J Musculoskelet Neuronal Interact 2013; 13(4):430-441

Original Article

Hylonome

Long-term effects of whole-body vibration on motor unit contractile function and myosin heavy chain composition in the rat medial gastrocnemius

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Abstract

Structural and physiological mechanisms underling functional adaptations of a muscle to chronic whole-body vibration (WBV) are poorly understood. The study aimed at examining the contractile properties of motor units and the myosin heavy chain (MHC) expression in rat medial gastrocnemius muscle in response to 3- or 6-month periods of the WBV. The three-month WBV induced modifications of contractile properties principally in slow (S) and fast resistant to fatigue (FR) motor units. In S units an increase in the maximum tetanus force, a reduction in the twitch force and a decrease in the twitch-to-tetanus force ratio were found. In FR units a shortening in the twitch time parameters, a decrease in the twitch-to-tetanus ratio and an increase in the fatigue resistance were observed. In addition, a decrease in the type I and an increase in the type IIax MHC content were revealed. The six-month WBV caused a decrease in the twitch-to-tetanus force ratio in S and FR units. Other structural and physiological changes in MU properties previously seen were no longer apparent. In conclusion, responses to the long-term WBV stimulus vary between particular types of motor units, what suggests that multiple adaptive processes in muscle tissue are involved.

Keywords: Training, Rate of Force Development, Fatigue, Medial Gastrocnemius, Rat

Introduction

Rhythmic oscillations evoked during whole-body vibration (WBV) trigger skeletal muscles to contract. The firing activity of low- and high-threshold motor units (MUs) has been detected at consistent phase angle with respect to the WBV waveform, implying reflexive muscle activity[1]. It has been hypothesized that one of the key functions of increased contractile activity of MUs during WBV exposure is to damp the soft tissue oscillations, the process referred to as muscle tuning[2,3]. It has been proposed that fast rather than slow MUs are more adjusted to minimize the soft tissue oscillations across the 10-50 Hz range of the vibration frequencies, due to brief contractile characteristics[2,3]. However, WBV exposition also influences the mechanisms responsible for the control of postural stability during stance[4], and activates presumably the low-threshold slow (S) MUs through the reflex vibratory mechanisms[1,2].

Short-term WBV treatment is able to provoke specific adaptations in mammalian skeletal muscle. In mice, the 6-week vertical vibratory stimulation has been shown to increase the total cross-sectional area of the soleus muscle as well as its type I and II muscle fibers[5]. It has also induced remodeling in the skeletal vasculature without any change in muscle fiber type percentage distribution[6]. In our previous study, specific functional adaptations have occurred in the fast fatigable (FF) and the fast fatigue-resistant (FR) MUs of rat medial gastrocnemius (MG) muscle following the 5-week WBV, principally manifested by an increase in the maximum tetanus force and a shortening of the twitch contractile characteristics, respectively[7]. Also, a trend toward the increase in the maximum force of S MUs has been noted. Yet, no changes have been found in the proportions of MU physiological types nor in myosin heavy

The authors have no conflict of interest.

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Edited by: M. Hamrick
Accepted 16 August 2013

chain (MHC) isoform content in studied fast-type muscle.

WBV has been frequently used as an alternative or additive to strength training but improvements in maximum explosive capabilities (i.e. vertical jump) rather than strength of skeletal muscle have been commonly observed in healthy non-athletic or physically active humans subjected to chronic WBV. Alterations in MU contractile properties and MHC isoform composition give important insight into mechanisms underlying skeletal muscle adaptations to exercise[8]. It is known that strength training imposes the mechanical loading that leads to increase in muscular force and fast-to-slow transformation of MHC isoforms[9,10], whereas loading exerted during dynamic (ballistic) training increases rate of MU force development and provokes slow-to-fast transformation of MHC[11,12]. However, the impact of mechanical oscillations produced by long-term WBV administration on the neuromuscular system is currently unknown.

The first purpose of this study was to determine the effects of long-term WBV treatment of high frequency (50 Hz) and magnitude (4.9 g) on slow- and fast-type MU contractile properties and MHC isoform composition of the fast-type MG muscle of rat. It has been reported in humans that early beneficial effects of WBV on muscle contractile performance may subside with longer exposition[13]. Therefore, the second aim was to determine how the reorganization in MU physiological parameters and MHC isoform composition evolves during the particular stages of long-term expositions to WBV (lasting 3 and 6 months). Uncovering modifications in MUs' characteristics serves for better understanding not only the clinical and sports applications of WBV, but also adaptive physiological behaviour to vibratory shocks experienced during natural activities[3]. The results will help in understanding the adjustment strategies of the neuromuscular system to mechanical loading exerted during particular stages of chronic WBV, and in verifying whether these strategies conform to presumed MU functions related to muscle tuning and maintenance of postural stability during the exposition to vibratory shocks.

Materials and methods

Animal groups, maintenance and care

Experiments were carried out on adult male Wistar rats. Two groups of 3 month old rats where whole-body vibrated for 3 months (WBV3 mo, *n*=6) and 6 months (WBV6 mo, *n*=5). Two age-matched groups of non-vibrated rats i.e. C3 mo (*n*=6) and C6 mo (*n*=5) served as controls. Therefore, at the time of testing the rats from the WBV3mo and their corresponding C3 mo group reached the age of 6 months, whereas those from the WBV6mo and their corresponding C6 mo group reached the age of 9 months. All the rats were housed in the standard cages (two per each), with the 12/12 h dark/light daily cycle, in the room with 22±2° temperature and 55±10% humidity, and were fed with standard food and water *ad libitum*. The experimental procedures were approved by the Local Ethics Committee and performed in agreement with the Guiding Principles for the Care and Use of Animals in the Field Physiological Sciences, the European Union guidelines, and the Polish Law on the Protection of Animals.

Whole-body vibration procedure

The whole-body vibration was carried out from Monday to Friday between 8.00-10.00 a.m. by placing the rats into the plastic, standard cage container, firmly attached to the vibratory platform (Power Plate®) by a pair of a rigid belts. Four consecutive 30 s runs of 50 Hz WBV, separated by 60 s rest intervals, were implemented in one session daily. The amplitude of sinusoidal vertical vibrations of the platform was set at 2.5 mm, and the average maximum acceleration of the bedding of the container was 4.9 g (determined with the use of the accelerometer ACL300, Biometrics LTD, UK). However, the peak-to-peak displacement calculated from the maximum acceleration was 0.98 mm for the bedding of a cage (with a rat inside) fixed on top of the platform. During the procedure animals were allowed to move freely inside the container, enabling the superposition of the WBV onto standard cage activity. Control rats were placed in the same room to assure that they experienced the similar noise from working WBV platform. This WBV protocol was identical to the one used in our previous study on effects of the 5-week WBV[7].

Surgical procedures

Deep anesthesia was induced twenty four hours after the last exercise session by intraperitoneal administration of sodium pentobarbital (initial dose 60 mg/kg, with supplementary doses approximately of 10 mg/kg, administered every hour). Throughout the entire acute experiment the depth of anesthesia was controlled by observation of pinna and limb withdrawal reflexes.

