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Effect of Aerobic Exercise with Blood Flow Restriction on Substrate

Utilization and Energy Expenditure

By

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Submitted in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science In the HTC Honors College at Coastal Carolina University

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Abstract

The purpose of this study was to examine the effects of intermittent blood flow restriction (BFR) compared to low- (LIIE) and high-intensity interval exercise (HIIE) on energy expenditure (EE) and substrate utilization. Participants randomly performed each interval exercise protocol, and then rested for a three-hour period, in which EE and substrate utilization were measured. Total EE was different between BFR (321.6 ± 30.1 kcals), HIIE (254.5 ± 33.5 kcals), and LIIE (287.1 ± 25.5 kcals). Fat oxidation (FatOx) in BFR ($1hr = 0.14 \pm 0.01$ g / min, $3hr = 0.11 \pm 0.01$ g / min) was greater than LIIE ($1hr = 0.08 \pm 0.02$ g / min, $3hr = 0.9 \pm 0.02$ g / min). This study suggests that the addition of intermittent BFR to LIIE may result in greater EE but similar substrate utilization compared to HIIE, albeit at a lower work rate.

Introduction

Regular exercise can help prevent and manage several health problems, however, according to the National Center for Health Statistics, only 21.7% of adults meet the physical activity guidelines for both aerobic and muscle strengthening activities (1). One of the most commonly reported obstacles to regular physical activity is a lack of time within their daily routine to include exercise (2). To manage this issue, different types, intensities, and duration of exercise have been utilized, such as interval training programs due to the decreased time commitment and significant cardiovascular improvements observed after such training. Typical high-intensity interval training involves alternating short intervals of high-intensity with lowintensity recovery intervals. While there is no universal interval training program, typically highintensity intervals are performed at near maximal to supramaximal intensity, followed by a lowintensity recovery or rest intervals (3). However, these high workloads may not be appropriate or well tolerated by everyone, such as those with low exercise capacities due to aging or injury. The inability of an individual to tolerate such a high workload may potentially further contribute to physical inactivity. Interestingly, recent studies have found positive physiological improvements when utilizing a much lower exercise intensity. These studies have used low intensities combined with a training technique called blood flow restriction (BFR) and have found significant improvements in aerobic capacity, aerobic performance, and muscular strength (4, 5, 6, 7) despite the relatively low intensities utilized. Therefore, BFR exercise has garnered a great deal of attention as a safe and potentially effective alternative to high-intensity exercise (8, 9, 10).

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While the adaptive benefits of BFR during resistance training have been well described (11), the potential for BFR training to enhance endurance is less explored. The few studies that have examined the effect of endurance training with BFR have demonstrated that low-intensity aerobic training with BFR has the potential to improve cardiovascular endurance, as well as muscle strength (12, 13, 14, 15). This improvement in muscle strength is not typically seen after endurance training, therefore making BFR that much more attractive. In addition to the typical improvements in cardiovascular fitness with aerobic training, metabolic adaptations associated with aerobic exercise training correlate with improved insulin action and glycemic control during recovery from exercise. Current recommendations for improving glycemic control involve performing moderate to vigorous-intensity aerobic and resistance exercise for an extended time. However, the general population fails to follow such time-consuming regimes, therefore potential alternatives should be explored, such as the application of BFR during aerobic-based exercise. Currently, there is a void in the literature pertaining to BFR training and energy expenditure, and substrate utilization (16, 17). Skeletal muscle is considered the major tissue responsible for glucose uptake and therefore is instrumental in glycemic control. Based on our current investigations on the acute effects of cycling exercise with BFR, there are alterations in muscle activation and metabolic stress during cycling with BFR compared to control conditions (16, 17). These alterations may have significant effects on glycogen degradation during exercise and impact substrate utilization and energy expenditure following exercise. This investigation will examine the acute effects of cycling exercise with the addition of BFR on energy expenditure and fuel usage.

