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Physiological responses to interval endurance exercise at different levels of blood flow restriction

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Running Title: Intermittent endurance blood flow restricted exercise.

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ABSTRACT

Purpose: We aimed identify a blood flow restriction (BFR) endurance exercise protocol that would both maximize cardiopulmonary and metabolic strain and minimize the perception of effort. Methods: Twelve healthy males (23±2 years, 75±7kg) performed five different exercises protocols in randomized order: HI, high-intensity exercise starting at 105% of the incremental peak power (P_{peak}); I-BFR30, intermittent BFR at 30% P_{peak}; C-BFR30, continuous BFR at 30% P_{peak}; CON30, control exercise without BFR at 30% P_{peak}; I-BFR0, intermittent BFR during unloaded exercise. Cardiopulmonary, gastrocnemius oxygenation (StO_2), capillary lactate ([La]), and perceived exertion (RPE) were measured. Results: $\dot{V}O_2$, ventilation ($\dot{V}_E$), heart rate (HR), [La] and RPE were greater in HI than all other protocols. However, muscle StO_2 was not different between HI (set1- 57.8±5.8; set2– 58.1±7.2%) and I-BRF30 (set1- 59.4±4.1; set2- 60.5±6.6%, $p<0.05$). While physiologic responses were mostly similar between I-BFR30 and C-BFR30, [La] was greater in I-BFR30 (4.2±1.1 vs 2.6±1.1 mmol.L^{-1}, p=0.014) and RPE was less (5.6±2.1 and 7.4±2.6; p=0.014). I-BFR30 showed similar reduced muscle StO_2 compared with HI, and increased blood lactate compared C-BFR30 exercise. Conclusion: Therefore, this study demonstrate that endurance cycling with intermittent BFR promotes muscle deoxygenation and metabolic strain, which may translate into increased endurance training adaptations while minimizing power output and RPE.

Keywords: Cycle ergometry; near-infrared spectroscopy; O_2 uptake; High-intensity exercise; Lactate; Rating of Perceived Exertion.

Abbreviations:

BFR - blood flow restriction

HI - high-intensity

P_{peak} - peak power

I-BFR30 - intermittent

C-BFR30 - continuous Blood Flow Restriction at 30% of peak power

CON30 - control exercise at at 30% of peak power

I-BFR0 - intermittent Blood Flow Restriction during unloaded exercise

50 StO_2 - oxygenation

51 [La] - capillary lactate

52 RPE - perceived exertion

53 $\dot{V}O_2$ - oxygen uptake

54 $\dot{V}_E$ - Ventilation uptake-

55 HR - Heart rate

56 OBLA - onset blood lactate accumulation

57 W - watts

58 O_2 - oxygen

59 CO_2 - dioxide of carbon

60 O_2Hb - oxyhemoglobin

61 HHb - deoxyhemoglobin

62 SD - standard deviation

63 ANOVA - Analysis of variance

64 mmHg - millimeters of mercury

65 HIF-1a - hypoxia inducible factor-1a

66 VEGF - vascular endothelial growth factor

67 COPD - chronic obstructive pulmonary disease

68 CHF - congestive heart failure

69

INTRODUCTION

In the last decade, exercise in combination with blood flow restriction (BFR) has emerged as an effective training paradigm to enhance improvements in muscle strength (Abe et al. 2006; Sumide et al. 2009; Takarada et al. 2000) and endurance (Abe et al. 2010a; de Oliveira et al. 2015), without the need for high muscle force generation. Exercise training with BFR is associated with greater muscle protein synthesis, lower proteolysis, and a greater hypertrophy (Fujita et al. 2007; Gualano et al. 2010; Laurentino et al. 2012; Ozaki et al. 2014) and with greater increase in muscle capillarity and aerobic capacity ($\dot{V}O_{2max}$) (Kacin and Strazar 2011; Larkin et al. 2012; Patterson and Ferguson 2010) compared to training without BFR.

BFR training is typically performed in association with resistance exercise, and the adaptive benefits are well-described (Pearson and Hussain 2015). However, the potential for BFR training to enhance $\dot{V}O_{2max}$ and exercise endurance is less well explored (Abe et al. 2006; de Oliveira et al. 2015). These studies demonstrated that endurance training performed with BFR resulted in significant increases in strength and hypertrophy compared to control training. Interestingly, BFR interval endurance training at low relative power output (e.g. 30% of incremental-test peak power; P_{peak}) is also associated with an increase in the onset blood lactate accumulation (OBLA) and $\dot{V}O_{2max}$ (de Oliveira et al. 2015; Park et al. 2010) improvements that are traditionally associated with high intensity interval training (HIT) (Gibala et al. 2006).

In the few studies to have investigated BFR endurance training using low relative power output, the aerobic adaptations are highly variable (Abe et al. 2010b; Ozaki et al. 2011). For example, following 6 weeks of cycle endurance training with intermittent BFR (at 90 mmHg found an increased P_{peak}, without an increase in $\dot{V}O_{2max}$ (Keramidas et al. 2012). Abe et al. (2010a), on the other hand, showed greater increases in quadriceps cross sectional area and volume, and $\dot{V}O_{2max}$

93 after 8 weeks of endurance training with continuous BFR (at 160-210 mmHg) compared with
94 unrestricted control. Park et al. (2010) also found an increase in $\dot{V}O_{2max}$ after 2 weeks of walking
95 training with blood flow occlusion, but no strength adaptations. Finally, de Oliveira et al. (2015)
96 showed that $\dot{V}O_{2max}$ and isometric quadriceps strength were increased, and blood lactate
97 accumulation was slowed, after 4 weeks of interval cycle-ergometer training with intermittent BFR
98 (at ~150 mmHg). Since that continuous BFR results in significantly greater ratings of perceived
99 exertion (RPE) and pain than intermittent BFR (Fitschen et al. 2014) and endurance outcomes
100 where similar between intermittent BFR training and HIT (de Oliveira et al. 2015), there may be
101 some optimal set of conditions that balance the physiological and perceptual responses to these
102 different approaches to exercise training.

103 While these studies clearly demonstrate the potential for BFR training to induce strength and/or
104 endurance adaptations using low power exercise, the relative cardiopulmonary and metabolic
105 strain and perceived exertion during low power BFR endurance exercise, in comparison to
106 traditional high power endurance training, remains unclear. We were therefore interested to
107 determine the cardiopulmonary (ventilation, and heart rate), metabolic (gas exchange, muscle
108 oxygenation, blood lactate), and perception of effort responses to a range of continuous and
109 intermittent BFR endurance exercise protocols compared with traditional high intensity exercise
110 training without BFR. We aimed to identify a BFR endurance exercise protocol that would
111 maximize cardiopulmonary and metabolic strain, while minimizing the perception of effort.

