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An Investigation into the Effect of Blood Flow Restriction on Pain and Muscular Endurance in Healthy Human Participants

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Abstract

Objective: This study compared the effect of exercise during full, partial (intermittent) and no BFR on pain and muscular endurance.

Design: Within-subject repeated measures cross-over study comparing full BFR (200 mmHg), partial BFR (100 mmHg) and no (control) BFR during hand-grip exercises of a bulb dynamometer (60 per minute) at 30% of their one-repetition maximum of grip strength.

Setting: Laboratory.

Participants: 20 student volunteers (male = 14, age = 22-29 years).

Main outcome measures: Time to exhaustion and pain perception at minute intervals during handgrip exercises.

Results: There were fewer (77.0 ± 34.7) handgrip exercise repetitions during full BFR compared with partial BFR (125.1 \pm 37.7, p < 0.001) and fewer repetitions for partial BFR compared with no BFR (147.6 \pm 11.3 repetitions, p = 0.026). Pain intensity was higher for full BFR compared with partial BFR (p = 0.045) and higher for partial BFR compared with no BFR (p < 0.001). Participants selected more total, sensory and affective pain descriptors of the Short-Form McGill Pain Questionnaire during full BFR compared with partial BFR and no BFR.

Conclusion: Full BFR produced severe exercise-induced pain so partial BFR may be a more acceptable training and rehabilitation aid.

Keywords

Blood flow restriction, Pain, Endurance, Exercise, Rehabilitation

Highlights

- Blood Flow Restriction (BFR) is used in sport training and clinical rehabilitation to increase muscle hypertrophy.
- This study found that full BFR (200 mmHg) produced more pain and less endurance to exercise than partial BFR (100 mmHg).
- Partial BFR (100 mmHg) may be a more acceptable training and rehabilitation aid.

Introduction

In sports training low-load resistance training under Blood Flow Restriction (BFR) is used for muscle fibre hypertrophy [1]. Elasticated wraps, Kaatsu apparatus used as part of a patented Japanese exercise method and pressure cuffs are used to restrict blood flow and training commonly involves three to five sets of exercises using loads of approximately 20% of the one-repetition maximum. Evidence suggests that ratings of discomfort during BFR exercise are greater at higher arterial occlusion pressures suggesting that lower relative pressures for BFR may be more acceptable and safer for training [2].

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Wernbom, et al. found that BFR reduced the number of repetitions of fatiguing low-load dynamic knee extension exercises compared with no BFR [3]. Ratings of perceived effort and acute pain were similar between groups but delayed onset muscle soreness was more severe in the no BFR leg. Weatherholt, et al. found that exercise during BFR increased perceived exertion, pressure and aching [4] and Loenneke, et al. found that that exercising with knee wraps placed around the upper thigh of each leg reduced the number of exercise repetitions to exhaustion and increased perceived exhaustion and pain [5]. Wide pressure cuffs (13.5 cm) caused greater perceived effort and pain than narrow cuffs (5.0 cm) [6]. However, BFR did not amplify discomfort experienced during lowload exercise to failure [7], with no differences in perceived exertion when arterial occlusion pressures were between 40% and 90% [8] suggesting that perceptual rating during exercise was unlikely to be a limiting factor when BFR was applied at higher pressures.

Blood flow restriction is also as an analgesic assay [9]. This Submaximal Effort Tourniquet Test (SETT) typically occludes arterial blood flow to the hand whilst a participant undertakes handgrip repetitions at a fixed load, often 30-33% of a single repetition maximal voluntary contraction. Time to exhaustion and pain severity during handgrip exercises are used as outcome measures. To our knowledge there are no studies that have undertaken detailed measurements of pain and muscle endurance during complete occlusion of arterial vessels (full BFR) and intermittent occlusion of arterial vessels whereby arterial vessel are open during systolic and closed during diastole (partial BFR). The aim of our study was to compare the effect of exercise during full, partial and no BFR on pain and muscular endurance.