All muscles of the thigh and leg of the left hindlimb were denervated by cutting branches of the sciatic nerve, except the one leading to the MG muscle. The MG was dissected from surrounding tissues and separated from neighboring muscles. The vessels providing blood supply to the MG muscle were left intact. The lumbar spinal cord over L2-S1 segments was exposed by the laminectomy and the dura mater was incised and removed. The L4-L5 ventral roots of spinal nerves were cut near the entry to the spinal cord.

The rat was mounted in the rigid metal frame and the skin around the laminectomy area was sutured to the metal frame forming a small pool. Then, the animal was positioned on a warm aluminum plate and firmly immobilized by clamping the Th12 and S1 spinal processes and the tibia. The exposed hind limb was immersed into the paraffin oil filling up the small aluminum container in order to cover the exposed spinal cord and ventral roots. The oil and body temperature were automatically maintained by a close-loop temperature controller at a constant level of 37±1°C. The distal tendon of MG muscle was cut near the calcaneus and attached via the low-compliance surgical thread to a force transducer. The muscle length was adjusted and kept at a passive force of 100 mN to allow production of maximum twitch amplitudes for the majority of

MG MUs[14]. Experiments were terminated by the administration of a lethal dose of sodium pentobarbital (180 mg/kg, i.p.).

Stimulation and recording

MUs were isolated by splitting the L4 and L5 ventral roots of the spinal nerves into fine filaments which were stimulated by bipolar silver-wire electrodes with rectangular electrical pulses of 0.1 ms duration and variable voltage (up to 0.5 V), produced by a dual channel square pulse stimulator (Grass Instrument Company, model S88). The criteria for isolation of a MU were the "all-or-none" type of an action potential and of a twitch response during repeated gradual increase and decrease of the stimulus voltage around the excitation threshold.

The electromyographic signals of MUs (EMG) were recorded with two non-insulated silver-wire (300 μm in diameter) electrodes inserted at the medial part of the muscle belly, perpendicularly to its long axis, and amplified by a low-noise multi-channel preamplifier (WPI, model ISO-DAM8-A), with the high and low-pass filters set at frequencies of 0.1 Hz and 3 kHz, respectively. The reference electrode was inserted into the denervated muscles of the opposite hind limb.

The force was measured under isometric conditions by a force transducer (deflection sensitivity of 100 μm per 100 mN). During experiments, the force amplitude and the EMG were monitored on an oscilloscope screen, and stored on a computer disc using the analogue-to-digital 12-bit converter (RTI-800) at the sampling rate of 1 kHz for the force records and 10 kHz for the action potentials.

Testing protocol and MU parameters

The stimulation protocol consisted of six successive steps: (1) five pulses at 1 Hz (5 twitches were recorded to calculate the averaged response); (2) 500 ms train of pulses at 40 Hz (the unfused tetanus was evoked); (3) 300 ms train of pulses at 150 Hz (the maximum tetanus was evoked); (4) 10 successive trains of pulses of 500 ms duration evoked at 1, 10, 20, 30, 40, 50, 60, 75, 100, and 150 Hz stimulation frequencies; (5) five pulses at 1 Hz; (6) the fatigue test: 40 Hz stimulation, lasting 333 ms, repeated every second[15], applied within 3 mins[16]. Each step in the protocol was separated by a 10 s interval.

MU classification as slow and fast ones was initially accomplished by the visual inspection of the presence or not of the sag in the force profile of the unfused tetani evoked at 40 Hz. MUs with sag were accepted as fast, while those without sag as slow (S) ones[16]. Then, fast units were further classified as fast fatigable (FF) if the value of the fatigue index was below 0.5 or as fast resistant to fatigue (FR) if the index exceeded 0.5[16,17].

For each MU, the twitch force (TwF), the contraction time (CT), the half-relaxation time (HRT) and the maximum tetanus force (TetF) were measured. The twitch post-tetanic force potentiation (TwPTP) was calculated as the reverse ratio of forces of twitch contractions generated prior and after the maximum tetanus contraction. The peak rate of force development (TwRFD), and maximum tetanus rate of force development (TetRFD) were measured at the initial rapid increment of force, and determined as the maximum force increase per one millisecond ($\Delta F \cdot \Delta t^{-1}$, mN/ms). The twitch-to-tetanus force ratio (Tw/Tet) was calculated as a ratio of the twitch to the tetanus forces.

From the peak forces obtained for the step 4 of the stimulation protocol force-frequency curves were plotted. The frequency required to evoke 60% of the maximum force was estimated by linear interpolation, and the slope of the force-frequency curve was calculated as a relative increase (percentage) of the tetanus force in response to 1 Hz increase in the stimulation frequency.

The fatigue index was calculated from the applied fatigue test as a reverse ratio of the peak force of the highest tetanus at the initial part of the test to the force generated two minutes later. In addition, for the comparison of changes in force profile of FR and FF MUs during the fatiguing stimulation, peak force responses of repeated tetani were measured at every 10 second time intervals during the entire period of applied fatigue test. The peak forces were then expressed as a percentage of force in relation to the peak force of the first tetanus at the beginning of the fatigue test.

Myosin heavy chain isoform quantification

The same WBV protocol was accomplished for the additional population of WBV 3 mo (n=9) and WBV 6 mo rats (n=9). From both WBV treated groups, and two additional aged-matched controls (n=9 for each group) MG muscles were dissected and were immediately frozen in liquid nitrogen and stored for further analysis. Muscle samples were subsequently homogenized in 10 volumes (w/v) of ice-cold 20 mM K_2HPO_4/KH_2PO_4 buffer, pH 7.2 containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and spun for 20 min at 10000×g at 0ºC. Then, the pellets were washed twice with the buffer and thoroughly suspended in 2% sodium dodecyl sulphate (SDS). After 10-min boiling followed by 20-min centrifugation at 10000×g at 25ºC, supernatants containing myofibril proteins were collected and further analysed. Myofibrils were further subjected to electrophoretic separation in 8% polyacrylamide gels according to Talmadge and Roy[18]. Usually 3-5 μg of protein was loaded. The bands corresponding to myosin heavy chains (MHC) of MG muscle served as the isoform markers. The percentage proportions of MHC isoforms in the analyzed muscles were estimated by comparing the degree of staining intensity with Coomassie brilliant blue using G:Box system from SynGene (Cambridge, UK) equipped with Gene Snap and GeneTools software. On the basis of electrophoretic technique used in this study we were not able to consistently separate the fast MHC-IIx type from the fast MHC-IIa type, as the two proteins comigrated. Therefore, in the entire paper one of the bands was called MHC-IIax. On the basis of immunohistochemical MHC recognition four main types of muscle fibres can be classified in rat hindlimb muscles: slow type I and fast type IIA, IIX IIB. The evidence has been provided that within the population of fast type muscle fibres those containing IIa, IIx and IIb MHC isoforms belong to fast MUs with high, intermediate and low fatigue indices, respectively[19].

