SECTION – EXERCISE SCIENCES

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THE EFFECT OF VIBRO-MASSAGE ON THE LEVEL OF SELECTED MARKER OF MUSCLE DAMAGEAND CONNECTIVE TISSUES AFTER LONG-TERM PHYSICAL EXERCISE IN MALES

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Authors' contribution:

A. Study design/planning
B. Data collection/entry
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D. Data interpretation
E. Preparation of manuscript
F. Literature analysis/search
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Abstract:

Study aim. The aim of the study was to examine the influence of using of vibro-massage on specific parameters of frequency, amplitude and pulsation sequence after prolonged physical exercise on post-workout restitution among men, based on the assessment of biochemical concentrations of indicators assessing the degree of damage to muscle cells.

Material and methods. A group of 20 men aged 22±2.5 years was selected for the study, with average values of: body height (BH) - 179±1.00 cm, body mass (BM) - 74.3±1.70 kg, percentage of fat content (PF) - 10.8±0.60%, and with a fairly high level of VO_2max 52.6±8.0 $ml \cdot kg^{-1} \cdot min^{-1}$

Results. In two groups - with the use of vibration procedures (VG) and placebo (PG), statistically significant differences were found for lactate concentration (LA) in blood samples (serum) collected after 15, 30 and 60 minutes, as well as MMP-2, Mb and IL-6 1h later and 24 hours following the exercise.

Conclusions. It has been shown that a single, strong physical stimulus causes large disturbances of homeostasis in the body, while the use of vibro-massage with the indicated parameters, during rest, results in an increase in the rate of the body's restitution during the post-exercise phase.

Introduction

A single, long-lasting physical effort with high energy expenditure is even restrictive for the body of professional athletes. In particular, this applies to skeletal muscles engaged during work. The effect of performing such physical work is often the occurrence of Delayed Onset of Muscle Soreness (DOMS), which is caused by mechanical damage to muscle cells [1]. It stimulates inflammatory response initiated by the movement of plasma proteins and leukocytes to the damaged space of metabolically active tissues and the initiation of a cascade of proinflammatory reactions, which, in turn, is manifested by an increase in the level of cytokines (IL-1α/β, IL-6), IL-8 and TNF-α- (tumor necrosis factor). At the same time, the production of cytokines with anti-inflammatory activity (IL-1Ra, IL4, IL-10) begins to limit the pro-inflammatory reaction [2]. Cytokines produced both by the damaged muscles and leukocytes transfer information between cells of various tissues and organs. The signal of muscle damage triggers an inflammatory response from the brain, liver and immune system. Muscle damage in athletes is associated with pain and edema, as well as with severe reduction in the efficiency of this tissue. This is accompanied by the release of intramuscular proteins from damaged muscle fibres into the blood, including creatine kinase (CK-M) and myoglobin (Mb). In addition, the high concentration of reactive oxygen and nitrogen (RONS) released during inflammation does not only have the effect of inhibiting the shortening of muscle cells by the mechanism controlling the flow of Ca^{2+} ions to the sarcoplasmic reticulum [3], but can also initiate a reduction in muscle mass and strength. This is related to the altered functioning of the mitochondrial respiratory chain and decreased ability of satellite cells to rebuild muscle fibres [4].

The physical training of professional athletes uses a model that takes a number of elements determining its effectiveness into account. The most important are knowledge of the potential and current exertion capacity of an athlete's body, susceptibility to physical loads of varying intensity and the speed of post-workout restitution. The latter can be accelerated after applying biological regeneration treatments. This interdisciplinary knowledge gives coaches and athletes the opportunity to effectively control the training process, including the optimisation of breaks between successive training units.

In scientific literature on the topic, there is an increase in interest regarding biological regeneration methods aimed at accelerating the rate of restitution of the body as a result of reduction of post-exercise skeletal muscle damage. Studies on the effects of systemic cryotherapy [5] and massage [6] are the best documented. There are single reports regarding the effects of vibration. There are, however, no research results on the impact of vibrations with well-defined parameters of frequency, amplitude and sequence of pulsations on post-workout restitution of the male body. This, in particular, refers to changes in the concentration of biochemical indicators assessing the degree of damage to muscle cells after prolonged physical effort, which was the objective of this study.

