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Periostin and sclerostin levels in individuals with spinal cord injury and their relationship with bone mass, bone turnover, fracture and osteoporosis status

Laurent Maïmoun[a,b,*], Fayçal Ben Bouallègue[a,b], Anthony Gelis[c], Safa Aouinti[d], Thibault Mura[d], Pascal Philibert[e], Jean-Claude Souberbielle[f], Marie Piketty[f], Patrick Garnero[g], Denis Mariano-Goulart[a,b], Charles Fattal[h]

[a] Département de Médecine Nucléaire, Hôpital Lapeyronie, CHU Montpellier, Montpellier, France
[b] PhyMedExp, INSERM, CNRS, Université de Montpellier, France
[c] Centre Mutualiste PROPARA, Montpellier, France
[d] Unité de Recherche Clinique et Epidémiologie, Hôpital La Colombière, CHU Montpellier, Montpellier, France
[e] Departement de Biochimie et d'Hormonologie, Hôpital Lapeyronie, CHU Montpellier, Montpellier, France
[f] Laboratoire des Explorations Fonctionnelles, Hôpital Necker, Paris, France
[g] INSERM Research Unit 1033-Lyos, Lyon, France
[h] Centre de Rééducation et Réadaptation Fonctionnelle La Châtaigneraie, Menucourt, France

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ABSTRACT

Background: Spinal cord injury (SCI) induces an acute alteration in bone metabolism. Although the aetiology of the bone disturbances is not precisely known, immobilisation reduces mechanical loading and the morphology of osteocytes, which are the primary mechanosensors. Periostin and sclerostin are secreted mostly by osteocytes and are involved in bone's mechanical response.

Objective: The present study was conducted to determine whether individuals with SCI present alterations in serum periostin and sclerostin and to assess their relationships with bone mineral density, bone turnover markers, fracture status, time since injury, densitometric osteoporosis and paraplegic vs. tetraplegic status.

Subjects and methods: One hundred and thirty-one individuals with SCI (96 males and 35 females; 42.8 ± 13.7 yr old) with a mean 14.2 ± 12.1 years since the time of injury were evaluated and compared with 40 able-bodied controls in a cross-sectional study. Periostin and sclerostin were assayed by ELISA from Biomedica® (Vienna, Austria), and bone turnover markers and areal bone mineral density (aBMD) were concomitantly analysed.

Results: Compared with controls, individuals with SCI presented higher periostin ($p < 0.01$), lower sclerostin ($p < 0.001$), similar markers of bone turnover levels and lower aBMD at the hip. Compared with chronic individuals, bone turnover markers, sclerostin excepted, values were higher as well as aBMD at hip in individuals with acute SCI. Moreover, the aBMD differences were more marked in tetraplegic than paraplegic individuals. Bone mineral density, fracture status, densitometric osteoporosis and paraplegia vs. tetraplegia did not seem to substantially influence the values of biological markers, sclerostin excepted.

Conclusion: This study showed for the first time that individuals with SCI presented higher periostin levels than healthy controls only during the acute phase. Conversely, sclerostin levels are lower whatever the post-injury time. Fractures and densitometric osteoporosis were not associated with differences in these two biological markers, whereas paraplegia vs. tetraplegia and fragility fracture status seemed to influence sclerostin levels only.

* Corresponding author at: Département de Biophysique, Université Montpellier, Service de Médecine Nucléaire, Hôpital Lapeyronie, 371, Avenue du Doyen Gaston Giraud, 34295 Montpellier Cedex 5, France.
E-mail address: l-maimoun@chu-montpellier.fr (L. Maïmoun).

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1. Introduction

Spinal cord injury (SCI) induces a deep alteration in bone metabolism, resulting in increased bone loss that is characterised by specificities in kinetics, severity and location [1]. The bone loss is generally described as having two phases: an acute period starting just after injury and peaking 3 to 5 months later [2–4] and a chronic period of progressive stabilisation of bone mass, which is usually reached 16 to 24 months post-injury [5,6].

The aetiology of the bone disturbances in individuals with SCI is not precisely known, but one of the consequences of the gradual loss of bone strength is an increasing risk of fracture after minor trauma [7]. Hormonal alterations, including a reduction in sexual hormones or growth factor levels, have been reported in these conditions, but these alterations do not completely explain the severe bone loss [8,9]. Immobilisation following a neurological lesion dramatically reduces mechanical loading, which is the main determinant of altered bone mass and architecture [1,10]. Osteocytes are now considered to be the primary mechanosensors that locally coordinate adaptive modelling responses to external mechanical strain [11]. Among the molecules produced by the osteocytes involved in mechanotransduction is sclerostin, which has a major role by inhibiting the canonical Wnt/β-catenin signalling pathway that then suppresses osteoblast activity [12]. Immobilisation after stroke induces an increase in sclerostin levels in aged postmenopausal women [13]. Moreover, experimental studies have shown that administering anti-sclerostin antibody preserves osteocyte morphology and structure and blocks the severe skeletal deterioration after motor complete SCI in rats [14]. Mice with sclerostin gene deletion are resistant to the major sublesional bone loss induced by SCI [15]. However, in humans, the effects of SCI on circulating sclerostin levels seem more complex because elevated [16], normal [4] and decreased [16–18] values have been reported.

