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Effects of immobilization and whole-body vibration on rat serum Type I collagen turnover

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ABSTRACT

Objective: The aim of this study was to investigate the effects of short-term, high-magnitude whole-body vibration (WBV) on serum type I collagen turnover in immobilized rats.
Materials and Methods: Thirty Wistar albino rats were randomly divided into the following 5 groups: immobilization (IS), immobilization + remobilization (IR), immobilization + WBV (IV), control (C), and WBV control (CV). Immobilization was achieved by casting from the crista iliaca anterior superior to the lower part of the foot for 2 weeks. The applied WBV protocol involved a frequency of 45 Hz and amplitude of 3 mm for 7 days starting a day after the end of the immobilization period. Serum type I collagen turnover markers were measured by using ELISA kits.
Results: Serum NH2-terminal propeptide of type I collagen (PINP) levels were significantly lower in the immobilization groups ($p < 0.02$) compared with the control groups. Although WBV improved PINP levels in the control groups, there were no differences in PINP levels among the immobilization groups. Similarly, serum COOH-terminal telopeptide of type I collagen (CTX) levels were higher in the WBV controls than their own controls ($p < 0,05$). Immobilization led to deterioration of tendon tissue, as observed by histopathological analysis with a transmission electron microscope.
Conclusion: Although 1 week of WBV had a positive effect on type I collagen turnover in controls, it is not an efficient method for repairing tissue damage in the early stage following immobilization.

Introduction

Despite its detrimental effects on the musculoskeletal system, immobilization is still an imperative and frequently used treatment protocol in sports injuries, particularly in those involving the lower extremities. It is a known fact that immobilization results in muscle atrophy, ultrastructural deterioration of the tendons, bone degeneration, joint stiffness, and functional limitations of the musculoskeletal system.[1–3] Therefore, providing the optimal treatment and rehabilitation post immobilization is essential in minimizing the harmful effects of immobilization on the components of the muscle-tendon unit.[4,5]

Recently, research has drawn attention to the therapeutic effects of vibration techniques.[6] Whole-body vibration (WBV) in particular has been shown to possess great potential in the treatment of several musculoskeletal system pathologies. Mechanical stimulation similar to that applied by WBV appears to be beneficial for the maintenance and/or enhancement of the trabecular bone volume and skeletal mass in individuals with low bone mineral density.[7–9] Experimental evidence indicates that rehabilitation protocols involving WBV may be useful in restoring muscle strength, balance, and mobility in the elderly and in patients with musculoskeletal problems.[10,11]

In light of these findings, WBV was thought to be an alternative treatment option for accelerated rehabilitation of athletes

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following immobilization. To show the effects of WBV on the musculoskeletal system, the levels of serum Type 1 collagen turnover, a major collagen in the bone and tendon tissue was evaluated. It was hypothesized that immobilization would result in the reduction of collagen synthesis and an increase in collagen degradation and that the application of WBV would have a positive effect on collagen turnover. The serum N-terminal propeptide of Type 1 collagen (PINP) level was used as a collagen Type 1 formation marker and the serum C-terminal telopeptide of Type 1 collagen (CTX) level was used as an indirect marker of collagen Type 1 degradation.[1,12]

Our objective was to evaluate how Type 1 collagen turnover was affected by immobilization and subsequent vibration.

Materials and Methods

Study design

Thirty Wistar albino female rats aged 4–6 months (mean weight: 230.9 ± 23.3 g, range: 200–250 g) were included in the study. The rats were housed for one week in an accredited animal facility room with controlled temperature (22 °C), humidity (50 ± 10%), and light (12-hr light/dark cycle) in order to adapt with the environmental conditions. They were then randomly divided into the IS, IR, IV, C and CV groups (Table 1), each consisting of six animals. Animals in each group were sacrificed at the end of the protocol.

Casting

Casts were applied from the anterior superior iliac crest to the lower part of the foot (pelvipedal cast). The animals were anesthetized with short-acting anesthesia just prior to the casting procedure (90 mg/kg ketamine + 10 mg/kg xylazine i.p.). While anesthetized, the hindlimbs were immobilized with the hip and knee joints fixed at approximately 160° and 180°, respectively, as previously described.[13] To induce deterioration in the Achilles tendon, the ankle joints were immobilized at 25°–30° of plantar flexion. To minimize movement in the casts, slight pressure was applied when wrapping the plaster on the body. Care was taken to minimize the weight of the cast, with a target weight of 90–120 g, and standardization in each group. The rats were checked on daily basis for chewed plaster, abrasions, venous occlusion, and fecal clearance. The rats were free to move using their forelimbs, and they ate and drank *ad libitum*. The casts were removed after two weeks. The length of the casting period was chosen to simulate the immobilization period after a sports injury or after a period of hospitalization.