Methods

Participants

There were 11 participants recruited for this study. Prior to participation, subjects were informed of the risks and benefits of the study and written informed consent was obtained from all individuals participating in the study. All procedures performed in this study were approved by the university's institutional review board. All participants recruited for this study were apparently healthy and recreationally active, based on their responses to a health history questionnaire. Any individual who self-reported a history of metabolic, pulmonary, cardiovascular disease, or an orthopedic related injury in the past 6-months was excluded. In addition, participants were asked to refrain from any unaccustomed strenuous physical activity, maintain their normal dietary habits, and not to take any anti-inflammatory drugs or nutritional supplements during the experimental period.

Participants were required to report to the laboratory on four separate visits. The first visit determined anthropometric measurements, an estimate of total energy expenditure, and peak exercise responses. The second, third, and fourth visits served as the experimental trials. The experimental trials used a repeated-measures crossover design, in which each subject performed three experimental trials: Low-intensity, Low-intensity with BFR, High-Intensity.

Visit 1

Upon arrival to the laboratory on the first visit, participants rested quietly in a seated position with legs uncrossed for 5 minutes. Following the 5 minutes of seated rest, the

participant's heart rate and blood pressure were measured on the right arm using an automated monitor (Omron, Model BP786N). Blood pressure was taken twice with the arm rested on a table at heart level and the average of the two measurements was used. Mid-thigh circumference was assessed midway between the patella (knee cap) and inguinal fold (crease at top of thigh). Additionally, a thigh skinfold was assessed on the mid-point of the anterior surface of the thigh. Mid-thigh circumference and skinfold were taken in duplicate. These measurements are standard measurements to report with BFR and NIRS data. Body composition was determined by wholebody densitometry using air displacement plethysmography (Bod Pod®, Cosmed, Concord, CA USA).

Subjects also performed a ramped exercise test on an electronically-braked cycle ergometer (Lode Corival, Netherlands) to determine peak exercise responses. The graded exercise test commenced at 20 W for four minutes followed by a ramped 20 W·min⁻¹ increase in work rate (WR) until volitional exhaustion. Breath by breath gas exchange (Quark CPET Cosmed, Rome, Italy) was collected throughout the test and averaged over 10-second intervals. The highest VO₂ averaged over a 10-second interval was taken as VO_{2peak}. The estimated VT was determined by visual inspection from gas exchange indices using the V-slope method, ventilator equivalents, and end-tidal pressures of oxygen and carbon dioxide.

Pre-Experimental Trial Protocol

The experimental trials were performed on different days separated by at least 48 hours and assigned in a randomized order (<u>www.randomization.com</u>). Each participant performed the experimental trials at approximately the same time in the morning. Three hours prior to each experimental trial, participants were asked to consume a standardized meal, based on an estimation of their total energy expenditure. For the purposes of the standardized meal, total energy expenditure consisted of estimations of resting energy expenditure via the Nelson equation and estimation of daily physical activity energy expenditure, as determined by responses to the International Physical Activity Questionnaire (IPAQ). The total caloric intake of the standardized meals was approximately 15-20% of the participant's total energy expenditure. This percentage is equivalent to the percentage of calories consumed during breakfast by an average adult. The standardized meal consisted of a nutritional shake (Ensure® Original Nutrition Shake., 220 calories per serving, 33 g carbohydrates, 6 g fat, and 9 g protein) and a cereal bar (Kroger® Fruit & Grain Cereal Bar 130 calories per serving, 25 g of carbohydrates, 3 g of fat, and 1 g of protein). The number of servings of the nutritional shake and cereal bars were adjusted, so each participant consumed between 15% and 20% of their estimated total energy expenditure. In addition to consuming the standardized meal, participants will be asked to avoid consumption of caffeine, alcohol, and tobacco for at least 12 hours prior to each visit and avoid vigorous activity at least 24 hours prior to each visit. Adherence to all pre-test requirements, including consuming the entire assigned meal, were verbally confirmed by the investigator prior to the start of the experimental trials.

In order to control for daily variations in resting oxygen consumption, the participants were asked to rest in a semi-recumbent position for 30 minutes for each experimental trial. After the first 15 minutes of the resting period, the participants were fitted with a face-mask. Breathby-breath gas exchange and HR, via a HR chest strap (Garmin) were continuously collected for the final 15 minutes of the resting period.