112 We, therefore, determined the cardiopulmonary, metabolic and perceptual responses during BFR
113 endurance exercise with continuous (just below passive occlusion pressure) and intermittent (just
114 above passive occlusion pressure) blood flow restriction, at very low (unloaded pedaling) and low

115 power output (30% P_{peak}). These were compared with responses to low- (30% P_{peak}) and high-
116 intensity (105% P_{peak}) interval cycling exercise without blood flow restriction.

117 Based on the findings of Fitschen et al. (2014) we hypothesized that endurance exercise with
118 intermittent higher-pressure BFR, would be better tolerated and induce a lesser cardiopulmonary
119 strain than continuous lower-pressure BFR . Based on de Oliveira et al. (2015), of similar adaptive
120 responses to intermittent BFR and HIT, we also hypothesized that intermittent higher-pressure
121 BFR would more closely mimic the magnitude of muscle deoxygenation observed in HIT lower-
122 pressure BFR. Confirming these hypotheses would provide new insights into the physiologic
123 conditions suitable for exploring low-intensity BFR endurance training benefit.

124

125 ***METHODS***

126 *Participants*

127 Twelve healthy male participants volunteered for the study (23 ± 2 years, 75 ± 7 kg, 177 ± 7 cm).
128 Participants were informed about the procedures and risks associated with the protocols and
129 provided written informed consent, in accordance with the latest revision of the Declaration of
130 Helsinki and the Belmont Report. This study was approved by the human subjects committee of
131 University of Santa Catarina State, Brazil. At the time of the study, none of the participants were
132 taking medications, nor were they in a structured training program.

133 *Experimental design*

134 Participants attended 7 laboratory visits, at the same time of day on each visit, with each visit
135 separated by 7 days. All exercise tests were performed on an electromagnetically-braked cycle

ergometer (Excalibur Sport, Lode, Groningen, NL), with breath-by-breath cardiopulmonary, muscle oxygenation, and capillary blood lactate ([La]) measurements. The first and last visit of the series was an incremental exercise test to the limit of tolerance to determine, amongst other variables, peak O_2 uptake ($\dot{V}O_{2peak}$) and P_{peak}. These tests were used to confirm that aerobic capacity was unchanged over the course of the experimental series. At each of the other 5 visits participants performed (in randomized order) one of 5 different intermittent exercise protocols.

Exercise protocols

Incremental exercise. The incremental cycling test began with 2 minutes at rest after which subjects began cycling at 1 $W.kg^{-1}$ for 3 minutes, followed by step-incremental increases of 35 W every 3 minutes until the limit of tolerance or the cadence decreased below than 60 rpm on three occasions. The subjects were asked to maintain a cadence of 70 rpm. P_{peak} was determined from the last uncompleted exercise stage, as: (W) + [t (s)/step duration (s) x step increment (W)]; where t is the duration of the uncompleted stage. After intolerance, the power on the ergometer was immediately reduced to 30 W and the participant was monitored during recovery for 5 minutes.

Intermittent exercise protocols. Initially, to determine the blood flow restriction pressure for each individual, participants lay in ventral position and a cuff was placed around the proximal portion of the thigh. The popliteal artery pulse was identified using Doppler auscultation (AV-800; Marted, Ribeirão Preto, São Paulo, Brazil). The thigh cuff was progressively inflated until the pulse was eliminated (confirmed by two investigators). The pressure associated with the cessation of pulse was taken as the pressure for blood flow occlusion in the passive state (Laurentino et al. 2012).

Following this, participants performed each of 5 intermittent exercise protocols in randomized order, with a range of power outputs and cuff inflation procedures. During intermittent exercise

158 with BFR, cuff belts (18 cm wide, aneroid auscultator Missouri®, Japan) were placed proximally
159 on both legs. Each intermittent exercise session followed the same pattern, with 2 sets of 5
160 repetitions each. Each repetition lasted 2 minutes, with 1 minute recovery between each repetition.
161 The rest interval between sets was passive and lasted 5 minutes. Subjects were asked to maintain
162 a cadence of 70 rpm during the exercise sets. The total duration of the exercise protocols was 35
163 minutes (bouts plus rest intervals). The intermittent exercise protocols were:

164 High-intensity exercise at 105% P_{peak} (HI) - Exercise repetitions were initiated at 105% P_{peak}, and
165 were decreased by 5% every 30 seconds of exercise until 2 minutes of exercise was completed i.e.
166 105%, 100%, 95%, and 90% P_{peak} for 30 s each [adapted from de Oliveira et al. (2015) (de Oliveira
167 et al. 2015)].

168 Intermittent BFR at 30% P_{peak} (I-BFR30) - Exercise repetitions were performed at 30% P_{peak} during
169 which thigh cuffs were inflated to 20 mmHg above the individual's passive occlusion pressure. In
170 the periods between the exercise repetitions, the thigh cuffs were deflated to 0 mmHg, and the
171 power output was decreased to 20 W.

172 Continuous BFR at 30% P_{peak} (C-BFR30) - Exercise repetitions were performed at 30% P_{peak}, and
173 thigh cuffs were inflated to 80% of the individual's passive occlusion pressure throughout the
174 entire 35 minute protocol (Brandner et al. 2015).

175 Control exercise at 30% P_{peak} (CON30) - Exercise repetitions were 30% P_{peak} without cuff inflation.
176 This protocol acted as a 'cuff control' for the I-BFR30 protocol.

177 Intermittent BFR at unloaded exercise (I-BFR0) - Exercise repetitions were performed at unloaded
178 cycling for 2 minutes. During the exercise the thigh cuffs were inflated to 20 mmHg above the

individual's passive occlusion pressure. In the periods between the exercise repetitions, the thigh cuffs were deflated to 0 mmHg and unloaded cycling was continued.

Measurements

Breath-by-breath cardiopulmonary measurements. Ventilatory and pulmonary gas exchange variables and heart rate (HR) were measured breath-by-breath using a commercial system (Quark PFTergo, Cosmed Srl, Rome, Italy). Before each test, the O_2 and CO_2 analysers were calibrated using ambient air and a gas of known O_2 and CO_2 concentration according to the manufacturer's instructions, while the turbine flow-meter was calibrated using a 3 L syringe over a range of different flow rates. Breath-by-breath $\dot{V}O_2$ and ventilation ($\dot{V}_E$) were averaged every 15 s to determine the greatest values during the incremental test for ($\dot{V}O_{2peak}$ and $\dot{V}_{Epeak}$, respectively) (Data Management Software, Cosmed, Rome, Italy).

Blood Lactate. Capillary blood samples were collected at discrete points during the exercise tests: every 3 minutes during incremental exercise, and at rest, 15 minutes (at the end of the first set) and 35 minutes (at the end of the second set) during the intermittent exercise protocols. Capillary blood was collected in a heparinized capillary tube from the earlobe. [La] was measured by an electrochemical method, which was calibrated with 5 $mmol.L^{-1}$ standards (YSI 1500 Sport, Yellow Springs Instrument, Yellow Springs, OH, USA).

