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Effect of treadmill gait on bone markers and bone mineral density of quadriplegic subjects

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Abstract

Quadriplegic subjects present extensive muscle mass paralysis which is responsible for the dramatic decrease in bone mass, increasing the risk of bone fractures. There has been much effort to find an efficient treatment to prevent or reverse this significant bone loss. We used 21 male subjects, mean age 31.95 ± 8.01 years, with chronic quadriplegia, between C4 and C8, to evaluate the effect of treadmill gait training using neuromuscular electrical stimulation, with 30-50% weight relief, on bone mass, comparing individual dual-energy X-ray absorptiometry responses and biochemical markers of bone metabolism. Subjects were divided into gait (N = 11) and control (N = 10) groups. The gait group underwent gait training for 6 months, twice a week, for 20 min, while the control group did not perform gait. Bone mineral density (BMD) of lumbar spine, femoral neck, trochanteric area, and total femur, and biochemical markers (osteocalcin, bone alkaline phosphatase, pyridinoline, and deoxypyridinoline) were measured at the beginning of the study and 6 months later. In the gait group, 81.8% of the subjects presented a significant increase in bone formation and 66.7% also presented a significant decrease of bone resorption markers, whereas 30% of the controls did not present any change in markers and 20% presented an increase in bone formation. Marker results did not always agree with BMD data. Indeed, many individuals with increased bone formation presented a decrease in BMD. Most individuals in the gait group presented an increase in bone formation markers and a decrease in bone resorption markers, suggesting that gait training, even with 30-50% body weight support, was efficient in improving the bone mass of chronic quadriplegics.

Key words
- Quadriplegic gait
- Electrical stimulation
- Osteoporosis
- Rehabilitation

Introduction

Many investigations (1-3) have been carried out in order to find methods for gait recovery after spinal cord injury. However, subjects who suffer a spinal cord injury present a significant reduction of physical capacity (4-7) resulting in a dramatic decrease in bone mineral density (BMD) (mainly 3-4 months post-injury) (8-10), which increases bone fragility and risk of fractures, even under minimum impact. The lack of muscle

contraction and mechanical load results in decreased BMD below the injury level (11).

Previous studies (12-17) have evaluated the effect of neuromuscular electrical stimulation (NMES) on bone density. Mohr et al. (12) and Bloomfield et al. (13), observed an improvement in BMD after NMES-cycling intervention. However, Leeds et al. (14) and Eser et al. (15) did not observe any differences after treatment. Different responses have been attributed to the different training protocols used (18).

Dual-energy X-ray absorptiometry (DEXA) has been extensively used to evaluate BMD and to monitor the effects of treatment on bone sites (19). Despite the high precision, low irradiation dose and fast scanning time, studies have suggested that even with a low precision error (20,21), depending on the expected rate of bone gain, a 1- to 2-year interval between exams would be required for an accurate identification of changes in bone mass due to the fact that densitometry is slow in revealing bone alterations (22-25). However, it is possible to use DEXA at short testing intervals to assess bone changes following spinal cord injury since the rate of bone loss is typically rapid and large relative to the precision error. Many studies have analyzed the effect of short periods of treatment on bone density in spinal cord-injured subjects using DEXA (13, 14,26).

Biochemical markers of bone turnover have been used to evaluate bone metabolism (27-29) since they respond to treatment more rapidly than bone density (30-32). Significant differences can be noted within 3-6 months of treatment (33). Bone markers measure the rate of bone turnover when the events of bone resorption and formation are coupled. However, when the events are uncoupled they reflect the increase in either bone formation or bone resorption (34). Hence, the uncoupled increase of osteocalcin, for instance, represents an increase in bone formation rate (30).

NMES allows the activation of paralyzed muscles in order to perform physical activities such as pedaling on a bicycle ergometer and gait (35,36). For quadriplegic subjects, gait training can be achieved using body weight support (BWS), which provides trunk stability and allows the control of the weight that can be supported by the lower limbs of each patient, thus reducing the risk of fracture in osteoporotic bones (3,37).

In view of the lack of studies on this topic, the purpose of the present investigation was to evaluate the effect of treadmill gait training with 30-50% of weight relief associated with NMES on the bone mass of quadriplegic subjects and to determine whether individual responses are similar when BMD measured by DEXA is compared with biochemical markers of bone metabolism.

