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ORIGINAL RESEARCH

Evaluation of longitudinal ligament of the spine of Wistar rats in an experimental model of Suit therapy

Avaliação dos ligamentos longitudinais da coluna de ratos Wistar em modelo experimental da terapia Suit

Evaluación de los ligamentos longitudinales de la columna vertebral de ratas Wistar desde el modelo experimental de la terapia Suit

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ABSTRACT | Ligaments adapt according to the intensity of physical activity and mechanical load to which they are subjected. In the last decade there have been methods and protocols in the field of infant neurofunctional physiotherapy, which have the term "suit" in common, to characterize the existence of suits with adjustable elastic bands and the possibility of applying load on the human skeleton. Since the mechanical load can produce fibrocartilaginous changes on the ligaments and also that no studies evaluating the effect of suit therapy on ligaments of the spine were found, research with experimental methods of load are justified. The aim of this study was to analyze thickness and morphology of longitudinal ligaments of the spine of Wistar rats when subjected to mechanical load by vertebral compression. Thirty animals were separated into five groups (G1 – control; G2 – simulation of the use of suit; G3, G4, and G5 – maintenance of the suit). The suit experimental model, in G4 and G5, were adapted weights or elastic bands arranged in "X" for 50% of spinal overload of the weight of the animal, who remained with the suit for 40 hours over four weeks of experiment, five days a week. There were no significant differences for thickness, and morphological changes of longitudinal ligaments were also not observed. We concluded that there were no changes in longitudinal ligaments of the spine in animals subjected to the experimental model of suit therapy.

Keywords | Cerebral Palsy; Child; Physical Therapy Modalities; Spine; Rats, Wistar.

RESUMO | Ligamentos adaptam-se de acordo com a intensidade da atividade física e carga mecânica a que são submetidos. Na última década, na área da fisioterapia neurofuncional infantil, têm surgido métodos e protocolos que possuem em comum o termo *suit* para caracterizar a existência de vestimentas com bandas elásticas ajustáveis e a possibilidade da aplicação de carga sobre o esqueleto humano. Visto que a carga mecânica pode produzir alterações fibrocartilaginosas sobre os ligamentos e que não foram encontrados estudos avaliando o efeito da terapia *suit* sobre os ligamentos da coluna, justificam-se pesquisas com métodos experimentais de carga. O objetivo deste trabalho foi analisar as espessuras e morfologia dos ligamentos longitudinais da coluna de ratos Wistar quando submetidos à carga mecânica por compressão vertebral. Trinta animais foram separados em cinco grupos (G1 – controle; G2 – simulação do uso de *suit*; G3, G4 e G5 – manutenção da vestimenta). Ao modelo experimental do *suit*, em G4 e G5, foram adaptados pesos ou elásticos dispostos em "X" para sobrecarga vertebral de 50% do peso do animal, que permaneceram com a vestimenta por 40 horas ao longo de 4 semanas de experimento, 5 dias por semana. Não houve diferenças significativas para a espessura, assim como não foram observadas mudanças morfológicas nos ligamentos longitudinais. Conclui-se que não houve alterações nos ligamentos longitudinais da coluna em animais submetidos ao modelo experimental de *suit* terapia.

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Descritores | Paralisia Cerebral; Criança; Modalidades de Fisioterapia; Coluna Vertebral; Ratos Wistar.

RESUMEN | Pueden adaptarse los ligamentos a la intensidad de la actividad física y a la carga mecánica sometida a ellos. En la última década, han surgido métodos y protocolos en el área de fisioterapia neurofuncional infantil que tienen en común el término "suit" para caracterizar la existencia de ropas con bandas elásticas ajustables y la posibilidad de aplicación de cargas en el esqueleto humano. Debido a que la carga mecánica puede producir alteraciones fibrocartilaginosas en los ligamentos y que no han sido encontrados estudios que evaluaron el efecto de la terapia *suit* en los ligamentos de la columna vertebral, las investigaciones con métodos experimentales de carga son necesarias. Este estudio tiene el propósito de evaluar las espesuras y la morfología de los ligamentos longitudinales de la columna vertebral de ratas Wistar cuando sometida a carga mecánica por compresión vertebral. Se dividieron treinta ratas en cinco grupos (G1 —grupo control; G2 —simulación de la utilización del *suit*; G3, G4, G5 —mantenimiento de la ropa). Desde el modelo experimental del *suit*, en el G4 y G5 se adaptaron pesos o elásticos puestos en "X" para la sobrecarga vertebral de 50% del peso de los animales, los cuales permanecieron con la ropa durante cuarenta horas, al largo de cuatro semanas de experimento, en cinco días semanales. No fueron observadas diferencias significativas para la espesura, tampoco cambios morfológicos en los ligamentos longitudinales. Se concluye que no se observaron alteraciones en los ligamentos longitudinales de la columna vertebral de los animales sometidos al modelo experimental de la terapia *suit*.