	C3 mo (n=6)	WBV3 mo (n=6)	C6 mo (n=5)	WBV6 mo (n=5)
Body weight (g)	503.3±62.4	496.7±23.4	473.0±27.7	478.0±36.5
Muscle weight (g)	1.17±0.09	1.30±0.13	1.27±0.09	1.35±0.17
Muscle/body weight (%)	0.23±0.04	0.26±0.02	0.27±0.01	0.28±0.02

No significant differences between control and experimental groups were noted. The mean values ± SD are presented. n – the number of animals studied within the particular groups.

Table 1. Body and muscle characteristics of studied animals.

Statistical analyses

Entire population of MUs sampled for each studied group was pooled together, assigned to three MU categories and compared with the use of the Chi-square test. Students and Cochrane-Cox *t*-tests served for statistical comparisons of body and muscle weights. Comparisons of MHC isoforms within the MG muscle, MU contractile and force-frequency parameters were made with the use of the Student's *t*-test or Mann-Whitney U test for the aged-matched experimental and control groups. The repeated measures analysis of variance (ANOVA) was used for the analysis of changes in the force-frequency relationship of MUs, and to test the hypothesis of main effect of time, interaction effect between time and the WBV, and effect of the WBV treatment on fast MU force profiles during the course of the fatigue test. In all cases of measured effects the sphericity assumption was violated, therefore the data was corrected using the Greenhouse-Geiser and Huynh-Feldt procedures. The $P < 0.05$ value was the level for accepting statistical significance.

Results

All animals withstood well the entire period of the WBV treatment. During initial days after the beginning of vibration programme some animals exhibited observable signs of nervousness, accompanied by increased frequency of defecation or urination. However, these signs withdrew quickly and after approximately a week their behavior did not differ from the behavior displayed by control animals during natural cage activity.

Following the 3 and 6 months of the WBV, no significant differences were observed between the body and muscle weights of control and vibrated rats ($P>0.05$, Table 1).

MU distribution and myosin heavy-chain proportions

Three-month WBV exposure changed proportions of the MHC in the MG muscle. The percentage content of the type I MHC was significantly decreased whereas the type IIax increased (Figures 1 and 2). There was no difference in the overall percentage proportions of three types of MUs, which were 16.0%, 40.8%, and 43.2% in C3 mo group, and 16.5%, 33.0%, and 55.5% in WBV3 mo group for S, FR, and FF MUs, respectively (Chi-square test, $P>0.05$). However, it is worth noticing that the relative number of FR units with a very high fatigue resistance indices increased within the population of these units (Figure 3b).

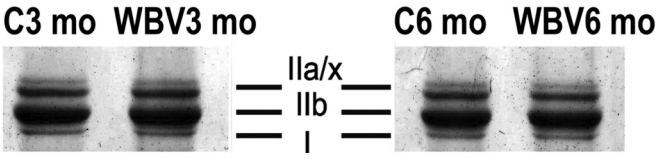

Figure 1. Electrophoregrams of MHC isoforms of MG muscles for control and WBV treated animals.

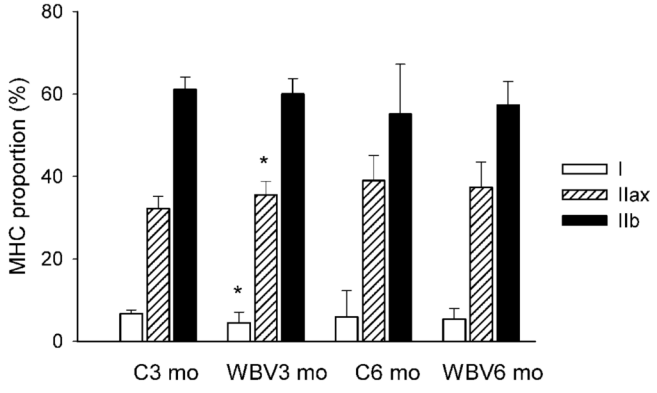

Figure 2. Percentage content of the MHC isoforms in the rat MG muscle. The asterisks above the bars denote significant differences ($P<0.05$) in comparison to the age-matched control groups.

Following the 6 months of the WBV exposure, no changes were observed in the distribution of the MHC isoforms (Figures 1 and 2). Nevertheless, when the percentage distribution of the three types of MUs was analyzed, the reduction in proportion of FR and the increase in proportion of FF units were observed in the WBV exercised rats (Chi-square test, $P<0.05$). The percentage distribution of S, FR and FF MUs in the MG muscle was: 16.0%, 45.4% and 38.7% in the C6 mo group, and 21.5%, 29.8% and 48.8% in WBV6 mo group. In addition, within the population of FR units there was a visible shift in the distribution

