Title: Neuromuscular electrical stimulation (NMES) combined with blood flow restriction increases fatigue and perceptual variables compared with NMES alone

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ABSTRACT

Context: Neuromuscular electrical stimulation (NMES) combined with blood flow restriction (BFR) has been shown to improve muscular strength and size greater than NMES alone. However, the previous studies use varied methodologies not recommended by previous NMES or BFR research. **Objective:** The present study investigated the acute effects of NMES combined with varying degrees of BFR, using research recommended procedures to enhance understanding and the clinical applicability of this combination. **Design:** Randomised crossover. **Setting:** Biomechanics laboratory. **Participants:** 20 healthy adults (age: 27 ± 4; height: 177 ± 8 cm; body mass: 77 ± 13 kg). **Interventions:** Six sessions separated by at least seven days. The first two visits served as familiarisation, with the experimental conditions performed in the final four sessions; NMES alone, NMES 40% BFR, NMES 60% BFR and NMES 80% BFR. **Main outcome measures:** Maximal voluntary isometric contraction (MVIC), muscle thickness, blood pressure, heart rate, rating of perceived exertion (RPE) and pain were all recorded before and after each condition. **Results:** NMES 80% BFR caused greater MVIC decline than any other condition (-38.9 ± 22.3 Nm, $p < 0.01$). Vastus medialis and VL muscle thickness acutely increased after all experimental conditions ($p < 0.05$). Pain and RPE ratings were higher after NMES 80% BFR, compared with all other experimental conditions ($p < 0.05$). No cardiovascular effects were observed between conditions. **Conclusion:** NMES combined with 80% BFR caused greater acute force decrement than the other conditions. Although, greater perceptual ratings of pain and RPE were observed with NMES 80% BFR. These acute observations must be investigated during chronic interventions to corroborate any relationship to changes in muscle strength and size in clinical populations.

Keywords: neuromuscular electrical stimulation; blood flow restriction; fatigue; muscle swelling

INTRODUCTION

Blood flow restriction (BFR) involves reducing arterial blood flow to a muscle and preventing venous return via the application of a pneumatic cuff or tourniquet around the proximal part of the target limb[1]. To date, BFR has been used in combination with low-load resistance exercise and aerobic exercise to enhance muscle strength and morphological adaptations compared with the same load of exercise without BFR, in both healthy and clinical populations[1,2].

However, in clinical practice voluntary movement may be contraindicated and immobilisation required for certain musculoskeletal disorders i.e. immediately post fracture or surgery. During disuse and immobilisation, skeletal muscle loss occurs at a rate of approximately 0.5% of total muscle mass per day[3], with strength declines between 0.3% and 4.2% each day[4]. When used passively, BFR has been shown to attenuate declines in muscle mass during periods of immobilisation[5–7], but unable to increase muscle strength and size[5–8].

Neuromuscular electrical stimulation (NMES) has also been shown to prevent disuse muscle atrophy[9], but there is inconsistent evidence regarding its efficacy in enhancing muscle adaptations[10]. More recently the combination of NMES with BFR has been investigated. The results of trials using NMES and BFR in humans are varied, with two studies reporting increased muscle strength and hypertrophy compared with NMES and BFR alone in healthy and spinal cord injured adults[11,12] and two others finding either within group changes only[13] or no added benefit[14]. Although mixed results have currently been observed, the clinical application for NMES and BFR increasing muscle strength

and size post-surgery or during immobilisation when voluntary exercise is contraindicated, is promising.

Varied methodologies have led to conflicting findings in studies investigating NMES and BFR, thus limiting the understanding of underlying physiological mechanisms that induce changes in muscle strength and hypertrophy. The NMES protocols currently utilised have considerable variability, with frequencies ranging from 20-100 Hz and unclear reporting of other parameters including stimulation intensities[11–14]. To maximise quadriceps strength after NMES it is recommended to use a frequency of 50 Hz, maximal tolerable intensities and to place stimulating electrodes over muscle motor points[15]. These parameters have not been utilised in previous NMES and BFR studies on the quadriceps[12,13]. Additionally, the vast majority of studies have implemented BFR by prescribing an arbitrary restrictive pressure[13,14,16,17] or based their occlusion pressure on systolic blood pressure (SBP)[11]. Recent findings indicate that neither of these approaches are effective for controlling the magnitude of BFR, with current recommendations suggesting that pressure should be prescribed via arterial occlusion pressure (AOP)[18].

The mechanisms by which NMES combined with BFR increases muscle strength and induces hypertrophy are currently unknown. Greater acute force decrement (fatigue) following NMES combined with BFR in a rat model correlated with increased hypertrophy compared with NMES alone[19]. Furthermore, resistance training with and without BFR that produces greater levels of fatigue (determined via reduced force production), results in larger improvements in muscle strength and size[20,21]. This evidence suggests that acute post-exercise decrements in force production could provide a

surrogate marker to optimise training programmes. However, there has been no direct comparison of the acute muscle responses to NMES in combination with varying levels of BFR.

