Title: The effect of blood flow restriction exercise on exercise-induced hypoalgesia and endogenous opioid and endocannabinoid mechanisms of pain modulation

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Abstract

Aim: This study aimed to investigate and compare the magnitude of exercise-induced hypoalgesia (EIH) with low intensity blood flow restriction (BFR) resistance exercise (RE) at varying pressures to other intensities of resistance exercise and examine endogenous mechanisms of pain reduction.

Methodology: Twelve individuals performed four experimental trials involving unilateral leg press exercise in a randomised crossover design: low load RE at 30% of one repetition maximum (1RM), high load RE (70% 1RM) and BFR-RE (30% 1RM) at a low and high pressure. BFR pressure was prescribed relative to limb occlusion pressure at 40% and 80% for the low- and high-pressure trials. Pressure pain thresholds (PPT) were assessed before, 5-min and 24-h following exercise in exercising and non-exercising muscles. Venous blood samples were collected at the same timepoints to determine plasma concentrations of beta-endorphin and 2-arachidonoylglycerol.

Results: High pressure BFR-RE increased PPTs in the exercising limb to a greater extent than all other trials. Comparable systemic EIH effects were observed with HLRE and both BFR-RE trials. PPTs in the exercising limb remained elevated above baseline at 24-h post-exercise following both BFR-RE trials. Post-exercise plasma beta-endorphin concentration was elevated during the BFR-RE trials. No changes to 2-arachidonoylglycerol concentration were observed.

Conclusion: High pressure BFR-RE causes a greater EIH response in the exercising limb that persists for up to 24-h following exercise. The reduction in pain sensitivity with BFR-RE is partly driven by endogenous opioid production of beta-endorphin. BFR-RE should be investigated as a possible pain-modulation tool in individuals with acute and chronic pain.

Keywords: Blood flow restriction, resistance exercise, hypoalgesia, beta-endorphin

New and noteworthy

We investigated the acute effect of light load resistance exercise with blood flow restriction (BFR) at different pressures on pain sensitivity and the mechanisms contributing to the exercise-induced hypoalgesia (EIH) response. For the first time we have shown that resistance exercise with high pressure BFR causes a greater hypoalgesia response in the exercising limb (48%) compared to low BFR pressure, light and heavy load resistance exercise (10-34%). Performing light load resistance exercise with BFR caused systemic hypoalgesia at both a low (11-17%) and high (11-21%) pressure that was comparable to heavy load resistance exercise (10-18%). BFR resistance exercise at both a low and high pressure prolonged the EIH response for up to 24-h in the exercising limb (15% and 24%, respectively). Activation of endogenous opioid production, reflected in the present study by increased plasma beta-endorphin concentration with low (21%) and high (23%) BFR pressure, and a conditioned pain modulation effect due to muscle discomfort during exercise, both appear to mediate the relationship between exercise and hypoalgesia.

****Introduction****

Exercise-induced hypoalgesia (EIH) describes an acute reduction in pain sensitivity following exercise (36). It is characterised by an elevated pain threshold or decreased suprathreshold pain intensity rating, assessed using noxious stimuli such as temperature, electrical and mechanical pressure stimulation (53). Reductions in pain sensitivity are of greatest magnitude in the exercising limb but also observed in remote non-exercising areas of the body (11, 68), suggesting both local manifestations and spinal/supraspinal nociceptive pathways are involved in pain inhibition with exercise.

Human and animal research models have identified several possible mechanisms thought to trigger EIH. This includes conditioned pain modulation (CPM) (68), recruitment of high threshold motor units (19), a link between baroreceptors and pain pathways (40) and ischemic and metabolite induced pain (56), which are collectively thought to trigger descending pain inhibitory pathways. EIH may also be driven by endogenously produced substances that have antinociceptive effects. One such substance is beta-endorphin (BE), an opioid neuropeptide that has been shown to increase in circulating concentration following exercise in humans (35, 66). This is thought to be caused by stimulation of group III and IV afferent fibres in the contracting muscle, activating the opioid system. This results in release of BE from the pituitary gland and peripheral neurons, which may serve as an agonist for opioid receptors found in abundance throughout the descending pain control circuits in the central and peripheral nervous system (50). Research demonstrating attenuation of EIH with administration of an opioid agonist such as naloxone supports the role of the opioid system in EIH (16).

Hypoalgesia that is insensitive to opioid antagonists suggests non-opioid mechanisms contribute to EIH (39, 71). One such mechanism is the endocannabinoid (ECB) system, which consists of cannabinoid receptors found at peripheral, spinal and supraspinal pain processing sites (63, 71) and endogenous ligand agonists, including ﻿anandamide and 2-arachidonoylglycerol (2AG). Endocannabinoids are synthesised and rapidly released from cells in response to stressful conditions such as exercise (10). Studies have reported increased concentrations of endocannabinoids following exercise in humans (10, 60), suggesting the ECB system might contribute to EIH. This hypothesis is supported by the dense expression of ECB receptors on A-delta and C-delta primary afferents (22), which are activated during muscle contractions to produce alterations in circulating concentrations of endocannabinoids (39).

The analgesic effect of exercise has become important in pain management programmes. Current evidence demonstrates the effectiveness of resistance exercise for reducing pain in individuals with chronic pain (6, 12, 33). The magnitude of EIH appears greater with higher intensity and prolonged exercise. For example, with resistance exercise a large effect (d=0.6-1.1) has been observed when using an external load of >75% of an individual’s one repetition maximum (1RM) (11, 38). However, individuals with chronic pain are often load compromised, and heavy loads may lead to hyperalgesia in these individuals (58). When exercising at lower intensities, prolonging the exercise duration by performing the task to failure can increase the magnitude of EIH, possibly by increasing the recruitment of fast twitch motor units at low exercise intensities (19). However, this may take a considerable amount of time and may therefore not be practical in individuals with chronic pain.