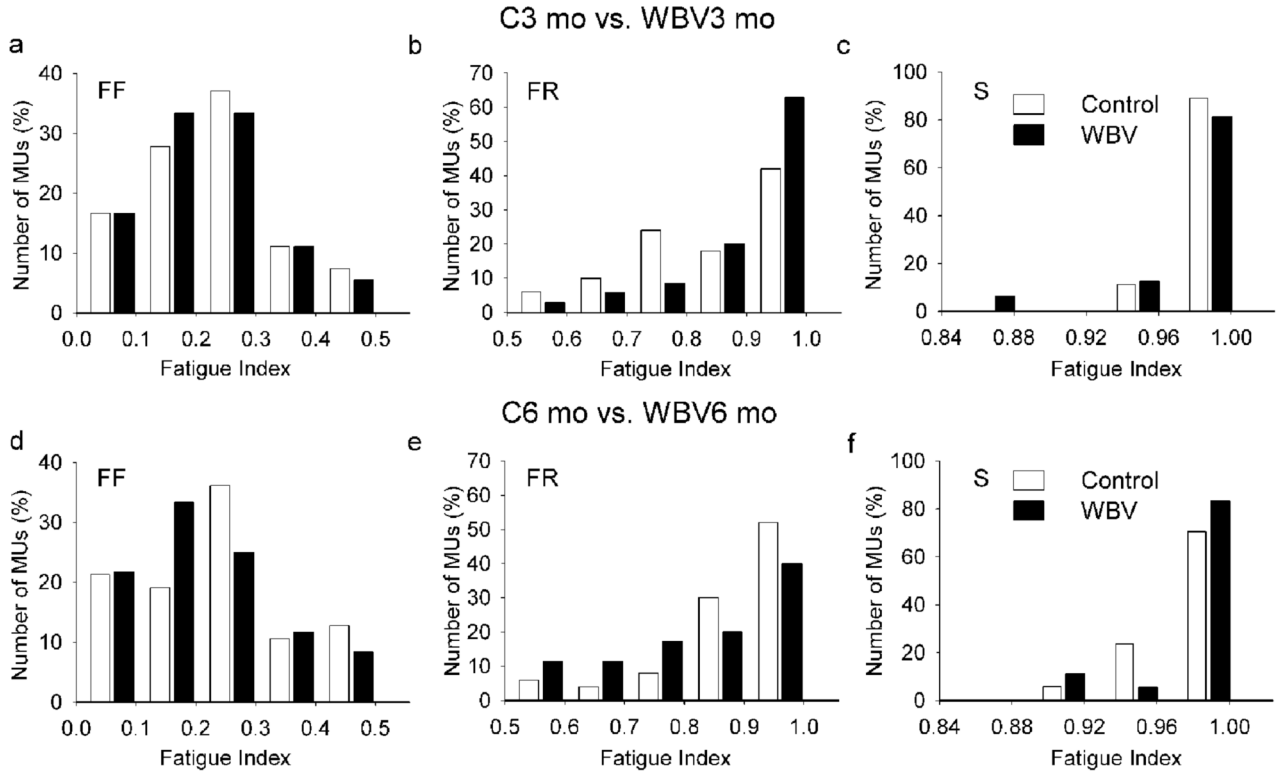

Figure 3. Distributions of fatigue indices within the populations of type-identified FF, FR and S units for WBV3 mo (a, b, c, respectively) and WBV6 mo (d, e, f, respectively) rats, and respective age-matched control animals. See text for explanation.

	CT (ms)	HRT (ms)	TwF (mN)	TetF (mN)	Tw/Tet	TwPTP	FatI
				FF			
C3 mo (n=55)	12.4±1.6	11.8±2.5	66.9±45.5	227.5±92.7	0.27±0.14	1.43±0.34	0.22±0.11
WBV3 mo (n=56)	12.7±2.1	12.5±3.5	78.9±37.2	278.0±118.0	0.29±0.08	1.37±0.22	0.21±0.10
P	0.7405	0.4875	0.1172	0.0590	0.4625	0.8008	0.6407
C6 mo (n=46)	13.1±2.1	12.8±3.3	77.6±41.8	253.3±132.5	0.31±0.11	1.37±0.20	0.23±0.13
WBV6 mo (n=54)	**13.8±1.9**	12.0±2.5	80.6±54.6	281.1±149.7	0.28±0.09	1.39±0.16	0.21±0.12
P	0.0335	0.3118	0.8161	0.3246	0.0996	0.4066	0.3247
				FR			
C3 mo (n=51)	13.5±2.2	16.0±3.8	23.1±18.2	110.7±47.5	0.19±0.08	1.23±0.21	0.84±0.13
WBV3 mo (n=36)	**12.3±2.4**	**14.8±5.0**	19.0±15.1	127.2±52.8	**0.14±0.07**	1.29±0.22	**0.92±0.13**
P	0.0103	0.0151	0.2589	0.1513	0.0009	0.1310	0.0052
C6 mo (n=55)	13.4±1.8	14.9±4.0	23.4±20.9	123.8±65.9	0.17±0.06	1.31±0.19	0.86±0.13
WBV6 mo (n=37)	13.6±1.9	15.1±3.5	19.8±15.8	138.1±75.2	**0.14±0.05**	1.35±0.21	0.83±0.16
P	0.8858	0.7116	0.4315	0.3415	0.0095	0.3145	0.5179
				S			
C3 mo (n=21)	22.5±2.1	36.2±4.7	7.5±3.7	48.0±16.3	0.15±0.05	0.93±0.09	0.99±0.02
WBV3 mo (n=22)	22.2±2.4	33.4±8.2	**5.3±3.7**	**69.9±35.0**	**0.08±0.04**	0.98±0.14	0.98±0.04
P	0.6866	0.2096	0.0392	0.0294	0.0000	0.1457	0.8809
C6 mo (n=19)	23.0±2.9	33.7±6.6	3.7±1.3	43.1±16.1	0.09±0.02	0.99±0.10	0.97±0.03
WBV6 mo (n=26)	23.4±3.8	30.1±5.4	3.4±1.5	44.9±15.4	**0.07±0.02**	0.97±0.15	0.99±0.03
P	0.8983	0.0507	0.3276	0.2553	0.0161	0.5378	0.0604

The mean values ± SD are presented. n – the number of MUs studied within the particular groups, CT – the twitch contraction time, HRT – the twitch half-relaxation time, TwF – the twitch force, TetF – the tetanus force, Tw/Tet – the twitch-to-tetanic force ratio, TwPTP - the twitch post-tetanic force potentiation, FatI – fatigue index. P indicates probability of Student's t-test for equal variances and U Mann-Whitney's test for un-equal variances for comparisons between WBV treated rats and the control animals.

Table 2. Motor units' contractile parameters.