Methods

Study design

A within-subject repeated measures study was designed to compare the effect of full BFR (200 mmHg cuff pressure), partial BFR (100 mmHg) and no (control) BFR (0 mmHg) on muscular endurance associated with completing grip strength repetitions at 30% of one-repetition maximum of grip strength and pain experience in healthy human volunteers. The study received institutional ethical approval from Leeds Beckett University and all participants provided signed consent. One investigator (Vincent Burnham) conducted all aspects of the experiment including outcome assessment and part of the data analysis was not blind to the conditions.

Recruitment

The sample size and power estimation were calculated using G'Power 3.1.9.2 for Windows based on the repetitions performed with and without occlusion at

30% of one-repetition maximum (1-RM) and ischaemic pain tolerance differences between male and female participants. For a power of 0.80 and a 0.05 alpha value, a minimum population of five participants was required in order to reach a difference when comparing occlusive intensity and 13 participants per group for sex differences.

Volunteers who expressed interest in the study were given a participant information sheet and contacted at least 48 hours later by principal investigator. Volunteers who invited them to take part in the study, which involved attending our laboratory on three occasions with at least one-week interval between visits to allow for muscle recovery. During the first study visit volunteers were orientated to the nature of the study and screened for eligibility using a self-screening eligibility form. Eligibility criteria were: Aged between 18 and 30 years of age, no previous experience of using BFR training techniques, injury-free, pain-free, no history of cardiovascular complications or skins conditions. Eligible volunteers provided written consent and were informed that they could withdraw from the study at any time and without reason. Each participant was assigned a study participant code so that data was anonymised.

Each visit involved taking measurements of muscular endurance and pain tolerance under one of three possible conditions:

- Full BFR using a cuff pressure of 200 mmHg.
- Partial BFR using a cuff pressure of 100 mmHg.
- No BFR (Control) using a cuff pressure of 0 mmHg.

Resting blood pressure was taken before the start of the experiment to ensure that a cuff pressure of 200 mmHg would occlude large arterial and venous vessels during systole and diastole and a cuff pressure of 100 mmHg would occlude large arterial and venous vessels during diastole but only occlude large venous vessels during diastole. Hence, there would be pulsate arterial flow during systole. A cuff pressure of 0 mmHg (deflated cuff) would not occlude large arterial and venous vessels during systole and diastole.

Each condition was presented at one of 3 experimental visits with the order of presentation of each condition determined using a computer-based block randomisation process with the sequence for each participant concealed within an opaque envelope which was opened before the start of the experiment during the first study visit [10]. Participants were not told which condition they were receiving during each visit.

Experimental procedure

During the first study visit anthropometric and baseline characteristics were recorded including body mass (Seca 761, Seca UK, Birmingham), height (Seca 223 tele-

scopic measuring rod, Seca UK) as well as information regarding sex, age and self-reported hand dominance.

Determination of one-repetition maximum grip strength

The one-repetition maximum grip strength of the non-dominant hand was determined during the first study visit. The participant was seated on a plinth with both feet on the floor with their forearm resting on a table in a neutral position (elbow flexed at 90°, 0° of shoulder flexion, abduction and rotation) [11]. The participant made three Maximal Voluntary Contractions (MVC) by squeezing a large-size hand bulb dynamometer (Martin Vigorimeter Measuring Instrument, GP Supplies UK) with a one minute interval between each maximal volun-



Figure 1: Experimental set-up.

tary contraction [11,12], (Figure 1). The one-repetition maximum grip strength was recorded as the maximum pressure achieved on any of the three attempts.

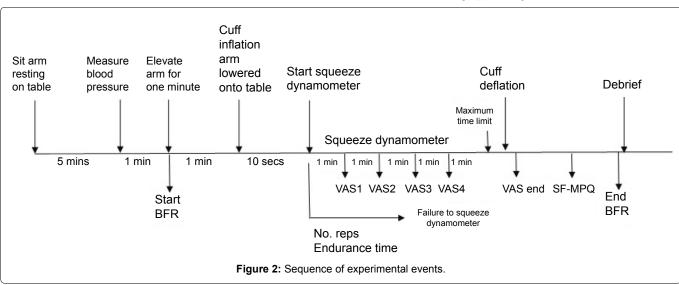
The experimental procedure was identical, except for condition under investigation, for each study visit (Figure 2). Participants were asked to sit for five minutes with their non-dominant arm resting at heart-level on a table [13]. Then brachial blood pressure was measured using an aneroid sphygmomanometer (Durashock DS54 Thumbscrew Hand Held, Welch Allyn (UK) Limited, Buckinghamshire, UK) paired with a blood pressure cuff (FlexiPort Reusable One-Piece Blood pressure cuff) of adult-size 11 (width 18.8 cm, height 15.5 cm and depth 4.1 cm). The cuff was positioned at a minimum of 2.5 cm above the antecubital fossa [13], in line with the brachial artery and a stethoscope (Littmann Classic II S.E., Medscope Limited, Cirencester, UK) used to listen for Korotkoff sounds.