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Exercise Protocols

The exercise protocols were matched for total work output and were performed on an electronically-braked cycle ergometer (Corival, Lode, Netherlands). Participants were instructed to maintain a pedal rate of 60-80 RPM throughout the protocol. Each exercise protocol started with a four-minute warm-up (20W). For the BFR and LI exercise trial, participants completed 10 two-minute work intervals interspersed with one-minute recovery. Work intervals were performed at a cycling workload corresponding to 70% of GET, as determined from the graded exercise test. The workload for the recovery intervals were 20 W. During the BFR trial, the BFR cuffs were inflated to 80% of limb occlusion pressure (LOP) at the start of the work intervals and remain inflated throughout each work interval. During all recovery intervals, the cuffs were rapidly deflated and remained deflated until the next work interval. The BFR cuffs were not worn by participants during the LI trial.

Participants completed five two-minute work intervals interspersed with one-minute recovery intervals. Each work interval lasted 2 minutes followed by 1 minute of recovery. Work intervals were performed at a cycling workload corresponding to 140% of GET, as determined from the graded exercise test. During all recovery intervals, the power output was reduced to 20 watts. The BFR cuffs were not worn by participants during the HI trial. Breath-by-breath gas exchange and HR were continuously collected throughout each of the exercise trials.

Recovery

Immediately following each of the exercise bouts, the participant assumed a semirecumbent position and were asked to remain seated for the 3-hour recovery period in order to measure EPOC. Immediately at the start of recovery, breath by breath gas exchange was collected continuously for the first 30 minutes of recovery, except for a short break at 15 minutes for the participant to drink water. Following the first 30 minutes, gas exchange was collected for 8 minutes of every 15-minute time period between 30 and 180 minutes. While the gas exchange was not being collected the participants could drink water ad-libitum while remaining in the semi-recumbent position. Participants were allowed two breaks from the semi-recumbent position at 60 minutes and 120 minutes to use the bathroom, if necessary.

BFR Application

To determine the BFR pressure for each participant, following the resting blood pressure measurement, cuffs (Hokanson, SC10D, Bellevue, WA, 10.0 cm width) were placed around the proximal portion of both thighs. Participants then laid supine on a treatment table and rested for five minutes. Then the popliteal artery pulse was identified on the participant's dominant leg using Doppler auscultation (Nicolet, Imex Pocket Dop II). Once the pulse was identified, the cuffs were progressively inflated until the pulse was eliminated. The pressure associated with the cessation of the pulse was taken as the limb occlusion pressure (LOP) (REFS). This procedure was performed prior to all exercise protocols that involved the application of BFR. During all protocols that involved BFR, the cuffs (Hokanson, SC10D, Bellevue, WA, 10.0 cm width) were placed proximally on both legs and inflated to each participants' custom pressure based on their LOP (80% LOP) (REF).

Enjoyment

Enjoyment of the exercise bout was measured using the Physical Activity Enjoyment Scale (PACES) (REF). During each experimental trial, the PACES scale was given to the subjects 15 minutes into recovery while they were seated (REF). The PACES is an 18-question survey with a 7- point bipolar scale (minimum score = 18 and maximal score = 126) measuring how the subject felt about the exercise they just completed.

Energy Expenditure

Energy expenditure during each exercise protocol was calculated from the absolute VO₂ (L·min⁻¹) averaged every 1 minute from the start of the first work interval to the end of the last work interval. Due to the limitations of using RER to calculate energy expenditure during high-intensity exercise, each absolute VO₂ (L·min⁻¹) was multiplied by a factor of 5 kcals \cdot L of O₂⁻¹. All of the energy expenditures during the exercise protocol were summed to calculate the total energy expenditure.

EPOC

The magnitude of EPOC was calculated over the first 90 minutes of recovery for each trial. The absolute VO2 (L·min⁻¹) was averaged over the final 10 minutes of the resting period and was considered the baseline measurement for each trial. During recovery, VO2 was averaged over 15-seconds during the times following times: 0-15 min, 17-30 min, 38-45 min, 53-60 min, 68-75 min, 83-90 min. For each 15-second VO₂, a netVO₂ was calculated by subtracting the

baseline VO₂ from the VO₂. The netVO2 was then plotted against the time during recovery and EPOC was calculated as the area under the curve via the trapezoidal rule.