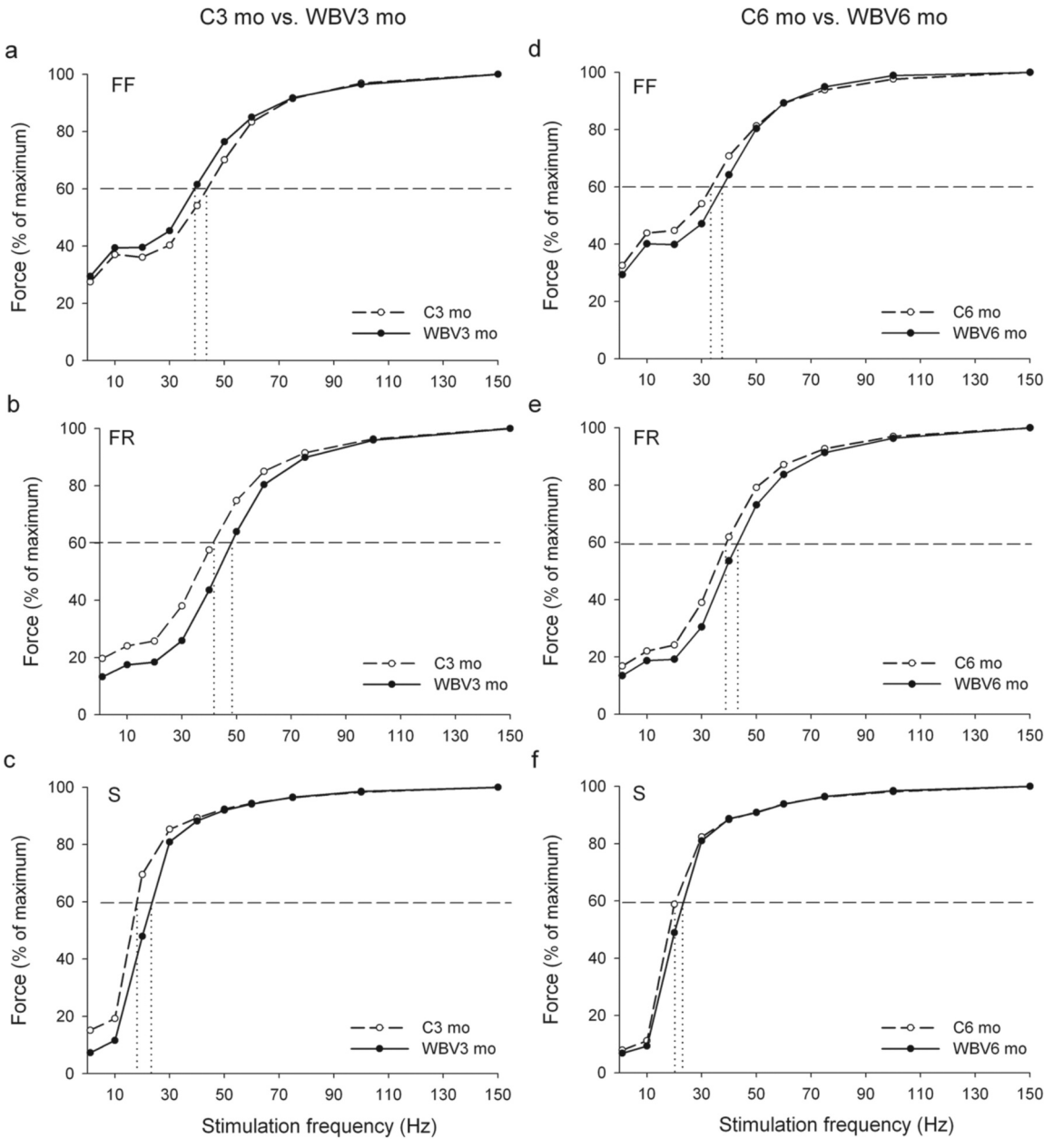

Figure 4. Force-frequency curves drawn for the FF, FR and S MUs for WBV3 mo (a, b, c, respectively) and WBV6 mo (d, e, f, respectively) rats, and respective control animals. The dashed horizontal and dotted vertical lines indicate the 60% of the MUs maximum force and the stimulation frequency required to evoke such force, respectively.

of units towards less fatigue resistant profile (Figure 3e). There were no differences in the relative MU distribution within the populations of the FF (Figure 3a, d) and S (Figure 3c, f) MUs.

It must be underlined that for type I MHC isoform (for comparisons made between C3 mo and WBV3 mo groups) the calculated power of *t*-statistics was 0.794, whereas for type IIax MHC isoform was 0.560 for accepted level of α. Therefore, for the latter type of MHC isoform a more numerous sample size should be used to overtake the potential type I statistical error when interpreting the histochemical findings.

Force- and time-related MU properties

S MUs reacted to 3 months of WBV basically with adaptive changes in the force-related parameters, as the twitch force was lower by 29.3% and the maximum tetanus force increased by 45.6% in comparison to the C3 mo group (Table 2). In consequence, the Tw/Tet ratio was significantly decreased (Table 2). The repeated measures analysis of variance revealed in S MUs a significant effect for WBV treatment on the relative forces generated at subsequent frequencies of stimulation [$F(1, 34)= 12.9$; $P<0.05$], as the forces were lower and the force-frequency curve

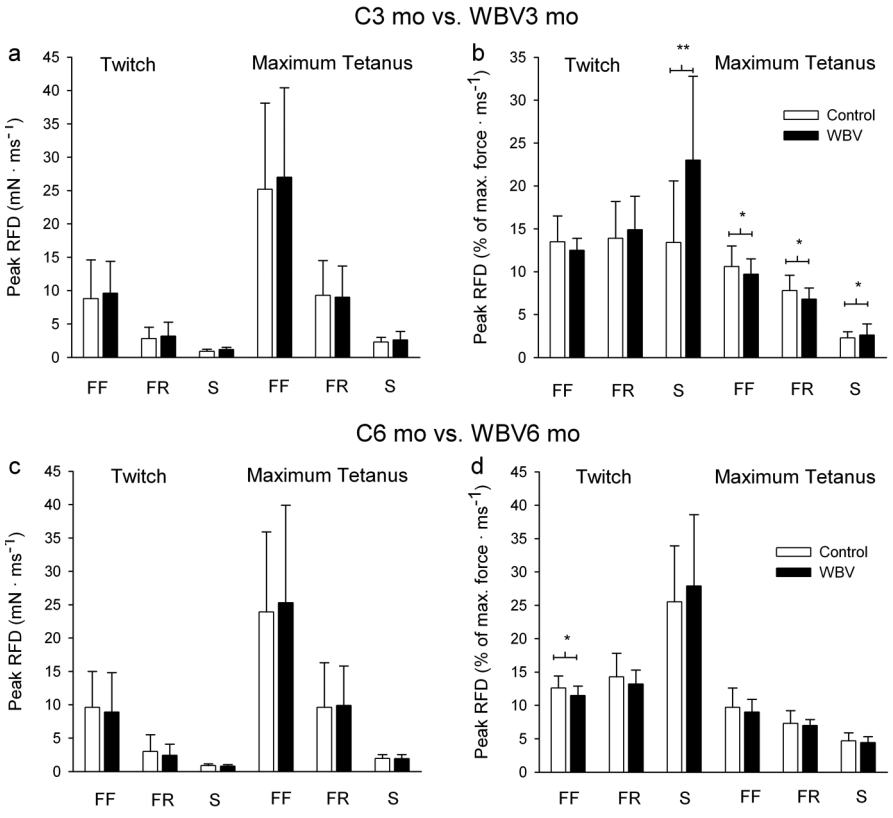

Figure 5. The twitch and maximum tetanic contraction peak rate of force development. The mean absolute and relative values±SD (error bars) are presented for C3 mo and WBV3 mo (a, b) as well as C6 mo and WBV6 mo (c, d) groups. Significant differences: * P<0.05, ** P<0.01 between the WBV and the respective control groups.

was shifted towards higher rates (Figure 4c). The stimulation frequency required to generate 60% of maximum force was increased by 21.9% (Table 3). Also, the twitch and maximum tetanic peak relative rate of force development were higher (Figure 5b).

In response to the same period of the WBV treatment the CT and HRT were shortened by 9.0% and 7.5%, respectively and the Tw/Tet ratio was reduced in FR MUs (Table 2). These changes coincided with the shift of the force-frequency curve towards the right (i.e., higher stimulation rates, Figure 4b). The stimulation frequency necessary to produce the 60% of the maximum force increased by 14.3%. The relative forces generated at the same stimulation frequencies were lower in the WBV3 mo group in comparison to the C3 mo one [repeated analysis of variance, significant effect, $F(1, 83)= 14.0$; $P<0.001$] (Figure 4b). Nevertheless, there was also a significant WBV treatment × frequency effect on the relative forces [$F(8, 664)= 7.8$; $P<0.001$; Greenhouse-Geisser $P<0.001$; Huynh-Feldt $P<0.001$]. The forces rose more steeply for FR units from the WBV3 mo group and the slope of the curve at the region around the 60% of the maximum force was increased (Figure 4b and Table 3).

