

# Factors related to decreased vitamin D levels in men with spinal cord injury living in a subtropical region

*Fatores relacionados a níveis reduzidos de vitamina D em homens com lesão da medula espinhal vivendo em uma região subtropical*

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## ABSTRACT

**AIMS:** To evaluate the levels of vitamin D, parathyroid hormone and calcium in individuals with spinal cord injury and to identify related factors.

**METHODS:** A cross-sectional study included men with spinal cord injury, living in the South Region of Brazil. A questionnaire was applied to evaluate socioeconomic data, life habits and health. The time elapsed since the spinal cord injury was considered. Vitamin D was evaluated in plasma via liquid chromatography; calcium and PTH were measured in serum by a colorimetric method and electrochemiluminescence respectively. For data analysis, linear regression and chi-square test were utilized. Values of  $p < 0.05$  were considered significant.

**RESULTS:** The study included 39 paraplegic or tetraplegic men with spinal cord injury. The mean age was  $35.52 \pm 9.78$  years, and the mean time since injury was  $6.09 \pm 5.55$  years. The mean biochemical levels were:  $19.0 \pm 6.98$  ng/mL for vitamin D;  $9.54 \pm 0.52$  mg/dL for total calcium; and  $34.81 \pm 10.84$  pg/mL for parathyroid hormone. Individuals with sun exposure for more than two hours daily had higher vitamin D levels than those exposed up to one hour per day ( $p = 0.001$ ). Linear regression identified an inverse relationship between lesion time and vitamin D levels (regression coefficient:  $-0.424$ ;  $p = 0.029$ ), while the other parameters did not show significant differences regarding the time elapsed since the lesion. Subjects with income above four minimum wages had higher vitamin D values (mean  $25.67 \pm 5.45$  ng/mL) when compared to those with income up to four minimum wages (mean  $18.43 \pm 6.79$  ng/mL) ( $p = 0.021$ ).

**CONCLUSIONS:** Levels of vitamin D were inversely correlated to the time elapsed since the spinal cord injury, probably due to insufficient sun exposure resulting from prolonged limitation of mobility. In addition, lower levels of vitamin D were associated to lower income. These factors may aggravate bone loss associated with decreased mobility resulting from spinal cord injury.

**KEYWORDS:** Vitamin D; parathyroid hormone; spinal cord injury; paraplegia; monthly income; socioeconomic status.

## RESUMO

**OBJETIVOS:** Avaliar os níveis de vitamina D, hormônio paratireoide e cálcio em indivíduos com lesão medular e identificar fatores relacionados.

**MÉTODOS:** Estudo transversal incluiu homens com lesão da medula espinhal, vivendo na Região Sul do Brasil. Foi aplicado um questionário para avaliar dados socioeconômicos, hábitos de vida e saúde. Foi investigado o tempo transcorrido após a lesão medular. A vitamina D foi avaliada no plasma por meio de cromatografia líquida; o cálcio e o hormônio paratireoideo foram dosados no soro por um método colorimétrico e eletroquimioluminescência respectivamente. Para a análise de dados, foram utilizadas regressão linear e teste do qui-quadrado. Valores de  $p < 0,05$  foram considerados significativos.

**RESULTADOS:** O estudo incluiu 39 homens paraplégicos ou tetraplégicos com lesão da medula espinhal. A média de idade foi de  $35,52 \pm 9,78$  anos e o tempo médio de lesão foi de  $6,09 \pm 5,55$  anos. As médias dos níveis bioquímicos foram:  $19,0 \pm 6,98$  ng/mL para vitamina D,  $9,54 \pm 0,52$  mg/dL para cálcio total e  $34,81 \pm 10,84$  pg/mL para hormônio paratireoideo. Indivíduos com exposição ao sol por mais de duas horas por dia apresentaram maiores níveis de vitamina D do que os expostos até uma hora por dia ( $p = 0,001$ ). A regressão linear identificou uma relação inversa entre o tempo de lesão e os níveis de vitamina D (coeficiente de regressão:  $-0,424$ ;  $p = 0,029$ ), enquanto os outros parâmetros não apresentaram diferença significativa em relação ao tempo decorrido desde a lesão. Os indivíduos com renda acima de quatro salários mínimos apresentaram maiores valores de vitamina D (média de  $25,67 \pm 5,45$  ng/mL) quando comparados com aqueles com renda até quatro salários mínimos (média  $18,43 \pm 6,79$  ng/mL) ( $p = 0,021$ ).

