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Bone Mass and Hormone Analysis in Patients With Spinal Cord Injury: Evidence for a Gonadal Axis Disruption

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Context: Bone loss is a constant finding in patients with spinal cord injury (SCI).

Objective: We sought to evaluate potential modifiable factors that could lead to bone loss in complete motor paraplegia by examining gonadal axis hormones, vitamin D status, and bone markers.

Design: This is a cross sectional.

Setting: It includes SCI Outpatient.

Patients and other Participants: Twenty-nine chronic male patients with SCI were compared with 17 age-matched, able-bodied men.

Main Outcome Measure: The bone mineral density (BMD) of lower limbs and lumbar spine were measured using dual x-ray absorptiometry. Parathormone, 25-hydroxyvitamin D [25(OH)D], collagen type I C-terminal telopeptide (CTX), and sexual hormone were measured.

Results: Patients with SCI had lower BMD at the inferior limbs sites. CTX showed an inverse relationship with the time since injury. Patients had lower free T levels (SCI, 12.00 $\pm$ 2.91 vs controls, 19.51 $\pm$ 5.72; $P \leq .001$), and the majority (72%) had normal/low levels of gonadotropins. Low T, however, was not related to low bone mass in patients with SCI. In the controls, the 25(OH)D level was positively correlated with the T and with the lumbar spine BMD, but these correlations were not observed in the SCI.

Conclusions: Impairment of testicular function after SCI was indicated by the low levels of T and the loss of correlation between T and 25(OH)D levels; this correlation was present in the able-bodied controls. Inappropriate levels of gonadotropins were identified in most patients, featuring a hypogonadotropic hypogonadism and suggesting a disruption of the pituitary-gonadal axis. T concentrations might not be an effective target for bone loss therapy. **(*J Clin Endocrinol Metab* 99: 4649–4655, 2014)**

Osteoporosis (OP) in patients with spinal cord injury (SCI) was described in 1948 by Abramson (1), and with the increased survival of patients with SCI, OP has become a relevant complication, leading to many new studies (2–4). SCI presents many factors that contribute to the physiopathology of bone loss, such as mechanical, neurovascular, hormonal, and genetic aspects (5–7). The first cause that is usually implicated in bone mass loss in this population is the reduced mechanical load, which is a fundamental stimulus that maintains normal bone

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Abbreviations: AP, alkaline phosphatase; ASIA, American Spine Injury Association; BMD, bone mineral density; CTX, C-terminal telopeptide; Ca, total Calcium; Crea, creatinine; CV, coefficient of variation; DF, distal femur; FN, femoral neck; FSH, follicle stimulating hormone; FTesto, Free testosterone; INSL-3, insulin-like 3; LS, lumbar spine; LH, lutein hormone; OP, osteoporosis; PRV, pseudorabies virus; RV, reference value; SCI, spinal cord injury; SHBG, sexual hormone binding globulin; TF, total femur; T, total testosterone.

strength (8). Nonetheless, the magnitude of bone loss in patients with SCI seems to be much higher than that observed following other immobilization conditions (9), leading us to believe that other factors are involved. Neurogenic and hormonal factors are currently the most studied.

The ascending and descending tracts that are involved in bone remodeling, particularly autonomic and sensory innervation and brain connections are interrupted by SCI (10). Bone cells, such as osteoblasts, have β_2 adrenergic receptors and are affected by the autonomic alterations of SCI, leading to bone loss (6). Neurological and hormonal (including T and leptin) control of bone remodeling has been proposed, as factors that most likely communicate between these systems (11).

Leydig cells interfere with bone metabolism because they produce insulin like-3, which modulates osteoblast activity (11); these cells also express CYP2R1, a gene that encodes 25-hydroxylase, which is responsible for the local production of 25(OH)D (12). Patients with SCI with complete paraplegia have altered testicular innervation and, most likely, Leydig cell dysfunction. Although the role of sex hormones and vitamin D are widely studied in the pathophysiology of OP, few researchers have assessed these variables in patients with SCI.

