Tissue oxygenation, strength and lactate response to different blood flow restrictive pressures

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Summary

This study aimed to determine whether changes in initial restrictive pressures (IRP, tightness of the cuff before inflation with air) affect tissue oxygenation, lactate production and leg strength before, during and after knee extension exercises. The cuff was positioned on the right thigh, and the IRP of either 40–45 or 60–65 mmHg were applied randomly prior to inflating the cuff to the final restrictive pressure (the pressure reached after inflating the cuff with air). Subjects performed four sets (30, 15, 15 and 15 reps) of isotonic knee extensions with 1-min rest between sets. Tissue oxygenation and blood lactate levels were assessed prior to, during and after exercise, and leg strength was assessed pre- and postexercise. There were significant condition by time interactions (P<0.01) and main effects for both condition (P<0.01) and time (P<0.01) for tissue oxygenation, deoxygenated haemoglobin, total haemoglobin. Significant main effects were detected for both condition (P<0.01) and time (P<0.01) for leg strength values. There was only a significant time main effect for lactate concentrations. This study is the first to show that a higher IRP had a significant impact on percent tissue oxygenation, leg strength and deoxygenated haemoglobin accumulation during exercise.

Introduction

Research indicates that blood flow restriction (BFR) training method greatly reduces the time requirements for each session of aerobic training to only 15–20 min to induce improvements in cardiorespiratory fitness (Abe et al., 2010b; Park et al., 2010) as well as being able to lower the resistance training intensity (20% of maximal strength) needed to elicit increases in muscular strength (Takarada et al., 2002; Karabulut et al., 2010a) and muscle size (Takarada et al., 2004; Abe et al., 2006). Therefore, the use of BFR training as an alternative form of exercise has gained in popularity across all populations, especially with older individuals (Abe et al., 2010a; Ozaki et al., 2011), individuals recovering from injury (Sata, 2005) or those with increased health-related risks associated with high intensity exercises (Nakajima et al., 2010; Fukuda et al., 2011).

Although numerous studies reported the efficacy of this novel training method to enhance muscular size (Takarada et al., 2002; Abe et al., 2006), muscular strength (Takarada et al., 2002; Karabulut et al., 2010a) and cardiorespiratory fitness (Abe et al., 2010b; Park et al., 2010), some studies have failed to observe significant improvements in muscle size (Teramoto & Golding, 2006) and strength (Burgomaster et al., 2003). It is crucial to know the details about the procedure used and follow standardized protocols to maximize beneficial training outcomes. It seems that the discrepancies in findings reported stem from the use of different methodologies and different equipment. A previous study (Loenneke et al., 2012) highlighted the importance of width of the restrictive cuffs used to restrict blood flow. It should also be noted that previous studies using different modalities to induce BFR, such as traditional blood pressure (BP) cuffs (Teramoto & Golding, 2006), elastic wraps (Loenneke et al., 2010) and nylon pneumatic cuffs (Cook et al., 2007) would have been unable to set and control the initial restrictive pressure (IRP; the amount of pressure created and applied by the tightness of cuffs before inflation with air).

An important variable (i.e. IRP) of BFR training protocols is often overlooked by researchers. It is a common misconception among researchers that when the final restrictive pressures (FRP; highest restrictive pressure reached after inflation with air) used during training are greater than IRPs or when FRPs were maintained throughout training sessions, IRP should not matter. Changes in pressures created by tightness of restrictive cuffs may have an impact on the diameter of veins leading to variation in blood accumulation in the limbs and in the amount of venous return. Reduced venous return in the exercising limbs increases the amount of the intramuscular accumulation of...
metabolic by-products such as lactate and $P_i$ (Baker et al., 1993), which may affect the level of stimulation of afferent nerve responses resulting in altered physiological responses (Taylor et al., 2000).

The details about BFR training protocol such as IRP have not been well described or explained in most previous studies. Variation in IRP may affect the flow and pooling of blood and may be one of the reasons causing inconsistencies in the level of BFR training-related adaptations. In addition, understanding the effect of IRP will help researchers adjust the current BFR training protocol or develop a new one. Therefore, it is essential to have a better understanding of the impact that different IRPs and the skeletal muscle pump (contraction and relaxation cycle) might have on the magnitude of tissue oxygenation, by-product production and blood pooling during low-intensity dynamic exercise in combination with BFR. The purpose of this study was to examine the impact of two different IRPs during low-intensity BFR knee extension exercise on thigh tissue oxygenation, oxygenated and deoxygenated haemoglobin, leg strength, and blood lactate responses.

