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Neuromuscular effects of dorsiflexor training with and without blood flow restriction

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ABSTRACT

Blood flow restriction training (BFRT) has been proposed for elderly and clinical populations with weakness. Before being used in these populations it is important to understand the neurological effects of, and subject perceptions to, BFRT. Seventeen healthy subjects were recruited and performed 2 experimental sessions, BFRT and training without blood flow restriction (TR-only), on separate days. Four sets of concentric/eccentric dorsiflexion contractions against theraband resistance were performed. Surface electromyography of the tibialis anterior was recorded during exercise and for the electrophysiological measures. At baseline, immediately-post, 10-min-post and 20-min-post exercise, motor evoked potentials (MEPs) from single pulse transcranial magnetic stimulation (TMS), paired-pulse TMS with interstimulus intervals of 2-ms (SICI) and 15-ms (ICF), and the M-max amplitude were recorded in the resting TA. Following training, subjects provided a numerical rating of the levels of pain, discomfort, fatigue, focus and difficulty during training. Muscle activation was higher in the last 20 contractions during BFRT compared to TR. There was no difference (time × condition interaction) between BFRT and TR for single-pulse MEP, SICI, ICF or M-max amplitude. There was a significant main effect of timepoint for single-pulse MEP and M-max amplitudes with both significantly reduced for 20-min-post exercise. No reductions were observed for SICI and ICF amplitudes. Taken together, BFRT and TR-only were only different during exercise and both regimes induced similar significant reductions in M-Max and MEP-amplitude post-training. Due to the lack of changes in SICI and ICF, it is unlikely that changes occurred in cortical sites related to these pathways. The increased surface electromyography activity in the last 20 contractions, indicate that the training regimes are different and that BFRT possibly induces more fatigue than TR. As such, BFRT could be used as an adjunct to conventional training. However, as subjects perceived BFRT as more painful, difficult and uncomfortable than TR-only, people should be selected carefully to undertake BFRT.

1. Introduction

Low load resistance blood flow restriction training (BFRT) regimes induce muscle fatigue and increase strength more effectively than comparative low-load resistance training in healthy populations [1]. In addition, other neuromuscular effects such as increased corticospinal excitability have been reported after BFRT [2, 3, 4] and may therefore be beneficial in increasing muscle activation to weak muscles in people with weakness due to brain injury and/or other neurological diseases.

Karabulut et al. [4], using twitch interpolation, investigated central fatigue during maximal voluntary contractions of the vastus lateralis before and after BFRT and compared this to training without BFR (TR) with matched load and repetitions. The maximum voluntary contraction reduced in both conditions, reduced more in BFRT however the interpolated twitch force only reduced after BFRT. The interpretation was that BFRT caused more fatigue and the location of the fatigue was centrally *and* peripherally mediated for BFRT while only peripherally mediated for TR.

Other studies, using transcranial magnetic stimulation (TMS), have shown an increase in corticospinal excitability following BFRT compared to TR [2, 3]. Although these studies investigated the biceps brachii [2] and tibialis anterior (TA) [3] using different exercise regimes, both studies reported higher corticospinal excitability in the short term (1 min after BFRT [3] or long term (up to 60 min after BFRT [2]) following BFRT

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compared to TR. The above studies indicate that adaptive changes to the corticospinal system can be different between BFRT and TR.

Although there are changes to the corticospinal system following BFRT compared to matched training, the location of changes within the corticospinal system have rarely been investigated. Short interval intracortical inhibition (SICI) was assessed following low load continuous BFRT, matched-repetition low load intermittent BFRT, matched-repetition low load training without BFRT and a high load training in the biceps brachii [2]. There was no difference in SICI between training regimes which indicates that the pathways and transmitter systems implicated in SICI, likely GABA mediated disinhibition [5, 6, 7, 8, 9], was not different between training paradigms. It is possible that changes occurred in multiple areas of the corticospinal system which when combined demonstrate a significant increase in corticospinal excitability but when assessed individually show no effect. Alternatively, changes to SICI may have occurred but were non-significant due to underpowered experimental designs or masked due to SICI measurements with an active contraction which can alter estimates [6]. In addition, the study was performed in a proximal upper limb muscle. As electrophysiological estimates could differ between proximal upper limb muscles and distal lower limb muscles [10, 11, 12], this finding may not be indicative of all limb muscles.

The purpose of the current study was to investigate neuromuscular activity during low load dorsiflexion training of the TA, using theraband training, with and without BFR. In addition, we would like to assess subject perceptions of pain, discomfort, fatigue, focus and difficulty during training. To elucidate on some of the possible neurological mechanisms following BFRT and matched TR we will measure, in the non-contracted TA muscle, corticospinal excitability using single pulse TMS, short interval intracortical inhibition (SICI) and intracortical facilitation (ICF) using paired pulse TMS. Likewise, peripheral nerve excitability will be measured using supramaximal electrical stimulation to the common peripheral nerve to measure maximal M-waves (M-max) of the TA. This is the first study to measure SICI and ICF in the TA following BFRT.

2. Methods

2.1. Subjects

Seventeen subjects participated in the study (8 males, 9 females; age: 35 ± 10 years (mean ± SD); height: 1.75 ± .08 m; weight: 73 ± 17 kg; systolic blood pressure: 117 ± 8 mmHg; diastolic blood pressure: 68 ± 8 mmHg). Fifteen subjects were right leg dominant. Training was performed on the dominant leg. Sixteen subjects were tested on two sessions spaced 7 ± 4 days apart and one subject was tested on one session (and withdrew from the experiment before the second session). The number of subjects were chosen based on a power analysis performed previously [3] which reported that ≥15 subjects were required for a power of ≥80%. The study conformed to the Declaration of Helsinki and was approved by the local scientific ethics committee (approval number: 1-16-02-520-14). All subjects provided written informed consent.

2.2. Experimental procedures

Subjects were randomly allocated to perform TR or BFRT on the first day. The remaining condition was performed on the second day. Prior to the experiment, brachial blood pressure was measured in lying using a sphygmomanometer (Riester®, 55 cm × 14.5 cm). Brachial blood pressure was measured on both testing sessions for each subject. Surface EMG (sEMG) was recorded using electrodes (Ambu Neuroline) placed over the trained TA in accordance with Cram et al. [13]. EMG data were amplified and filtered (bandwidth: 10 Hz–1000Hz) using custom made amplifiers and collected using Mr Kick II software. This was recorded during the training protocols and for baseline and post-training measurements.

2.2.1. TMS measurements

TMS was performed using a 110-mm double cone coil (Magstim 200). Current was applied in the posterior to anterior direction. Stimuli were applied at ~50% of the maximal stimulator output while the coil was moved grossly around the approximate hotspot location. Once the approximate hotspot had been located, the coil was moved systematically and stimuli were delivered every 5–7 seconds for ~4 stimuli per location. The hotspot was established as the coil location that produced the largest peak-to-peak motor evoked potential (MEP) amplitude and was used for all subsequent TMS measurements. The resting motor threshold was determined by delivering stimuli every 5–7 seconds, from 30% of the maximum stimulator output, in increments of 5% of the maximum stimulator output, until the peak-to-peak MEP amplitude for 5/10 MEPs were >50 μV. The corresponding maximum stimulator output percentage was deemed the resting motor threshold (rMT). This process was performed independently on each day.