Palabras clave | Parálisis Cerebral; Niño; Modalidades de Fisioterapia; Columna Vertebral; Ratas Wistar.

INTRODUCTION

The joints between the vertebrae are strengthened and supported by ligaments, and among them are the anterior longitudinal ligament (ALL) and the posterior longitudinal ligament (PLL), extending from the cervical spine to the sacrum[1]. Ligaments adapt to the intensity of physical activity, and may hypertrophy or atrophy, in such a way that they may change the resistance in response to exercise or immobilization[2]. This dynamic behavior suggests that cells are able to detect changes in the mechanical load and to coordinate their response to change the composition of the extracellular matrix (ECM). One of the ways in which ECM can be changed is by the formation of a fibrocartilaginous matrix where ligaments are under compression[3]osteotendinous junctions, osteoligamentous junctions. Individuals with vertebral slip feature ossification of the ligamentum flavum and infiltrate with chondrocytes, suggesting the involvement of mechanical load on morphological changes[4].

Ligaments have low supply of oxygen and nutrients, low cell density, and poor regenerative capacity. However, they experience some of the highest mechanical loads of the body. When these loads exceed a critical threshold, injuries may occur, resulting in morphofunctional changes and disorder of movements[5]. Ligaments are functional (effective) under stress or stretch, however, without compression or functionality when shortened beyond their rest threshold[2].

Mechanical stimuli, when provided in a proper manner, induce the growth and better alignment of cells, improving the quality of ECM[5,6], however, the frequency as well as the intensity of these stimuli are not yet well known[2].

In the last decade, methods have emerged in the field of neurofunctional physiotherapy, which have the term "suit" in common, in their classifications, to characterize the existence of suits that function as dynamic orthotics[7,8]. The basic concept is to create a support unit to align the body as close to normal, improve and adjust the proprioceptive system through pressure exerted on joints, ligaments, and muscles, reducing pathological reflexes, and restoring muscle synergies that are translated into appropriate patterns of movement[7]. Elastic bands are adjustable, which means a discharge of 15 to 40 kg can be axially applied in the body[8]. However, the suit suffered changes compared with its original form, to adapt the therapeutic needs within the neurological rehabilitation, and little attention has been given to the effects of this device on the musculoskeletal system, in particular spinal stabilizers ligaments.

Since the mechanical load may produce alterations on the ligaments and the lack of studies evaluating the effect of suit therapy on ligaments of the spine, and also scientific reports are rare on the technique, experimental methods of load, in suit model type, on the longitudinal ligaments of the spine of rats are justified. Thus, the aim of this study was to analyze longitudinal ligaments of the spine of Wistar rats submitted to mechanical load produced by an experimental model of suit therapy.

METHODOLOGY

Animals

The sample was composed of 30 Wistar rats, males, with eight weeks of age. There were no restrictions on food, being feed and natural water *ad libitum*, with controlled temperature (24ºC±1ºC) and light/dark cycle of 12 hours. The research was approved by the Ethics Committee on the Use of Animals (CEUA) of the State University of Western Paraná – Unioeste.

Materials and experimental protocol

An experimental model, of therapeutic suit type, was made based on the model used in suit therapy. This suit was composed of upper and lower parts, closed with Velcro, prepared with raw cotton 180-thread fabric, in which were adapted two elastic bands arranged in "X" for vertebral approximation, with 50% of the load weight of the animal, as previously described[9].

For the calculation of body load, done by the displacements of the elastic bands, they were analyzed per load cell, with capacity up to 100 kgf (SB-100 of LYNX®). Through a system of biological data collection (BioEMG1100, LYNX®, Brazil), it was possible to display the obtained data every two centimeters of displacement. The collected data were arranged in scatter plots (kgf versus displacement) and it was possible to find the polynomials that have modeled the data and allowed calculating the displacements of the elastics in centimeters. The elaboration of spreadsheets, scatter graphs, and polynomial interpolation were built with the Excel program.