Substrate Oxidation Calculations

From the gas exchange, the fat oxidation rate and carbohydrate oxidation rate were calculated from the averages VO₂ and VCO₂ from the final 10 minutes of rest and 10-minute averages during recovery between 50-60 minutes (1HR), 110-120 minutes (2HR) and 170-180 minutes (3HR). Calculating fat oxidation and carbohydrate oxidation by indirect calorimetry assumes steady-state conditions which may not be present during the first 60-120 minutes of recovery from high-intensity exercise. Bicarbonate buffering and non-metabolic CO₂ has been shown to be no different from resting control conditions from 60 to 120 minutes after high-intensity exercise. Therefore, exercise and data early in recovery were excluded from the analysis. Fat and carbohydrate oxidation rates were calculated using the following equation: Fat oxidation rate = $1.695*VO_2 - 1.701*VCO_2$

Carbohydrate oxidation rate = $4.585*VCO_2 - 3.226VO_2$

Statistical Analysis

Statistical analyses were completed on IBM SPSS statistical software (Version 25.0; SPSS, Inc., Chicago, IL). A two-way (trial [BFR, HI, LI by time [Rest, Ex, 1HR, 2HR, 3HR) repeated measures ANOVA was used to compare the physiological responses absolute VO₂ (L·min⁻¹), relative VO₂ (ml·kg⁻¹·min⁻¹), RER and HR). A two-way (trial [BFR, HI, LI by time [Rest, 1HR, 2HR, 3HR) repeated measures ANOVA was used to compare the fat oxidation rate and carbohydrate oxidation rates between trials. A one-way repeated measures ANOVA was used to compare energy expenditure during exercise, EPOC, and post-exercise enjoyment (PACES) between trials. Subsequent Bonferroni pairwise post-hoc comparisons were made when necessary. Cohen's d was used as an estimate of effect size (7). Effect size was interpreted as where small effect < 0.4, medium effect = 0.40-0.75, large effect = 0.75-1.1, very large effect = 1.1-1.45, and huge effect > 1.45. Statistical significance was established if p ≤ 0.05 .

Results

Participants

Ten of the participants recruited for this study were included in the analysis. One participant's data was excluded due to an equipment error during one of their visits. Of the ten participants included in the study, there were seven male and three female participants. The subjects were 25.1 ± 6.0 years old, 172.4 ± 4.1 cm tall and weighed 75.8 ± 12.9 kg. The BMI of the subjects was 25.4 ± 3.6 kg·m-2 and had 21.8 ± 7.5 % body fat. From the graded exercise test, the VO2peak was 2.74 ± 0.82 L·min-1 (36.0 ± 7.5 ml·kg-1·min-1) and WRpeak was 252.3 ± 59.1 W. Therefore, the subjects total work output of 2421.6 ± 567.6 W in each exercise protocol. The equal total work outputs were part of the design of the study.