Muscle oxygenation. An index of muscle tissue oxygen saturation (StO_2, %) was calculated from signals obtained using a continuous-wave near-infrared spectroscopy (NIRS; PortaMon, Artinis Medical Systems, Elst, The Netherlands). Light diode emissions at three wavelengths (905, 850, and 770 nm) were intensity-modulated at a frequency of 1 MHz, across 3 channels (3 equivalent pulsed light sources, and 1 avalanche photodiode detector with shielding from ambient light).

Emitters were spaced at 30, 35 and 40 mm from the detector. The intensity of received light was used to resolve the relative absorption (expressed as change from baseline, expressed in arbitrary units) of oxyhemoglobin (O_2Hb) and deoxyhemoglobin (HHb) and their sum (tHb) within the tissues under the probe and sampled at 10 Hz. From these, the StO_2 index was calculated using spatially resolved spectroscopy. NIRS is unable to separate signals from hemoglobin and myoglobin (Mb), therefore all signals represent some weighted contribution from both Hb and Mb chromophors. The NIRS system was placed on the medial gastrocnemius at the point of the largest circumference of the calf secured on the skin with tape, covered with a dense black vinyl sheet, and wrapped with an elastic bandage. This aimed to minimize light interference and movement of the equipment during cycling exercise.

Perception of effort (RPE). The Borg category-ratio CR-10 scale was used to quantify the perception of effort during the intermittent exercise protocols (Borg 1998). Participants were instructed "Rate the intensity of your effort", and coached to integrate sensations of pain (e.g. from compression of the tissues during cuff inflation) and effort (e.g. from exercise) into a single overall rating based on the perception of the tolerability of the exercise.

Statistical procedures

Data are presented as mean and standard deviation (SD). Normality was verified using Shapiro-Wilk's test. Comparisons of $\dot{V}O_{2max}$ and P_{peak} at the beginning and end of the study (visit 1 and visit 7) were made by paired Student's t-test. For physiologic variables during intermittent exercise protocols, the final 30 seconds of each 2 minute repetition was averaged to produce a mean (SD) for each set (sets 1 and 2), and the differences within variables were compared using mixed-model ANOVA (SPSS, v19.0, IBM Corporation, New York, USA), with the participant included as a random effect. Condition (5 intermittent exercise protocols) and set (2 sets per visit) were fixed

224 effects. When differences were indicated, *post hoc* analyses were performed using the Bonferroni
225 adjustment for multiple comparisons. The uncertainties in the effects were expressed as 95%
226 confidence limits and significance was accepted at an alpha ≤ 0.05.

227

228 ***RESULTS***

229 *Incremental exercise*

230 $\dot{V}O_{2peak}$ averaged 47.9 ± 5.8 mL.min^{-1}.kg^{-1} at visit 1, and was not different at visit 7 (47.9 ± 5.9
231 mL.min^{-1}.kg^{-1}; p = 0.80). P_{peak} was also not different between visits 1 and 7 (252 ± 29 vs. 249 ± 28
232 W, respectively; p = 0.26). These data confirmed that aerobic fitness remained stable during the
233 study.

234 *Passive occlusion pressure*

235 Passive occlusion pressure was 129 ± 16 mmHg among participants, resulting in an average
236 pressure of 149 ± 16 mmHg during protocols with intermittent cuff inflations (I-BFR30 and I-
237 BFR0) and 103 ± 14 mmHg during the protocol with continuous cuff inflation (C-BFR30).

238 ***- INSERT FIGURE 1 -***

239 *Intermittent exercise protocols*

240 An example of the $\dot{V}O_2$ responses to the 5 intermittent exercise protocols for a representative
241 participant is shown in Figure 1. The group mean gastrocnemius StO_2, O_2Hb, HHb and tHb
242 responses to the 5 intermittent exercise protocols are presented in Figure 2. As expected, the
243 transient changes in $\dot{V}O_2$ and StO_2 were greatest for HI, with the peak $\dot{V}O_2$ and nadir StO_2 rapidly
244 reaching an approximately-stable fluctuation by approximately the 3rd repetition of the first set.

Overall, while $\dot{V}O_2$ was strongly dependent upon power output (105% P_{peak}, 30% P_{peak} or unloaded), the muscle StO_2 and HHb responses, in particular, were strongly influenced by the cuff protocol (intermittent occlusion, continuous restriction or free flow).

- ***INSERT FIGURE 2*** -

Pulmonary responses. The group average physiologic responses to the 5 intermittent exercise protocols are presented in Figure 3. Within protocols there was no effect of set on $\dot{V}O_2$ (i.e. $\dot{V}O_2$ x set; $p > 0.05$). Conversely, $\dot{V}_E$ showed a significant set effect in the HI exercise protocol only, resulting in a greater $\dot{V}_E$ in set 2 compared with set 1 (156. ± 34 vs. 137 ± 25 $L.min^{-1}$; $p < 0.01$). Between protocols group mean $\dot{V}O_2$ and $\dot{V}_E$ followed a similar pattern (Figure 3A and 3B); $\dot{V}O_2$ and $\dot{V}_E$ were greater in HI in both exercise sets compared to all other protocols ($p < 0.05$), there were no differences among C-BFR30, CON30 or I-BFR30 conditions ($p > 0.05$), and I-BFR0 resulted in a significantly lower responses compared to all other conditions ($p < 0.05$).

Cardiometabolic responses. Group mean HR, [La] and muscle StO_2 responses are shown in Figure 3. Within protocols there was no set effect for HR. [La] was significantly increased compared with baseline in both the HI and I-BFR30 protocols during set 1 ($p < 0.01$), and between set 1 and set 2 ($p < 0.01$) (Figure 3F). In the C-BFR30 protocol [La] significantly increased between baseline and set 2 ($p < 0.01$), whereas [La] remained unchanged from baseline throughout the CON30 ($p > 0.05$) and I-BFR0 ($p > 0.05$) protocols. There was no effect of set on StO_2, except for during I-BFR0 where StO_2 in set 1 was less than in set 2 (60.1 ± 7.9 vs 63.2 ± 7.0 %, $p = 0.03$).

Between protocols, the HR (Figure 3C) and [La] (Figure 3F) responses were similar: HR and [La] were greater in the HI compared to all other protocols ($p < 0.05$), and were lower in CON30 and I-BFR0 compared with all other protocols ($p < 0.05$). HR and [La] were not different between I-

BFR30 and C-BFR30 protocols, except for [La] at the end of set 1, where [La] I-BFR30 was greater than C-BFR30 (4.2 ± 1.1 vs 2.6 ± 1.1 mmol.L^{-1}) ($p = 0.014$).