**CONCLUSÕES:** Os níveis de vitamina D foram inversamente correlacionados com o tempo decorrido desde a lesão da medula espinhal, provavelmente devido à exposição insuficiente ao sol resultante da limitação prolongada da mobilidade. Níveis mais baixos de vitamina D foram associados a menor renda. Estes fatores podem agravar a perda óssea associada à diminuição da mobilidade resultante da lesão da medula espinhal.

**DESCRIPTORIOS:** vitamina D; hormônio paratireoideo; traumatismos da medula espinhal; paraplegia; renda mensal; situação socioeconômica.

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**Abbreviations:** HPLC, high performance liquid chromatography; PTH, parathyroid hormone; PTHi, intact parathyroid hormone; SCI, spinal cord injury.

## INTRODUCTION

Worldwide incidence of spinal cord injury (SCI) is 3.6 to 95.4 patients per million [1] and in Brazil, although it is underreported, 6.000 to 8.000 cases are estimated per year [2]. SCI is predominantly caused by automobile accidents, falls, practice of different sports, and acts of violence [2].

Spinal trauma has been determined as an important factor to bone loss immediately after injury due to changes generated by immobility and reduced impact activity [3,4]. These changes include increased bone resorption, hypercalciuria and suppression of parathyroid hormone (PTH) [5]. It is estimated that 21 to 81% of individuals with this lesion will be diagnosed with osteoporosis [3] and have a 5 to 23% greater chance to present fractures when compared to individuals without physical limitations [6]. Fractures after spinal trauma also present additional complications, such as pain, pressure ulcers, and spasticity, among others [6]. They also lead to decreased quality of life and prolonged hospitalization. However, there are no protocols to detect, prevent or treat bone loss after spinal trauma [6,7].

Although immobilization due to SCI is the main triggering factor for bone loss, neural lesions, circulatory problems and hormonal changes are also implicated in the pathogenesis of osteoporosis [8]. Vitamin D plays an important role in calcium homeostasis [9], and when in low concentrations it is associated with myalgia and muscle weakness [10]. Factors such as low sun exposure, advanced age, dietary habits, genetic changes, skin color, use of sunscreens and geographic location influence the serum concentrations of 25-hydroxyvitamin D (25OHD), a portion that reflects the status of this element in the organism [11,12]. PTH, another hormone related to calcium metabolism, is important to balance bone remodeling because it maintains adequate levels of circulating calcium [4].

Individuals with SCI may have an inadequate or severely deficient level of vitamin D, which can result mainly in secondary hyperparathyroidism, decreased bone mineral density, osteoporosis and increased risk of fractures [13,14]. Secondary hyperparathyroidism and vitamin D deficiency also play an important role

in the development of post SCI osteoporosis [13]. Complete lesions, low body mass index, age, history of previous fracture, alcohol use, paraplegia, and longer time elapsed from the injury are considered risk factors for developing osteoporosis in individuals with SCI [1].

The association of secondary hyperparathyroidism with vitamin D deficiency may represent a modifiable risk factor for bone loss and fractures in individuals with SCI [13]. The prevention and treatment of post-injury bone loss also has a major impact on quality of life and prognosis for these patients. Therefore, evaluation of bone metabolism markers in order to identify the profile of individuals with SCI may contribute to the establishment of a strategy for the prevention and/or treatment of bone loss.

The purpose of this study was to verify the serum levels of vitamin D, PTH and calcium in individuals with SCI and their relationships with the time elapsed since the injury, time of sun exposure and socioeconomic level. To our knowledge, this is the first study that includes individuals with SCI from the southern region of Brazil in order to evaluate parameters related to bone metabolism.

## METHODS

The study was approved by the Research Ethics Committee of Pontificia Universidade Católica do Rio Grande do Sul under the protocol of number 11/05716. All participants signed an informed consent term, in compliance with the guidelines and standards for research on human beings of the Resolution 466/2012 of the National Health Council of Brazil and of the Helsinki Declaration.

The study included male individuals with SCI, attended in institutions of support or rehabilitation from the metropolitan region of Porto Alegre, Rio Grande do Sul state, which is located in the South Region of Brazil, within a subtropical area. Unlike most of Brazil, the southern region has a temperate climate, with well-defined seasons and many sunless days.

Individuals were invited by the coordinators of the supporting or rehabilitation centers to participate in the study. Those who met the inclusion criteria and who accepted to participate were then contacted by the researchers, and interviews and sample collection were scheduled.

A structured questionnaire composed by 17 questions, elaborated by the authors, was applied. The questionnaire included information such as age, injury time, cause of injury, general lifestyle habits

(exercise practice, use of tobacco, and time of sun exposure), income, general health, medications in use, and food. The questionnaire was applied by two of the authors, in a standardized manner. Application of the questionnaire was performed in the institutions facilities frequented by the volunteers.