Other essential mediators of the bone response to mechanical loading, such as periostin [19], may also be affected by the reduced mechanical loading due to immobilisation after SCI. This matricellular protein of 90kD is secreted by osteocytes and osteoblasts [20,21] and plays a crucial role in bone formation partly by promoting osteoblast differentiation and proliferation [22]. Experimental preclinical studies have demonstrated that unloading causes reduced periostin expression [19]. Conversely, an increase in mechanical constraints using, for example, an axial compression load on mouse tibia [21] or intense training [20] results in overexpression of periostin and the down-regulation of sclerostin [21]. In humans, circulating periostin levels have been reported to be either weakly or not correlated with areal bone mineral density (aBMD) [23–25], although higher serum periostin levels have been associated with a higher fracture risk and fragility fractures in postmenopausal women [24,25]. However, the implication of periostin in the extensive bone loss and bone remodelling alteration in individuals with SCI remains to be elucidated.

Therefore, the present study was conducted in individuals with SCI in order to determine (1) the effects of SCI on periostin and sclerostin levels, (2) the relationship of periostin and sclerostin levels with aBMD, bone turnover markers, and the following clinical factors: time interval from SCI onset, fractures, densitometric osteoporosis and paraplegic vs. tetraplegic status.

2. Participants and methods

2.1. Participants

This study followed a cross-sectional design. One hundred and thirty-one individuals with SCI and neurological lesion exclusively due to trauma were consecutively recruited from the Centre Mutualiste PROPARA, a specialised spinal cord injury clinic (Montpellier, France). According to the American Spinal Injury Association Impairment Scale (ASIA), they were classified as complete (AIS A), incomplete with no motor function below the neurological level of injury (AIS B), or incomplete with incomplete motor recovery (AIS C and D). All skeletal fractures at SCI onset and post-SCI were recorded during the clinical history. The patient's clinical history was determined by both interviewing and reading the patient record. Spasticity and its location were also recorded. All individuals with SCI used a manual or electrically assisted wheelchair as their primary means of mobility.

Individuals with SCI were compared to a control group of 17 healthy males and 23 healthy females. None of the participants had a history of a neuromuscular disease like multiple sclerosis, had an evident secondary cause of osteoporosis (i.e. osteogenesis imperfecta or known endocrine disorders associated with bone loss), or was taking medication known to affect bone metabolism, including bisphosphonates, within the 3 years before inclusion. They had to be 18 years or older.

Study approval was obtained from the Regional Research Ethics Committee (Comité de Protection des Personnes Sud-Mediterranee IV, Montpellier, France). Participants were given oral and written information and then delivered written consent.

2.2. Methods

All participants were evaluated in a single session after an overnight fast. Height was measured with a stadiometer. Weight was determined using a weight scale with a precision of 0.1 kg. BMI was calculated as weight (kg) divided by the square of height (m).

2.2.1. Bone mineral density, body fat and fat-free soft tissues

DXA (Hologic QDR-4500A, Hologic, Inc., Waltham, MA) measured the areal bone mineral density (aBMD; g/cm^2) at specific bone sites: the anteroposterior lumbar spine (L1-L4), the dominant arm radius, and the hip. When an individual with SCI presented with a fracture, osteosynthesis material or heterotopic ossification, the contralateral hip and radius were assessed. All scanning and analyses were performed by the same operator to ensure consistency after following standard quality control procedures. Quality control for DXA was checked daily by scanning a lumbar spine phantom consisting of calcium hydroxyapatite embedded in a cube of thermoplastic resin (DPA/QDR-1; Hologic x-caliber anthropometrical spine phantom). The least significant change (LSC) in our Department of Nuclear Medicine was calculated and was determined at 0.013 g/cm^2 for the lumbar spine and 0.018 g/cm^2 at the hip.

2.2.2. Assays

Fasting blood samples (25 ml) were collected in the morning (08 h30–10 h00) in sterile chilled tubes by standard venipuncture technique. The samples were left to clot at room temperature and were then centrifuged at 2500 rpm for 10 min at 4 °C. Serum samples were stored at −80 °C until analysis. All samples were run in duplicate and, to reduce inter-assay variation, they were analysed in a single session.

Concerning bone metabolism, serum samples were assayed by Cobas 6000 (Roche Diagnostic, Mannheim, Germany) for osteocalcin (OC), procollagen type I N-terminal propeptide (PINP), and type I—C telopeptide breakdown products (sCTx). The inter- and intra-assay CVs for the latter three parameters were lower than 7%. As previously described [26], serum sclerostin and periostin were measured with quantitative sandwich ELISA kits from Biomedica (Bioactive Sclerostin ELISA BI 20472 and periostine BI 20433; Vienna, Austria). The intra-assay and inter-assay CVs were < 3% and ≤6%, respectively. The periostin ELISA assay uses a mouse monoclonal antibody directed against the mid-region and a goat polyclonal antibody directed against epitopes that spread across the whole periostin molecule and are mostly conserved between the isoforms. The range for the assay is 125 to 4000 pmol/l.

3. Statistical analysis

The characteristics of the individuals with SCI are described with proportions for categorical variables and with means and standard deviations (SD) for quantitative variables. Comparisons between groups were performed using Student's *t*-test for quantitative variables and the Chi-square or Fisher's exact test for qualitative variables. Adjustments for age and gender were performed using multivariate linear regression models.

