)	0.377±0.223

^aBased on Z- or T-score at the total hip or femoral neck

^b*MPa* megapascal

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Table 2

Correlation between bone density and distal femur stiffness or maximal load

Bone density (g/cm ²)	<u>Distal femur stiffness (MPa^a)</u>		<u>Distal femur maximal load (kg)</u>	
	<i>r</i>	<i>p</i> Value	<i>r</i>	<i>p</i> Value
Distal femur	0.58	0.002	0.83	<0.0001
Proximal tibia	0.52	0.007	0.76	<0.0001
Femoral neck	0.40	0.04	0.57	0.002
Total hip	0.35	0.07	0.59	0.001

^aMPa megapascal

Table 3

Univariate models for distal femur stiffness and maximal load

Variable	Distal femur stiffness (MPa ^a)		Distal femur maximal load (kg)	
	$\beta \pm SE$	<i>p</i> Value	$\beta \pm SE$	<i>p</i> Value
Age (years)	0.049 $\pm$ 0.972	0.96	0.110 $\pm$ 0.741	0.88
Injury duration (years)	-2.487 $\pm$ 0.815	0.005	-1.967 $\pm$ 0.613	0.004
25 OH Vitamin D (ng/ml)	-0.096 $\pm$ 1.145	0.93	0.434 $\pm$ 0.870	0.62
BMI (kg/m ²)	2.653 $\pm$ 1.734	0.14	2.823 $\pm$ 1.263	0.03
Total lean mass (kg)	1.331 $\pm$ 1.028	0.21	1.905 $\pm$ 0.710	0.01
Total mass (kg)	0.533 $\pm$ 0.532	0.33	0.706 $\pm$ 0.388	0.08
Lower extremity lean mass (kg)	6.310 $\pm$ 2.5	0.02	6.711 $\pm$ 1.7	0.0005
Osteocalcin (ng/ml)	1.136 $\pm$ 1.481	0.45	1.118 $\pm$ 1.120	0.33
C-telopeptide (ng/ml)	36.34 $\pm$ 49.61	0.47	36.81 $\pm$ 37.54	0.34
Adiponectin (ng/ml)	-0.016 $\pm$ 0.005	0.002	-0.013 $\pm$ 0.004	0.002

^aMPa megapascal

Table 4

Multivariate models for distal femur stiffness and maximal load

	$\beta \pm \text{SE}$	<i>p</i> Value	<i>R</i> ²
<i>(A) Distal femur stiffness (MPa^a)</i>			
Adiponectin (ng/ml)	-0.013±0.005	0.01	0.44
Injury duration (years)	-1.707±0.756	0.03	
<i>(B) Distal femur maximal load (kg)</i>			
Adiponectin (ng/ml)	-0.00719±0.003	0.05	0.58
Injury duration (years)	-0.995±0.55	0.05	
Lower extremity lean mass (kg)	3.855±1.74	0.04	

^aMPa megapascal

Table 5

Factors associated with history of post-SCI osteoporotic fracture

	Fracture (n=6)	No fracture (n=21)	p Value
<i>Bone mineral density (BMD) (g/cm²) [mean ± SD]</i>			
SCI-specific skeletal sites			
Distal femur	0.458±0.10	0.797±0.25	0.007
Proximal tibia	0.453±0.10	0.809±0.27	0.01
<i>Traditional skeletal sites</i>			
Total hip	0.621±0.15	0.835±0.23	0.04
Femur neck	0.646±0.15	0.856±0.24	0.05
<i>Hip bone density classification^a</i>			
Normal BMD	0 (0.0 %)	10 (47.6 %)	0.06
Osteopenia/Osteoporosis/BMD lower than expected for age	6 (100.0 %)	11 (52.4 %)	
<i>Distal femur strength</i>			
Distal femur stiffness (MPa ^b)	99.5±56.3	160.6±49.0	0.01
Distal femur maximal load (kg)	38.4±14.6	91.1±40.8	0.005
<i>Biomarkers</i>			
Adiponectin (ng/ml)	5,656.7±3,003.3	3,802.2±1,380.4	0.04
Osteocalcin (ng/ml)	20.47±3.1	22.46±1.6	0.57
CTX (ng/ml)	0.307±0.09	0.398±0.05	0.39

^aBased on Z- or T-score at the total hip or femoral neck^bMPa megapascal