The present study aimed to standardise and provide a better understanding of how muscular, cardiovascular and perceptual variables are acutely affected by NMES alone and combined with varying levels of BFR, using previously established protocols. It was hypothesised that muscular fatigue, muscle swelling and perceptual variables (i.e. pain and exertion) would be higher with NMES and BFR compared with NMES alone.

METHOD

Participants

Twenty recreationally active (3.1 ± 1.4 h/week), healthy males (n = 15) and females (n = 5) (age: 27 ± 4; height: 177 ± 8 cm; body mass: 77 ± 13 kg, and body mass index: 25 ± 3 kg/m^2) volunteered to participate in this study. The sample size was calculated using G*Power software and the effect sizes of previous research assessing the same outcomes[22]. Inclusion criteria were: (a) absence of lower-limb injury, (b) negative answers in the PAR-Q questionnaire, (c) no personal history of cardiovascular or metabolic disease, (d) non-smokers, (e) resting SBP < 140 mmHg and (f) normal range on the ankle brachial index (ABI) test (0.9-1.4)[23]. Participants were instructed to maintain their usual level of physical activity throughout the study. All participants provided written informed consent and the study was approved by St Marys University ethics sub-

committee (SMEC_2016-17_104) and conducted in accordance with the Declaration of Helsinki.

Study design

The study followed a randomised crossover design, generated via online software (http://www.randomization.com). All testing was undertaken at the University's temperature-controlled laboratory (21-22°C). Participants were required to visit the laboratory on six occasions, separated by at least 7 days to prevent a training effect and at the same time of day (± 1 h) to minimise the circadian effect. All participants were tested at least 2 h postprandial and were instructed to avoid caffeine and exercise prior to testing. The first two visits served as familiarisation sessions, with the experimental conditions performed in the final four sessions. During the first visit, height, weight, ABI, knee extension maximal voluntary isometric contraction (MVIC), vastus medialis (VM) and vastus lateralis (VL) muscle thickness, AOP and NMES maximal tolerable intensity were measured. During the second visit, MVIC, muscle thickness, AOP and NMES maximal tolerable intensity were repeated[15]. After the familiarisation sessions, participants were randomly allocated to perform the experimental conditions, with the same trained researcher performing all outcome measurements (Fig 1):

1) NMES and cuff not inflated (NMES alone)
2) NMES and 40% BFR (NMES 40)
3) NMES and 60% BFR (NMES 60)
4) NMES and 80% BFR (NMES 80)

******* Insert Figure 1 here *******

PROCEDURES

ABI

ABI was measured using recommended procedures[23]. A standard blood pressure cuff and a handheld Doppler probe (Hi-Dop, Ana Wiz ltd, Surbiton, London, UK), were used to measure SBP of the arm (brachial artery) and of the ankle (posterior tibial artery). All participants had a normal ABI 1.1 ± 0.1. Test–retest (intra session) reliability across three sessions on 20 adults for ABI was 0.9% coefficient of variation (CV) and 0.02 minimum detectable change (MDC).

NMES

The familiarisation sessions were used to determine each participants maximal tolerable NMES intensity. In subsequent sessions, participants then performed four identical NMES protocols under varying levels of BFR (0%, 40%, 60% and 80% AOP). During all sessions, participants were seated, fixed to a strain gauge and underwent 8 min and 10s of NMES at a fixed knee joint angle of 90°. The NMES protocol used a bi-phasic rectangular pulse, 50 Hz stimulation frequency, duty cycle was 5 s of stimulation followed by a 5 s pause, ramp up 1.5 s and ramp down 0.5 s, 400μs pulse width for 40 repetitions and intensity at the maximum tolerated for each participant. Quadriceps muscles were stimulated using three self-adhesive electrodes (Axion Medical, Axion GMBH, Villengen-Schwennigen, Germany) (2 mm thick) linked to a portable battery-powered

186 neuromuscular electrical stimulator (Mi-Theta 600; Cefar Compex; Medicompex,
187 Ecublens, Switzerland). The negative electrode (10 x 5 cm) was positioned proximally
188 13.4 cm (BFR cuff width) below the inguinal crease, which was the most proximal thigh
189 position possible due to the cuff size. The other two (positive) electrodes (5 x 5 cm) were
190 placed over the motor points of the VM and VL muscles. Muscle motor points were
191 identified using a pen electrode (Compex; Medicompex, Ecublens, Switzerland) and a
192 large reference electrode placed over the proximal quadriceps[15]. The pen electrode was
193 moved slowly over the skin, with the stimulatory current gradually increased until a clear
194 muscle twitch was observed. The electrode was placed over the point that caused the
195 largest visible twitch[15]. Throughout the study, the electrode location was recorded,
196 marked and applied at the same motor point sites during every session. Participants were
197 instructed to relax their thigh muscles throughout. Vastus medialis and VL maximal
198 tolerable intensities equalled 67.1 ± 44.1 mA and 70.7 ± 44.7 mA, respectively.