The aim of this study was to evaluate potential modifiable factors that could interfere with bone mass in young patients with traumatic, complete paraplegia and exclude spinal cord injury by examining gonadal axis hormones, 25(OH)D status, and bone metabolism markers.

Materials and Methods

Subjects

This cross-sectional study was performed at an outpatient rehabilitation center. Our institution's ethics committee, which is based on the Human Subjects Institutional Review Board, approved the protocol, and all subjects provided written informed consent.

The following inclusion criteria were used in this study: male, age 18–50 years, diagnosis as a traumatic SCI subject according to American Spine Injury Association (ASIA) criteria A and B (A, complete; and B, incomplete sensitive patients) for greater than 6 months. The following exclusion criteria were implemented for the subjects with SCI: use of any type of medication that could interfere with bone metabolism (corticosteroids, calcitonin, or bisphosphonates), any history of bone metabolic diseases, spasticity above grade 3 on the Ashworth modified scale (13), presence of spinal instrumentation in more than two vertebrae, or heterotopic ossifications in the left hip.

Thirty-two males with SCIs at the T2–T12 level were recruited, and three were excluded (because of heterotopic ossification at both proximal femur sides) prior to analysis; these subjects were the last and not most recent consecutive subjects with SCI observed in the SCI ambulatory rehabilitation center. No patient with previous traumatic brain injury was included. Subjects were analyzed according to the different measured variables. An additional 17 able-bodied male adults were compared as the control group and were paired by age and sex (one was excluded after recruitment). All subjects with SCI were examined and classified prior to all exams by the same physical medicine rehabilitation physician following the ASIA classification (14), and spasticity was classified according to the Ashworth modified scale (13).

The subjects with SCI also completed a questionnaire regarding fractures and were subjected to lumbar spine and inferior limb radiographies to evaluate possible fracture occurrence and bone deformities. No fractures were detected on the x-rays. Three individuals had heterotopic ossification at the right hip that did not interfere with the data analysis.

The subjects were asked about the time since injury, smoking habits, the use of anticonvulsants, dairy product intake, sports activity, and standing. Standing was considered positive if a subject stood at least three times per week for a minimum of 30 minutes with an orthosis or stand-in-table (15). All data were collected during the spring. The dairy intake was adequate for both groups.

Wheelchair weight was measured, on a special scale, with and without the subject, and the difference between these weights was considered the subject's weight. Height was not measured, but patients were asked about this parameter. Body mass index was calculated using the weight divided by the height squared (16).

Calcium intake was estimated based on information from the summarized questionnaire concerning dairy milk product consumption, and participants who ingested at least 800 mg/d were considered normal consumers. In Brazil, the average nondairy calcium intake is 448 mg/d (17).

Dual x-ray absorptiometry

The subjects (SCI and controls) were subjected to dual x-ray absorptiometry exams (Discovery A) that provided the areal BMD (g/cm^2), and the z score was calculated using the means-national health and nutrition examination survey (NHANES) III database (18). In vitro quality control (long-term precision) was performed daily using a reference phantom for the lumbar spine (LS) and total body, according to the manufacturer's instructions. The in-vivo short-term precision was calculated by repeatedly scanning hospital patients, according to the convention of the International Society for Clinical Densitometry (19).

The following bone sites scanned were: femoral neck (FN), total femur (TF), LS, and distal femur (DF). The Quality Control Program showed coefficients of variation (CV) of 0.8% for LS and TF and 1.2% for FN. The DF was analyzed in the anterior-posterior position using the image from the total body scan, and the CV was 2.2% (20).

The evaluation included the four lumbar vertebral bodies (L1–L4). In the case of technical limitations for all four vertebrae analysis (scoliosis, spine instrumentation, or lumbar osteodegenerative process), at least two vertebrae were included. For the DF analysis, the subject was positioned in dorsal decubitus with the lower limbs in extension; this position was obtained using the proper equipment. A special region of interest was created based on prior studies (20). The BMDs of the FN and TF of the right and left femurs of patients with SCI were compared, and no

significant differences were found. Thus, data from the left femur were selected for analysis.