Recent research suggests that performing light load blood flow restriction resistance exercise (BFR-RE) may have a pain-modulation effect. BFR-RE involves partial and full restriction of arterial and venous blood flow, respectively, in the active limb when exercising using loads of 20-30% 1RM (26). It is widely known for increasing muscle hypertrophy and strength adaptations compared to an equivalent exercise intensity without BFR (45). BFR-RE also provides an acute EIH effect (41, 42) and has been shown to reduce pain across a training programme in individuals with a knee pathology (9, 15, 31). For load compromised and rehabilitating populations with chronic pain that cannot withstand high intensity exercise or low intensity exercise to failure, BFR-RE would therefore have a dual effect for pain and muscular adaptations. However the magnitude of EIH with BFR-RE compared to an equivalent form of exercise at both low and high intensities has not been examined, and the effect on the opioid and ECB systems is unknown (27). In addition, research shows that higher BFR pressures augment various acute responses to BFR-RE compared to lower pressures (32), however it is not yet known if pressure affects the acute EIH response to BFR-RE. Therefore, the aim of this study was to compare the magnitude of EIH with BFR-RE using a low and high pressure to resistance exercise at both low and high intensities. It was hypothesised that BFR-RE would increase the EIH response compared to LLRE, which would be augmented to a similar level as HLRE by use of a higher pressure. To understand the mechanisms driving EIH, we examined the response of the opioid and ECB systems and muscle discomfort during exercise and employed mediation analyses to assess how much of the measured effect of the independent variable (exercise intervention) on the dependent variable (hypoalgesia) is attributable to each of the potential mediator variables (opioid production, endocannabinoid production and muscle discomfort).

Materials and methods

## ****Participant information****

Twelve recreationally active individuals (mean ± standard deviation: age = 29 ± 6 y; height = 1.78 ± 0.08 m; weight = 81.1 ± 10.7 kg; body mass index = 26.07 ± 3.29; mean arterial pressure = 76 ± 25 mmHg; 1RM = 156 ± 62 kg;  *n* males = 10, *n* females = 2) were recruited to participate in this study. All protocols were approved by the University Ethical Committee and participants provided written informed consent in compliance with the Declaration of Helsinki, 7th version, October 2013 (72). Participants refrained from strenuous exercise, caffeine and alcohol in the 24h prior to experimental sessions and maintained normal dietary habits for the study duration. All were non-smokers free from neurological, cardiovascular, pulmonary and metabolic diseases and musculoskeletal injuries in the previous 12 months.

## Sample size calculation

The primary outcome measure of pressure pain threshold (PPT) was used to calculate the required a priori sample size for a repeated measures ANOVA using G\* Power Version 3.1 (13). This was based on the effect size of d=0.6 for change in pressure pain threshold with dynamic resistance exercise reported in a previous meta-analysis (53). To achieve a power of 95% at an alpha level of 0.05, including four conditions and three measurement timepoints, a total of 12 participants was required.

## Experimental design

A randomised crossover design was used that involved 8 acute experimental trials. All participants first completed a familiarisation session involving a medical health screening, collection of anthropometric data, calculation of unilateral 1RM strength and familiarisation to all protocols. Participants then attended 4 experimental trials in a randomised order, determined using a random number generator (https://www.random.org). For each trial participants attended a 24h follow up trial and were instructed to refrain from any exercise in the period between the trial and follow-up. All experimental trials were separated by a minimum of 72h.

## Experimental protocol

Participants arrived at the laboratory having fasted for 2h prior. Following 5min of rest in a seated position, a venous blood sample was collected from an antecubital vein in a plasma separator tube. Haematocrit was measured to determine plasma volume. PPT was then assessed at multiple body sites using a handheld pressure algometer (Wagner Instruments, CT, United States). Following this, participants performed a warm-up of 2min of unloaded cycling on a stationary ergometer followed by 10 repetitions of unilateral leg press exercise on the dominant limb at 15% 1RM. Following 2min of rest, participants performed the resistance exercise protocol assigned to that trial. PPT assessment and venous blood sample collection were repeated at 5min and 10min post-exercise, respectively. During the 24h follow up trial, PPT assessment and venous blood sample collection were repeated (Figure 1).

## Resistance exercise protocols

Participant’s unilateral concentric 1RM was assessed during the familiarisation as previously described (30). Four resistance exercise protocols were used in a randomised order, including: 1) light load resistance exercise (LLRE); 2) BFR-RE with low pressure (BFR40); 3) BFR-RE with high pressure (BFR80) and 4) heavy load resistance exercise (HLRE). All exercise was performed with the dominant lower limb. Contractions were performed through 0-90° range of knee flexion through a contraction cycle of 1.5s concentric and 1.5s eccentric, assessed using a metronome. During the LLRE and BFR40 and BFR80 trials, participants performed 4 sets (30, 15, 15 and 15 repetitions, respectively) of unilateral leg press exercise at 30% 1RM with 30s inter-set rest periods. During the BFR40 and BFR80 trials, BFR was applied continuously throughout all exercise and rest periods at 40% and 80% of limb occlusion pressure (LOP), respectively. During the HLRE trial, participants performed 4 x 10 repetitions of unilateral leg press exercise at 70% 1RM. An interset rest period of 53s was used for the HLRE trial, to ensure that the overall exercise duration was equal across all protocols and thus post-exercise measures were conducted at the same time relative to exercise completion. Both protocols were designed consistent with recommendations for each type of exercise (14, 54). Exercise volume (kg) was calculated as: repetitions x exercise load (kg).

## Blood flow restriction

An automatic Personalised Tourniquet system (Delfi Medical Inc, Vancouver, BC, Canada) was used to apply BFR. This system is designed to automatically calculate LOP, defined as the minimum pressure required for full arterial occlusion of a limb (1), and has clinically acceptable accuracy and high reliability (24, 49). This system has a dual purpose variable contour cuff (11.5 x 86 cm) connected by airtight hose tubing to a Personalised Tourniquet device and automatically regulates pressure within acceptable limits (30). With participants positioned on the leg press (24) the cuff was applied to the most proximal portion of the limb and LOP was calculated. LOP was calculated at both the BFR40 and BFR80 trial. Following 2min rest, the cuff was inflated prior to beginning exercise.

