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Original Article

The role of androgens or growth factors in the bone resorption process in recent spinal cord injured patients: a cross-sectional study

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Study design: This cross-sectional study compared the androgen and growth factor profiles and the bone turnover of patients with spinal cord injury (SCI) *versus* able-bodied controls (AB).

Objective: Determine whether androgens, GH, or either IGF-I or IGFBP-3, are implicated in bone turnover alteration in patients with recent SCI.

Setting: Propara Center, Montpellier, France.

Methods: In all, 16 men (31.3 years) with complete SCI, seven paraplegics and nine tetraplegics, who had sustained injury an average of 3 months earlier, and 12 AB who served as controls (27.5 years) participated. Androgens, growth hormone and its mediators were investigated. The bone resorption process was evaluated by urinary and plasma type I collagen C-telopeptide (CTXu, CTXp), while bone formation was evaluated by osteocalcin (OC) and bone alkaline phosphatase.

Results: Total testosterone (TT) and the free androgen index (FAI) were significantly lower in the SCI patients, whereas FSH was significantly higher ($P<0.05$). These hormonal variations were not related to the level of neurological lesion. There was no significant difference in GH, IGF-I, or IGFBP-3 levels. CTXu and CTXp indicated high bone resorption activity in the SCI patients ($P<0.05$). Regarding bone formation markers, only OC was affected by neurological lesion ($P<0.05$). Basal hormone levels did not correlate with markers of bone turnover.

Conclusion: The high bone resorption process observed in SCI patients did not seem directly related to testicular endocrine abnormalities or an altered growth factor profile. Nevertheless, the reduced TT and FAI levels could be aggravating factors in the development of acute bone loss.

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Keywords: patient with spinal cord injury; bone loss; androgens; growth factors; markers of bone turnover

Introduction

Disuse osteoporosis is defined as a decrease in the amount of normal bone tissue that occurs as a consequence of clinical or experimental immobilization.[1,2] In patients with spinal cord injury (SCI), bone loss begins just after injury and peaks 3–5 months later.[1,3,4] This acute phase of bone loss has been characterized principally by a marked increase in bone resorption activity[3–5] that induces an alteration in bone mineral density (BMD) in the bone sites located below the neurological lesion.[1,6] In an earlier study[5] using dual-energy X-ray absorptiometry, however, we were unable to detect an alteration in bone status in recent SCI patients. We thus hypothesized that bone biochemical markers would be more specific to evaluate the bone resorption process in this period.[5] After approximately 2 years,[1,6] the metabolic process tends towards a new steady state, but BMD is still estimated to be only 50–70% of the normal values of healthy subjects. This reduction in bone mass is very likely at the origin of the

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pathological fractures of long bones occurring after minor trauma.[7] The reasons for the bone loss in patients with SCI remain unclear, although a marked decrease in the mechanical strain (muscle contractions and weight bearing) that is normally applied to bone is considered to be the major causal factor.[8] However, this decrease alone cannot explain the difference in bone loss magnitude observed between patients with SCI and able-bodied subjects (AB) undergoing prolonged intervals of bed rest.[9] Other nonmechanical factors related to neurological lesion that may affect bone integrity have been proposed such as the vasomotor paralysis that slows intraosseous circulation[10] or the modification in the body blood volume distribution.[11] Moreover, various hormonal abnormalities – and, in particular, the androgen and growth hormone (GH) deficiency known to be implicated in bone loss and bone turnover alteration in AB men[12,13] – have been highlighted in chronic SCI patients.[14–17] In the skeleton, GH is known to stimulate the proliferation and the differentiation of osteoblasts and to increase the synthesis of type I collagen, alkaline phosphatase and osteocalcin.[18] GH modulates the osteoblast production of insulin-like growth factor-I (IGF-I) which is an important mediator for GH-induced bone cell proliferation.[19,20] In addition, the expression of the androgen receptor in the osteoblasts suggested that bone is a target tissue for testosterone.[21] The impact of hormonal alterations on bone have nevertheless not been investigated in this clinical context.

