**Title: Aerobic exercise with blood flow restriction causes local and systemic hypoalgesia and increases circulating opioid and endocannabinoid levels**

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**Abstract**

**Aim:** This study examined the effect of aerobic exercise with and without blood flow restriction (BFR) on exercise-induced hypoalgesia and endogenous opioid and endocannabinoid systems.

**Methodology:** In a randomised crossover design, pain-free individuals performed 20 min of cycling in four experimental trials: 1) Low intensity aerobic exercise (LI-AE) at 40% V̇O2max; 2) LI-AE with low pressure BFR (BFR40); 3) LI-AE with high pressure BFR (BFR80); and 4) High intensity aerobic exercise (HI-AE) 70% V̇O2max. Pressure pain thresholds (PPT) were assessed before and 5 min post-exercise. Circulating concentrations of beta-endorphin and 2-arachidonoylglycerol were assessed before and 10 min post-exercise.

**Results:** In the exercising legs, post-exercise PPTs were increased following BFR40 and BFR80 compared to LI-AE (23-32% vs 1-2% increase, respectively). The increase in PPTs were comparable to HI-AE (17-20% increase) with BFR40 and greater with BFR80 (30-32% increase). Both BFR80 and HI-AE increased PPTs in remote areas of the body (Increase of 26-28% vs 19-21%, respectively). Post-exercise circulating beta-endorphin concentration was increased following BFR40 (11%) and HI-AE (14%), with the greatest change observed following BFR80 (29%). Post-exercise circulating 2-arachidonoylglycerol concentration was increased following BFR40 (22%) and BFR80 (20%), with the greatest change observed following HI-AE (57%).

**Conclusion:** Addition of BFR to LI-AE can trigger both local and systemic hypoalgesia that is not observed follow LI-AE alone and activate endogenous opioid and endocannabinoid systems of pain inhibition. Compared to HI-AE, local and systemic hypoalgesia following LI-AE with high pressure BFR is greater and comparable, respectively. LI-AE with BFR may help pain management in load compromised individuals.

**New & noteworthy**

We have shown that performing BFR during low intensity aerobic exercise can trigger local and systemic hypoalgesia, which is not typically observed with this intensity of exercise. High pressure BFR triggers greater and comparable hypoalgesia than high intensity aerobic exercise in the exercising limbs and remote areas of the body, respectively. Performing BFR during low intensity aerobic exercise activates the opioid and endocannabinoid systems, providing novel insight into potential mechanisms of hypoalgesia with BFR exercise.

**1. Introduction**

Exercise-induced hypoalgesia (EIH) describes an acute reduction in pain sensitivity and/or perception of pain intensity to a noxious stimulus following exercise (52). It is a transient effect occurring as a consequence of neurophysiological mechanisms involved in processing of noxious stimuli (33). One hypothesised mechanism is enhanced descending pain inhibition via activation of endogenous pain inhibition systems, which secrete substances that have antinociceptive effects (38). These substances include endorphins (e.g. beta-endorphin ﻿[β-EP] within the opioid system) and endocannabinoids (e.g. 2-arachidonoylglycerol [2AG] within the endocannabinoid system), which have both been found to increase in circulating concentration following exercise (14, 17, 38). While the mechanisms are not well established, it is clear that a single bout of either resistance or aerobic exercise triggers hypoalgesia for up to 30 minutes in healthy pain-free individuals (52). The EIH response is more variable in individuals with chronic pain, with pain sensitivity and intensity either decreasing, remaining unchanged or even increasing (i.e. hyperalgesia) in response to exercise (38). Nevertheless, exercise is widely used as a therapeutic strategy for individuals with chronic pain (15).

Aerobic exercise is most commonly studied in relation to EIH (52). It can reduce pain sensitivity in both exercising (i.e. a local effect) and non-exercising (i.e. a remote/systemic effect) areas of the body (32, 51). Larger hypoalgesia responses are consistently observed in areas closer to the exercising muscle (52) and higher intensity, longer duration aerobic exercise appears to maximise the hypoalgesia effect (19, 32–34, 51). Hoffman et al. (19) compared pain intensity ratings during a pressure-pain stimulus before and after cycling for 30 min at 75% of maximal oxygen consumption (V̇O2max), 10 min at 75% V̇O2max and 30 min at 50% V̇O2max. The authors found that pain ratings reduced only after 30 min of cycling at 75% V̇O2max. In consonance with this, Naugle et al. (33) reported that larger effect sizes were found for change in pressure pain threshold when aerobic exercise was performed at a high intensity (i.e. 75% V̇O2max) and for a longer duration (i.e. >10 min), while lower intensities (e.g. 50% V̇O2max) and shorter durations produced a comparatively smaller effect. However, for load compromised individuals such as those with chronic pain or following surgery, higher intensity and longer duration exercise may not be feasible.

