



The effect of peripheral nervous system in growing bone biomechanics. An experimental study

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ABSTRACT

Objective: There are several factors which affect bone growth. One of them is the peripheral nervous system whose effect on the biomechanics has not been extensively studied. The purpose of this study is to assess the effect of peripheral nervous system in bone biomechanics in an experimental rat model.

Materials & methods: 27 male Wistar rats were used. In all animals, the roots of the right brachial plexus were dissected and after that the animals were divided into three groups A, B and C. The animals were sacrificed six, nine, and twelve months respectively after the denervation. Both humerus were resected and biomechanical analysis was performed.

Results: According to the findings of the present study the denervated bones sustain less loading before fracture and they become also more elastic. Additionally, in greater time after denervation plastic deformity is noticed.

Conclusion: Apart from structural changes, the peripheral nerves are responsible for biomechanical changes in the bones such the greater elasticity of the bone and the reduced strength

1. Introduction

The metabolism of the bone depends on several factors which begin the regulation of bone formation in utero.¹ These factors include genetic factors such as hormones, mechanical factors such as loading and tensile forces, as well as anatomical factors such as the innervation of the bone.² Bone is a living tissue, which must be stiff, flexible enough to absorb energy and light enough to allow mobility.^{1,3} Additionally, in bone tissue continuous remodeling is noticed.⁴ Concerning the role of peripheral nervous system in bone metabolism, it seems that its role is double: firstly the regulation of the mechanical forces applied to the bone and secondly the provision of crucial trophic factors for the functionality and the structure of the bone as well.⁵ Both neuropeptides, such as calcitonin-gene related peptide (CGRP) and vasoactive intestinal peptide (VIP), as well as neurotrophins such as nerve growth factor (NGF), contribute in the metabolism of the bone.⁶

The muscle system also affects the bone metabolism as an indirect effect of the peripheral nervous system. Muscles are responsible for the mechanical forces applied to the bones which are strongly correlated with bone growth. Since ancient years the idea that compression inhibits bone growth has been established.⁷ In 19th century Hueter

–Volkman law was introduced which consists the basis of growth modulation for correcting angular deformities.^{8,9} Later, at the end of the 20th century, Frost proposed that both mild compression and tension promote bone growth. Nevertheless, when these two forces exceed a certain limit, they inhibit bone growth.¹⁰ In molecular level, it seems that the differentiation of bone cells, such as osteoblasts and osteoclasts, is controlled by leptin which is produced in the muscle system and has positive effect on bone metabolism.^{11–13}

The purpose of this study is to examine the biomechanics of denervated bone and to determine if the peripheral nervous system affects apart from the growth of the bones their biomechanical behavior.

2. Materials & Methods

The experimental study took place in the Microsurgery laboratory of the University of Ioannina. The study protocol was approved by the institutional review board as well as by the animal care committee.

Twenty-seven male Wistar rats aged 3 weeks old were used. The mean weight of the animals was 85 gr (67–102gr). The rats were divided into 3 groups (A, B, C). In all three groups ketamine was used for anaesthesia. Ketamine was administered intraperitoneal (87 mg/kg)

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Fig. 1. Placement of the humerus on the 3-point bending device.

and the roots of the brachial plexus of the right upper limb were identified and all the five roots (C5-T1) were cut. Wound closure followed and the Wistar rats were placed in the cages. In the experimental group A, the rats were sacrificed six months after the denervation of the right upper limb and both humerus, the denervated one as well as the unilateral one were extracted. In the experimental group B the rats were sacrificed nine months after the denervation and both humerus were extracted. In group C both humerus were extracted after the sacrifice of the Wistar rats, nine months after the denervation of the left upper limb.

The animals were hosted in the microsurgery lab of the University of Ioannina, under standard laboratory conditions (room temperature $20 \pm 3^\circ\text{C}$, humidity $55 \pm 5\%$, and cycling change of light and dark every 12 h). They were housed in individual cages, and they were fed unrestrictedly with standard laboratory animal chow.

All the bones, which were extracted, were subjected to

biomechanical examination in a three-point bending strength device (Fig. 1). The mounting of the bones on the bending apparatus was done similarly for all specimens in a 3-point configuration. The distance between the two lower supports was 55 mm. The bone was fixed on them (with the aid of a scotch tape) in order to be in contact with the two lower supports of the bending apparatus (i.e. at the bottom side of the jig). The loading cell of the bending apparatus had a round-shaped tip and was symmetrically centered over the bone gap. Its size was chosen in order to be in contact only with the bone. The bone was loaded in a vertical (top-bottom) direction. This configuration resulted in zero rotational moment during the entire test.