Single pulse stimulation was delivered at 120% of the rMT. Paired pulse stimulation was delivered with the first pulse at 80% rMT and the second pulse at 120% rMT. For paired pulse stimulation, the pulses were spaced with an interstimulus interval of 2 ms (SICI) and 15 ms (ICF, [14]. At each measurement timepoint, single pulse, paired pulse (SICI) and paired pulse (ICF) stimulus types were delivered randomly every 5–7 seconds for 12 trials per stimulus type. Measurements were collected in lying, with a small wedge placed under the knees and the head tilted up resting on a pillow. TMS measurements were collected at baseline, immediately-post, 10-min-post and 20-min-post training.

2.2.2. Supramaximal peripheral electrical stimulation

Electrical stimulation of the common peroneal nerve was applied using 100 μs single rectangular pulses at the fibula head. The optimal location of M-max stimulation was determined as the stimulus intensity that produced the largest M-wave with the smallest possible stimulus artefact. This location was marked and used as the location for the remainder of the experiment. The M-max was determined by increasing the stimulus intensity by 5 mA until the M-wave peak-to-peak amplitude ceased to increase. Following this, the M-max was increased a further 10 mA and decreased until the M-wave began decreasing. The preceding stimulus intensity was deemed the M-max. For testing, 1.5 × the stimulus intensity used to elicit M-max was used. The supramaximal stimuli were delivered every 2–2.5 seconds for 10 stimuli per timepoint. M-max measurements were collected immediately after the TMS measurements at baseline, immediately-post, 10-min-post and 20-min-post training.

2.2.3. Training set up and protocol

For training, subjects were seated comfortably with their foot resting on a board with an average resting ankle angle of 130 ± 7° (mean ± SD). The maximum comfortable dorsiflexion range for the subject was established and a stop was placed so that subjects were unable to dorsiflex past that point. For each contraction, subjects were required to dorsiflex the foot to the stop. Following this, the foot was placed under a blue theraband which was affixed to the board and provides resistance as described by Page et al. [15]. The subjects practised dorsiflexing the foot, with the resistance of the theraband, to the stop, approx. 4–5 times using the training paradigm. Once subjects were comfortable with the paradigm, the knee and ankle angle were measured to facilitate testing in the same position between days.

During BFRT, a cuff (Reister®, either 70 × 22 cm (for thigh diameters <50cm) or 100 × 26 cm (for thigh diameters >50 cm)) was placed around the thigh of the trained leg and inflated to 1× systolic blood pressure. The cuff was inflated over a period of 1-min. Training commenced when the blood pressure cuff was inflated and had stabilized at the testing pressure.

Subjects performed four sets with 30 repetitions for the first set and 15 repetitions for the three remaining sets with 30 seconds between sets. This training paradigm has been used previously during blood flow restriction for other muscle groups by other laboratories [2, 16, 17].

Subjects concentrically contracted the TA for 1 s, held the foot at the stop for 3 s and lowered the foot over 0.5 seconds so that the sole of the foot touched the board. In the final set, some subjects were unable to touch the foot stop for the last repetitions. If this occurred, subjects were asked to dorsiflex the foot as much as able.

Following training and all post measurements, subjects were asked to provide a numeric rating of the training regime indicating levels of pain, discomfort, fatigue, focus and difficulty. For this '0' indicated 'no pain/discomfort/fatigue/focus/difficulty and '10' indicated 'the highest imaginable level of pain/discomfort/fatigue/focus/difficulty'.

2.3. Data analysis

During training, sEMG measurements were collected and the amplitude of the contraction was determined for each contraction. To facilitate the determination of onset for each contraction, data were smoothed using a 1st order, 1 Hz low pass butterworth filter. Contraction onset was defined as when the sEMG exceeded 50 μV for greater than 10 ms. Once contraction onset had been established, the unsmoothed data were smoothed using a 1st order 20 Hz low pass Butterworth filter. The root mean square (RMS) of the sEMG amplitude was measured in the first 3 seconds of each training contraction. The sEMG amplitudes of the 75 contractions were averaged in blocks of 5 contractions for a total of 15 blocks per subject per training condition.

Data were rectified before analysis. For MEPs, the sEMG amplitude (μV.ms) of individual trials was defined as the area under the curve, 25–70 ms following the stimulus. The M-max sEMG amplitude was defined as the area under the curve, 3–28 ms following the stimulus. These time windows were chosen as they encompassed the response for all subjects.

2.4. Statistical analysis

TMS and M-max sEMG amplitudes trials were assessed for normality as previous studies have reported that the sEMG amplitude of these trials could be log-normally distributed [18, 19]. To do this, for each timepoint, for each condition and for each subject z-scores were calculated. These z-scores were combined for TMS trials, and M-max trials. MEP sEMG amplitudes were consistent with log-normal distribution and M-max trials were consistent with normal distribution. Given the distribution, single pulse, SICI and ICF MEP sEMG amplitudes were log transformed and averaged for each timepoint, for each condition for each subject. These averaged data were then back transformed (exponentiated).

For the single pulse MEP sEMG amplitude, the exponentiated data were expressed as a proportion of the average M-max sEMG amplitude for the corresponding timepoint. For SICI and ICF MEP sEMG amplitude, the exponentiated data for the SICI and ICF sEMG amplitude were expressed as a proportion of the exponentiated single pulse MEP sEMG amplitude for the corresponding timepoint. As these proportions are also consistent with log-normal distribution [18, 19], these were further log-transformed for statistical analysis. Following this, linear mixed models were performed on the 1) single pulse, 2) SICI and 3) ICF proportions with subject and subject × condition (BFR, TR) as random factors and timepoint (baseline, immediately-post, 10-min-post and 20-min-post), condition and timepoint × condition as fixed factors. When appropriate, the estimates and 95% confidence intervals of the linear mixed models were exponentiated and reported. The above analysis dealing with log-normally distributed response data has been proven previously [18, 19].

As the sEMG amplitude of M-max trials were consistent with normal distribution, analysis of M-max sEMG amplitudes were averaged for each timepoint, for each condition, for each subject. Log transformation did not occur as the trials were normally distributed. A linear mixed model was performed with subject and subject × condition as random factors and timepoint, condition and timepoint × condition as fixed factors. Data were expressed as mean differences and 95% confidence intervals of the mean difference.

Baseline TMS rMT, stimulation intensity to elicit M-max, M-max amplitude, t × 120, SICI and ICF MEP sEMG amplitudes were compared using paired t-tests.

For the sEMG amplitude during training, linear mixed models were performed with subject and subject × condition (BFRT and TR) as random factors and contraction-block, condition and contraction-block × condition as fixed factors.

Numeric rating scores were compared between BFRT and TR conditions for subjects tested on both days using paired t-tests.

