Serum leptin, bone mineral density and the healing of long bone fractures in men with spinal cord injury

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Abstract

Previously reported fracture rates in patients with spinal cord injury range from 1% to 20%. However, the exact role of spinal cord injury in bone metabolism has not yet been clarified. In order to investigate the effects of serum leptin and bone mineral density on the healing of long bone fractures in men with spinal cord injury, 15 male SCI patients and 15 matched controls were involved in our study. The outcome indicated that at 4 and 8 weeks after bone fracture, callus production in patients with spinal cord injury was lower than that in controls. Besides, bone mineral density was significantly reduced at 2, 4 and 8 weeks. In addition, it was found that at each time point, patients with spinal cord injury had significantly higher serum leptin levels than controls and no association was found between serum leptin level and bone mineral density of lumbar vertebrae. Moreover, bone mineral density was positively correlated with bone formation in both of the groups. These findings suggest that in early phases i.e. week 4 and 8, fracture healing was impaired in patients with spinal cord injury and that various factors participated in the complicated healing process, such as hormonal and mechanical factors.

KEY WORDS: leptin, long bones fracture, spinal cord injury, bone mineral density
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INTRODUCTION

Among humans who suffer from three major disorders, including adipose tissue decomposition, neuroendocrine alteration and abnormal skeletal remodeling, spinal cord injury (SCI) is a long-standing and pressing public health problem. Recently, studies have focused on abnormal bone metabolism in patients with SCI [1,2].

As documented complications of SCI, reduction in bone mineralization and deterioration of skeletal microarchitecture occur predominantly in long bones of lower limbs [3,4]. However, other studies have demonstrated that SCI may result in the rising of various hormones that significantly increase serum osteo-inductive factors, such as osteocalcin and leptin [5,6]. Initially discovered as a satiety factor regulating food intake and energy expenditure, Leptin is the circulating protein product of the obesity (Ob) gene, which is synthesized and secreted by adipocytes [7,8]. As an important hormonal regulatory factor, leptin not only has influence on lipid metabolism, but also has positive effects on peripheral bone metabolism [9,10]. Therefore, in SCI patients with bone fractures, serum leptin can be a crucial linking molecule among changes in fat tissue, the neuroendocrine system and the osseous system [11]. A number of investigations have supported the positive effect of leptin on bone mineral density (BMD). It has been shown that leptin not only promotes osteoblastic differentiation [12,13], but also inhibits osteoblast apoptosis and osteoclastogenesis [14,15]. In addition, accelerated fracture healing and earlier osseous union were reported in rats who sustained limb fractures with associated SCI in previous studies [16,17]. Nevertheless, other results have been conflicting: numerous animal model investigations have revealed malunion, delayed union, or non-union sublesional limb fractures in mice with SCI [18,19], and osteoporosis in persons with SCI will increase the risks of lower limb fractures [20]. These findings indicate that pathophysiologic mechanisms of bone remodeling after SCI are more complicated than previously thought.

To investigate a link between serum leptin concentration, BMD and fracture healing response we investigated 30 consecutive male patients with long bone fractures with or without SCI. We collected relevant data in different phases of fracture

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healing and analyzed possible mechanisms involved in fracture healing to explore the effects of SCI on bone formation.

MATERIALS AND METHODS

Participants

Between January 2009 and December 2013, 15 male patients with SCI (six cervical, four thoracic, and five lumbar spine injuries) with lower extremity long bone fractures (six femurs, and nine tibias) were recruited from the Affiliated People's Hospital of Jiangsu University, China. The patients' demographics are presented in Table 1. The patients ranged from 23 to 64 years old with an average age of 46.60 years. The type and level of SCI were determined by computerized axial tomography and magnetic resonance imaging. SCI levels varied from C5-L3. On admission, all patients were evaluated by the American Spinal Cord Injury Association (ASIA) score. Besides, all SCI patients were unable to walk. In addition, a matched group of 15 consecutively admitted male patients (Table 1) with long bone fractures (seven femurs and eight tibias) and without SCI were included in the study. The age ranged from 25 to 68 years with an average age of 44.47 years. This group had similar fractures to the patients with SCI. Our exclusion criteria were polytrauma, significant soft tissue injury, open fracture, fracture with large bone loss, diabetes, bone-related diseases, immunosuppression, endocrinology disease, and use of medications, such as glucocorticoids and hormones.