## Pressure pain threshold

All PPT measurements were conducted with the participants seated with both arms resting on the thighs (67–70). PPTs were assessed using a handheld pressure algometer (Wagner Instruments, CT, United States) with a stimulation area of 1cm2 at a pressure rate increase of approximately 1kgf/s. Assessment of PPT using a handheld algometer has previously been shown to be valid and reliable (34, 52). PPT assessment sites were located and marked in the middle of the dominant and non-dominant quadriceps muscles (20cm proximal to the base of the patella), the middle of the dominant biceps brachii muscle (10cm proximal to the cubital fossa) and in the non-dominant upper trapezius muscle (10cm for the acromion in direct line with the neck) (67–70). Participants were instructed to give a verbal notification of “now” when the pressure was first perceived as painful, and the PPT was defined as the kgf at this point. Two PPT assessments were completed at each site, with 20s intervals between assessments, and the average was used for analysis (67–69). All measurements were taken by the same assessor. During the familiarisation, PPTs were assessed 3 times at 10min intervals at each site to estimate intrarater reliability using intraclass correlation coefficients (ICC). Minimum detectable change (MDC) and standard error of measurement (SEM) were calculated (Table 1).

## Rating of perceived exertion and pain

Participant’s rating of perceived exertion (RPE) and pain were assessed using Borg’s scales (4) immediately following each set of exercise as previously described (30). ﻿For RPE, it was explained to participants that a rating of 6 meant they felt no exertion, and 20 meant they were giving maximal effort and could not exert themselves any further (30). ﻿For pain, participants were informed that 10 was their reference point, and a score of 11 (absolute maximum, highest possible intensity pain) could be given if the pain was worse than they had ever felt before (30). Participants were asked to score the pain experienced in the quadriceps of the exercising limb only.

## Beta-endorphin and 2-arachidonoylglycerol levels

Venous blood samples were collected into vacutainers containing EDTA-Na2 (6mg) and immediately centrifuged for 15min at 1000 x g at 4°c. Plasma supernatant was aliquoted to eppendorf tubes and stored at -80°c until assays were conducted for analysis.

Plasma BE levels were determined using a commercially available enzyme immunoassay kit following standard published procedures (Diagenics, Milton Keynes, London, UK). The detection range was 15.652-1000pg/ml with a sensitivity of <9.375pg/ml and no observed significant cross-reactivity or interference with analogues. The inter- and intra-assay coefficients of variation were <10% and <8% respectively. All BE assays were run in duplicate.

Plasma 2AG levels were determined using a commercially available enzyme immunoassay kit following standard published procedures (MyBioSource, San Diego, CA, USA). The detection range was 3.70-300ng/ml with a sensitivity of <1.44ng/ml and no observed significant cross-reactivity or interference with analogues. The inter- and intra-assay coefficients of variation were <12% and <10% respectively. All 2AG assays were run in duplicate.

## Plasma volume

Plasma volume was determined to correct blood marker concentrations for any changes in plasma volume with exercise. A capillary blood sample was taken, and haematocrit was measured using a micro-haematocrit centrifuge and a haematocrit reader (Hawksley, Sussex, UK). The percent changes in plasma volume were calculated using the following formula: % change in plasma volume = (100/[100 Hct pre])\*100([Hct pre - Hct post]/Hct post) (3).

**Mediation analyses**

We conducted mediation analyses with a multicategorical independent variable (exercise intervention) to examine the impact of change in plasma beta-endorphin, 2AG and muscle discomfort during exercise on the effect of exercise intervention on change in PPT from pre-exercise to 5 min post-exercise (Figure 1). Pathway C, referred to as the “total effect”, describes the observed effect of the independent variable on the dependent variable. The total effects are comprised of a direct effect pathway (Pathway C’) of the independent variable on the dependent variable, and a total indirect pathway (mediated pathway A+B) of the independent variable on the dependent variable through the mediator. We employed the Baron and Kenny method (2) which involves conduction of several regression analyses, and examined the relevant coefficients at each step. With this method, mediation can be said to be present when the following conditions hold in sequential regression analyses: 1) The independent variable (exercise intervention) is associated with the dependent variable (PPT) in the absence of the mediator; 2) The independent variable (exercise intervention) is associated with the mediator variable; 3) The mediator variable is associated with the dependent variable (PPT); 4) the observed effect of the independent variable on the outcome shrinks when the mediator is added to the model. Using this method, it is possible to estimate the coefficient for the indirect effect by calculating the product of the standardised regression coefficient (Path A) of exercise intervention on the mediator with the regression coefficient (Path B) of the mediator variable on the dependent variable (Path A\*B). The regression coefficient for path A\*B represents the change in the dependent variable related to the independent variable that is mediated by the mediator. Bootstrapping methods, which involved resampling of the data 5,000 times and is considered to be the least vulnerable to type I errors, were used to calculate the 95% confidence intervals (CIs) of the coefficients for the total, indirect and direct effect (57).