Determination of intact PTH serum levels (PTH<sub>i</sub>) was done by electrochemiluminescence (ECLIA) (Roche®), with reference values between 15 and 65 pg/mL. Total calcium, creatinine, urea and albumin were measured in serum by dry chemistry on Vitros® 950 Johnson & Johnson equipment, using the reference values of 8.4-10.2 mg/dL for total calcium, 3.5-5.0 g/dL for albumin, 19-43 mg/dL for urea and 0.65-1.25 mg/dL for creatinine.

Plasma levels of 25-OH-vitamin D<sub>3</sub> were determined by high performance liquid chromatography (HPLC) adapted from the Kandár and Záková method. After plasma deproteinization with ethanol, the metabolite was isolated from the matrix by solid phase extraction in octysilic cartridges containing 500 mg stationary phase. In this procedure, the extraction cartridges were conditioned with methanol and water, washed with ultrapure water and a mixture of methanol and water and finally eluted with methanol, which was evaporated to dryness. The dried extract was taken up with a methanolic solution of the internal standard (dodecanophenone). The resulting solution was injected onto a Lichrospher C18 (Merck®) column which was maintained at 52°C. The elution was performed with mobile phase consisting of acetonitrile and ultra-purified water (77:23, v/v), with a flow of 1.2 ml/min<sup>-1</sup> and chromatographic monitoring at 264 nm. Under these conditions, the total time of chromatographic analysis was 20 min, eluting 25-OH-vitamin D<sub>3</sub> in 7.6 min and dodecanophenone in 15.1 min. The method is linear between 5 ng/mL and 100 ng/mL. For each batch of 12 clinical samples commercial controls provided by Chromsystems (Munich, Germany) were processed.

The levels of PTH<sub>i</sub>, calcium, and vitamin D levels were demonstrated by percentage, mean and standard deviation. Linear regression and the Chi-square test were performed in the IBM SPSS 18.0 program to evaluate correlation and association between the variables of interest. Values of  $p \leq 0.05$  were considered significant.

## RESULTS

Forty individuals were evaluated and one was excluded from the analyses because renal function

markers and PTH levels were consistent with disorders that could interfere with bone metabolism. The general characteristics of the included subjects are shown in **Table 1**.

**Table 1.** General characterization of the sample and according to the type of paralysis in men with spinal cord injury.

Variable	Total 39 (100%) mean±SD	Paraplegic 30 (78%) mean±SD	Tetraplegic 9 (22%) mean±SD
Age (years)	35.52±9.78	34.41±9.82	39.33±9.15
Weight (kg)	74.71±15.92	73.53±13.85	78.78±22.16
Drug use (units per day)	2.55±2.19	2.35±1.88	3.22±3.07
Calcium (mg/dL)	9.54±0.52	9.53±0.51	9.60±0.57
Vitamin D (ng/mL)	19.04±6.98	20.13±7.18	15.27±4.92
PTH (pg/mL)	34.81±10.84	33.43±8.61	39.59±16.19
Injury time (years)	6.09±5.55	6.07±5.87	6.17±4.55

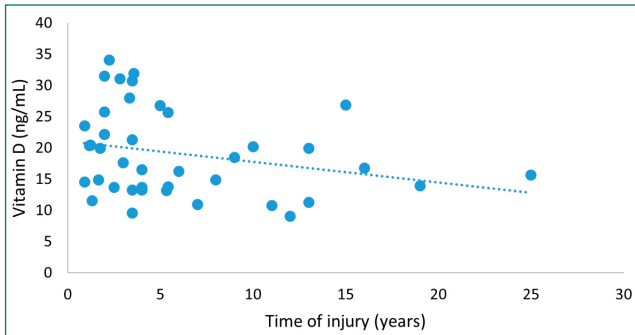
SD, standard deviation.

The age of the 39 participants varied between 18 and 58 years. Thirty (78%) were paraplegic and nine (23%) were tetraplegic. The youngest tetraplegic had 25 and the oldest 55 years. The shortest time elapsed since injury among the paraplegic group was 11 months and the longest was 25 years. In the tetraplegic group, the shortest time was 14 months and the longest 13 years. Regarding medications, 34 subjects (87%) used some type of medicine of continuous use.