Performing blood flow restriction (BFR) during low intensity exercise may augment the hypoalgesia response (21, 22, 30, 44). BFR exercise involves partial and full restriction of arterial and venous blood flow, respectively, in the active limb during exercise (23). This is achieved by placing a pneumatic tourniquet cuff proximally on the exercising limb and inflating it to a pre-determined level of limb occlusion pressure (LOP) (35). Using a unilateral lower limb exercise model, we recently examined the EIH response to resistance exercise with and without BFR in pain-free individuals using a mechanical noxious stimulus (22). Compared to low intensity resistance exercise alone, BFR exercise produced greater local and remote hypoalgesia that was comparable to the effects of high intensity resistance exercise (22). Interestingly, a higher BFR pressure (i.e. 80% LOP) produced the greatest hypoalgesia effect, suggesting a possible effect of BFR pressure, however this was found to the exercising limb only. Circulating concentration of β-EP increased following BFR exercise while no changes were observed in 2AG, suggesting that hypoalgesia with BFR exercise may be driven by opioid-related mechanisms. Furthermore, the level of muscle discomfort generated during BFR exercise partially mediated the magnitude of the hypoalgesia response, possibly suggesting a conditioned pain modulation effect (51).

If addition of BFR to low intensity aerobic exercise (BFR-AE) can augment the hypoalgesia effect in a similar manner, this may be favourable for load compromised populations who cannot tolerate high intensity aerobic exercise for long durations. This would be particularly valuable in conjunction with the potent muscular and aerobic adaptations that occur with BFR-AE training (35). Therefore, the aim of this study was to compare the magnitude of EIH with BFR-AE using different BFR pressures, to aerobic exercise at low and high intensities without BFR. We also examined muscle discomfort, β-EP and 2AG as possible mechanisms. It was hypothesized that: 1) BFR-AE would increase the EIH response compared with LI-AE and would be comparable to HI-AE; 2) A higher BFR pressure would lead to greater EIH response compared to a lower BFR pressure; and 3) BFR exercise would increase circulating concentrations of β-EP but not 2AG.

**2. Materials and methods**

**2.1 Participants**

Twelve recreationally active males were recruited to participate in this study (mean ± standard deviation: age = 27 ± 6 y; height = 182.3 ± 6.8 cm; weight = 79.4 ± 10.6 kg; body mass index = 23.5 ± 2.5 kg/m2; V̇O2max = 47.26 ± 5.91 ml/kg/min. As some studies suggest that there are sex differences in the EIH response (2, 39), and the purpose of this study was to investigate the effect of a novel exercise intervention, only males were included in the present study. All participants provided written informed consent in compliance with the Declaration of Helsinki (57) and all protocols were approved by the University Research Ethics Committee. All were non-smokers free from metabolic, cardiovascular, neurological and pulmonary disease and musculoskeletal injury in the previous 12 months. Participants refrained from caffeine, alcohol, and strenuous exercise in the 24 h prior to experimental trials and maintained normal dietary habits throughout the study duration.

**2.2 Power calculation**

The primary outcome measure of mechanical pressure pain threshold (PPT) was used to calculate the required sample size a priori using G\* Power (11). An effect size of d=0.46 was used for this calculation, which is the effect size for the difference in PPT between BFR exercise and low intensity exercise reported previously (22). To achieve a power of 80% at an alpha level of 0.05 with a two way (4 x 2) repeated measures ANOVA, and account for a 10% attrition rate, a total of 12 participants were required.

**2.3 Experimental design and protocol**

Participants were required to complete five exercise trials consisting of a lactate threshold and graded exercise test followed by four experimental trials in a randomised, counterbalanced, crossover design. Participants were familiarised to all testing protocols in the first session prior to the graded exercise test. Experimental trials were separated by 72 h. During the first session, participants completed a warm-up of 5 min cycling at a workload of 75 W with a pedalling frequency of 75 rpm. This was followed by a lactate threshold and graded maximal exercise test on a cycle ergometer to determine V̇O2max using an automated metabolic system (Oxycon Pro, Erich Jaeger GmbH, Hoechberg, Germany) (Figure 1). V̇O2 and respiratory exchange ratio (RER) were determined from expired air (breath-by-breath). Before each trial, the analyser was calibrated using oxygen and carbon dioxide gases of known concentrations and the flowmeter was calibrated using a 3-litre syringe (Viasys Healthcare GmbH, Hoechberg, Germany). During the trial subjects breathed room air through a facemask (Cranlea Human Performance Ltd, Birmingham, UK) that was secured in place by a head-cap assembly (Cranlea Human Performance Ltd, Birmingham, UK). Heart rate was monitored continuously using a heart rate monitor (Polar s610, Polar Electro Oy, Kempele, Finland). Beginning at 100 W, participants performed 6 x 4 min stages with a 25W increment per stage (Figure 1). Heart rate, rating of perceived exertion (RPE), V̇O2, blood lactate and respiratory exchange ratio were measured during the final 30 s of each stage. Blood lactate was measured from a 20μl capillary blood sample using Biosen C-line (EKF Diagnostic, Ebendorfer Chaussee 3, Germany). Following 5 min rest, participants performed a graded maximal exercise test (Figure 1). Beginning at the workload at which they reached their lactate threshold, participants cycled continuously at 75 rpm and the workload was increased by 25W increments every 60 s until volitional exhaustion. V̇O2 was averaged every 30 s for V̇O2max determination. V̇O2max was met if V̇O2 plateaued or failed to increase with progressive work rate, respiratory exchange ratio was >1.1, RPE was >18, heart rate was within 10 b.min-1 of age predicted maximum, and blood lactate concentration was ≥ 8 mmol/L.