Blood analysis

Morning fasting blood samples were collected from all subjects on the day that the dual x-ray absorptiometry was performed. Creatinine (Crea), urea, aspartate transaminase, alanine transaminase, alkaline phosphatase (AP), and total calcium (Ca) levels were obtained using a colorimetric method (ADVIA 1650). The PTH level was measured using an in-house electrochemiluminescence immunoassay with an intra-assay error of 5% and interassay error of 13.4% (20, 22). The 25(OH)D level was quantitatively determined using chemiluminescence immunoassay technology (Liaison, DiaSorin) with an intra-assay CV of 4.6% and interassay CV of 8.2%. Total testosterone (T) was measured using a RIA (in-house method) with an intra-assay CV of 8.0% and interassay CV of 12.1% (23). Prolactin (PRL), lutein hormone (LH), follicle stimulating hormone (FSH), and sexual hormone binding globulin (SHBG) levels with intra-assay CVs of 1.4, 2.0, 3.7, and 2.6%, respectively, and interassay CVs of 8.45, 8.69, 11.6, and 17.8%, respectively and CTX with an interassay CV of 4.7% and intra-assay CV of 4.6% were measured using commercial chemiluminescence immunoassays (Elecsys Analyzers, Roche). Free testosterone (FTesto) was calculated using SHBG, T, and an albumin value of 4.3 g/dl.

Subjects were classified regarding vitamin D status into the following categories: deficient for 25(OH)D levels <20 ng/ml, insufficient for levels of 20–29 ng/ml, and normal for levels at least 30 ng/ml (24).

Statistical analysis

The data are described using numerical summaries (mean ± SD) for continuous variables and frequencies for all categorical variables. The right and left femurs were compared using Student paired *t* test. A nonpaired *t* test was used to evaluate group differences for age, height, and BMD. Blood sample analyses were compared with a Mann-Whitney nonparametric test. Correlations were performed with the Pearson or Spearman tests when applicable. Nonlinear exponential regression was performed to analyze the CTX and time given that/because injury. The data were tested for normality. Statistical analysis was performed using SPSS version 18.0 software (SPSS) and STATA version 12 (STATA). The level of significance was set at $P < .05$.

Results

The mean time of SCI was 63.8 months (median, 36 mo; range, 7–288 mo). There was no significant difference between the subjects with SCI and the controls regarding age (32.7 ± 6.9 and 31.9 ± 5.8 y, respectively; $P = .910$) or height (177.5 ± 6.3 and 177.0 ± 6.3 cm, respectively; $P = .728$). The body mass index was 26.8 ± 3.4 kg/m^2 for the control group and 23.7 ± 3.3 kg/m^2 for the subjects with SCI ($P = .004$). None of the patients were performing physical activities, and the controls were considered sedentary. None of the participants currently smoked. The level of injury varied from T2–T12. Twelve patients (41.37%) were flaccid, whereas 17 (58.63%) were spastic. Thirty-two percent of the subjects with SCI used anticonvulsants (gabapentin or carbamazepin), but with heterogeneous compliance. Kidney and liver functions were evaluated, and there was only a significant difference between the groups for Crea and AP (Table 1), which could be explained by the lower lean mass and the possible presence of heterotopic calcification, respectively.

Table 1. CTX, AP, Crea, and 25(OH)D Levels for the Control and Patients With SCI

Variable	Group	N	Mean	SD	*P*	RV
CTX, ng/ml	Control	17	0.439	0.212	.235	0.20–0.70
	SCI	29	0.475	0.556		
AP, U/L	Control	17	58.12	11.91	<.001	50–250
	SCI	28	89.19	10.59		
Crea, mg/dl	Control	17	1.03	0.15	<.001	0.8–1.2
	SCI	29	0.74	0.21		
25(OH)D, ng/ml	Control	17	25.81	7.26	.075	≥30
	SCI	28[a]	22.15	10.17		

[a] One patient was excluded due to a blood sample problem.

