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ARTICLE

Factors associated with osteocalcin in men with spinal cord injury: findings from the FRASCI study

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Abstract

Study design Cross-sectional study.

Objective To assess the association between clinical and demographic factors, bisphosphonate use, and circulating total osteocalcin levels in men with chronic spinal cord injury.

Setting Veteran Affairs Medical Center.

Methods As part of an epidemiological study assessing SCI-related health conditions, 214 men with chronic spinal cord injury underwent a DXA scan and provided a blood sample and information regarding SCI, medication use, and fracture history. General linear models were used to assess clinical/demographic factors of osteocalcin, and if significant, were included in multivariate model.

Results We found that total osteocalcin levels increased 1.0 ng/ml for every kilogram increase in lean mass ($p = 0.05$) and increased 4.53 ng/ml for every ng/ml increase in C-telopeptide level ($p < 0.0001$). Osteocalcin levels were greater in people reporting no alcohol consumption compared with drinkers (15.49 ng/ml versus 18.58 ng/ml, $p < 0.0002$), lower in diabetics compared with nondiabetics (15.23 ng/ml versus 18.92 ng/ml, $p = 0.0001$), and lower in bisphosphonate users compared with nonusers (15.50 ng/ml versus 18.58 ng/ml, $p < 0.03$). The association between age and osteocalcin was not significant ($p = 0.06$). This model explained 58% of the variation in ln osteocalcin levels (model $p < 0.0001$, $r^2 = 0.58$).

Conclusions Total osteocalcin levels vary based on health habits, body composition, comorbid illnesses, and bisphosphonate use in men with chronic spinal cord injury.

Introduction

Spinal cord injury (SCI) is associated with marked decline in physical function, bone loss, infertility, fat accumulation, insulin resistance, and the development of metabolic syndrome. Based on extensive preclinical data in rodent models, this multiorgan decline is thought to be largely under the control of the skeleton [1–3]. Osteocalcin is a hormone produced exclusively in the bone microenvironment by osteoblasts and mature osteocytes. Sequestered osteocalcin is released from the extracellular matrix during bone remodeling, circulates in blood, and signals via functional receptors in the pancreas, muscle, adipose tissue, testis, and brain. One study reported that ~20% of the circulating osteocalcin is undercarboxylated [4].

Undercarboxylated osteocalcin regulates glucose metabolism by increasing β-cell proliferation and insulin synthesis, muscle metabolism by increasing insulin sensitivity in myocytes, fat metabolism by increasing insulin sensitivity and adipokine production (adiponectin) in adipocytes, and reproductive status by stimulating testosterone production in the testis [1–3, 5–7]. It was recently reported [8] that osteocalcin injection recovers male fertility in osteocalcin deficient mice. Based on this wide-reaching activity, it is now believed that bone metabolism contributes to the control of total body metabolism and energy balance via carboxylation status of osteocalcin. Hind limb unloading in rodents leads to reduced undercarboxylated osteocalcin and associated dysfunction in downstream signaling leading

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to impaired glucose metabolism and reproductive dysfunction [9]. Moreover, it has been shown that exercise, mechanical loading, extracellular matrix pH, and osteoanabolic medications can all alter the carboxylation status of osteocalcin, suggesting that therapeutic interventions promoting bone health, including parathyroid hormone analogs or bisphosphonates, may alter energy balance and reproductive function.

Osteoclastic bone resorption is required for the hormonal activation of osteocalcin [7]. Bisphosphonates are antiresorptive drugs that block osteoclastic bone resorption and suppress bone formation rates. While there have several clinical trials testing bisphosphonates in SCI [10, 11], change in osteocalcin is rarely reported as an outcome in these trials. Therefore, there is limited information on the impact of bisphosphonate use on osteocalcin levels after SCI. The goal of this study was to determine the association between bisphosphonate use and circulating levels of total osteocalcin after SCI. We also sought to identify additional clinical and/or demographic factors associated with circulating total osteocalcin levels after SCI.

