



# Blood flow restriction increases myoelectric activity and metabolic accumulation during whole-body vibration

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## Abstract

**Purpose** Whole-body vibration (WBV) training is frequently applied in sports and rehabilitation with the aim of inducing beneficial functional and structural adaptations. In the past decades, blood flow restriction (BFR) training has received increasing attention by enhancing the effectiveness of several low-load exercise regimens. The objective of this study was to evaluate the additional effect of BFR on myoelectric activity and metabolic accumulation during WBV training.

**Methods** Fifteen active men performed three sessions in a counterbalanced order on three different days: whole-body vibration exercise (WBV), whole-body vibration exercise with blood flow restriction (WBV + BFR), and a control session (CON) with neither WBV nor BFR. Electromyographic (EMG) activity was measured in six lower limb muscles throughout each exercise session; lactate and reactive oxygen species (ROS) concentrations were determined prior to, immediately after and 15 min after the exercise sessions.

**Results** EMG amplitudes increased from CON ( $29 \pm 13\%$  MVC) to WBV ( $45 \pm 20\%$  MVC) to WBV + BFR ( $71 \pm 37\%$  MVC) conditions ( $p < 0.05$ ). Likewise, lactate concentrations increased in a similar manner, demonstrating significantly higher increases in the WBV + BFR session compared to WBV and CON. Furthermore, significant correlations between lactate concentration and EMG amplitude were detected. ROS concentration did not change significantly between the conditions.

**Conclusions** The findings of the present study emphasize that the addition of BFR increases the acute effects beyond WBV treatment alone which becomes manifested in both neuromuscular and metabolic adaptations. Further research is needed to identify potential long-term effects of the combination of these two training regimens.

**Keywords** Blood flow restriction · Electromyography · Metabolic accumulation · Whole-body vibration · Myoelectric activity

## Abbreviations

1RM	One repetition maximum
AOP	Arterial occlusion pressure
BFR	Blood flow restriction
BP	Blood pressure
CON	Control
CSA	Cross-sectional area
EMG	Electromyography
EPR	Electron paramagnetic resonance
MVC	Maximum voluntary contraction

RMS	Root mean square
ROS	Reactive oxygen species
WBV	Whole-body vibration

## Introduction

Whole-body vibration (WBV) is a frequently prescribed training regimen in sports and rehabilitation. It has previously been reported that WBV increases strength (Delecluse et al. 2003; Marin and Rhea 2010; Roelants et al. 2006), functional performance (Cochrane and Stannard 2005; Marshall and Wyon 2012; Perchthaler et al. 2015), bone mineral density (Verschuere et al. 2004; Ward et al. 2004) and postural stability (Ritzmann et al. 2014). Moreover, recent investigations have shown that WBV training has positive effects on muscle mass (Bogaerts et al. 2007; Ebing et al. 2018; Machado et al. 2010). However, these studies were

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conducted in sedentary participants (Ebing et al. 2018) or individuals who already had a deficit in skeletal muscle mass (Bogaerts et al. 2007; Machado et al. 2010). For young and active individuals, there is to date no convincing evidence demonstrating the hypertrophic effects of WBV training. This lack of structural adaptation might result from a muscular activation that is not high enough to elicit anabolic stimuli. This is supported by a study from Pollock et al. (2010) revealing that muscle activity even at the highest vibration frequencies and amplitudes ranged between 5 and 50% of the maximum voluntary contraction (MVC) and is thus similar to that during fast walking (~1.7 m/s) (Masamoto et al. 2004).

In the past two decades, the topic of blood flow restriction (BFR) training has received increasing attention in the scientific community (Bittar et al. 2018; Centner et al. 2019; Hughes et al. 2017; Loenneke et al. 2012b; Scott et al. 2015; Slys et al. 2016). Combining low-load (20–30% of one repetition maximum/1RM) resistance training with BFR has been shown to facilitate superior gains in muscle mass and strength, compared to the same exercise without BFR (Patterson and Ferguson 2010; Slys et al. 2016; Takarada et al. 2000b; Yasuda et al. 2015). Additionally, several meta-analyses (Centner et al. 2019; Lixandrao et al. 2018) revealed that low-load BFR training promotes comparable increases in muscle mass compared to conventional high-load resistance training (> 70% 1RM) which is of particular relevance for patients in rehabilitation or elderly. The underlying potential mechanisms are not fully elucidated but several factors such as an increased intramuscular metabolic stress (Suga et al. 2012; Takarada et al. 2000a), augmented muscle fiber recruitment (Takarada et al. 2000a; Yasuda et al. 2009; Yasuda et al. 2014), cell swelling (Loenneke et al. 2012a) and an enhanced activation of muscle protein synthesis (Fry et al. 2010; Fujita et al. 2007) have been assumed to be involved. Especially, the accumulation of metabolites, including an increased production of blood lactate and reactive oxygen species (ROS), has repeatedly been reported to stimulate muscle growth (Pearson and Hussain 2015; Powers et al. 2010; Schoenfeld 2013). Both, increased metabolic stress as well as reduced oxygen availability have been identified as important mediators of an increased recruitment of higher threshold motor units (MU) (Moritani et al. 1992; Suga et al. 2012; Yasuda et al. 2010). Indeed, evidence suggests that the addition of BFR enhances muscle excitation as shown by an increase in electromyography (EMG) amplitude when compared with conventional low-load training (20–30% 1RM) (Takarada et al. 2000a; Yasuda et al. 2009).