The mean PTH level was 29.77 ± 8.09 pg/ml and 34.26 ± 18.19 pg/ml for the controls and the patients with SCI, respectively ($P = .472$), and the Ca level was 9.49 ± 0.35 mg/dl and 9.38 ± 0.48 mg/dl, respectively ($P = .585$) (RV 8.0–10.5); these values were all within the normal range.

The mean value of the bone resorption marker CTX did not differ between the groups (Table 1); nevertheless, there was an inverse relationship between the CTX values and the duration of injury ($r = -0.60$; $P < .001$, Figure 1).

The mean levels of 25(OH)D were not significantly different between the groups (Table 1), although the mean value for the subjects with SCI was lower than that for the controls ($P = .075$). Nevertheless, there was a significant difference in the vitamin D status distribution: 12 (44.4%) subjects with SCI were vitamin D deficient, whereas only four subjects (23.5%) were deficient in the control group

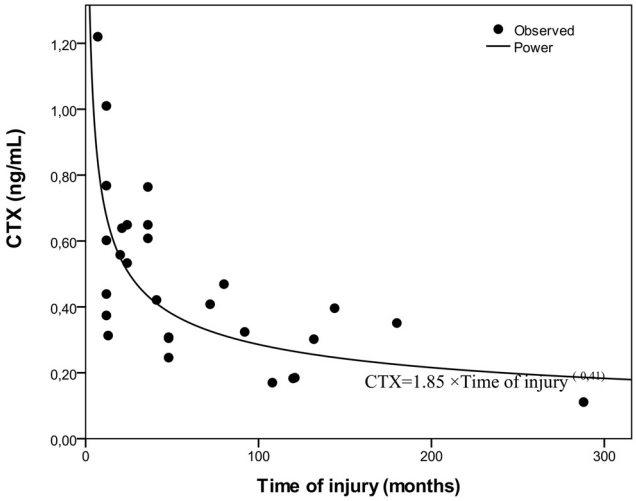

Figure 1. Negative correlation between CTX and time since injury in patients with SCI.

Table 2. Comparison Between the Hormone Values of the Control Group and the SCI Subjects

Variable	Group	Mean	SD	Minimum	Maximum	RV	*P*
LH, mUI/ml	Control	4.57	1.97	1.43	7.35	3.0–10.0	.060
	SCI	6.57	3.99	1.78	18.36		
FSH, mUI/ml	Control	3.41	2.02	1.29	7.64	0.3–10.0	.002
	SCI	6.09	4.32	1.19	21.40		
SHBG, nmol/L	Control	29.75	14.08	7.31	54.63	6–50	.170
	SCI	36.26	15.50	10.72	70.67		
T, ng/dl	Control	798.76	271.68	391.00	1406	350–1000	.009
	SCI	589.10	177.73	278.00	1009		
FTesto, pg/ml	Control	19.51	5.72	11.6	31.6	13–35	<.001
	SCI	12.00	2.91	6.74	18.8		

(P = .01). Insufficiency was found in eight (47.05%) individuals from the control group and 10 (37.03%) subjects with SCI. There was no significant difference between users and nonusers of anticonvulsants regarding 25(OH)D levels (18.16 ± 11.46 and 23.05 ± 7.32 ng/ml, respectively; P = .21).

A significant difference between groups was found for FSH, T, and FTesto (Table 2). The median PRL level did not differ between the subjects with SCI and the controls (8.21 ng/ml and 7.51 ng/ml, respectively).

Three patients with SCI (12%) showed T levels below the normal values (276, 318, and 320 ng/dl), and 18 (72%) subjects with SCI had low FTesto values. Although the mean FSH value was significantly higher in the patients with SCI, only three subjects with SCI (16.7%) with low FTesto levels had an FSH level that was above the normal limits (Figure 2), and two (11.1%) of these subjects had high LH levels.