The aim of this study was to clarify the endocrine basis of the acute and early bone resorption process following SCI and, specifically, to determine the importance of androgens and growth factors in the bone turnover of young male patients with recent complete neurological lesion. Moreover, we investigated whether the endocrine profiles of these patients were related to the level of injury.

Materials and methods

Subjects

In all, 16 male patients with SCI were recruited from PROPARA, a specialized SCI clinic (Montpellier, France). Ages varied from 21 to 44 years, with a mean of 31.3 ± 1.8 years. All patients had traumatic injury and the mean time since the event was 3 months (range 2.5–4). The neurological level varied from C4 to D12, and all patients displayed a complete motor lesion as defined by the American Spinal Injury Association.[22] Nine patients were tetraplegics and seven were paraplegics. Treatment of the spinal injury was carried out by osteosynthesis. At 3 months, all the patients with SCI were using manual or electrical wheelchairs. The able-bodied control group comprised 12 age-matched healthy males with a mean age of 27 ± 4.2 years (range 22–35).

None of the subjects had a history of metabolic bone disease or were taking medication known to affect bone metabolism or reproductive function. Other exclusion criteria were pathological fractures or heterotopic ossification, smoking, excessive alcohol intake, eating disorders, diabetes mellitus, hyperparathyroidism, thyroid dysfunction, liver disease and renal disorders.

The protocol was reviewed and approved by the Regional Research Ethics Committee (Languedoc Roussillon, France), and each subject gave informed consent before the study.

Biochemical measurements

The urine was collected during a 24-h period from 8:00 a.m. Intake of coffee, tea, tobacco and alcohol was prohibited for the 48 h before the day of investigation. Blood samples (20 ml) were taken between 8:00 and 9:00 a.m. and then centrifuged at 3000 r.p.m. for 10 min at 4°C. Serum and urine samples were stored at −80°C until analysis. All samples were run in duplicate and, to eliminate inter-assay variation, all the serum or urine samples were analyzed in a single session.

Bone resorption markers Urinary and plasma serum type I-C telopeptide breakdown products (CTXu and CTXp) were measured by ELISA (CrossLaps™ ELISA, OSTEOMETER A/S® Rodovre, Denmark). All data obtained from the 24-h urine samples were corrected by the urinary creatinine (Cr) concentration measured by standard colorimetric method. The reference range for urinary CTX is 71–279 ng/mmol/Cr in our laboratory, whereas reference range for serum CTX is <5500 pmol/l (manufacturer's specification).

Bone formation markers Intact osteocalcin (OC) was measured with an immunoradiometric assay (IRMA) (Elsa-OST-NAT™ CIS Bio International®, Gif/Yvette, France). Serum bone alkaline phosphatase (B-ALP) was measured by IRMA (Tandem®-R Ostase® Hybritec Inc.®, San Diego, CA, USA). The reference range for serum OC in our laboratory is 5–20 and 4–15 ng/ml for B-ALP.

Calcium homeostasis Calcium and phosphorus were determined by routine methods. Intact parathormone (iPTH) was measured by IRMA (N-tact® PTH SP Diasorin, MN, USA) and 1.25 $(OH)_2$ vitamin D ($1.25(OH)_2$ vitamin D) was measured by radioimmunoassay. The reference range for iPTH in our laboratory is 10–55 and 20–66 pg/ml for $1.25(OH)_2$ vitamin D.

Sex hormones Total testosterone (TT) and sex hormone-binding globulin (SHBG) concentrations were determined by IRMA (Immunotech, Marseille, France; and Cis Bio International, Gif-sur-Yvette, France, respectively). Serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were determined by immunofluorescent assays (Kriptor®, BRAHMS, Sartrouville, France). Estradiol (E) was determined by

enzyme-linked fluorescent assay (ELFA) (BioMerieux, Marcy-l'Etoile, France). The reference range for TT in our laboratory is 11–35, 10–50 nmol/l for SHBG, 1–5 mIU/ml for LH, 2–4 mIU/ml for FSH and <60 pg/ml for E.