To determine the relationships between bone markers and time since injury, we first computed the z-scores of bone markers for age using the control group data. We then examined the relationships between these z-scores and the time since injury using a linear spline regression model with a single free knot. This method enabled us to distinguish two periods showing different changes in the bone markers over time and to locate the breakpoint between these periods (the location of the free knot was identified according to the method presented by Molinari et al. [27]. Relationships between bone markers and between the bone markers and aBMD were analysed with Spearman's correlation coefficient. The age-adjusted Spearman partial correlation coefficient was used in the stratified analysis according to time since injury (> and ≤2 years).

All analyses were conducted by the Medical Statistics Department of the Montpellier University Hospital using SAS software (V.9.3, SAS Institute, Cary, NC, USA, and R V.3.2.3). A two-sided p value of < 0.05 was considered to indicate statistical significance.

4. Results

Patient characteristics are shown in Table 1. All were Caucasian with ages ranging from 20 to 80 years. The time from the initial injury varied from 0.1 to 49.1 years with a median of 11.8 years. Most individuals with SCI presented a complete medullar motor lesion (AIS A or B, n = 117); 74.1% were paraplegic and 25.9% were tetraplegic. Seventy-two post-SCI fractures were counted with a similar prevalence for men and women (p = 0.895). They occurred on average 14.5 ± 12.0 years (range: 0.5–48.8 years) following SCI. Fractures were mostly observed in femoral and tibial regions and in chronic patients compared with acute patients. Prevalence of fracture did not seem to be affected by the paraplegia vs. tetraplegia status (Table 2), or gender (Supplemental file).

Menopausal status was similar between individuals with SCI and

Table 1
Clinical characteristics and biochemical profiles of individuals with spinal cord injury and able-bodied controls.

Variables	Controls (n = 40)	Individuals with SCI (n = 131)	Acute SCI individuals (≤2 years) (n = 23)	Chronic SCI individuals (> 2 years) (n = 108)
Age, years	42.6 ± 13.3	42.8 ± 13.7	34.6 ± 12.7	44.6 ± 13.3###
Men/women (n)	17/23	96/35***	18/5	78/30
BMI, kg/m^2	23.6 ± 2.4	23.1 ± 4.6	22.5 ± 4.1	23.2 ± 4.7
Menopausal status n, (%)	9 (39.1)	10 (28.6)	1 (20.0)	9 (30.0)
Characteristics of SCI				
Duration of SCI, years [min;max]	–	14.2 ± 12.1 [0.1;49.1]	0.7 ± 0.6 [0.1;2.0]	17.1 ± 11.5 [2.2;49.1]###
Paraplegia/tetraplegia (%)	–	74.1/25.9	78.3/21.7	73.1/26.9
Complete injury, n (%)	–	117 (89.3)	20 (87)	97 (89.8)
Spasticity, n (%)	–	75 (59.1)	7 (31.8)	68 (64.8)##
Post-SCI fracture history				
Fracture (n)	–	72	4	68###
Patients with 1 fracture, n(%)		36 (27.4)	2 (8.6)	34 (31.4)
Patients with 2 fractures, n(%)		12 (9.1)	1 (4.3)	11 (10.2)
Patients with 3 fractures, n (%)		4 (3.0)	0 (0)	4 (3.7)
Post-SCI occurrence[d] (years) [min;max]	–	14.5 ± 12.0 [0.5;48.8]	0.7 ± 0.2 [0.5;0.9]	15.3 ± 11.8 [0.7;48.8]###
Fractures location				
Femur	–	35	3	32
Tibia	–	22	–	22
Other[e]	–	15	1	14
aBMD				
Total hip[a] (g/cm^2)	0.925 ± 0.147	0.690 ± 0.221***	0.920 ± 0.258	0.639 ± 0.176###
Z-score total hip[a] (SD)	−0.16 ± 0.96	−2.34 ± 1.49***	−0.89 ± 1.56	−2.64 ± 1.30###
Lumbar spine[b] (g/cm^2)	0.986 ± 0.158	1.015 ± 0.168	0.982 ± 0.115	1.02 ± 0.18
Z-score lumbar spine[b] (SD)	−0.23 ± 1.46	−0.34 ± 1.53	−0.89 ± 0.86	−0.21 ± 1.63
Radius[c] (g/cm^2)	0.597 ± 0.067	0.613 ± 0.072	0.623 ± 0.072	0.611 ± 0.072
Osteoporosis status, n (%)	1 (2.5)	76 (60.8)***	6 (28.6)	70 (67.3)###
Bone turnover markers				
Osteocalcin, ng/ml	24.6 ± 9.5	25.9 ± 10.4	33.2 ± 11.5	24.3 ± 9.5###
PINP, ng/ml	60.4 ± 26.9	83.5 ± 63.1	161.8 ± 80.5	66.2 ± 42.5###
sCTx, ng/ml	0.48 ± 0.21	0.56 ± 0.37	1.01 ± 0.53	0.45 ± 0.22
Sclerostin, pmol/l	71.4 ± 34.4	53.7 ± 38.2***	49.9 ± 33.4	54.6 ± 39.2
Periostin, pmol/l	726.6 ± 166.6	854.7 ± 253.8**	1176.0 ± 314.1	784.9 ± 174.2###

Data are presented as mean ± SD. SCI: spinal cord injury; BMI: body mass index; SD: standard deviation; aBMD: areal bone mineral density; PINP: propeptide amino-terminal of type I procollagen; sCTx: serum carboxy-terminal telopeptide of type I collagen.

