Effect of eccentric exercise and whole body vibration on muscle micro structure and energy metabolism

Doctoral thesis

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Introduction

The eccentric exercise caused muscle fiber micro-injury and the muscle fiber composition

It is well documented in the literature that strenuous, unaccustomed, mostly eccentric exercise causes delayed onset of muscle soreness (DOMS) indicating muscle fiber damage. The indirect markers of DOMS are the muscle pain (Armstrong et al. 1991, Lieber and Friden 1999, McNeil and Khakee 1992), damage of sarcolemma resulting in leakage of creatine kinase (Brown et al. 1997, Clarkson et al. 1986, Kirwan et al. 1986, MacIntyre et al. 2001, Sorichter et al. 1997), the LDH activity increase in the venous blood, decreased control of the motor units (Leger and Milner 2001, Miles et al. 1997, Pearce et al. 1998). The mechanical consequence of these alterations is the depressed force generation (Cheung et al. 2003, Clarkson et al. 1992, Connolly et al. 2003, Gibala et al. 1995, Hortobagyi et al. 1998, Nosaka and Clarkson 1995).

The direct markers of delayed onset of muscle soreness (DOMS), are the Z line streaming, dissolution and damage of sarcolemma (Friden and Lieber 1998, Friden et al. 1983b), changes in cell membrane integrity (Armstrong et al. 1991, Lieber and Friden 1999, McNeil and Khakee 1992), desmin loss (Friden and Lieber 1998, 2001, Lieber et al. 1996, Small et al. 1992), decrease of energy supply (Evans 1991, Ferry et al. 1992), abnormally accumulated calcium ions due to sarcoplasmic reticulum injury (Armstrong 1990) and the fibronectin appearance in the damaged muscle fibers (Crenshaw et al. 1993, Holmbom 1997, Lieber et al. 1996, Thornell et al. 1992, Yu et al. 2002).

The soreness can be elicited the easiest in the non anti-gravity muscles in untrained individuals applying large range of motion (Chleboun et al. 1998, Howell et al. 1993, Jones et al. 1987, Murayama et al. 2000, Nosaka et al. 2001a). The repeated eccentric exercise has a protective effect to stretching, the muscles adapt to muscle stretch and DOMS symptoms are depressed. After the micro-injury, the regeneration of the muscle structure begins immediately and it has hormonal and gene expression background (Yu et al. 2002, 2003).

The motor units are turned on, regardless of fiber composition, based on the size principle (Henneman et al. 1965). However, from others test results concluded that during eccentric contractions of motor units order is changed, which means the selective connection of large, fast motor units (Howell et al. 1995, Nardone and Schieppati 1988, Nardone et al., 1989).

Basically the ultra-structure of the fast and slow fibers is similar, but there are some differences. "Z" disc of the fast fibers is thinner (30-50 nm) than that ofslow fibers (100 nm) (Eisenberg 2010, Friden et al. 1983b, Horowits 1992) and the cross bridge cycles of the two fiber types are different (Reggiani et al, 1997; He et al 2000). Consequently, it can be assumed that the reaction of the two muscle fiber types to muscle stretch is different.

A number of researches has been carried out to prove this theory. Some researchers have reported that the fast fibers (Cermak et al. 2013 Friden et al. 1983b, Friedmann et al. 2004, Jones et al. 1986 Macaluso et al. 2012) and others that the slow fibers are damaged predominantly (Armstrong et al. 1,983, Hody et al. 2013, Mair et al. 1,995, Vijayan et al. 2001a). In these studies, mostly fast, or in slow muscle fibers were examined. Up to now, a human research has been published, which studied the relationship between indirect indicators of DOMS and the percentage of muscle fiber composition (Magal et al. 2,010). Significant relationship was found between pain and muscle fiber composition only.

Mechanism of whole body vibration

The vibration causes the tonic vibration reflex (Hagbarth et al. 1976) that would result in an increase of muscle activation and thereby increases the muscle spindle excitability, which is explained increased performance during vibration (Bosco et al. 1999a, Cardinale and Lim 2003 Cardinale et al. 2006, 2005 Issurin, Issurin et al. 1994) and directly after vibration (Cochrane et al. 2004, Delecluse et al. 2003, Issurin and Tenenbaum 1999, Bosco et al. 1998, Cormin et al. 2006). Nevertheless, researchers have reported force and power reductions after the vibration, too (de Ruiter et al. 2003, Erskine et al. 2007, Herda et al. 2009).

During the vibration very small amplitude, but very fast stretching reaches the muscle fibers, so the vibration training can be imagined as a special eccentric exercise that can increase the muscle tension during vibration via neural system.