Occlusion procedure

The non-dominant arm was elevated and supported in a vertical position for one minute to promote venous drainage after which the blood pressure cuff was inflated to the appropriate pressure. Then, the arm was lowered onto the table and the hand-bulb dynamometer placed in the hand.

Measurement of muscular endurance

The participants were asked to squeeze the dynamometer once every 2 seconds (i.e. 0.5 Hz, 30 times per minute) in time with a metronome that clicked every second (i.e. one second squeeze followed by one second of relaxation) to 30% of their pre-determined one-repetition maximum grip strength which was marked on the goniometer dial. Time to exhaustion was measured as the time at which the participant volitionally stopped the exercise or were unable to reach 30% of their one-repetition maximum grip strength on three successive at-



tempts with time taken at the first failed of the three failed attempts. The number of repetitions was also recorded using a counter clicker and recorded on countback to the final of the three successful attempts. A time limit of five minutes was set to reduce the risk of adverse effects associated with excessive blood flow occlusion. Upon completion of the repetitive grip strength exercises, the cuff was deflated at a rate of 2 mmHg per second to limit rapid reperfusion [14].

Measurement of pain intensity

Participants provided a verbal rating of their present pain intensity using a Visual Analogue Scale (VAS) at the following time points [15]:

- Arm resting on table before cuff inflation (VAS baseline)
- Arm on table immediately following cuff inflation but before start of repetitive grip strength exercises (VAS occlusion)
- At one minute intervals during repetitive grip strength exercises (VAS 1, 2, 3, 4)
- Arm on table immediately after cuff deflation (VAS end)

The VAS was anchored at 0 mm with the term "No Pain" and at 100 mm and the term "Worst Pain Imaginable", and participants were not given access to their previous VAS responses.

Participants also completed a Short-Form McGill Pain Questionnaire immediately after cuff deflation by recalling attributes of their pain experience during repetitive grip strength exercises. The Short-Form McGill Pain Questionnaire (SF-MPQ) has a list of 15 words used to describe of the quality of pain and its psychometric properties have been shown to be valid and reliable [16]. Eleven of these descriptors are related to sensory dimensions of pain and 4 of these words are related to affective dimensions of pain. The SF-MPQ is completed by selecting descriptors appropriate to pain experienced. In clinical practice individuals would rate the intensity of each word as none, mild, moderate or severe to enable calculation of a score for Pain Rating Index (PRI). In our study instructed participants to select descriptors but not to rate the intensity of each descriptor. At the end of the BFR intervention we asked participants to rate 'overall pain severity' during grip-exercises selecting one of the following descriptors no pain, mild, discomforting, distressing, horrible, excruciating. We also asked them to rate the overall duration of their pain during BFR condition as either continuous or intermittent or brief.

Data analysis

Independent-samples t-tests were used to compare baseline sex differences for age, height, weight, BMI,

hand grip strength and hand dominance. Repeated measures one-way ANOVA for Mean Arterial Pressure (MAP), diastolic and systolic blood pressure were used to determine whether resting blood pressure was similar prior to each session. Mean arterial pressure was estimated at rest [17] as:

$$MAP \approx \frac{(2 \times DP) + SP}{3}$$

Continuous data was analysed using repeated measures analysis of variance (ANOVA). If Mauchly's test of Sphericity was not assumed, then a Greenhouse-Geisser correction was used for the data set. Alpha was set at 0.05 and adjustment made for multiple comparisons using the Bonferroni correction. Data for muscular endurance (i.e. number of repetitions and endurance time) and for pain intensity ratings at the end of the first minute during repetitive grip strength (VAS1) was analysed using a two-way mixed ANOVA to assess the effect of condition (within-subject, 3 levels: Full BFR, Partial BFR, No BFR (control) and sex (between-subject, 2 levels: Male; Female).