[a] n: 126 for all SCI individuals (91 males and 35 women).

[b] n: 79 for all SCI individuals (58 males and 21 women).

[c] n: 127 for all SCI individuals (94 males and 33 women).

[d] The post-SCI fracture occurrence is the time in years after injury when the fracture occurred.

[e] Other fractures included: scapula, sacrum, tarsus, ankle and pelvis. Densitometric osteoporosis was defined as a T-score < −2.5 DS measured at total hip.

** Indicates a significant difference with controls for $p < 0.01$.

*** $p < 0.001$.

Indicates a significant difference with individuals with acute SCI (< 2 years) for $p < 0.01$.

$p < 0.001$.

Table 2
Clinical characteristics and biochemical profiles of individuals with spinal cord injury according to paraplegic or tetraplegic status.

	Individuals with paraplegia (n = 97)	Individuals with tetraplegia (n = 34)	p-Values
Age, years	44.0 ± 14.2	39.4 ± 11.7	0.090
Men/women (n)	75/22	21/13	0.078
BMI, kg/m^2	23.6 ± 4.7	21.5 ± 4.0	0.021
Menopausal status n (%)	6 (27.3)	4 (30.8)	1.000
Characteristics of SCI			
Duration of SCI, years [min;max]	14.3 ± 12.7 [0.1; 49.1]	13.91 ± 10.36 [0.3; 35.8]	0.865
Complete injury, n (%)	84 (86.60%)	33 (97.06%)	0.113
Spasticity, n (%)	53 (56.38%)	22 (66.67%)	0.301
Post-SCI fracture history			
Fracture, n	51	21	–
Individuals with 1 fracture, n (%)	22 (22.7)	14 (41.1)	
Individuals with 2 fractures, n (%)	10 (10.3)	2 (5.8%	
Individuals with 3 fractures, n (%)	3 (3.1)	1 (2.9)	
Post-SCI occurrence[a] (years) [min;max]	13.9 ± 13.0 [0.5; 48.8]	15.6 ± 9.8 [3.1; 35.0]	0.318
Fractures location			
Femur	26	9	–
Tibia	13	9	–
Other[b]	12	3	–
aBMD			
Total hip (g/cm^2)	0.720 ± 0.196	0.610 ± 0.265	0.004
Z-score total hip (SD)	−2.13 ± 1.23	−2.91 ± 1.96	0.010
Lumbar spine (g/cm^2)	1.047 ± 0.155	0.961 ± 0.177	0.026
Z-score lumbar spine (SD)	−0.07 ± 1.38	−0.79 ± 1.68	0.044
Radius (g/cm^2)	0.623 ± 0.065	0.580 ± 0.085	0.003
Osteoporosis status, n (%)	54 (58.7%)	22 (66.7%)	0.421
Bone turnover markers			
Osteocalcin, ng/ml	26.2 ± 10.5	24.88 ± 9.99	0.471
PINP, ng/ml	82.6 ± 63.4	86.29 ± 62.92	0.853
sCTx, ng/ml	0.51 ± 0.29	0.69 ± 0.52	0.176
Sclerostin, pmol/l	57.3 ± 41.5	43.3 ± 24.0	0.014
Periostin, pmol/l	865.0 ± 266.2	824.7 ± 214.8	0.764

Data are presented as mean ± SD. SCI: spinal cord injury; BMI: body mass index; SD: standard deviation; aBMD: areal bone mineral density; PINP: propeptide amino-terminal of type I procollagen; sCTx: serum carboxy-terminal telopeptide of type I collagen.

[a] The post-SCI fracture occurrence is the time in years after injury when the fracture occurred.

[b] Other fractures included: scapula, sacrum, tarsus, ankle and pelvis.

controls and between individuals with paraplegia and tetraplegia.

4.1. Sclerostin, periostin, bone turnover markers and areal bone mineral density

Sclerostin levels were significantly lower and periostin levels were significantly higher in individuals with SCI (Table 1; Fig. 1) than in healthy controls.

Concerning bone metabolism markers, when all samples were studied, no difference between persons with SCI and controls was observed (Table 1). In both groups, values of sclerostin, periostin and bone turnover markers did not differ with gender. Conversely, aBMD appeared higher in men than women, both in individuals with SCI and healthy controls (see Supplementary materials).

The aBMD measurements were not available for 5 individuals with SCI at the hip, for 52 individuals at the lumbar spine, and for 4 individuals at the radius (Table 1). The main reasons were the presence of osteosynthesis material, heterotopic ossifications and muscle contractures. The individuals with SCI had noticeably lower aBMD at the hip, with a z-score representing only −2.3 SD of normal values and ranging from −6.3 SD to +1.7 SD. Lumbar spine and radius aBMD values in the individuals with SCI were similar to the values in healthy controls. The difference between men and women for aBMD at the total hip was similar in individuals with SCI and healthy controls, suggesting that SCI had no gender effect on aBMD at this bone site (see Supplementary materials). Individuals with SCI presented a higher prevalence of osteoporosis (60.8%) than controls (2.5%) and individuals with chronic SCI were more affected than individuals with acute SCI. Paraplegia vs. tetraplegia status (Table 2) and gender did not affect the prevalence of osteoporosis.