Materials and methods

Participants

We assessed men with chronic SCI enrolled in the longitudinal Fracture Risk after SCI (FRASCI) Study. Participants with SCI were eligible if they were 22 years of age or older, one or more years after injury, were not ventilator dependent, did not have a tracheostomy, and had no other neuromuscular disease. Three hundred and forty-eight participants with SCI were enrolled in this cohort between August 2009 and December 2014. We excluded 48 participants because bone density, body composition, or biomarker results were not available. Because acute SCI is associated with uncoupled bone remodeling and accelerated osteoclastic resorption, for the current analysis we excluded participants with injury duration less than 3 years ($n = 28$). We excluded women with SCI ($n = 52$), as there were too few to make meaningful comparisons based on gender. We also excluded six participants with SCI actively taking medications known to influence bone metabolism, including statins or warfarin. The final cohort for this osteocalcin sub study consisted of 214 men with chronic SCI. Data from baseline testing were used in the current cross-sectional analysis. The Institutional Review Boards approved all protocols prior to initiation of the study, and all participants gave their written informed consent to participate.

Motor score

A trained rater confirmed level and completeness of injury at study entry by physical exam according to the American Spinal Injury Association Impairment Scale (AIS). We considered injury severity in two categories: motor complete (AIS A/B) or motor incomplete (AIS C or D).

Dual X-ray absorptiometry (DXA) for bone mineral density (BMD) and body composition

Bone mineral density (BMD) and body composition were determined by dual X-ray absorptiometry (DXA) scanner (5th generation GE Healthcare iDXA with enCore configuration version 12.3). Body composition variables (total fat mass (kg) and total lean mass (kg)) were calculated by the system software from the whole body scans. We measured a phantom daily to confirm scanning accuracy (within 0.003 g/cm^2). The root-mean-square coefficient of variation (RMS-CV) was 2.3%, and root-mean-square standard deviation (RMS-SD) was 0.012 g/cm^2 at the distal femur. At the proximal tibia, the RMS-CV was 2.4%, and the RMS-SD was 0.028 g/cm^2. Bone density could not be determined at the distal femur ($n = 6$), proximal tibia ($n = 7$), total hip ($n = 12$), or femoral neck ($n = 12$) due to knee replacement, hip replacement, severe contractures preventing proper positioning, prior fracture with instrumentation, or heterotopic ossification.

Biochemical analyses

Participants were asked to undergo testing in a fasting state and efforts were made to collect samples in the morning before a meal. Information was collected on time since last meal or snack to determine if biomarker levels varied based on this factor. Plasma samples were drawn into an EDTA tube and processed immediately. Samples were centrifuged for 15 min at 2600 rpm ($1459 \times g$) 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. Total osteocalcin was measured by electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN) with a detection limit of 0.50 ng/ml. C-telopeptide was measured by electrochemiluminescence immunoassay (Roche Diagnostics) with a detection limit of 0.01 ng/ml. 25 OH vitamin D was quantified by high performance liquid chromatography tandem mass spectrometry with a detection limit of 1.0 ng/ml. Interleukin-6 (IL-6) was determined by ultrasensitive ELISA (R & D Systems, Minneapolis, MN) with a sensitivity of 0.094 pg/ml and day-to-day variability of 9.6, 7.2, and 6.5% at concentrations of 0.49, 2.78, and 5.65 pg/ml, respectively. Total adiponectin

was measured by ELISA (ALPCO Diagnostics Inc., Salem, NH) with a detection limit of 0.075 ng/ml and day-to-day variability less than 15% at various concentrations for all forms of adiponectin. Assays were performed in duplicate and any duplicate with >10% CV was repeated.