The StO_2 responses between the 5 intermittent exercise protocols are presented in Figure 3D. During intermittent restriction protocols the set 1 value of StO_2 (59.4 ± 4.1 and 60.1 ± 7.9 %, for I-BFR30 and I-BFR0 respectively) were similar to one another and were not different from HI (57.8 ± 5.8 %; $p > 0.05$). While StO_2 in I-BFR30 and I-BFR0 were similar, as expected the HHb amplitude was less in I-BFR0 than I-BFR30 (Figure 2). During set 2 however, StO_2 remained low during HI (58.1 ± 7.2 %) and I-BFR30 (60.5 ± 6.6 %) protocols, and was greater during I-BFR0 (63.2 ± 7 %, $p < 0.01$). During the continuous restriction protocol (C-BFR30) and the control protocol with free flow (CON30) the StO_2 was greater than all other protocols (StO_2 averaged in sets 1 and 2 was 64.6 ± 4.5 and 67.3 ± 4.2 %, respectively; $p < 0.05$) and did not differ from each other ($p = 0.58$).

Perception of effort (RPE). Broadly, the sense of effort responded similarly to the pattern observed in [La]. Within protocols there was a significant effect of set during HI, I-BFR30 and C-BFR30, with RPE in set 1 (8.5 ± 1.4; 4.3 ± 2.1 and 5.0 ± 2.2 respectively) lower than set 2 (9.8 ± 0.5; 5.6 ± 2.1 and 7.4 ± 2.6 respectively; $p < 0.05$). Between protocols, RPE was greatest during HI and lowest during CON30 and I-BFR0. The two cuff protocols at 30% P_{peak} (I-BFR30 and C-BFR30) resulted in RPE values that were intermediate (Figure 3E), being similar in set 1 and becoming different from one another in set 2 ($p = 0.01$).

- ***INSERT FIGURE 3*** -

DISCUSSION

Only a few studies have investigated the efficacy of BFR endurance training, and the training responses in these studies have been highly variable (Abe et al. 2010a; Abe et al. 2006; Abe et al. 2010b; Park et al. 2010; Sundberg 1994). This variability is likely due to the different combinations of exercise training and blood flow restriction or occlusion protocols used. Our study aimed to identify key features of combined endurance exercise and BFR that would provide a high cardiopulmonary and metabolic strain, and thus be expected to provide a strong adaptive stimulus during exercise training, while minimizing the perception of effort. We found that a 35 minute intermittent exercise protocol at 30% peak aerobic power with intermittent BFR at ~150 mmHg resulted in a significantly increased blood lactate, was well tolerated by all participants, and resulted in a muscle StO_2 that was not different from high-intensity interval exercise exceeding 100% peak aerobic power.

While our results confirm that pulmonary $\dot{V}O_2$ is predominantly dependent on the power output, they also show that blood lactate and muscle oxygenation could be modulated using the various BFR protocols investigated. Specifically, some participants struggled to complete the required 10 intervals of traditional high intensity exercise (HI). The perceived exertion was maximal at the end of HI, whereas RPE was significantly less during all BFR protocols (reflected in low $\dot{V}O_2$, HR and ventilatory demands of all BFR exercise tasks). As expected, the control conditions, either with (I-BFR0) or without BFR (CON30), elicited only minor perturbations in cardiopulmonary, metabolic or perceptual strain. Our primary hypothesis that intermittent higher-pressure BFR (I-BFR30) would be better tolerated than continuous lower-pressure BFR (C-BFR30) was supported: RPE was significantly lower during I-BFR30 (at ~150 mmHg) than C-BFR30 (at ~100 mmHg) despite a greater increase in blood lactate and a greater decrease in muscle StO_2 in the former. Our secondary hypothesis was also supported, as we found that muscle deoxygenation profiles in I-

311 BFR30 and HI were similar, whereas C-BFR30 resulted in a smaller degree of muscle
312 deoxygenation. A greater muscle hypoxia is associated with an enhanced peripheral adaptive
313 stimulus supporting endurance exercise performance (Sundberg 1994; Takarada et al. 2000;
314 Sumide et al. 2009; Abe et al. 2006). Therefore, our findings reinforce the notion that moderate-
315 intensity endurance exercise with intermittent higher-pressure BFR is both well tolerated and
316 provides a large muscle deoxygenation similar to that seen in traditional, maximal effort, high-
317 intensity exercise.

318 *Metabolic responses to blood flow unrestricted and restricted endurance exercise*

319 We tested a range of blood flow unrestricted and restricted endurance exercise protocols to better
320 understand the combination of cuff duration, cuff pressure, relative aerobic power that was both
321 well tolerated and resulted in a strong metabolic perturbation. The reference protocol was 10
322 repetitions of high-intensity intermittent (2 min exercise, 1 min recovery) cycling exercise at
323 ~100% peak power (HI), which is well known to provide robust training adaptations (de Oliveira
324 et al. 2015). While all participants completed this protocol, such high-intensity interval exercise
325 may be contraindicated in some elderly, patients, or athletes with injuries. Therefore we tested
326 whether endurance exercise at 30% P_{peak} with continuous or intermittent BFR could elicit
327 metabolic responses that are expected to be conducive to promoting peripheral adaptations.

328 Participants were able to tolerate a greater cuff pressure (~150 mmHg) using intermittent
329 restriction compared with continuous restriction (~100 mmHg). This seems important because the
330 blood lactate and muscle StO_2 response were actually less perturbed during continuous restriction,
331 despite a greater RPE. Our data suggest that ~100 mmHg pressure applied to both thighs by wide
332 pressure-cuffs during endurance cycle ergometry was insufficient to have a major impact on
333 muscle StO_2 and lactate accumulation in healthy young subjects. In fact, StO_2 was not different

between continuous restriction (C-BFR30) and without restriction (CON30) during exercise at 30% P_{peak}. This is likely due to a relatively low cuff pressure, an increase in perfusion pressure above resting, and the action of the muscle pump, each of which may have contributed to maintaining O_2 delivery during C-BFR30 at the control rate. However, these effects were not sufficient to restore O_2 delivery to control rates during I-BFR30, where cuff pressures were greater.

Although StO_2 was not different between I-BFR0, I-BFR30, and HI during set one, the relative increase in HHb was less in I-BFR0 than I-BFR30. I-BFR0 was also accompanied by a lower cardiopulmonary ($\dot{V}O_2$, HR) and metabolic strain (lactate, RPE), as expected from this condition, which had low power output demands. Differences in perfusion pressure, vascular distension and longitudinal capillary recruitment between the two intermittent BFR conditions, which differed in the influence of muscle pump, muscle O_2 consumption, and systemic blood pressure responses during exercise, likely contribute to the similar set 1 StO_2 responses. Nevertheless, the greater $\dot{V}O_2$ and HHb responses in I-BFR30 than I-BFR0 are consistent with a greater physiologic perturbation throughout the I-BFR30 condition.

