INVESTIGATION OF OSTEOMETABOLIC AND CARDIO-RESPIRATORY CHANGES OCCURRING AFTER GAIT TRAINING UNDER NEUROMUSCULAR ELECTRIC STIMULATION IN QUADRIPLEGIC PATIENTS

DANIELA CRISTINA LEITE DE CARVALHO¹, ALBERTO CLIQUET JR ²

SUMMARY

This study was aimed to evaluate the effects of gait training with neuromuscular electric stimulation (NMES) on skeletal and cardio-respiratory systems of full quadriplegic patients (C4-C8). Aerobic power (VO₂), carbon dioxide production (VCO₂), minute ventilation (VE), heart rate (HR), blood pressure (BP), energy consumption, bone markers analysis (osteocalcin, bone alkaline phosphatase, pyridinoline, and deoxypyridinoline) and bone densitometry (DEXA) of the femoral neck and total femur were performed at baseline and six months later. Eleven patients walked on ergometric wake, with NMES and discharge of 60 – 70% of body weight, during six months, twice a week, 20 minutes daily. Ten patients did not perform the gait. Within the gait group, 81.8% showed significant increases of formation markers, of which 72.7% also presented with reduced bone resorption. Within the control group, 20% showed increased bone formation. DEXA results were, in general, opposite to those of bone markers. Cardio-respiratory tests showed a significant increase for VO₂ l/min (36%), VCO₂ (42.97%), VE (30.48%), SBP mmHg (4.8%) and energy consumption kcal/min (37.68%). In the control group, only VO₂ l/min showed a significant increase (26.29%). Gait training with NMES was more efficient in increasing bone formation rates and aerobic power in quadriplegic patients.

Keywords: Quadriplegia; Gait; Electrical stimulation, Bone mineral density; Oxygen consumption; Metabolism; Respiration.

INTRODUCTION

Recently, osteoporosis and cardiovascular diseases have emerged as important public health problems. Patients with spinal cord injuries present with a strong bone mass loss during the first 3 months after injury, as well as being at a higher risk for cardiovascular diseases than normal population¹. Thus, rehabilitation programs in spinal cord injured patients are important to improve or maintain a healthy skeletal and cardio-respiratory systems and, as a result, to reduce the risk of fractures on osteoporotic bones and the risk of cardiovascular diseases, making them also able to walk again as soon as possible, since new evidences have shown that gait training with neuromuscular electric stimulation (NMES) regularly performed may contribute to motion recovery, either through locomotion generator pattern or through neuroplasticity. The potential of functional recovery may also occur through medical (stem cells studies)² or technological advancements (creation of electric stimulators that could be used for patients’ daily life activities).

Osteoporosis is a usual complication related to spinal cord injury, because palsy causes a mechanical stress reduction over bones. The absence of muscular contraction and of weight support increases bone resorption by osteoclasts³,

Study conducted at the Laboratory of Biomechanics and Locomotive Apparatus Rehabilitation, Department of Orthopaedics and Traumatology, Medical Sciences College, Campinas State University (UNICAMP), Campinas, Brazil, and Department of Electric Engineering, University of São Paulo (USP), São Carlos, Brazil.

Correspondences to: Prof. Dr. Alberto Cliquet Jr. - Departamento de Ortopedia e Traumatologia / F.C.M. / UNICAMP. Rua Alexander Fleming, 181, Cidade Universitária “Zeferino Vaz” s/n - 13083-970 – Campinas, SP / Brasil - E-mails: cliquet@fcm.unicamp.br, dclcarvalho@yahoo.com.br


2 – Chairman EESC– USP / FCM - UNICAMP.