Somatotropic hormones Growth hormone (GH) concentrations were measured by IRMA (HGH-CTK IRMA, Diasorin, Sallugia, Italy); insulin-like growth factor-I (IGF-I) was measured by IRMA (DSL, Diagnostic Systems Laboratories, Webster, TX, USA); and insulin-like growth factor binding protein-3 (IGFBP-3) was determined by IRMA (IGFBP-3 IRMA, Immunotech, Marseille, France). The reference range for GH in our laboratory is 0–5, 100–500 ng/ml for IGF-I and 2000–4500 for IGFBP-3.

Statistical analysis
The results are expressed as means and standard deviations. For continuous variables (age, weight, etc.), the distribution was tested by the Shapiro-Wilk statistical method. The comparisons of baseline levels among the two groups of SCI patients (tetraplegic and paraplegic) and the AB group were performed using Student's *t*-test. To correct for multiple testing, the significance level was adjusted according to the Bonferroni correction (=significance level/number of tests). Spearman correlation was performed to examine the degree of association between two parameters. A level of $P<0.05$ was accepted as significant. Version 8.2 SAS software (SAS Institute, Cary, NC, USA) was used for statistical analysis.

Results

Anthropometric characteristics
The anthropometric characteristics of the patients with SCI and the AB controls are presented in Table 1. No differences were found between patients and controls for age, weight, height, and body mass index (BMI).

Table 1 Anthropometric characteristics of patients with spinal cord injury and able-bodied controls

Parameters	*SCI Group* (n = *16*)		*Able-bodied Group* (n = *12*)
	Paraplegics (n = *7*)	*Tetraplegics* (n = *9*)	
Age (year)	35.8 ± 9.0	27.8 ± 8.1	26.9 ± 4.2
Weight (kg)	68.4 ± 9.7	66.5 ± 8.4	69.9 ± 7.6
Height (m)	1.75 ± 0.08	1.75 ± 0.07	1.76 ± 0.04
BMI (kg/m^2)	22.5 ± 3.2	21.8 ± 3.9	22.6. ± 1.9
DOI (days)	94 ± 10.6	106 ± 12.8	—

Data are presented as means ± SD. Patients with spinal cord injury (SCI), body mass index (BMI), duration of injury (DOI)