Since the discomfort associated with continuous restriction was greater than intermittent, continuous, lower-pressure, BFR may be a sub-optimal approach for the design of BFR endurance training protocols. On the other hand, the greatest effect of all the BFR protocols investigated was that muscle StO_2 was not different between I-BFR30 and HI, despite a wide difference in $\dot{V}O_2$ (40% versus 95% $\dot{V}O_{2max}$). Local muscle hypoxia (amongst other variables) is known to be an important component of the angiogenic stimulus, resulting an increase in hypoxia inducible factor-1a (HIF-1a) and consequent transcription of vascular endothelial growth factor (VEGF) (Egginton 2009), (Hudlicka and Brown 2009). Therefore, the relative deoxygenation, combined with the low relative workload, in I-BFR30 may act as an efficient trigger promoting angiogenesis (Evans et al.

2010), (Hunt et al. 2013; Kacin and Strazar 2011; Larkin et al. 2012). The increase in muscle capillary density is a well-established adaptation following high intensity interval training (Daussin et al. 2008) and may underlie a major component of the increase in aerobic capacity and high-intensity exercise tolerance (Hoppeler et al. 1985; Wagner 1996). Similarly, muscle metabolic stimuli associated with high rates of lactate production and accumulation, as well as tissue hypoxia, contribute to regulation of the training response (Brooks 2016; Niooie and Samaneh 2016; Wahl et al. 2011; Terrados et al. 1990). Therefore, the greater lactate accumulation and StO_2 reduction during intermittent, higher-pressure, BFR likely indicate enhanced conditions for muscle remodeling compared with continuous, lower-pressure, BFR. Furthermore, while the central cardiopulmonary strain to I-BFR30 remained low, these data also provide an indication that this protocol may generate a muscle adaptive stimulus that is similar to that traditionally associated with high intensity interval training. These suggestions remain to be tested.

By restricting O_2 delivery during our intermittent BFR protocol (I-BFR30), we significantly increased [La] accumulation and non-invasively estimated O_2 extraction (StO_2), compared to the same power output without BFR (CON30). Ozaki et al. (2014) also found a greater lactate accumulation during walking exercise with BFR, but this is unsurprising considering the cuff pressure was much greater in their protocol (240 mmHg). The addition of BFR during endurance exercise may promote a greater activation of higher-order motor units to compensate for increased fatigue development during restricted blood flow (Karabulut et al. 2014; Moritani et al. 1992; Sundberg 1994). If so, the observed earlier onset of lactate accumulation during I-BFR30 compared with C-BFR30 may be in part due to an increased contribution to power production from less oxidative muscle fibers, despite the power output and $\dot{V}O_2$ response remaining low (30% peak power). Thus, increasing the activation of higher-order poorly-oxidative muscle fibers during BFR

endurance exercise, as well as increasing the metabolic perturbations in lower-order fibers, may underlie the endurance training benefits of the BFR paradigm (Moritani et al. 1992; Takarada et al. 2000; Sundberg 1994). Additional studies of muscle activation, muscle fatigue and fiber-specific metabolic perturbations using the I-BFR30 protocol are needed to confirm these suggestions.

Cardiopulmonary responses to blood flow unrestricted and restricted endurance exercise

Recent studies have investigated the potential efficacy of BFR training during endurance exercise tasks using cycling (Abe et al. 2010a; de Oliveira et al. 2015; Keramidas et al. 2012) and walking (Abe et al. 2006; Abe et al. 2010b; Ozaki et al. 2014; Park et al. 2010; Renzi et al. 2010). Using photoplethysmography, Renzi et al. (2010) showed a greater increase in HR and mean arterial pressure, and smaller decrease in total peripheral resistance, during BFR walking exercise compared to control, consistent with the expected reduction in venous return in BFR (Iida et al. 2007). However, the overall increase in central hemodynamics (HR and estimated cardiac output) during BFR exercise remained low compared to predicted maxima. We compared directly the cardiopulmonary responses during BFR endurance exercise and traditional high-intensity interval exercise. HI provided a frame of reference to better understand the central cardiopulmonary strain of BFR endurance exercise. We found HR was approximately 16% greater in both BFR protocols (I-BFR30 and C-BFR30) compared to the same power output without blood flow restriction (CON30), consistent with previous reports (Abe et al. 2006; de Oliveira et al. 2015; Renzi et al. 2010). Because we found a greater blood lactate concentration in BFR, the greater HR response may also be consequent to chemoreceptor stimulation causing increased sympathetic outflow (Hayes et al. 2009). Nevertheless, peak HR remained low during BFR (65-76% of HR_{max}), in

comparison with HI where HR_{max} was reached at the end of the training session in all participants. Thus, our I-BFR30 design provided conditions associated with a strong peripheral adaptive stimulus (a raised blood lactate, and a StO_2 of similar magnitude to that in HI) but with a relatively low central cardiopulmonary demand, illustrated by the moderate elevation in HR and RPE. Whether this combines with a mean arterial pressure response to reduce cardiac power demands compared with HI, and therefore provide a promising adjunct for rehabilitation endurance training in cardiac disease, remains to be determined. Further studies are required to establish the safety and efficacy for BFR endurance training in cardiac diseases.

Because it is known that HR, blood lactate and pain from the cuff are increased by BFR, we were interested in whether there was a significant effect on ventilation ($\dot{V}_E$) during BFR endurance exercise. The rationale for BFR exercise is that it is a beneficial paradigm for exercise training in vulnerable populations such as the elderly (Abe et al. 2010b; Vechin et al. 2015; Yasuda et al. 2014) or patients with chronic disease (Gualano et al. 2010; Madarame et al. 2013; Mattar et al. 2014). However, lung function declines with age (Campbell and Lefrak 1978; Mahler et al. 1986) meaning that healthy elderly are more likely to have a low breathing reserve during exercise than young participants at a similar relative power output, particularly in women (Guenette et al. 2007). Interestingly, despite a greater HR, blood lactate, and RPE in BFR exercise, $\dot{V}_E$ remained very low during both I-BFR30 and C-BFR30 protocols (21-30% of peak $\dot{V}_E$), and far below the value reached during HI (52-90% of peak $\dot{V}_E$). Indeed the $\dot{V}_E$ response was not significantly greater in BFR compared with the control condition at the same power output without blood flow restriction (CON30). The $\dot{V}_E$ response followed closely the dynamics of $\dot{V}O_2$ in the BFR endurance protocols, and was not greatly increased by the enhanced metabolic acidosis. Looking forward, the similar $\dot{V}_E$ response to the control condition may be important considering clinical populations with

limited ventilatory capacity, such as in chronic obstructive pulmonary disease, where expiratory flow limitation is associated with dynamic hyperinflation, an increased work of breathing, and low ventilatory capacity (O' Donnell & Laveneziana, 2007; Casaburi & ZuWallack, 2009). Pulmonary rehabilitation is one of the most effective treatments for COPD, largely because of training effects that promoting the oxidative phenotype in skeletal muscle (Maltais et al. 2015). Training approaches that promote peripheral muscle adaptations while reducing the ventilatory demands of the exercise (such as isolated-muscle training, hyperoxic gas breathing or non-invasive ventilation) have been shown to be efficacious in COPD (Emtner et al. 2003; Dolmage & Goldstein 2006; Vogiatzis 2011). In this regard, BFR appears to have promise. Nevertheless, additional studies are required to confirm the safety and efficacy of BFR exercise in these clinical populations.