Methods

Study design

A randomized within-subjects study design was used to test the effects of IRP on the dependent variables. To test the research questions, participants were asked to return to the laboratory on two separate days, separated by at least 48 h. After standard skin preparation, the sensor of tissue spectrometer system was placed at the midpoint of the right thigh to detect the amount of tissue oxygenation during the rest, exercise and recovery periods of each testing session. The load lifted during dynamic exercises was same for both sessions (~41–68 Nm). The BFR cuff was placed on the upper most portion of the right thigh, and initial pressures of either 40–45 or 60–65 mmHg were applied in random order. Following the assessment of the premaximum voluntary contraction (MVC) values, subjects performed four sets of knee extension exercises (30, 15, 15 and 15 reps) with 1-min rest between sets. The peak postexercise MVC was assessed immediately after performing knee extension exercises. A drop of blood from a finger prick was collected to analyse blood lactate levels at baseline, during the rest period between second and third sets, immediately after, 5 min-post, 10 min-post, and 20 min-postexercise. The cuff was deflated and removed immediately upon the completion of the session. Except for the IRPs, the exercise bouts were identical and the same standardized testing procedure was performed for both sessions.

Subjects

Twenty young males (25 ± 4 years) volunteered to participate in this research study. The study protocol (protocol number: 2011-006-IRB) was approved by the University of Texas at Brownsville Institutional Review Board for Human Participants and adhered the Declaration of Helsinki. Participants were required to visit the research laboratory on three separate days to complete this study. On the first day, all participants read and signed an informed consent document and completed a health status questionnaire and a Physical Activity Readiness Questionnaire (PAR-Q) before participation in the study. After completing the necessary paperwork and confirming each participant’s eligibility to take part in the study, participants’ height (175.2 ± 46.9 cm) and body mass (83.3 ± 414.9 kg) were measured. Forty percentage of the participants were classified as ‘sedentary’ (little or no physical activity) and 60 percentage of the participants were classified as ‘recreationally active’ (physically active but not participating in regular structured exercise training).

Procedures

A calibrated Biomed System 4 Pro isokinetic dynamometer (Biodex Medical Systems, Inc., Shirley, NY, USA) was used to assess isometric MVC torque of the right leg extensors before (pre) and after (post) the knee extension exercises. Each participant was seated in an upright position in the dynamometer chair and secured with restraining straps around the trunk and hips in accordance with the Biodex User’s Guide (Biodex Pro Manual, Applications/Operations. Biodex Medical Systems, Inc.). The right knee joint was aligned with the rotational axis of the dynamometer, and the lever arm was secured to the leg just superior to the malleoli. The knee joint angle was set at 60° below the horizontal plane for all isometric testing, and participants were instructed to sustain 5 s maximal isometric actions to determine peak MVC torque. Strength of the right leg extensors was determined before (pre) and after (post) the dynamic knee extension exercises. The pre-MVC values were measured about three min after the procedure of proper cuff placement and pressure setting.

Participants then completed one set of 30 repetitions and three sets of 15 repetitions of knee extension exercises with a 1-min rest period between sets. As the BFR training method generally uses load about 20% of maximum strength, the relative low load lifted by each participant was determined by multiplying the pre-exercise MVC value obtained during the first session by 0.2. The number was adjusted by rounding up or down to the nearest whole, and the relative load was set between 41 and 68 Nm. For the following session, each participant lifted the same load used for the first session. The load lifted was approximately 15–25% of each participant’s maximum leg strength. The velocities of the concentric or eccentric movements were standardized to 1.2 s using a metronome. As soon as the peak postexercise MVC was determined, the restriction cuff was deflated and removed. The only difference between two sessions was the IRP used.

Tissue oxygen saturation of the quadriceps muscle was monitored with a near-infrared spectrometer (InSpectra™ Tissue Spectrometer System – Model 325) by placing the reflectance probe at 50% of the distance between the anterior
superior iliac spine and the superior part of the patella on the quadriceps muscle. The area of the skin was shaved and cleaned with alcohol before placing the optically dense rubber holder at the exact same spot during both visits. Tissue oxygenation was recorded before the cuff was placed for the baseline values. After participants were seated in the Biodex chair and the cuff was placed, the probe was connected to the rubber holder and tissue oxygenation was continuously monitored during exercises with the cuff. The sensor was kept in place after the cuff was removed, and the changes in tissue oxygenation were documented for about 3 more min after exercises. NIRS is a noninvasive method that utilizes a 25 mm reflectance probe to measure the absorption of light photons in the 680–800 nm spectrum. The absorption spectrum of light remitted from a tissue sample varies mainly with oxyhaemoglobin (HbO₂) and deoxyhaemoglobin (HHb) levels. The optical attenuation at 720 nm is responsive to oxyhaemoglobin, and the attenuation at 760 nm is responsive to oxyhaemoglobin and deoxyhaemoglobin absorption (Skarda et al., 2007). The spectrometer system automatically provided an average of every 9 s data before, during and after knee extension exercises for each dependent variable measured. Please refer to the manuscripts by Skarda et al. (2007) and Myers et al. (2005) for more detailed information about how the changes in haemoglobin levels were determined.