## Statistical analysis

All statistical analysis was performed with IBM SPSS Statistics Version 24.0 (IBM Corp, Chicago, IL, USA). Data are presented as mean ± SD with 95% CIs unless stated otherwise. The effect of the number of experimental trials on baseline PPTs was analysed with a two-way repeated measures ANOVA with session (1-4) and assessment site (dominant quadriceps, non-dominant quadriceps, dominant biceps and non-dominant trapezius) as within subject factors. The effect of resistance exercise on PPTs was assessed using a three-way repeated measures ANOVA with condition (LL, BFR40, BFR80, HL-RE), time (pre-exercise, 5min post and 24h post) and assessment site (dominant quadriceps, non-dominant quadriceps, dominant biceps and non-dominant trapezius) as within-subject factors. If a statistically significant three-way interaction was found, two-way repeated measures ANOVAs with Bonferroni correction were used to examine the effect of exercise condition on PPT, at each assessment site. RPE and perceived pain were each assessed using repeated measures ANOVA with condition (LL, BFR40, BFR80, HL-RE) and time (Set 1-4) as within-subject factors. Plasma BE and 2AG concentration were each assessed using repeated measures ANOVA with condition (LL, BFR40, BFR80, HL-RE) and time (pre-exercise, 5min post and 24h post) as within-subject factors. Exercise volume was assessed using repeated measures ANOVA with condition as the within subject factor. For any statistically significant interaction, paired sample t-tests with Bonferroni correction were used for post-hoc analysis. Alpha significance was set a priori p<0.05. Effect sizes were described using Cohen’s d, calculated by dividing the mean difference between two measures by the pooled SD of the differences, and described as: weak < 0.2, weak to moderate 0.2–0.4, moderate 0.4–0.65, moderate to strong 0.65–0.7 and strong > 0.8 (59). For mediation analyses, the SPSS macro PROCESS (Model 4) was applied with the three mediators. Dummy coding was employed as the independent variable (exercise intervention) is multicategorical, and analysis was run to account for dependency of individuals across exercise conditions. The regression/path coefficients are all presented in unstandardised form, as recommended by Hayes (17). Coefficients were considered statistically significant if the confidence intervals did not cross zero (57).

Results

## Exercise parameters

All 12 participants completed all experimental trials with no adverse events. Exercise load and total exercise volume were greater in the HLRE trial. Exercising BFR pressure was greater in BFR80 compared to BFR40 (Table 2).

## Baseline PPTs

No differences in baseline PPTs were found between the 4 experimental trials (*F* (3,33) = 0.098, p=0.981, d=0.1). A main effect of assessment site was found (*F* (3,33) = 99.136, p<0.01, d=0.9). PPTs were higher at the dominant and non-dominant quadriceps sites compared to the biceps brachii and trapezius sites (both p<0.01, d=0.9).

## Change in PPTs with resistance exercise

A three-way interaction effect was found for condition, assessment site and time (*F* (18,198) = 13.492, p<0.01, d=0.6). There was a two-way interaction effect for the dominant quadriceps site (Figure 2). At 5 min post-exercise PPTs were increased compared to pre-exercise in all conditions (Figure 2). Post-hoc analysis showed that, compared to LLRE, PPT was 2.43 ± 1.60 kgf higher following BFR40 (p<0.01, d=0.25), 3.72 ± 2.24 kgf higher following BFR80 (p<0.01, d=0.46) and 1.68 ± 1.53 kgf higher following HLRE (p<0.05, 0.18). Following BFR 80, PPT was 1.29 ± 1.18 kgf higher compared to BFR40 (p<0.01, d=0.11) and 2.04 ± 1.75 kgf higher compared to HLRE (p<0.01, d=0.39). At 24h post-exercise, PPTs were 1.39 ± 0.74 kgf (p<0.01, d=0.5) and 2.44 ± 0.65 kgf (p<0.01, d=0.7) higher than pre-exercise values in the BFR40 and BFR80 trials, respectively, whereas PPTs had returned to baseline in the LLRE and HLRE trials.

There was a two-way interaction effect for the non-dominant quadriceps site (Figure 2). At 5 min post-exercise PPTs were increased compared to pre-exercise in all conditions (Figure 2). Compared to LLRE, the increase in PPT was greater following BFR40 (1.49 ± 0.70 kgf, 95%CI: 0.246 to 2.730, p<0.01, d=0.54), BFR80 (1.82 ± 0.60 kgf, 95% CI: 0.333 to 3.300, p<0.01, d=0.65) and HLRE (1.34 ± 0.92 kgf, 95% CI: 0.181 to 2.856, p<0.05, d=0.46). At 24h PPTs had returned to baseline following LLRE (p=0.62, d=0.022), BFR40 (p=0.71, d=0.01), BFR80 (p=0.50, d=0.02) and HLRE (p=0.48, d=0.04).

There was a two-way interaction effect at the biceps site (Figure 2). At 5 min post-exercise PPTs were increased compared to pre-exercise in all conditions (Figure 2). Compared to LLRE, the increase in PPT was greater following BFR40 (0.28 ± 0.13 kgf, 95% CI: 0.157 to 0.391, p<0.01, d=0.21), BFR80 (0.64 ± 0.42 kgf, 95% CI: 0.235 to 1.259, p<0.01, d=0.43) and HLRE (0.34 ± 0.20 kgf, 95% CI: 0.158 to 0.517, p<0.01, d=0.25). At 24h PPTs had returned to baseline following LLRE (p=0.28, d=0.01), BFR40 (p=0.33, d=0.01), BFR80 (p=0.23, d=0.01) and HLRE (p=0.78, d=0.04).

There was a two-way interaction effect at the trapezius site (Figure 2). At 5 min post-exercise PPTs were increased compared to pre-exercise in all conditions (Figure 2). Compared to LLRE, the increase in PPT was greater following BFR40 (0.31 ± 0.27 kgf, 95% CI: 0.057 to 0.563, p<0.05, d=0.25), BFR80 (0.30 ± 0.17 kgf, 95% CI: 0.139 to 0.458, p<0.01, d=0.23) and HLRE (0.26 ± 0.13 kgf, 95% CI: 0.139 to 0.371, p<0.01, d=0.20). At 24h PPTs had returned to baseline following LLRE (p=0.21, d=0.07), BFR40 (p=0.64, d=0.09), BFR80 (p=0.23, d=0.01) and HLRE (p=0.85, d=0.04).