WBV protocol

To evaluate the immediate effects of vibration after two weeks of immobilization (IV group) and effects of vibration on control subjects (CV group), WBV with a frequency of 45 Hz and an amplitude of 3 mm was applied. The WBV platform, which directly provided mechanical stimulation on the animals' feet, was designed by the mechanical engineers of Hacettepe University, School of Sports Sciences and Technology. For the animals to adapt, intermittent vibration was used with cycles of vibration and rest periods. The WBV application was initiated with a duration of 15 min on the first day and then was gradually increased by 5 min each day until it reached a maximum of 30-min application a day. A detailed explanation of the WBV protocol is provided in Table 2. Animals in the IR group were kept on the vibration platform for the same duration as those in the IV and CV groups, but they did not undergo WBV.

Measurement of serum Type 1 collagen turnover

Just before the animals were sacrificed, blood samples (2 ml) were collected from the aorta, following a high-dose of anesthesia. Serum samples were centrifuged for 10 min at 3500 g in a frigofric centrifuge and stored at −80 °C until analysis. PINP (CSB-E12774r; Cusabio Biotech Co., Wuhan, Hubei Province, China) and CTX (CSB-E12776r; Cusabio Biotech Co.) were measured using a commercial sandwich ELISA assay kit, following the manufacturer's instructions.[12] The enzyme–substrate reaction was terminated by the addition of 50 μl stop solution, and the optical density and color change of each well was measured using a microplate reader spectrophotometer (SpectraMax Plus 384 Microplate Reader; Molecular Devices, LLC, Sunnyvale, CA, USA) at a wavelength of 450 ± 2 nm. The PINP and CTX levels in the samples were determined by comparing the optical density of the samples with the standard curve. The detection ranges were 62.5–4000 pg/ml for PINP and 0.47–30 ng/ml for CTX.

Histopathology

The Achilles tendon was dissected from the calcaneus and musculotendinous junction via an incision over 2 cm proximal to the Achilles tendon insertion for histopathological analysis under a transmission electron microscope (TEM). Tissue samples taken from the Achilles tendons of both limbs were fixed in 2.5% glutaraldehyde for 24 h, washed in Sørensen's phosphate buffer (pH 7.4), and postfixed in 1% osmium tetroxide in a phosphate buffer (pH 7.4) for one hour. Then, a third fixative, 10% formaldehyde, was applied to the samples for one hour before they were dehydrated in increasing concentrations of alcohol (25%, 50%, 75%, and pure alcohol). After this procedure, the samples were washed with propylene oxide and embedded in an epoxy-resin-containing media. Semi-thin sections of approximately 2 μm thickness and ultra-thin sections of approximately 60 nm thickness were cut with a glass knife on an LKB Nova ultramicrotome (LKB, Bromma, Sweden). Ultra-thin sections were collected on copper grids, stained with uranyl acetate and lead citrate, and examined with JEM-1200EX TEM (JEOL Ltd., Tokyo, Japan).[14]

In addition to these evaluations, the soleus muscles (which can be easily differentiated from the tendon tissue) were carefully dissected from the proximal and distal musculotendinous complexes from both hindlimbs, and the wet weights were calculated

Table 1
Study design (WBV: Whole-body vibration, S: Sacrification).

Day	1–14 days	15 day	16–21 days	22 day
1. Group IS	Casting immobilization	S		
2. Group IR	Casting immobilization		Remobilization	S
3. Group IV	Casting immobilization		WBV	S
4. Group C	–	S		
5. Group CV	–		WBV	S

Table 2
WBV application protocol.

Day	Frequency	Type of application	Total time
1	45 Hz	1 min. WBV–1 min. rest	15 min
2	45 Hz	2 min. WBV–1 min. rest	20 min
3	45 Hz	2 min. WBV–1 min. rest	25 min
4	45 Hz	2 min. WBV–1 min. rest	30 min
5	45 Hz	2 min. WBV–1 min. rest	30 min
6	45 Hz	2 min. WBV–1 min. rest	30 min
7	45 Hz	2 min. WBV–1 min. rest	30 min