Data gathered from the SF-MPQ was analysed using descriptive statistics in the first instance. The mean tally of SF-MPQ descriptors chosen by each individual was calculated and a two-way mixed ANOVA used to analyse the effect of condition (within-subject, 3 levels: Full BFR, Partial BFR, No BFR (control) and sex (between-subject, 2 levels: Male; Female). The score for global pain intensity was calculated for each participant for each condition (no pain = 0, mild = 1, discomforting = 2, distressing = 3, horrible = 4, excruciating = 5) and two way mixed ANOVA used to analyse the effect of condition (within-subject, 3 levels: Full BFR, Partial BFR, No BFR (control))) and sex (between-subject, 2 levels: Male; Female).

Results

Characteristics of the participants

Twenty participants were enrolled and all completed the study (14 male, 6 female, age = 22-29 years), with no statistically significant differences in age, weight, Body Mass Index (BMI) or hand dominance between males and females. Males were taller than the females and had a greater maximal voluntary contraction (Table 1). There were no statistically significant differences in baseline resting blood pressure between conditions (Table 2).

Analysis of muscular endurance

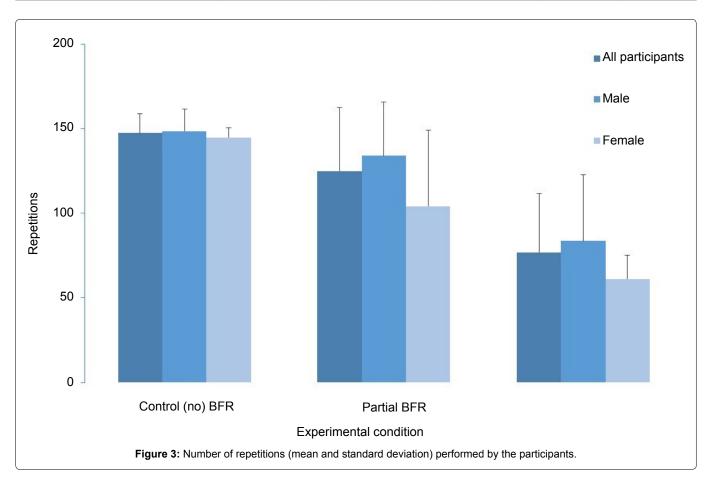
Two-way mixed ANOVA found main effects for the number of repetitions performed (F(2,36) = 36.94, p < 0.001), (Figure 3) but not for the interaction between the number of repetitions and sex (F(2,36) = 1.16, p = 0.324). Post-hoc analysis found that fewer repetitions were performed for full BFR (mean \pm SD = 77.0 \pm 34.7 repetitions)

Table 1: Mean (standard deviation) characteristics of participants by sex (n = 20).

	Male (n = 14)	Female (n = 6)	P value
Age (years)	24.7 (0.5)	23.3 (0.4)	0.09
Hand dominance (n = right:left)	14:0	6:0	
Body weight (kg)	77.0 (2.3)	75.0 (3.7)	0.64
Height (m)	1.79 (0.02)	1.71 (0.01)	0.005
Body mass index (kg/m²)	24.2 (0.8)	25.8 (1.3)	0.27
Maximal voluntary contraction (KPa)	112 (6)	71 (3)	< 0.001

Table 2: Mean (standard deviation) baseline resting blood pressure (mmHg, n = 20).

	Full BFR	Partial BFR	No BFR	F score	P value
Systolic pressure	119.6 (6.4)	118.7 (7.7)	120.3 (8.6)	0.24	0.24
Diastolic pressure	72.3 (6.7)	74.7 (4.5)	75.8 (5.7)	2.93	0.07
Mean arterial pressure	88.6 (4.6)	89.4 (4.6)	90.6 (5.9)	1.22	0.31