Variable definition

Information regarding SCI, medical history, medication use, and fracture history was obtained by questionnaire at the time of DXA scan. Participants completed a health questionnaire based on the American Thoracic Society adult respiratory disease questionnaire [12]. Smokers were defined as smoking 20 or more packs of cigarettes or using 336 g (12oz) of tobacco or more in a lifetime or smoking 1 or more cigarettes a day for at least 1 year. Current smokers reported cigarette use within 1 month of testing. Smoking status was considered dichotomously (current smoker versus never/past smoker) and smoking exposure was considered continuously. Lifetime alcohol consumption and alcohol consumption after SCI were calculated based on report of average daily, weekly, or monthly quantity and frequency of alcohol consumption and duration of alcohol use before and after injury. Each glass of wine (4 ounce = 10.8 g), beer (12 ounce = 13.2 g), and shot of liquor (1.5 ounce = 15.1 g) was converted to grams of alcohol [13]. Participants were asked to report physician-diagnosed hypertension or diabetes. Heart disease was defined as receiving treatment for "heart trouble" in the 10 years prior to study entry. These disease definitions were validated in prior studies [14]. Current bisphosphonate users were defined as those using a bisphosphonate for at least 3 months prior to testing. Participants were weighed and supine length measured for the calculation of body mass index (BMI). BMI was considered as a continuous variable and in obesity categories based on SCI-specific cut points, where obesity was defined as having a BMI ≥ 25 [15]. Age and 25 OH vitamin D levels were considered as continuous variables. Vitamin D deficiency was defined as having a 25 OH vitamin D level less than 20 ng/ml. For fracture history, information was collected on timing (before SCI, at time of SCI, or after SCI), location, and cause of fracture. Fractures were then categorized as osteoporotic (ie., those occurring from standing height or less or in the absence of trauma) or traumatic as previously described [16]. Fractures occurring prior to testing were considered prevalent. Hand, foot, skull, sternum, and rib fractures were excluded. When available, medical records were used to confirm self-reported fracture history (29 out of 65 post-SCI osteoporotic fractures). For men age 50 or older, *T*-score was used to classify hip BMD according to the World Health Organization definitions of normal (*T*-score > −1), osteopenia (*T*-score < −1 and > −2.5), and osteoporosis (*T*-score < −2.5). For men under the age of 50, *Z*-score was used to classify BMD at the hip as normal (*Z*-score > −2) or as lower than expected for age and sex (*Z*-score < −2). Mobility mode (more than 50% of the time) was considered in the following two categories: wheelchair use (motorized wheelchair or hand-propelled wheelchair) or walking (with aid such as crutch, cane, or walk without assistance). Remodeling index was calculated as a ratio of C-telopeptide to osteocalcin [17].

Statistical analysis

All analyses were performed using SAS 9.4 (SAS Institute, Inc., Cary, NC). *T*-tests or χ^2 tests were used to compare participant characteristics as appropriate. Since the distribution of osteocalcin was skewed, natural log-transformation was used to normalize the distribution of the outcomes and stabilize the variance for the comparisons. General linear models (PROC GLM) were applied to assess associations between clinical/demographic factors and ln osteocalcin. Factors with a p value of <0.10 in the univariate models, as well as factors that were deemed clinically significant (age, injury duration, bone density, total body bone mineral content, % total fat, % lean mass), were included in the multivariable models assessing the association of bisphosphonate use and ln osteocalcin (PROC GLM). Factors with a p value of <0.05 were considered statistically significant.

Results

Participant characteristics

Participant characteristics are presented in Table 1, based on active bisphosphonate use. Twelve (6%) participants were active bisphosphonate users (taking a bisphosphonate for at least 3 months prior to testing). Alendronate was the most commonly used bisphosphonate (83.4%), followed by ibandronate and zoledronic acid (8.3% for both). The mean duration of bisphosphonate use was 3.48 years and ranged from 0.5 to 7 years. Overall, participants were aged 54.7 ± 13.9 (SD) years (ranged from 22.8 to 87.6) years, and were 19.3 ± 12.6 (ranged from 3.0 to 60.8) years post injury. All were male and most were white (85.5%). Eighteen percent of participants were active smokers, 15% reported a history of diabetes, and 10% reported a history of heart disease. Sixty-two percent of participants used a wheelchair as their primary mode of mobility and 48% had a motor complete injury. The mean BMI was 27.4 ± 5.5 kg/m^2 and 66.4% of participants had normal 25 OH vitamin D levels (≥20 ng/ml). No participants reported active heterotopic ossification. Mature heterotopic ossification was noted by DXA in 12 participants in the no bisphosphonate group. Forty-six