Material and Methods

Twenty-one male quadriplegic subjects (mean age 31.95 ± 8.01 years) participated in the study. The injury level varied between C4 and C8 (C4, N = 4; C5, N = 4; C6, N = 9; C7, N = 3; C8, N = 1). In the gait group (GG), all individuals had a complete lesion. In the control group (CG) all individuals had an incomplete lesion (ASIA Impairment Scale: B). Mean time post-injury was 66.42 ± 48.23 months (range 25-180 months). Mean body mass and height were 63.52 ± 9.41 kg and 176.28 ± 5.28 cm, respectively. The study was approved by the local Ethics Committee and all subjects gave written informed consent to participate. Inclusion criteria were intact lower motor neurons required for muscle contraction using surface electrical stimulation and to permit walking for treadmill gait, with 30-50% BWS for 20 consecutive min, with no skin damage or ulcers. Another requirement was no history of cardiopulmonary disease. Radiological and clinical examinations of the lower limbs were performed to guarantee that no subject had

fractures, joint degeneration changes or clinical joint instability.

The individuals were divided into two groups: GG and CG. GG individuals (N = 11) were submitted to treadmill gait training provided by NMES for 6 months, twice a week, 20 min per session, whereas CG individuals (N = 10) were not submitted to gait training. GG subjects had their quadriceps and tibialis anterior muscles stimulated for at least 5 months before beginning gait training (twice a week) in order to be able to walk for 20 min, as well as to support at least 50% of their body weight through knee extension provided by NMES (pregait training). A 4-channel electrical stimulator delivered a 25-Hz signal with monophasic rectangular pulses of 300-µs duration and a maximum intensity of 200 V (1 kΩ load).

BWS was provided by a harness hanging from an overhead support, and the support vest allowed free movement of the lower limbs. The body weight support was 30 to 50%. The four-channel electrical stimulator was used to provide the stance gait phase through quadriceps muscle activation and the swing phase by the withdrawal reflex (stimuli to the common peroneal nerve). Manual assistance during treadmill walking was provided to all patients in order to allow a safe and closer to normal pattern of gait. Surface electrodes (5 x 9 cm^2) were placed on the quadriceps and over the common peroneal nerve (round electrodes 3.2 cm in diameter). The stimulation unit was triggered by hand switches controlled by the staff.

BMD (g/cm^2) was determined by DEXA using a DPX-ALPHA Lunar Apparatus (DeWitt, MI, USA). The BMD of the lumbar spine (L2-L4), proximal femur (femoral neck and trochanteric area) and total femur was analyzed. The precision error was 1.0% for the lumbar spine, 1.37% for the femoral neck, 1.7% for the trochanteric area, and 1.5% for the femur. The least significant changes (LSC) were 2.77% for the lumbar spine, 3.80% for the femoral neck, 4.59% for the trochanteric area, and 4.15% for the total femur. The coefficient of variation was determined from three repeated measurements of the 5 spinal cord-injured subjects included in the study on 3 separate days (95% confidence interval) (12,13,15). All exams were performed by the same technician at the beginning of the study and 6 months later. Biochemical markers of bone turnover were also evaluated. Serum osteocalcin (OC) and bone alkaline phosphatase (B-ALP), both markers of bone formation, and free pyridinoline (PYD) and free deoxypyridinoline (DPD), both markers of bone resorption, were analyzed. PYD and DPD were corrected for creatinine and are reported as nmol/mmol. Bone markers were measured by an immunoassay method (Metra Biosystems, Mountain View, CA, USA). Serum and urine samples were collected between 8:00 and 10:00 am after an overnight fast and stored at -80ºC until analysis. The measurements were made at the beginning of the study and 6 months later.

Data were analyzed separately for each individual, because each subject presented different bone responses, since BMD is influenced by many factors as nutrition, bone condition before lesion, physical activity, and body weight, which is an important factor for bone mass variability (15-32%) (38).

Bone markers and BMD results were considered to be significant when differences between the initial and final values were higher than the LSC (34). Bone marker values were analyzed based on the study of Hannon et al. (33), who observed that the LSC, within a single individual, was 21% from baseline values for OC, 28% for B-ALP, 36% for PYD, and 26% for DPD (95% confidence interval).