The sample was divided in five groups (from G1 to G5), with six animals each:

- G1 (Group 1) – absolute control;
- G2 (Group 2) – putting the suit following removal;
- G3 (Group 3) – length of stay of 2 hours with the suit only;
- G4 (Group 4) – length of stay of 2 hours a day with the suit and weights adapted;
- G5 (Group 5) – length of stay of 2 hours a day with the suit and traction bands;

For G4 and G5, a load of 50% of the body weight of the animal was given. In G4, the load, through the pellets of fishing, was divided in two units coupled to the suit (±25% load on each bag); and for G5, two elastics pulled and arranged in "X" on the back of the animal (±25% load on each elastic). To this end, the animals previously weighted before the beginning of each week. The displacement of each elastic was measured with a digital caliper (Digimess® – São Paulo/Brazil).

During the length of stay with the suit, the animals were kept in boxes (three animals per box). The researchers avoided the postural accommodation of the animals, in such a way that they would keep moving during the 2 hours proposed. Before the beginning of the study, the rats were adapted for three days for putting the suit.

The experiment lasted four weeks, performed in five consecutive days, with two days off, totaling length of stay of 40 hours with the suit (from G3 to G5) and without it (G1 and G2). On the day following the last day of experiment, the animals were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine (10 mg/kg), then beheaded and the thoracic segment of the vertebral column dissected.

Histomorphometry of ligaments

Histomorphometric analysis was performed on a vertebra in the thoracic region (T3-T6), in the interscapular region, without a specific segment of this region. The choice of the sample considered the visual and structural quality that best make the analyses feasible.

The bones were fixed in buffered formalin at 10% for 24 hours at room temperature. Then, the decalcification in trichloroacetic acid at 5% was carried out for about ten days. After this time, the bones were washed, longitudinally leaved (Figure 1), dehydrated, cleared, and embedded in histological paraffin to be sectioned into thickness of 7 micrometers (μm), using a histologic microtome. After making the blades, they were stained in Hematoxylin-eosin (HE). The images were obtained for photomicrography, recorded in JPEG format, and analyzed in the program Image-Pro Plus version 6.0 (Media Cybernetics, Inc.).

Figure 1. Sagittal section of the vertebral segment with presentation of vertebral bodies and spinal cord

Each vertebra was analyzed in four quadrants: two quadrants for PLL (upper and lower) and two for ALL (upper and lower). The images were obtained on increase of 400× immediately below the articular cartilage and the apparent concentration of chondrocytes for upper images and immediately above the articular cartilage and the apparent concentration of chondrocytes for inferior images. The thicknesses of longitudinal ligaments were obtained by drawing a line to measure their width in micrometers (μm) and positioned in the center of the image at 50% zoom. In the presence of ligament spaces resulting from the artifacts, they were not considered in full measure. The morphological analysis was performed in each quadrant observing the presence of chondrocytes between the fibers of connective tissue and presence of neovascularization (Figure 2).

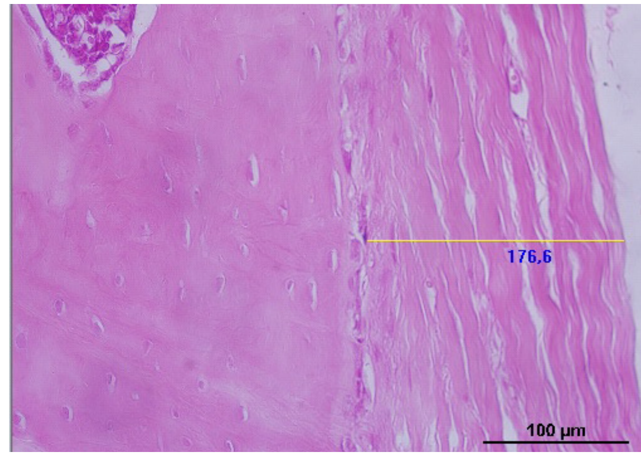

Figure 2. Morphological analysis of the anterior longitudinal ligament

Statistical analysis

The Shapiro-Wilk test was used to verify the normality of the data. Afterwards, one-way ANOVA was applied for analysis between groups, along with Tukey test. The significance level was 5%.