3. Results

3.1. Baseline variables

There was no difference in TMS resting motor threshold (as a percentage of maximum stimulator output) ($P > 0.99$; BFR: 52 ± 9%, TR: 52 ± 10%; mean difference: 0%, $CI_{95\%}$: -1 to 1%) or stimulus intensity to elicit M-max ($P = 0.570$; BFR: 38 ± 14 mA, TR: 36 ± 10 mA; mean difference: 2 mA, $CI_{95\%}$: -4 to 7 mA) between conditions. There was no difference in baseline t × 120 MEP sEMG amplitude ($P = 0.872$; BFR/TR estimate: 1.02, $CI_{95\%}$: 0.75 to 1.40), SICI MEP sEMG amplitude ($P = 0.192$; BFR/TR estimate: 1.28, $CI_{95\%}$: 0.87 to 1.87) or ICF MEP sEMG amplitude ($P = 0.182$; BFR/TR estimate: 1.19, $CI_{95\%}$: 0.91 to 1.57). There was a significant difference in baseline M-max sEMG amplitude between conditions ($P = 0.044$; BFR: 12085 ± 3109 μV ms, TR: 14100 ± 3992 μV ms; mean difference: -2014 μV ms, $CI_{95\%}$: -3966 to -62 μV ms).

3.2. Muscle activity during training

For one subject the sEMG during training (on the TR day) was removed due to a faulty connection rendering the data unusable (which was repaired on completion of the training). Data for this subject during training on the TR day were treated as missing values. Fig. 1 shows example sEMG data for one subject during BFRT and TR. For the sEMG amplitude during testing, there was a significant interaction effect between condition × contraction-block ($P = 0.009$). As training progressed the sEMG amplitude was significantly higher for the last 4 contraction-blocks (consisting of the last 20 contractions) in the BFRT compared to the TR condition (Fig. 2).

3.3. TMS measurements – single pulse

Fig. 3 shows example and summary data for single pulse stimulation. For MEP sEMG amplitude, there was no significant interaction effect for condition × timepoint ($P = 0.234$) or main effect of condition ($P = 0.220$, Fig. 4). There was a significant main effect of timepoint ($P < 0.001$) with MEP sEMG amplitudes significantly lower immediately-post, 10 min-post and 20 min-post training compared to baseline (Fig. 4).

3.4. TMS measurements – paired pulse

Fig. 5 shows example and summary data for SICI and ICF. For SICI there was no significant interaction effect for condition × timepoint ($P = 0.704$) or main effect of condition ($P = 0.269$) or timepoint ($P = 0.804$, Fig. 6). It should be noted that for some subjects at some timepoints and conditions the conditioning pulse conditioned the test pulse sufficiently that the test pulse elicited no visible response. In such cases, the area was still taken between the two timepoints and included in the sEMG amplitude calculation. For ICF, there was no significant interaction effect for condition × timepoint ($P = 0.213$) or main effect of condition ($P = 0.189$). There was a significant main effect of timepoint ($P = 0.025$) meaning ICF changed significantly over timepoints (Fig. 6). ICF sEMG amplitudes were significantly higher immediately post-training than baseline but were not different 10 min-post or 20 min-post training

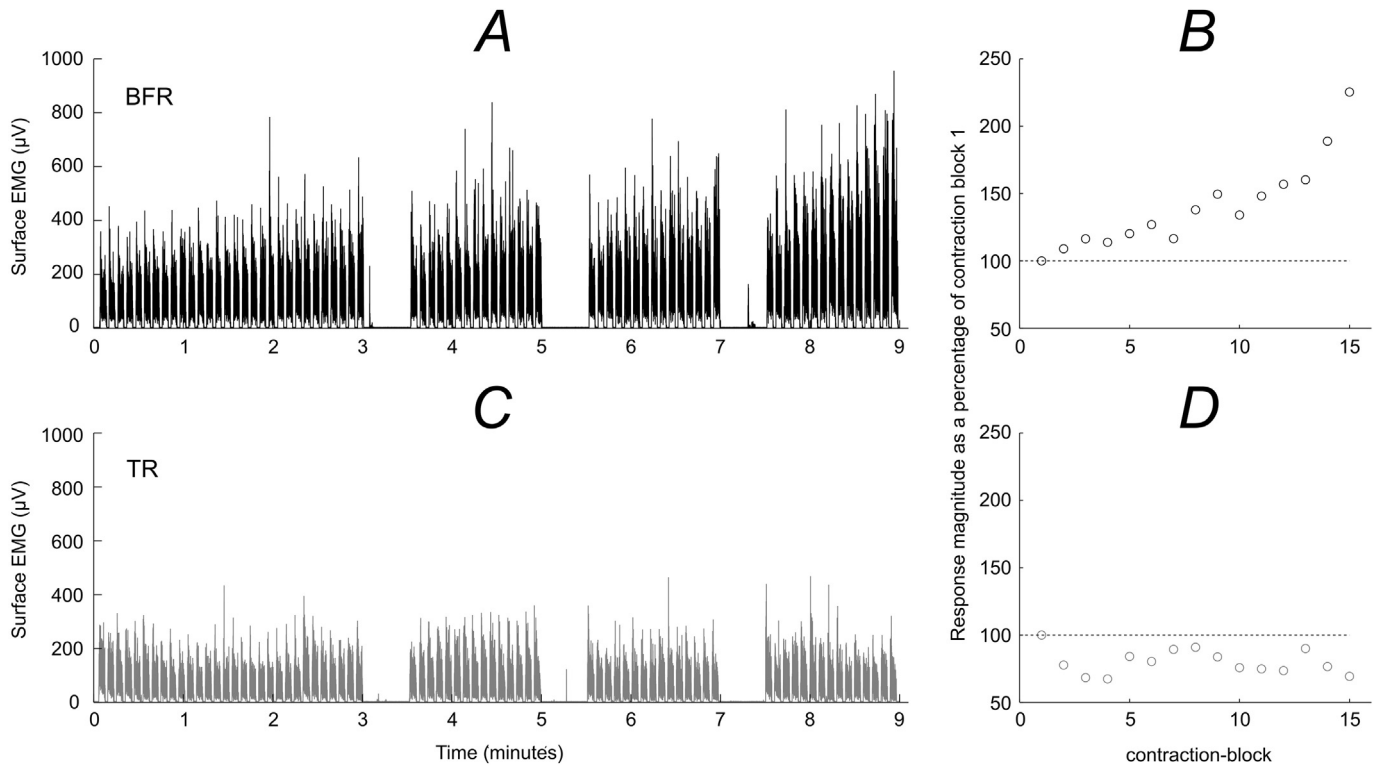

Fig. 1. *A and C.* Example traces of the surface EMG of the 75 contractions during BFR (*A*) and TR (*C*). Traces were rectified and smoothed with a 20 Hz low pass Butterworth filter. *B and D.* Summary of the data shown in figures *A* and *C* separated into the 15 contraction-blocks (an average of 5 contractions per block) for the BFR and TR condition, respectively. The response magnitude for each contraction-block are reported as a percentage of contraction-block 1.

compared to baseline (Fig. 6).

3.5. Supramaximal peripheral electrical stimulation

There was no significant interaction effect for condition × timepoint ($P = 0.822$). There was a significant main effect of timepoint ($P < 0.001$) meaning that M-max sEMG amplitude was significantly reduced immediately-post, 10-min post and 20-min post training compared to baseline (Fig. 4). There was a significant main effect of condition ($P = 0.034$) and M-max sEMG amplitude was significantly higher for the TR than BFRT condition (Fig. 4). This difference was likely due to the difference in baseline levels and M-max sEMG amplitudes relative to these baseline levels. As subject × condition was treated as a random factor, the model allowed each subject to have their own baseline level for each condition.