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which promotes a reduction of bone mass, weakening bones and making them more susceptible to fractures. The most extensive bone loss is seen between the first four to six months after trauma. After that period, bone loss ratio is lower and, within sixteen months, bone mass is reduced to 2/3 of the original amount\(^{[3,4]}\). With the full cervical injury, in addition to the significant bone mass loss, a shutdown occurs between the central command of sympathetic system and its peripheral portion\(^{[9]}\). The absence of sympathetic nervous system changes cardiovascular responses during exercise, jeopardizing chronotropic and inotropic responses. Exercises associated to NMES on lower limbs recruit large muscular groups, activate lower limbs’ venous pump, facilitate venous feedback, increase systolic volume, improve heart contraction force, and, as a result, enhance \(O_2\) supply to active muscles\(^{[5,6]}\). Additionally, NMES promotes muscular changes including increased muscle resistance, hypertrophy and histochemical changes (increased number of myoglobins, mitochondria and capillary density), which increases aerobic power in patients with spinal cord injury. Besides, a muscular improvement may benefit bones, since a strengthened muscle generates a stronger mechanical stress on the bone where it is inserted, enhancing bone formation stimulus\(^{[6]}\).

The present study analyzed the effect of gait training yielded by NMES, on ergometric belt with 60-70% of body weight discharge, during 6 months, twice a week, on bone mass and cardio-respiratory system of quadriplegic patients with full injuries.

**METHOD**

The clinical trial was conducted in quadriplegic patients \((n=21)\) with full injuries, injury level ranging from C4 to C8, who suffered rachimedullary trauma, belonging to Laboratory of Biomechanics and Locomotive Apparatus Rehabilitation, HC/UNICAMP. The study was divided into 2 groups: a gait group, constituted of 11 patients (age \(= 33.83 \pm 8.73\) years old; time of injury \(= 78.58 \pm 56.20\) months; body mass \(= 64.91 \pm 12.68\) kg and height \(= 178 \pm 8.92\) cm) and a control group, formed by 10 patients (age \(= 30.9 \pm 7.72\) years old; time of injury \(= 51.4 \pm 29.37\) months; body mass \(= 65.1 \pm 10.57\) kg and height \(= 76.6 \pm 4.81\) cm).

Patients included in gait group (GG) were submitted to at least 5 months of knee extension exercises through NMES on quadriceps muscles with the objective of increasing strength and resistance of quadriceps muscles and, thus, allowing for the maintenance of an orthostatic position during the 20 minutes of ambulation.

Gait training on ergometric belt was performed with combined use of 4-channel electric stimulation to enable functional gait, and of dynamic suspension with discharge between 60% and 70% of body weight to avoid excessive mechanical load on lower limbs, but also to allow heel’s touch during gait. The electric stimulator used releases a bipolar single-phase wave, in a 25 Hz frequency, with a pulse train lasting 300\(\mu\)s and amplitude of up to 200 V (1 k\(\Omega\) load). Treatment was delivered during 6 consecutive months, twice a week, 20 minutes a day, being rest determined by the very patient.

In control group (CG), patients were not submitted to gait training induced by NMES. Patients who could not participate on proposed treatment due to lack of time or transportation difficulties were included in this group. CG patients were submitted only to traditional physical therapy twice a week (kinesiotherapy: range of motion maintenance, stretching and active exercises on preserved muscles), with the majority of treatments also including the maintenance of orthostatic posture (by means of orthostatic tables).

Those patients presenting with involvement of lower motor neuron, medical and/ or psychological instability, other diseases/ injuries or in the presence of a medical or drug condition that could interfere on bone formation were excluded. This study was approved by the Committee on Ethics in Research of the Medical Sciences College, UNICAMP.

At the moment of admission and 6 months later, all patients were submitted to bone densitometry analysis, bone metabolism biochemical markers collection and cardio-respiratory test.

Bone densitometry was performed at femoral neck region and total femur, using the DEXA technology, model DPX-ALPHA, Lunar brand. The minimal significant variation for femoral neck was 3.80% and for total femur, 4.15%.