RESULTS

The findings allowed comparing the averages of the ligament thickness per group of the upper and lower regions of the vertebrae, as well as the average for each ligament, as shown in Table 1. There was no statistical difference in the comparison between groups, or in the comparison within the groups between lower and upper parts.

Table 1. Means and standard deviations of the thicknesses of longitudinal ligaments per upper and lower spine region and total means per ligament

	Thicknesses	G1	G2	G3	G4	G5
ALL	Upper	119.13±32.43	140.53±61.50	144.51±20.71	127.34±41.81	164.59±34.90
	Lower	119.66±44.11	136.71±61.97	130.48±60.06	161.48±29.47	150.02±41.37
PLL	Upper	65.61±20.17	104.76±79.31	86.59±46.83	60.89±37.92	65.59±22.15
	Lower	73.32±40.88	67.75±25.46	64.5±18.90	66.60±26.14	56.78±22.61
Total	ALL	119.39±10.71	138.62±53.21	137.49±29.12	144.40±17.90	157.30±31.73
	PLL	69.47±29.10	86.25±51.28	75.55±31.04	63.74±27.60	61.19±15.11

No morphological changes were found in the ligaments, such as presence of chondrocytes or other cells between fibers of the connective tissue. Neovascularization was also not observed in such regions.

DISCUSSION

The methods that use the suit therapy, were designed in the project known as "Penguin suit", developed by the space program of Russia that was used by astronauts in space flights to counteract the harmful effects of weightlessness and hypokinesia. The fact that it could be used for long periods of time was the basis of the creation of the intensive therapy with the use of the suit[8,10], which involves a program of 80 hours of body activity (therapeutic exercise), performed four hours a day, on five consecutive days, for a period of four weeks and the patient staying up to three hours with the suit. Some of the concepts advocate the use of these suits with traction bands outside the clinical environment by encouraging its handling by direct caregivers, favoring their use for longer periods of time[7]. However, when searching in the literature, we observed that it is still a technique lacking scientific depth, considering the difficulty in finding research regarding its use, both clinical and experimental.

The experimental method of this research aimed to approach the most of the intensive therapy protocol, however, the animals remained only supported by their members with volunteer movements without remaining lay down. We did not determine a protocol of exercises as a way to increase body activity. Thus, there was no musculoskeletal requirement beyond that imposed by the suit and the elastics bands adapted to the load of 50% of the weight of the animal, which may have contributed to have no deformation of the longitudinal ligaments by mechanical stress.

In bipedal posture, the distribution of gravity on the spine works in equilibrium with muscular forces, which is directly proportional to vertebral direction and pelvic obliquity[10]. In the four supports posture, there is no one-way gravitational acting with muscle fibers of the torso, and muscle activity might have supplied the load imposed by traction bands, without enough load in the longitudinal ligament to generate morphological changes in these structures. Solomonow[2] describes that muscles associated with joints have an important role as movement limiters and, therefore, as joint stabilizers. In some joints, such as in intervertebral ones, the muscle function as stabilizer is amplified. Since the region of collection was interscapular, in addition to the direct effect on the spine, promoted by the suit, a possible altered muscle action of those caused (or inserted) on the scapula, could have produced influences on the ligaments.

The musculoskeletal system is able to respond to changes in the conditions of mechanical load[2,3,11,12], and this functional and structural adaptation is already well understood to muscle and bone. However, little is known about the effects of exercise on ligaments[13]. Tendons and ligaments have very similar characteristics, both are recognized for presenting great tensile strength and are composed of collagen fibers. Therefore, their cells behave dynamically with the ability of reorganizing before mechanical stimuli, generating changes in ECM[14].

The morphology of the connective tissue can be influenced especially during motor growth and development. A study by Kasashima et al.[15] with ponies demonstrated that there was an increase in the thickness of the superficial digital flexor tendon in groups that exercised daily with load for short periods, however long lasting. Previously, Fujie et al.[16] had already observed in rabbits aging one to three months that the changes in mechanical dimensions and properties of patellar tendons, induced by mechanical stress, were higher in younger animals. Wearing et al.[13], in a review on the impact of children's obesity on the musculoskeletal apparatus, comment that many orthopedic conditions that manifest in obese adults may be a consequence of excessive and prolonged load imposed on young tissues. Thus, it was believed that the mechanical load imposed by the suit associated with overload (of the weight or elastic) could produce some type of change to the fibrocartilaginous tissue, a fact that did not occur, as pointed to bone tissue[9] and intervertebral disc[17].

