

Experimental Model of Suit Therapy with Traction Bands in Vertebral Bone Remodeling in Wistar Rats

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Abstract

Objectives: Osteopenia and osteoporosis are quite prevalent in children with Cerebral Palsy and the percentage of these children with a history of fractures is high. In the past decade there have been methods and protocols within the suit therapy characterized by garments with adjustable elastic bands and the possibility of applying load on the human skeleton for improved bone mineral density. The aim of this study was to analyze the histomorphometric bone of dorsal and lumbar vertebrae, and to quantify serum levels of osteocalcin, after using an experimental model of suit therapy in Wistar rats.

Methods: The sample included 30 male Wistar rats. It was made for animals, an experimental model of the “suit”, adapted and arranged in two elastic “X” to approach the spine by means of traction load resulting in 50% of the weight of the animal. They remained two hours daily with the suit, 5 consecutive days and four weeks of trial, a total of 40 hours.

Results: There was no statistical result for cortical thickness, and osteocytes counts and percentages of the trabecular porous area in the dorsal and lumbar vertebrae.

Conclusion: There was no change in bone tissue of the dorsal and lumbar vertebrae with the spinal compression performed by the traction bands of the suit in this experimental model.

Keywords: Therasuit; Pediasuit; Adelisuit; Intensive therapy; Bone mineral density; Cerebral palsy

Introduction

There are several children neurological manifestations with motor impairments. Among the most prevalent is the Cerebral Palsy (CP), being reported as 1.2 to 3.6 per 1,000 live births [1]. In these infants, the slowed growth decreases bone mineral density (BMD) below the normal range [2,3] which added to the absence or reduction of weight-bearing and poor muscles load results in significant bone fragility [4,5]. Thus, osteopenia and osteoporosis, are very prevalent in this category of patients [3].

Due to the importance of determining effective means for bone development, preventing weight loss and the incidence of fractures in these patients [5,6], exercise has been recognized as an effective strategy for the adequacy of bone mass through bone tissue metabolic changes [7–11] and so it becomes logical to introduce the weight support on the skeleton and exercise, aiming the improvement of bone health in children with CP [1,12,13].

In the last decade there have been methods and protocols in the area of neurofunctional physiotherapy, which have in common the term “suit” in its nomenclatures to characterize the existence of garments that work as dynamic orthoses, which were developed from an original model for astronauts [14–16]. The basic concept of this therapy is to create a support unit to align the body as close as possible to normal, improve and adjust the proprioceptive system using the pressure exerted on the skeleton, muscles and ligaments by means of adjustable elastic bands [14,15]. Thus, it can be applied axially on the body, a load of 15 to 40 kg [14,16]. Therefore, the available methods that use the suit therapy, are unanimous in pointing the improvement in BMD [14–17]. However, the suit therapy has undergone many changes from the original form to fit within the therapeutic needs of neurorehabilitation, and at present, little attention has been given to the therapeutic effects of this therapeutic device regarding the changes on the skeletal system of children with neuromotor dysfunction.

Animals used in experiments, are extremely useful tools to analyze morphological features that could hardly be observed in human

studies, not only because of technical impracticability, but, especially in regard to the principles of bioethics, facing the investigation need of some condition of human life. Given the above, the objective of this study was to analyze histomorphometrically the bone tissue of dorsal and lumbar vertebrae, as well as to quantify serum levels of Osteocalcin (OC), after using an experimental model of suit and suit therapy associated with mechanical load for vertebral compression in rats and also provide a model to suit therapy in animal studies as the lack of studies with such characteristics.

Materials and Methods

Animals

The sample consisted of 30 male Wistar rats, approximately eight weeks old. There were no restrictions on feeding, being offered food and natural water ad libitum, with controlled temperature ($24 \pm 1^\circ\text{C}$) and 12-hour dark/light cycle. This research was approved by the Ethics Committee on Animal Use (CEUA), --- University -----. It is included only animals that did not show any noticeable change in behavior or physical.

Experimental model of suit therapy

It was made for the animals, an experimental model of therapeutic jumpsuit dress type, based on the original model used in suit therapy.

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This garment is composed of an upper and a lower part, closed with Velcro, made of raw cotton 180, in which two elastic bands were adapted, arranged in “X” for vertebral approach through traction and load of 50% of the animal weight (Figure 1).