[INSERT FIGURE 1 HERE]

For the experimental trials, participants arrived at the laboratory fasted. A venous blood sample was collected from an antecubital vein in a plasma separator tube and haematocrit was measured to determine plasma volume. PPT was then assessed at multiple body sites using a handheld pressure algometer. Participants performed a warm-up identical to the first trial. Following 2 min rest, participants then performed the aerobic exercise protocol assigned to that trial. At 5 min and 10 min post-exercise, PPT assessment and venous blood sample collection were repeated, respectively.

**2.4 Aerobic exercise protocols**

Four aerobic exercise protocols were used in a randomised order for the four experimental trials: 1) low intensity aerobic exercise (LI-AE); 2) BFR-AE at 40% LOP (BFR40); 3) BFR-AE at 80% LOP (BFR80) and 4) high intensity aerobic exercise (HI-AE). Participants cycled continuously for 20 min with a pedalling frequency of 75 rpm. Exercise intensities corresponding to 40% V̇O2max (LI-AE, BFR40 and BFR80 trials) and 70% V̇O2max (HI-AE trial) were estimated individually by interpolating the linear relationship between V̇O2 and the exercise intensity. During the BFR trials, BFR was applied to the lower limbs bilaterally at 40% and 80% LOP for BFR40 and BFR80, respectively. Pressure was set relative to each limbs LOP. The BFR cuffs were inflated prior to beginning exercise, followed by intermittent cycles of 3 min inflation and 1 min deflation, totalling 5 cycles over 20 min of continuous cycling.

**2.5 Blood flow restriction**

An automatic personalised tourniquet system (Delfi Medical Inc, Vancouver BC, Canada) was used to perform BFR. This system has a dual-purpose tourniquet cuff (11.5 x 86 cm) connected by airtight hose tubing to a personalised tourniquet device. This system automatically measures LOP and regulates pressure during exercise within clinically acceptable limits (23). With participants seated upright to reflect the position they would be cycling in (20), the cuffs were applied bilaterally to the lower limbs, and LOP was measured in each limb unilaterally.

**2.6 Pressure pain threshold**

All PPT measurements were taken with the participant seated with both arms resting on the thighs (22, 49–51, 53). PPT assessment sites were located and marked in the quadriceps muscles of both lower limbs (20 cm proximal to the base of the patella), the dominant biceps brachii muscle (10 cm proximal to the cubital fossa) and the non-dominant upper trapezius muscle (10 cm from the acromion in direct line with the neck) (22, 49–51, 53). PPTs were assessed using a handheld mechanical pressure algometer (Wagner Instruments, CT, United States) with a stimulation area of 1 cm2 at a pressure rate of approximately 1 kgf/s. Participants gave the verbal notification “now” when the pressure was first perceived as painful, and PPT was quantified as the kgf applied at this point. Two PPT assessments were completed at each site, with 20s intervals between assessments, and the average was used for analysis (22, 49–51). All measurements were taken by the same assessor. During the familiarisation, PPTs were assessed 3 times at 10min intervals at each site and standard error of measurement minimum detectable change (MDC) were calculated. The MDC was 0.82, 0.88, 0.62 and 0.80 kgf for the right and left quadriceps, bicep, and trapezius, respectively).

**2.7 Perceptual response**

Participant’s rating of perceived muscle discomfort and RPE were assessed as previously described (22) using Borg’s RPE and pain scales (4). Briefly, muscle discomfort and RPE were assessed in the final 15 s of each BFR inflation cycle, and at the corresponding timepoint in the non-BFR trials. A muscle comfort score was obtained for each lower limb individually.

**2.8 Beta-endorphin and 2-arachidonoylglycerol levels**

Vacutainers containing EDTA-Na2 (6mg) were used to collect venous blood samples which were immediately centrifuged for 15 mins at 1000 x g at 4°c (Mikro 220R Hettich centrifuges, Tuttlingen, Germany). Plasma supernatant was aliquoted to eppendorf tubes and stored at -80°c until analysis. Plasma β-EP and 2-AG concentrations were determined using commercial available enzyme immunoassay kits as previously described (22). To correct concentrations for any changes in plasma volume with exercise, a capillary blood sample was taken pre- and post-exercise and haematocrit was measured using a micro-haematocrit centrifuge and reader (Hawksley, Sussex, UK). Percent change in plasma volume was calculated using the following formula: % change in plasma volume = (100/[100 Hct pre])\*100([Hct pre - Hct post]/Hct post) (54).