4.2. Effect of SCI duration on bone parameters

Individuals with SCI were subdivided into two groups (acute ≤2 years vs. chronic > 2 years) according to the time since neurological injury (Table 1). Individuals with acute SCI were younger than individuals with chronic SCI but presented similar BMI and similar percentages of paraplegia and tetraplegia or motor complete injury. Individuals with chronic SCI clearly presented significantly lower aBMD at the total hip compared with individuals with acute lesion and healthy controls, while no difference was observed for aBMD evaluated at the lumbar spine and radius. OC, PINP, sCTx and periostin levels were significantly higher in individuals with acute SCI than in those with chronic SCI, while sclerostin levels were similar in the two groups. Age adjustment did not modify the differences between groups. Individuals with acute lesion presented higher values of OC, PINP, sCTx and periostin and lower values of sclerostin than healthy controls, while individuals with chronic lesion presented only lower sCTx and sclerostin values (data not shown). Fig. 2 presents the periostin and sclerostin levels according to post-SCI duration and gender.

Fig. 3 shows the schematic representation of z-scores for all bone markers, according to the time since injury. Two phases were clearly identified: (1) a phase of decreasing values for all bone parameters, showing specific kinetics, and (2) a phase of stabilisation or slight increase, with values between −1 and +1 standard deviation. These two periods were dissociated by a breakpoint that was 5.2 years for OC, 4.3 years for PINP, 2.6 years for sCTx, 3.8 years for sclerostin, and 2.4 years for periostin.

4.3. Comparison between individuals with paraplegia and tetraplegia

Data showing the characteristics of individuals with SCI according to paraplegic or tetraplegic status are presented in Table 2. When the two subgroups were examined, the duration of SCI and age were similar, although the men were younger in the tetraplegia subgroup. All aBMD parameters were lower in the individuals with tetraplegia compared with those with paraplegia, but with specific gender differences. Total hip aBMD was lower only in women with tetraplegia compared with women with paraplegia, while aBMD at lumbar spine and radius were lower in men with tetraplegia compared with men with paraplegia. Whatever the gender, total hip aBMD was lower in both subgroups compared with healthy controls, while aBMD at the radius was increased in individuals with paraplegia compared with healthy controls (data not shown). Both subgroups presented similar values for markers of bone turnover except for sclerostin levels, which were lower in individuals with tetraplegia, mainly in men ($p = 0.077$), and for osteocalcin, which was lower only in women with tetraplegia compared with individuals with paraplegia. Age adjustment did not modify the results.

4.3.1. Relationship between fractures and densitometric osteoporosis

We evaluated the potential link between fractures (concomitant to SCI or after SCI) and levels of periostin and sclerostin according to the

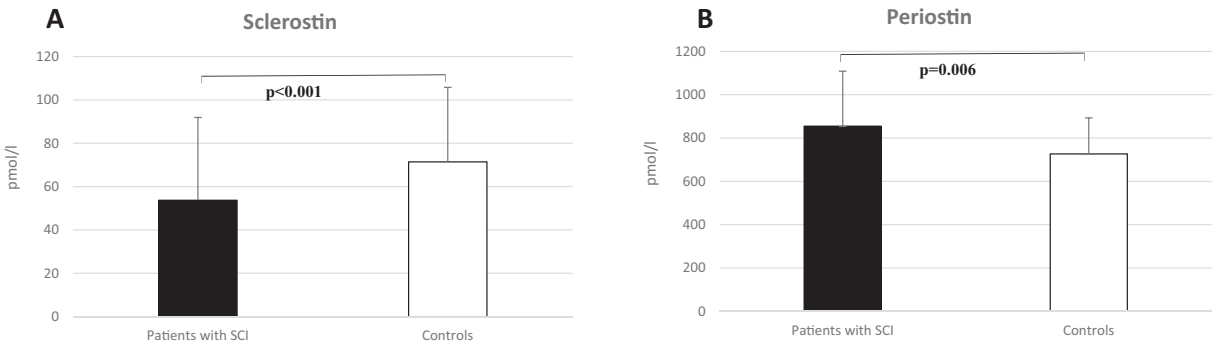

Fig. 1. Sclerostin (A) and periostin (B) levels in patients with SCI and in controls.
Legend: Data are represented by mean ± SD. SCI spinal cord injury.

acute or chronic phase. After age adjustment, acute individuals with SCI and fracture presented periostin and sclerostin levels similar to those with no fracture. Among individuals with chronic SCI, only sclerostin levels appeared lower in those with fracture than without fracture (43.5 ± 5.1 vs. 63.4 ± 4.5 pmol/l, $p < 0.01$). When individuals with SCI were subdivided according to densitometric osteoporosis, no difference was observed for periostin (759.9 ± 20.4 pmol/l with osteoporosis vs. 809.22 ± 29.0 pmol/l without osteoporosis; $p = 0.17$) or sclerostin (51.0 ± 4.2 pmol/l with osteoporosis vs. 63.1 ± 6.0 pmol/l without osteoporosis).