There was a high positive correlation between FTesto and 25(OH)D in the control group (r = 0.66, P = .004), but this relationship was not observed in the subjects with SCI (Figure 3).

Bone mineral density

The subjects with SCI had lower bone mineral density (BMD) values than those of the controls at all femoral sites, although there was no significant difference in the LS BMD between groups (Table 3). The mean DF BMD from patients with SCI was 29.1% lower than that of the controls.

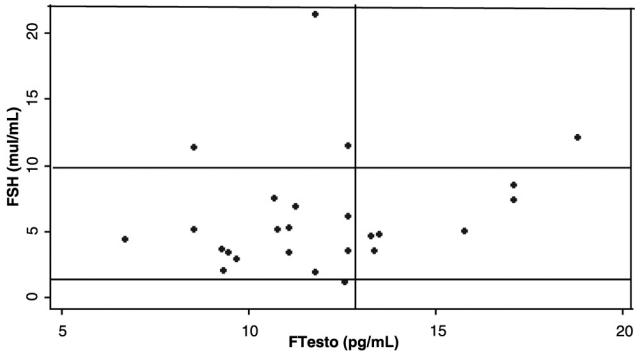

Figure 2. Distribution of FSH and free T in patients with SCI. The medium horizontal lines represent the normal range limits for FSH and the medium vertical line represents the lower limit for free testosterone levels.

BMD and modifiable factors

There was no significant difference between the standing (n = 26, 88%) and nonstanding (n = 3, 12%) groups regarding DF (0.880 ± 0.159 and 0896 ± 0.159 g/cm^2, respectively; P = .704), TF (0.752 ± 0.128 and 0825 ± 0.124 g/cm^2, respectively; P = .225) and FN (0.716 ± 0.119 and 0.807 ± 0.210 g/cm^2, respectively; P = .431).

There was a significant positive correlation between the LS BMD and 25(OH)D levels in the control group (r = 0.60; P = .01) but not in the SCI group.

There was also a positive correlation between FTesto and LS BMD (r = 0.63, P = .007) for the control group but not for the subjects with SCI. For T and BMD, there was no correlation for any site in the control group (DF, r = 0.17, P = .550; TF, r = 0.23, P = .376; FN, r = 0.04, P = .886; and LS, r = 0.46, P = .065). Unexpectedly, for the

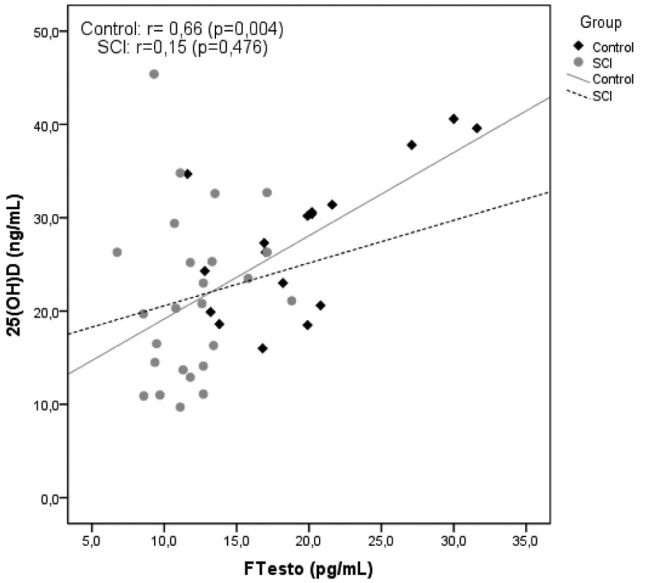

Figure 3. Correlation between free T and 25(OH)D in controls and patients with SCI.