The effect of mechanical vibration depends upon the vibration load consisting of magnitude of vibration frequency, vibration amplitude and acceleration (Luo et al. 2005). Further factors are the posture during vibration training (Gyulai et al. 2013), the type of muscle contraction (isometric or dynamic), the duration of the vibration and the rest period between each application. The effects of vibration can also affected by fitness and health status of the people (Delecluse et al. 2003,Tihanyi J. et al. 2010, Tihanyi T. K. et al. 2007).

Few studies have focused on energy consumption during the vibration. Rittveger et al. (2002) found association between oxygen uptake and increase in the vibration frequency. However, the amplitude of vibration resulted in greater oxygen demand then an increased vibration frequency. Direct determination of macro-erg phosphate concentration, using MR spectroscopy, is very rare in relation to mechanical vibration.

Up to now there has been only one study published which investigated the effect of the 20 Hz vibration on bioenergetics in gastrocnemius muscle (Zange et al. 2008). They found that the artery occlusion combined with vibration resulted in increase of ATP and decrease of PCr. However, vibration without arterial occlusion did not altered none of the high energy phosphate compounds. Eccentric exercise, however, increased the creatine phosphate and inorganic phosphate ratio (PCr / Pi) and the creatine phosphate and ATP ratio (PCr / ATP) significantly, but no significant effect was observed when concentric exercise was applied (Walker et al. 1998).

In summary there is no agreement between researchers whether reduction of force and physical performance after whole body vibration can be attributed to muscle or/and neural fatigue. Also, it is not known if magnitude of vibration frequency influence muscle bioenergetics differently.

Aims of the studies

The aim of the first study was to investigate the effect of six day eccentric exercise on the changes of the direct and indirect markers of DOMS and the alteration of torque production of the knee extensor muscle in relation to fiber distribution of vastus lateralis muscle. Also, our goal was to find out if fast or slow muscle fibers are more susceptible to muscle damage using immune histochemical fibronectin staining.

The aim of the second study was to investigate the effect of 20 and 40 Hz frequency whole body vibration on the energy metabolism of the knee extensor muscles using 31P-MR spectroscopy.

Methods

In our first study, fifteen healthy sedentary males were recruited (age: 22.5±1.6 years, mass: 82.2±8.0 kg, height: 180.0±5.1 cm), nine subjects belonged to eccentric exercise group (EC), and six were in control group (KC). After warming up, the eccentric exercise protocol consisted of six sets of 15 repetitions for six consecutive days. During eccentric contractions the range of motion for the knee joint was 120°. The subjects mark they muscle pain every day. We recorded torque produced by the quadriceps muscle during each repetition and determined the peak and mean torque form the torque-time curves by Multicont II computerized dynamometer.

On Days 1, 3 and 6 venous blood was collected to measure CK and LDH concentration. Muscle biopsy samples were taken three days before the intervention and on Days 3 and 7 of the exercise protocol.

The paraffin-embedded samples were 4 micrometers thick serial sections were prepared by microtome. The sections were placed on glass slides for immunohistochemical staining. Antibodies used for separation the fast and slow muscle fibers (Monoclonal anti-Myosin, Skeletal, Fast; antibody produced in mouse, 1:400, Cat. number: M4276, Sigma, secondary antibody Alexa Fluor 488 goat anti-rabbit IgG, Alexa Fluor 546 goat anti-mouse), and to detect fibronectin (polyclonal, rabbit antihuman, DakoCytomation, A 0245). The LSM510 META confocal fluoresens microscope was used to analysis the samples.

In our second study 15 trained subjects (seven females and eight males) took part (age: 19.7±0.8 years, height: 175.3±9.2 cm and body mass: 68.8±9.7 kg) and divided into three groups. Ten subjects participated in vibration training (Nemes Bosco - vibration platform), 5 of them were trained 3x1 min on 20Hz and 40Hz vibration frequency, five were trained first 40Hz frequency then 20Hz vibration frequency. Five people were in the control group standing in the same position as the vibration group (half squat) for 3x1 min twice. ^{31}P -MRS was used to determine the spectra areas for PCr, αATP, βATP, γATP, summed ATP, and Pi/PCr and PCr/ATP ratio before the vibration/control training , between the trainings and after the second session.

Statistica 7.0 software was used for the statistical analysis. Mean and standard deviation (SD) were calculated for each variable. Non parametric Kruskal-Wallis

ANOVA was used to determine the significant differences between variables. The level of significance was set at $p < 0.05$.

Results

The eccentric exercise and muscle fiber type composition

The muscle soreness increased significantly until the third day then decreased gradually and on the seventh day disappeared almost completely.

The mean and peak torque was significantly lower (38.8%; 39.4%; $p < 0.001$) on Day 3 compared with Day 1. The mean and peak torque elevated from Day 3 to Day 6 significantly (39.6%, 37,0%, $p<0,02$). The mean torque on Day 6 was 15.6% lower compared to that calculated on Day 1. However, the difference was not significant.