Urine and blood samples were collected for bone markers analysis. Two biochemical markers of bone resorption were analyzed: pyridinoline and free deoxypyridinoline, which were adjusted by urinary creatinin. Urine samples, light-protected, were stored at -20\(^\circ\) C until the moment of analysis. For the analysis, the immunologic method based on antibody against free pyridinoline (PYD; Pyrilinks, Metra Biosystems) and free deoxypyridinoline (DPD; Pyrilinks-D, Metra Biosystems) was employed. Other two bone formation biochemical markers have also been analyzed: bone alkaline phosphatase (B-ALP; Alkphase-B, Metra Biosystems) and osteocalcin (OC; Osteocalcin, Metra Biosystems). Serum samples were stored until the moment of analysis at -70\(^\circ\) C. All samples were collected between 8 AM and 10 AM, with patients submitted to a 12-hour fasting. For bone metabolism markers, the minimal
significant variation was 21% for OC, 28% for B-ALP, 36% for PYD and 26% for DPD (7).

The cardio-respiratory test was conducted in a direct and non-invasive form, at a room temperature of 25°C, by gas analyzer Vmax, model 29c, SensorMedics brand. The equipment was calibrated prior to each test using reference gases. The following parameters were analyzed: aerobic power (VO\(_2\)), minute ventilation (V\(_E\)), carbon dioxide production (VCO\(_2\)), heart rate (HR) and respiratory exchange ratio (R = VCO\(_2\)/VO\(_2\)). Values were recorded at each 30 seconds and averages were obtained from the last 5 values recorded in each phase. With VO\(_2\) and R values achieved, we could analyze energy (in Kcal) spent by minute during rest and exercise. From the R value, the caloric equivalent is obtained (kcal of energy by liter of consumed oxygen). The energetic consumption was achieved by multiplying VO\(_2\) l/min average value by Kcal average value by liter of consumed O\(_2\) (Kcal/lO\(_2\)) obtained from R, by the equation: R = Kcal/lO\(_2\) and Energetic consumption (Kcal/min) = VO\(_2\) l/min x Kcal/lO\(_2\).

All the patients repeated cardio-respiratory test protocol three times before the official test to become familiar with the equipment and to enable the achievement of reliable data. Heart rate was measured during the test, at intervals of successive R waves of the SensorMedics electrocardiogram. In GG, the test was performed during gait induced by NMES on ergometric belt, aided by dynamic suspension, before and after treatment completeness. The test constituted of 8 minutes of rest, 10 minutes of gait with NMES and 10 minutes for recovery. Blood pressure measurements were collected at rest phase (seated position), gait phase (after 10 minutes of gait) and recovery phase (soon after seated again). All patients performed the initial test at a speed of 0.5 km/h. The final test was performed at the maximum speed reached by each individual, ranging from 1.0 to 1.3 km/h.

In CG, the test constituted of 8 minutes of rest, 10 minutes of bilateral knee extension exercise at seated position using a 2-channel stimulator and 10 minutes for recovery. GG was submitted to gait training sessions for 6 months, while CG was only submitted to electric stimulation at the moment of cardio-respiratory test, with the objective of producing the acute effect of electric stimulation for both groups (10 minutes of NMES for both groups). Furthermore, it must be mentioned that CG did not follow the gait protocol during cardio-respiratory test due to the fact that those patients were not submitted to previous NMES 5-month training, which was required to qualify them to perform gait.

In order to evaluate changes on metabolic and cardio-respiratory parameters (VO\(_2\), VCO\(_2\), V\(_E\), FC and R) and values for blood pressure and energetic consumption, the Variance Analysis with 2 repeated factors was applied for conducting the statistical analysis, with significance levels of 5% (p<0.05) being used.

RESULTS

Bone Mass

Figure 1 shows the results of formation and resorption bone markers for each individual in GG. After 6 months of gait training (GG), the results showed a significant increase on formation markers in 81.8% (M=9) of patients, with 72.7% also presenting a significant reduction of resorption markers. In addition, one subject presented a reduction on bone remodeling rate (subject 10) and one presented a reduction of bone resorption ratio (subject 11).

Figure 2 shows formation and resorption bone markers for each CG individual. After 6 months, 30% of patients did not present significant changes on bone markers and 20% presented with a significant increase of bone formation markers. Furthermore, 30% of patients presented with re-
duced bone resorption markers. One subject presented an increased bone resorption rate and another one presented reduced bone formation markers.

Table 1 shows DMO results (g/cm²) at baseline and after 6 months for the gait group (GG) and control group (CG), with differences in percentage between baseline and end results.