All patients sustained an isolated shaft fracture of the femur or tibia and were treated with a closed, antegrade, reamed intramedullary nailing technique. These patients were followed up in the same rehabilitation unit. Ethical approval for this study was granted by the Affiliated People's Hospital of Jiangsu University, China, and all patients or authorized relatives gave informed consent.

Radioimmunoassay

Blood samples were taken from all subjects between 7:00 AM and 8:00 AM after an overnight fast at 2, 4, 8 and 16 weeks after treatment. Plasma or sera was obtained and assayed by the Linco RIA kit (LINCO Corporation, St Louis, MO, USA). Serum leptin concentration was detected by radioimmunoassay. For the radioimmunoassay, a fixed concentration of labeled tracer antigen was incubated with a dilution of ^{125}I-labeld leptin antiserum, so as to limit the concentration of antigen binding sites on the antibody. Provided that testing serum (unlabeled antigen) was added, the labeled tracer antigen and testing serum would compete for the limited antigen binding sites on the antibody. In the next step, antibody-bound tracer could be separated from unbound tracer. Finally, an instrument (DFM-96 radioimmunity-counter, Nanjing Medical University, China) was applied to determine the radioactivity and quantify the leptin concentration. The results were expressed in ng/ml.

Clinical and radiographic follow-up

At 2, 4, 8 and 16 weeks after the treatment, the fracture healing process was assessed clinically and radiologically. During the usual follow-up examinations, standard anteroposterior and lateral radiographs were taken for all patients. In addition, fracture consolidation was scored by 2 blinded radiologists who did not participate in the study and bone formation was evaluated by the Lane-Sandhu Score (Table 2) [21].

BMD measurements

BMD was assessed at 2, 4, 8 and 16 weeks after the treatment by dual-energy X-ray absorptiometry (Lunar Corporation, Madison, Wisconsin, USA). All scans were performed as per a standardized procedure and T and Z scores of the lumbar vertebra (L1-L4) were recorded. If patients underwent lumbar surgery, the involved vertebrae would be excluded and the upper or lower non-involved vertebrae

TABLE 1. Patients' demographic characteristics and the differences between SCI group and control group (mean±S.D.)

Variables	SCI group (n=15)	Control group (n=15)	p value
Age (y)	46.60±11.88	44.47±12.09	0.51
Weight (kg)	70.73±7.85	69.13±7.23	0.49
Height (cm)	173.73±7.28	172.27±7.34	0.57
BMI (kg/m^2)	23.62±1.63	24.06±2.72	0.21
Time to operation (h)	72.3±11.32	69±17.40	0.06
Fracture localization (%)			0.13
Femur	6 (40)	7 (46.7)	
Tibia	9 (60)	8 (53.3)	
Injury level (%)		-	
Cervical	6 (40)		
Thoracic	4 (26.7)		
Lumbar	5 (33.3)		
ASIA score (%)		-	
A	4 (26.7)		
B	6 (40)		
C	3 (20)		
D	2 (13.3)		

ASIA, American Spinal Cord Injury Association; BMI, body mass index,
Note: BMI was calculated as body weight (in kilograms) divided by height (in meters) squared

TABLE 2. Fracture formation was scored according to the Lane-Sandhu score by two independent radiologists.

Points	Callus and fracture line
0	No callus tissue, fracture line clear
1	25% callus tissue, fracture line still clearly visible
2	50% callus tissue, fracture line blurred
3	75% callus tissue, fracture line barely visible
4	100% callus tissue, no remaining fracture line visible

would be considered in the analysis. According to diagnostic categories of the World Health Organization [16], osteoporosis was defined as a T-score ≤-2.5, osteopenia was defined as a T-score <-1 and >-2.5 and normal bone mass was defined as a T-score ≥-1.

Statistics

All statistical analyses were performed using SPSS 12.0 (SPSS, Inc., Chicago, IL, USA) The clinical data were expressed as mean ± standard deviation, including BMD, bone callus volume, serum leptin level values and demographic parameters. All variables were normally distributed (Shapiro-Wilk test). Differences between the two groups were detected through the application of t test and one-way analysis of variance (ANOVA). The sample size is small, therefore Fisher's exact x^2 test was chosen. Pearson correlation was used to investigate correlations between variables along with linear regression. A P value of less than 0.05 was considered to be statistically significant. In order to investigate the relationship of serum leptin concentration with weight, BMI, and age, leptin-relative factors were controlled using a partial correlation coefficient.