199

200 **Determination of blood flow restriction pressure**

201 A handheld vascular Doppler probe (8 Hz) was placed 3 cm proximal from the end of the
202 medial malleolus and over the posterior tibial artery to determine AOP. A pneumatic cuff
203 (PTS tourniquet system, Delfi medical innovations, Vancouver, Canada) (width 13.4 cm;
204 length 58 cm) was placed around the most proximal portion of each participant's right
205 thigh. The pneumatic system connected to the tourniquet cuff, increased the cuff pressure
206 in stepwise increments, and when no auscultatory pulse was detected by the Doppler
207 probe, this determined AOP[24]. The BFR pressures used during the experimental
208 conditions were 0%, 40%, 60% and 80% of AOP in a resting condition, which matched
209 the body position in which the intervention was carried out[18]. The BFR pressure was

maintained throughout the NMES session, including rest periods and released immediately upon completion. The mean AOP observed was 168.9 ± 12.1 mmHg.

MVIC

Knee extension MVIC was measured using a custom-made strength chair and a digital strain gauge (Interface SSM-AJ-500 Force Transducer, Interface, Scottsdale, USA) to assess peak force production. Prior to testing, calibration of the strain gauge with a known mass allowed conversion from voltage to Newtons. Participants were seated with the backrest at 80˚. Straps were placed across the torso and hips to prevent any unwanted movement. Knee extension MVIC was determined for the right leg, with the load cell fixed at an angle corresponding to 90^0 of knee flexion (goniometer) and the resistance pad fastened 2 cm above the lateral malleolus. Chair set-up was recorded and standardised for each session. The pre-intervention MVIC began with a warm up of 3 x 5 s submaximal contractions at 25%, 50% and 75% of each participant's voluntary maximal effort, followed by 3 x 5 s maximal contractions, with 30 s rest between repetitions[25]. The same procedure was also used during the familiarisation sessions. Participants were instructed to exert maximum force as fast as possible and peak torque was defined as the highest MVIC value observed, multiplied by shank length (Nm). Verbal encouragement was provided throughout. Three contractions were initially performed. Where two measurements differed by >5%, an additional contraction was performed. Post-intervention MVIC's were conducted 60 s post-NMES intervention and cuff deflation. All raw MVIC signals were low-pass filtered using a zero-lag fourth order Butterworth filter with a 11 Hz cut-off frequency, determined from a residual analysis. Reliability for MVIC measurements was 3.8% CV and 9.6 Nm MDC.

Muscle thickness

Quadriceps muscle thickness was measured using B-mode ultrasonography (Echoblaster 128 EXT-1Z, Telemed, Lithuania; 60mm linear scanning probe, 7 MHz transducer scanner) at the sites of the VM and VL muscles. MTH of VM was measured at 20% of this distance and VL at 50% of the distance between the patella and anterior superior iliac spine. The VM measurements were taken from 12.5% of thigh circumference in the medial direction from the midpoint of the thigh, and the VL measurements were taken from 10% of thigh circumference in the lateral direction, which represent the location of the maximum cross-sectional area of these muscles. The ultrasound probe was placed over the VM and VL musculature in two separate trials. Before all scans, the participants lay for 5 min in a supine position. The measurement sites were marked by indelible ink and determined by the NMES electrodes marking the reference location. With the leg in full knee extension, the deep and superficial aponeurosis of each muscle was identified, and the distance between the two interfaces calculated as muscle thickness. The mean of three measurements from the centre of each image was used for data analysis[12]. Reliability for VM and VL muscle thickness measurements were 3.2% CV, 0.6 mm MDC and 5.2% CV, 0.6 mm MDC, respectively.

Blood pressure

Systolic and diastolic blood pressure (DBP) were measured using an automatic blood pressure monitor (Omron M3-IT, Omron Healthcare UK ltd, Milton Keynes, UK). Blood pressure measurements were performed after 5 min of supine rest and were assessed

twice, if variability was > 5 mmHg, a third measure was taken and the mean recorded. Reliability for SBP and DBP were 3.3% CV, 2.5 mmHg MDC and 5.1% CV, 2.3 mmHg MDC, respectively.

Heart rate

Heart rate was measured using a heart rate monitor, coded transmitter and chest strap placed underneath each participants xyphoid process (Polar TY1, Polar, Kempele, Finland). Heart rate was taken after 5 min of supine rest, pre and post experimental conditions, and also recorded following each set (10 repetitions) of the NMES protocol. Reliability at rest was 5.2% CV and 3 beats/min MDC.

Rating of perceived exertion

Rating of perceived exertion was taken following each set (10 repetitions) of the NMES protocol using the standard Borg 6–20 scale[26]. Participants confirmed that they fully understood how to rate RPE prior to testing.