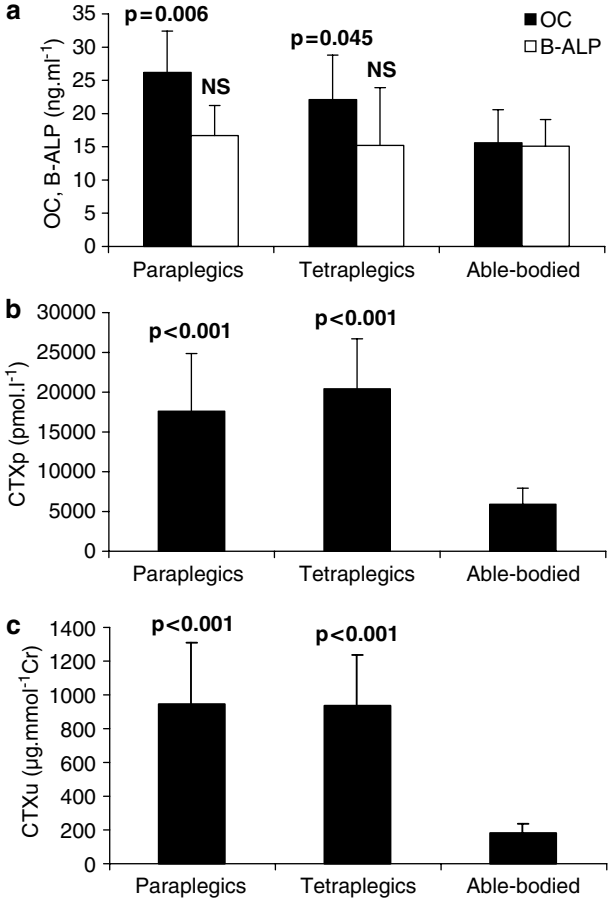

Figure 1 Bone biochemical marker levels in patients with spinal cord injury and able-bodied controls. Osteocalcin (OC) and bone alkaline phosphatase (B-ALP) (**a**), plasma type I collagen C-telopeptide (CTXp) (**b**) and urinary type I collagen C-telopeptide (CTXu) (**c**). The levels of bone urinary resorption marker were obtained from 24-h fasting urine samples after urinary creatinine correction. Data are presented as means ± SD. *P*-value represents the statistically significant difference *versus* able-bodied controls. NS: non significant

Bone biochemical markers
The biochemical marker levels are presented in Figure 1. Urinary and serum CTX indicated a dramatic increase in bone resorption ($P<0.05$) in the paraplegic and tetraplegic patients. The two markers of bone formation, OC and B-ALP, were differently affected by immobilization. OC was significantly higher ($P<0.05$) in patients with SCI than in the AB controls, while no variation was observed for B-ALP, which remained within the reference range.

Calcium homeostasis
Parameters of calcium homeostasis are shown in Figure 2. Serum phosphate concentrations were significantly higher ($P<0.05$) in persons with SCI than in the AB controls, while no significant difference was noted for serum calcium. The calciotropic hormones (iPTH and 1.25(OH)$_2$vitamin D) were suppressed in patients and were below the reference range.

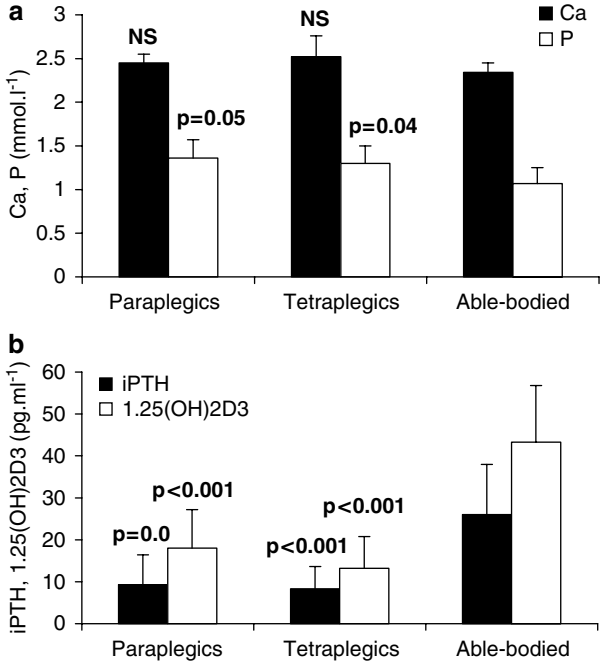

Figure 2 Parameters of calcium homeostasis in patients with SCI and able-bodied controls. Calcium (Ca) and phosphorus (P) (**a**), intact parathormone (iPTH) and $1.25(OH)_2$ vitamin D ($1.25(OH)_2D$) (**b**). Data are presented as means ± SD. *P*-value represents the statistically significant difference *versus* able-bodied controls. NS: non significant

Androgen and growth factor status

The mean hormone levels are listed in Figure 3. The TT concentration was lower in the SCI patients, being only 75% of the concentration found in AB, while the SHBG concentration was not significantly different between patients and AB controls. The free androgen index (FAI) was calculated as the molar ratio of TT to SHBG. FAI was significantly reduced ($P<0.05$) by 37% in the paraplegics and 25% in the tetraplegics. There were no significant differences between the patients and AB for E or LH plasma levels, while FSH was significantly higher in patients ($P<0.05$). All the testicular hormone values were within the normal clinical range.