Material and methods

The study involved 20 male volunteers aged 22±2.5 years, with the average body height (BH) of - 179±1.00 cm, body mass (BM) - 74.3±1.70 kg, percentage of fat content (PF) - 10.8±0.60% and VO_2max 52.6±8.0 $ml \cdot kg^{-1} \cdot min^{-1}$, who have recreationally cycled at least three times a week during past 3 years. All the men expressed their written consent to participate in the study and were informed about the possibility to resign at any stage without giving a reason. The approval of the Bioethical Commission No. 83/KBL/OIL/2011 for research was obtained. The procedures did not violate the assumptions of the Declaration of Helsinki. During the entire study period, the men were advised not to change their current diets but were to refrain from drinking alcohol and undertaking additional forms of physical activity.

The experimental protocol included 2 series of tests: preliminary and basic.

During the preliminary part, the men's body build and body composition parameters were evaluated (BH, BM, PF, LBM) using a body composition analyser (JAWON MEDICAL IOI-353 - CE0197-Korea) and measurements of selected circulatory indices were performed - heart rate (HR) and respiratory exchange (oxygen consumption per minute (VO_2), maximum oxygen uptake (VO_2max), respiratory exchange ratio (RER) as well as the amount of total work (TW) in the graduated test. The following set of measuring devices was used: ER 800D - 72475 BIT2 (ERG) cycloergometer from Jaeger (Germany), Cortex MetaLyzer ergospirometer (Germany) and Polar RS 400 cardiomonitor by Polar Elektro (Finland). All of the tests took place in the morning in the air-conditioned laboratory of the Department of Physiology and Biochemistry at the University of Physical Education in Krakow.

Basic research, aimed at showing the effect of lower limb vibro-massage on post-workout restitution in men, was designed as an alternate experimental study and was performed in the following order. The men were randomly divided into 2, 10-subject groups: A and B. In the first part, group A underwent vibratory treatments, while group B was the control group (resting time on placebo mattresses). After 2 weeks, the protocols were changed and participants from group A received treatment on the placebo mattresses, while participants from group B were subjected to vibro-procedures. Placebo treatments were carried out on specially designed Vitberg devices, which in terms of shape, appearance and equipment, were identical to those for vibrations. They produced the same sound signals at the various phases of the placebo procedure, but they were not a source of vibrations with the tested parameters.

All the men were asked to perform a 120-minute physical effort at the relative intensity of 50%VO_2max, with a cadence of 90 RPM on a foot-pedal ergometer, along with max 30-second cadence accelerations (RPM-max) in the final 30, 60, 90 and 120 minutes of the exercise, after a 10-min warm-up at the same intensity.

Immediately after the effort, after drying the body and being re-weighed (BM), as well as undergoing a 4-minute spray under water at 21±2°C, the subjects from the experimental group underwent a 60-minute vibro-massage in a semi-recumbent position via the Vitberg+ Massager (RAM Vitberg+ Base module + Knee module) [Poland]. At each stage of the procedure, the parameters smoothly changed from minimum to maximum values. The therapeutic stimuli generated by the device were cycloidal vibrations (CV) directed in 3 perpendicular directions (3D), small amplitude, low and medium frequency and variable pulse sequences (f=20-52 Hz, A=0.1-0.5 mm, a=6.9-13.5 m/s^2). During the 60-minute treatment, the vibrations were paused at different values of frequency, amplitude and acceleration.

Blood collection from the ulnar vein in the volume of 15 ml for biochemical determinations took place before (I) and immediately after exercise (II), as well as 1 hour (III) and 24 hours after completion of the stress test (IV). 8 venous blood samples were collected from all of the participants. All biochemical analyses were performed at the Laboratory of the Department of Physiology and Biochemistry, University of Physical Education in Krakow. The following were determined in the blood sample: hemoglobin (HGB) via the Drabkin method and hematocrit (HCT) by the microhematocrit method. Plasma volume changes (%ΔPV) were estimated using the Dill and Costill equation [7] modified by Harisson et al. [8].