Results achieved with bone markers, within GG, showed that patients 1, 6, 7, 8, 10 and 11 were not correlated to the results achieved by DEXA. From patients showing an increased bone formation rate (9 patients), 4 presented with increased DMO at femoral neck region and/ or total femur, 5 presented with lost DMO at femoral neck and/ or total femur.

In control group, one subject could not perform femoral DEXA due to an intense flexor contracture of the hip. From 2 patients presenting increased bone formation markers, one presented with increased DMO and another one presented with DMO reduction.

From those not presenting changes on bone markers (3 patients), two patients presented a DMO reduction and one presented steady DMO on regions analyzed.

Cardio-respiratory test

At baseline test, in GG, the patients presented the following average values during gait: VO₂ = 0.430 ± 0.09 l/min, VO₂ = 6.96 ± 1.79 ml/kg/min, VCO₂ = 0.356 ± 0.08 l/min, HR = 106.23 ± 17.31 bpm, V̇E = 18.93 ± 2.95 l/min and R = 0.81 ± 0.06. After 6 months of training, the average values achieved were as follows: VO₂ = 0.586 ± 0.14 l/min, VO₂ = 9.57 ± 2.54 ml/kg/min, VCO₂ = 0.509 ± 0.13 l/min, HR = 109.23 ± 15.79 bpm, V̇E = 24.70 ± 4.62 l/min and R = 0.85 ± 0.04. Results showed significant increases for VO₂/min (36%), VCO₂ (42.97%), V̇E (30.48%) and R (4.93%). HR did not present significant changes. The values obtained during rest did not significantly change after training.

Before the beginning of gait training, patients showed average systolic blood pressure (SBP) and diastolic blood pressure (DBP) values, at rest, of 94.16 ± 5.15 mmHg and 65.83 ± 5.15 mmHg respectively. During gait, SBP significantly raised to 105 ± 5.22 mmHg, with no changes in DBP (70 ± 7.38 mmHg).

After the 6-month period of training, patients presented SBP and DBP, during rest, of 101.67 ± 8.35 mmHg and 65.83 ± 9.0 mmHg respectively, with a significant SBP rise. During gait, SBP significantly raised to 110.83 ± 6.68 mmHg (p<0.05), with no changes in DBP (69.16 ± 7.92 mmHg).

Figure 3 shows the values obtained before and after 6 months of training for VO₂, VCO₂, V̇E, HR and SBP during gait phase. Rest SBP is also shown.

In the CG, at the moment of study admission, during the knee extension exercise, the patients presented with the following average values: VO₂ = 0.232 ± 0.05 l/min, VO₂ = 3.45 ± 0.66 ml/kg/min, VCO₂ = 0.190 ± 0.04 l/min, HR = 67.9 ± 11.72 bpm, V̇E = 10.96 ± 1.75 l/min and R = 0.82 ± 0.03. After 6 months, the values obtained were as follows: VO₂ = 0.293 ± 0.09 l/min, VO₂ = 4.62 ± 1.30 ml/kg/min, VCO₂ = 0.223 ± 0.07 l/min, HR = 73.32 ± 12.49 bpm, V̇E = 11.65 ± 2.60 l/min and R = 0.76 ± 0.07. The results showed an increase of 26.29% for VO₂ l/min, 17.37% for VCO₂, 7.98% for HR, 6.29% for V̇E, only with VO₂ showing a significant increase (p<0.05) during knee extension exercise. Rest VO₂ has also significantly risen after 6 months.
During physical activity, GG presented significantly higher values (for all parameters analyzed) when compared to values obtained in CG (during knee extension exercise).

During gait, the average energetic consumption obtained before training was 2.07 ± 0.48 kcal/min and after training, it significantly increased to 2.85 ± 0.71 kcal/min (p = 0.003). At baseline, during gait, the energetic consumption was 2.4 times higher than the energetic consumption at rest. After 6 months, with gait being performed at the fastest speed as possible, the energetic consumption was 3.8 times higher than at rest.