Given the fact that muscular activation during WBV is equivalent to that during low-load exercise (Pollock et al. 2010) and that low-load exercise performed with BFR showed augmented MU recruitment (Takarada et al. 2000a; Yasuda et al. 2009; Yasuda et al. 2014) and

metabolic response (Fujita et al. 2007; Takarada et al. 2000a), we hypothesized that the combination of WBV and BFR may also positively influence EMG activity levels and be associated with increased metabolic stress.

Thus, the main objective of this study was to evaluate the additional effect of BFR on myoelectric activity during WBV training. Therefore, WBV training under free-flow conditions was compared to WBV with BFR. To control for the influence of the vibration stimulus, a third session was implemented with the same exercise condition but neither WBV nor BFR. We expected a gradual increase in EMG activity of the lower extremities concomitant with an elevation in blood lactate and ROS concentrations from CON to WBV to WBV + BFR. Furthermore, we hypothesized that neuromuscular changes are related to increased metabolic accumulation.

## Methods

### Subjects

Fifteen men ( $24.9 \pm 3.5$  years) were recruited to participate in this study. All subjects were healthy and required to be experienced in resistance training (> 1 year) to avoid learning effects that could occur between the sessions. Participants with chronic illnesses such as coronary heart disease, diabetes mellitus, kidney diseases, a history of deep vein thrombosis, uncontrolled hypertension, neurologic diseases or smoking were excluded from the study. Furthermore, volunteers were advised not to take antioxidative or anti-inflammatory supplementations during their time of participation. Participants' characteristics are presented in Table 1.

The study was approved by the local ethics committee (455/18) and conducted in accordance with the latest revision of the Declaration of Helsinki. Experimental procedures and potential risks were explained, before informed consent was obtained prior to inclusion.

**Table 1** Participant characteristics ( $N=15$ )

Variable	Mean $\pm$ SD	MIN	MAX
Age (years)	24.9 $\pm$ 3.5	19	30
Height (cm)	179.9 $\pm$ 6.1	172	198
Weight (kg)	80.2 $\pm$ 11.3	67	106
BMI (kg/m <sup>2</sup> )	24.7 $\pm$ 3.1	22.0	33.5
AOP (mmHg)	234.0 $\pm$ 20.9	190	262
RT experience (years)	5.5 $\pm$ 3.0	2.5	14

AOP arterial occlusion pressure, BMI body mass index, RT resistance training

## Experimental design

To examine the acute effects of whole-body vibration with blood flow restriction on neuromuscular activity, a repeated measures cross-over design was implemented. All subjects completed three different exercise sessions in an isometric squat position with the knee joint flexed at 120° (Figueroa et al. 2012; Gómez-Bruton et al. 2017; Machado et al. 2010), on three different days in a counterbalanced order: (1) WBV without BFR; (2) WBV with BFR; and (3) a control condition in which the same exercise was performed with neither WBV nor BFR.

All sessions were conducted at the same time of the day (between 8 a.m. and 11 a.m.) and were separated by a wash-out period of at least 1 week to ensure adequate recovery. Prior to measurements, all participants were instructed to abstain from unaccustomed strenuous exercise for 72 h and follow a 12-h fasting period.

## Procedure

The experimental procedure for the three testing sessions was similar, except for the exercise protocol.

Before each session, EMG electrodes were placed on six muscles of the right leg: M. soleus (SOL), M. gastrocnemius medialis (GM), M. tibialis anterior (TA), the M. rectus femoris (RF), the M. vastus medialis (VM) and lateralis (VL). For normalization of the EMG data, prior to the measurements, subjects performed two isometric MVCs for each recorded muscle; we used the trial with the highest EMG for data normalization. The MVCs were executed according to Roelants et al. (2006) and Wiley and Damiano (1998), performed against resistance and held for 3 s with 1 min in-between. Body position during MVCs was strictly controlled and standardized by means of supervision by the authors and by goniometric recordings of ankle and knee joint angles. Antagonistic muscle activity was monitored and trials repeated when antagonists were activated. Baseline activation was recorded in a standing position without vibration or blood flow restriction. Muscle excitation was then continuously measured during the whole length of each set.

Additionally, capillary blood was taken at the right index fingertip prior to the MVCs as well as immediately and 15 min after the exercise session and cuff deflation. These blood samples were processed immediately and used for measuring lactate concentration and total reactive oxygen species production (Fig. 1).