4.3.2. Correlation of bone turnover markers, aBMD and SCI-related parameters

As the variation in biological parameters clearly followed two phases (see Fig. 1) with a "normalisation" of values beyond 2 years, correlations between parameters were analysed independently before and after 2 years of SCI.

Briefly, in individuals with acute lesion (Table 3), PINP levels were positively correlated with OC, sCTx and periostin levels. PINP, sCTx, periostin, and aBMD at the total hip were negatively correlated with lengthening time since the initial injury. Only sCTX was positively correlated with aBMD at the total hip.

In individuals with chronic lesion (Table 3), OC, PINP and sCTx levels were positively correlated, as were sclerostin with periostin and PINP with periostin. aBMD measured at all sites was positively correlated with sclerostin levels. Only aBMD at the total hip and radius was negatively correlated with the time since the initial injury.

In controls (Table 3), no correlation between bone markers and sclerostin or periostin was observed. Periostin levels were positively correlated with aBMD at all bone sites.

5. Discussion

In the present study, we examined the effects of SCI on bone metabolism in individuals with a wide range of SCI durations (from 0.1 to 49.1 years). We found that individuals with SCI had higher periostin levels than healthy controls during the acute phase and similar levels in the chronic phase, while sclerostin levels were systematically suppressed in this population.

Recent studies have attempted to identify the underlying biochemical mechanisms of bone loss due to SCI and have particularly focused on sclerostin. Morse et al. [17] reported low sclerostin levels in a limited number of chronic men (n = 30) with a mean post-SCI duration of 22.4 years (range 4.1–42.6 years). In our larger study, we confirmed the low sclerostin levels in this population of individuals with paraplegia and tetraplegia [17]. In addition, sclerostin levels appeared positively correlated with aBMD at all bone sites only in those with chronic lesion. Interestingly, these levels were similar in individuals with short intervals (≤2 years) and long intervals (> 2 years) following SCI onset, suggesting that sclerostin production is chronically suppressed in individuals with SCI. Although high values have been reported [28], low values of sclerostin are generally found during the chronic phase [16–18], which may result from the reduced number of sclerostin-producing cells (osteocytes) due to the dramatic sublesional low aBMD. It has been suggested that circulating sclerostin is a biomarker of osteoporosis severity in individuals with chronic SCI, but it cannot be considered a mediator of ongoing bone loss [16,17]. Our finding of concomitant lower sclerostin levels and bone mass in individuals with tetraplegia compared with paraplegia reinforces this hypothesis. In the same way, the fact that patients with chronic SCI and fractures have lower sclerostin levels than chronic SCI without fracture may also attributed to a lower aBMD or a higher bone microarchitecture

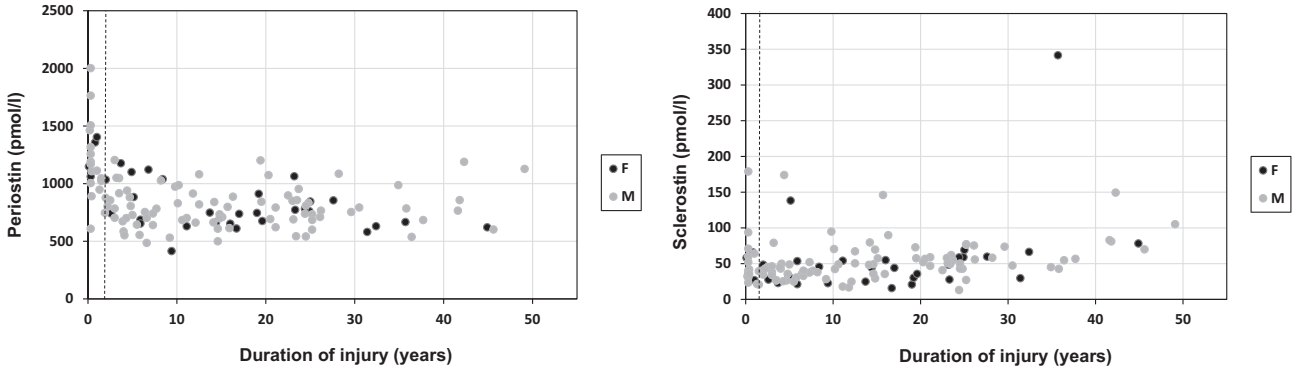

Fig. 2. Periostin and sclerostin values according to the duration of spinal cord injury and gender.
Legend: F: females; M: males.
A vertical line at 2-years is presented to differentiate acute and chronic phases.