Results

Table 1 presents the biochemical markers of bone formation and resorption for

each GG and CG subjects. The results showed a significant increase in bone formation markers after gait training in 81.8% (9 individuals) of the subjects, with 66.7% (8 individuals) presenting a significant decrease in bone resorption markers. However, in many cases, despite the increased bone formation rate and decreased bone resorption markers, resorption marker values were higher than reference values. Reference values were 3.7-10.0 ng/mL for OC, 12-41 U/L for B-ALP, 12-37 nmol/mmol for PYD/Cr, and 2-7 nmol/mmol for DPD/Cr. Moreover, one subject presented a decrease in bone turnover and one presented a decrease in bone resorption rate.

In the CG, no alterations were observed in 30% of the individuals (3 individuals), while 20% (2 individuals) presented a significant increase in bone formation markers. CG individuals who presented an increase of formation markers increased their independence during daily activities (they managed to obtain driver licenses and became less dependent on accompanying persons) during the 6-month period. A decrease in bone resorption markers was noted in 30% of subjects (3 individuals). One subject showed increased bone resorption markers and another showed decreased bone formation markers.

Table 2 presents the results of BMD (g/cm^2) at the beginning and after 6 months of GG and CG. The results obtained with biochemical markers were not always reproduced in BMD. Of the individuals who presented an increase in bone formation markers (N = 9), 3 presented a BMD gain at most of the sites analyzed (individuals 3, 4, and 5), 4 presented loss of BMD at most of the sites analyzed (individuals 1, 6, 7, and 8), 1 (individual 9) presented maintenance of BMD except for total femur, which lost bone mass, and 1 (individual 2) presented maintenance of BMD except for the femoral neck, which gained bone mass. The total femur of individual 10 was not evaluated.

In the CG, at the beginning of the study and 6 months later, one subject could not have his femur evaluated because of a flexor contracture of his hip. Of the two individuals who presented a significant increase in bone formation markers, one presented an increase and the other a decrease in BMD. Even among those who did not present any alteration of bone markers (when comparing ini-

Table 1. Biochemical markers of bone formation and resorption for each subject of gait group (GG, N = 11) and control group (CG, N = 10) before and after 6 months.

Subjects	Osteocalcin (ng/mL)				Bone alkaline phosphatase (U/L)				Free pyridinoline (nmol/mmol)				Free deoxypyridinoline (nmol/mmol)			
	GG		CG		GG		CG		GG		CG		GG		CG	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	5.23	6.55	4.45	4.50	28.98	25.13	15.58	13.28	55.30	30.52	57.65	38.05	9.98	8.24	5.26	11.49
2	8.40	23.61	4.56	8.25	34.05	33.24	23.55	26.96	85.02	41.88	62.41	39.04	5.95	5.79	8.61	8.36
3	4.42	8.89	4.48	3.92	22.70	28.59	19.79	17.95	52.60	37.04	44.30	29.65	6.11	5.66	6.38	5.36
4	4.52	6.55	4.58	4.60	28.23	32.08	16.47	14.76	50.22	38.24	38.80	27.34	6.07	4.15	16.39	6.42
5	4.42	7.16	4.43	4.84	21.57	27.44	12.66	10.26	25.04	16.94	48.35	35.76	5.75	2.82	18.24	12.46
6	7.80	13.81	6.18	17.47	25.50	23.58	21.38	28.16	203.47	161.26	56.58	52.06	27.3	24.34	9.23	10.47
7	6.79	10.81	4.49	5.01	19.75	18.42	25.77	27.74	179.64	96.72	69.17	58.25	30.33	18.28	13.85	11.37
8	5.79	11.19	4.38	4.37	22.04	26.19	31.49	27.80	40.87	40.92	43.11	35.44	22.82	8.20	12.06	9.38
9	4.51	7.13	4.58	4.48	16.08	15.29	31.10	31.26	41.86	27.54	43.41	22.89	4.39	2.69	7.45	4.99
10	6.81	4.57	4.45	3.47	18.67	17.08	10.44	7.02	60.74	35.17	55.95	37.02	8.17	6.95	6.30	7.61
11	6.72	6.72	-	-	19.05	23.41	-	-	56.59	46.84	-	-	14.64	10.02	-	-

tial and final values), some presented a gain of BMD and some lost or maintained their BMD.