A Nova Biomedical lactate analyzer (Nova Biomedical Corporation, Waltham, MA, USA) was calibrated once a day prior to testing using the standard control solutions. Subjects were asked to wash their hands with soap and water at least 5 min before testing started, and isopropyl alcohol wipes were used to clean the puncture site before the collection of blood. A 1.8-mm lancet was used to prick the subject’s finger and collect a drop of blood into the lactate analyser strip at before, during the 60 s rest period between second and third sets, immediately postexercise, 5 min-post, 10 min-post, and 20 min-postexercise. The minimum difference for lactate values to be considered ‘real’ was 1.11 (Weir, 2005).

Arm BP was measured following at least 5 min rest in the sitting position by an automated device (Omron HEM-773AC, Vernon Hills, IL, USA), and the BP value was used to set the FRP for the first testing session. The same FRP as the prior visit was utilized to keep the final pressure consistent across sessions. The BFR device (Kaatsu-Master; Sato Sports Plaza, Tokyo, Japan) consists of two elastic belts (5·5 × 90 cm) with a pneumatic bag along the inner surface of the cuffs and an electronic air pressure control system that monitors the restriction pressures set by the investigator. The elastic cuff was placed in the most proximal portion of the leg around the inguinal area. The initial tightness of the cuff is measured by the electronic device as the IRP and was randomly set at either 40–45 or 60–65 mm Hg. Then, the cuffs were inflated to reach the approximate normal resting systolic BP (120 mmHg) for a healthy adult. The pressure was held at 120 mmHg for 30 s and released for 10 s. Then, the pressure was increased by 20 mmHg while holding for 30 s at each pressure and releasing for 10 s between increments until the FRP was reached (Final Restrictive Pressure = Arm Systolic Blood Pressure × 1.44).

**Statistical analyses**

Two separate two-way repeated measures ANOVAs were used to examine mean differences in MVC [initial pressure (40–45 versus 60–65 mmHg) × time (pre- versus postexercise)] and lactate [initial pressure (40–45 versus 60–65 mmHg) × time (pre-exercise, internset, postexercise, 5 min, 10 min, 20 min)]. In addition, four separate two-way repeated measures ANOVAs [initial pressure (40–45 versus 60–65 mmHg) × time (average of every 9 s data before, during, after knee extension exercises)] were utilized to test hypotheses about differences in tissue oxygenation, oxygenated haemoglobin, deoxygenated haemoglobin, and total haemoglobin. Post hoc comparisons were performed with Bonferroni corrections, when a significant effect was detected. For the follow-up analyses, paired t-tests were used for IRP differences at each time point and one-way repeated measures ANOVA was used for time effects within treatment. An α value of 0.05 was used to determine statistical significance. Data were analysed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA) and reported as means ± SE in all figures.

**Results**

There were significant differences in percent tissue oxygenation during the knee extension exercises within each condition (P<0.001) and significantly lower tissue oxygenation values when using IRP of 60–65 mmHg compared with the session using IRP of 40–45 mmHg (P = 0.01; Fig. 1a). A significant interaction between IRP and time for tissue oxygenation was also observed (P = 0.02), indicating that the IRPs are changing tissue oxygenation over time in different ways. There was a significant interaction between IRP and time for total haemoglobin (P<0.001). The session with IRP of 60–65 mmHg resulted in significantly greater total haemoglobin values compared with the session with IRP of 40–45 mmHg (P<0.001), and total haemoglobin values also changed significantly within each condition (P<0.001; Fig. 1b). There were no significant changes in oxygenated haemoglobin values between IRPs; however, a significant difference in the amount of oxygenated haemoglobin values was observed within each condition (P<0.001; Fig. 2a). There was a significant interaction between IRP and time (P = 0.001) and significant main effects of IRP (P = 0.02) and time (P<0.001) for deoxygenated haemoglobin. When using IRP of 60–65 mmHg, the amount of deoxygenated haemoglobin relative to baseline was significantly greater compared with the session using IRP of 40–45 mmHg (P = 0.02). The pattern of changes in deoxygenated values was displayed in Fig. 2b.