Waugh et al.[18] evaluated the calcaneal tendon of children subjected to resisted plantar flexion exercises, noting that there were changes in mechanical properties of tendons, with 29% increase of rigidity. Again it should be noted that in this study we used animals with healthy central nervous system, and therefore with normal muscle adaptive capacities. When considering the reduction of muscle strength in children with neuromotor dysfunction, associated with the load imposed by traction bands for long periods, the vertebral slip, as well as damage to the connective tissue due to lack of efficient muscular stability, would be a reasonable assumption, especially if analyzed that such loads are imposed without a validated quantitative control by methods using suit therapy.

Prolonged exposure to mechanical load can lead to chronic inflammation and degeneration of tissues of the spinal ligaments as a result of a cumulative disorder[19]. Hypertrophy and failure of longitudinal ligaments – especially the PLL – results in spinal cord compression with strong potential for the development of myelopathy[20,21], causing ossification of this ligament[22,23]. Such thickening may be the result of a process of ligament degeneration caused by metaplasia of collagen fibers giving rise to infiltrators of chondrocytes, proliferation of fusiform cells similar to fibroblasts, infiltration of vessels and small ossifications[23]. In the research by Kasashima et al.[15], the results support the hypothesis that the imposition of additional physical activity, even after a long period of rest with little activity, did not result in compensatory activity of tendon tissue of ponies, but cumulative instead. This allows the reasoning of the stay of individuals, with little musculoskeletal capacity, before loads and in long periods, also considering suit therapy in home environment. It is worth mentioning that research on tendons previously cited had the infant population as a target, and this study was composed of young adult animals, which may have influenced the results.

King et al.[24] and Pinski et al.[19] confirmed in their investigations the presence of inflammatory cytokines in lumbar ligaments of cats, after cyclic movements of high frequency associated with the load. These findings may not exclude the hypothesis of tissue microinjury in the ligaments investigated in this study, although they have not presented indicative of thickening and morphological changes, however, it must be considered that the sample did not carry out any repeated and long lasting spinal movement.

It is important to note that the emphasis on research on the spine has been focused on disc changes, degenerations of joint cartilage, and myelopathies associated with PLL ossification[25,27]. We did not find, both for the experimental demarcation and for this discussion, research covering issues related to suit therapy or mechanical loads as a way of vertebral joint approach on the anterior and posterior longitudinal ligaments.

Suit therapy has as its main focus neuropediatric physiotherapy, which in turn focuses on the treatment of motor disorders, such as non-progressive encephalopathy cases (Cerebral Palsy). In such cases, there are changes in muscle tone, as well as inadequate recruitment of motor units to generate reaction force suited to the loads imposed on the skeleton[28]. Thus, there is a limitation of this study, which was the use of healthy animals, not subjected to brain injury models.

This study was conducted to analyze the effects of suit therapy with traction bands on the PLL and ALL of the spine, using as samples Wistar rats. Considering this, there were other limitations to this study, such as the animals being on four supports with the torso with different gravitational load from the human bipedal posture and the sample being composed of young adult rats making the comparison with the population with the highest indication to suit therapy (children) difficult, yet, in addition to not performing sample size calculation for greater certainty of the exact size of the groups, cytokines that could give indications of reshaping process have not been evaluated either. Another significant fact was the scarce research on the subject, having no guiding methodologies to the experimental process, thus requiring the elaboration of the experimental model of the suit and methods of adapted histological analysis.

Therefore, there is strong evidence of the need for quantitative and qualitative research in the field of suit therapy, with broader investigations that may include other structures of the musculoskeletal apparatus. It is important to note that the discussion here proposed does not aim to induce, neither to conclude, that suit therapy is not a safe method to be used in the field of children's neurological rehabilitation. What we propose is the beginning of a discussion, based on scientific evidence directing physical interventions, since they are techniques routinely used in clinics and rehabilitation centers and that must be supported on scientific bases.

There were no changes in the thickness of longitudinal ligaments of the spine of Wistar rats, with spinal compression performed by traction bands, in this experimental model. Hence, no morphological changes were observed in the collagen fibers and/or presence of chondrocytes and neovascularization that could indicate an inflammatory or degenerative process of these ligaments. Thus, we can see that with applied loads the method does not seem to interfere with the characteristics of the ligaments, not being indicated such times and loads, without the evolution of human studies.

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