RESULTS

General observations

No neurovascular complications or wound infections were observed in either group. After the surgery, hospitalization ranged from 9 to 22 days and no patients were lost to follow-up. With or without the assistance of a cane, 4 patients (2 patients with an ASIA score of C and 2 with an ASIA score of D) were able to walk at 8 weeks and 9 patients (4 patients with an ASIA score of B and all patients with an ASIA score of C or D) could walk at 16 weeks in the SCI group. However, all patients were able to walk within 2 weeks in the control group. At 2, 4, and 8 weeks after the operation, the average body mass index (BMI) of the SCI group was significantly lower than that of the control group (P<0.05) (Table 3).

Leptin serum level

From 2 to 16 weeks after the operation, the SCI group had significantly higher serum leptin levels than the control group (p<0.05) (Table 3). In the SCI group, the serum leptin level was significantly higher at 2 weeks than at all the other time points (p<0.05), whereas in the control group, no statistically significant differences were observed in serum leptin level at the different time points. Furthermore, there was a positive correlation between serum leptin level and BMI in the control group (p=0.001, r=0.604) instead of the SCI group (p=0.29) (Fig. 1).

TABLE 3. Comparison of values for BMI, BMD, leptin, and fracture formation in various phases in SCI group and control group (mean±S.D.)

Variables	Time (weeks)	SCI group (n=15)	Control group (n=15)	p value
BMI (kg/m^2)	2	20.17±1.05	22.34±2.62	0.000
	4	19.32±2.08	22.49±3.45	0.000
	8	22.09±1.28	23.03±0.86	0.038
	16	23.40±0.93	24.11±0.95	0.086
BMD (g/cm^2)	2	–0.382±0.40	0.363±0.38	0.000
	4	–0.671±0.52	0.417±0.21	0.000
	8	0.034±0.58	0.434±0.24	0.011
	16	0.164±0.59	0.412±0.36	0.21
leptin (ng/ml)	2	11.78±4.37	4.48±2.49	0.000
	4	9.15±2.42	3.56±1.85	0.000
	8	7.88±2.67	3.64±1.72	0.001
	16	7.20±4.10	3.86±2.41	0.013
Lane-Sandhu scores	2	0.67±0.62	0.73±0.70	0.67
	4	1.53±0.74	2.20±0.77	0.012
	8	2.40±0.73	2.93±0.59	0.027
	16	3.33±0.90	3.60±0.74	0.334

Note: The basal values of BMI were 21.7±1.05 kg/m^2, BMD were 0.441±0.31 g/cm^2 and leptin were, 3.40±1.825 ng/ml, according to our hospital data (the Affiliated people's Hospital with Jiangsu University, 303 local male health volunteers, age range: 19 to 62 years)

BMD measurement

At 2, 4, and 8 weeks after the operation, lumber vertebrae BMD of the control group was significantly higher than that of the SCI group (Table 3). After adjusting for BMI, this difference was still significant. In addition, serum leptin level was positively correlated with BMD in the control group (p=.002, r=0.384). But in the SCI group, no positive or negative associations between serum leptin level and BMD were observed (p=0.81) (Fig. 2).

Fracture formation

At 4 and 8 weeks after the operation, the mean Lane-Sandhu scores at the fracture healing area were significantly higher in the control group when compared with the SCI group. At both time points, the control group exhibited greater bone mass and better fracture healing than the SCI group. Moreover, serum leptin level had a positive correlation with fracture formation in the control group (p=0.037, r=0.27), which was observed in the SCI group only at 16 weeks (p=.011, r=0.63) (Fig. 3). In addition, it was found that higher BMD was correlated with better fracture formation in the control and SCI groups (p=0.000, r=0.85; p=.000, r=0.56, respectively) (Fig. 4).