## Exercise protocols

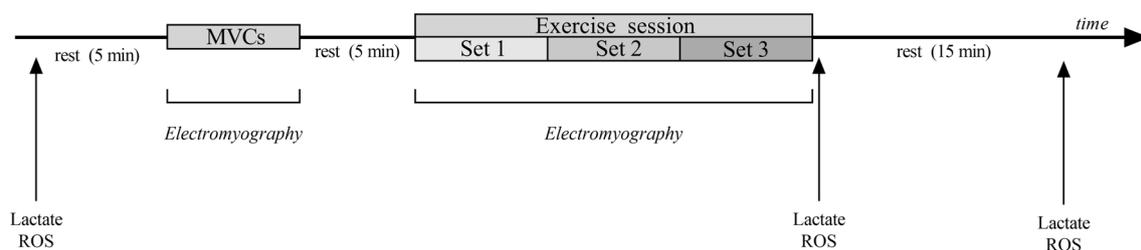
Protocols were performed in a random and counterbalanced order.

### Protocol 1: control session (CON)

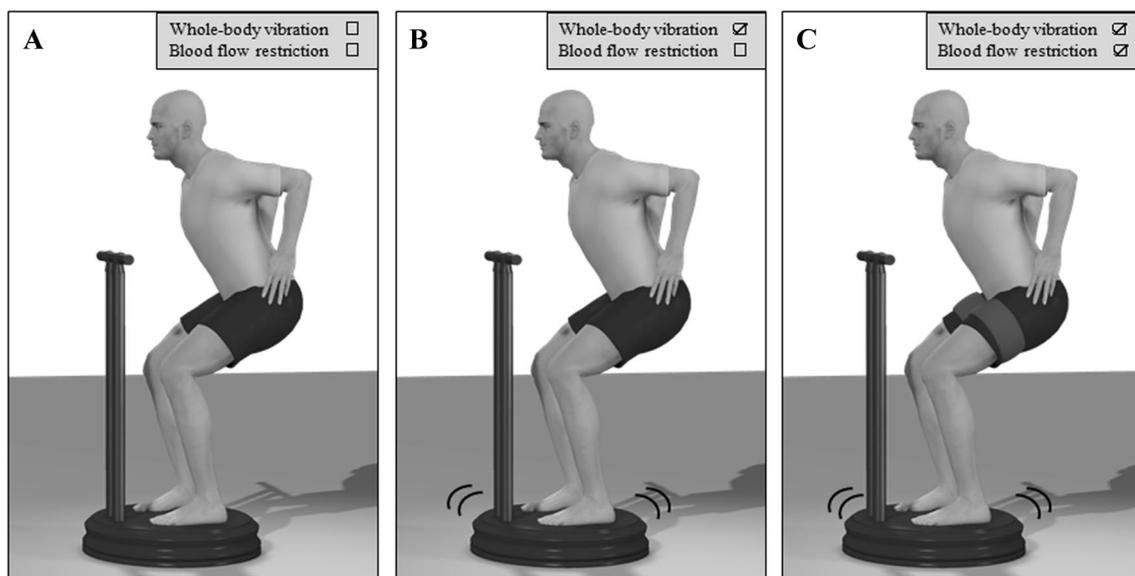
In this protocol, subjects were instructed to perform an isometric squat exercise (Fig. 2a) with a knee angle of 120°, as this exercise position has frequently been used in the scientific literature (Figueroa et al. 2012; Gómez-Bruton et al. 2017; Machado et al. 2010). Participants performed three sets with 120 s and an inter-set-rest period of 60 s. Additionally, the weight was equally distributed on both feet with the head and eyes directed in forward position. The foot-to-foot distance was set to 42 cm. Biofeedback of the degree of knee flexion was provided by a screen. At the beginning of each set, an electronic trigger [Stimuli, Pfittec Biomedical Systems, Endingen, Germany] was set simultaneously to the start of the exercise, which automatically marked the timeframe of interest (120 s) in the raw EMG file and could thus be used for later data processing and analyses. During the control condition, neither a vibration stimulus nor blood flow restriction was applied. All individuals were able to successfully complete the pre-defined length of isometric contractions.

### Protocol 2: whole-body vibration exercise (WBV)

In this protocol, subjects performed the same exercise regime (Fig. 2b) on a side-alternating vibration platform [Novotec Medical, Pforzheim, Germany]. The vibration frequency was set to 30 Hz, to elicit a sufficient degree of muscle activation (Ritzmann et al. 2013). During the exercise, subjects were barefoot to prevent potential dampening



**Fig. 1** Experimental procedure (min minutes, MVC maximum voluntary contraction, ROS reactive oxygen species)



**Fig. 2** Overview of the three different exercise protocols. In the control (CON) condition, (a) subjects were standing in an isometric squat position ( $120^\circ$ ) on a non-vibrating platform without blood flow restriction. In the WBV condition, (b) subjects performed the same

exercise with superimposed vibration stimuli, whereas in the third condition (WBV + BFR), (c) a pressurized cuff (60% of each individual's arterial occlusion pressure) was applied during exercising under whole-body vibration

effects that might occur with footwear. Additionally, the weight was equally distributed on both feet with the head and eyes directed in forward position. The foot-to-foot distance was set to 42 cm resulting in a vibration amplitude of 4 mm (Ritzmann et al. 2013).

All subjects successfully completed the prescribed time of isometric contraction per set.