## RPE

RPE increased after each set of exercise compared to the previous set for all conditions (all p<0.01, d range = 0.72-0.88). There was a two-way interaction for RPE (*F* (9,99) = 6.080, p<0.01, d=0.36) (Figure 3). After set 1, compared to LLRE RPE was greater in BFR40 (p<0.05, d=0.56), BFR80 (p<0.01, d=0.79) and HLRE (p<0.05, d=0.61). RPE was greater in BFR80 compared to BFR 40 (p<0.05, d=0.42). After set 2, compared to LLRE RPE was greater in BFR40 (p<0.05, d=0.44), BFR80 (p<0.01, d=0.77) and HLRE (p<0.05, d=0.63). RPE was greater in BFR80 compared to BFR40 (p<0.05, d=0.42). After set 3, compared to LLRE RPE was greater in BFR40 (p<0.01, d=0.65), BFR80 (p<0.01, d=0.82) and HLRE (p<0.05, d=0.73. RPE was greater in BFR80 compared to BFR40 (p<0.01, d=0.68) and HLRE (p<0.01, d=0.64). After set 4, compared to LLRE RPE was greater in BFR40 (p<0.01, d=0.68), BFR80 (p<0.01, d=0.85) and HLRE (p<0.05, d=0.69). RPE was greater in BFR80 compared to BFR40 (p<0.01, d=0.71) and HLRE (p<0.01, d=0.69).

## Muscle discomfort

Muscle discomfort increased after each set of exercise compared with the previous set for all conditions (all p<0.01, d range = 0.70-0.82). There was a two-way interaction for muscle discomfort (*F* (9,99) = 19.072, p<0.01, d=0.63) (Figure 3). After set 1, compared to LLRE muscle discomfort was greater in BFR40 (p<0.01, d=0.35) and BFR80 (p<0.01, d=0.39). Muscle discomfort was greater in BFR80 compared to HLRE (p<0.01, d=0.32). After set 2, compared to LLRE muscle discomfort was greater in BFR40 (p<0.01, d=0.48) and BFR80 (p<0.01, d=0.55). Muscle discomfort was greater in BFR40 (p<0.01, d=0.46) and BFR80 (p<0.01, d=0.57) compared to HLRE. Muscle discomfort was greater in BFR80 compared to BFR40 (p<0.01, d=0.36). After set 3, compared to LLRE muscle discomfort was greater in BFR40 (p<0.01, d=0.53) and BFR80 (p<0.01, d=0.69). Muscle discomfort was greater in BFR40 (p<0.01, d=0.50) and BFR80 (p<0.01, d=0.59) compared to HLRE. Muscle discomfort was greater in BFR80 compared to BFR40 (p<0.01, d=0.48). After set 4, compared to LLRE muscle discomfort was greater in BFR40 (p<0.01, d=0.71) and BFR80 (p<0.01, d=0.82). Muscle discomfort was greater in BFR40 (p<0.01, d=0.59) and BFR80 (p<0.01, d=0.66) compared to HLRE. Muscle discomfort was greater in BFR80 compared to BFR40 (p<0.01, d=0.59).

## Plasma BE and 2AG levels

There were no changes in plasma volume following exercise across all trials (all p<0.05). There was a two-way interaction for BE (*F* (1.545,16.991) = 82.552, p<0.01, d=0.88). There was an increase in BE at 5 min post-exercise in BFR40 (p<0.01, d=0.55), BFR80 (p<0.01, d=0.59) and HLRE (p<0.01, d=0.24). The increase in BE was significantly greater following BFR40 (p<0.01, d=0.47) and BFR80 (p<0.01, d=0.49) compared to HLRE. There were no differences in BE at 24h post-exercise compared to pre-exercise following LLRE (p=0.72, d=0.01), BFR40 (p=0.62, d=0.01), BFR80 (p=0.47, d=0.02) and HLRE (p=0.60, d=0.01).

There was no two-way interaction for 2AG (*F* (1.679,18.472) = 0.640, p=0.698, d=0.06). There were no changes in 2AG across timepoints in any condition (all p>0.05, d range = 0.01-0.06) except for HLRE, where 2AG level at 24h post-exercise was lower compared to pre-exercise (p<0.05, d=0.1).

**Mediation analyses**

For beta-endorphin, the bootstrap CI for estimate of the coefficient for the indirect effect of beta-endorphin on change in PPT with exercise was above zero for the BFR40, BFR80 and HLRE trials, indicating a statistically significant mediation (Table 3). The model analyses suggest that plasma beta-endorphin mediates approximately 42% of the total effect of exercise intervention on change in PPT. As repeated measures ANOVA demonstrated no significant within- or between-group changes in 2AG concentration, mediation analyses were not run for this mediator due to the lack of variance. For muscle discomfort, the bootstrap CI for estimate of the coefficient for the indirect effect of muscle discomfort on change in PPT with exercise was above zero for the BFR40 and BFR80 trials, indicating a statistically significant mediation (Table 3). The model analyses suggest that muscle discomfort mediates approximately 58% of the total effect of BFR exercise on change in PPT.

Discussion

There are several novel findings in the present study. Both low and high pressure BFR and HLRE augmented the increase in pain threshold in the exercising limb compared to LLRE (26-48% vs 10%). High pressure BFR resulted in the greatest increase in PPT in the exercising limb (48%). A systemic effect was observed to less extent than the exercising limb, which was comparable to HLRE (10-18%) with both low and high pressure BFR-RE (11-17% and 11-21%, respectively). Whereas PPTs had returned to baseline at 24 hours following LLRE and HLRE, following low and high pressure BFR-RE the PPTs were elevated relative to baseline in the exercising limb only (15% and 24%, respectively). No alterations in circulating 2-AG concentration were found; however, post-exercise BE concentration was increased for both low (21%) and high (23%) pressure BFR-RE conditions compared to LLRE (0.4%) and HLRE (5%). Finally, beta-endorphin and muscle discomfort were found to mediate the relationship between exercise intervention and change in PPT.