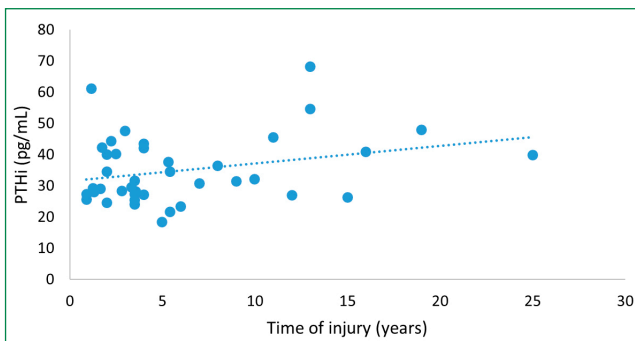
When assessed for vitamin D levels, individuals with a time of sun exposure greater than two hours per day had higher levels of vitamin D (mean 31.77±9.78) than those exposed up to one hour per day (mean 16.95±4.98) ( $p=0.001$ ).

From all the evaluated patients, 33 (85%) had an income up to four minimum wages, three (7.5%) between five and 10 minimum wages, and three (7.5%) greater than 10 minimum wages. Subjects with income above four minimum wages had higher vitamin D levels (mean 25.67±5.45 ng/mL) when compared to those with income up to 4 minimum wages (mean 18.43±6.79 ng/mL) ( $p=0.021$ ).

In the linear regression analysis corrected for hours of sun exposure, a negative relation was found between vitamin D values and time of SCI. The longer time from the injury, the lower vitamin D values ( $p=0.029$ ) (**Figure 1**). Regarding the time of SCI and PTH levels, there was a positive regression coefficient without statistical significance ( $p=0.070$ ) (**Figure 2**). When evaluated by type of injury (complete or incomplete) the biochemical parameters did not present significant difference (**Table 2**).



**Figure 1.** Linear regression of values of vitamin D and time elapsed since the lesion in men with spinal cord injury, corrected for hours of sun exposure. Univariate model; regression coefficient:  $-0.424$ ,  $p=0.029$ .



**Figure 2.** Linear regression of values of intact parathyroid hormone (PTHi) and time elapsed since the lesion in men with spinal cord injury. Univariate model; regression coefficient:  $0.253$ ,  $p=0.381$ .

**Table 2.** Vitamin D, parathyroid hormone and total calcium levels according to complete or incomplete spinal cord injury.

Variable	Complete injury n=23	Incomplete injury n=16	P
	mean±SD	mean±SD	
Vitamin D (ng/mL)	20.0±6.54	17.5±7.58	0.276
PTHi (pg/mL)	34.3±10.39	35.47±11.80	0.756
Total calcium (mg/dL)	9.47±0.50	9.65±0.54	0.316

SD, standard deviation; PTHi, intact parathyroid hormone.

## DISCUSSION

According to published literature, a large proportion of people suffering from SCI are young adults, which was confirmed in this sample, whose mean age was 35 years. Nogueira et al. [15], in a study conducted with 59 subjects in the city of São Paulo, showed that the participants had an average age of 37 years. Opperman et al. [16] observed similar results in relation to the mean age of individuals with SCI.

A mean of 2.5 drugs taken per day was found in this sample. A study conducted in Minas Gerais, which

evaluated hospitalized patients with SCI, found that the daily average of drug use was greater, approximately 10 [2]. One possible cause for this difference is that the subjects in the present study were outpatients, while the other were inpatients. Pharmacological therapy should be carefully monitored, since it involves risk of adverse reactions and longer hospital stay [2].

The mean levels of vitamin D were 19.04 ng/mL, which, according to a guideline published by Hollick et al. [11], are considered as deficient. It's important to emphasize that this study was performed in a subtropical region, what could contribute with the low vitamin D levels. This finding is similar to the data published by Bauman et al. [17] that also evaluated subjects with SCI. These authors detected a mean concentration of vitamin D of 14 ng/mL in the assessed group. Likewise, Gifre et al. [3] also found a high incidence of hypovitaminosis D, reaching 60% in individuals with SCI.

An important and somewhat expected result was that the time elapsed since injury was inversely proportional to the vitamin D levels. This finding is probably related to the shorter time of sun exposure due to limited mobility. SCI is a complex condition that leads to biopsychosocial and lifestyle changes [15, 17].

This low solar exposure in patients with SCI could be related with longstanding hospitalization, immobilization, as well as the lack of financial conditions, which entails the impossibility of hiring a professional who could assist the patient's locomotion, in order to increase the time of sun exposition [18]. The association between low income and vitamin D deficiency found in this study is relevant because intervention of public policies could assist these patients in providing them with more outdoor living opportunities. As far as we know, there are no studies that found relationship between vitamin D levels and low income, which may represent another risk factor for hypovitaminosis D.