### Protocol 3: whole-body vibration exercise with blood flow restriction (WBV + BFR)

For this session, each participant performed the same exercise protocol that was completed during the WBV session. However, a 12-cm-wide pneumatic nylon tourniquet [Zimmer Biomet, Warsaw, IN, USA] was placed around the most proximal portion of each thigh. Cuff pressure was set to 60% (Giles et al. 2017; Loenneke et al. 2013) of each individual's arterial occlusion pressure and kept inflated by a computerized tourniquet system [A.T.S. 3000, Zimmer Biomet, Warsaw, IN, USA] during the entire session including rest periods (Fig. 2c). Arterial occlusion pressure was recorded in a standing position at both legs of each participant. Cuff pressure was successively increased until a pulse was no longer detected at the posterior tibial artery by Doppler ultrasound [Handydop, Kranzbühler, Solingen, Germany]. At this point, an arterial occlusion of 100% was assumed. The mean absolute occlusion pressure during exercise was  $140 \pm 13$  mmHg.

The average time of isometric contraction was  $120 \pm 0$  s,  $119.8 \pm 0.5$  s and  $114.7 \pm 15.4$  s for the first, second and third sets, respectively.

### Instruments

#### EMG recordings

After shaving the skin and carefully cleaning it with an alcoholic solution, two bipolar surface EMG electrodes [Ambu Blue Sensor P, Bad Nauheim, Germany] were positioned on the muscle belly of each of the six muscles. The longitudinal axes of the electrodes were, therefore, aligned with the fiber orientation of the respective muscle. Additionally, a reference electrode was placed on the tibia. Impedance was kept below  $2 \text{ k}\Omega$  with a mean interelectrode resistance of  $1.53 \pm 0.22 \text{ k}\Omega$ ,  $1.56 \pm 0.24 \text{ k}\Omega$  and  $1.57 \pm 0.24 \text{ k}\Omega$  for CON, WBV and WBV + BFR sessions, respectively.

All signals were pre-amplified ( $1000 \times$ ), band-pass filtered (10–1000 Hz) and sampled with 2 kHz. Cables were carefully taped to the skin to minimize motion artifacts.

#### Electrogoniometry

Ankle (dorsiflexion and plantar flexion) and knee (flexion and extension) joint kinematics in the sagittal plane were recorded by electro-goniometers (Biometrics, Gwent, UK). The ankle goniometer was fixed at the lateral aspect of the right ankle, with its movable endplates attached

parallel to the major axis of the foot in line with the fifth metatarsal and the major axis of the leg in line with the fibula. The knee goniometer was placed over the lateral epicondyle of the femur, with one endplate attached to the shank and aligned to the lateral malleolus of the fibula and the other to the thigh aligned to the greater trochanter. The knee flexion angle was set to zero at 0° during normal upright stance, and joint flexion was reflected by an angle of 0°. An angle of 90° between the fifth metatarsal and the fibula corresponded to a 90° ankle angle; an angle of 90° reflected plantar flexion. Signals were recorded with a sampling frequency of 2 kHz.

### Blood analysis

Capillary blood was obtained from the right index finger before, as well as immediately and 15 min after the exercise condition.

The production of ROS was measured by electron paramagnetic resonance (EPR) imaging. A benchtop EPR spectrometer [E-Scan M, Bruker, Germany] was equipped with a temperature controller BIO-III to detect ROS production under in vivo conditions at 37 °C. Total ROS production was analyzed from 20 µl of capillary blood, sampled directly from the right index finger of each participant using a standard Eppendorf pipette. After adding 20 µl of oxygen-sensitive label (NOX 15.1–5 µmol/l) to the 20 µl blood sample, the mixture was vortexed and placed on ice. Utilization of this oxygen-sensitive label allows monitoring of cellular oxygen consumption (Mariappan et al. 2009). Subsequently, 15 µl of the blood-label solution was combined with 15 µl of Krebs-HEPES buffer diluted spin probe 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH, 400 µmol/l), obtained from Noxygen Science Transfer and Diagnostics GmbH [Elzach, Germany]. Under these conditions, released ROS in capillary blood interacts in the intracellular and extracellular space with CMH to form the stable radical CM° (Mrakic-Sposta et al. 2012). The following settings were used for the EPR spectrometer: center field:  $g = 2.011$ , sweep width: 60 G, frequency = 9.76 GHz, power = 20.97 mW, gain:  $1 \times 10^3$ , modulation amplitude: 1.06 G, sweep time: 5.24 s, number of scans: 10. The EPR signal was calibrated using a standard concentration of CM° (10 µmol/l) filled in a 50-µl glass capillary.

Furthermore, the capillary blood was used for measuring lactate concentration. Therefore, 20 µl of capillary blood was obtained from the right index finger and the blood-filled plastic capillary was placed in a 1000-µl cup with haemolysis solution. The mixture was then analyzed by an enzymatic–amperometric method using a Biosen S-Line Lab + lactate and glucose analyzer [EKF Diagnostics, Cardiff, UK].