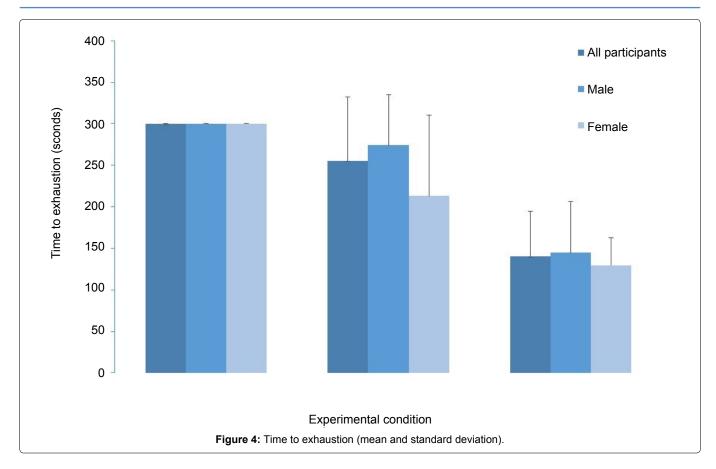
compared with partial BFR (125.1 \pm 37.7 repetitions, p < 0.001); That fewer repetitions for full BFR compared with no BFR (147.6 \pm 11.3 repetitions, p < 0.001); and that fewer repetitions for partial BFR compared with no BFR (p = 0.026), (Figure 3).

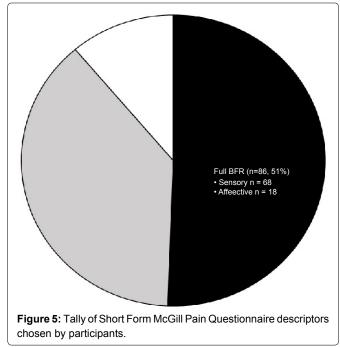
Two-way mixed ANOVA found main effects for time to exhaustion (F(2,36) = 47.33, p < 0.001) but not for the interaction between time to exhaustion and sex (F(2,36) = 1.75, p = 0.194). Post-hoc analysis found that the time to exhaustion was lower for full BFR (mean \pm SD = 140.35 \pm 53.84 seconds) compared with partial BFR (255.70 \pm 76.62 seconds, p < 0.001); was lower for full BFR compared with no BFR (300.0 \pm 00.00 seconds, p <

0.001); and was lower for partial BFR compared with no BFR (p = 0.016), (Figure 4).

Analysis of pain response

Pain intensity: The data set for pain intensity rating was incomplete due to participants stopping hand-grip exercises by their own volition or by failing to reach 30% of their one-repetition maximum grip strength on three successive attempts. At the end of the fourth minute of hand-grip exercises there were only 2 data points for full BFR, 15 data points for partial BFR and 20 data points for no BFR. Therefore, pain intensity ratings at the end of the first minute during repetitive grip strength (VAS1)





were used in the analysis as there were full data sets for each condition.

Two-way mixed ANOVA found main effects for pain intensity (VAS1) at the end of the first minute (F(2,36) = 18.73, p < 0.001) but not for the interaction between pain intensity and sex (F(2,36) = 0.532, p = 0.507). Post-hoc analysis found that pain intensity was higher for full BFR

(mean \pm SD = 28 \pm 22 mm) compared with partial BFR (16 \pm 11 mm, p = 0.045); Higher for full BFR compared with no BFR (3 \pm 5 mm, p < 0.001); and higher for partial BFR compared with no BFR (p < 0.001).

Pain quality: Participants selected more total, sensory and affective pain descriptors of the Short Form-McGill Pain Questionnaire following full BFR than partial BFR and no BFR (Figure 5). The most common sensory pain descriptors selected following full BFR were Hot-burning (n = 13), Aching (n = 12), Throbbing (n = 12), and Cramping (n = 10); Following partial BFR were Throbbing (n = 13) and Aching (n = 13); and following no BFR were Tender (n = 11). The most common affective descriptors selected following full BFR were Tiring-Exhausting (n = 7) and Sickening (n = 5); following partial BFR were Tiring-Exhausting (n = 9) and following no BFR were Tiring-Exhausting (n = 3).