There were no significant differences in the mean serum levels of GH, IGF-I or IGFBP-3 between the patients and AB controls (Figure 4). The ratio of IGF-I to IGFBP-3 was used as a marker of IGF-I bioavailability and was not found to differ between patients and controls. In SCI patients and AB, the hormonal concentrations were within the normal range for men, except in two patients who had low basal IGF-I levels and three who had low IGFBP-3 levels.

Relationship between neurological level and androgen and growth factor status

The values of hormonal and bone biochemical markers found in the paraplegic patients did not differ significantly from the values in tetraplegic patients. Although

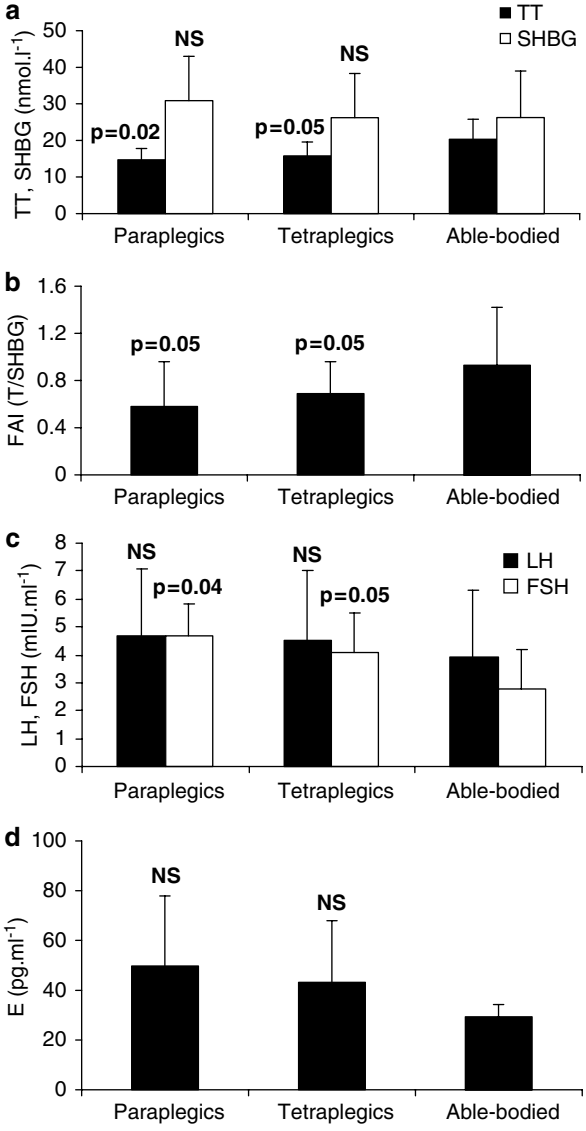

Figure 3 Sexual hormone in patients with spinal cord injury and able-bodied subjects. Testosterone (TT) and sex hormone-binding globulin (SHBG) (**a**), free androgen index (FAI) (**b**), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (**c**), estradiol (E) (**d**). Data are presented as means ± SD. *P*-value represents the statistically significant difference *versus* able-bodied controls. NS: non significant

the results regarding BII (IGF-I/IGFBP-3) tended to be higher in tetraplegic compared to paraplegic patients, they were not statistically different ($P=0.1$).

Relationship between androgen and growth factor status and bone homeostasis disturbance

As the level of neurological lesion did not affect the hormonal parameters, the groups of tetraplegic and paraplegic patients were combined to increase the statistical power. Nevertheless, no correlation was found between calciotropic hormone, androgen, or growth factor levels and markers of bone turnover.