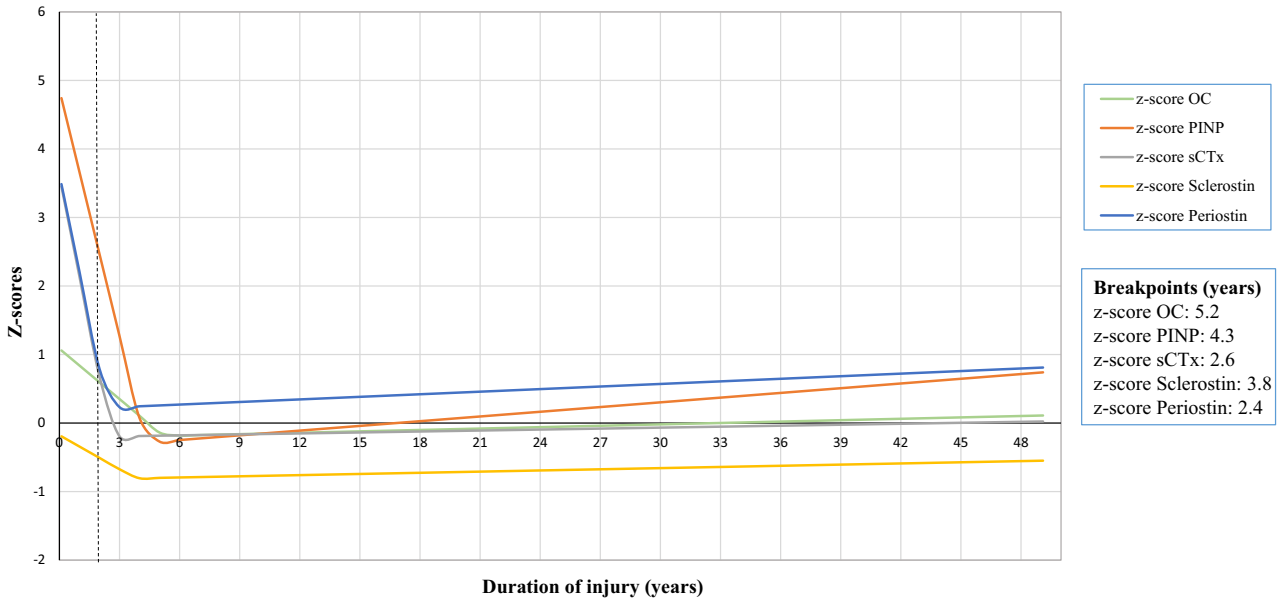

Fig. 3. Variation in z-score values for each marker of bone turnover according to the time since the initial injury.
Legend: Data are represented by z-score values. OC = osteocalcin, PINP = propeptide amino-terminal of type I procollagen, sCTX = serum carboxy-terminal telopeptide of type I collagen. For each bone marker, a breakpoint was determined to discriminate the two phases of variation (i.e. (1) a phase of decreasing values for all bone parameters and (2) a phase of stabilisation or slight increase, with values included between −1 to +1 standard deviation).
A vertical line at 2-years is presented to differentiate acute and chronic phases.

deterioration. Nevertheless, it should be noted that serum sclerostin does not necessarily reflect sclerostin levels in the osteocyte microenvironment. Thus, the canonical Wnt signalling may be repressed in these cells by sclerostin during all stages of bone loss after SCI, as suggested by the rise in rat bone mass following initiation of sclerostin inhibitory antibodies at 12 weeks post-SCI, a time when bone mass has reached a new lower equilibrium [14].

To our knowledge, only one study evaluated sclerostin levels in individuals with SCI as a function of the time since the initial injury [16]. The results demonstrated that those with acute SCI had higher sclerostin values than those with chronic SCI. The discrepancy in sclerostin levels between this study and ours may be attributed to the definition of the acute period: < 2 years as opposed to < 5 years for Battaglino [16], which may already correspond to the chronic phase of the disease. Conversely, the threshold at 2 years is generally acknowledged as the point at which the relative normalisation of aBMD occurs [5,6], even though residual bone loss was reported in the chronic phase [29]. We used z-score values and our results suggested that a stabilisation of aBMD occurred beyond of 2.6 years as indicated by the normalisation of sCTx levels. Moreover, in our study, the reduced levels of sclerostin in the acute phase might explain the concomitant increase in bone formation markers (OC and PINP). However, the variation in sclerostin levels during the acute phase remains subject to debate, since Gifre et al. [4] reported no variation in the first 12 months following SCI. The post-injury duration, as well as the lesion characteristics, including motor complete or incomplete SCI [17] and paraplegia or tetraplegia status observed in our study, might explain these divergent results in the acute phase. Nevertheless, we should note that the individuals with acute SCI were younger than those with chronic SCI, had sustained fewer fractures, and presented a lower prevalence of densitometric osteoporosis, which may also have modified the sclerostin levels. In the present study, only fragility fractures in the chronic phase seemed to influence sclerostin levels. It was also demonstrated that sclerostin levels are considerably influenced by the type of assay technique [30].

This is the first study to report periostin levels in individuals with SCI. In contrast to sclerostin, periostin levels were higher in individuals with SCI than in healthy controls. This finding was somewhat unexpected because in experimental studies mechanical loading induced an overexpression of periostin [21,31,32], while mechanical unloading, as observed in tail-suspension or sciatic neurectomy, was associated with a concomitant deterioration in bone structure (trabecular and cortical) and a decrease in periostin gene expression [19,33]. Nevertheless, data on the effect of reduced mechanical loading in humans are relatively sparse and a recent study in cosmonauts after a 4- to 6-month space flight, which is yet another condition of mechanical unloading, showed no variation in periostin levels despite the observation of a deterioration in bone mass and microarchitecture [34]. It is important to distinguish two periods for periostin evaluation: elevated values during the acute phase when bone resorption is intense and normal values in the chronic phase when bone mass tends to stabilise. It is interesting to note that, in patients with SCI, both periostin and PINP markedly decreased with time since injury which is responsible for the negative correlation seen in the acute phase and were positively intercorrelated in agreement with the fact that these two biological markers reflect the changes in the bone anabolic response following the trauma.