Physiological Responses

The physiological responses (absolute VO₂ ($L \cdot min^{-1}$), relative VO₂ ($ml \cdot kg^{-1} \cdot min^{-1}$), RER, and HR) to BFR, HI, LI are shown in Table 1. Absolute VO₂ ($L \cdot min^{-1}$), relative VO₂ ($ml \cdot kg^{-1} \cdot min^{-1}$) and RER did not meet the assumption of sphericity, so a Greenhouse-Gasser test was run. There was a significant trial by time interaction for absolute VO₂ ($L \cdot min^{-1}$), relative VO₂, HR and RER. Subsequent post-hoc testing showed significant trial effects and time effects for each variable. Specifically, absolute VO₂ (L·min⁻¹) and relative VO₂ (ml·kg⁻¹·min⁻¹) were similar at rest between trials, but all trials had significantly different absolute VO₂ (L·min⁻¹) and relative VO₂ (ml·kg⁻¹·min⁻¹) during exercise (for all comparisons; $0.61 \le d \le 1.86$). In addition, BFR trial had a greater absolute VO₂ (L·min⁻¹) and relative VO₂ (ml·kg⁻¹·min⁻¹) at 2HR compared to HI (d = 0.84 and d = 0.79) and LI (d = 1.068 and d = 0.87); the differences between HI and LI at 2HR were not significant. There were no differences between trials for absolute VO₂ (L·min⁻¹) and relative VO₂ (ml·kg⁻¹·min⁻¹) at 1HR and 3HR. Post-hoc testing also revealed significant time effects for absolute VO₂ (L·min⁻¹) and relative VO₂ (ml·kg⁻¹·min⁻¹). Within all trials, absolute VO₂ (L·min⁻¹) and relative VO₂ (ml·kg⁻¹·min⁻¹) was greater during exercise than rest and all points in recovery, as expected. The only other significantly time effect was observed within the BFR trial; absolute VO₂ (ml·kg⁻¹·min⁻¹) at rest and 2HR during the BFR trial did not reach significance (p = 0.08).

The RER at rest was not different between trials, but the RER during exercise was significantly different between all trials (BFR vs HI; d = 3.89, BFR vs LI; d = 1.14, HI vs LI; d = 5.03). The only differences in RER between trials during recovery occurred at 1HR. Specifically, the RER during BFR was significantly lower than the RER during HI (d = 0.69) and LI (d = 0.40). Within all trials, the RER during exercise was significantly greater than rest and all points during recovery, as expected. Additionally, the RER at rest was significantly greater than all points during recovery within all trials (for all comparisons; $1.54 \le d \le 2.66$). Within all trials, RER at each point in recovery were not significantly different from each other.

There were no differences between groups for HR at rest. There was a significantly greater HR during exercise in the BFR and HI trials compared to LI (d = 1.83 and 1.82,

respectively); the difference in HR during exercise between BFR and HI was significant. During recovery, HR at 2HR in BFR was significantly greater than at 2HR in HI (d = 0.38); the HR at 1HR and 3HR were not different between BFR and HI. Additionally, HR during BFR was significantly greater than HR during LI at all points during recovery (1HR d = 0.90; 2HR d = 0.57; 3HR d = 0.48). The only difference in HR during recovery between HI and LI occurred at 1HR d = 0.95). Within all trials, HR during exercise was significantly different from rest and all points during recovery, as expected. There were no other significant differences within the BFR trials. Within HI, the HR at 1HR was significantly greater than HR at rest (d = 0.60 and 2HR (d = 0.82) and 3HR (d = 0.72). During LI, HR at rest was significantly greater than HR at 1HR (d = 0.33), 2HR (d = 0.59) and 3HR (d = 0.51). There were no differences in HR between time points in recovery during LI.

Fat and Carbohydrate Oxidation Rate

Fat oxidation rate at rest and during recovery for each trial are shown in Figure 1. There was a significant trial by time interaction for fat oxidation rate. Subsequent post hoc testing showed significant group and time effects. At rest, there were no significant differences in fat oxidation rate between trials. Fat oxidation rate was significantly greater in BFR compared to HI at 2HR d = 1.13). There were no other differences in fat oxidation rate between BFR and HI; the difference at 1HR approach but did not reach significance (p=0.065; d = 0.92). Additionally, fat oxidation was significantly greater in BFR compared to LI at 1HR (d = 1.93) and 2HR (d = 1.70); the difference at 3HR was not significant. Fat oxidation rate was similar between HI and LI during all points in recovery. Within all trials, fat oxidation rate during rest was significantly

lower than points during recovery (for all comparisons; $3.56 \le d \le 5.70$). The only other difference in fat oxidation rate was between 1HR and 3HR during LI trial (d = 1.94).



Figure 5. Fat oxidation rate. †- significantly different than LI, ‡- significantly different than HI

Carbohydrate oxidation rate responses at rest and during recovery for each trial are shown in Figure 2. The trial by time interaction and the main effect of trial was no significant. There was a significant main effect of time. Specifically, the carbohydrate oxidation rate was significantly lower during rest than all points during recovery.