In the present study there was an increase in PPT in exercising and non-exercising muscles across all exercise conditions (43, 67–70). Findings from the present study also support the notion that both local and central inhibitory nociceptive pathways contribute to systemic hypoalgesia following exercise (27, 70). As hypothesised, greater increases in PPTs were observed with HLRE (10-26%) compared to LLRE (1-10%) (20, 37, 38). Performing LLRE with low and high pressure BFR resulted in greater increases in PPTs (11-34% and 11-48%, respectively), which is in line with previous research demonstrating greater pain reduction during BFR-RE compared to LLRE in individuals with anterior knee pain (42). The increase in PPTs during both BFR-RE conditions were similar to HLRE at all assessment sites, except for the exercising limb where high pressure BFR appeared to augment the change (48% vs 26% for BFR80 and HLRE, respectively). This may partially explain the findings of recent studies in load compromised populations where high pressure BFR (60-80% LOP) has been shown to reduce pain more than HLRE over a training programme (9, 15, 28, 31).

The hypoalgesia response to exercise is typically diminished at 30-45 minutes following exercise (53). A reduction in pain sensitivity at 45 minutes following BFR-RE has been observed in individuals with anterior knee pain (42), and recent evidence in patients following knee ligament surgery suggests pain may be reduced for up to 24 hours post-exercise (25, 29). A particularly novel finding in the present study is that PPT in the exercising limb remained elevated above baseline at 24 hours following BFR-RE only (15% and 24% for low and high pressure BFR-RE). This may explain previous findings where patients have reported reduced pain the day after performing BFR-RE (25, 29). However, this persistent elevation in PPT is likely not related to endogenous opioid production, which had returned to baseline at 24 hours post-exercise in the present study. Moreover, though it was not measured we did not anticipate muscle discomfort to be present in the exercising limb the following day, therefore it is unlikely that the prolonged hypoalgesia at 24 hours post-exercise is mediated in a CPM manner. Limitation of this effect to only the limb that the BFR stimulus was applied to suggests that prolonged hypoalgesia is primarily related to activation of local or segmental pain inhibitory mechanisms, rather than a systemic mechanism.

Several mechanisms of EIH have been proposed, including recruitment of high threshold motor units (19), a link between baroreceptors and pain pathways (40), ischemic and metabolite induced pain (56) and conditioned pain modulation (68). The intensity of exercise is acknowledged as an important determinant of the magnitude of EIH, with higher intensity exercise resulting in greater EIH (20, 37, 38). The present study provides evidence of greater EIH with BFR-RE compared to LLRE without BFR, for which several explanations are possible. The BFR stimulus may have acted as a noxious conditioning stimulus in a CPM mechanism, whereby ‘pain inhibits pain’. Perception of discomfort and pain is exacerbated during BFR-RE (44) to a similar or greater extent as HLRE (23, 48). This is often attributed to stimulation of group III and IV afferent fibres and a subsequent increase in sympathetic nervous activity and perception of pain and discomfort (65). Muscle discomfort was greater in the exercising limb during both BFR-RE conditions, and results of the mediation analysis demonstrate that muscle discomfort mediates the relationship of exercise intervention on change in PPT, which indicates a link between the pain and discomfort generated during exercise and the EIH response. The greater increase in PPT alongside greater discomfort scores in the exercising limb within the high pressure BFR condition compared to the low pressure BFR condition would support this. This would also agree with existing research which shows the magnitude of EIH to be greater following painful compared to non-painful exercise (8, 19). BFR-RE is often associated with a sensation of numbness; it has been suggested that pressure applied to the peripheral nerve results in ischemia and nerve conduction blockage (46), which could possible alter the sensation of pain with BFR-RE. Though we did not measure nerve conduction velocity in the present study, Clark et al. (5) found no changes in nerve conduction velocity before and after 4 weeks of BFR-RE resistance training. Furthermore, as any side effects of nerve compression are rapidly reversible (46) it is unlikely that the elevation of PPTs at 24h following BFR-RE in the present study is due to alterations in neural function. Other possible mechanisms of a greater EIH response with BFR-RE include increased blood pressure, metabolite accumulation and recruitment of fast-twitch fibres, all of which are associated with BFR exercise (27); however this cannot be determined from the present study.

Consistent with previous human research (35, 66) the current study demonstrated significant elevation of peripheral blood BE concentration following low (21%) and high (23%) pressure BFR-RE, suggesting involvement of an opioid-mediated mechanism in EIH with BFR-RE. The results of the mediation analyses would support this. Activation of the endogenous opioid system and stimulation of BE production may contribute to the EIH response (39, 62) by inhibiting noxious-evoked activity (61). The body produces BE in response to physiological stressors such as pain and exercise; the latter is thought to be caused by stimulation of group III and IV afferent fibres in contracting muscle during exercise. A high level of metabolic stress is generated during BFR-RE, indicated by ischemia, hypoxia, intramuscular accumulation of metabolites and a reduction in tissue pH (64). This may activate group III and IV afferent fibres and is thought to drive the greater perception of intensity and discomfort associated with BFR-RE. Indeed, in the present study participants reported greater RPE during the BFR-RE conditions compared to LLRE. Increased perception of exercise with BFR exercise is often attributed to stimulation of group III and IV afferent fibres and afferent feedback (23). However, as highlighted by Marcora (47), a significant body of evidence suggests that perception of effort is independent from afferent feedback, and the exact mechanism during BFR exercise has not been objectively examined to date. Nevertheless, alongside an increased perception of discomfort in the exercising muscle, increased perception of effort may have contributed to stimulation of endogenous opioid production. The findings of Heyman et al. (18) would support this notion, as the authors observed an increase in BE concentration only when the exercise intensity was high. It is important to note that endorphins do not appear to cross the blood-brain barrier therefore cannot be regarded as indicative of central effects, which is a limitation of examining BE in systemic circulation in relation to EIH.

Contrary to our hypothesis, we did not observe a change in peripheral blood 2-AG concentration following exercise in any of the experimental conditions. Previous research has shown no changes in circulating 2-AG concentration following intense exercise (18) when another endocannabinoid, known as anandamide, has been found to increase (18), which may be determined by their different metabolic pathways (55). Furthermore, animal research shows that by manipulating the duration and intensity of the exercise stressor, it is possible to elicit either opioid or non-opioid mechanisms of hypoalgesia (7, 21, 36, 51). Therefore, the properties of the exercise itself may be an important factor in determining activation of endogenous pain inhibitory mechanisms.