Table 1 FRASCI cohort baseline participant characteristics

Variable	Active bisphosphonate use ($n = 12$)	No bisphosphonate use ($n = 202$)	p	Total cohort ($n = 214$)
Demographics				
Age (years), [mean ± SD]	54.3 ± 14.4	54.7 ± 13.9	0.91	54.7 ± 13.9
Age at injury (years), [mean ± SD]	32.6 ± 17.1	35.5 ± 15.8	0.53	35.3 ± 15.9
Years since injury, [mean ± SD]	21.6 ± 11.1	19.2 ± 12.6	0.50	19.3 ± 12.6
White, *n* (%)	12 (100)	171 (84.7)	0.22	183 (85.5)
Motor complete injury, *n* (%)	8 (66.7)	95 (47.0)	0.19	103 (48.1)
Wheelchair user, *n* (%)	10 (83.3)	123 (60.9)	0.14	133 (62.2)
Vitamin D normal (≥20 ng/ml), *n* (%)	12 (100)	130 (64.4)	0.009	142 (66.4)
Body composition				
Total fat (%), [mean ± SD]	33.0 ± 9.1	35.9 ± 8.3	0.25	35.7 ± 8.3[c]
BMI (kg/m^2), [mean ± SD]	24.1 ± 5.2	27.6 ± 5.5	0.03	27.4 ± 5.5
Total lean mass (kg), [mean ± SD]	47.3 ± 10.9	53.5 ± 8.0	0.01	12.4[c]
Areal bone mineral density (aBMD, g/cm^2), [mean ± SD]				
SCI-specific skeletal site				
Distal femur	0.59 ± 0.12	0.78 ± 0.22	0.0004	0.77 ± 0.22[a]
Proximal tibia	0.53 ± 0.18	0.82 ± 0.29	0.0003	0.80 ± 0.29[b]
Traditional sites				
Radius	0.96 ± 0.11	0.98 ± 0.11	0.48	0.98 ± 0.11[c]
Total hip	0.72 ± 0.14	0.86 ± 0.23	0.03	0.85 ± 0.22[d]
Femoral neck	0.71 ± 0.12	0.85 ± 0.20	0.01	0.84 ± 0.20[d]
Hip bone density classification, *n* (%)				
Normal/Osteopenia	2 (16.7)	101 (50.0)	0.01	103 (48.1)
Osteoporosis/BMD lower than expected	10 (83.3)	89 (44.1)		99 (46.3)
Hip BMD not available	0 (0)	12 (5.9)		12 (5.6)
Post-SCI osteoporotic fracture, *n* (%)	2 (16.7)	39 (19.3)	1.0	41 (19.2)
Health habits and comorbidities				
Current smoker, *n* (%)	1 (8.3)	37 (18.3)	0.70	38 (17.8)
Cigarette exposure (pack-years) [mean ± SD]	72.40 ± 59.36	28.81 ± 30.86	0.18	30.53 ± 33.14[e]
Lifetime alcohol consumption (kg-years) [mean ± SD]	257.65 ± 327.84	177.23 ± 383.03	0.48	181.74 ± 379.93
Post-SCI alcohol consumption (kg-years) [mean ± SD]	654.44 ± 1059.28	389.57 ± 627.16	0.40	404.43 ± 657.91
Diabetes, *n* (%)	2 (16.7)	31 (15.4)	1.0	33 (15.4)
Hypertension, *n* (%)	2 (16.7)	80 (39.6)	0.14	82 (38.3)
Heart disease, *n* (%)	3 (25.0)	19 (9.4)	0.11	22 (10.3)
Biomarkers				
25 OH vitamin D (ng/ml), [mean ± SD]	25.3 ± 6.2	23.6 ± 10.6	0.39	23.7 ± 10.4
Adiponectin (ng/ml), [mean ± SD]	8565.0 ± 5803.2	5048.6 ± 2748.7	0.06	5245.8 ± 3086.5
IL-6 (pg/ml), [mean ± SD]	3.2 ± 3.8	4.0 ± 5.2	0.57	4.0 ± 5.2
C-telopeptide (ng/ml), [mean ± SD]	0.13 ± 0.07	0.35 ± 0.17	<0.0001	0.34 ± 0.17
Osteocalcin (ng/ml), [mean ± SD]	11.9 ± 4.0	21.0 ± 9.7	<0.0001	20.4 ± 9.7
Bone remodeling index, [mean ± SD]	0.01 ± 0.01	0.02 ± 0.01	0.001	0.02 ± 0.01