Table 3. Comparison Between SCI Subjects and Controls for All BMD Sites

BMD g/cm²	Group	Mean	SD	Minimum	Maximum	n	*P*
BMD DF	Control	1.245	0.191	1.007	1.663	17	<.001
	SCI	0.884	0.169	0.610	1.281	28[a]	
BMD TF	Control	1.066	0.144	0.798	1.427	17	<.001
	SCI	0.765	0.128	0.479	0.994	28[a]	
BMD FN	Control	0.960	0.143	0.685	1.267	17	<.001
	SCI	0.721	0.114	0.542	1.092	28[a]	
BMD LS	Control	1.102	0.122	0.954	1.417	17	.500
	SCI	1.062	0.154	0.798	1.395	27[a]	

[a] Subjects were excluded because of technical problems (<2 vertebrae to analyze and technical problems when performing measurements at femoral sites due to an unsuitable position).

subjects with SCI, we observed an inverse relationship between T and the BMDs of the TF ($r = -0.49$, $P = .007$) and LS ($r = -0.35$, $P = .043$), but not for the BMDs of the DF ($r = 0.34$, $P = .074$) and FN ($r = -0.36$, $P = .062$).

Discussion

Loss of bone mass is a common and serious SCI complication, and this loss can result in fractures (25, 26). Time since injury, level of injury, and age are nonmodifiable factors for bone mass loss, but other factors can be modified, such as standing, the levels of 25(OH)D, and sexual hormones (27). Having a better understanding of all these contributing factors can help establish efficient interventions to prevent or diminish bone loss in this population.

Our results have shown low bone mass in the inferior limbs, but not in the LS of patients with SCI. The resorption marker (CTX) was inversely associated with the time since injury, suggesting that a high loss of bone mass occurs early after the lesion. The CTX level is useful for demonstrating the inverse relationship between the time since injury and bone loss, suggesting that early intervention would prevent greater losses (28).

Compared with the controls, the studied patients had a higher prevalence of vitamin D deficiency, which was determined using the 25(OH)D levels; however, 25(OH)D concentrations less than 20 ng/ml were found in both groups. The high prevalence of vitamin D deficiency in patients with SCI has been reported by others (29) and is partly due to the low accessibility to the outdoors and decreased opportunity for exposure to sunlight. The use of anticonvulsants (30) also causes low 25(OH)D levels, but this association was not found in our study population. Another important factor that could contribute to the low observed levels, even in the controls, in the present study was that the blood samples were obtained in the spring; vitamin D levels are lowest during this season (31).

A positive correlation between the 25(OH)D level and bone mass at LS was observed in the controls. 25(OH)D is a modifiable factor and as such, could be involved in the bone mass loss in subjects with SCI; and it is advisable, for bone health and muscle performance, that this deficiency is treated.

In addition, the discovery that the testis, particularly the Leydig cells, can be a source of 25-hydroxylation of vitamin D in men (32) and that the concentrations of 25(OH)D are normally positively related to T levels, opens another perspective to understand our findings because low T levels were found in most of our patients with SCI. Confirming this hypothesis, we observed an important positive correlation between the T and 25(OH)D levels in the control subjects. However, this correlation was absent in the patients with SCI, suggesting a possible disruption of testicular function. These subjects had lower sexual hormone production and impaired 25-hydroxylation of vitamin D, which are both potential markers for Leydig cell function.

Low free T concentrations were observed in 72% of our study population, most of whom had inappropriate (normal/low) levels of gonadotropins, which is a characteristic of a hypogonadotropic hypogonadism state. Evidence supports the existence of a neural central-gonadal axis that directly controls the Leydig cell production of T. Supporting our findings, Lee et al proposed, in a very elegant article, the existence of a neural brain-testicular pathway that regulates T release function independently of LH release by studying rats that were subjected to a spinal cord transection (33). Furthermore, they demonstrated the presence of a neuroanatomical connection between the testicles and central brain using a transneuronal labeling technique based on injecting pseudorabies virus (PRV) into the gonads of intact animals; the connection was disrupted by spinal cord injury. This disruption of the hypothalamic-pituitary-gonadal axis after an SCI has been suggested by others (34, 35). Naftchi and colleagues (36) also demonstrated a persistent low T level and inappropriate gonadotropin level in tetraplegic patients with SCI. These findings, however, are not agreed upon in the literature;