After 3 months of the WBV there were no significant differences between the contractile parameters (Table 2) and between the relative forces produced during the stimulation protocol applied for testing the force-frequency relationship in the FF units (Figure 4a). However, there was the significant WBV treatment × frequency effect on the force-frequency relationship [$F(8, 808)= 3.7$; $P<0.001$; Greenhouse-Geisser $P<0.05$; Huynh-Feldt $P<0.05$]. The course of the curve was less steep in the WBV3 mo group than in the C3 mo one (Figure 4a) and the slope of the steepest part of the curve was significantly decreased in comparison to the C3 mo animals (Table 3). The stimulation frequency required to achieve 60% of maximum force was lower (Table 3).

In addition, the peak relative rate of force development of maximum tetanic contraction was reduced in FR and FF units in response to 3 months WBV exposition (Figure 5b).

Only few apparent effects of the WBV on contractile properties emerged after the 6-month treatment. Significantly lower values of the Tw/Tet ratios in FR and S MUs as well as prolongation of the twitch contraction (Table 2) and a decrease in the peak relative rate of twitch force development of FF units were observed (Figure 5d). No other changes in the basic contractile properties were detected (Table 2).

Similarly as after the completion of the 3-mo WBV treatment, the course of the force-frequency curve was horizontally shifted rightward (Figure 4e) and the 60% Fmax frequency was increased (Table 3) in the FR units. The repeated measures analysis of vari-

	60% Fmax frequency (Hz)	Slope (% Fmax/1Hz)
FF		
C3 mo (n=55)	42.4±13.1	2.1±0.6
WBV3 mo (n=56)	**39.1±12.2**	**1.8±0.5**
P	0.0165	0.01734
C6 mo (n=46)	33.0±18.1	1.8±0.5
WBV6 mo (n=54)	**36.4±13.0**	**2.0 ± 0.4**
P	0.0123	0.0055
FR		
C3 mo (n=51)	40.0±8.3	2.4±0.7
WBV3 mo (n=36)	**46.7±8.4**	**2.6±0.6**
P	0.0011	0.0366
C6 mo (n=55)	38.8±5.8	2.4±0.5
WBV6 mo (n=37)	**43.1±8.5**	2.4±0.6
P	0.0383	0.6813
S		
C3 mo (n=21)	18.7±3.0	4.8±1.2
WBV3 mo (n=22)	**22.8±4.4**	4.4±1.6
P	0.0012	0.6121
C6 mo (n=19)	20.9±2.8	4.1±1.0
WBV6 mo (n=26)	22.8±3.2	4.4±1.0
P	0.05172	0.9324

The mean values ± SD are presented. n – the number of MUs studied within the particular groups. P indicates probability values of Student t-test and U Mann-Whitney test for unequal variances for comparisons between the WBV treated rats and the control animals.

Table 3. Force-frequency relationship parameters.

ance showed that the forces generated at the same stimulation frequencies were lower in the WBV6 mo group as compared to the C6 mo group [significant effect, F(1, 85)= 8.1; *P*<0.05]. However, there was a significant WBV treatment × frequency effect on the force-frequency relation [F(8, 680)= 4.2; *P*<0.001; Greenhouse-Geisser *P*<0.05; Huynh-Feldt *P*<0.05]. The course of the force-frequency curve was steeper for the WBV6 mo group with respect to the C6 mo one (Figure 4e). Similarly, the significant effect for WBV treatment × frequency, manifested by an increased slope of the of the force-frequency curve, was found in FF units [F(8, 808)= 5.0; *P*<0.001; Greenhouse-Geisser *P*<0.05; Huynh-Feldt *P*<0.05] (Figure 4d). The slope of the steepest part of the curve (i.e. around 60% of maximum force) was increased in comparison to the C6 mo group (Table 3). The stimulation frequency required to produce 60% of maximum force was higher (Table 3). No differences in the force-frequency relationship was noted in S units following 6 months of WBV (Table 3, Figure 4f).

Finally, no changes in the peak absolute rate of force development of the maximum tetanic contraction were found in all types of MUs after 3 or 6 months of the WBV (Figure 5a, c).

Resistance to fatigue of MUs

It was observed that after the completion of 3 months of WBV the FatI of FR units increased as compared to the non-treated animals (Table 2). Also, the repeated measures analysis of variance revealed in this type of units a significant main effect for WBV [F(1, 81)=4.3; P<0.05], time [F(17, 1377)=27.4; P<0.000; Greenhouse-Geisser P<0.000; Huynh-Feldt P<0.000], and for WBV treatment × time [F(17, 1377)= 3.7; P<0.000; Greenhouse-Geisser P<0.05; Huynh-Feldt P<0.05] on the relative force generation during the fatigue test. In the WBV3 mo group the force potentiated more, declined less and was better maintained from 30 to 180 s time period (Figure 6b) during the fatiguing stimulation, in comparison to the C3 mo group. No other effects for the WBV treatment and for the WBV treatment × time were noted in fast units following both periods of the WBV (Figure 6a, c, d). Also, there were no differences in the fatigue indices of the FF and S MUs after the employed WBV protocols (Table 2).

Discussion

The mechanical loading exerted repeatedly during the long-term WBV treatment induced modifications of MHC content and contractile properties principally in S and FR MUs. Some of the changes observed after 3 months of the WBV, e.g. shortening of twitch time parameters and the rightward shift of the force-frequency curve in FR units and reduction in the twitch-to-tetanus ratio in FR and S units followed short-time WBV effects found previously after 5 weeks of the identical WBV programme[7]. In response to the 3-month WBV, structural alterations suggesting transition of type I MHC content towards faster profile and increase in strength of type S units were found in the MG muscle that were not seen following the short-term WBV. Additionally, a very interesting result was enhanced fatigue resistance of FR units. Nevertheless, increase in the maximum tetanus force which occurred in FF following 5 weeks of WBV[7] was not observed after 3 months of treatment. Also, after completion of 6 months of WBV, almost all structural and physiological changes in MU properties seen previously were no longer apparent.

Effects of the 3-month WBV

In the present study repeated boats of WBV with high magnitude of acceleration (4.9 g) were used. In the previous studies determining effects of WBV onto the musculoskeletal system lower magnitudes of acceleration (<1 g) have been reported during 15 min of continuous stimulation[5,6]. The peak acceleration ≤1.0 g is recognized as the low magnitude[20], however, using the higher magnitudes is acceptable for short, lasting not longer than 1 minute WBV[21]. Therefore, our short-lasting (four 30-s bouts) WBV protocol presumably induced considerably high mechanical loading on the musculoskeletal system.