In CG, during exercise at baseline tests, the energetic consumption was 1.12 ± 0.25 and, after 6 months, it increased to 1.39 ± 0.46 (non-significant: p = 0.06). At study baseline, during the knee extension exercise, the energetic consumption was 1.4 time higher than the energetic consumption at rest (0.76 ± 0.27). After 6 months, the energetic consumption remained 1.4 times higher than at rest (0.95 ± 0.32). Figure 4 shows the energetic consumption (kcal/min) at the moment of admission in the study and after 6 months obtained for GG (during gait) and CG (during knee extension exercise).

**Table 1** - DMO (g/cm²) values obtained from femoral neck and total femur regions, at baseline and after 6 months, in gait group (GG) and control group (CG). Here, differences in percentage between baseline and final values are also described.

<table>
<thead>
<tr>
<th>DMO nr.</th>
<th>Group</th>
<th>Femoral neck</th>
<th>Total femur</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td>Baseline</td>
<td>Final</td>
</tr>
<tr>
<td>1</td>
<td>GG</td>
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<td>0.863</td>
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<tr>
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<tr>
<td>12</td>
<td>CG</td>
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<td>NA</td>
</tr>
<tr>
<td>13</td>
<td>CG</td>
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<td>CG</td>
<td>0.603</td>
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</table>

NA = not assessed

Figure 3 – Values obtained on cardio-respiratory test before and after 6 months of gait training. Values expressed in average ± SD. A) VO₂ (ml/kg/ min), B) VCO₂ (l/min), C) V̇E (l/min), D) HR (bpm), E) SBP (mmHg) at rest, F) SBP (mmHg) during gait.
DISCUSSION

The results with bone markers showed that gait training was effective in increasing the bone formation rate in 81.8% of patients included in GG. The analysis of bone markers is useful to evaluate current behavior of bone metabolism, enabling the evaluation of therapies effects on bone mass in a short period of time(5). Bone densitometry, when employed to monitor treatments, is recommended to be performed in a time interval of 1 to 2 years between baseline and final test in order to enhance the reliability of the results(7).

Bone monitoring in spinal cord injured individuals, by means of DEXA technique, should be made very carefully, because in addition to an accuracy error of the equipment, patients may present spasticity during tests (which displaces reference markers of bone regions of interest), as well as for being difficult to reproduce the same position of lower limbs(8). The changed position of markers may cause significant percentage differences on results achieved, especially those of the femoral proximal region(8).

Mohr et al.(4) and Bloomfield et al.(9) observed raises in DMO after one training session using ergometric bicycle associated to NMES (between 10% - 30% in DMO after 6 or 12 months of training), which were highly superior to the results achieved in post-menopausal women as a result of drug therapies. Increases of 1 – 3% a year are expected with hormonal replacement therapies and alendronate(10). Although the absence of mechanical load seems to be much more harmful to skeleton (reduction of about 22% on the first three months after injury) than the absence of that ovarian hormone (DMO reduction of 4-5% a year), one must still question if exercises using NMES and, consequently, create a mechanical stimulus on bones below injury level, are efficient to promote such intense bone gains in a short period of time.

Furthermore, another aspect suggesting DEXA technique inaccuracy is the fact that many patients from gait group have lost significant amounts of DMO, with all patients having more than 24 months of injury and, based on literature, the major bone loss occurs during the first 3-4 months after injury (22-27% bone reduction), reaching a new permanent regimen after 16 months of injury (about 40% of bone loss).

Garland et al.(3) did not find any difference on DMO between 16 months and 10 years of injury.

The efficiency of aerobic training programs is usually determined by aerobic power measurements (11), with activities such as bicycle and arm ergo meter cycle showing to be efficient in increasing aerobic power. However, exercises with arm ergo meter cycle promote less aerobic power increase, due to an intense blood trapping on lower limbs, as a result of the absence or reduction of the venous sympathetic tonus (5).

Metabolic and cardio-respiratory results showed that, after 6 months of gait training, a significant increase occurred on oxygen consumption, with average VO$_2$ rise of 36.28% (from 0.430 ± 0.09 l/min to 0.586 ± 0.14 l/min), corroborating the studies by Mohr et al.(12) and Goss et al.(11) who also observed increases after a proposed training, although these were of lower proportions (increase of 18% and 28%, respectively).