### Data processing

The respective raw EMGs for the recorded muscles in each condition were rectified and the root mean square (RMS) values of the first and last 5 s of each of the three sets were calculated in all trials. In this regard, the electronic trigger was used for orientation purposes. To make EMG activity comparable across different muscles and participants, the raw EMG signal was normalized to the maximum EMG activity that was recorded during the MVCs. For this purpose, a RMS of 100 ms was calculated at the maximum EMG signal of the MVC recordings. The mean of the two MVCs for each muscle was used for the normalization procedure.

Additionally, mean knee and ankle joint angles were calculated during each condition for every subject.

### Statistics

Software package SPSS version 24.0 [IBM, Armonk, USA] was used for all analyses. Normal distribution as well as homogeneity of variances were checked for all variables. Subsequently, a two (time)  $\times$  three (condition) repeated measures ANOVA (rmANOVA) was performed to test for interaction effects in the level of myoelectric activity and a 3 (time)  $\times$  3 (condition) rmANOVA was used to analyze changes in metabolic parameters. The level of significance was set to  $p < 0.05$  for all tests. For further interpretation of significant interactions, contrast analyses were conducted. To correct for multiple comparisons, Bonferroni correction was applied, and the significance level was subsequently adjusted.

Bivariate, two-tailed Pearson correlation analyses were conducted to determine the strength of linear relations between the two variables myoelectric activity of VM and VL and lactate concentration. All data are presented as mean  $\pm$  standard deviation.

### Results

All subjects completed the investigations and no dropouts were reported. Mean knee and ankle angle were  $122^\circ \pm 3^\circ$  and  $75^\circ \pm 5^\circ$  during the WBV + BFR condition,  $122^\circ \pm 4^\circ$  and  $76^\circ \pm 4^\circ$  in the WBV condition and  $121^\circ \pm 2^\circ$  and  $76^\circ \pm 4^\circ$  during the CON session.

### Myoelectric activity

#### Set 1

During the first set, the calculation of a 2  $\times$  3 rmANOVA revealed a significant main effect of time for SOL ( $p < 0.05$ ;

$\eta^2=0.509$ ), TA ( $p < 0.05$ ;  $\eta^2=0.655$ ) and RF ( $p < 0.05$ ;  $\eta^2=0.297$ ), with the myoelectric activity being higher at the end of the set compared to the beginning. For the VM ( $p=0.10$ ;  $\eta^2=0.186$ ), VL ( $p=0.16$ ;  $\eta^2=0.134$ ) and GM ( $p=0.17$ ;  $\eta^2=0.129$ ), there was no significant main effect of time. Additionally, the rmANOVA demonstrated a significant main effect of condition with WBV + BFR presenting significantly higher overall EMG amplitudes in all muscles compared to CON ( $p < 0.05$ ). No significant interaction effect was observed in any of the muscles (Fig. 3).

## Set 2

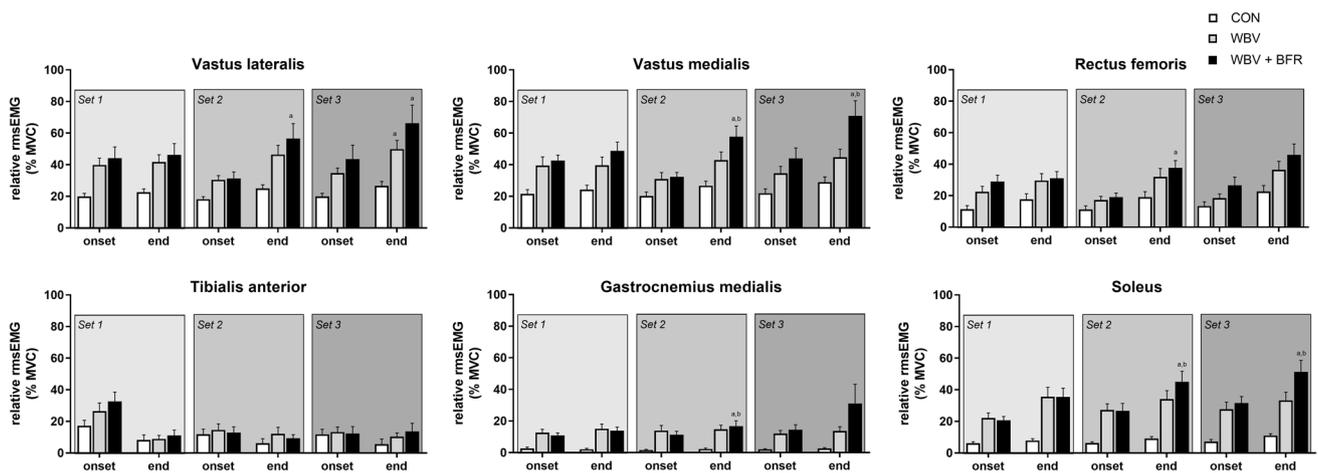
During the second set, the statistical analyses showed a significant main effect of time for SOL ( $p < 0.05$ ;  $\eta^2=0.539$ ), GM ( $p < 0.05$ ;  $\eta^2=0.299$ ), RF ( $p < 0.05$ ;  $\eta^2=0.795$ ), VL ( $p < 0.05$ ;  $\eta^2=0.660$ ) and VM ( $p < 0.05$ ;  $\eta^2=0.833$ ) with a higher myoelectric activity at the end compared to the beginning of the set. However, no significant time effect was observed for the TA muscle ( $p=0.18$ ;  $\eta^2=0.126$ ). Moreover, the rmANOVA revealed significantly higher overall EMG amplitudes for SOL ( $p < 0.05$ ;  $\eta^2=0.487$ ), GM ( $p < 0.05$ ;  $\eta^2=0.566$ ), RF ( $p < 0.05$ ;  $\eta^2=0.345$ ), VL ( $p < 0.05$ ;  $\eta^2=0.378$ ) and VM ( $p < 0.05$ ;  $\eta^2=0.357$ ) comparing WBV + BFR with CON. Again, there was no main effect of condition for the TA muscle ( $p=0.18$ ;  $\eta^2=0.117$ ) comparing WBV + BFR with CON. The interaction of both factors (time  $\times$  condition) reached statistical significance for SOL ( $p < 0.05$ ;  $\eta^2=0.327$ ), GM ( $p < 0.05$ ;  $\eta^2=0.231$ ), RF ( $p < 0.05$ ;  $\eta^2=0.200$ ), VM ( $p < 0.05$ ;  $\eta^2=0.391$ ) and VL ( $p < 0.05$ ;  $\eta^2=0.318$ ), but not for TA ( $p=0.71$ ;  $\eta^2=0.025$ ). After contrast analyses, WBV + BFR showed a greater increase in EMG activity than the CON session for the SOL ( $p < 0.05$ ), GM ( $p < 0.05$ ), RF ( $p < 0.05$ ), VM ( $p < 0.05$ )