The experiments were carried out with a SHIMADZU Autograph tester (AGS-500NJ standard Unit, Shimadzu Corporation, Tokyo, Japan) with precision of indicated values $< 1\%$. The displacement speed was set to 1 mm/min. The resistance force developed by the bone (i.e. actually the applied load), and the displacement of the head were continuously recorded with a AGS-J data processing software (Trapezium Lite Software 345-47147, Shimadzu Corporation, Tokyo, Japan). The head movement and the recording came to a stop whenever a failure of the bone took place.

The detachment, fixation, and final preparation of the bone construct from the rat body were performed within 1 h postmortem. The specimens were kept moist with 0.9% normal saline throughout the preparation and testing time. The ambient temperature during experimentation was 20°C .

Every denervated humerus was compared with the contralateral one of the same rat and the results are expressed in graphs depicting the load that was applied on the bone as a function of the displacement of the head of the device until fracture occurred.

For the statistical analysis SPSS v. 19 was used.

3. Results

In group A (6 months after the denervation), the loading forces on the denervated humerus were reduced compared to the contralateral limbs. Moreover, the denervated bones underwent greater displacement

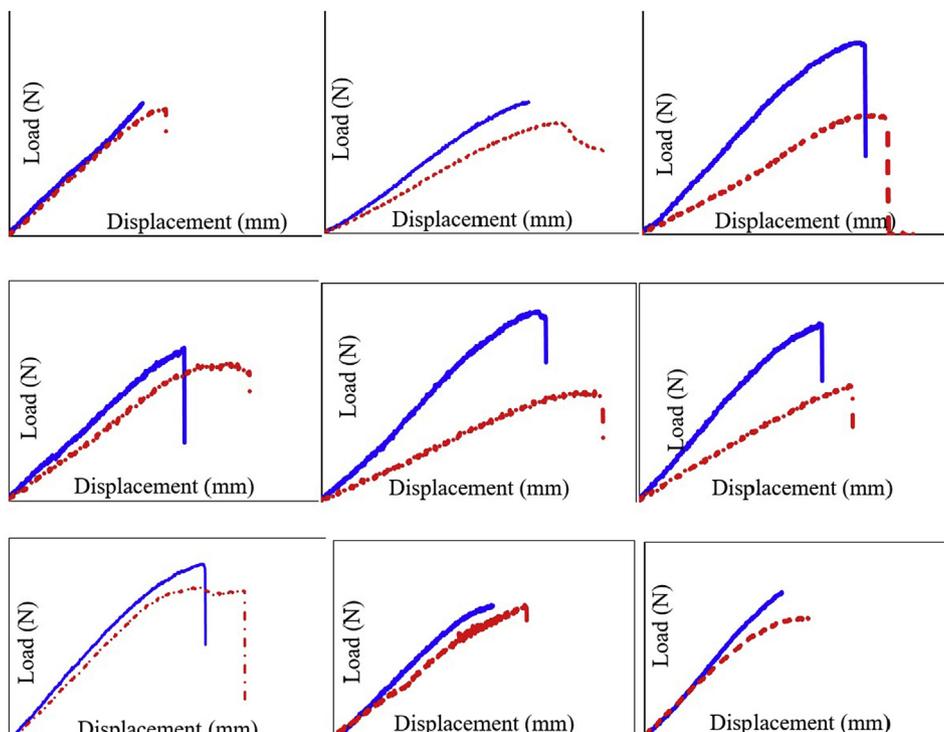


Fig. 2. Graphs of experimental group A. The vertical axis depicts the load and the horizontal axis depicts the displacement of the bone. The blue continuous line represents the normal humerus and the red interrupted line depicts the contralateral denervated humerus.