DISCUSSION

This study demonstrated the effects of SCI on the relationships among serum leptin level, BMD and fracture healing

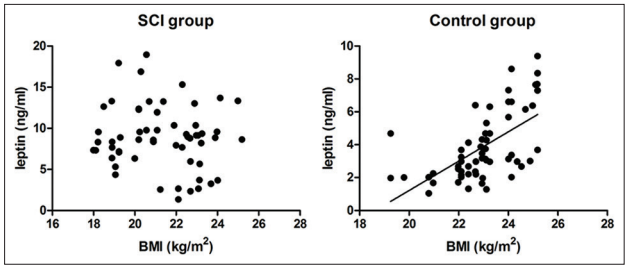

FIGURE 1. Scatter plots of serum leptin concentration changes and BMI at all time points. Positive correlation was observed between serum leptin and BMI in the control group (p=0.001, r=0.604, n=60) but not in the SCI group (p=0.29, n=60).

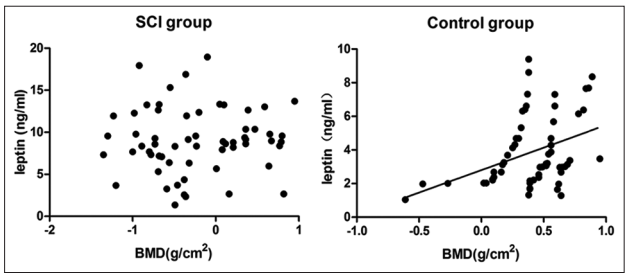

FIGURE 2. Scatter plots of serum leptin concentration changes and BMD at all phases. Positive correlation was observed between serum leptin and BMD in the control group (p=0.002, r=0.384, n=60) but not in the SCI group (p=0.81, n=60).

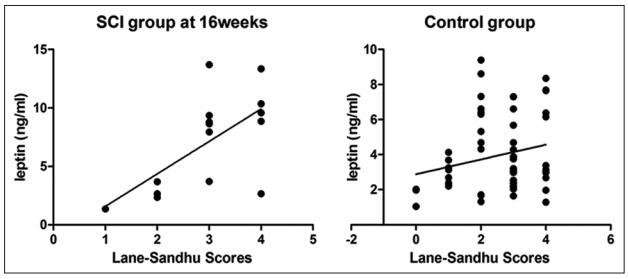

FIGURE 3. Serum leptin level was had a weak positive correlation with fracture formation in the control group (p=0.037, r=0.27, n=60), and in SCI group, this correlation was observed only at 16 weeks (p=0.011, r=0.63, n=15).

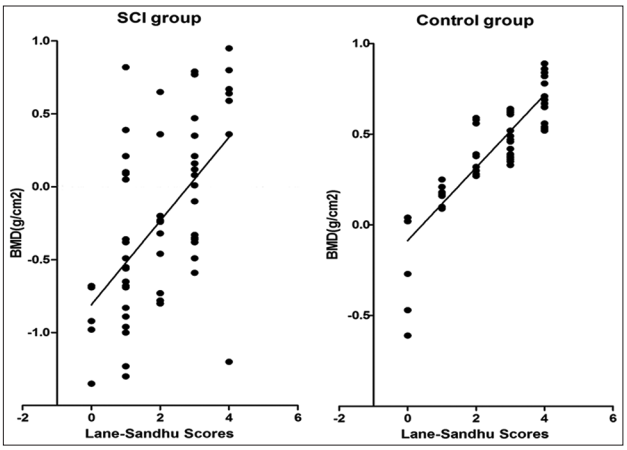

FIGURE 4. Higher BMD was correlated with better fracture formation in both control and SCI groups at all phases (p=0.000, r=0.85, n=60; p=0.000, r=0.56, n=60, respectively).

in clinical patients. When compared with the control group, the SCI group displayed impaired fracture healing, increased serum leptin level and reduced BMD. These clinical findings are consistent with the results of previous mice femoral fractures studies [18,19].