In the project, changes in the following were analysed: myoglobin, Interleukin 6 (IL-6) and MMP-2 via the ELISA enzyme immunoassay technique, using the E-LizaMat3000 (DRG Instruments Gmbh, Germany). EIA-3955, EIA-4640, ENZ-5814 (DRG), 201-15-0159 (Sunredbio) kits were used.

Micro blood samples for the determination of lactate concentration (LA) were taken from the earlobe before, immediately after and after 15, 30 and 60 minutes of exercise (5 blood draws). The blood was analysed using the Mini-photometer plus DR Lange, type LP-20, Dr. Lange (Germany). The concentration of biochemical markers in blood samples taken after exercise was corrected according to the change in plasma volume (%ΔPV).

Methods of statistical analysis

The obtained results were blocked giving the following groups: experimental: vibro-massage (VG) and placebo (PG). The STATISTICA 13.1 (StatSoft Polska) package was used for statistical analysis of the results. The results are presented as arithmetic mean and standard deviation. Normality of distribution was examined using the Shapiro-Wilk test. In order to assess the significance of differences between means, the Student's *t* test for dependent samples or the Wilcoxon matched pairs signed rank test was used, depending on the fulfilment of the normality criterion. A significance level (α) of 0.05 was assumed.

Results

After prolonged physical exercise in the men with from the VG and PG, dehydration, determined via body mass measurements before and after exercise (ΔBM), was 1.2±0.10 and 1.1±12 kg, respectively, and did not differ significantly. The results of concentrations of individual blood biochemical indicators are presented as arithmetic means and standard deviations. These data are presented in Tables 1, 2, 3, 4 and 5.

The concentration of IL-6, Mb and MMP-2 in the blood samples of men from the VG and PG taken before (I) and immediately after exercise (II), did not significantly differ (Tab. 2). In contrast, in the blood samples collected after 1 hour (III) and 24 hours (IV), significant differences were found in the results. Thus, the average values of IL-6 and MMP-2 were significantly lower in men from the VG than in the PG. In contrast, the results

Table 1. Concentration of lactate (LA) in blood samples taken immediately after exercise (0) and during the 15th, 30th and 60th minute of vibro-massage in men from the experimental group (VG) and at the same intervals in the placebo group (PG)

Time [min]	LA [mmol/l]		*p*
	VG	PG	
0	1.99 ± 0.39	2.17 ± 0.57	0.263
15	1.72 ± 0.38	2.03 ± 0.59	0.049*
30	1.41 ± 0.24	1.62 ± 0.25	0.021*
60	1.36 ± 0.17	1.65 ± 0.42	0.018*

The results are presented as mean values and standard deviation.
* significant differences ($p < 0.05$)

of Mb concentration in the blood samples taken after exercise were significantly higher in the VG (Tab. 2).

In the group of men subjected to vibrotherapy (VG) after exercise, faster elimination of lactate concentration (LA) levels was observed than in the case of the placebo group (PG) ($p<0.05$). Significant differences were found in the blood samples collected during the 15th, 30th and 60th minute of restitution (Tab. 1).

The effectiveness of vibratory procedures in accelerating the rate of restitution of the body after prolonged

Table 2. Concentration of selected biochemical markers before (I) and immediately after (II) 1h after (III) and 24h after exercise (IV) in men from the experimental (VG) and placebo group (PG)

Parameter	Group	I	II	III	IV
IL-6 [pg/ml]	PG VG	5.69 ± 2.61 7.56 ± 4.95	9.31±5.04 10.02±4.65	13.96 ± 4.11 # 10.44 ± 3.02 *	10.35 ± 2.51 # & 7.48 ± 3.08 & *
Mb [ng/ml]	PG VG	15.34 ± 5.44 15.53 ± 5.58	31.10±19.67 30.81±21.14	31.38 ± 25.86 # 34.07 ± 28.25 # *	13.95 ± 5.67 & 16.41 ± 7.25 & *
MMP-2 [ng/ml]	PG VG	6.61 ± 3.76 6.85 ± 3.84	10.02±7.11 9.98±8.92	8.95 ± 5.49 # 6.92 ± 3.48 *	8.99 ± 3.95 # 6.17 ± 4.40 *