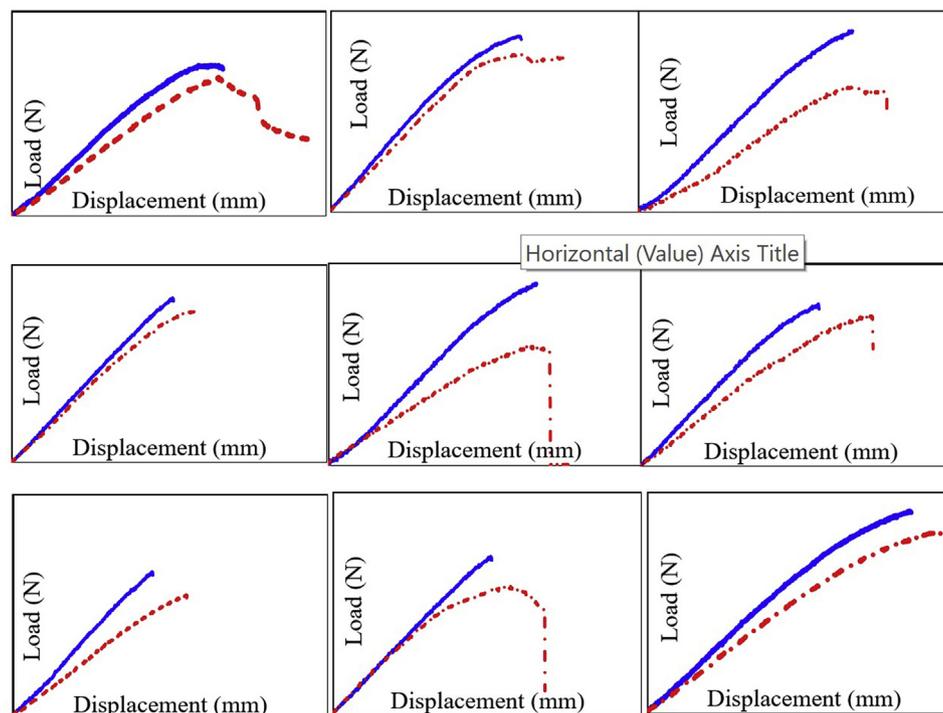


Fig. 3. Graphs of experimental group B. The vertical axis depicts the load and the horizontal axis depicts the displacement of the bone. The blue continuous line represents the normal humerus whereas the red interrupted line depicts the contralateral denervated humerus.

compared to the contralateral ones. More explicitly, the loading forces were 20.5% reduced in the denervated bones. On the other hand, the displacement was increased by 22.73% ($p < 0.05$). All the graphs of the experimental group A are shown in Fig. 2.

In the experimental group B (9 months after the denervation), the results were similar to those of the experimental group A. The loading forces were decreased in the denervated limbs by 19.79%, whereas the displacement showed an increase by 16.18% ($p < 0.05$) (Fig. 3).

The experimental group C included the rats whose humerus underwent biomechanical evaluation 12 months after the denervation of the left upper limb. In this experimental group a decrease in the maximum load force of 32.94% was noticed in the denervated humerus. Similarly, to the previous two groups the displacement of the denervated limbs was increased by 31.1% ($p < 0.05$). Additionally, in approximately half of the animals of this group plastic deformity was noticed in the denervated bones after the yield point, which was determined on the stress curves, where an alteration was noted in the behavior of the bone under loading. (Fig. 4).

4. Discussion

Bone metabolism is regulated mostly by neuropeptides and neurotrophins. Starting from the neuropeptides, calcitonin-gene related peptide (CGRP), vasoactive Intestinal Peptide (VIP) and substance P (SP) seem to be of highest importance. As far as it concerns the neurotrophins, nerve growth factor (NGF) has been investigated extensively.⁶

The effect of peripheral nervous system in bone growth has gained popularity over the years. Several authors examined the results of denervation on bone development both in experimental as well as in clinical aspect.^{14–16}

In the late 80's, Dysart et al.,¹⁷ after the denervation of the humerus in 25 days old rats, concluded that denervated humeral bones were less developed concerning their length compared to the normal ones. Later in 1997 Edoff et al.¹⁵ concluded that after dissection of the sciatic and the saphenous nerve, the length of metatarsal bones in immature rats

was shorter compared to the normal ones. 17 years later, Zamarioli et al.¹⁸ concluded that after provoking spinal cord injury, both macroscopic as well as microscopic structures of the bone are altered.

Additionally, there are clinical studies emphasizing on the impact of peripheral nervous system on the skeletal structures. In 2012, Reading et al.¹⁹ showed that humeral head deformity in neonatal brachial plexus palsy is correlated with glenohumeral dysplasia. One year later van Gelein Vitringa et al.²⁰ concluded that ventral and dorsal side of denervated scapulas were statistically significant smaller compared to the normal ones.