and VL ( $p < 0.05$ ). For the muscles SOL ( $p < 0.05$ ), GM ( $p < 0.05$ ) and VM ( $p < 0.05$ ), the WBV + BFR session showed greater increases in EMG amplitude over time compared to the WBV session.

## Set 3

In the last set, the calculation of a  $2 \times 3$  rmANOVA revealed a significantly higher EMG activity at the end of the set for the SOL ( $p < 0.05$ ;  $\eta^2=0.511$ ), RF ( $p < 0.05$ ;  $\eta^2=0.789$ ), VM ( $p < 0.05$ ;  $\eta^2=0.870$ ) and VL muscle ( $p < 0.05$ ;  $\eta^2=0.761$ ) but not for TA ( $p=0.08$ ;  $\eta^2=0.199$ ) and GM ( $p=0.15$ ;  $\eta^2=0.145$ ). When ignoring the time factor and looking at the main effect of condition, all muscles except the TA ( $p=0.50$ ;  $\eta^2=0.048$ ) showed statistically significant differences between conditions. In this case, a higher overall muscle activation was apparent in SOL ( $p < 0.05$ ;  $\eta^2=0.573$ ), GM ( $p < 0.05$ ;  $\eta^2=0.363$ ), RF ( $p < 0.05$ ;  $\eta^2=0.321$ ), VM ( $p < 0.05$ ;  $\eta^2=0.378$ ) and VL ( $p < 0.05$ ;  $\eta^2=0.374$ ) comparing WBV + BFR and CON. Two muscles (SOL and VM) demonstrated significantly higher EMG amplitudes comparing the WBV + BFR session with WBV alone ( $p < 0.05$  and  $p < 0.05$ , respectively).

Regarding an interaction of time and condition, the statistical analyses revealed a significantly higher myoelectric activity over time for the SOL ( $p < 0.05$ ;  $\eta^2=0.244$ ), VM ( $p < 0.05$ ;  $\eta^2=0.480$ ) and VL ( $p < 0.05$ ;  $\eta^2=0.253$ ). Thereby, WBV + BFR showed higher increases in EMG signals in SOL and VM compared to CON ( $p < 0.05$  and  $p < 0.05$ , respectively) and WBV session ( $p < 0.05$  and  $p < 0.05$ ). In the VL muscle, only the difference between WBV + BFR and CON reached statistical significance ( $p < 0.05$ ), whereas WBV + BFR and WBV ( $p=0.21$ ) did not differ significantly.



**Fig. 3** Normalized EMG (%MVC) data of all muscles and conditions. The root mean square (RMS) of the first (onset) and last 5 s (end) of each set was used for analyses. Data are presented as mean  $\pm$  SEM.

<sup>a</sup>Significantly different from CON following rmANOVA and post hoc contrasts ( $N=15$ ), <sup>b</sup>significantly different from WBV following rmANOVA and post hoc contrasts ( $N=15$ )

For the GM ( $p=0.19$ ;  $\eta^2=0.113$ ), TA ( $p=0.21$ ;  $\eta^2=0.107$ ) and RF ( $p=0.13$ ;  $\eta^2=0.134$ ), no significant interaction effect was detected.

### Metabolic parameters

Blood lactate analyses demonstrated a significant main effect of time ( $p<0.05$ ;  $\eta^2=0.771$ ), condition ( $p<0.05$ ;  $\eta^2=0.223$ ) as well as time  $\times$  condition interaction ( $p<0.05$ ;  $\eta^2=0.214$ ) (Table 2). The calculation of post hoc contrasts revealed significant higher increases from pre- to post-exercise in the WBV + BFR group compared to the WBV ( $p<0.05$ ) and CON groups ( $p<0.05$ ).