PG – placebo group; VG – vibro-massage group, IL-6 – interleukin 6; Mb – myoglobin; MMP-2 – metalloproteinase 2. The results are presented as mean values and standard deviation.
* significant differences ($p < 0.05$) compared to the placebo group; # significant differences ($p < 0.05$) compared to the values immediately after exercise; & significant differences ($p < 0.05$) compared to the values 1h after completing exercise.

Table 3. Decrease/increase in myoglobin (Mb) in the blood 1h (III) and 24h (IV) after exercise compared to the value of this indicator immediately after exercise in men from the experimental (VG) and placebo group (PG)

Time [h]	Δ Mb [ng/ml]		*p*
	VG	PG	
After 1 h (III)	-2.97± 1.76	-0.57 ± 0.71	0.029*
After 24h (IV)	14.69 ± 9.22	12.89 ± 8.87	0.018*

The results are presented as mean values and standard deviation.
* significant differences ($p < 0.05$).

Table 4. Decrease/increase in blood-IL-6 concentration 1h (III) and 24h (IV) after exercise compared to the value of this indicator immediately after exercise in men from the experimental group (VG) and placebo (PG)

Time [h]	Δ IL-6 [pg/ml]		*p*
	VG	PG	
After 1h [III]	-1.13 ± 0.91	-3.94 ± 1.14	0.004*
After 24h [IV]	1.83 ± 1.21	-0.33 ± 1.28	0.008*

The results are presented as mean values and standard deviation.

Table 5. Decrease/increase in MMP-2 blood concentration 1h (III) and 24h (IV) after exercise compared to the value of this indicator immediately after exercise in men from the experimental (VG) and placebo group (PG)

Time [h]	Δ MMP-2 [ng/ml]		*p*
	VG	PG	
After 1h [III]	0.59± 1.11	-1.78 ± 1.42	0.036*
After 24h [IV]	1.52 ± 1.32	-2.03 ± 1.61	0.006*

The results are presented as mean values and standard deviation.
* significant differences ($p < 0.05$).

physical exercise was assessed on the basis of analysing the dynamics of changes in the concentration of biochemical indicators (Δ) noted immediately after exercise and 1 h as well as 24 h after restitution. The average values of increases/decreases in the analysed variables (ΔMb, ΔIL-6 and ΔMMP-2) in the males subjected to vibration (VG) were significantly different compared to the PG (Tab. 3, 4 and 5), which indicates their faster recovery after prolonged physical exercise.

Discussion

The positive effects of vibration on health have been known for a long time, however, the exact mechanisms and effectiveness of its operation are only now being studied. Most publications concern Whole Body Vibration, but in a standing position and via the feet. It is indicated that WBV improves the perfusion of muscle tissue with blood, lymph flow conditions also improve, which significantly affects the delivery of oxygen and other components important for the muscle at work [9-11]. Such a type of vibrotherapy has already been studied as a form of biological regeneration used in post-exercise restitution [12]. Similarly to our research, single-session whole body vibration (12 Hz, 4 mm, 3 series after 1 minute, 30 seconds of passive intervals between sets) after eccentric exercise caused the levels of creatine kinase (CK) 24 and 48 hours following exposure to be lower than in the control group. As in our research, a wider palette of muscle damage markers indicates the usefulness of vibration in accelerating post-exercise restitution. In the research by Timon et al. [12], it was also indicated that muscle pain assessed using the Visual Analogue Scale (VAS) was reduced. In the subject-based literature, the effectiveness of vibrotherapy counteracting DOMS [13-15] is indicated.