**2.9 Mediation analysis**

Mediation analysis was conducted with a multicategorical independent variable (exercise intervention) to test whether the effect of exercise on PPT is mediated by circulating β-EP and 2AG concentrations and muscle discomfort during exercise, as described previously (22) (Figure 2). Bootstrapping method involving resampling of the data 5,000 times was used to calculate 95% confidence intervals (CI) of the coefficients for the total, indirect and direct effects (37) (Figure 2).

[INSERT FIGURE 1 HERE]

**2.10 Statistical analysis**

All statistical analysis was performed using IBM SPSS Statistics Version 26.0 (IBM Corp, Chicago, IL, USA). Data are presented as mean ± SD with 95% CI unless stated otherwise. The effect of the number of experimental trials on baseline PPTs was analysed with a two-way repeated measures ANOVA with trial (LI-AE, BFR40, BFR80 and HI-AE) and assessment site (right quadriceps, left quadriceps, bicep and trapezius) as within-subject factors. The effect of aerobic exercise on PPTs at each assessment site was assessed using a two-way repeated measures ANOVA with trial and time (pre- and post-exercise) as within-subjects factors. Resting levels of plasma β-EP and 2-AG concentration between trials were each assessed using one-way repeated measures ANOVA. Exercise workload and BFR pressures were each compared using paired t-test. Plasma β-EP and 2-AG concentration, haematocrit, muscle discomfort and RPE were each assessed using two-way repeated measures ANOVA with trial and time as within-subject factors. If appropriate, 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 measured by partial Eta squared (*ηp2*) for ANOVAs and by Cohen’s D for pre-post changes and post-hoc analyses (40). For mediation analysis, the SPSS macro PROCESS (Model 4) was applied with the three potential mediators. As the independent variable (exercise intervention) is multicategorical, dummy coding was employed, and analysis was run accounting for dependency of individuals across trials. The regression/path coefficients are all presented in unstandardized form (18) and were deemed statistically significant if the confidence intervals did not cross zero (37).

**3. Results**

**3.1 Participants**

All participants completed all trials with no adverse events. There were no differences in LOP between the right and left legs for the BFR40 trial (199 ± 15 vs 194 ± 22 mmHg, respectively) or the BFR80 (201 ± 20 vs 203 ± 15 mmHg, respectively) (both p>0.05). There was no difference in LOP between the BFR40 and BFR80 trials for the right leg (199 ± 15 vs 201 ± 20 mmHg, respectively) or the left leg (194 ± 22 vs 203 ± 15 mmHg, respectively) (both p>0.05). BFR pressure was higher in the BFR80 trial compared to the BFR40 trial in both legs (p<0.05). The workload corresponding to 40% V̇O2max was lower than 70% V̇O2max (64 ± 22 W vs 187 ± 20 W, respectively, p<0.05).

**3.2 Baseline PPTs**

No differences in baseline PPTs were found between the four experimental trials (*F*(9,99)=1.493, p=0.161, *ηp2*=0.3). A main effect of assessment site was found (*F*(3,33)=66.277, p<0.01, *ηp2*=0.8). Baseline PPTs were higher in the right and left quadriceps sites compared to the bicep and trapezius sites (both p<0.01).

[INSERT TABLE 1 HERE]

**3.3 Change in PPTs with exercise**

Compared to pre-exercise values, PPTs were increased at each assessment site at 5 min post-exercise in all trials except LI-AE. In the right quadriceps, PPT was higher following BFR40 (p<0.05, d=0.41), BFR80 (p<0.01, d=0.78) and HI-AE (p<0.05, d=0.17) compared to LI-AE (Figure 2). In the left quadriceps, PPT was higher following BFR40 (p<0.05, d=0.34), BFR80 (p<0.01, d=0.82) and HI-AE (p<0.05, d=0.39) compared to LI-AE (Figure 2). PPT was higher following BFR80 compared to BFR40 (p<0.01, d=0.43) and HI-AE (p<0.05, d=0.43). In the bicep, PPT was higher following BFR80 (p<0.05, d=0.45) and HI-AE (p<0.05, d=0.23). In the trapezius, compared to LI-AE, PPT was higher following BFR80 (p<0.05, d=0.45) (Figure 3).

[INSERT FIGURE 3 HERE]

**3.4 Muscle discomfort and RPE**

Compared to LI-AE, muscle discomfort and RPE were higher in all other trials at all timepoints (all p<0.05). RPE was higher in BFR80 and HI-AE compared to BFR40 at 7 min, 11 min, 15 min and 19 min (Table 2). Muscle discomfort in the right quadriceps was higher in BFR80 compared to BFR40 at 7 min, 11 min, 15 min and 19 min (all p<0.05) and compared to HI-AE at 3 min and 19 min (Table 2). Muscle discomfort in the left quadriceps was higher in BFR80 compared to BFR40 at all timepoints and compared to HI-AE at 3 min, 7 min and 19 min (Table 2).