The analysis of ROS production showed neither a significant main effect of time ( $p=0.051$ ;  $\eta^2=0.192$ ) or condition ( $p=0.57$ ;  $\eta^2=0.039$ ), nor a statistically significant interaction ( $p=0.36$ ;  $\eta^2=0.073$ ).

### Correlations

After the examination of the relationship between EMG amplitude and blood lactate concentrations, we found a significant correlation for the VL ( $p<0.05$ ,  $r=0.71$ ) and VM ( $p<0.05$ ,  $r=0.62$ ) muscle in the WBV + BFR condition (Fig. 4a, b).

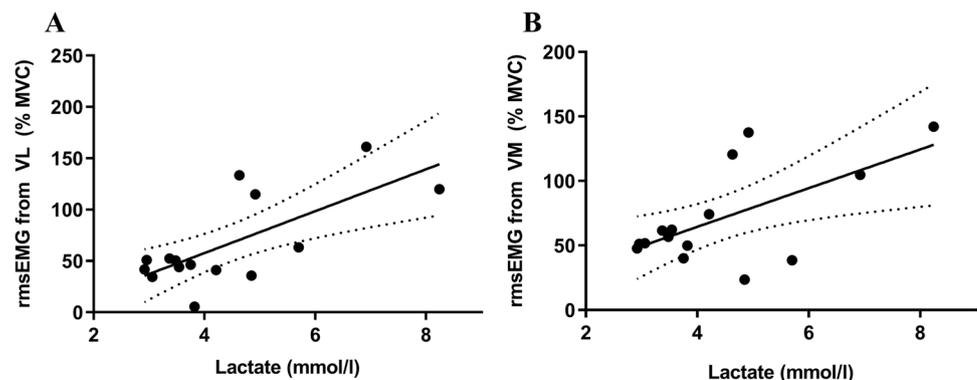
**Table 2** Changes in metabolic parameters during each condition ( $N=15$ )

Variable/time point	CON	WBV	WBV + BFR	rmANOVA ( $3 \times 3$ )
Lactate (mmol/l)				
Pre	1.79 $\pm$ 0.96	1.68 $\pm$ 0.83	1.53 $\pm$ 0.63	
Post	3.05 $\pm$ 1.32	3.18 $\pm$ 1.07	4.42 $\pm$ 1.53	$p<0.05$
15 post	1.90 $\pm$ 0.76	2.02 $\pm$ 0.84	2.75 $\pm$ 1.33	
ROS ( $\mu\text{mol/l/min}$ )				
Pre	0.78 $\pm$ 0.13	0.81 $\pm$ 0.15	0.86 $\pm$ 0.16	
Post	0.76 $\pm$ 0.17	0.85 $\pm$ 0.29	0.89 $\pm$ 0.33	$p=0.36$
15 post	0.75 $\pm$ 0.22	0.79 $\pm$ 0.20	0.73 $\pm$ 0.10	

Values are expressed as mean  $\pm$  SD

CON control group, rmANOVA repeated measures ANOVA, ROS reactive oxygen species, WBV whole-body vibration group, WBV + BFR whole-body vibration and blood flow restriction group

**Fig. 4** Scatter plots with regression line and 95% confidence intervals (dotted line) of lactate concentration and EMG activity during the last set of the VL (a) and VM (b) muscle during in the WBV + BFR condition ( $N=15$ )



### Discussion

The present study permits major insights into adaptations to BFR applied during WBV exercise with an emphasis on the conjunction of metabolic and neural parameters. BFR was effective in increasing myoelectric activity and metabolic demand in several muscles of the lower extremity when superimposed to WBV exercise. These effects became more distinct with prolonged exercise duration and reached up to  $71 \pm 37\%$  of maximal voluntary activation intensities. Thereby, augmented lactate accumulation correlated with the gain in myoelectric activity in the WBV + BFR condition, strengthening the positive relationship between metabolic and neuronal markers.

In general, exposure to WBV has been shown to result in an increased EMG activity by up to 50% of MVC which seems to be dependent on several factors including vibration frequency (Cardinale and Lim 2003; Pollock et al. 2010; Ritzmann et al. 2013), amplitude (Pollock et al. 2010; Ritzmann et al. 2013) or joint angles (Abercromby et al. 2007; Ritzmann et al. 2013). The increase in EMG activity is in line with the findings from the present study demonstrating relative EMG values up to  $45 \pm 20\%$  and in the WBV condition compared to only  $29 \pm 13\%$  in CON.