Limitations

The study was performed in healthy young individuals, thus caution should be taken to extrapolate these findings to different populations. In addition, further work is needed to establish the safety of BFR endurance exercise in vulnerable patient groups. This study was only designed to measure acute physiological responses to BFR endurance exercise. Whilst the efficacy of I-BFR30 (with high BFR pressure) to enhance aerobic and strength parameters was previously verified (de Oliveira et al. 2015). It is important note that the exercise protocol present here, was an optimization of that used previously (de Oliveira et al. 2015). In this study, we individualized the cuff pressures for the BFR conditions (Loenneke et al. 2013) to better normalized among individuals. As consequence, a lower absolute pressure was utilized in this study (150 mmHg) compared to those used in de Oliveira et al. (2015) (140-200 mmHg throughout 4 weeks of training). The physiologic responses in both studies were largely similar in metabolic (lactate) and cardiovascular parameters (percentage of $\dot{V}O_{2max}$ and HR_{max}), suggesting that even with the lower

449 cuff used pressures herein, we anticipate positive training adaptations similar to de Oliveira et al.
450 (2015). Because the cuff pressure during training is one of the exercise intensity variables and the
451 subjects seem to adapt to the occlusion stimulus during the early phase of the training, previous
452 aerobic BFR training designs (Abe et al. 2006; Abe et al. 2010; de Oliveira et al. 2015) have
453 progressively increased the cuff pressure to keep inducing a training overload. Nevertheless,
454 further work is still needed to establish the efficacy of I-BFR30 or other BFR endurance training
455 protocols and to find the best manner to adjust the workload over the training program (if
456 increasing volume, intensity or restriction pressure).

457 Another limitation of this study is that we did not quantify the local muscle conditions during the
458 different protocols. We were limited to interpretation of whole-body metabolism ($\dot{V}O_2$, capillary
459 blood lactate) and peripheral relative deoxygenation (gastrocnemius medialis StO_2). Although
460 blood lactate (at least within individuals) may provide an index of metabolic strain, caution is
461 warranted in interpreting our results due to cuff-induced changes in blood flow that influence
462 lactate release and clearance within working muscles and throughout the body, that will have
463 differed among the different protocols. Similarly, we measured muscle oxygenation in the
464 gastrocnemius medialis during each condition as a representative muscle contributing to force
465 production and whole-body metabolism during cycling. This muscle was chosen for practical
466 reasons because we were unable to have sufficient space in all participants to implement both the
467 BFR thigh cuffs and the NIRS probe on the vastus lateralis or medialis. Therefore, while the
468 gastrocnemius medialis does not provide the majority of the power production during cycling we
469 chose this NIRS site on the basis that it could be reliably accessed in all participants and the
470 gastrocnemius medialis and vastus lateralis each show similar profiles of deoxygenation during
471 incremental cycle ergometry (Takagi et al. 2013). Thus, oxygenation changes in the gastrocnemius

medialis during the different free-flow and blood flow restricted protocols are assumed to be representative of conditions within the quadriceps – the primary power producing muscles for cycling. However, there is a wide heterogeneity of muscle oxygenation both within and between muscles during cycling (Koga et al. 2014; Okushima et al. 2015). Therefore future studies, with regional muscle measurements, are needed to better understand the impact of different BFR protocols on muscle oxygenation and metabolism during cycling exercise. Regional measurements of muscle oxygenation (e.g. multisite NIRS), muscle activity (e.g. multisite electromyography) and muscle metabolism (e.g. by biopsy) will enhance our understanding of the adaptive stimuli under different conditions of blood flow restriction during cycling exercise.

Practical application and perspectives

The I-BFR30 protocol appeared to have balanced well the competing needs to have low power output and high cuff-pressures (to optimize the efficacy of BFR), with a tolerable perception of pain and effort, while maintaining a physiological response profile consistent with producing adaptive stimuli (increased blood lactate and low muscle S_tO_2). In order to complete the same intermittent exercise protocol with continuous BFR, we found during pilot testing that lower restriction pressures were needed: otherwise participants stopped early due to pain. The continuous, lower cuff pressure, however, was insufficient to increase lactate, or decrease S_tO_2, to the same extent. Therefore, our intermittent BFR protocol performed with higher pressures (20 mmHg above pressure needed for resting limb occlusion) seems to be a pragmatic basis on which to begin the optimization of a personalized BFR endurance exercise training program (Brandner et al. 2015; de Oliveira et al. 2015; Sumide et al. 2009).

BFR may be an advantageous alternative for endurance training in populations where high loads or intensities are contraindicated. Individuals that require avoidance of high mechanical impact

(elderly, athletes recovering of injury) may be still able to maintain or increase endurance fitness using the BFR approach. That cycling is body-weight supported (unlike walking) strengthens the rationale for its use in these vulnerable groups. The ability to stimulate muscle angiogenesis with low metabolic and ventilatory demands may be particularly advantageous for some patient populations where muscle capillary rarefaction is particularly significant (e.g. COPD, CHF) (Gouzi et al. 2013; Wagner 1996).

Conclusion

Intermittent low power output exercise combined with intermittent blood flow restriction at 20 mmHg above of the passive restriction pressure (I-BFR30) was well tolerated, and increased muscle metabolic strain (increased lactate and reduced StO_2) compared to exercise with spontaneous blood flow. Interestingly, with this protocol, muscle oxygenation was not significantly different from high-intensity intermittent exercise that is known to provide a strong adaptive stimulus to muscle remodeling. Thus, a low power intermittent exercise protocol (30% P_{peak}) with combined intermittent blood flow restriction (~150 mmHg) appeared to generate a muscle metabolic stain that was similar to traditional high-intensity aerobic interval exercise, but which relies upon a far greater power output for training efficacy. Therefore, endurance cycling with intermittent BFR promotes muscle deoxygenation and metabolic strain, which may translate into increased endurance training adaptations while minimizing power output and RPE.