Three months of WBV induced some structural and functional adaptations not seen after the short-term treatment[7]. An adaptive increase in force and the relative rate of force development of the maximum tetanic contraction were observed in S MUs in the WBV3 mo group. It is likely that the increase in force was caused by a hypertrophic response of slow muscle fibers, since the maximum tetanic force is a function of the total number of cross

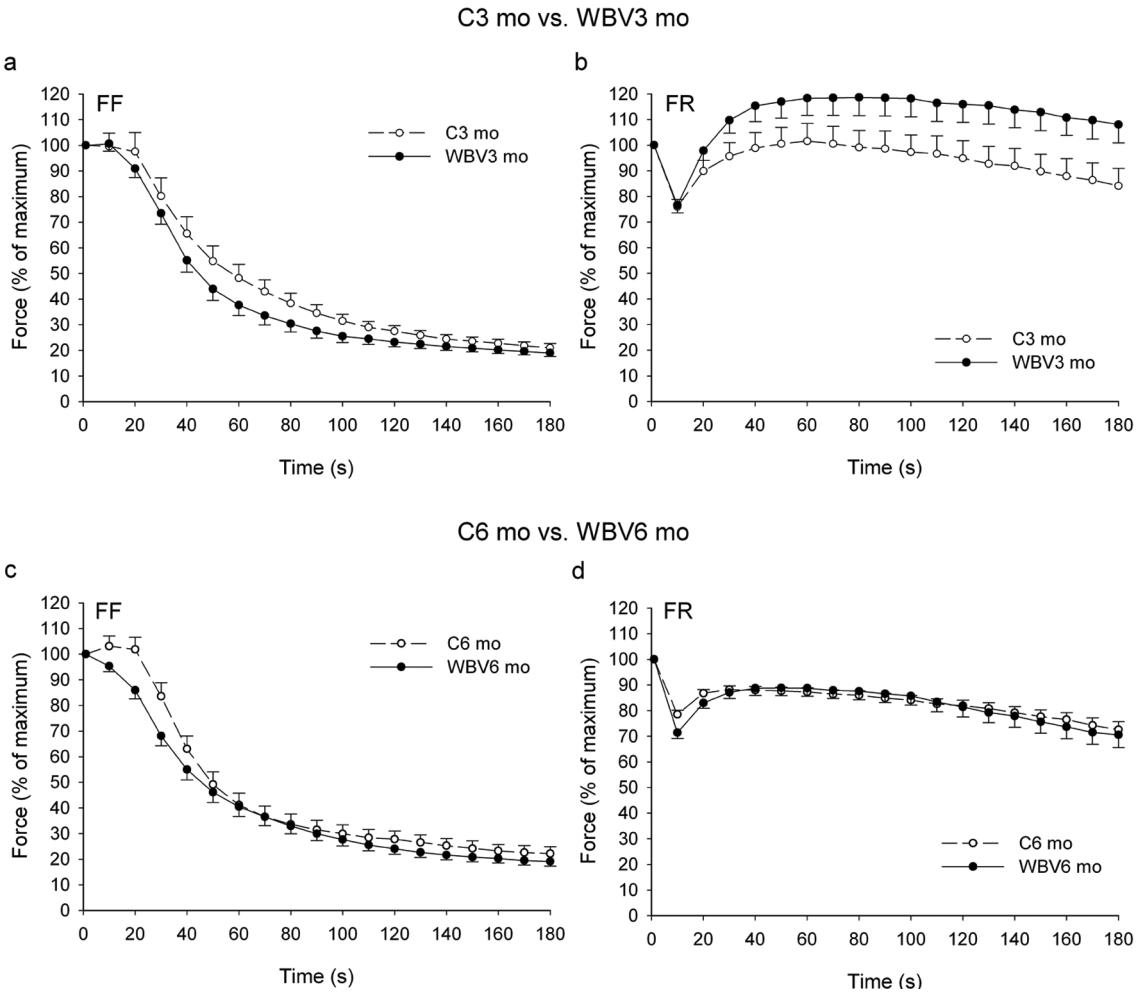

Figure 6. Effects of 3 mo and 6 mo WBV on the course of the fatigue test of fast MUs. The peak relative forces generated throughout 180 seconds of fatiguing protocol by FF and FR MUs for WBV3 mo (a, b) and WBV6 mo (c, d) and the respective control animals were presented. The forces of unfused tetanic contractions evoked by 40 Hz stimulation were measured every 10 seconds and expressed in percentage of the force of first tetanus generated at the beginning of the test. Values are means±SE. See Results section for description of findings.

bridges acting in parallel. Such response was observed by Xie et al.[5] in type I muscle fibres in mouse soleus muscle after 6 weeks of the 45 Hz frequency WBV (15 minutes daily).

The type I muscle MHC isoform content decreased in favor of type IIax MHC and this coincided with a shift in the distribution of fatigue resistance of FR units towards higher values. In this study we were not able to separate the IIa and IIx MHC isoforms, due to the methodological limitations. Nevertheless, IIa MHC FR units are characterized by very high fatigue indices (over 0.7), whereas IIx MHC units much lower (around 0.5)[19]. Therefore, it can be suggested that the transition from the type I to type IIa MHC took place, and the proportion of the type IIa MHC FR units was raised in the MG muscle. Such type I to IIa transition might partly improve the oxidative energy metabolism of FR units, as they became more fatigue resistant. However, the enhancement in the activity-dependent force potentiation of FR units during early (when fatigue did not yet emerged) and late course of the applied fatigue test suggests that also some positive alterations in the excitation-contraction coupling mechanisms responsible for regulation of both force potentiation and fatigue processes took place simultaneously[22]. It must be emphasized that the applied chronic WBV induced similar alterations in the MHC expression like observed in response to training modes employing ballistic and stretch-shortening movements[12]. Yet it is unknown, whether it was caused by the WBV-specific firing activity of MUs or was a characteristic effect of mechanical loading exerted by the WBV.

Low threshold MUs, most probably S, are readily activated during WBV and tonic vibration reflex[1,2] and their firing activity is well maintained during tens of seconds of vibratory stimulation[23]. They can also be activated with high-frequency burst during transient body mass supporting actives[24,25]. It has been suggested that S MUs are appreciably involved in posture maintenance during WBV as their contractions contribute to large in-

crease in muscle tendon complex stiffness which opposes the external forces acting on joints[26]. Adaptive increase in the maximum force implies that S MUs might experience high mechanical loading during WBV and develop higher forces than that required to maintain muscle length in standing posture. It is possible that due to inherent time-related properties, S MUs exposed to the 50 Hz WBV might develop relatively high-force contractions with respect to their maximum which corresponded to the steep portion of the force-frequency curve.

One of key functions of fast MUs during WBV is to actively damp the induced soft tissues oscillations[2]. The contractile element can ideally dissipate the power from soft tissue oscillations when it is able to cyclically perform the contraction and relaxation cycles within the time period of vibration cycle and modulate its force at the same rate as the vibration frequency (when natural frequencies of soft tissues overlap on the same excitation frequencies induced by WBV)[2]. During WBV MUs fire at subharmonics to the excitation frequencies[1] and activated by the tonic vibration mechanisms presumably fire tonically at low rates, i.e. 3-10 Hz[27,28]. In the present study the 50 Hz WBV frequency was employed and it has been estimated that natural frequencies of oscillations encountered by rat MG during the natural activities are in the range of 30-50 Hz[29]. In response to 3 months of WBV, similarly as after completion of the same but 5-week programme[7], we observed in FR MUs an adaptive shortening of the twitch time parameters and a shift of the force-frequency curve to the right. FR units could work at frequencies corresponding to initial flat portion of the force-frequency curve suggesting that during the applied WBV they rather generated twitches or brief unfused tetanic contractions. It is conceivable, that shorter MU contraction-relaxation period enables FR MUs work at higher rates and still produce unfused tetanic contractions optimal for damping process. Possibly, FR units could then respond to increased number of vibration cycles within the unit of time, and hence, increase the effectiveness of muscle tuning. This mechanism will work only if firing rates of reflexively activated MUs are also increased. Indeed, it was observed that the slope of the steady-state frequency-current curve increases in fast-type motoneurons in response to the 5-week WBV[30].