Pain

A rating of pain was taken following each set (10 repetitions) of the NMES protocol as well as 24 and 48 hours post the final set, using the 0-10 numeric rating pain scale (NRPS), with "0" representing no pain and "10" the worst pain imaginable"[27]. Participants confirmed that they fully understood how to rate pain prior to testing.

Statistical Analysis

A two-way repeated-measures analysis of variance (ANOVA) was used to determine the effects of condition (0%, 40%, 60% and 80% BFR) and time; MVIC, muscle thickness, SBP, DBP, heart rate across two time points (pre and post), HR, RPE, Pain across four time points (set 1, set 2, set 3, set 4). If the assumptions of ANOVA were violated, the Greenhouse–Geisser correction factor was applied. Significant interactions and main effects were followed with appropriate *post-hoc* analyses and Bonferroni adjustments. Statistical significance was set at $p < 0.05$. Statistics were computed using SPSS Statistics software package version 24.0 (SPSS, Chicago, USA). Data are presented as means ± SD unless otherwise stated.

RESULTS

No differences were observed between baseline values across the four experimental conditions ($p > 0.05$). No adverse events occurred.

MVIC

There was a main effect of time ($F_{(1,19)} = 37.2$, $p < 0.001$), no condition effect ($p > 0.05$) and a condition × time interaction ($F_{(3,57)} = 10.6$, $p < 0.001$) for MVIC decline (Fig 2). Post-hoc pairwise Bonferroni comparisons confirmed greater MVIC decline after NMES 80% BFR compared with NMES alone ($p < 0.001$), NMES 40% BFR ($p < 0.001$) and NMES 60% BFR ($p = 0.001$) (Fig 2). All differences were above the 9.9 Nm MDC, error of measurement.

******* Insert Figure 2 here *******

Muscle thickness

There was a main effect of time ($F_{(1,19)}$=43.1, $p < 0.001$; $F_{(1,19)}$=92.1, $p < 0.001$) for VM muscle thickness and VL muscle thickness increase, respectively (Table 1). However, there was no condition effect or condition × time interaction observed ($p > 0.05$).

Blood pressure

A main effect of time ($F_{(1,19)}$= 12.1, $p = 0.002$) was observed for SBP. There was no condition effect or condition × time interaction ($p > 0.05$) shown for SBP. There were no effects observed on DBP ($p > 0.05$) (Table 1).

******* Insert Table 1 here *******

Heart rate

There was a main effect of time ($F_{(1.4,26.7)}$=54.8, $p < 0.001$), condition effect ($F_{(3,57)}$=4.1, $p = 0.010$) and condition × time interaction ($F_{(6.6,125.2)}$=3.9, $p = 0.001$) for heart rate (Table 1 and 2). Post-hoc pairwise comparisons revealed after set 1, NMES alone was lower than NMES 80 ($p = 0.019$); after set 2, NMES 80 was higher than NMES alone ($p = 0.019$); after set 3, NMES 60 and NMES 80 were higher than NMES alone ($p = 0.026$ and $p = 0.01$, respectively); after set 4, NMES 80 was higher than NMES alone ($p = 0.019$) (Table 1 and 2). However, all differences were below the 3.2 bpm MDC, showing no meaningful change.

Rating of perceived exertion

There was a main effect of time ($F_{(1.1,21.3)}$=11.9, p = 0.002), condition effect ($F_{(3,57)}$=7.7, p < 0.001) and condition × time interaction ($F_{(3.8,72.4)}$=3.4, p = 0.015) for RPE (Table 2). Post-hoc pairwise comparisons confirmed RPE to be higher; after set 1 of NMES 80 compared with NMES alone (p = 0.006), after set 2 of NMES 80 compared with NMES alone, NMES 40 and NMES 60 (p = 0.018; p = 0.027; p = 0.005, respectively), after set 3 of NMES 80 compared with NMES alone, NMES 40 and NMES 60 (p = 0.002; p = 0.002; p = 0.038, respectively). Finally, RPE was higher after set 4 of NMES 80 compared with NMES alone, NMES 40 and NMES 60 (p = 0.001; p = 0.001; p = 0.041, respectively).

Pain

There was a main effect of time ($F_{(1.6,31.2)}$=13.6, p < 0.001), condition effect ($F_{(3,57)}$=19.6, p < 0.001) and condition × time interaction ($F_{(5.3,100.3)}$=4.8, p < 0.001) for pain (Table 3). Post-hoc pairwise comparisons revealed ratings of pain were higher; after set 1 of NMES 80 compared with NMES alone, NMES 40 and NMES 60 (p = 0.006; p = 0.001; p = 0.027, respectively), after set 2 of NMES 80 compared with NMES alone, NMES 40 and NMES 60 (p < 0.001; p < 0.001; p = 0.010, respectively), after set 3 of NMES 80 compared with NMES alone, NMES 40 and NMES 60 (p < 0.001; p < 0.001; p = 0.001, respectively). Finally, pain ratings were higher after set 4 of NMES 80 compared with NMES alone, NMES 40 and NMES 60 (p < 0.001; p < 0.001; p = 0.003, respectively) and lower after set 4 of NMES alone compared with set 4 of NMES 60 (p = 0.039).