Previous studies report that the application of a pressurized cuff/tourniquet increases EMG activity compared to the same exercise under free-flow conditions (Cook et al. 2014; Karabulut et al. 2010; Loenneke et al. 2015; Takarada et al. 2000b; Yasuda et al. 2009). Although, to date, the combined effect of whole-body vibration with BFR has not been investigated, Chen et al. (2018) have demonstrated that an exposure to local vibration (35–40 Hz) on the mid-thigh in combination with BFR induced greater increases in myoelectric activity compared to local vibration alone. This is in line with our results suggesting that the relative EMG activity increases up to  $71 \pm 37\%$  of MVC, when the venous return is restricted. Thus, a net increase of  $\sim 26\%$  was observed compared to performing WBV exercise under free-flow conditions. The fact that BFR induces augmented fiber type II recruitment has been considered as an important variable to explain the potent hypertrophic effects of this training regime (Loenneke et al. 2011; Pearson and Hussain 2015). According to the *size principle* (Henneman et al. 1965), there is an intensity-dependent recruitment from slow twitch (type I) to fast twitch (type II) fibers, with the latter being primarily recruited under highest exercise intensities or movement velocities. However, under conditions with BFR, an increased lactate accumulation (Takarada et al. 2000a) and reduced oxygen availability (Moritani et al. 1992) might be responsible for the involvement of higher threshold motor units (MU) even at very low intensities (Moritani et al. 1992; Takarada et al. 2000b; Yasuda et al. 2014).

Interestingly, our results demonstrate that lactate concentration is significantly elevated when exercising with BFR compared to CON or WBV alone. After correlating the EMG parameters with these metabolic variables, a moderate positive relationship was identified for the VL and VM muscles in this condition. The exact underlying mechanism on how metabolic accumulation may influence neural drive remains poorly understood. Previous studies speculate that the metabolic accumulation stimulates group III and IV afferents and thus inhibits the excitability of alpha motoneuron pools leading to enhanced fiber recruitment to maintain force output (Yasuda et al. 2010). Additionally, metabolites including inorganic phosphate and hydrogen ions have been shown to prevent proper cross-bridge cycling and thus inducing fatigue (Debold 2012), which in turn might be compensated with an increased neuromuscular drive. Besides lactate concentration, ROS production was measured by means of electron spin resonance since the acute generation of ROS is believed to be involved in cell signaling and muscle remodeling (Powers et al. 2010, 2011a; Valko et al. 2007). Although no significant time  $\times$  condition interaction was revealed in this study, both exercising groups (WBV + BFR vs. WBV) demonstrated descriptive increases in ROS production leading to a trend towards a significant time effect in the rmANOVA. A study from

Item et al. (2013) combined BFR with high-load resistance training ( $> 70\%$  1RM) and WBV and found an increase in the antioxidant enzyme manganese superoxide dismutase (MnSOD) and xanthine dehydrogenase (XD) compared to resistance training alone (Item et al. 2013). XD is known to be a precursor of xanthine oxidase (Droge 2002), which is an important source of superoxide radicals, especially under conditions of ischemia reperfusion (Droge 2002). The inconsistent results from this study and the study from Item et al. (2013) might be explained by different exercise protocols (weighted vs. un-weighted) and cuff pressure intensities (absolute pressure vs. relative pressure). Evidence on this topic suggests that the amount of generated ROS seems to be intensity dependent, with higher exercise intensities leading to higher ROS formations (Alessio et al. 2000; Finaud et al. 2006). In a recent study from Mrakic-Sposta et al. (2012), ROS generations exceeding 2–2.1  $\mu\text{mol/l/min}$  have been indicated to induce oxidative protein and lipid damage, whereas lower ROS concentrations did not result in macromolecular damage (Mrakic-Sposta et al. 2012). Therefore, we speculate that the moderate ROS production in the present study (0.73–0.89  $\mu\text{mol/l/min}$ ) was not high enough to cause oxidative stress but might instead be involved in cell signaling and muscle remodeling (Powers et al. 2011b; Sauer et al. 2001).

## Practical implications

The outcomes of the present study can be of major relevance for athletic training, fitness and rehabilitative settings aiming to achieve beneficial functional and structural adaptations of the neuromuscular system without excessive loading.

To increase metabolic and hormonal stress, recent investigations have applied pneumatic cuffs around the most proximal portion of the respective limb (Fry et al. 2010; Nielsen et al. 2017; Reeves et al. 2006; Suga et al. 2012; Takarada et al. 2000a). The combination of WBV together with partial vascular occlusion in particular could, therefore, enhance the effects of WBV alone. Both increased metabolic stress and reduced oxygen availability have been identified as important mediators of an increased recruitment of higher threshold MU (Moritani et al. 1992; Suga et al. 2012; Yasuda et al. 2010) related to a hypertrophic effect in long-term training regimens (Aagaard et al. 2001). Our results revealing a significant association between the myoelectric activity and metabolic accumulation may confirm previous findings indicating that physiological factors enhance neural drive. Based on these results, further research is needed to verify these findings for clinical populations and identify potential long-term effects of the combination of these two training regimens. Using merely body weight exercises with no additional load is of special relevance for patients in

rehabilitation or elderly people who are due to comorbidities such as diabetes, coronary heart diseases or joint impairments, not capable of lifting heavy loads. With reference to an increased adaptive response, such a training hybrid could also be highly beneficial for athletes and/or sportive orthopedic patients with a progressed recovery state. As the exercise has been executed in a static squat, high-impact loads and angular excursion can be avoided. This highlights again the relevance of the investigated research question.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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