[a]DXA available for $n = 208$

[b]DXA available for $n = 207$

[c]DXA available for $n = 212$

[d]DXA available for $n = 202$

[e]$n = 127$ individuals with a smoking history

percent of all participants and 83% of active bisphosphonate users had osteoporosis based on BMD at the hip. Bisphosphonate users had lower BMI and total lean mass than nonusers, were more likely than nonusers to have normal 25 OH vitamin D levels and to be osteoporotic based on BMD at the hip, had lower BMD at the hip and knee, and had

lower levels of both C-telopeptide and osteocalcin levels with lower remodeling indices than nonusers. There were no other significant differences in clinical or demographic characteristics between the two groups.

Clinical factors associated with osteocalcin levels

Osteocalcin ($p = 0.35$), adiponectin ($p = 0.22$), IL-6 ($p = 0.51$), and C-telopeptide levels ($p = 0.16$) did not vary significantly based on time since last meal or snack. C-telopeptide and osteocalcin levels were highly correlated ($r = 0.74$, $p < 0.0001$). In univariate analyses (Table 2), ln osteocalcin levels were positively associated with C-telopeptide and IL-6 levels ($p < 0.0001$ and $p = 0.01$, respectively). Osteocalcin levels were greater in people reporting no alcohol consumption compared with drinkers ($p < 0.0001$), lower in diabetics compared with nondiabetics ($p = 0.0002$), and lower in bisphosphonate users compared with nonusers ($p < 0.0001$). There was no association between osteocalcin and any lower extremity bone density site tested or total body bone mineral content ($p = 0.14$–0.83).

In multivariable models adjusting for age and lean mass, the association between IL-6 and ln osteocalcin was no longer significant ($p = 0.14$), while the associations between ln osteocalcin and diabetes, alcohol consumption, and bisphosphonate use remained significant (Table 3). Osteocalcin increased 1.0 ng/ml for every kilogram increase in lean mass ($p = 0.05$) and increased 4.53 ng/ml for every ng/ml increase in C-telopeptide level ($p < 0.0001$). Osteocalcin levels were greater in people reporting no alcohol consumption compared with drinkers (15.49 ng/ml versus 18.58 ng/ml, $p < 0.0002$), lower in diabetics compared with nondiabetics (15.23 ng/ml versus 18.92 ng/ml, $p = 0.0001$), and lower in bisphosphonate users compared with nonusers (15.50 ng/ml versus 18.58 ng/ml, $p < 0.03$). The association between age and osteocalcin was not significant ($p = 0.06$). This model explained 58% of the variation in ln osteocalcin levels (model $p < 0.0001$, $r^2 = 0.58$). The results remained unchanged when removing the 12 participants with mature heterotopic ossification from the analysis. BMI ($p = 0.73$), % gynoid fat ($p = 0.56$), and % total body fat ($p = 0.10$) were not significantly associated with ln osteocalcin when each variable was separately introduced into the models instead of lean mass.

Discussion

The FRASCI study is the largest longitudinal study of disuse osteoporosis and the first to assess the impact of multiple risk factors for bone loss during the chronic phase of SCI. Here we prospectively assessed factors associated