Recently, a large number of in vitro and in vivo studies have shown that by regulating bone mass and promoting bone mineralization through the hypothalamus and the sympathetic nervous system, leptin plays a significant role in bone metabolism [22,23]. In our study, serum leptin was significantly higher in the SCI group than that in the control group at all-time points after the operation and the peak serum leptin concentration in the SCI group occurred at 2 weeks., Previous studies demonstrated that SCI could result in direct damages, such as diffuse axonal injury, contusions and intracranial hematomas. Besides, pathophysiology was amplified by secondary or delayed events, including hypoxia, hypotension, ischemia, edema, increased intracranial pressure. All of these would lead to HPA axis damage and dysfunction [24]. In addition, adrenergic stimulation increased leptin expression and secretion through adipocytes [25], which might cause the observed elevation in serum leptin level during the acute period (2weeks). At 4 weeks after the operation, the internal milieu was relatively stable and HPA axis dysfunction was recovered, therefore the serum leptin level decreased. BMI was considered as a marker of adipose tissue and a number of studies indicated that serum leptin concentration was strongly associated with BMI [4,26,27]. In our study, serum leptin concentration was strongly associated with BMI in the control group instead of the SCI group. As the stress response of the neuroendocrine system led to accelerated decomposition of fat mass, BMI of SCI men was reduced at 2, 4, and 8 weeks after the operation. However, serum leptin did not decrease at those time points. This implied that leptin serum level was influenced by not only changes in fat mass distribution but also neurohumoral regulation after SCI.

As an important factor in bone remodeling, BMD reflects the quality of bone mineralization, which has been shown to be associated with physique, nutrition, weight loading, lifestyle, etc [28]. SCI is characterized by obesity and disruption of mechanisms that link fat mass with BMD. In general, greater body fat mass is related to greater BMD [29,30]. Therefore, as the obesity gene product, leptin has been suggested to have a protective effect on bone and increase bone mineralization. Nevertheless, previous results have been controversial. Some studies reported a positive correlation between serum leptin level and BMD [31,32], some reported a negative correlation [33,34] and even some reported no association [35,36]. These different results demonstrated that a large number of factors having an influence on the relationship between leptin and

BMD. Our results showed that at 2, 4 and 8 weeks after the operation, BMD decreased in the SCI group. The reason was that the majority of these patients (11 of 15) were wheelchair users with lesser mechanical loading than other patients. In the lower extremities, an alteration in fat mass distribution was observed in wheelchair individuals who led to muscle and adipose tissue atrophy. In the SCI group, there was no correlation between BMD and leptin. Although serum leptin level increased after SCI, the protective effect of leptin on male patients was insignificant, which was consistent with the results of Sabour et al [37]. For the control group, BMD did not decrease and was positively associated with serum leptin level, which indicated that the ability to walk contributed to body weight bearing and muscular stimulation and thus prevented BMD reduction [38].

In this study, our results indicated that fracture healing was adversely affected by SCI at 4 and 8 weeks. This finding was in line with that of Garland D, which was supported the hypothesis that SCI impairs fracture healing [39]. Due to low levels of physical activity, the mechanism involves denervation and disuse, which contributes to osteoclastic activity increase and enhances bone resorption. At the same time, SCI-induced vitamin D deficiency may alter calcium metabolism and increase osteoclastic activity as well [40]. These processes may decrease bone turnover and bone mineralization and impair fracture healing. At 16 weeks, mechanical loading enhanced bone formation, because more SCI patients (9 of 15) were able to walk. Consequently, Lane-Sandhu scores were still low in the SCI group at that time point. However, this difference was not statistically significant when compared to the control group. In addition, the osteoinductive potential of serum leptin showed possible activity in the SCI group and the control group at 16 weeks, which suggested that the positive effect of serum leptin on bone formation was related to the level of weight-bearing activities and normal adipose metabolism during fracture healing. Previous studies demonstrated that low BMD bone could be caused by disturbed metabolism of bone remodeling and led to a qualitatively reduced healing process [41]. Here, the same result was found in both control and SCI groups: higher BMD was correlated with better fracture formation. In addition, regular weight-bearing activity and functional exercises could increase bone strength. Therefore, efforts to osteoporosis management in SCI persons will reduce the risks of lower limb fractures [42]. Nevertheless, this investigation has several limitations, such as the small number of cases and the diversity of the patients with SCI injury. This was owing to strict adherence to the exclusion criteria. In consideration of premenopausal and postmenopausal factors, a comparative group of female patients was not available in our study and these would have been confounding factors on BMD and serum leptin level.

CONCLUSION

Our study presented impaired fracture healing and decreased callus production in SCI patients. Furthermore, BMD decreased after SCI. When compared to the controls, men with SCI had significantly higher serum leptin levels; however, these results revealed no correlation between serum leptin level and BMD of lumbar vertebras in SCI patients. As the effect of SCI on fracture healing is complicated and the sample is small, the identification of relevant mechanisms and factors requires further investigation.

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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