To calculate the body overload, performed by means of the elastic displacements, it was analyzed by the load cell with a capacity up to 100 Kgf, SB-100 Lynx[®] (sensitivity 1.4622 mV/V and 12 V dc). The values were obtained every two centimeters of displacement. The collected data were arranged in scatter diagrams (kgf versus displacement), and by using the technique of polynomial interpolation was possible to find polynomials that shaped the data and allowed the displacements calculation of the elastics in centimeters. The preparation of spreadsheets, scatter graphics and polynomial interpolation were built with Excel program.

Division of the groups

The sample was divided into five groups, with six animals each:

- Group 1 (G1) – absolut control;
- Group 2 (G2) – suit placement and then removal;
- Group 3 (G3) – permanence for two hours with the suit;
- Group 4 (G4) – permanence for two daily hours with the suit and adapted weights;
- Group 5 (G5) – permanence for two daily hours with the suit and traction bands.

For G4 and G5 was provided a load of 50% of the animal body mass. In G4 the load by means of fishing sinkers, was divided into two bags attached to the suit (25% load on each side) and to G5, two elastics stretched and arranged in “X” on the animal dorso (25% load on each elastic). For this purpose, the animals were pre-weighed before the beginning of each week. The displacement of each elastic was measured with a digital pachymeter of Digimes[®] brand (São Paulo –Brazil) for better precision of the calculated measure and consequent accuracy on the animals load. The G2 suffered the same stress of suit placement than the other groups (G3 to G5) and so, even not remaining with it, they were equalized in the experimental manipulation.

During the time spent with the suit, the animals were kept in accommodation boxes with three animals per box. Researchers prevented postural accommodation of the animals, so that they would remain in motion during the two hours proposed. Before starting the



Figure 1: Experimental model of therapeutic jumpsuit showing the placement form and arrangement of the elastics for vertebrae compression by means of traction.

study, all the rats were adapted for three days regarding the placement of the garment.

The experiment lasted four weeks, held on five consecutive days, with a break of two days, totaling 40 hours of permanence with the suit (G3 to G5) and without the garment (G1 and G2). On the day following the last day of the experiment, the animals were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg), and under the effect of these anesthetics, were decapitated at the guillotine for vertebral and blood analysis.

Bone histomorfometry

Histomorphometric analysis was performed on thoracic (T3-T6) and lumbar region vertebrae (L4-L6), without having a specific segment of these regions. The bones were fixed in 10% buffered formalin for 24 hours at room temperature. Then descaling was performed in 5% trichloroacetic acid for ten days. After this time, the bones were washed, cleaved longitudinally, dehydrated, diaphanized, and embedded in histological paraffin for sectioning in seven micrometers thickness (μm), using a histological microtome (Olympus CUT 4055). After making the slides, it was stained with hematoxylin-eosin (HE). The images were obtained by photomicrography, saved in JPEG format and analyzed with Image-Pro Plus version 6.0 (Media Cybernetics, Inc.).

For osteocytes counting and cortical thickness measurement, each vertebrae was photographed in four quadrants: two posterior (upper and lower) and two anterior (upper and lower). The images of the four quadrants were obtained at 400X magnification, immediately below the articular cartilage and the apparent concentration of chondrocytes to upper images, and immediately above the articular cartilage and the apparent concentration of chondrocytes to lower images.

The counting of osteocytes number was adapted from Ando et al. [18] and Hagiwara et al. [19]. Therefore, it was chosen an interest area (rectangle of 100 μm width by 200 μm length) which was overlaid on the cortical layer. The counting was performed only in cells that were within this space, considering the top and left edges of the rectangle, as lines of exclusion and the right and inferior edges, lines of inclusion for this analyzes.

For measurement of the cortical layer, a line was drawn in this area, keeping a distance of 5.195 μm from the image top edge to the upper quadrants and the same distance from the lower edge for the analysis of images of the lower quadrants. The four values from both analysis (osteocytes counting and cortical thickness) were summed to provide a final value per vertebrae for each category of analysis.

The percentage measurement of the porous area and trabecular area followed the methodology adapted from Carvalho et al. [20]. For this, the images were obtained at 100X magnification, upper quadrant below the articular cartilage and the lower quadrant above the articular cartilage of each vertebrae, being attached to them, a grid containing 150 points. Medullar regions (porosity) and trabecular which have been under the points were marked and counted, and their measurements (%) were determined according to the following formulas:

$$\text{Porosity} = \frac{\text{number of points in the cavity} \times 100}{\text{Total number of points}}$$

$$\text{Trabecular bone density} = \frac{\text{number of points on the trabecular} \times 100}{\text{Total number of points}}$$

Serum level of osteocalcin

For the OC analysis, a blood sample was collected from each

animal at the euthanasia time, with a minimum fasting of eight hours. Subsequently, the blood was centrifuged to separate the serum. Once separated and frozen it was analyzed by Immulite®/Immulite® 1000 osteocalcin (Siemens Medical Solutions Diagnostics – USA) kit, considering the reference value of < 2.0 to 21 ng/ml.