Table 2 Univariate factors associated with ln osteocalcin in men with SCI

Variable	β ± SE	*p*
Age (years)	−0.001 ± 0.002	0.52
Years since injury (years)	−0.001 ± 0.002	0.51
BMI (kg/m^2)	−0.004 ± 0.005	0.40
Total fat (%)	−0.005 ± 0.003	0.11
Distal femur BMD (g/cm^2)	−0.15 ± 0.13	0.26[a]
Proximal tibia BMD (g/cm^2)	−0.08 ± 0.10	0.40[b]
Distal radius BMD (g/cm^2)	−0.17 ± 0.25	0.51[c]
Total hip BMD (g/cm^2)	−0.08 ± 0.12	0.50[d]
Femoral neck BMD (g/cm^2)	−0.12 ± 0.14	0.38[d]
Total body bone mineral content (g)	−0.00004 ± 0.00005	0.48
Total lean mass (kg)	0.001 ± 0.003	0.71
Leg lean mass (kg)	0.002 ± 0.006[e]	0.68
Adiponectin (ng/ml)	0.000004 ± 0.000009	0.66
C-telopeptide (ng/ml)	1.658 ± 0.114	<0.0001
IL-6 (pg/ml)	0.013 ± 0.005	0.01
25 OH vitamin D (ng/ml)	−0.002 ± 0.003	0.36
Smoking exposure (pack-years)	−0.001 ± 0.001	0.23[e]
Alcohol consumption post-SCI	−0.0001 ± 0.00007	0.15
Alcohol consumption lifetime	−0.00004 ± 0.00004	0.29
	LSMeans ± SE	*p*
Vitamin D deficiency (<20 ng/ml)		
Yes	2.952 ± 0.048	0.57
No	2.919 ± 0.034	
Walking status		
Wheelchair user	2.942 ± 0.035	0.59
Walk with or without aid	2.911 ± 0.045	
Injury completeness		
Motor complete	2.944 ± 0.040	0.65
Motor incomplete	2.919 ± 0.039	
Injury level		
Tetraplegia	2.948 ± 0.040	0.55
Paraplegia	2.915 ± 0.038	
Obesity status		
Obese	2.931 ± 0.035	0.96
Not obese	2.929 ± 0.047	
Current smoker		
Yes	2.992 ± 0.066	0.31
No	2.917 ± 0.031	
Current alcohol user		
Yes	2.874 ± 0.030	<0.0001
No	3.171 ± 0.061	
Heart disease		
Yes	2.811 ± 0.087	0.15
No	2.944 ± 0.029	
Diabetes		
Yes	2.686 ± 0.069	0.0002
No	2.975 ± 0.029	
Hypertension		
Yes	2.890 ± 0.045	0.25
No	2.956 ± 0.036	
Bisphosphonate use		
Active use	2.410 ± 0.112	<0.0001
No active use	2.962 ± 0.027	
Post-SCI osteoporotic fracture		
Yes	3.016 ± 0.064	0.14
No	2.910 ± 0.031	

[a]DXA available for $n = 208$

[b]DXA available for $n = 207$

[c]DXA available for $n = 212$

[d]DXA available for $n = 202$

[e]$n = 127$ individuals with a smoking history

Table 3 Multivariable model of factors associated with ln osteocalcin in men with SCI

Variable	β ± SE	e^{β}	*p*
Age (years)	0.003 ± 0.001	1.00	0.06
Total lean mass (kg)	0.004 ± 0.002	1.00	0.05
C-telopeptide (ng/ml)	1.51 ± 0.11	4.53	<0.0001
	LSMeans ± SE	$e^{lsmeans}$	*p*
Bisphosphonate use			
Yes	2.741 ± 0.085	15.50	0.03
No	2.922 ± 0.031	18.58	
Diabetes			
Yes	2.723 ± 0.064	15.23	<0.0001
No	2.940 ± 0.045	18.92	
Alcohol use			
Yes	2.740 ± 0.046	15.49	0.0002
No	2.922 ± 0.061	18.58	

with circulating total osteocalcin levels in 214 men with chronic SCI. We considered data obtained at baseline testing in this cross-sectional analysis. We found that bisphosphonate users were more likely to have normal 25 OH vitamin D levels and to have osteoporosis based on BMD at the hip and had significantly lower BMD at both SCI-specific and traditional skeletal sites than nonusers. Bisphosphonate users also had lower bone remodeling indices than nonusers. Total osteocalcin levels were significantly lower in diabetics, alcohol drinkers, and bisphosphonate users, and were positively associated with circulating C-telopeptide levels and total lean mass. These associations are independent of mature heterotopic ossification, total body bone mineral content, and bone density at the distal femur, proximal tibia, total hip, or femoral neck. The mean circulating total osteocalcin levels (mean 18.58 ng/ml) that we report are consistent with other recent reports in adults with type 1 diabetes (mean 14.8 ng/ml) [18], healthy adults (mean 15.92 ng/ml) [19], postmenopausal women (mean 18.6 ng/ml) [20], and males with SCI (mean 21 ng/ml) [21].