Two-way mixed ANOVAs on the mean tally of SF-MPQ descriptors for Total scores found that there were significant main effects for condition (F(2,36) = 32.07, p < 0.001), (Table 3), but not for the interaction between condition and sex (F(2,36) = 0.29, p = 0.705). Pairwise comparisons found that the mean tally for Total scores was higher for full BFR compared with partial (p = 0.039) and with no BFR (p < 0.001), and that the mean tally for Total scores was higher for partial BFR compared with no BFR (p < 0.001). Two-way mixed ANOVAs on the mean tally of SF-MPQ descriptors for Sensory scores

Table 3: Mean (standard deviation) tally of McGill Pain Questionnaire responses (

	Full BFR	No BFR	Partial BFR	F score	P value
Total score	4.3 (2.3)	0.95 (1.0)	3.25 (1.3)	32.07	< 0.001
Sensory score	3.40 (1.76)	0.80 (0.95)	2.70 (1.26)	29.00	< 0.001
Affective score	0.90 (0.85)	0.15 (0.37)	0.55 (0.60)	6.66	0.006

chosen found that there were significant main effects for condition (F(2,36) = 29.00, p < 0.001) but not for the interaction between condition and sex (F(2,36) = 0.195, p)= 0.82). Pairwise comparisons found that the mean tally for Sensory scores was higher for full BFR compared with no BFR (p < 0.001), and for partial BFR compared with no BFR (p < 0.001), but there were no differences between full and partial BFR (p = 0.115). Two-way mixed ANOVAs on the mean tally of SF-MPQ descriptors for Affective scores chosen found that there were significant main effects for condition (F(2,36) = 6.66, p = 0.006) but there were no significant interactions for condition x sex (F(2,36) = 0.13, p = 0.84). Pairwise comparisons found that the mean tally of Affective scores was higher for full BFR compared with no BFR (p = 0.021) but there were and no differences between full BFR compared with partial BFR (p = 0.144) or for partial BFR compared with no BFR (p = 0.176).

Two-way mixed ANOVAs on overall pain severity found significant main effects for condition (F(2,36) = 35.79, p < 0.001) and but not for the interaction between condition and sex (F(2,36) = 1.21, p = 0.31). Pairwise comparisons found that overall pain severity was higher for full BFR compared with no BFR (p < 0.001), and for partial BFR compared with no BFR (p < 0.001), but there were no differences between full and partial BFR (p = 0.148). Sixteen participants reported pain as 'continuous' rather than 'intermittent' or 'brief' following the full BFR condition; 15 participants reported pain as 'continuous' during the partial BFR condition; and 10 participants reported pain as 'continuous' during the no BFR condition.

Regression analysis: It was not possible to analyse the relationship between pain intensity and endurance for the no BFR condition because all participants reached the 300 second maximum time limits for grip-exercises. The linear regression analysis of partial BFR data found that time to endurance was explained by pain intensity at the end of the first minute (VAS1: B = -0.479, R = 0.71, t(19) = -4.279, p < 0.001) and by SF-MPQ overall pain severity (B = -47.1, R = -0.500, t(19) = -2.447, p = 0.025). The linear regression analysis of full BFR data found that the time to endurance was not explained by pain intensity at the end of the first minute (VAS1:B = -7.25, B = -0.291, t(19) = -1.292, p = 0.213) nor SF-MPQ global pain intensity (B = -21.14, B = -0.344, D = -1.552, D = 0.138).

Discussion

This study found that full BFR reduced muscular endurance and increased the severity of sensory and affective components of pain. Time to muscle endurance was explained by SF-MPQ overall pain severity and by pain intensity one minute after starting exercise for partial (intermittent) BFR but not full BFR. Women rated pain during exercise with BFR more severe than men, consistent with a large body of evidence that women are more sensitive to noxious stimuli [9].

Our findings that exercise during BFR reduces muscle endurance times and repetitions of low-load exercise and increases perceived exertion and pain are consistent with previous studies some of which have found concurrent gains in muscle hypertrophy [2-5,18-25], although some studies fail to observe such effects [26,27]. It has been suggested that ischemic muscle pain generated during moderate-load BFR training may limit use to highly motivated individuals, although BFR-induced sensations may not be the limiting factor during low-load exercise [8,9].