**3.5 Haematocrit and circulating beta-endorphin and 2AG concentration**

There was no change in haematocrit as a result of exercise in any trial (Table 2). There were no differences in resting circulating β-EP concentration(*F*(3,33)=2.008, p=0.132, *ηp2*=0.15) or 2AG concentration (*F*(1.649,18.135)=1.167, p=0.34, *ηp2*=0.10) between trials. There were two-way interaction effects for circulating β-EP and 2AG concentration (Table 2). Circulating concentrations of β-EP and 2AG increased from pre-to post-exercise in all trials except LI-AE. Post-exercise circulating β-EP and 2AG concentration was greatest following BFR80 and HI-AE, respectively (p<0.05) (Table 2).

[INSERT TABLE 2 HERE]

**3.6 Mediation analysis**

There was no statistically significantly mediation found for any of the potential mediators (Supplementary Data File 1).

**4. Discussion**

This study is the first to report EIH in response to BFR-AE in pain-free individuals, with several notable findings. For the first time, this study shows that combining BFR with LI-AE triggers both local and systemic hypoalgesia that is not observed following LI-AE alone. Compared to HI-AE, the magnitude of this effect is greater in the exercising limbs with high pressure BFR, and comparable in remote areas of the body. Low pressure BFR-AE results in hypoalgesia that is comparable to HI-AE in the exercising limbs but does not appear to trigger hypoalgesia in remote areas of the body. Furthermore, our data show that BFR-AE and HI-AE activates the opioid and endocannabinoid systems, which are involved in pain inhibition, leading to an increase in circulating βEP and 2AG concentration.

In the present study, HI-AE increased PPTs compared to LI-AE in the exercising limbs (17-20% vs 1-2%, respectively) and remote non-exercising areas of the body (19-21% vs 2%, respectively). It is well-documented that higher intensity aerobic exercise results in a greater EIH response both locally and systemically (19, 32, 34, 51). Notably, there was no change in PPTs with LI-AE. Previous research suggests that the exercise intensity of 40% V̇O2max in the present study may have been insufficient to trigger EIH with aerobic cycling exercise. For example, Hoffman et al. (19) reported no change in pain ratings following 30 min of cycling at 50% V̇O2max in pain-free individuals. Similarly, Vaegter et al. (51) found no change in PPTs in both the exercising muscles and remote non-exercising body sites following 2 x 10 min bouts of cycling at 50% V̇O2max. Together, the present study and previous studies (19, 51) suggest that there may be a minimum threshold of exercise intensity to elicit a EIH response with aerobic cycling exercise. Importantly, our data shows that this threshold can be reached when BFR is performed during low intensity aerobic exercise which ineffective in isolation. BFR elicited a hypoalgesia response at an exercise low aerobic exercise intensity which was ineffective without BFR. Furthermore, as very short duration high intensity aerobic exercise can elicit EIH (41), this implies that the exercise intensity, or a combination of intensity and duration, may be a more principal determinant of the magnitude of the EIH response after aerobic exercise. Interestingly, Micalos et al. (32) found a decrease in PPTs in the legs following 30 min of cycling at 30% V̇O2peak, which has also been reported with other forms of low intensity exercise (31). It is important to note that in the present study, a decrease in PPTs was observed following LI-AE in some individuals (Figure 3). It is possible that low-to-mild intensity aerobic exercise may facilitate ascending afferent signalling and the somatosensory system compared to moderate-high intensity aerobic exercise which activates descending pain inhibitory systems (32). Further research is needed to determine the minimal exercise intensity threshold for triggering EIH with both BFR and non-BFR exercise and the underlying mechanisms.

A growing body of evidence demonstrates that BFR exercise can have a therapeutic effect on pain in chronic pain populations (12, 16, 24, 29, 30). It was recently shown that addition of BFR to low intensity resistance exercise increased PPTs at local and remote areas of the body (22). Similarly, in the present study addition of BFR to LI-AE resulted in a greater increase in PPTs in the exercising limbs (23-32% vs 1-2%). A BFR pressure of 40% LOP resulted in EIH comparable to HI-AE in the exercising limbs whilst 80% LOP produced the greatest EIH effect. This is in line with our previous findings (22) and provides further support for the notion that there may be a dose-response effect of pressure on EIH with BFR exercise. Our data shows that HI-AE increases PPTs in remote non-exercising areas of the body (i.e. the bicep and trapezius sites), supporting the hypothesis that both local and central inhibitory nociceptive pathways contribute to EIH (21, 53). Notably, BFR-AE also increased PPTs in the bicep and trapezius sites, similarly to resistance exercise with BFR (22). However, contrary to our previous data (22) only BFR-AE at 80% LOP increased PPTs in remote areas of the body. A lower BFR pressure may be insufficient to elicit systemic EIH with LI-AE, and a higher pressure may be required. Further research is needed to investigate this and the relationship between exercise intensity, duration and BFR pressure with respect to EIH with different modes of exercise. A particularly important observation in the present study is that BFR at 80% LOP resulted in systemic hypoalgesia comparable to HI-AE at a lower exercise intensity (40% vs 70% V̇O2max). Previous research suggests that low-moderate intensity aerobic exercise is insufficient for systemic EIH in non-exercising limbs (34, 51). For example, Naugle et al. (34) found that cycling at 50-55% heart rate reserve was insufficient to increase PPT at the non-exercising sites, but was adequate when performed at 70% heart rate reserve. Similarly, Vaegter et al. (51) found that 2 x 10 min bouts of cycling at 75% V̇O2max increased PPTs in the bicep and trapezius, while no change was observed following the same exercise at 50% V̇O2max. Our finding that LI-AE at 40% V̇O2max did not result in EIH in the bicep and trapezius would agree with such data. Therefore, a particularly important finding in the present study is that addition of high pressure BFR to LI-AE can increase PPTs in remote non-exercising areas of the body at an aerobic exercise intensity that does not typically influence pain sensitivity. This may have important implications for pain management programmes in individuals with chronic pain who are extremely load compromised.