It is important to acknowledge some limitations in the present study. Assessment of pressure pain threshold and thus EIH was conducted using a mechanical noxious stimulus, which is one of several possible methods. The results of the present study may therefore not be considered generalisable to other measures of EIH; however, similar EIH responses are observed across different methods of stimulation (53). We only examined a single biomarker from the opioid and ECB systems, therefore the effect of BFR-RE on other opioid and ECB substances remains unknown. Finally, the results are generalisable to non-injured and pain-free adults only, and it is unclear whether chronic pain populations would yield similar results. However, despite these limitations several important implications can be drawn from the present study. Though EIH is typically greatest with higher intensity and prolonged exercise, our results show that addition of BFR to LLRE can maximise the EIH response achieved during low intensity exercise to a similar or greater extent as HLRE. This has important implications for individuals with acute and chronic pain who may be load compromised, i.e. following surgery or older adults with chronic pain. Our results also provide evidence of systemic hypoalgesia after unilateral exercise, which suggests load compromised individuals with acute or chronic pain may gain a pain relief benefit by exercising unaffected or pain-free limbs. Finally, our results suggest that BFR-RE may prolong the hypoalgesia effect in the limb to which BFR is applied for up to 24 hours. This could be important for allowing other rehabilitation training to take place and to improve the quality of life of patients in the days between exercise training.

In conclusion, high pressure BFR-RE caused the greatest EIH response in the exercising limb and a systemic effect was observed to less extent, which was comparable to HLRE with both low and high pressure BFR-RE. BFR-RE with a low and high pressure prolonged the hypoalgesia effect for up to 24 hours in the limb to which BFR was applied. The mechanisms underlying EIH with BFR-RE may be linked to the opioid system and a conditioned pain modulation effect rather than endocannabinoid-mediated mechanisms and associated with the augmented physiological stress of BFR-RE. Overall, for the first time this study provides evidence for the mechanisms and hypoalgesic effect of BFR-RE and highlights it’s possible usefulness as an exercise intervention for load compromised, acute and chronic pain populations.

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Author contributions

LH and SDP designed the study. LH recruited and tested participants. Data was analysed by LH and SDP. Both LH and SDP contributed to the manuscript, reviewed it and approved the content of the final version.

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**Figures legends**

**Figure 1.** Model demonstrating the potential mediating effect of beta-endorphin, 2-arachidonoylglycerol and discomfort on the relationship between exercise intervention and pressure pain threshold. M1-3 = mediator; A1-3 = effect of independent variable on the mediator; B1-3 = effect of mediator on dependent variable; C’ = direct effect. BE = beta-endorphin; 2AG = 2-arachidonoylglycerol; PPT = pressure pain threshold.

**Figure 2.** A-D) Mean ± SD at each timepoint and E-H) individual change in PPT, at each assessment site, for each experimental condition (n=12). † = significantly increased compared to pre-exercise (p<0.01); \* = significantly greater than LLRE (p<0.01); ‡ = significantly greater than other conditions (p<0.01); # = significantly higher than LLRE and HLRE (p<0.01). PPT; pressure pain threshold; LLRE; light load resistance exercise; BFR40; blood flow restriction at 40% limb occlusion pressure; BFR80; blood flow restriction at 80% limb occlusion pressure; HLRE; heavy load resistance exercise.

**Figure 3.** A-B) Mean ± SD after each set of exercise and C-D) individual change in RPE and muscle discomfort for each experimental condition (n=12). † = significantly greater than LLRE (p<0.01); ‡ = significantly greater HLRE (p<0.01); # = significantly higher than BFR40 (p<0.01). RPE; rating of perceived exertion; LLRE; light load resistance exercise; BFR40; blood flow restriction at 40% limb occlusion pressure; BFR80; blood flow restriction at 80% limb occlusion pressure; HLRE; heavy load resistance exercise.

**Figure 4.** A-B) Mean ± SD and C-D) individual change in blood plasma concentration of beta-endorphin and 2-arachidonoylglycerol at pre-exercise, 5min and 24h post-exercise for each condition (n=12). \* = significantly different to pre-exercise (p<0.01); † = significantly greater than HLRE (p<0.01). LLRE; light load resistance exercise; BFR40; blood flow restriction at 40% limb occlusion pressure; BFR80; blood flow restriction at 80% limb occlusion pressure; HLRE; heavy load resistance exercise.

**Table legends**

**Table 1.** Baseline blood plasma biomarker and pressure pain threshold values (Mean ± SD).

**Table 2.** Exercise parameters and BFR pressures (Mean ± SD).

**Table 3.** Coefficients, confidence intervals and significance statistics for mediation analyses.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Baseline pressure pain threshold (kgf/cm2)** |  |  |  |  |
| **Assessment site**  Dominant quadriceps  Non-dominant quadriceps  Dominant biceps brachii  Non-dominant trapezius | **Value**  9.64 ± 2.32  9.49 ± 2.54  3.93 ± 1.27  4.57 ± 1.20 | **ICC**  0.97  0.96  0.94  0.95 | **SEM**  0.10  0.12  0.21  0.15 | **MDC**  0.27  0.33  0.58  0.41 |
| **Resting blood plasma markers** |  |  |  |  |
| BE concentration (pg/mL)  2-AG concentration (ng/mL) | 90.29 ± 16.98  3.99 ± 0.78 | - | - | - |

ICC, intraclass correlation coefficient; SEM, standard error of measurement; MDC, minimal detectable change; BE, beta-endorphin; 2-AG, 2-arachidonoylglycerol.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **LLRE** | **BFR40** | **BFR80** | **HLRE** |
| 1RM (kg)  Exercise load (kg)  Exercise volume (kg)  BFR pressure (mmHg)  LOP  Exercising pressure | 156 ± 52  47 ± 16  3516 ± 1168  -  - | 156 ± 52  47 ± 16  3516 ± 1168  200 ± 16  80 ± 6 | 156 ± 52  47 ± 16  3211 ± 1206  197 ± 13  158 ± 10\* | 156 ± 52  109 ± 36\*  4375 ± 1454\*  -  - |