In general, it has been shown that bone resistance is influenced by bone macroarchitecture. The bone size and shape directly affect the bone strength, whereby the periosteal radius plays an important role in the bone resistance against bending loads.^{21,22} More explicitly if the periosteal radius increased by 8% the bone strength would be greater by 36%.²² Despite the analysis of bone strength regulation, there is no documented evidence, to our knowledge, concerning the elasticity of denervated bones.

The present study is differentiated due to the fact that we did not emphasize on the structural changes of the denervated bones, but on their biomechanical behavior was noticed and to our knowledge it is the first one focused on this aspect. According to our findings, mechanical strength is reduced in the denervated bones, compared to healthy bones. Moreover, all denervated bones sustained greater displacement before fracture. These findings were observed in all tested intervals after denervation, 6, 9 and 12 months. The results from the group with longer period of time after denervation, showed that the denervated bones display a longer regime of plastic deformation.

The predominant presence of elastic deformation (compared to plastic deformation) is a great biomechanical advantage of healthy bones, because, regardless the fracture strength, they are able to re-establish their initial shape when the applied force is removed, with no loss of their elastic properties, at all. In the contrary, plastic deformation actually means that the sample obtains a permanent deformation in its shape, owing to the application of the force; in other words, when it is in the plastic deformation regime, it is totally unable to re-establish

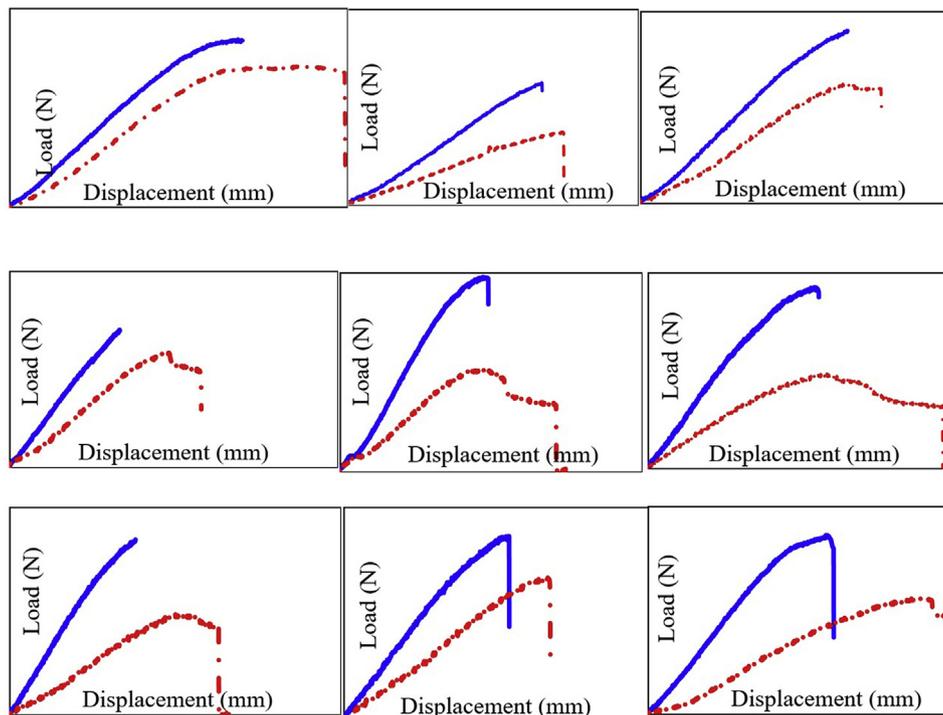


Fig. 4. Graphs of experimental group C. There are bigger differences concerning the maximum load strength and displacement between the normal and denervated bones. Additionally, plastic deformity is noticed in some denervated bones of this group after the yield point.

its initial shape, when the load is removed. In the light of the experimental findings of the present study, this means that the long-term denervated bones are prone to permanent deformation, without being able to return in its previous condition. Consequently, denervation results in a decay of the mechanical properties of the bones, reflected both in the reduction of the mechanical strength and the expansion of plastic deformation regime.

5. Conclusions

The experimental results of this study suggest that apart from structural changes, the peripheral nerves are responsible for biomechanical changes in the bones as well, since the denervated bones are mechanically weaker and have a longer plastic deformation regime, than the healthy ones. In addition, as time passes, these changes may be even more obvious. Nevertheless, more research is needed in order to be able to extract more safe results.

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None.

Conflicts of interest

All authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving animals were in accordance with the ethical standards of the institutional and/or national research committee.

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