******* Insert Table 2 here *******

DISCUSSION

The purpose of this study was to standardise and determine if varying BFR pressures induce different acute effects when combined with NMES. The main findings were that the addition of BFR (40-80%) to NMES was required to acutely affect torque output (fatigue). Furthermore, NMES 80% BFR caused greater fatigue (16.2%) than NMES alone (3.5%) (Fig 2), with no deleterious cardiovascular effects (Table 1 and 2).

The impairment of the force generating capacity of a muscle is defined as muscle fatigue[28]. Our result that NMES combined with 80% BFR induced the greatest acute fatigue (torque decrements) is consistent with findings after BFR alone and combined with low-intensity voluntary isometric contractions[29,30], demonstrating that the addition of BFR acutely reduces force generating capacity and the level of force reduction is dependent on the pressure applied to the limb. For example, Pierce et al[29] applied BFR (163 mmHg) passively for 5 x 5 min and produced equal knee extension torque decrements (16%) to the present study. Our results are also in accordance with prior BFR investigations that found 80% actual and estimated AOP induced acute decrements in MVIC torque[22,29,31,32]. The acute decrement in MVIC shown here with the addition of BFR (18%) is also similar to that observed after a single bout of resistance exercise (20%), which has correlated with increased muscular strength and size of the VL after training protocols lasting 6 weeks[20,33]. Furthermore, animal models have shown that NMES combined with BFR causes significantly greater torque decrements than NMES alone, which also led to greater muscle growth[19,34]. Nakajima et al[19] reported NMES force to rapidly decrease during a combined intervention of NMES and BFR compared to NMES

alone in a rat model. Their acute findings correlated with increased muscle size with NMES and BFR vs. NMES alone (11.0% vs. 6.2%), after 3 weeks of training[19]. Furthermore, Natsume et al[34] also found greater fatigue and muscle weight after NMES and BFR vs. NMES alone in a rat model[34]. If acute fatigue is desirable for long term muscular adaptations, our findings provide stronger support for combining NMES with 80% BFR, compared with 40% and 60% BFR and no support for NMES alone (Fig 2).

Although mechanistic reasons for our findings were not investigated, torque decrements will have occurred due to a number of physiological processes. For example, increases in intramuscular inorganic phosphate concentration have been reported after BFR[35–37] and are a known cause of peripheral fatigue[38,39]. Indeed, others have reported that a combination of submaximal exercise with arterial occlusion rapidly depletes type I and type II muscle fibres of phosphocreatine[40], leading to increases in inorganic phosphate concentration[41]. Decreases in blood flow/O^2 delivery associated with BFR, exacerbate this rate of peripheral fatigue[39,42]. Muscle fatigue can be compensated for by increased motor unit activation in an effort to maintain force output[43]. Hence, during fatiguing muscle contractions there is an increased activation of motor units that innervate type II fibres, thus increasing the potential for muscle fibre hypertrophy[44]. This provides one potential reason for the reported relationships between fatiguing tasks (induced by NMES and BFR) and muscle growth[19].

No previous NMES and BFR research has used AOP to determine BFR pressures in humans. However, in animal models Natsume et al[34] stated that they used a cuff pressure approximately 40-60% of AOP and Nakajima et al[19] used a BFR pressure that lowered

O^2 partial pressures considerably but blood flow was not completely occluded. This could be interpreted as above 60% AOP in line with previous research on humans finding the level of muscle oxygenation/deoxygenation during 40% AOP is not substantially different from that seen during non-BFR[45]. Reis et al[45] concluded that 60% AOP appears to represent a threshold required to induce higher deoxygenation and decreased tissue oxygenation levels[45]. The present findings found increased acute fatigue when adding 40-80% BFR to NMES. This is consistent with the previously mentioned animal model data finding acute fatigue caused significant hypertrophy[19]. This relationship needs to investigated in humans to determine what optimal BFR pressures are required when combined with NMES to enhance muscle strength and hypertrophy in rehabilitation settings.

Muscle swelling was measured by changes in muscle thickness in the present study. The acute increases in VM and VL muscle thickness observed (Table 1), were similar to previous studies that applied BFR combined with resistance exercise using pressures from 40% AOP to 150% SBP[46–48]. However, there was no condition effect or condition × time interaction observed. Our findings also support previous BFR data, showing no greater muscle swelling effect utilising higher BFR pressures > 40% AOP[48,49]. Muscle swelling has been argued to trigger the proliferation of satellite cells, thus contributing to the hypertrophic response to exercise[50]. Although, it is currently unknown if acute muscle swelling contributes to hypertrophy observed with NMES combined with BFR. The present study supports the use of NMES alone and combined with BFR (40-80%) to induce acute muscle swelling (Table 1).