Statistical analysis

The Shapiro-Wilk test for verification of data normality was used. Then, one-way ANOVA for analysis between groups was applied, with t post hoc. The significance level was 5%.

Results

Body mass

Only G4 showed significant variation in body weight, with 65.6 grams less than G2, and 62.76 grams less than G1 (Table 1).

There was a significant increase in total body weight of the animals until the fourth week of intervention. At the end of the fourth week the weight remained with no significant difference between groups.

Bone histomorfometry

There were no significant differences between the groups in comparison to the percentage of the porous and trabecular area, as well as the osteocytes count. For cortical thickness, G3 showed significant difference from the G4 to the dorsal vertebra (Table 2).

Bone tissue morphology

No morphological changes were found in vertebral bone tissue in these regions, as acellular spaces, neovascularizations or nonspecific cellular infiltrates (Figure 2).

Groups (G)	Groups comparison	Difference	Error	(p)	Weeks (S)	Week of comparison	Difference	Error	(p)
G5	G4	41,133	16,167	0,178	S1	S2	-6,227	1,932	0,036*
	G3	-3,000	16,167	1,000		S3	-20,000	1,892	0,000*
	G2	-24,467	16,167	1,000		S4	-27,067	2,386	0,000*
	G1	-21,627	16,956	1,000		S5	-14,867	12,173	1,000
G4	G5	-41,133	16,167	0,178	S2	S1	6,227	1,932	0,036
	G3	-44,133	16,167	0,117		S3	-13,773	1,623	0,000
	G2	-65,600	16,167	0,005*		S4	-20,840	1,738	0,000
	G1	-62,760	16,956	0,011*		S5	-8,640	12,005	1,000
G3	G5	3,000	16,167	1,000	S3	S1	20,000	1,892	0,000
	G4	44,133	16,167	0,117		S2	13,773	1,623	0,000
	G2	-21,467	16,167	1,000		S4	-7,067	1,568	0,001
	G1	-18,627	16,956	1,000		S5	5,133	12,163	1,000
G2	G5	24,467	16,167	1,000	S4	S1	27,067	2,386	0,000
	G4	65,600	16,167	0,005*		S2	20,840	1,738	0,000
	G3	21,467	16,167	1,000		S3	7,067	1,568	0,001
	G1	2,840	16,956	1,000		S5	12,200	11,979	1,000
G1	G5	21,627	16,956	1,000	S5	S1	14,867	12,173	1,000
	G4	62,760	16,956	0,011*		S2	8,640	12,005	1,000
	G3	18,627	16,956	1,000		S3	-5,133	12,163	1,000
	G2	-2,840	16,956	1,000		S4	-12,200	11,979	1,000

* Statistical difference of G4 compared to G2 and G1 and difference on evolution between the first four weeks.

Table 1: Body mass: comparison between groups (G1, G2, G3, G4 and G5) and weekly evolution (S1, S2, S3, S4 and S5).

DORSAL	G1	G2	G3	G4	G5
Thickness of Cortical layer	79,70 ±29,68 µm	78,53 ±15,81 µm	130,75* ±41,90 µm	110,15 ±33,80 µm	83,35 ±14,75 µm
Counting of Osteocytes	19,88 ±4,77	19,04 ±4,79	16,62 ±2,79	22,54 ±5,75	18,75 ±2,79
% porosity	62,162 ±7,524	63,830 ±5,026	63,995 ±6,422	65,163 ±6,141	68,712 ±4,103
% trabecular	37,83 ±7,5241	36,16 ±5,027	35,99 ±6,422	34,832 ±6,1418	31,277 ±4,101
LUMBAR					
Thickness of Cortical layer	113,631 ±42,275	119,696 ±31,101	130,163 ±47,536	111,965 ±45,821	123,949 ±18,620
Counting of Osteocytes	13,875 ±7,186	14,791 ±3,128	18,583 ±2,648	17,958 ±2,466	17,125 ±4,56
% porosity	66,33 ±2,818	62,385 ±6,419	66,22 ±2,714	66,052 ±5,477	62,885 ±8,201
% trabecular	33,664 ±2,817	37,607 ±6,420	33,774 ±2,712	33,940 ±5,479	36,718 ±8,282

* Difference of G3 compared to G4

Table 2: Means and standard deviations of groups for each vertebral histomorphometric evaluation.