The CK concentration was in the range of normal values at baseline but on Days 3 and 7 it was 70.6 and 75.5 fold greater compared to Day 1 (both $p<0.001$). The LDH concentration was in the range of the normal values at baseline and increased 1.5 fold on Day 3 ($p = 0.013$) and was still elevated on Day 6 ($p = 0.014$).

There was no association between muscle fiber type composition and changes in CK and LDH activity at any time point.

The magnitude of reduction in mean and peak torque from Day 1 to Day 3 significantly correlated with ST% ($r=0.81$, $p<0.01$; $r = 0.76$, $p<0.02$). Also, there was a significant association between the recovery of mean and peak torque and fiber distribution ($r=-0,80$, $p<0,01$; $r = -0.76$, $p < 0.02$).

The amount and density of fibronectin was not possible to quantify. There was only minor fibronectin staining in the sarcoplasm on Day 3 in subjects with higher fast fiber %. In the subjects who have higher slow twich fibre ratio, the fibronectin appeared in the fast fibres only on the Day 3, and appeared in slow and fast fibres on Day 7. Three subjects with high FT% showed fibronectin in FT fibers only on Day 7. In two subjects who had 32% and 36% ST fibers, the FT fibers did show sarcoplasmic fibronectin antibodies.

Whole body vibration and energy metabolism

In the vibration study, after the vibration/control workout the inorganic phosphate spectral area decreased in vibration and control group. The rate of change was significant only in the V20-40 group ($F = 8.8$, $p = 0.005$) in the second ($p <0.01$) and third ($p<0.01$) measurements compared to a result of the first measurement. After the one minute isometric test exercise carried out in the MR we did not found any significant changes between the groups or measurements.

When the two vibration group was merged, significant decrease in Pi was observed after the first and the second treatment ($F = 5.5$, $p <0.01$), whereas the control group had no significant change.

Creatine phosphate showed high stability during the measurements. PCr spectra area did not show significant differences between groups and measurements.

The Pi/CrP ratio decreased in all three groups after vibration/control treatment. The rate of decrease in the control group was 26.75% and 25.9%, in the V20-40 group was 22.7% and 25.4%, in the V40-20 group was 18.2% and 22.4%. The change was not significant in either case. In the combined vibration group significant decrease was observed in Pi/CrP ratio (20.5% and 24.0%) after the first and second vibration intervention.

The change in αATP, βATP and γATP spectrum area after vibration/control training or after the one minute long isometric test exercise were minimal, there was no significant difference between the groups or measurements. There were no significant changes in the spectral area of ATP in the combined vibration group. When αATP, βATP and γATP spectral areas were summed again we did not observed significant difference either between groups or measurements.

Conclusion

The eccentric exercise and muscle fiber type composition

Our results are in a good agreement with the previous findings, i.e. the fast muscle fibers are more susceptible to muscle damage due to eccentric training than slow fibers are.

The results have also shown that the repeated bouts of eccentric exercise applied during six consecutive did not augment the muscle damage and did not delayed muscle regeneration despite the extremely elevated CK concentration in the blood.

Our results suggest that muscles with fast fiber dominance lose out less force production capacity and the regeneration of the muscle fibers in these muscles faster than in slow fiber dominance muscles.

The results indicate that the eccentric exercise induced muscle damage is not only fiber type dependent, but also muscle fiber composition dependent that determines whether only fast fibers or both fast and slow fibers are damaged transiently.

The results, also, suggest that sarcoplasmatic fibronectin appearance is a good indicator for muscle regeneration.

Whole body vibration and energy metabolism

Due to the limitations of this study the following cautious conclusions can be drawn from the results.

The vibration intervention applied in this study resulted in minor alteration in bioenergetics of the vastus lateralis muscle which is probably the most touched by the vibration.

It seems that 20 Hz vibration frequency results in greater change in Pi and Pi/PCr than 40 Hz vibration. However, it is difficult to conclude that the decrease of Pi and Pi/PCr indicate muscle fatigue that could be the reason the reduction in force and physical performance.

It is probable that rather vibration load (frequency, amplitude, muscle tension and duration) than vibration frequency itself is the major influencing factor on alteration of macroerg phosphate compounds.

In practical point of view, it should be emphasized that decrease in Pi spectra and force after vibration can be a trigger for chronic adaptation, i.e. strength gain

Own publication list

Ureczky D, Vacz G, Costa A, Kopper B, Lacza Z, Hortobagyi T, Tihanyi J. The effects of short-term exercise training on peak-torque are time- and fiber-type dependent. J Strength Cond Res 02/2014; DOI:10.1519/JSC.0000000000000414

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Ureczky D, Vácz G, Costa A, Lacza Zs, Tihanyi J. Az izom nyújtására bekövetkező mikrosérülést jelző fibronectin és a rostösszetétel közötti kapcsolat. Magyar Sporttudományi Szemle 12(45): 21-24. (2011)