Discussion

The present results show that treadmill gait training was efficient in increasing the rate of bone formation, even with 30-50% of BWS, since most individuals presented a significant increase of OC (a bone formation marker) and a decrease of PYD and/or DPD (bone resorption markers). Since the results for the bone markers presented a dissociation of bone resorption and formation events, they represented an increase of bone formation rate (30,34). The increase in bone formation rate is associated with gait training and not with pregait training since NMES used to provide knee extension without any resistance has been shown not to improve bone density in many studies (13,39).

All individuals had sustained their injuries at least 25 months before the study and, based on the literature, bone loss occurs dramatically during the first 3-4 months post-injury (22-27% depletion) and bone mass achieves a new steady state by 16 months after the injury, with approximately 37% bone depletion. Garland et al. (40) did not observe any significant differences in bone mass between 16 months and 10 years post-injury.

Some individuals included in our study (6 months between DEXA measurements) presented a significant decrease of bone mass, i.e., a reduction of 20.46% in the femoral neck and of 29.95% in the trochanteric area. These dramatic decreases of bone mass may reflect the imprecision of DEXA due to the short time between measurements or due to the problems that occur during densitometry exams in spinal cord-injured persons (spasticity and lack of reproducibility of lower limb position).

Bloomfield et al. (13) also observed that some quadriplegic individuals submitted to NMES-cycle ergometer training presented a bone loss at one or more bone sites analyzed during 9 months of physical activity. They hypothesized that insufficient calcium intake could have been responsible for the increased bone loss since the maintenance of mineral homeostasis (extracellular calcium ion concentration) is essential for body function.

Table 2. Bone mineral density (BMD) values (g/cm^2) obtained before and after 6 months for each subject of gait group (GG, N = 11) and control group (CG, N = 10) for lumbar spine (L2-L4), femoral neck, trochanteric area, and total femur.

Subjects	Lumbar spine				Femoral neck				Trochanteric area				Total femur			
	GG		CG		GG		CG		GG		CG		GG		CG	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	1.353	1.345	1.456	1.402	0.923	0.863	NE	NE	0.723	0.652	NE	NE	0.935	0.851	NE	NE
2	1.447	1.473	1.239	1.197	0.756	0.795	0.759	0.692	0.574	0.561	0.461	0.438	0.686	0.682	0.628	0.570
3	0.902	0.866	1.278	1.283	0.253	0.334	0.795	0.679	0.319	0.386	0.592	0.554	0.389	0.385	0.763	0.627
4	1.303	1.333	1.184	1.170	0.674	0.673	0.790	0.815	0.461	0.458	0.529	0.497	0.686	0.721	0.663	0.670
5	0.993	0.983	1.099	1.110	0.677	0.699	0.647	0.669	0.483	0.516	0.453	0.445	0.638	0.654	0.543	0.537
6	1.173	1.180	1.143	1.144	1.114	0.886	0.422	0.523	0.694	0.554	0.285	0.416	0.953	0.774	0.557	0.513
7	1.132	1.132	0.996	1.011	0.977	0.904	0.573	0.568	0.723	0.610	1.740	1.702	0.863	0.831	1.156	1.175
8	1.240	1.287	1.707	1.708	0.856	0.796	1.115	1.068	0.603	0.535	0.793	0.770	0.801	0.760	0.973	0.961
9	1.092	1.085	1.102	1.085	0.603	0.600	0.641	0.644	0.523	0.522	0.539	0.547	0.608	0.592	0.717	0.717
10	1.221	1.221	1.062	1.033	1.079	1.022	0.603	0.654	0.685	0.655	0.491	0.479	NE	NE	0.603	0.586
11	1.158	1.162	-	-	0.838	0.692	-	-	0.681	0.477	-	-	0.840	0.647	-	-

NE = not evaluated.

As shown in Table 1, it is clear that there was a reduction in bone marker resorption in the GG subjects, meaning that the calcium intake was not the reason for the bone loss observed by DEXA.

The present results show that treadmill gait provided by NMES associated with partial body weight support was efficient in increasing the bone formation markers together with a decrease of bone resorption markers in quadriplegic subjects.

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