3.6. Numerical rating scales

Fig. 7 shows the numerical rating scores for all subjects and each condition. Subjects perceived that BFRT was more difficult, induced more pain, discomfort and fatigue than the TR protocol. Subjects were more focussed while performing BFRT compared to TR.

4. Discussion

Dorsiflexion resistance training of the TA with a theraband has not previously been investigated for BFRT *and* TR. The current study showed that the sEMG amplitude during BFRT was higher than during TR for the last 4 contraction-blocks (last 20 contractions) of the training regime. Although there was no difference between BFRT and TR in post-training corticospinal or peripheral nerve elicited sEMG amplitude, both conditions showed a long-term reduction in MEP sEMG amplitude and M-max sEMG amplitude. There were no differences in SICI and ICF between conditions suggesting that these pathways are not differently altered during BFRT.

4.1. Muscle activity during training

The sEMG amplitude increased during BFRT compared to TR. Similar findings have been observed in other muscles using low load BFRT with repetition and load matched TR [20, 21, 22, 23, 24]. Increased voluntary muscle activation is commonly observed in moderately fatigued muscles [25, 26, 27, 28]. The increased sEMG amplitude could indicate that BFRT is more effective at fatiguing the muscle than matched TR. Corroborating this finding, subjects also reported that BFRT was more fatiguing and difficult than TR. As training progresses, it is possible that contractions with BFRT required a greater amount of voluntary activation, as indicated by greater sEMG amplitude, for the same contraction force than TR, as has been observed elsewhere [28]. Our findings are also consistent with load-matched exercise paradigms to volitional failure that demonstrate that BFRT fatigues muscles more effectively than TR [29, 30, 31].

Another possibility is that the increase in sEMG amplitude was due to deafferentation. There was no difference between BFRT and TR protocols in post-training TMS measures which were administered after cuff removal. With high pressures, deafferentation results in higher sEMG amplitude compared to unrestricted muscles. For example, removing input from the III and IV afferents during fatiguing contractions of the knee extensors, higher sEMG amplitude in the vastus lateralis compared to the unrestricted muscle has been observed [32]. Although lower pressures were used in the current study, the deafferentation would have occurred more gradually and hence, the deafferentation increase in sEMG amplitude would have been more prominent towards the end of the protocol. One problem with this explanation, is that extremely high pressures are often used in the deafferentation paradigms. Using lower pressures, would not selectively block the group III and IV afferents. In addition, the de-recruitment of afferents during our blood flow restriction protocol would be related to afferent size, with larger afferents being derecruited first. As it takes approximately 20 min to begin to de-recruit

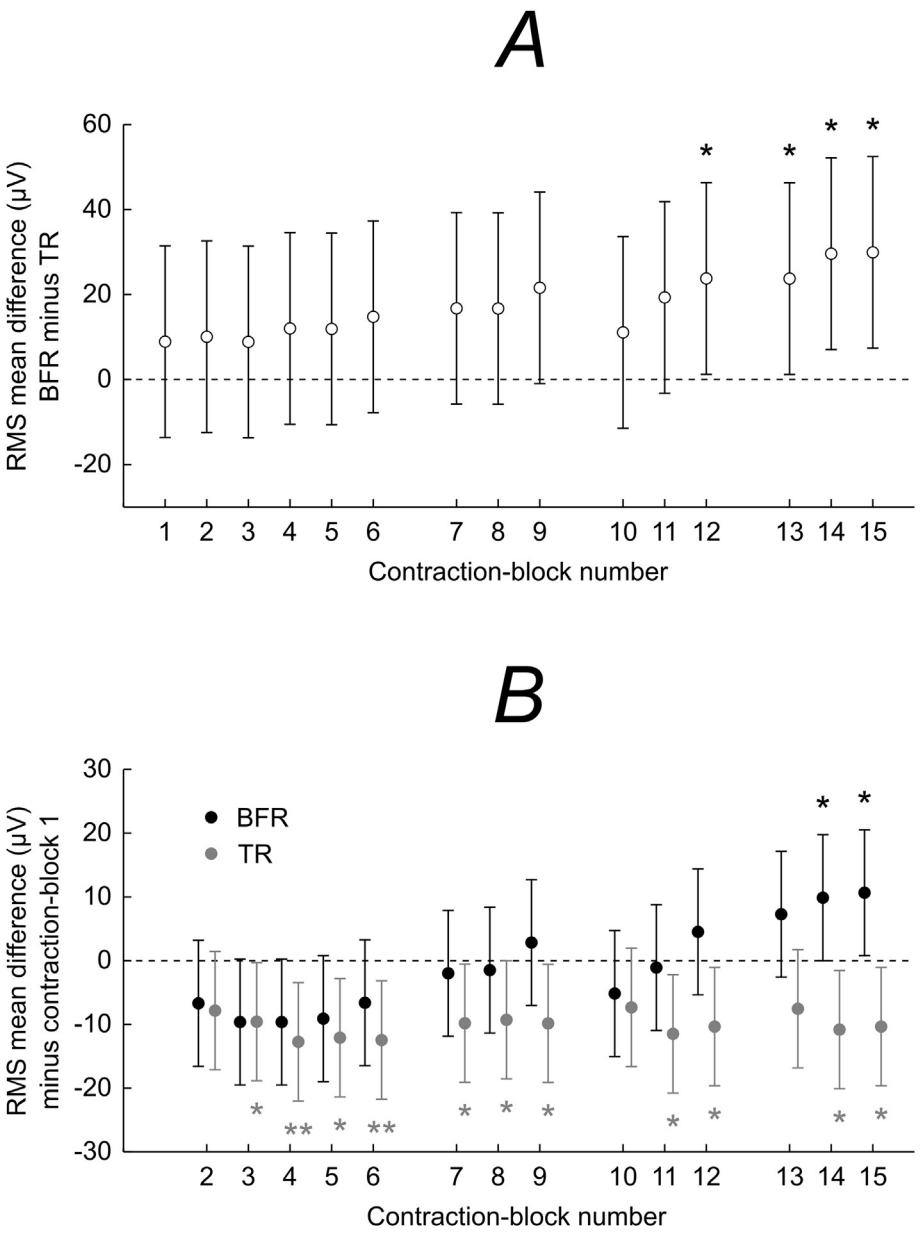

Fig. 2. *A and B.* Post hoc contrasts performed due to a significant condition × contraction-block interaction. *A.* Modelled mean differences and 95% confidence intervals of the RMS for the BFR minus TR condition for each contraction-block. '*' indicates significant differences between BFR and TR to $P < 0.05$. *B.* Modelled mean differences and 95% confidence intervals compared to baseline values for the contraction-block RMS amplitude for the BFR condition (black filled circles and lines) and TR condition (grey filled circles and lines). '*' and '**' represent significant differences to the first contraction-block for the BFR condition (black stars) and TR condition (grey stars) to $P < 0.05$ and $P < 0.01$, respectively.

group I afferents at 200 mmHg [33], it is unlikely that the smaller diameter afferents would have been derecruited with the pressures, and within the timeframes of the current study.