Exercising during BFR restricts oxygenation and reduces the efficiency of exercising muscle fibres resulting in the recruitment of additional fibres to sustain the activity, thus increasing the potential for muscle fibre hypertrophy [28,29]. BFR is associated with lower O₂ saturation and higher deoxyhemoglobin concentrations at oblique fibres of vastus medialis with lower rates of increase of tissue oxyhemoglobin concentrations during recovery between exercise sets [3]. Changes in blood lactate, intramuscular phosphocreatine, diprotonated phosphate, pH and noradrenaline have a role in muscle hypertrophy and pain [20,22,30-32].

Muscle endurance was longer with partial compared with full BFR, probably due to in part to tissue perfusion and oxygenation during systole. Venous occlusion occurs in systole and diastole during partial BFR which would impede by-product clearance. In the absence of BFR tissue perfusion is impaired when exercising at high maximal voluntary contractions creating a metabolic stress similar to BFR. Low-load resistance training under BFR produces similar muscle hypertrophy and strength increases to conventional high-load resistance training without BFR regardless of age and training status [1,23,33,34]. Hence, for a consistent number of repetitions individuals may be unable to use high resistance training loads under full BFR.

Experimental models of pain associated with muscle ischaemia are used to elucidate mechanisms of nociception, to assess factors influencing pain sensitivity response (e.g. sex, gender, ethnicity etc.) and as analgesic assays to evaluate treatment response [16]. The Submaximal Effort Tourniquet Technique (SETT) uses full BFR to induce tonic ischaemic pain arising from skin and muscle nociceptors and measuring pain intensity and time to pain tolerance as outcomes [31,35]. During SETT exercises can only be performed for short periods of time due diminished muscle endurance. The use of partial rather than full BFR would increase time to muscle endurance enabling participants to undertake longer exercises, such as those that simulate activities of daily living including repetitive grasping, holding, reaching, opening jars, drying dishes, picking up objects, typing and writing. This would improve the ecological validity of the SETT. Moreover, lower cuff pressure used during partial BFR would reduce the likelihood of nerve compression that can markedly influence muscle endurance.

Study Limitations

Only immediate and short-term effects of BFR were measured and it would have been interesting to observe potential residual effects over a longer measurement time span and/or over a course of BFR training at different intensities. In particular, evaluating the effect of BFR on delayed onset muscle soreness would have been useful especially as Wembom et al. [3] has reported that the incidence and severity of delayed onset muscle soreness is higher following no BFR. It was noteworthy that no participants reported residual and/or adverse effects associated with full or partial BFR in the follow-up period of one-week post experiment. The disproportionate number of men and women in our study reduces our confidence in the finding that pain associated with BFR was rated more severe by women than by men, although the direction of this finding is consistent existing evidence [10].

We did not gather robust data on the reason for failure to continue grip-exercises (i.e. whether the participant 'gave-up' or whether they were unable to achieve sufficient muscular power to reach 30% of their one-repetition maximum grip strength). We intend to conduct a follow-up study to investigate whether perceptual experience or motor output contributes to the failure to adhere to exercises. A comparison between competitive sports people and sedentary people could provide insights on psychological factors that influence response to pain in different populations (e.g. fear-avoidance, anxiety, catastrophizing and the meaning of pain). Moreover, there is merit in establishing the reliability and validity of incorporating movement-related tasks in a modified SETT design that uses partial rather than full BFR.

Conclusion

Full BFR produced greater severity of exercise-induced pain and more sensory and affective pain descriptors than partial and no BFR. It seems logical to recommend the use of partial occlusion if BFR is to be used for training purposes because of the lower pain associated with exercise making partial BFR a more acceptable training aid. In addition, consideration should be given to the use of partial BFR during experimentally induced pain using SETT as it would enable the assessment of movement related tasks associated with activities of daily living because the time to muscle endurance is longer and there is less chance of causing nerve compression block.

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Conflict of Interest

None declared.

Ethical Approval

The study received institutional ethical approval from Leeds Beckett University and all participants provided signed consent. Experiments were carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Contributions of Authors

Study design: Gareth Jones, Vincent Burnham, Mark I. Johnson.

Data collection: Vincent Burnham.

Data analysis: Mark I. Johnson, Gareth Jones, Vincent Burnham.

Preparation of the manuscript: Mark I. Johnson, Gareth Jones, Vincent Burnham, Peter Francis.

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