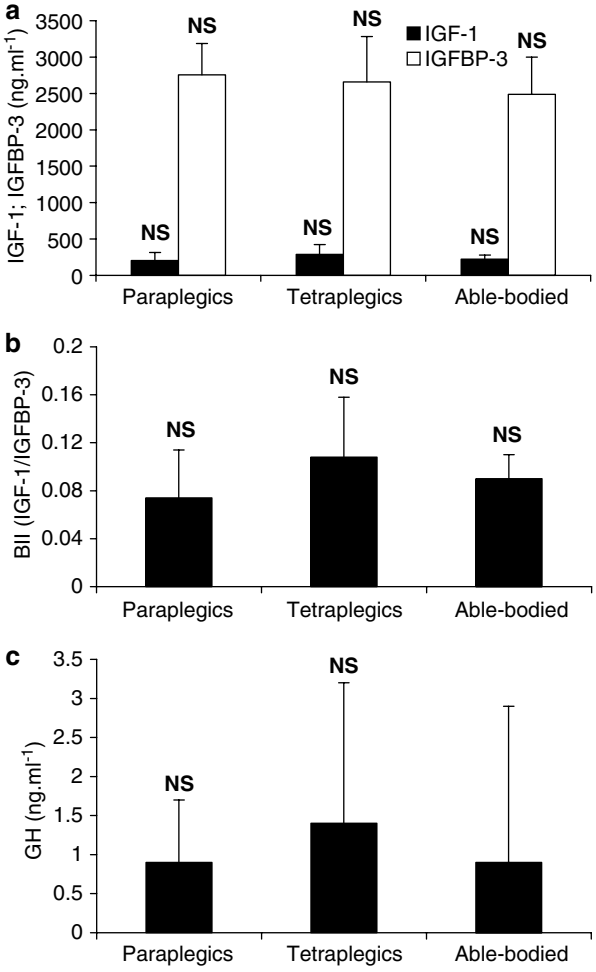

Figure 4 Somatotropic hormone in patients with spinal cord injury and able-bodied subjects. Insulin-like growth factor-I (IGF-I) and insulin-like growth factor binding protein-3 (IGFBP-3) (**a**), bioavailability IGF-I index (BII) (**b**), growth hormone (GH) (**c**). Data are presented as means $\pm$SD. NS: non significant

Discussion

Our results confirmed that bone homeostasis and bone turnover were altered in patients with recent SCI. This was demonstrated by a dramatic reduction in the calciotropic hormones associated with high-resorption activity.[3–5] In addition, our findings showed a disturbance in the plasma androgen profiles characterized by significantly lower TT and FAI levels in comparison with AB control levels. The studies investigating the effects of SCI on the hypothalamic-pituitary-gonadal (HPG) axis have shown conflicting results. Elevated levels,[23] normal concentration[24,25] and various degrees of decreased serum total and free testosterone[14,15,26,27] have been reported, without an alteration in SHBG[23] or estradiol level.[24,26] It seems likely that the postinjury duration[28,29] and the anatomical lesion level[25–27] would account for these conflicting data. Brackett *et al*,[25] and more recently Safarinejad *et al*,[27] reported that the incidence of androgen abnormalities in the men with an injury between T8 and T10 or T11 was significantly higher than in those with an injury at any other level. Our data indicated that the function of the HPG axis is altered early after the neurological lesion. Claus-Walker *et al*[28] found lower TT values for the first 18 months after paralysis, and a tendency toward normalization with time. We found no significant endocrine difference as a function of lesion level, possibly because our classification into paraplegic and tetraplegic groups was not specific enough. The mechanism(s) underlying the perturbations in the HPG axis and its clinical significance remain open to speculation. Several pathophysiological mechanisms have been proposed. For some,[14,26,27] the low testosterone level found in chronic patients with SCI could be due to diminished LH and FSH levels. Our results conversely suggest that the primary defect is testicular, with moderate alterations in endocrine function (as shown by low T and normal LH) and exocrine function (germinal cells), as suggested by the high FSH level. A possible role of hyperprolactinemia in testicular steroidogenesis has also been suggested,[15,24,26] and other nonhormonal factors could be involved. Wimalawansa *et al*[30] showed that strict immobilization in rat induced a suppression of testosterone production. Moreover, this alteration was considered to be partly responsible for the loss in weight-bearing bone, since testosterone replacement therapy prevented the bone loss.