Two-way repeated measures ANOVAs [pressures (40–45 versus 60–65 mmHg) × strength (pre- versus postexercise]
MVC) did not detect any significant interactions for IRP and MVC, but there were significant main effects for IRP \((P = 0.004, \eta_p^2 = 0.36)\) and MVC values \((P < 0.001, \eta_p^2 = 0.47)\). The session with IRP of 60–65 mmHg resulted in a significantly greater fatigue response (lower MVC values) compared with the session with IRP of 40–45 mmHg \((P < 0.004)\). The pattern of changes in knee extension strength was shown in Fig. 3a. No significant interaction for IRP and lactate level and no main effect for IRP were detected, but there was a main effect for lactate concentration during testing \((P < 0.001; \text{Fig. 3b})\).

**Discussion**

The results of this study verify that even when FRPs during exercise are not changed, different IRPs can have significant effects on blood accumulation and leg strength; therefore, IRPs must be set at standardized tensions, 35–45 mmHg for the initial stages and 55–65 mmHg for later stages of training, to obtain desired training-induced changes (Karabulut et al., 2011). It should be noted that this study used Kaatsu-Master to restrict blood flow, and initial pressures used for other types of equipment might be different. Further, this study is the first to show quantitatively that higher IRP results in significant increases in the amount of blood pooling and increases in the accumulation of deoxyhaemoglobin and total haemoglobin levels during exercise with BFR. Interestingly, higher IRPs do not result in significant changes in the flow of oxygenated haemoglobin to the limbs during the exercise protocol; however, it is important to highlight that accumulation of deoxygenated haemoglobin is indicative of a decreased oxygen supply when the period of exercise with BFR is increased. The other key finding of this study is the significantly greater fatigue response when a higher IRP was used and could represent another factor responsible for variations in physiological adaptations following BFR training protocols.

The information reported about the belt pressures used has only been about the pressure reached after cuff inflation (FRP), and it was defined as ‘final belt pressure’ (Abe et al., 2010a,b), ‘final occlusion pressure’ (Abe et al., 2006) etc. Several previous studies investigated the effects of different FRPs on specific variables such as haemodynamic parameters.
in nonexercising supine subjects (Iida et al., 2007) and skeletal muscle function (Cook et al., 2007). However, most of the published studies (Abe et al., 2006, 2010a; Ozaki et al., 2011) have not even mentioned IRPs when the BFR protocols were described. To date, there is only one published study (Karabulut et al., 2011) investigating the effects of varying IRPs on tissue oxygenation during rest. The current findings are also in agreement with the previous findings (Karabulut et al., 2011) that a greater IRP results in significantly lower tissue oxygenation.

The unique finding of the present study is that IRP is very critical in determining the level of tissue oxygenation and blood pooling that occurs during exercise with BFR. The findings from the present study also provide evidence that a greater hypoxic condition is created when a greater IRP is applied as demonstrated by increased amounts of accumulated deoxyhaemoglobin. As the current study used the same FRPs for both days of testing and as the pressure cuff was not deflated during rest periods, it appears that IRP was vital in determining the level of blood accumulation and skeletal muscle fatigue. It should be noted that even though significant impacts of IRPs on tissue oxygenation and leg strength were detected, there were no significant changes in lactate concentration. The findings may indicate that mechanisms responsible for fatigue may be a combination of both central and peripheral mechanisms, which also confirms a similar finding from a previous study (Karabulut et al., 2010b) reporting that BFR-induced fatigue might be due to both central and peripheral mechanisms.

One limitation of the present study was that the use of arm systolic BP for each individual to determine and set the cuff pressure used. The findings from previous studies highlighted the importance of thigh circumference to occlude arterial flow (Loenneke et al., 2012) and the importance of leg subcutaneous fat, leg lean body mass and thigh circumference for tissue oxygenation (Karabulut et al., 2011); therefore, future studies are needed to determine a standardized way of setting cuff pressures using thigh circumference and limb composition.

This study suggests that increases in IRPs resulted in decreases in tissue oxygenation, increases in blood and deoxyhaemoglobin accumulations, and an increased fatigue response demonstrating the importance of using proper IRPs and

![Figure 2](image.png)

Figure 2 (a) Changes in oxygenated haemoglobin (a. u.; arbitrary units relative to baseline). (b) Changes in deoxygenated haemoglobin (a. u.; arbitrary units relative to baseline). * represents significant differences ($P<0.05$) between haemoglobin values during each session. # represents significant difference between initial pressures ($P<0.05$). Values reported as Mean ± SE.
standardized protocols to obtain desired and reliable physiological outcomes. This is the first study to show the importance of tightness of the blood flow restriction cuffs during BFR training and to provide detailed information about the technique and procedure for future studies. Setting IRPs too low or high will change the amount of blood accumulation and possibly cause changes in overall training response. Future BFR training studies should find a way to set IRPs properly or use equipment that can detect and set IRPs at desired pressure levels with standardized training protocols.

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Conflict of interest

The authors declare that they have no conflict of interest.

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