Acute SCI is associated with "uncoupled remodeling" with rapidly increasing bone resorption in the face of relatively normal bone formation rates [22]. This persists for 2–3 years post injury. The current analysis was restricted to individuals 3 years or more post injury to avoid this period of uncoupled remodeling. We report that osteocalcin and C-telopeptide levels were strongly correlated (0.74, $p < 0.0001$), suggesting a return to more "coupled" remodeling. Based on our findings, C-telopeptide levels had a greater impact on variations in osteocalcin levels than all other factors that we studied. This is likely due to the release of sequestered osteocalcin from the extracellular matrix during bone remodeling. Our finding of decreased osteocalcin in diabetics is consistent with other reports in the general population. Accumulating data suggest that osteocalcin plays a significant role in glucose and energy metabolism and that reductions in osteocalcin can lead to elevated glucose, insulin resistance, increases in body fat, and a higher risk of type 2 diabetes [4, 5, 23–28]. Similarly, the positive association between total osteocalcin and lean mass agrees with reports in obese children [29], obese adolescents undergoing weight loss therapy [30], pubescent male elite soccer players [31], and in obese adults [32]. Osteocalcin is known to target muscle to improve insulin sensitivity and glucose metabolism [33, 34]. More recently, osteocalcin was shown to be a myogenic factor by stimulating myoblast proliferation via PI3K/Akt and p38 MAPK signaling [35]. We found that prevalent alcohol use was also associated with lower osteocalcin levels. It was recently reported that alcohol consumption suppresses both osteocalcin and C-telopeptide levels in postmenopausal women [36].

After adjusting for C-telopeptide levels, lean mass, diabetes, and prevalent alcohol use, bisphosphonate users had significantly lower osteocalcin levels than nonusers. It has previously been shown that bisphosphonate use leads to lower osteocalcin levels in rodent models [37]. Similarly, observational and experimental studies in humans, including randomized placebo-controlled trials, indicate that bisphosphonate administration leads to lower osteocalcin levels over time [38–44]. Results have not been entirely consistent though, as some human studies have failed to find that bisphosphonate administration significantly alters osteocalcin levels [45, 46]. Although these prior studies provide valuable data on the bisphosphonate-osteocalcin association, they did not specifically assess this association after SCI. Examining this association is warranted as SCI alters the physiologic milieu resulting in one that is quite unique compared with other populations. In particular, individuals with SCI experience rapid bone loss and muscle atrophy after injury, as well as loss of supralesional neuronal input to bone, mechanical unloading of limbs, increased adiposity and high rates of obesity, and vascular dysfunction below the injury site. This suggests that prior data on bisphosphonates and osteocalcin in non-SCI populations may not be generalizable to people with SCI.

While there have been several clinical trials assessing the impact of bisphosphonate use on bone in SCI, few of these studies report change in osteocalcin level as an outcome [11]. Because there is great variation in the markers of bone formation used in clinical trials, limited information exists regarding the impact of bisphosphonate treatment specifically on total osteocalcin levels in SCI. A two-year course of alendronate plus calcium treatment

was associated with decreased osteocalcin levels [11]. While both groups (alendronate plus calcium versus calcium alone) experienced significant decreases in osteocalcin levels over the two-year period, the alendronate plus calcium group experienced a significantly greater decline in total osteocalcin levels. The study population was heterogeneous in terms of years post injury (ranging from 0.1 to 29.5 years) and the analysis did not adjust for potential confounding factors. Therefore, it is difficult to make conclusions regarding the effect of alendronate treatment on osteocalcin levels based on this study. As such, the present study adds to the existing literature by demonstrating that, after adjusting for confounding factors, bisphosphonate use is associated with lower osteocalcin levels in men with SCI.