Kang et al., similarly as in our study, also investigated the application of vibrations with variable amplitude in a seated position [16]. In this project, vertical, sinusoidal vibrations (10-50 Hz, amplitude: 1-5 mm) were generated by a special armchair with eight stimulus centres (arms, lumbar spine, thigh, shank). The applied vibratory factor caused a significant decrease in lactate concentration compared to the control group. It was also noted that this factor allowed heart rate values to return to baseline faster. The only work so far investigating the usefulness of cycloidal vibrations in post-exercise restitution indicated a lack of significant differences between vibration therapy and the recognised as well as applied muscle massage used to date or stretching of muscles previously subjected to eccentric work [17]. The exercise applied in this project was eccentric in nature, and the vibrational stimulus used was applied twice a day for 20 minutes, in a seated position, thanks to the use of a vibrating pad placed under the right thigh (73 Hz frequency, maximum amplitude of 0.5 mm). Differences in relation to our project resulted from other characteristics of the vibratory stimulus and the size of the body area subjected to vibro-massage.

In our research, the use of cycloidal vibrations with variable parameters of intensity, amplitude and frequency during rest also contributed to the restitution acceleration of the body, which was manifested by faster reduction in blood lactate level in the VG blood samples (serum) than in the PG collected during the 15th, 30th and 60th minute of vibro-massage following the prolonged effort. This demonstrates faster removal and redistribution of LA to tissues capable of metabolising it. However, in the venous blood samples (in serum) collected immediately after long-term exercise and 1 h as well as 24 hours from its completion, significantly larger changes (Δ) in Mg, IL-6 and MMP-2 concentrations were found than in the PG. They indicate the effectiveness of the vibrating procedure applied in accelerating the rate of post-exercise body restitution. The design of the experiment allowed for the maximum use of the available research materials and a long period between tests (wash out period) secured against overlapping of biochemical changes generated by physical effort for project participants. Myoglobin level has been chosen as a marker for the assessment of the level of myocyte cell damage, and the dynamics of its changes after long-term efforts are already quite well known [18-20]. The authors deliberately did not determine changes in the level of creatine kinase (CK) as a marker with large fluctuations in the population or dependence on a large number of modifiable and non-modifiable factors [21].

Among the cytokines mediating inflammation, Interleukin 6 was chosen – changes in its level are one of the strongest tools used to assess the effectiveness of methods improving muscle regeneration [22]. In our research, its concentration was significantly increased after exercise in both groups (VG and PG), while the VG group showed its significantly lower concentration 1 h and 24 h after the completion of exercise. The dynamics of changes in the concentration of this interleukin are complex, but its persistent high serum concentrations are a strong proinflammatory factor that may indicate the on-going process of muscle cell destruction [23, 24]. In future research, we plan to take markers of plasma antioxidant status into account, which will allow for more comprehensive interpretation of post-exercise biochemical changes.

An innovative indicator used in our study to control the dynamics of the restitution process was the MMP-2 extracellular matrix metalloproteinase. This enzyme is involved in the remodelling of the intercellular matrix and its connection with various forms of physical activity and skeletal muscle work is only now being studied [25-27]. Significant differences in the concentrations of this enzyme in men in VG and PG immediately following exercise as well as 1 h and 24 h after its completion, can indicate a beneficial effect of vibration on connective tissue protection, which, apart from muscle cells, is the basic tissue involved in the pathogenesis of DOMS [28]. On the other hand, it can be interpreted as a reduced number of factors responsible for angiogenesis. The answer to this question, however, requires further experimental research in this area.

Conclusions

Our research indicates that even a single, long-lasting physical effort causes significant disturbances in the homeostasis of the male body, which last up to several tens of hours after its completion. Therefore, it is reasonable to use biological regeneration treatments to improve the restitution of the body. Vibro-massage with the indicated parameters significantly accelerated regeneration of the men's bodies after exercise. This is especially important for high-performance athletes who are exposed to overloading conditions on a daily basis. The observed significant changes in the concentration of metalloproteinase in men from the VG and PG after 1 h and 24 h also indicate the beneficial effect of vibration on the protection of connective tissue, which, alongside muscle cells, is the basic tissue involved in the pathogenesis of DOMS.

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