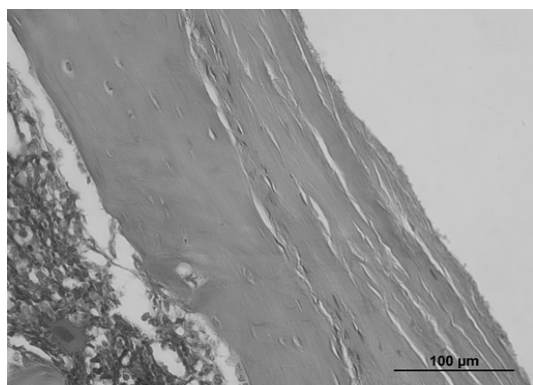


Figure 2: Photomicrograph of a lumbar vertebrae of Wistar rat showing bone tissues without morphological changes: in A, cortical area in 400x magnification and B, trabecular area in 100x magnification.

Quantitative analysis of serum osteocalcin

There was no estimated OC quantity on blood serum that could indicate changes in bone metabolism. All samples presented results of <2.0 ng/mL.

Discussion

The suit therapy was designed in the project known as “Penguin suit” developed by the Russian space program. This garment was used by astronauts on long space voyages to neutralize the effects of lack of gravity and consequent body hypokinesia [14]. The fact that it could be worn for long periods of time was the basis for creation of the intensive therapy using the suit [16,17], which consists of 80 hours of therapeutic exercises program performed in four hours daily over five consecutive days, for a period of four weeks. During this period the patient remains for up to three hours with the garment [14].

The experimental method of this research aimed to approximate at maximum to the existing protocol of intensive therapy, to do so, the animals remained in quadrupedal and bipedal support, without remaining lying. It was not given a protocol of body activity to be performed during the two hours proposed. Therefore, there was no musculoskeletal demand beyond the one imposed by the garment and elastics, which may contribute for not having muscle and bone overload enough to interfere with bone remodeling. Thus, it was found no change in the osteocytes number, trabecular volume and presence of serum expression of OC.

According to Maimoun and Sultan [18], as the changes in bone mass result from slow metabolic action, biochemical bone markers are useful to investigate the acute effects of mechanical loading on bone remodeling, however, the results of OC on serum in this study, confirm the absence of bone formation even in initial process. In contrast, G3, showed an increase to cortical layer thickness compared to G4. This finding may be a chance occurrence, considering the other results presented and the lack of change in body mass for this group pointing to an increase in muscle mass due to higher contraction labor and consequent influence on bone formation. Another hypothesis could be the higher animal body activity of G3, once that without the bands and weights adapted to the suit, and with the increase in sensory inputs, mainly tactile and proprioceptive provided by the suit, the animals may have felt freer and sensorially stimulated to movement.

On bipedal posture, the gravity distribution on the spine works

in balance with the muscular forces that are directly proportional to the spine direction and pelvic tilt [19]. Solomonow [21], describes that the muscles associated with the joints have an important role as limiters of the movement and therefore as joint stabilizers, and in some joints, such as intervertebral, the muscle function as stabilizer is amplified. Based on this assumption, on all fours posture, there is no unidirectional gravitational action with the muscle fibers of the trunk, neither unloading of this weight compatible with the spinal direction. Thus, the muscular activity could supply the load imposed by the traction bands, without there being vertebral compression sufficient to generate changes in the bone tissue metabolism of these structures.

In a retrospective analysis of researches about bone density regarding the frequency and duration of mechanical stimuli, can be highlighted the study carried out by Iwamoto et al. [22], which in a sample of 40 rats, found that after eight and 12 weeks of treadmill walking there was a significant increase in the rate of mineral apposition and bone formation at the proximal and distal tibia shafts of these animals. They also observed that the rate increasing was greater after 12 weeks of physical activity. In a study carried out by Boreham and McKay [23], with humans, it became evident an increase of 1.2% BMD at the femoral trochanteric region after eight months of jumps incorporated to the school physical education program. Likewise, Gunter et al. [24], reported that children 7-9 years of age showed 7.9% higher bone mineral content at the spine and 8.4% more in BMD at the hip than the control group, after an intervention with jumps for seven months. Finally, Meyer et al. [25], observed that children after nine months of exercises, including in physical education at least 10 minutes of jumping or strength training, showed BMD 4.7% higher at the spine and 5.4 % at the hip than the control group. In this sense, the researches have shown that changes in bone tissue by physical interventions occur with short stimuli for prolonged periods and at high frequency. Comparing with the present study, the time of this experiment was four weeks and may be an inefficient period to occur morphological changes in bone tissue.