Despite the above explanations, the differences in sEMG amplitude between BFRT and TR were not reflected post-exercise and indicates that any peripheral differences that occurred between training paradigms were not maintained after training. Therefore, training using this paradigm may have been large enough to result in changes during exercise, but the training stimulus was not large enough to result in post-exercise differences. Another explanation is that removal of the cuff restored afferent feedback which eliminated post-exercise sEMG amplitude differences. Considering this, using protocols similar to those in the present study, we may need to consider the differences between BFRT and non-BFRT training regimes and determine if these differences are large enough to warrant using BFRT.

4.2. TMS measurements

The reduction in MEP sEMG amplitude in both training paradigms potentially indicates that the current training paradigms are effective for fatiguing the TA. Following fatiguing isometric exercise in the TA, single pulse MEP sEMG amplitudes were significantly depressed for 20 minutes [34]. In other muscle groups, reduction in MEP amplitudes following fatiguing training has also been observed [35] and can last for up to 20–30 minutes [36, 37, 38, 39, 40]. Given that both regimes were effective in reducing the TA MEP sEMG amplitudes (which could indicate fatigue), this type of theraband training, over multiple sessions, could be used to increase strength of the TA in healthy and clinical populations.

The post exercise SICI and ICF were not different between BFRT and TR. The one previous study that compared SICI sEMG amplitude between BFRT and TR (in the biceps brachii) also showed no differences in SICI [2] between interventions however this study recorded SICI sEMG amplitude in the active muscle (which can alter estimates [6]). It was also performed on a proximal upper limb muscle which could differ between distal lower limb muscles [10, 11, 12]. It has been suggested that SICI may measure inhibitory activity in a combination of neural receptors and cortical neurotransmitters (GABA mediated disinhibition [5, 6, 7, 8, 9]). As SICI sEMG amplitude is not altered post training and not different

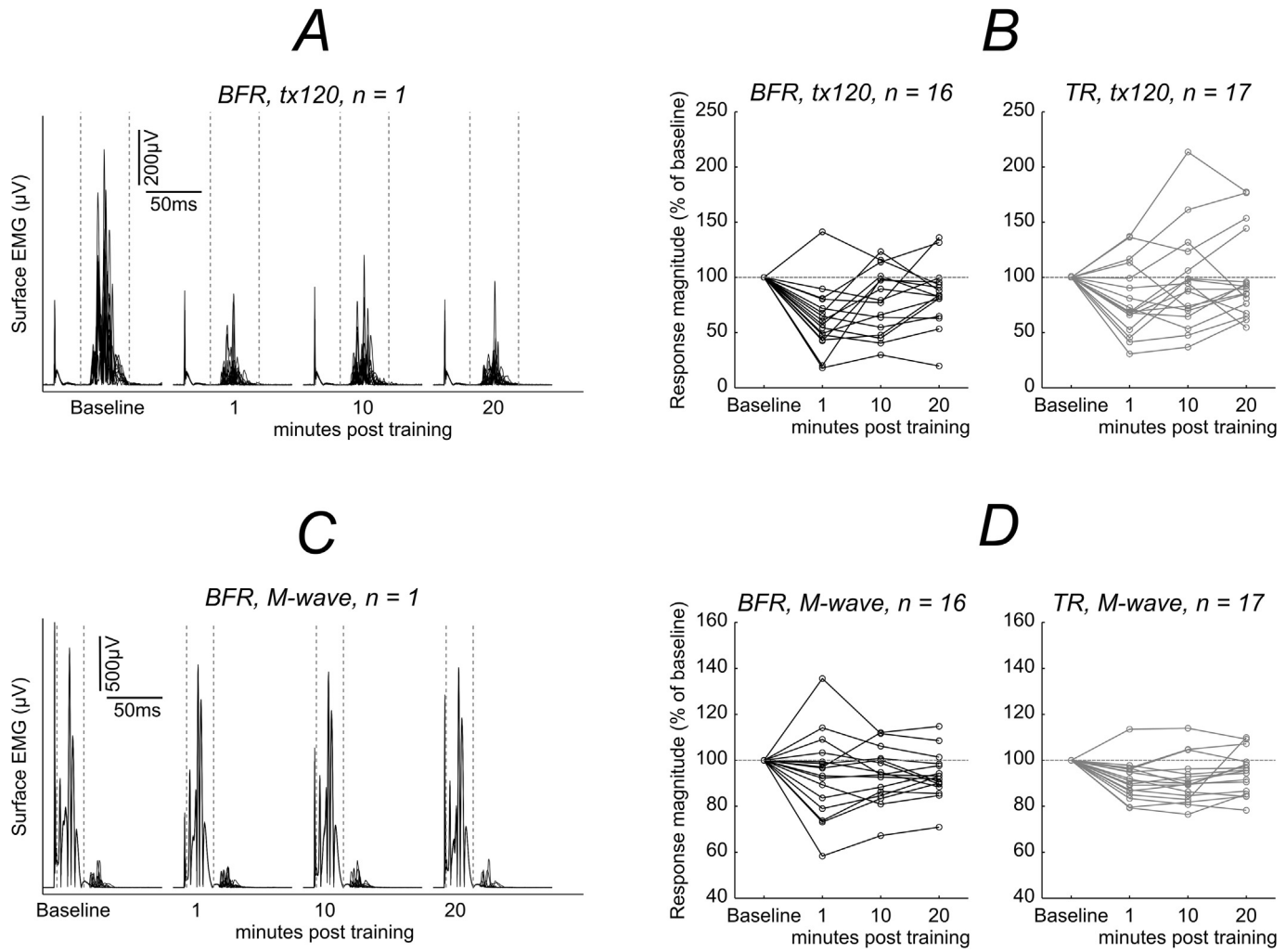

Fig. 3. *A and B.* Example rectified traces of the surface EMG of 12 MEPs from single pulse TMS stimulated at t×120 (*A*) and 10 M-max traces (*C*) for the BFR condition for one subject. Data are shown at baseline, 1-min, 10-min and 20-min post training. The vertical dotted lines indicate the analysis window for each timepoint. *B.* MEP surface amplitude (normalized to M-max) for the t×120 stimulation intensity for each timepoint as a percentage of baseline for the BFR condition (black lines) and TR condition (grey lines) for all subjects. The data summarizes the response magnitude (as a percentage of baseline) for each subject prior to the final log transformation used in the linear mixed models. *D.* M-max magnitude for each timepoint as a percentage of baseline for the BFR condition (black lines) and TR condition (grey lines) for all subjects.

between training protocols it shows that this pathway is unlikely affected due to TA training with or without BFR.

The mechanisms involved in ICF are not completely understood [41, 42], however it has been proposed that it could be due to the activation of glutaminergic mediated cortico-cortical pathways [43]. Similarly to SICI, this was also not different between BFRT and TR. However, both BFRT and TR had a slight but significant increase in ICF after training. This indicates that the training itself, induced changes in ICF or altered the relationship between the ICF mediated MEP sEMG amplitude and the single pulse MEP sEMG amplitude which the ICF MEP sEMG amplitude was expressed as a proportion of. These sEMG amplitude changes were transient and returned to baseline levels 10 minutes after the intervention.