Figure 6. Carbohydrate oxidation rate

Energy Expenditure

Total energy expenditure during exercise in BFR, HI and LI are shown in Figure 3. The one-way ANOVA showed a significant effect. Specifically, BFR trial expended more energy compared to HI (d = 2.25) and LI (d = 0.77). Additionally, LI produced a greater energy expenditure compared to HI (d = 1.48).



Figure 3. Energy expenditure (EE) during exercise. *†*- significantly different than LI, *‡*- significantly different than HI.

EPOC

The magnitude of EPOC in each trial is shown is Figure 4. The one-way ANOVA showed a significant effect. The magnitude of EPOC following the BFR protocol was similar to HI, but was greater than LI (d = 0.97). Additionally, the magnitude of EPOC was significantly greater in HI compared to LI (0.97).



Figure 4. Excess post-exercise oxygen consumption (EPOC). ‡- significantly different than LI.

Enjoyment

Enjoyment of the trial was measured by the PACES scale. The score on the PACES scale following the BFR, HI, and LI protocols were 85.2 ± 18.5 , 89.9 ± 10.3 , 86.9 ± 22.1 , respectively. There were no differences in scores between trials.

Discussion

The results of this study suggest that, by utilizing BFR, individuals can participate in lowintensity exercise and achieve greater energy expenditure than if they would've exercised at a significantly higher work rate, as during the high-intensity is this study. This is an important consideration for individuals who are interested in losing weight or altering their body composition, as by increasing energy expenditure a caloric deficit, which is an important aspect of weight loss, maybe facilitated. Therefore, our results provide preliminary evidence for the potential use of BFR during low-intensity cycling to aid in weight loss.

When we start exercising the aerobic energy system is not capable of immediately meeting the body's energy demand, so the anaerobic energy system must contribute to help meet the initial energy demand. This reliance on the anaerobic energy system during this time results in an oxygen deficit or O_2 debt, being accrued, which must be paid back following exercise. This reimbursement is what we refer to as excess post-exercise oxygen consumption (EPOC), during which oxygen consumption remains elevated above resting levels after the completion of the exercise. So being that the results of this study showed similar EPOC in high intensity and BFR conditions, it would suggest that there were similar anaerobic contributions between the two conditions during exercise. This is particularly interesting considering that the high-intensity condition was performed at double the intensity of exercise as the BFR condition.

As we know, our body cannot store an endless amount of carbohydrates to be utilized as fuel. We can find carbohydrates as glucose in the blood and stored as glycogen in the muscles and liver, but this supply is limited. On the other hand, our body can store a significantly larger amount of fats to be used for energy. We also know that at lower intensities of exercise a greater percentage of our energy demand comes from fats, whereas at higher intensities our body relies more on carbohydrates. That being said, when we consider that our study showed increased fat oxidation following exercise with the BFR condition, it suggests that more carbohydrates were utilized during exercise. This increased utilization of carbohydrates suggests that by utilizing BFR during low-intensity exercise, substrate utilization shifts to more carbohydrates despite the low intensity of exercise. This increased utilization of carbohydrates during BFR cycling may have been due to the reduced oxygen availability to the exercising muscle caused by the BFR application. Carbohydrates can be utilized anaerobically (without oxygen) and aerobically, whereas fats can only be utilized in the aerobic energy system. Therefore, the reduction in oxygen in the exercising muscle from the BFR may have limited the oxidation of fats. Additionally, since we reduced our carbohydrate storage during exercise, we will rely more heavily on the aerobic energy system (fats) following exercise, as evident from the greater fat oxidation observed after BFR exercise.

In conclusion, our study suggests that utilizing blood flow restriction during lowintensity exercise may result in energy expenditure, excess post-exercise oxygen consumption, and fat oxidation similar to or greater than that of high-intensity exercise, albeit at a much lower work rate. Going forward, it would be interesting to test these conditions in a training study and determine whether or not blood flow restriction training will actually result in greater weight loss and changes in body composition (i.e. lower body fat percentage) than traditional high-intensity training or moderate intensity.

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