Androgens are known to play a major role in the regulation of bone metabolism[31] and a reduction in TT level could be implicated in the bone resorption process observed in both the present and previous investigations.[1,6] However, no relationship was found between androgens and the markers of bone turnover. This apparently limited effect of hormonal abnormalities on bone health might be explained by the fact that, despite being low, the TT levels were within the normal adult physiological range, and a major alteration in gonadal function and long-term hypogonadism are necessary to induce osteoporosis.[12] Moreover, epidemiological studies have highlighted the importance of estrogens in regulating bone homeostasis in men.[32] Ongphiphahanakul *et al*[32] showed that BMD and bone biochemical markers were correlated with 17 β-estradiol rather than testosterone. As the estrogen values were slightly elevated in the SCI patients, an estrogen increase might thus compensate the reduced action of TT on the bone cells. Another argument in favor of the limited effect of the low TT level is that the bone loss observed in patients with SCI and AB with endocrine disorders affects different bone regions.[33] In castrated patients[12] and in men with acquired hypogonadism,[33,34] a progressive loss of BMD in the lumbar spine has been observed, while in patients with SCI this specific bone site was not affected.[6,9,33] Our data suggest that the low TT level in the patients with SCI was not the major factor affecting the early bone loss, but it might have been an aggravating factor. The absence of published reports of vertebral fractures in these patients lends support to our findings. Moreover, the disturbance in

hormone secretion could be implicated in other mechanisms such as abnormalities in spermatogenesis[24] and it may exacerbate the adverse lipid profile and body composition changes,[35] since testosterone replacement therapy was demonstrated to attenuate the alterations in myofibrillar proteins from SCI.[36]

Our data showed that GH-IGF-I levels were not altered during the early stages following SCI. This result was unexpected because, just as physical activity stimulates the secretion of GH,[37] physical inactivity reduces its release.[17] Similar results have been reported regarding basal plasma GH, although basal determination is of limited clinical interest.[17,24] However, despite the fact that the level of IGF-I, we found differs from the reports of previous studies,[16,17,24] we cannot exclude a modification in IGF-I and IGFBP-3 autocrine/paracrine activity. Decreased local production of IGF-I by osteoblasts in patients with recent SCI should not affect circulating IGF-I levels, because these systemic levels are mainly related to the large production of IGF-I by the liver. Shetty *et al*[16] reported a low level of IGF-I in chronic quadraplegic men. Bauman *et al*[17] confirmed a depressed plasma IGF-I level in younger individuals, but in both studies the mean postinjury duration in the SCI patients was longer (11.5 and 15 years) than in the present investigation (3 months). No data concerning the alteration in IGF-I in patients with recent SCI were available and it is probable that this endocrine disorder involves a long-term immobilization and inactivity period. Nevertheless, it appears that immobilization is not the only factor inducing a variation in the hormonal levels of patients with SCI because an increase in IGF-I and its binding protein (IGFBP-3) was observed in healthy subjects submitted to prolonged experimental bed rest.[8] This finding was interpreted as a possible compensatory effect of resistance to IGF-I in bone.[8]

Conclusion

In conclusion, this study demonstrates that plasma TT and FAI were reduced in patients with recent SCI, while the GH-IGF-I levels were not affected. No relationship was found between the low TT and bone biochemical markers, suggesting, however, that gonadal dysfunction has no direct effect on bone remodeling. This hormonal alteration might thus only be considered as an aggravating factor for bone resorption. Our results also confirmed that the neurological lesion induces a marked increase in bone resorption activity and an alteration in calcium homeostasis.

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