Taken together, the neurological processes within the cortex that contribute to SICI and ICF, with the training paradigm used, are not different between BFRT and TR, as measured by sEMG amplitude. It was hypothesized that these could be altered as following prolonged BFRT with high pressure to motor block there is a reduction in GABA neurotransmitter concentrations [44] in the motor areas of the deafferented areas and a reduction in SICI in the biceps brachii, a muscle immediately proximal to the nerve block [45]. However, in the current study the restriction pressure was presumably too low and for too short a time for any potential effect. Although training itself has the potential to reduce SICI [46, 47] and GABA [48] this is not seen universally [49] and was not seen in the current study.

4.3. Location of training induced changes

As there were no changes in SICI sEMG amplitude, a small transient increase in ICF sEMG amplitude until 10 min after training and long term significant reductions in the single pulse MEP (relative to M-max) and M-max sEMG amplitudes, it could suggest that post-exercise effects do not occur at the cortical level and that these changes occur, in part, spinally *and*, in part, peripherally. The hypothesis of spinally mediated fatigue has been inferred by a process of elimination. Findings from other laboratories indicate that following fatiguing training regimes of the TA, electrically evoked potentials applied at the thoracic spine (TMEPs), a stimulus that bypasses the cortex, caused a reduced TMEP sEMG amplitude in the relaxed TA for at least 10 minutes [50]. As the M-max sEMG amplitude remained unchanged in that study, it is likely that the sole change in TMEP sEMG amplitude due to the fatiguing contraction occurred at the spinal level. Another study demonstrated short term reduction in F-wave sEMG amplitude to the relaxed TA following fatiguing training regimes indicating reduced excitability at the spinal

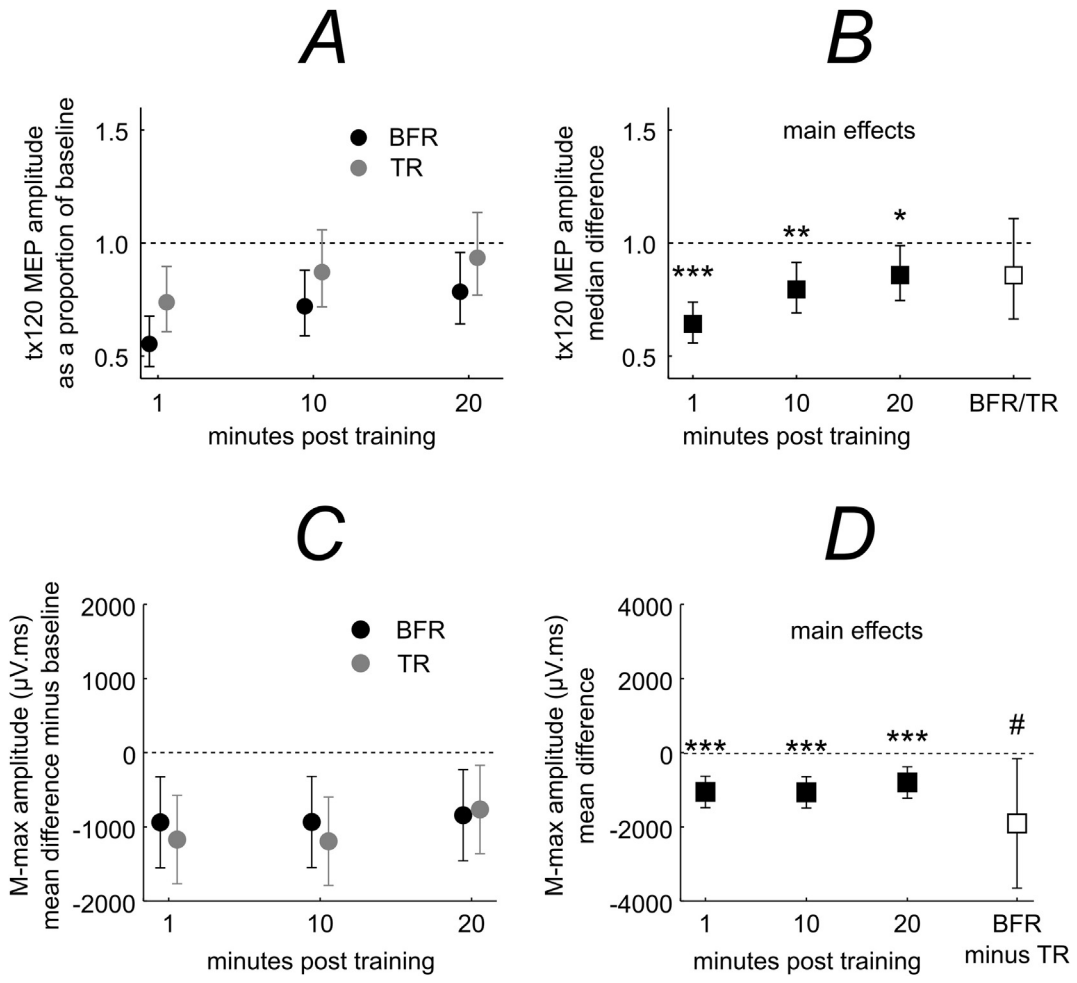

Fig. 4. *A and B.* Modelled data for the linear mixed model used to assess MEP surface EMG amplitude (normalized to M-max). The horizontal dotted lines indicate no difference between the compared estimates. *A.* Modelled median differences and 95% confidence intervals compared to baseline values for the MEP amplitude for the BFR condition (black filled circles and lines) and TR condition (grey filled circles and lines). *B.* Modelled median differences and 95% confidence intervals for the main effect of timepoint as a proportion of baseline values (black filled squares) and condition with TR as a proportion of BFR (white filled square). *C and D.* Modelled data for the linear mixed model used to assess M-max amplitude. The horizontal dotted lines indicate no difference between the compared estimates. *C.* Modelled mean differences and 95% confidence intervals compared to baseline values for the M-max amplitude for the BFR condition (black filled circles and lines) and TR condition (grey filled circles and lines). *D.* Modelled mean differences and 95% confidence intervals for the main effect of timepoint minus baseline values (black filled squares) and condition with BFR minus TR (white filled square). '***', '**' and '*' indicates significant differences to baseline estimates to $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively. '#' indicates a significant difference between BFR and TR estimates to $P < 0.05$.

level [51]. In light of these results, the reduction in M-max sEMG amplitude may indicate peripheral (muscle) changes and the reduction in MEP sEMG amplitude may indicate spinal changes (although not directly tested in the current paradigm).

4.4. Subject perceptions of blood flow restriction training

Subjects perceived BFRT as more painful, more difficult and more uncomfortable than TR. This must be considered when using BFRT as a training method in healthy and clinical populations. If people perceive training as painful, difficult and uncomfortable, despite its potential benefits, exercise compliance might be reduced. In populations where exercise adherence is necessary to attain functional goals (such as patients with sarcopenia, patients post-surgery or patients following acquired brain injury), the benefits of BFRT must be weighed with any potential non-compliance issues. As such, people should be selected carefully for BFRT, with BFRT being used on a case-by-case basis.