In agreement with previous research (1, 13, 43), BFR-AE induced elevations in RPE and muscle discomfort compared to LI-AE that were similar to those observed during HI-AE with high pressure BFR. The elevation in RPE suggests that the perceived intensity of BFR-AE was greater than LI-AE, which may contribute to the greater EIH effect as high intensity aerobic exercise maximises the hypoalgesia effect (19, 32–34, 51). During the last minute of exercise, muscle discomfort was greater in high pressure BFR-AE; however, these values are lower than those observed with high pressure resistance exercise with BFR (22) and are therefore unlikely to be a factor limiting completion of BFR-AE. It is hypothesised that the local ischemia, hypoxia, venous blood pooling and metabolite accumulation during BFR-AE (6, 25) stimulates group III and IV afferent fibres, leading to a subsequent increase in sympathetic nervous activity and perception of pain and discomfort (48). The ischemia and metabolite-induced pain, along with mechanical compression of the underlying tissues during BFR, may contribute to EIH through a conditioned pain modulation effect whereby the pain/discomfort generated during BFR exercise inhibits reduced the perception of pain to a mechanical noxious stimulus. (21). In contrast to our previous investigation (22), muscle discomfort was not found to be a mediator of the relationship between exercise intervention and PPT in the present study. Therefore, the level of muscle discomfort during exercise may not be a primary mechanism of hypoalgesia with BFR-AE. However, the results of the present study do provide further support for evidence showing a greater EIH effect following painful compared with non-painful exercise (3).

In consonance with previous research (36, 42) the current study demonstrated an elevation of circulating βEP concentration following HI-AE. In the peripheral nervous system, beta-endorphin produces analgesia by binding to opioid receptors and inhibiting the release of substance P, a key protein involved in the transmission of pain (46, 55). Most notably, whilst there was no change following LI-AE, circulating βEP concentration increased following both low and high pressure BFR-AE at a matched external workload. This is the second study demonstrating activation of the endogenous opioid system with BFR exercise (22), providing further support for the potential role of opioids in BFR-induced hypoalgesia. Interestingly, the greatest increase in circulating βEP concentration occurred following BFR-AE at 80% LOP, suggesting that the applied pressure may influence the level of opioid release. Our finding that HI-AE activated the endocannabinoid system and increased circulating 2AG concentration is in line with several previous investigations demonstrating increases in circulating 2AG concentration following aerobic exercise at 70-75% V̇O2max or 70-80% maximum heart rate (5, 7–9, 45). Importantly, BFR-AE at both 40% and 80% LOP increased circulating 2AG level while no change was found with LI-AE at a matched external workload (however, these were small effects). Interestingly, our previous investigation found no change in circulating 2AG concentration with resistance exercise with or without BFR at a low or high intensity (22). This may be explained by the mode of exercise (aerobic vs resistance), duration (20 min vs 5-6 min) and intensity (40-70% V̇O2max vs 30-70% one repetition maximum) of exercise. Previous research suggests manipulation of the mode, intensity and duration of exercise can elicit either opioid or endocannabinoid mechanisms of hypoalgesia (10, 27).

It is thought that activation of the endogenous opioid and endocannabinoid systems and stimulation of βEP and 2AG production may contribute to EIH by inhibiting noxious-evoked activity (28, 46, 47, 56). These substances are produced in response to pain and other physiological stressors including exercise. Therefore, the increase in circulating βEP and 2AG concentration with BFR-AE may be driven by the augmented perception of exercise intensity and greater muscle discomfort observed during BFR-AE compared to LI-AE. Furthermore, Feuerecker et al. (14) found that an increase in 2AG with walking exercise was augmented by hypoxic stress when walking at altitude. Although speculative at this time, as performing BFR-AE results in greater hypoxia in the exercising muscle tissue (6, 25) it is plausible that this may contribute to activation of the endocannabinoid system specifically. In contrast to our previous investigation (22), neither circulating βEP or 2AG concentration were found to mediate the relationship between exercise intervention and PPT. This may be explained by the difference in exercise mode (i.e. aerobic vs resistance). Therefore, while the activation of these systems and release of their respective ligand agonists are likely involved in hypoalgesia, this may not be the primary mechanism for BFR-AE.