the results regarding sexual hormone levels in this population are debated. Some have reported hypergonadotropic hypogonadism, revealing a primary gonadal failure (36), similar to our observations in three of our patients, whereas others have observed normal T levels (34, 35). Some of these studies lack control groups (38), and in other studies, the severity of the patient injuries was heterogeneous and included partial and total patients with SCI (35). The differences in the study designs could partially explain the contradictory results found in the literature. In our casuistic study, we only included patients with complete motor SCI (ASIA A and B).

Our results with inappropriate levels of gonadotropins and disruption in vitamin D and T correlation suggests that neurological dysfunction that occurs after SCI might affect different pathways (central and peripheral) that could potentially affect bone mass. Androgens can act directly on bone tissue by binding to specific receptors on bone cells or can act indirectly by producing estrogens via aromatization (39). In contrast, orchiectomy stimulates osteoclast proliferation through receptor activator of nuclear factos-Kappa B-ligand expression, increasing bone resorption (39). Nevertheless, simple hormonal replacement in hypogonadic patients may not completely reverse bone loss (40).

A recent study showed that testicular function could affect bone metabolism in ways other than via T levels. Leydig cells also produce insulin-like 3 (INSL), which has a role in osteoblast function (Ferlin, SElice et al, (11)]. INSL-3 is a member of the insuline-like hormone superfamily, is mainly produced in gonadal tissues, and is considered a biomarker of Leydig cell function.

In addition, the decreased testicular function suggested by the low T levels found in our study population was not sufficient to explain the low bone density that was only found in the inferior limbs and not at the LS segment, which was also below the SCI level. In fact, we found an unexpected inverse correlation between the total T levels and BMD in the patients with SCI, but not in the controls. This relationship was not observed for free T, and the meaning of these findings is difficult to determine at this moment. The consistency of these data must be confirmed, but for now, we conclude that the T concentrations in this population are not as relevant as we previously believed for the maintenance of bone mass. Consequently, T concentrations would not be an effective target for bone loss therapy, although important for other purpose, such as sexual dysfunction.

Recent data suggested that there is a neurological control of bone remodeling, and this homeostasis may be lost after a complete SCI (44). Leptin is one of the adipokines that could be the key to link bone metabolism and sexual hormones and could inhibit bone formation [Karsenty and Oury, (41)]. The peripheral and central actins of leptin appear to differ, and leptin seems to have a neurogenic influence [Elefteriou, (5); Turner, Kalra et al, (42)]. However, leptin levels were not assessed in our study.

The lack of a mechanical load on the inferior limbs in SCI paraplegic subjects most likely plays a role in bone loss (8); however, we could not demonstrate any difference in BMD between patients who were able to stand and those who were not.

This study has limitations. Although the included population was very homogenous, the sample size is relatively small, particularly because it was difficult for these individuals to participate in all procedures. It is a cross-sectional study and, as such, does not allow us to determine the probable cause. We did not measure the level of adipokines or INSL-3, and these factors may be important to shed light on the etiology of bone loss in this population. These measurements could be performed in future studies. We could not find any evidence that T concentrations would be an effective target for bone loss therapy in these patients, as was expected. Further investigation of the central control of bone remodeling and testis function might help to elucidate SCI bone mass loss.

In conclusion, patients with SCI have low BMD in their inferior limbs, and the loss rate is inversely related to the time given that/because injury (as shown by the biomarker CTX); thus, earlier antiresorptive interventions should be considered, along with the correction of vitamin D levels. An impairment of testicular function was demonstrated by the low T levels, and a hypogonadotropic hypogonadism was identified in most patients, suggesting a disruption of the central-gonadal axis, most likely due to the loss of innervation below the level of the SCI.

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