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517

518 ***CONFLICT OF INTEREST***

519 The authors declared have no conflict of interest.

520

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697 ***Figure Legends***

698 **Figure 1**. Pulmonary O_2 uptake ($\dot{V}O_2$) responses to five different intermittent exercise protocols,
699 with and without blood flow restriction (BFR), in a representative participant. Intermittent exercise
700 was two sets of 2 minutes exercise and 1 minute recovery, with 5 repetitions per set, and 5 minutes
701 resting recovery between sets. A) HI, high-intensity exercise starting at 105% P_{peak}. B) I-BFR30,
702 intermittent BFR (~150 mmHg) at 30% P_{peak}. C) C-BFR30, continuous BFR (~100 mm Hg) at
703 30% P_{peak}. D) CON30, control exercise without BFR at 30% P_{peak}. E) I-BFR0, intermittent BFR
704 (~150 mmHg) in unloaded exercise.

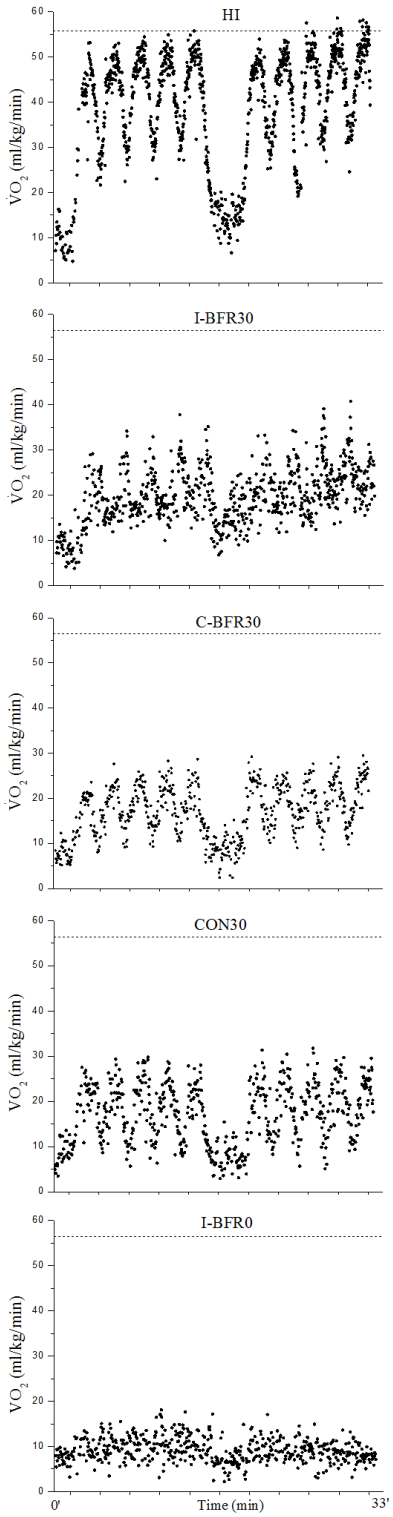
705 **Figure 2**. Group mean muscle oxygenation (StO_2) and relative change from baseline of
706 deoxygenated hemoglobin+myoglobin (HHb), oxygenated hemoglobin+myoglobin (O_2Hb) and
707 total hemoglobin+myoglobin (tHb) in response to five different intermittent exercise protocols,
708 with and without blood flow restriction (BFR). Intermittent exercise was two sets of 2 minutes
709 exercise and 1 minute recovery, with 5 repetitions per set, and 5 minutes resting recovery between
710 sets. A) HI, high-intensity exercise starting at 105% P_{peak}. B) I-BFR30, intermittent BFR (~150
711 mmHg) at 30% P_{peak}. C) C-BFR30, continuous BFR (~100 mm Hg) at 30% P_{peak}. D) CON30,
712 control exercise without BFR at 30% P_{peak}. E) I-BFR0, intermittent BFR (~150 mmHg) in
713 unloaded exercise.

714 **Figure 3**. Group mean cardiopulmonary and metabolic responses to five different intermittent
715 exercise protocols, with and without blood flow restriction (BFR). Intermittent exercise was two
716 sets of 2 minutes exercise and 1 minute recovery (at 20W), with 5 repetitions per set, and 5 minutes
717 resting recovery between sets. Protocols were: HI, high-intensity exercise starting at 105% P_{peak};
718 I-BFR30, intermittent BFR (~150 mmHg) at 30% P_{peak}; C-BFR30, continuous BFR (~100 mmHg)
719 at 30% P_{peak}; CON30, control exercise without BFR at 30% P_{peak}; I-BFR0, intermittent BFR (~150
720 mmHg) in unloaded exercise. A) Pulmonary O_2 uptake ($\dot{V}O_2$). B) Ventilation ($\dot{V}_E$). C) Heart rate
721 (HR). D) Gastrocnemius oxygenation by near-infrared spectroscopy (S_tO_2). E) Rating of perceived
722 exertion (CR10, RPE). F) Capillary blood lactate ([La]). Panels A-B-D (VO_2, V_E, StO_2) are
723 represented by the average of the last 30 seconds of each repetition. Panels C and E (HR, RPE)
724 show the greatest value in the last 10 seconds of each repetition. Panel F ([La]) was measured at
725 rest, and immediately at the end of each exercise set. [a] $p<0.05$ vs. I-BFR30, C-BFR30, CON30, I-

726 BFR0. [b] $p<0.05$ vs. I-BFR0. [c] $p<0.05$ vs. CON30. [d] $p<0.05$ vs. C-BFR30. [e] $p<0.05$ vs. set 1. [f]
727 $p<0.05$ vs. from rest.