Synthesis and metabolism of vitamin D largely depends on exposure to short wave ultraviolet B (UVB) rays, which will initiate the entire process of activation of 25-OH-vitamin D in 1,25(OH)<sub>2</sub>-vitamin D, also known as calcitriol, via its substrate 7-dehydrocholesterol, into the epidermis [11]. In addition to a negative-acting on bone tissue, hypovitaminosis D may impair other aspects such as muscle strength, and cause complications related to different organs [19]. According to a study by Nemunaitis et al. [14], people with tetraplegia and those with pressure ulcer had severely decreased vitamin D values, with a mean below 10 ng/mL.

In the comparison between serum levels of PTH and time of injury we can observe a tendency for a positive correlation, which might indicate that the longer the injury time, the higher levels of PTH were found, although this finding was not statistically significant. A possible explanation for the lack of significance could be the short average injury time of the study sample. Compared to the mean baseline PTH parameters obtained by Bauman [12] (mean of 70.0 pg/mL), lower levels (mean of 34.81 pg/mL) were found in the present study. In contrast, in another study by Bauman et al. [20], mean PTH values were 38.0 pg/mL. The average injury time of the participants in our study was 6.09 years, lower than the average of 20 years found by Bauman et al. [20]. A study by Mechanick et al. [21], showed a lower mean PTH in individuals with complete lesion when compared to those with incomplete lesion, a relationship not found in the present study.

The suppression of the PTH-vitamin D axis is due to prolonged immobilization, when a process of accelerated bone demineralization occurs, increasing the calcemia and consequently reducing the secretion of PTH and conversion of the bioactive portion of vitamin D [17]. However, Karapolat et al. [8] highlight that in the acute period after the injury there is suppression of the PTH-vitamin D axis, while during the chronic phase, a reverse activity of PTH can be observed. PTH values gradually increased from the first days until 36 months after the SCI [22]. This may have possibly influenced the PTH values found in the present study.

Albumin, creatinine and urea were evaluated because renal function may be affected by variations in total calcium concentrations. No changes in albumin concentrations were identified, as occurred in the study by Bauman et al [20]. It is worth mentioning that ionized calcium is the one that best demonstrates the calcemic status [23], but we chose to use the total calcium dosage because it presents greater stability, is widely used in clinical practice, and provides a good information [23].

The technique used for measure the serum vitamin D is one of the most precise. The technique of 25-hydroxyvitamin D by HPLC-DAD, based on the study of Kich et al. [24], showed to be precise and accurate with adequate sensitivity. There are several commercial vitamin D dosing methods cited in the literature, more specifically 25-hydroxyvitamin D [25], presenting pros and cons. However, studies have shown a high variability between these techniques, which may lead to misinterpretations [26]. Although there are publications with divergent opinions about the most appropriate method, in most of them efficiency liquid

chromatography is defined as one of the best methods, because it presents a high accuracy [26]. Some other methods, such as competitive binding protein assays, may overestimate the values [27].

The comorbidities of the acute phase of SCI have been reduced due to advances in care, however there is still much to progress in preventing sequels in the chronic phase of SCI. One of these sequels is osteoporosis, which currently lacks specific protocols for rehabilitation therapies [28]. Understanding the complications of SCI allows the development of therapeutic approaches that can reduce comorbidities and improve quality of life of the patients.

We can conclude that the levels of vitamin D in paraplegic or tetraplegic individuals with SCI were inversely correlated to the time elapsed since the injury. This may be due to insufficient sun exposure resulting from prolonged limitation of mobility. In addition, lower levels of vitamin D were associated to lower income. These factors may aggravate bone loss associated with decreased mobility and low impact activity resulting from SCI.

Information should be given to persons with reduced mobility about the benefits of sun exposure and how sun exposure should be practiced as a prevention for vitamin D deficiency, since it doesn't have costs and because the literature demonstrates that normal levels of vitamin D prevent the complications described above and improve quality of life.

This the first study carried out in a group of individuals with SCI in the south region of Brazil that evaluates parameters related to bone metabolism. Follow-up of the studied sample including evaluation of bone mineral density should be performed.

## NOTES

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### Conflicts of interest disclosure

The authors declare no competing interests relevant to the content of this study.

### Authors' contributions

All the authors declare to have made substantial contributions to the conception, or design, or acquisition, or analysis, or interpretation of data; and drafting the work or revising it critically for important intellectual content; and to approve the version to be published.

### Availability of data

The authors declare to have had access to all available data and assume full responsibility for the integrity of these results.

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