Given the data available on the periostin response to mechanical loading, it is difficult to interpret the higher values of periostin in individuals with SCI. This might indicate a protective mechanism of the skeleton because periostin is required for adaptive bone modelling and injury responses [35]. Nevertheless, as bone loss is present in individuals with SCI, an increase in periostin expression that drives an increase in bone formation would not be sufficient to compensate the high bone resorption. Kim et al. [25] similarly reported an increase in periostin levels in postmenopausal women with lower aBMD and osteoporotic fracture and suggested that this might reflect the presence of compensatory mechanisms in which periostin expression is increased to overcome poor bone health. Rousseau et al. [24] observed that high periostin levels are independently associated with increased fracture risk in postmenopausal women. They interpreted their findings by recalling that women with lower bone mass have higher mechanical strain on the remaining bone, which may increase periostin expression.

Moreover, although we do not exclude a transient mechanism of

Table 3
Correlation analysis of sclerostin and periostin with biochemical markers of bone turnover, areal bone mineral density, and time since injury in individuals with SCI and controls.

Parameters	Sclerostin	Periostin
Individuals with acute SCI (DOI ≤ 2 years)		
OC, ng/ml	−0.023	0.251
PINP, ng/ml	0.217	0.662***
sCTx, ng/ml	0.074	0.184
Sclerostin, pmol/l	–	0.322
Periostin, pmol/l	0.322	–
aBMD total hip, g/cm^2	0.272	0.304
aBMD lumbar spine, g/cm^2	0.013	0.449
aBMD radius, g/cm^2	−0.155	0.010
Duration of injury, years	−0.213	−0.510**
Individuals with chronic SCI (DOI > 2 years)		
OC, ng/ml	−0.144	0.146
PINP, ng/ml	−0.117	0.242*
sCTx, ng/ml	−0.129	0.004
Sclerostin, pmol/l	–	0.190*
Periostin, pmol/l	0.190*	–
aBMD total hip, g/cm^2	0.374***	0.197*
aBMD lumbar spine, g/cm^2	0.348**	0.172
aBMD radius, g/cm^2	0.262**	0.036
Duration of injury, years	−0.007	−0.142
Healthy controls		
OC, ng/ml	−0.029	−0.062
PINP, ng/ml	0.084	−0.095
sCTx, ng/m	−0.090	−0.160
Sclerostin, pmol/l	–	0.038
Periostin, pmol/l	−0.038	–
aBMD Total hip, g/cm^2	0.116	0.400*
aBMD Lumbar spine, g/cm^2	0.241	0.513***
aBMD Radius g/cm^2	0.160	0.316*
Age, years	0.669***	−0.234

Data are presented as coefficients of correlation (adjusted by age in persons with SCI). DOI: duration of injury; SCI: spinal cord injury; OC: osteocalcin; PINP: procollagen type I N-terminal propeptide; sCTx: serum type I−C telopeptide breakdown products; aBMD: areal bone mineral density.

* Indicates a significant correlation for $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

bone adaptation to the new mechanical constraints imposed by SCI, other factors may be responsible for the higher periostin levels during the acute phase. In particular, SCI due to a traffic accident or a fall is generally associated with multiple fractures. Fracture healing and bone repair have been shown to increase periostin levels [25,31,36,37] and upregulate periostin mRNA [38], which can be observed up to one year following the fracture [37], mainly after osteoporotic non-vertebral fracture [25]. In this specific SCI population, age, gender, paraplegic or tetraplegic status, fragility fracture, and osteoporosis status did not seem to influence the periostin levels, suggesting that other factors related to SCI that were not identified in our study may act on periostin production.

The present study confirmed that individuals with SCI present lower aBMD at the hip, while aBMD at the lumbar spine is not altered compared with healthy controls [1–6,39]. Interestingly, when subgroup analysis was performed according to paraplegia vs. tetraplegia status, individuals with tetraplegia showed lower aBMD at the hip, lumbar spine and radius compared with those with paraplegia.

This study has several limitations, including its cross-sectional design and the difference in gender ratio between the group of individuals with SCI and the healthy controls. However, these limitations are mitigated by the large population of individuals with SCI with a broad range of times since injury and the limited effect of gender on periostin and sclerostin levels. Moreover, the analysis of the effect of fractures and osteoporosis status, and the analysis of aBMD and markers of bone turnover in both individuals with SCI and age-matched healthy controls, are strengths of this study. Nevertheless, as previously reported for sclerostin in healthy subjects during bedrest, we cannot totally exclude the possibility that an initial modification in sclerostin or periostin occurs at a very early stage in the first days following SCI onset [40,41]. Moreover, it would be interesting to conduct a longitudinal investigation into the relationship between fracture repair and periostin levels in the acute phase.

In conclusion, this study showed for the first time that individuals with SCI had higher periostin levels than healthy controls during the acute phase and normalised levels in the chronic phase. Conversely, sclerostin levels were suppressed whatever the duration post-injury. The presence of densitometric osteoporosis does not seem to be associated with specific values of these parameters, whereas paraplegia vs. tetraplegia and fragility fractures seem to influence sclerostin levels only.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2019.07.019.

Declaration of Competing Interest

I certify that neither I nor my co-authors have a conflict of interest that is relevant to the subject matter or materials included in this work.

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