Erinckson and Vukovich [26], in a pilot study with men, evaluated changes in bone markers during a eight week jump training program. The results showed that the bone tissue of adult men, did not respond to high-impact exercises. Furthermore, they concluded that the use of recovery periods between exercise sessions performed on the same day can result in positive changes in bone renovation. This conclusion was based on a previous study carried out by Robling et al. [27] who investigated the recovery time to restore the mechano sensitivity in bone dynamic loads. For these authors, given the knowledge that the bone cell is able to sense and respond to mechanical forces, starting to reduce the mechano sensitivity right after starting the stimulus, under constant mechanical stimulation, the bone tends to become insensitive to morphological and chemical changes. In this study, the mechanical stimulus was constant (two hours), daily and with high magnitude load (50% of the animal weight) which may have provided, desensitization of mechanoreceptors.

Some treatment methods that employ suit therapy advocate the use of these garments with traction bands out of the clinical environment, encouraging its manipulation by caregivers, providing the use of therapeutic device for a longer time [15]. This statement may be more effective for the quality of bone remodeling, since it increases the time and period of mechanical stimulation on bone tissue compared to the protocol of intensive therapy that uses the suit for a maximum time of three hours for a limited period of four weeks. Apart from longer stimulus time, should be considered that in order to allow normal

domestic activities, the mechanical load will be applied in a mild to moderate way and in various directions.

According to Wallace et al. [28], in an experimental study with rats, boosting usual activities with bone loading in different directions can positively affect a growing skeleton, suggesting that low-intensity activities that produce loads in various directions may represent an alternative more feasible for the quality of bone tissue development than strenuous high-impact exercises. Still, Chen et al. [29] found in a home therapy program of 12 weeks for CP, with light increasing loads, that there was an increase in femoral BMD by increasing muscle strength, and claim that physical exercise in a natural environment, can increase the participation of children. Therefore dynamic activities with daily doses of short duration, low magnitude, high frequency, with rest breaks [2,30,31], different mechanical stimuli in different directions [20], are more effective for increasing bone strength.

It is important to consider all aspects of bone response to the exercise so that they can be reliably used as a treatment in a clinical environment. Given this, any form of scientific evidence that may result from the use of physical activity protocols in living beings should be carefully analyzed and adequate to a musculoskeletal system with disordered development. It is worth remembering that, as confirmed by Riquelme et al. [32], lesions in the central nervous system, such as the CP, the change in muscle tone will be present, as well as impaired recruitment of motor units to generate adequate reactive power to the loads imposed daily to body. Given this assumption, appropriate muscular strengthening exercise programs for this population, rather than providing direct mechanical load on skeletal, may contribute indirectly to the desired bone formation. According Mullender et al. [33], the forces generated by the muscles during exercise, sufficiently overload the bone tissue, producing adaptation with increasing in bone metabolism. This hypothesis was recently reinforced by Noble et al. [34], in a study with CP in which they observed the relation of bone endurance with muscle volume, concluding that there is significance in the development of therapeutic programs for strengthening and improvement in muscle volume to improve bone strength and reduce fracture risk in CP.

There were some limitations to this study, as the animals present themselves on all fours being the trunk with different gravitational loads than in the bipedal posture of the human; and the scarce researches that addressed issues related to mechanical loads or suit therapy as vertebral articular approximation forms, with no guiding methodologies for the experimental process, requiring not only the creation of an experimental suit model, but also experimental parameters which were based on the existing suit therapy protocol and on methods of histological analyzes adapted. After searching the literature it was found that this is an unpublished study, pointing to the need for quantitative and qualitative research regarding the suit therapy, with extensive investigations and that can include other structures of the locomotor system, especially the muscles for checking its response to this method of physical therapeutical intervention.

Important to stress that the discussion generated here aims neither to induce nor to conclude that the concepts of suit therapy have no scientific and secure basis for its results not be considered a reliable and efficient intervention. What is proposed is the beginning of a discussion based on scientific evidences guiding to physical interventions where there is the qualitative and quantitative knowledge necessary to achieve real goals.

Conclusion

There were no changes in bone tissue of the dorsal and lumbar

vertebras of Wistar rats with the spinal compression performed by the traction bands of this experimental model of suit and suit therapy. Similarly, no morphological changes that indicate some form of degenerative process of the bone tissue were observed.

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