A link between bisphosphonate use and reduced osteocalcin is biologically plausible. Osteoblasts express osteocalcin, and thus interventions that inhibit bone remodeling in general, or osteoblast activity specifically, could presumably suppress osteocalcin production as well [47]. Evidence suggests that bisphosphonates inhibit osteoblasts in two ways. First, bisphosphonates inhibit osteoblasts indirectly, by suppressing osteoclast activity. By suppressing osteoclasts, however, bisphosphonates also suppress the ability of osteoclasts to secrete "coupling" factors that activate bone-forming osteoblasts. These help osteoblasts to replace bone that is lost due to osteoclast-induced resorption. Bisphosphonates may also inhibit osteoblasts through more direct, toxic effects on osteoblasts. In particular, in vitro studies indicate that bisphosphonates can directly inhibit osteoblast activity, migration, and survival [48]. In each of these studies cytotoxic effects on osteoblasts were obtained with micromolar (1–10 μM) doses of zoledronic acid. Thus, clinical doses of bisphosphonates could inhibit osteoblasts and ultimately suppress osteocalcin production, providing a plausible mechanistic link for the association between bisphosphonate use and reduced osteocalcin observed in this study. While bone biopsies are challenging to obtain in the SCI population, additional work focused on osteoblast activity in response to treatment with bisphosphonates would help clarify this relationship.

Bisphosphonate-induced reductions in osteocalcin could have a number of downstream negative implications following SCI. To the extent that osteocalcin is involved in bone formation, any bisphosphonate-induced reductions in osteocalcin could potentially suppress bone turnover in individuals over time. However, in addition to having adverse effects on bone, reductions in osteocalcin could adversely affect a variety of other physiologic processes as well. In addition to the metabolic effects described in detail above, osteocalcin appears to have an impact on brain function, as low osteocalcin levels have been linked to cognitive problems such as anxiety and defects in learning and memory [6]. Investigators have also demonstrated that osteocalcin plays a role in fertility. In particular, studies in rodents and humans show that osteocalcin can promote testosterone synthesis [1]. This increases concern that suppression of osteocalcin could have wide-ranging adverse health effects following SCI.

In this study we identified clinical factors, health behaviors, and medications that are associated with total osteocalcin levels in men with chronic SCI. Strengths of this study include the focus on individuals with SCI, use of a reliable osteocalcin assay, and information on demographic and clinical factors that enabled assessment of bisphosphonate's independent association with osteocalcin. However, this study also had several limitations to consider. First, the number of bisphosphonate users in this study was relatively small, and the study population only included men. As such, these findings should be confirmed in women with SCI. Second, the study used an observational cross-sectional study design, which precludes the study from establishing causality between bisphosphonate use and reduced osteocalcin levels. Moreover, the relationship between osteoporosis and bisphosphonate use in this study is unclear. It is likely that pharmacological intervention was initiated by treating physicians in response to osteoporosis, fracture history, or other clinical consideration. Indeed, there are several clinical trials testing bisphosphonate use in SCI that have reported either improvement in BMD or mitigation of bone loss [11, 49]. However, it is also possible that bisphosphonate use causes bone loss, as suggested by a recent article by Bauman et al. [10]. In addition, some studies suggest that undercarboxylated osteocalcin may be the form of osteocalcin most strongly related to osteocalcin's metabolic effects [24]. However, the present study only measured total osteocalcin, and so was unable to assess the relationship of bisphosphonate use to undercarboxylated osteocalcin. Diabetes diagnosis was by self-report and we could not distinguish between the two types (type 1 versus type 2). Similarly, prevalent fractures were verified by medical record review in only 50% of the cases. Despite these limitations, the present study identifies several factors, including modifiable risk factors, which are associated with osteocalcin levels in men with SCI, including bisphosphonate use. These associations could have implications for bone formation after SCI, but may have more wide-ranging implications involving glucose metabolism, body composition, cognitive health, and fertility. Additional research using larger sample sizes and more detailed assessments of bisphosphonate use and osteocalcin could further our understanding of the bisphosphonate-osteocalcin association and its effects on individuals with SCI.

Data archiving

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Authors contributions RAB, NN, MS, and LRM were responsible for: integrity of the study; study design and conduct; data collection, analysis, and interpretation; drafting of the manuscript; revision of manuscript content and approval of the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Statement of ethics We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this research.

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