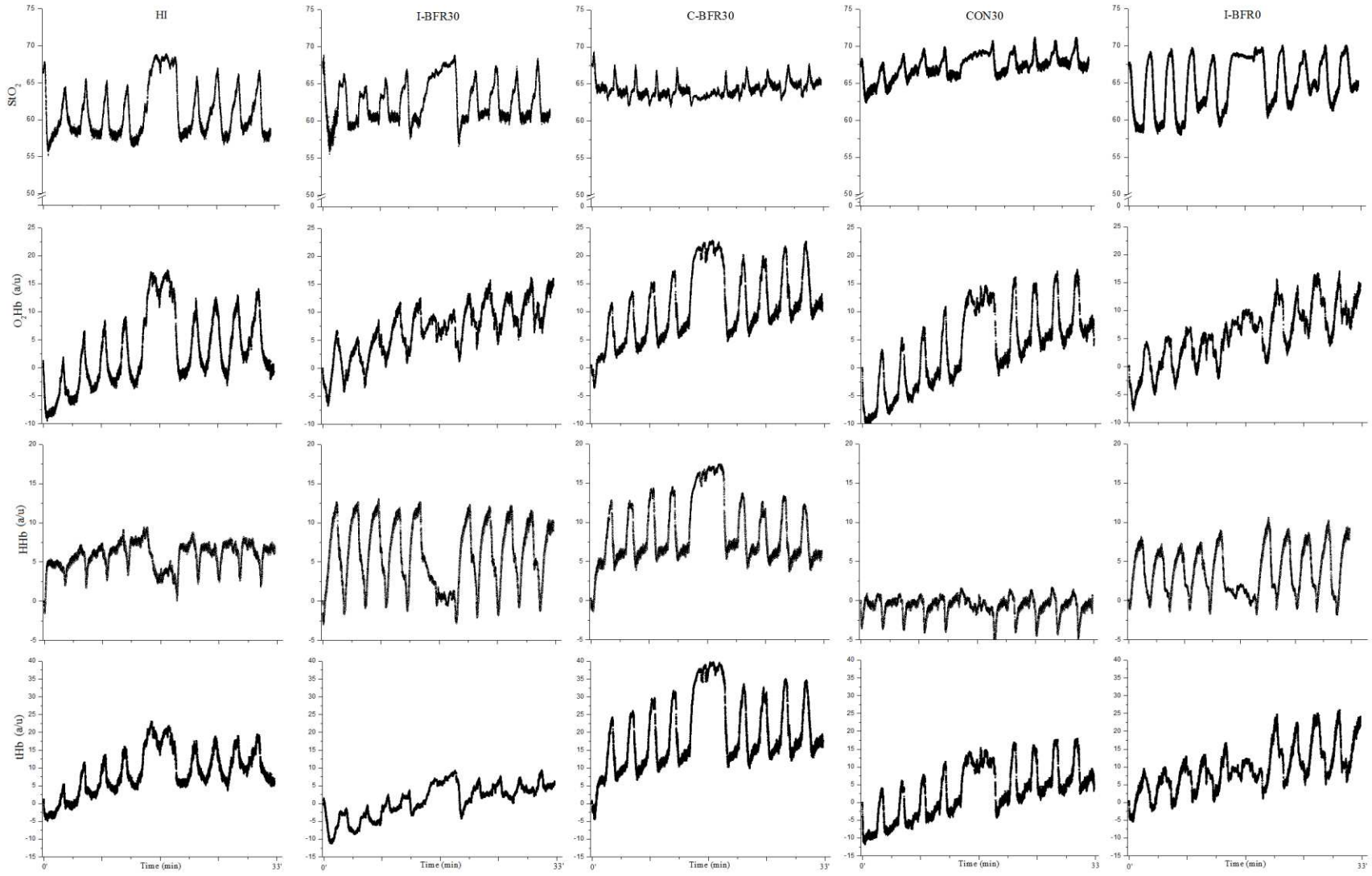
728

729 ***Figures***



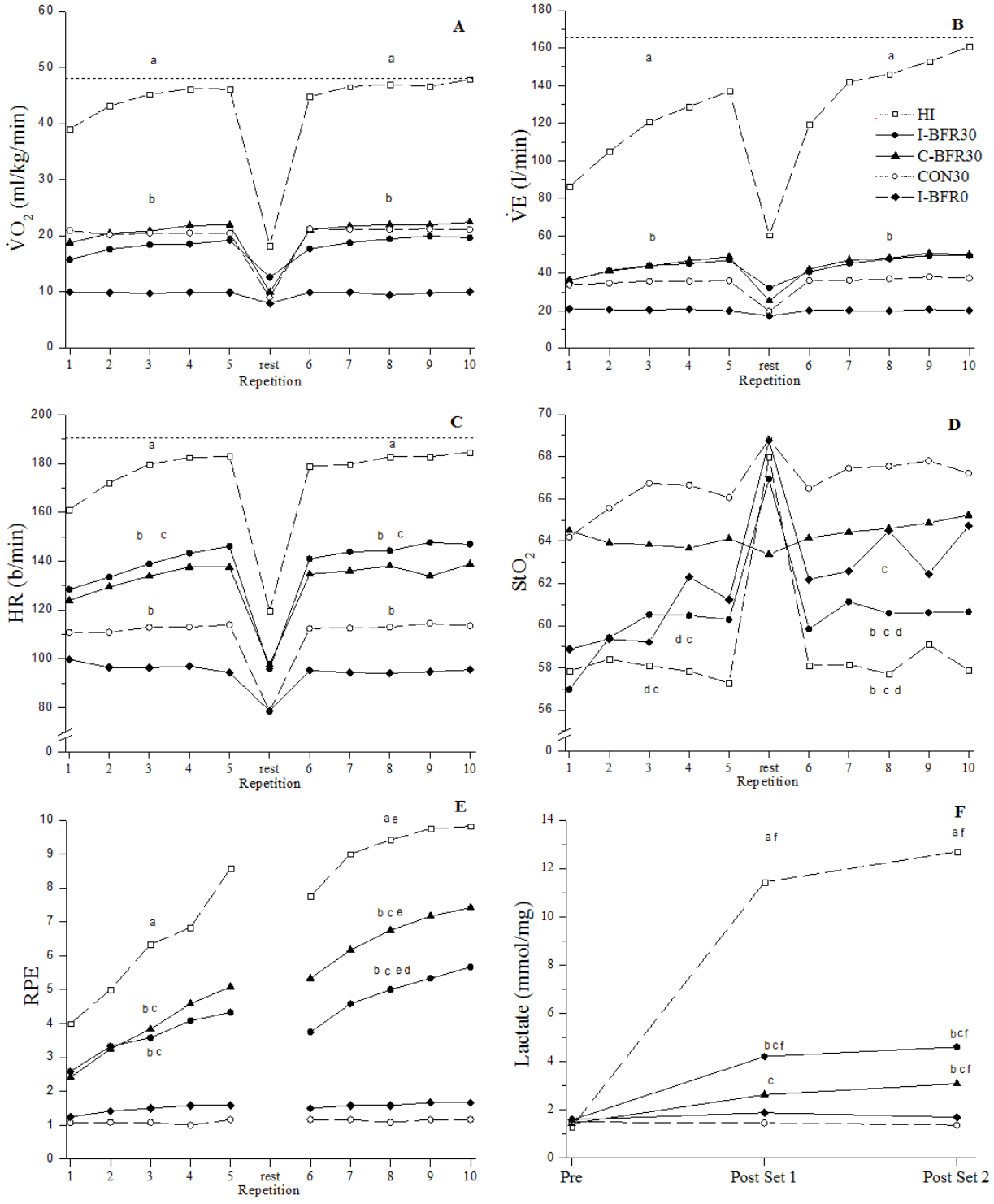
730

731 **Figure 1**



732

733 **Figure 2**



734

735 **Figure 3**