Contrary to the short-term effects of WBV[7], the improvement in the maximum tetanic force of FF units in the WBV3 mo group did not reach the established level of statistical significance to be considered as being not by a chance alone. The explanation of this discrepancy is not obvious. There is ample evidence that high-threshold (fast) MUs are activated reflexively[1] and by feed-forward anticipatory mechanisms[2]. High-threshold motoneurones and converging on them group Ia and II polysynaptic pathways receive high facilitatory input from the supraspinal vestibulospinal and rubrospinal tracts which mediate commands involved in postural tasks[31]. Therefore, at initial stages of the chronic WBV FF units might be highly activated during expositions, since high-threshold MUs effectively participate in brief postural corrective movements[32] or activities related to body weight bearing[25]. On the other hand, it has been documented that during sustained muscle vibration of voluntarily contracting muscle, the firing rate of activated high threshold MUs starts to decline abruptly within seconds after the onset of vibratory stimulation[23]. This effect has been attributed to depression of synaptic transmission through monosynaptic and polysynaptic pathways mediating the tonic vibratory-induced volleys[33]. Therefore, it is likely that the WBV, chronically repeated for an extensive period, might induce activity-dependent spinal neuronal plasticity manifested by changes in synaptic transmission making high threshold FF MUs gradually less responsive to the WBV excitation.

Effects of the 6-month WBV

The picture of adaptive changes after 6 months of WBV was substantially different, as most of the changes in contractile parameters observed in the WBV3 mo group were no longer apparent. Similarly, Torvinen et al.[13] have reported for humans that the beneficial increase in knee extension force seen after 2 months of WBV was not longer observed when the training was further continued until the 4 months period was reached. Some positive changes in human muscle strength and explosive force have also been found for shorter periods (up to 3 months) of WBV exposition[34,35], but longer treatments (4 months and over) have not provided a reliable proof of any additional effects of WBV on muscle performance[8].

It is highly unlikely that the neuromuscular system ceases to respond to excitation frequencies and does not adapt somehow to the mechanical loading exerted by the WBV after very long-term applications. In this study we observed decreased ratios of the twitch-to-tetanus forces in S and FR MUs in response to both 3 and 6 months WBV. This parameter is strongly positively correlated to the isometric twitch forces of S and FR MUs and partly depends from muscle-tendon complex viscoelastic properties[14]. It is know that with increase in the series elastic compliance of the muscle the twitch amplitude is diminished[36] and its contraction and relaxation times are lengthened[37]. Therefore, we hypothesise that the stiffness of muscle fibres contained in FR and S units might decrease with time of the long-term WBV treatment. This would explain the steadily observed decrease in the twitch-to-tetanus force ratio and the withdrawal of observed adaptive shortening of twitch time parameters seen in FR and S MUs in response to identical but shorter WBV expositions[7]. However, the parameters of the maximum tetanic contractions and the force-frequency relationship could be also affected because decrease in muscle series elastic stiffness results in reduction of tetanic contraction amplitude, slowing of its force rise and relaxation[38].

Although, the direct effect of chronic WBV treatment on muscle-tendon complex stiffness is unknown, some studies have documented an increase in soft tissues elasticity in response to long-term WBV. For instance, Fagnani et al.[39] have found an increase in lower limb movement range in female competitive athletes after 8 weeks of WBV. In addition, Sands et al.[40] have found that long-term WBV enhances the range of a lower limb motion in male gymnasts beyond that obtained for static stretching only. In our study the adaptive decrease in the twitch-to-tetanus ratio concerned only S and FR units, which on the basis of previously noted adaptations are actively

involved in the contractile activity during the WBV (twitch-to tetanus ratio did not changed in FF units). It is rather impossible that within a muscle tendon complex, the passive contractile element in series or in parallel could be selectively modified, and thus account only for change in contractile stiffness produced by FR and S units. During the muscle-tendon vibration a considerable amount of energy is taken up and absorbed by elastic deformation of cyclically active cross bridges[29]. Therefore, supposedly series elastic compliance of the myofibrillar apparatus responsible for cross- or non-cross bridge stiffness[41,42] might be affected in those units. For instance, Bellafiore et al.[43] have found an increased titin content in the mouse gastrocnemius muscle after the short-term endurance training. The authors have postulated that this effect increases muscle elasticity and extensibility and is caused by high number of stretch-shortening cycles encountered during endurance training. Since the WBV also provokes the contractile element to frequently oscillate in the stretch-shortening cycle manner, it is likely that structure of the muscle fibres of the activated MUs can accordingly be modified by mechanism similar to the above mentioned.

It is beyond the scope of this study to discuss potential physiological consequences of these observations, but the suggested decrease in the stiffness of S and FR units can potentially influence muscle tuning and force generation processes through alterations in the storing and releasing of the elastic energy[37] and transmission of forces between the active and passive muscle fibres of MUs[26]. Future studies are required to clarify effects of chronic WBV on muscle-tendon unit and motor unit structural remodelling as well as on passive and active stiffness. Novel experimental methods should also be employed to differentiate, whether the effects of WBV on the neuromuscular system are mediated by reflexive or direct action of mechanical shocks onto muscle tissue. Finally, recruitment and firing behaviour of motor units following chronic applications of WBV should be delineated for better understanding an adaptive physiological response of the neuromuscular system to experienced WBV.

In summary, the 3-month WBV period shortened the twitch time parameters and enhanced fatigue resistance of FR units, increased the force of S units, decreased type I MHC and increased type IIax MHC isoform muscle content. Nevertheless effects on MHC were quite modest and might have little functional relevance. It is proposed, that these contractile modifications point out that FR MUs adapt to optimize muscle tuning of vertical vibrations, whereas S units to be more effectively involved in opposing the external forces acting on joints and maintaining joint position during the WBV. We provided some evidence that after 6-month period of the WBV treatment, the stiffness of S and FR units decreases and presumably contributes to the concealing of time- and force-related contractile modifications observed in these units following the shorter WBV expositions. Albeit, an increase in the elasticity of S and FR MUs likely lessens the demands on active muscle tuning. Since the applied WBV program was composed of relatively low-volume WBV, further studies using progressively increasing in volume and intensity WBV protocols are required for better understanding physiological adaptations of the neuromuscular system to chronic vertical oscillations.

Acknowledgements

The study was supported by the Polish Ministry of Science and Higher Education, Grant No. N N404 042136.

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