4.5. Limitations

There are some limitations to our study. We took the blood pressure at the arm, and used this pressure as the occlusion pressure for the leg. In healthy subjects, blood pressure in the arm provides a good estimate of systolic, diastolic and mean arterial pressure in the leg [52, 53]. Blood pressure taken at the arm and thigh are highly correlated [52, 53]. In addition, brachial blood pressure is easier to perform/measure. Despite this, some studies have suggested that the blood pressure should be taken at the occluded limb [54]. However, given the high correlation between brachial and popliteal blood pressure measurements, we believe brachial blood pressure is an appropriate indication of popliteal blood pressure [52, 53].

The variable that the study was powered for was the MEP sEMG amplitude at t × 120 which was based on interaction effect over four time points (1 min, 10 min, 20 min and 30 min post-training) and three training conditions (BFRT with high pressure, blood flow restriction without training and training without BFR). Although this is different to our current protocol, we felt it was the best power calculation that we had from which to base our subject numbers. Despite this, we cannot rule out that the non-significant interaction effects weren't due to an underpowered experimental design as 1) the conditions are slightly different to those in which the power calculation was performed and 2) The variable used in the power calculation (t × 120) is different for the M-max, ICI and

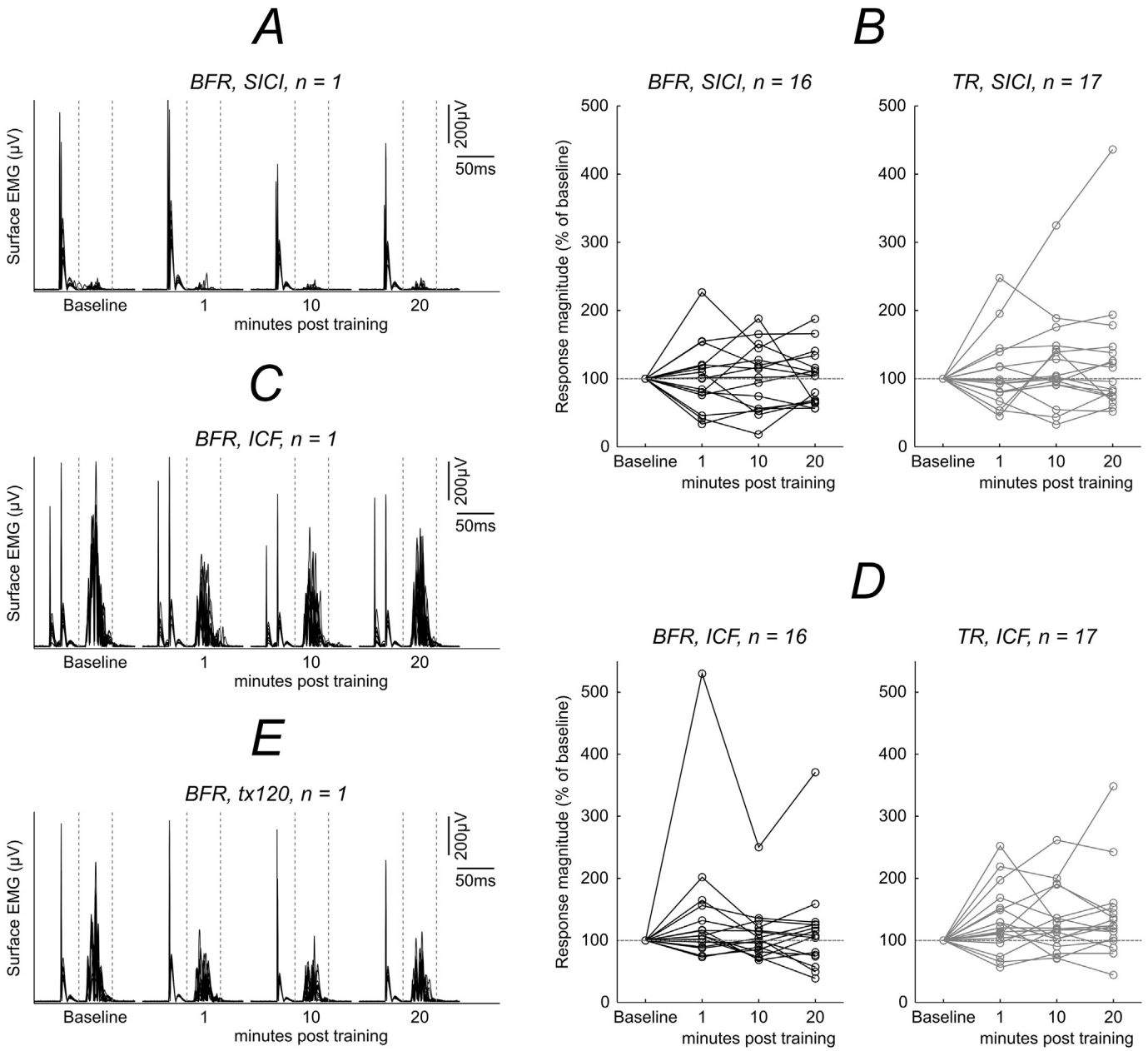

Fig. 5. *A and C.* Example rectified traces of the surface EMG of the 12 MEPs from paired pulse TMS stimulated with the first pulse at t × 80% and the second pulse at t × 120% for the BFR condition for one subject. The interstimulus interval was 2 ms (SICI, *A*) and 15 ms (ICF, *C*). Data are shown at baseline and 1-min, 10 min and 20 min post training. For comparison, the 12 single pulse MEPs stimulated at t × 120% are shown *(E)*. The vertical dotted lines indicate the analysis windows for each timepoint. *B and D.* SICI (*B*) and ICF (*D*) response magnitudes (normalized to the single pulse MEP magnitude stimulated at t × 120%) for each timepoint as a percentage of baseline for the BFR condition (black lines) and TR condition (grey lines) for all subjects. The data summarizes the response magnitude (as a percentage of baseline) for each subject prior to the final log transformation used in the linear mixed models.

ICF variables.

Lastly, it is important to note that the current study measure sEMG amplitude and that mechanisms can only be inferred from this. As highlighted by Vigotsky et al. [55], the sEMG amplitude is the net effect of changes in neural excitation/inhibition, activation (excitation-contraction) dynamics, muscle activation and muscle contraction dynamics. We managed to control for some of these factors by the non-changing of sEMG electrodes within the session and treating subject and subject × condition (BFR, TR) as random factors in the statistical analysis. Despite this, our interpretation is still based on a net effect, that could be altered at a number of levels. For example, in using sEMG amplitude we are not assessing firing rates of individual motor units and cannot measure the extent of overlapping motor unit firing. This may limit the generalisability of the findings in the current study.