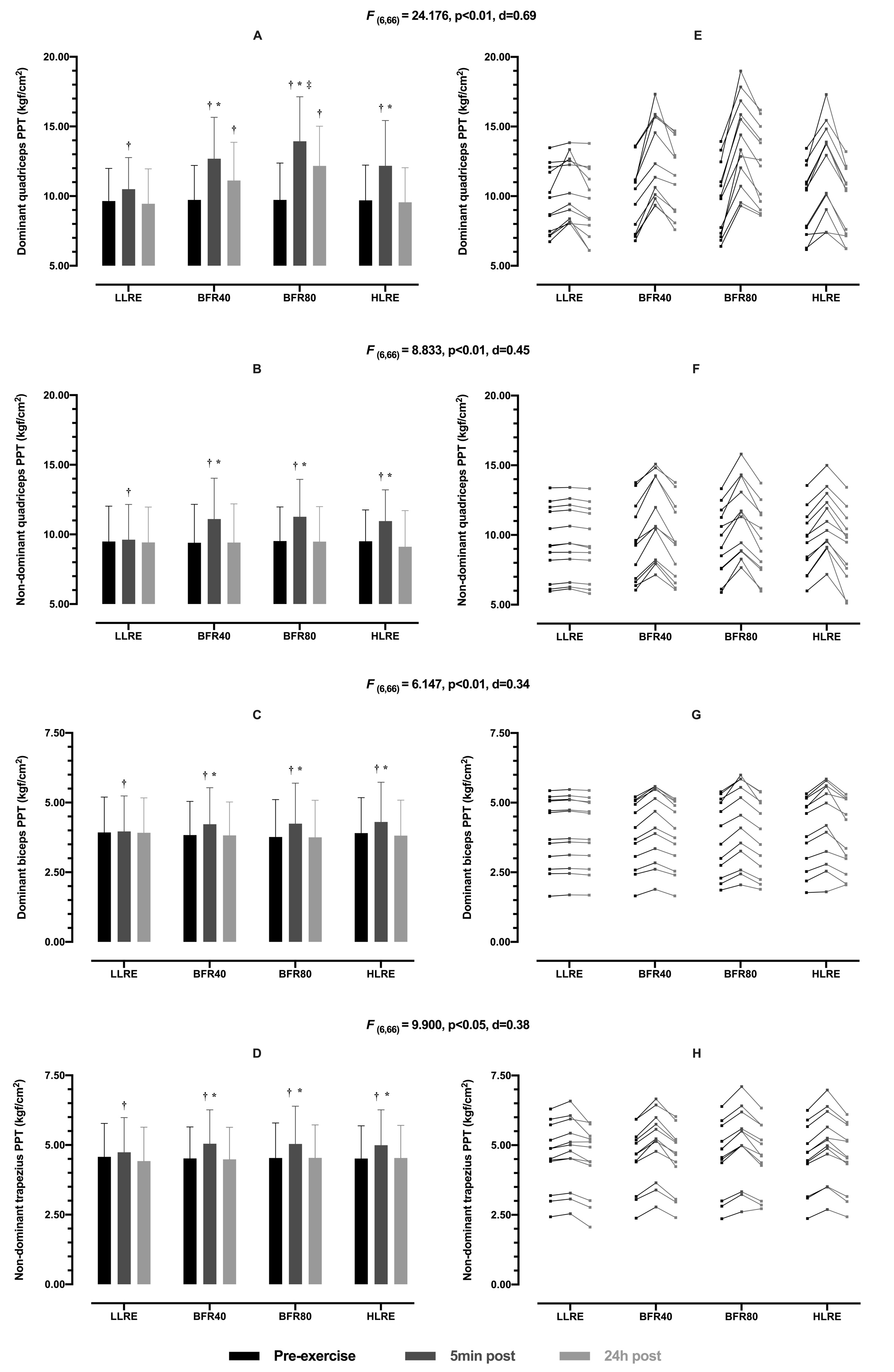
\* = significantly greater compared to other trials (p<0.05). 1RM; One repetition maximum; BFR; Blood flow restriction; LOP; Limb occlusion pressure; LLRE; Light load resistance exercise; BFR40; Blood flow restriction exercise at 40% limb occlusion pressure; BFR80; Blood flow restriction exercise at 80% limb occlusion pressure; HLRE; Heavy load resistance exercise.

|  |  |  |  |
| --- | --- | --- | --- |
| **Outcome measure** | **Total effect (C path)** | **Direct effect (Path C’)** | **Indirect effect (Path A+B)** |
| **Beta-endorphin**  LLRE (Constant)  BFR40  BFR80  HLRE  **Muscle discomfort**  LLRE (Constant)  BFR40  BFR80  HLRE | 2.35 (1.01, 3.68)\*  3.64 (2.31, 4.98)\*  1.62 (0.29, 2.96)\*  2.35 (1.01, 3.68)\*  3.64 (2.31, 4.98)\*  1.62 (0.29, 2.96)\* | -0.34 (-2.60, 1.91)  0.78 (-1.57, 3.14)  1.09 (-0.20, 2.38)  -0.54 (-1.81, 0.74)  -2.78 (-4.84, -0.60  1.30 (0.92, 1.69) | 2.68 (0.99, 5.68)\*  2.86 (1.10, 6.09)\*  0.54 (0.18, 1.23)\*  2.88 (1.31, 5.12)\*  6.36 (3.74, 9.24)\*  0.33 (-0.27, 1.04) |

\* = p<0.05. LLRE; light load resistance exercise; BFR40; blood flow restriction at 40% limb occlusion pressure; BFR80; blood flow restriction at 80% limb occlusion pressure; HLRE; heavy load resistance exercise.

A close up of a map

Description automatically generated



A screenshot of a cell phone

Description automatically generated

A screenshot of a cell phone

Description automatically generated