The current study is not without limitations. We were only able to examine a single biomarker for each of the opioid and endocannabinoid systems; future studies should examine additional opioid and endocannabinoid receptor agonists for a more comprehensive analysis. It was not possible to measure central opioid and endocannabinoid responses, therefore we only examined these systems via circulating concentrations. Endorphins do not appear to cross the blood-brain barrier and therefore cannot be regarded as indicative of central effects. However, there is pre-clinical evidence to suggest that increases in peripheral endocannabinoid concentrations can influence central responses (26). The EIH response was only quantified using a mechanical pressure stimulus; while similar EIH responses are observed across different methods of stimulation (33, 52), future research should include several methods of quantifying exercise-induced hypoalgesia. Finally, the results are only generalizable to pain-free male adults.

In conclusion, the addition of BFR to LI-AE leads to greater local and systemic hypoalgesia at an exercise intensity which does not typically induce hypoalgesia. Furthermore, the magnitude of this effect is greater and comparable to HI-AE in the exercising limbs and remote areas of the body, respectively, when a higher BFR pressure (i.e., 80% LOP) is used. BFR-AE activates the endogenous opioid and endocannabinoid systems, which are involved in pain inhibition, however this may not be the primary mechanism of EIH with BFR-AE. The findings of the present study may have important implications for pain management in load compromised individuals with acute and chronic pain. However, it is important to note that it may not be feasible to engage all patient types in greater discomfort from BFR exercise for short-term pain relief post-exercise.

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**Author contributions**

LH and SDP designed the study. LH and IG recruited and tested participants. LH performed ELISA analysis. Data was analysed by LH and SDP. All authors contributed to the manuscript and approved the final version.

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**Figure and table legends**

**Figure 1.** Illustration of the design and experimental protocols for the lactate threshold and graded exercise test. RPE, rating of perceived exertion; V̇O2, oxygen consumption; RER, respiratory exchange ratio.

**Figure 2.** Overview of mediation analysis. The total effect (Path C, dashed line) represents the

observed effect of the exercise intervention on PPT. Path A represents the effect of the exercise

intervention on the mediator. Path B represents the effect of the mediator on PPT. The total

effects are comprised of a direct effect pathway (Path C’, solid line) of exercise intervention on

PPT, and a total indirect path (mediated path A\*B) of exercise intervention on PPT through the

mediator.

**Figure 3.** Changes in pressure pain thresholds, mean ± SD (top row) and individual change

(bottom row). \* = significantly greater than LI-AE (p<0.05); † = significantly greater than all other

trials (p<0.05). PPT, pressure pain threshold; LI-AE, low intensity aerobic exercise; BFR40, BFR-

AE at 40% LOP; BFR80, BFR-AE at 80% LOP; HI-AE, high intensity aerobic exercise (males,

n=12). Two-way repeated measures ANOVA.