Oxygen consumption increase observed in this study probably occurred due to peripheral adjustments, although it could have been influenced by cardiovascular system improvement (central adjustment). Peripheral adjustment has a faster effect and occurs due to an increased muscular force and resistance, which enables the muscle to extract a larger amount of oxygen (5). For central adjustment [related to heart rate (HR) and systolic volume (SV)] to occur, it is required that the physical activity performed stresses cardiovascular system. By recruiting large muscles groups, NMES on lower limbs activates those limbs’ venous pump, facilitates venous feedback, increases cardiac deficit, increasing the contractile ability of the heart through Frank-Starling mechanism(5,13). After 6 months of training, quadriplegic patients’ HR did not significantly increase during physical exercise due to a compromised sympathetic autonomous system. The moderate increase during gait probably occurred due to the parasympathetic inhibition, not due to sympathetic activation.

Thus, the probable heart’s increased ability to pump blood to tissues in activity after training was due to an increased venous feedback (induced by NMES), with a resulting increase of systolic volume and of myocardial
contraction force, with no bias from HR increase, once a significant increase of SBP at rest occurred (from 94.16 ± 5.14 to 101.67 ± 8.35 mmHg) and during gait (from 105 ± 5.22 to 110.83 ± 6.68 mmHg), with no changes in DBP and HR. Dela et. al. also observed during exercises in bicycle associated to NMES, in quadriplegic patients, an increased cardiac deficit (CD) due to an increased VS, while, in paraplegic patients, the CD increase occurred mostly due to a HR rise. Faghri et al. have also observed a significant SBP increase at rest, after a training program in ergometric bicycle combined to NMES (36 sessions, during 12 weeks), in chronic spinal cord injured patients. They theorized that the SBP increase after training may have occurred due to renal renin-angiotensin hormonal system adjustment, which stimulates arterioles vasoconstriction, increasing blood volume.

The SBP rise during physical activity reflects an increase of cardiovascular activity (HR and/or CD increase), which may occur with the use of NMES on lower limbs. However, the rise occurred in a moderate level, because quadriplegic patients with full injuries present with an extensive muscle palsy combined to a compromised sympathetic nervous system, jeopardizing cardiovascular response during gait. Patients belonging to control group also presented improvements on aerobic power (although at lower levels), with a significant VO₂ increase at rest and during the knee extension exercise.

According to Janssen et al., the improvement of physical capacity occurs after injury’s acute phase lasting up to about 48 months after rachimedullary trauma due to the adjustment phase of the individual to his/her new condition, which involves mobility enhancement by the use of preserved muscles. All patients included in control group were submitted to traditional physical therapy during the 6-month period of the study, with 6 out of 10 patients having an injury time below 48 months, 2 having an injury time of 50 months and only 2 patients with injury time above 96 months. Thus, metabolic and cardiovascular improvements seen may have occurred both due to spontaneous healing and due to traditional physical therapy, which involved exercises for preserved muscles and transfers training.

Although patients from control group have also presented a significant VO₂ increase during physical exercises, their values were significantly lower than those achieved during gait, even at a speed of 0.5 km/h. After 6 months of training, GG patients presented a significant energetic consumption increase during gait. According to Blair et al., low to moderate exercises levels, by enhancing energetic consumption, are already enough to reduce the risk of cardiovascular diseases, even in healthy patients. The low energetic consumption may be one explanation for the low oxygen consumption achieved during physical activity. Thus, rehabilitation program in patients with spinal cord injuries are important to improve or maintain the health of skeletal and cardio-respiratory systems, and, as a result, to reduce risks of fractures on osteoporotic bones and to reduce risks of cardiovascular diseases, in addition to make them able to walk again as soon as possible.

CONCLUSIONS

The results showed that gait training was efficient in increasing bone formation rate (observed through bone metabolism markers), and also that even quadriplegic patients, with full injuries, may benefit from regular physical activity, improving metabolic and cardio-respiratory responses.

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