Pain was increased with the addition of 80% BFR to NMES compared to all of the other conditions in the present study (Table 2). Additionally, NMES combined with 60% BFR produced greater ratings of pain than NMES alone (Table 2). This indicates that the pain experienced is mostly attributable to the level of occlusive pressure (60-80%). Exercise-induced muscle pain can be generated by stimulation of group III and IV muscle afferents, elicited by metabolic perturbations of the working musculature. It is generally accepted that BFR reduces metabolite clearance, thus inducing greater pain compared to non-occluded exercise[51]. Cuff inflation at higher pressures (80% AOP) has been previously characterised as moderately painful[52], which supports the lower pain ratings observed after NMES and 40% BFR (Table 2). The lower pain and RPE scores reported with the addition of 40% compared with 80% BFR to NMES in the present study, may lead to greater clinical applicability, due to NMES BFR 40% inducing significant fatigue (Fig 2) with reduced pain and RPE scores.

There were no unanticipated effects on the cardiovascular system during any of the trials (Table 1 and 2). This supports previous NMES research using maximal tolerable intensities[53,54] and BFR research using 70% BFR pressures[55,56]. In agreement with the current findings, no adverse events have occurred in healthy and spinal cord-injured adults previously[11–14]. The present findings support the use of NMES and BFR on the selected cardiovascular measures (Table 1 and 2).

The current study has some limitations, such as the sample, which was restricted to young, healthy men and women. Thus, we acknowledge that our findings may not apply to other populations. Also, the measurements were taken immediately pre and post every

experimental condition. Therefore, the time-course of change in the period of time after the intervention is unknown. The investigator and participants were not blinded to experimental conditions. Blinding aims to prevent biased assessment of outcomes and ascertainment bias after randomisation[57]. Future research should, therefore, consider evaluating the time-course responses to BFR and NMES interventions among a wider range of clinical populations who are likely to benefit from its application.

CONCLUSION

This is the first study to standardise the BFR pressure using a percentage of AOP when combining it with NMES. To determine which protocol would be best suited for rehabilitation settings, we evaluated several factors, including muscle fatigue, muscle swelling, cardiovascular response and perceptual responses. On the basis of our results, we recommend combining NMES with 80% BFR for the quadriceps muscle group. However, NMES combined with 40% BFR cannot be excluded, due to lower perceptual ratings than 80% BFR and acutely inducing fatigue (Fig 2; Table 1), which may be a surrogate marker for muscle hypeetrophy[19]. We can only speculate that the increased metabolic stress associated with BFR has led to the increased fatigue, RPE and pain ratings observed with the addition of 40-80% BFR to NMES in the present study (Fig 2; Table 2). Of course, these acute observations must be expanded upon during chronic training interventions to corroborate any relationship to changes in muscle strength and size. The combination of NMES and BFR has the potential to assist the rehabilitation of skeletal muscle in post-surgery patients and during immobilisation, when voluntary exercise is not possible.

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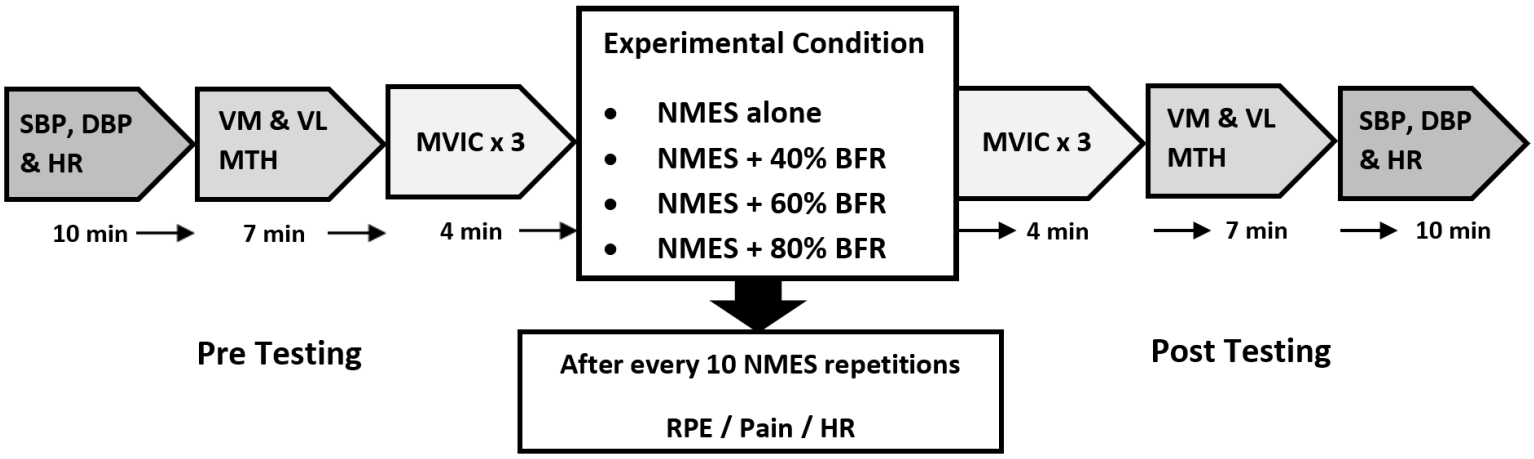