**Table 1.** Ratings of muscle discomfort and perceived exertion during exercise (mean ± SD).

**Table 2.** Haematocrit and circulating plasma β-EP and 2AG concentration (mean ± SD).

**Supplementary data**

**1. Table of mediation analysis results:**

URL: <https://figshare.com/s/2a15d17ddda74200499a>

DOI: <https://doi.org/10.6084/m9.figshare.15202326.v1>

**Exercise completion:**

Final blood lactate

Final heart rate

 Final RPE

Final V̇O2

Final RER

**~ 29**

**Rest 5 min**

**Rest 2 min**

**Warm-up**

75 W

75 rpm

**0**

**5**

**Lactate Threshold**

Start at 100 W

75 rpm

~ 6 x 4 min stages

↑25W each stage

**7**

**~ 24**

**Final 30 s each stage:**

Blood lactate

Heart rate

RPE

V̇O2

RER

**Graded Exercise Test**

Start at W at lactate threshold

75 rpm

↑25W every 1 min

Until volitional exhaustion

**Exhaustion**

**Time (min)**





|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **3 min** | **7 min** | **11 min** | **15 min** | **19 min** |  |  |  |  |
| **RPE** |  |  |  |  |  |  | ***F*** | **p** | ***ηp2*** |
| LI-AE | 8 ± 2 | 8 ± 2 | 9 ± 3 | 9 ± 3 | 9 ± 3 | **Condition x Time** | 3.737 | <0.01 | 0.25 |
| BFR40 | 10 ± 2\* | 11 ± 2\* | 11 ± 2\* | 12 ± 2\* | 12 ± 2\* | **Time** | 32.649 | <0.01 | 0.75 |
| BFR80 | 12 ± 2\* | 13 ± 3\*‡ | 14 ± 3\*‡ | 14 ± 3\*‡ | 15 ± 2\*‡ | **Condition** | 27.118 | <0.01 | 0.71 |
| HI-AE | 12 ± 2\* | 13 ± 1\*‡ | 14 ± 2\*‡ | 15 ± 2\*‡ | 16 ± 2\*‡ |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
| **Discomfort****(Right leg)** |  |  |  |  |  | ***F*** | **p** | ***ηp2*** |
| LI-AE | 0.5 ± 0.9 | 0.7 ± 1.1 | 0.8 ± 1.1 | 0.9 ± 1.3 | 1.1 ± 1.9 | **Condition x Time** | 6.594 | <0.01 | 0.38 |
| BFR40 | 1.9 ± 1.2\* | 2.2 ± 1.2\* | 2.5 ± 1.3\* | 2.9 ± 1.5\* | 2.8 ± 1.6\* | **Time** | 21.231 | <0.01 | 0.66 |
| BFR80 | 3.4 ± 1.3\*† | 3.8 ± 1.3\*‡ | 4.7 ± 1.7\*‡ | 5.0 ± 1.7\*‡ | 5.7 ± 1.8\*†‡ | **Condition** | 27.487 | <0.01 | 0.71 |
| HI-AE | 1.6 ± 1.3\* | 2.3 ± 1.7\* | 2.9 ± 2.3\* | 3.4 ± 2.5\* | 3.6 ± 2.6\* |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
| **Discomfort****(Left leg)** |  |  |  |  |  | ***F*** | **p** | ***ηp2*** |
| LI-AE | 0.5 ± 0.9 | 0.7 ± 1.1 | 0.7 ± 1.1 | 1.0 ± 1.6 | 1.1 ± 1.9 | **Condition x Time** | 6.383 | <0.01 | 0.37 |
| BFR40 | 1.7 ± 1.1\* | 2.0 ± 1.2\* | 2.3 ± 1.2\* | 2.9 ± 1.6\* | 3.0 ± 1.5\* | **Time** | 27.492 | <0.01 | 0.71 |
| BFR80 | 3.5 ± 1.5\*†‡ | 3.8 ± 1.5\*†‡ | 5.0 ± 1.9\*‡ | 5.3 ± 1.9\*‡ | 6.0 ± 1.8\*†‡ | **Condition** | 29.295 | <0.01 | 0.73 |
| HI-AE | 1.7 ± 1.4\* | 2.3 ± 1.7\* | 2.9 ± 2.3\* | 3.2 ± 2.4\* | 3.5 ± 2.2\* |  |  |  |  |

\* = significantly greater than LI-AE (p<0.05); † = significantly greater than HI-AE (p<0.05); ‡ = significantly greater than BFR40 (p<0.05). RPE, rating of perceived exertion; LI-AE, low intensity aerobic exercise; BFR40, BFR-AE at 40% LOP; BFR80, BFR-AE at 80% LOP; HI-AE, high intensity aerobic exercise.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Pre-exercise** |  | **Post-exercise** |  |  | ***F*** | **p** | ***ηp2*** |
| **β-EP (pg/mL)**LI-AEBFR40BFR80HI-AE**2AG (ng/mL)**LI-AEBFR40BFR80HI-AE**Hct (%)**LI-AEBFR40BFR80HI-AE | 547.38 ± 256.07546.49 ± 228.58586.42 ± 265.79556.02 ± 270.2725.13 ± 19.6325.36 ± 20.1626.02 ± 20.3624.45 ± 18.1247.80 ± 2.9048.40 ± 3.6647.92 ± 2.6449.20 ± 2.94 |  | 548.78 ± 262.63625.51 ± 292.47\*†759.26 ± 353.51\*†‡609.61 ± 278.38\*†24.64 ± 19.5229.03 ± 21.42\*†29.17 ± 21.14\*†33.35 ± 20.24\*†‡47.90 ± 3.9047.90 ± 3.5548.50 ± 2.8149.10 ± 3.54 | **dΔ**0.010.300.550.200.030.180.150.460.020.140.210.03 | **Condition x Time****Time****Condition****Condition x Time****Time****Condition****Condition x Time****Time****Condition** | 18.16722.11012.73158.73662.1716.8771.2150.0071.208 | <0.01<0.01<0.01<0.01<0.01<0.010.3200.9350.322 | 0.620.670.540.840.850.390.100.000.10 |

\* = significantly greater than pre-exercise (p<0.05); † = significantly greater than LI-AE (p<0.05); ‡ = significantly greater than all other trials (p<0.05). β-EP, beta-endorphin; 2AG, 2-arachidonoylglycerol; LI-AE, low intensity aerobic exercise; BFR40, BFR-AE at 40% LOP; BFR80, BFR-AE at 80% LOP; HI-AE, high intensity aerobic exercise; dΔ, effect size of within-group change.