4.6. Implications and conclusions

The training regimes used in the current study were effective in inducing post-exercise electrophysiological changes, as indicated by sEMG amplitude, regardless of the training regime. Therefore, if coaches or clinicians would like to train the TA specifically, with potential progression, the training device and training set up (in terms of sets and repetitions) could be used. Over time and with appropriate progression, this could result in training mediated adaptations. If the increase in sEMG amplitude during training was due to peripheral fatigue and not due to deafferentation, adding BFR allows subjects to train at a higher intensity

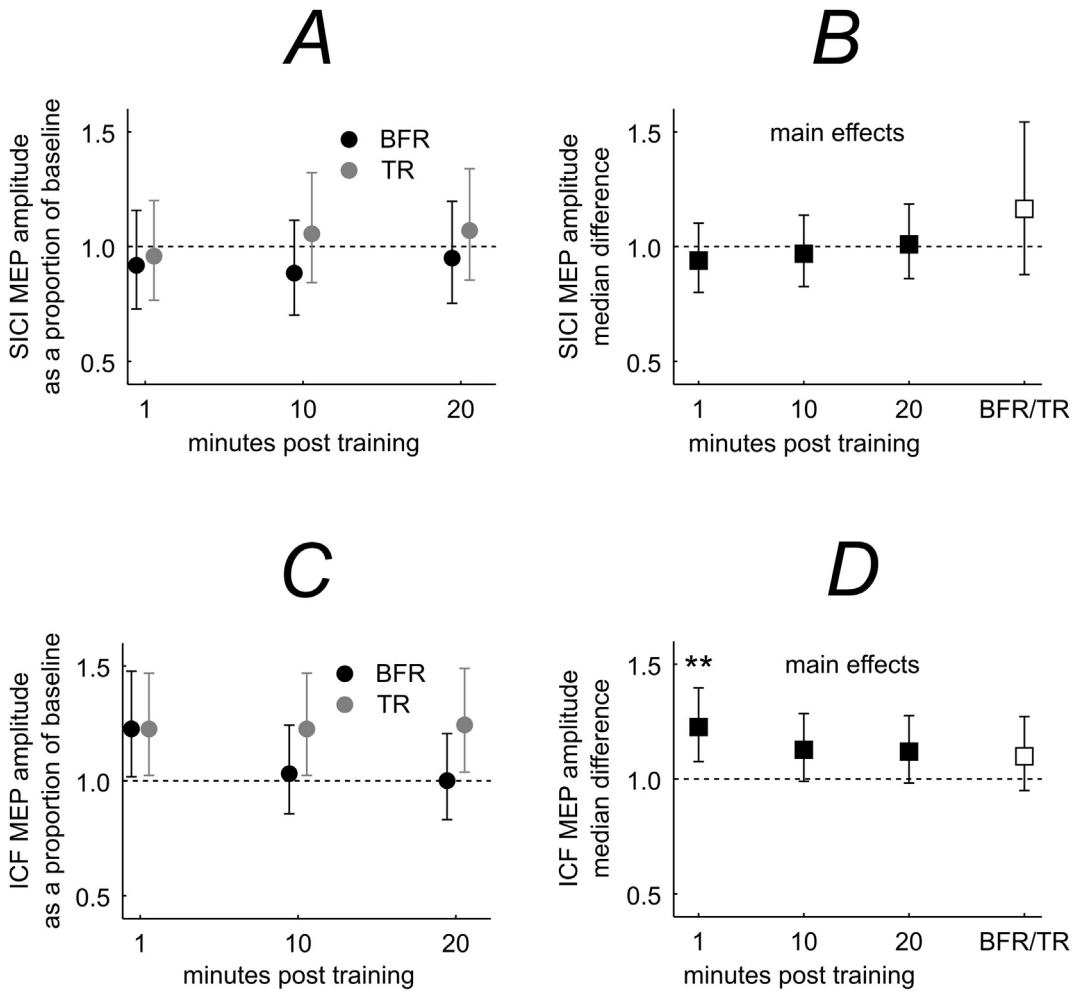

Fig. 6. *A and C.* Modelled median differences and 95% confidence intervals compared to baseline values for the SICI (*A*) and ICF (*C*) surface EMG amplitude for the BFR condition (black filled circles and lines) and TR condition (grey filled circles and lines). *B and D.* Modelled median differences and 95% confidence intervals for SICI (*B*) and ICF (*D*) for the main effect of timepoint as a ratio of baseline values (black filled squares) and condition with the TR condition as a proportion of BFR condition (white filled square). '**' indicates significant differences to the baseline estimates to $P < 0.01$.

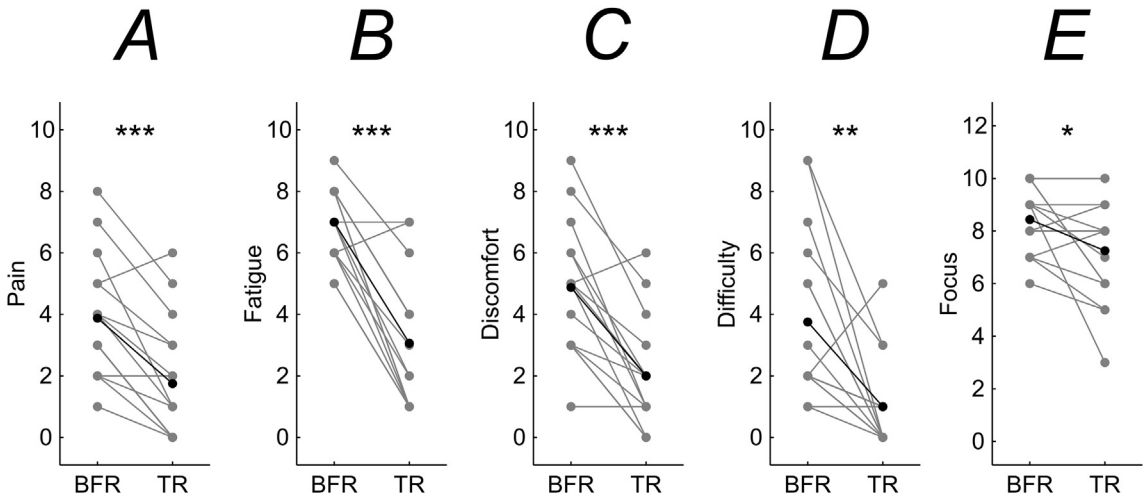

Fig. 7. *A–E.* The results of the numeric rating scores (0–10) for pain (*A*), fatigue (*B*), discomfort (*C*), difficulty (*D*) and focus (*E*) following the BFR and TR condition. The individual filled grey circles indicate individual responses for each subject. The grey lines join the responses for each subject on the BFR-session and TR-session. Note that some lines and filled circles are superimposed. The black filled circles indicate the means for the BFR and TR sessions. '***', '**' and '*' indicate differences between BFR and TR to $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively.

for the same amount of time which will not significantly alter the motor/muscular system after training. This is promising if this type of training is transferred to other populations as the aftereffects to training may not interfere in post-exercise activities, such as walking, any more than conventional training. Despite this, subjects perceived BFRT as more painful, more uncomfortable and more difficult that TR. As such, the possibility of exercise non-compliance if BFRT is used must be considered and people should be selected for BFRT on a case-by-case basis. In addition, before this training can be mainstream in clinical populations more research in controlled laboratory settings is required.

Declarations

Author contribution statement

Simon Svanborg Kjeldsen, Erhard Trillingsgaard Næss-Schmidt, Peter William Stubbs: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Gunhild Mo Hansen: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Jørgen Feldbæk Nielsen: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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