Fig 1. Experimental protocol. All participants performed the same neuromuscular electrical stimulation (NMES) protocol under four different blood flow restriction (BFR) pressures (0, 40, 60 and 80%). Outcome measures; systolic blood pressure (SBP); diastolic blood pressure (DBP); heart rate (HR); vastus medialis (VM) and vastus lateralis (VL) muscle thickness (MTH); knee extension maximal voluntary isometric contraction (MVIC) were assessed before (pre) and after (post) each experimental condition. Outcome measures assessed after every 10 NMES repetitions included; rating of perceived exertion (RPE), pain and HR. See abbreviations throughout.

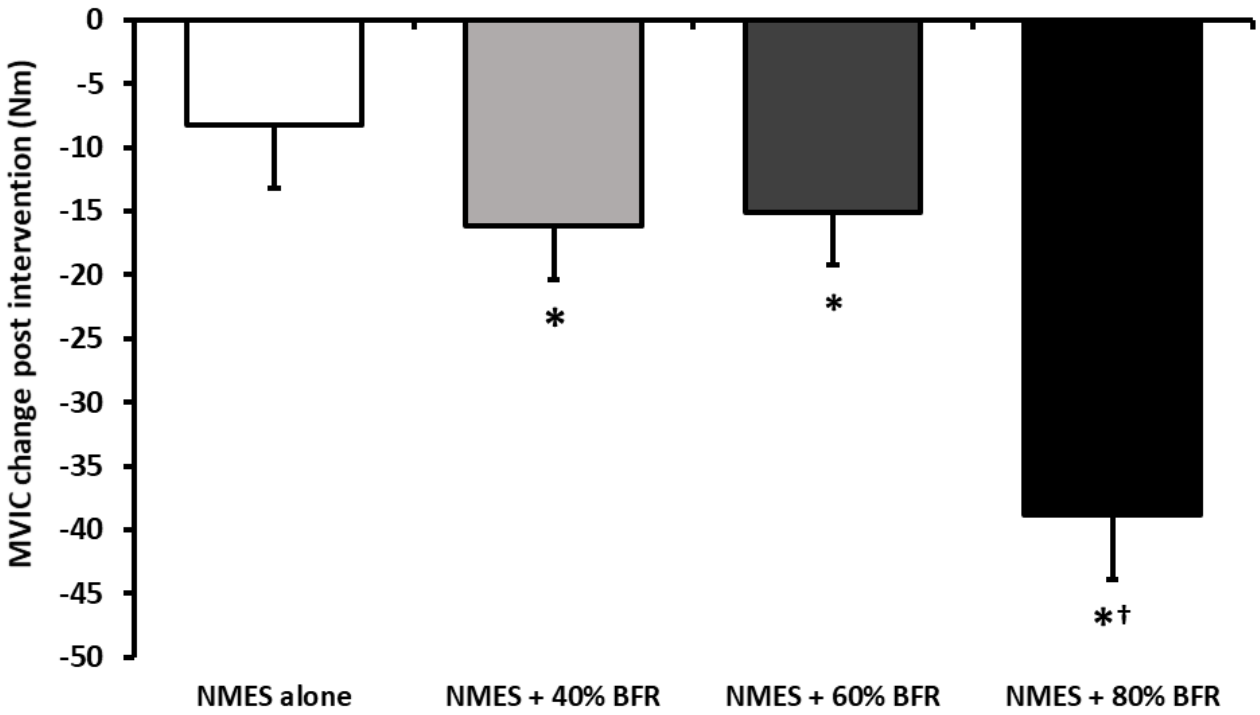

Fig 2. Knee extension maximal voluntary isometric contraction (MVIC) pre-test to post-test change Δ; values as mean ± SEM. Significant differences were set at $p < 0.05$; * = significant difference between pre-test and post-test; † = significantly greater change compared to all other experimental conditions

Table 1. Knee extension MVIC, muscle thickness and cardiovascular pre-test and post-test measurement values; mean (SD) [95% Confidence Interval]

	NMES alone			NMES +40% BFR			NMES + 60% BFR			NMES + 80% BFR		
	Pre	**Post**	**C [95% CI]**	**Pre**	**Post**	**C [95% CI]**	**Pre**	**Post**	**C [95% CI]**	**Pre**	**Post**	**C [95% CI]**
MVIC	239.8	231.5	-8.3 [-18.5;	240.3	224.1	-16.2 [-25.0;	240.4	225.4	-15.1 [-23.8;	242.6	203.8	-38.9 (-49.3;
(Nm)	(51.3)	(57.1)	1.9]	(48.3)	(46.8)*	-7.3]	(52.3)	(55.7)*	-6.4]	(55.1)	(52.1)*†	-28.3]
VM MTH	25.0	25.6	0.6 [0.3;	25.2	26.0	0.8 [0.3; 1.2]	25.0	25.8	0.8 [0.4; 1.3]	24.7	25.9	1.2 [0.8; 1.5]
(mm)	(2.7)	(2.6)*	0.9]	(2.9)	(2.8)*		(2.9)	(2.9)*		(2.7)	(2.9)*	
VL MTH	17.2	17.9	0.7 [0.5;	16.6	17.7	1.0 [0.6; 1.5]	16.9	18.0	1.1 [0.7; 1.6]	17.0	18.4	1.4 [0.9; 1.9]
(mm)	(2.8)	(2.8)*	1.0]	(2.4)	(2.9)*		(2.5)	(3.0)*		(2.9)	(3.2)*	
SBP	122.8	125.2	2.3 [0.7;	121.9	123.9	1.9 [-1.4;	123.4	124.7	1.4 [-0.6;	123.0	125.5	2.5 [0.8; 4.1]
(mmHg)	(8.7)	(9.2)*	4.0]	(8.5)	(7.8)	5.2]	(9.3)	(8.1)	3.3]	(8.1)	(7.8)*	
DBP	69.4	71.1	1.7 [-0.9;	70.2	71.4	1.3 [-0.6;	71.2	71.2	0.1 [-2.2;	70.7	71.6	0.9 [-1.8;3.5]
(mmHg)	(6.7)	(5.3)	4.4]	(6.2)	(7.6)	3.1]	(7.1)	(6.3)	2.4]	(6.0)	(6.5)	
HR	61.0	60.7	-0.3 [-2.2;	60.7	61.2	0.5 [-1.1;	60.6	58.3	-2.4 [-4.6; -	62.2	59.5	-2.7 [-6.5;
(bpm)	(9.3)	(9.6)	1.6]	(9.3)	(8.6)	2.1]	(8.8)	(9.5)*	0.2]	(9.1)	(9.7)	1.1]

Significant differences were set at $p < 0.05$; * = significant difference between pre-test and post-test; † = significantly greater change compared to all other experimental conditions. C = change from pre to post

Table 2. Measurement values after every set (10 contractions) of the interventions; mean (SD)

	NMES alone				NMES + 40% BFR				NMES + 60% BFR				NMES + 80% BFR			
	Set 1	**Set 2**	**Set 3**	**Set 4**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 1**	**Set 2**	**Set 3**	**Set 4**
HR	71.1	71.9	71.8	72.2	74.2	74.6	75.1	74.5	73.6	76.4	77.0	76.5	77.1	79.3	79.4	78.8
(bpm)	(9.1)	(9.7)	(8.4)	(8.7)	(9.8)	(9.1)	(10.4)	(9.7)	(11.6)	(10.5)	(9.6)	(11.4)	(11.8)	(11.4)	(11.3)	(12.2)
RPE	11.0	11.0	11.1	11.1	10.5	10.8	11.3	11.3	10.6	11.1	11.9	12.1*	12.1	12.9	13.4#	13.7†
(6-20)	(3.1)	(3.0)	(2.9)	(2.7)	(2.8)	(2.8)	(3.0)	(3.0)	(2.5)	(2.6)	(3.0)	(3.1)	(3.3)	(3.5)	(3.3)	(3.5)
Pain	3.6	3.5	3.6	3.5	3.4	3.7	3.8	3.9	3.6	4.2	4.6	4.8*	5.3	6.0#	6.6†	6.7^
(0-10)	(1.9)	(1.8)	(1.8)	(1.7)	(1.7)	(1.9)	(1.9)	(2.0)	(1.9)	(2.0)	(1.9)	(1.8)	(1.5)	(1.3)	(1.3)	(1.6)

Significant differences were set at $p < 0.05$; RPE results (* = significant difference between set 1 and set 4; # = set 3 of NMES 80 significantly larger than all sets of NMES alone, NMES 60 and set 1 of NMES 40; † = set 4 of NMES 80 significantly larger than all sets of NMES alone, NMES 60 and set 1 and 2 of NMES 40); Pain results (* = significant difference between set 1 and set 4; # = set 2 of NMES and 80% BFR significantly larger than all sets of NMES alone, NMES and 60% BFR and set 1 of NMES and 40% BFR; † = set 3 of NMES and 80% BFR significantly larger than all sets of NMES alone, NMES and 60% BFR and set 1 and 2 of NMES and 40% BFR; ^ = set 4 of NMES and 80% BFR significantly larger than all sets of NMES alone, NMES and 60% BFR and set 1 of NMES and 40% BFR)