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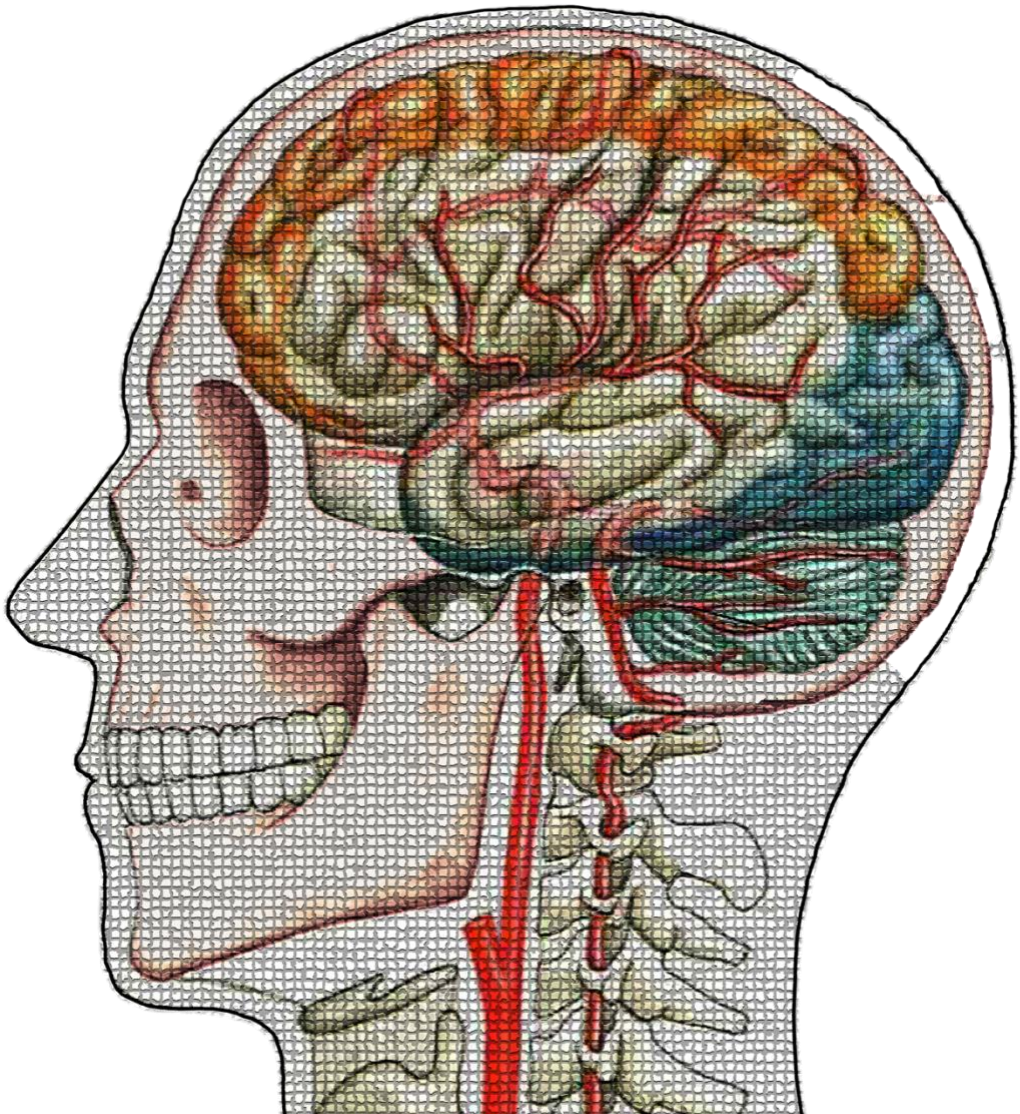
Blood Flow Restriction Training Physiological Effects



Effects of training with flow restriction on the exercise pressor reflex

Abstract

Purpose We hypothesized that 5 weeks of endurance training with blood flow restriction (R-training), providing relative ischemia and stimulation of the muscle chemoreflex, would decrease the exercise pressor reflex (EPR) when compared to training with the same workload in a free-flow condition (NR-training).

Methods 10 subjects performed one-leg knee-extension training four times a week during a 5-week period. Both legs were trained with identical workload, with one leg being trained during flow-restriction induced by lower body positive pressure. The EPR was assessed by measuring the increase in heart rate (HR) and mean arterial pressure (MAP) during an isometric knee extension of 35% of max torque for 90 s, this was done before (C), and after training in each leg (R and NR, respectively).

Results At the end of isometric contraction, the increase in mean AP (MAP) in the NR-trained leg and in the control condition were 41 ± 4 and 38 ± 4 mmHg, respectively, whereas the increase in the R-trained leg was 30 ± 4 mmHg ($p < 0.05$ R vs C and NR), corresponding to a decrease of about 25%. A similar patter was observed with respect to responses in HR, where the increase was 28 ± 3 and 28 ± 3 bpm in the NR and C, and 22 ± 4 in the R condition ($p < 0.05$ R vs C and NR).

Conclusions Peripheral metabolic changes induced by relative ischemia are important in modifying the EPR in response to exercise training.

Keywords Exercise · Arterial pressure · Flow restriction · Pressor reflex

Abbreviations

ANOVA	Analysis of variance
AP	Arterial pressure
C	Control
EPR	Exercise pressor reflex
HR	Heart rate
MAP	Mean arterial pressure
MSNA	Muscle sympathetic nerve activity
MVC	Maximal voluntary contraction
NR	Non flow-restricted
R	Flow-restricted

Introduction

It is generally admitted that the cardiovascular response to physical exercise implies the concerted action of a central command mechanism, and of the exercise pressor reflex (EPR), with influence from the cardiopulmonary reflex (Rowell and O'Leary 1990; Michelini et al. 2015; Fadel and Raven 2012). As hypothesized, the EPR implies mechano- and chemo-sensitive receptors that affect afferent nerve traffic in c-fibers, which in turn increase the sympathetic outflow to the heart and resistance vessels (Rowell and O'Leary 1990; Seals et al. 1988). This is possibly accompanied by a postulated simultaneous withdrawal of vagal tone to the heart, which occurs in the first seconds of exercise (Fagraeus and Linnarsson 1976; Lador et al. 2008). The ensemble of these responses lead to increased heart rate (HR) and arterial pressure (AP), and is important for a proper hemodynamic response during physical exercise in healthy humans (Amann et al. 2011). The relative role of these mechanisms in generating the cardiovascular response to exercise is still matter of debate. Insight on the role of the EPR may come from endurance training, as long as it was previously shown

that endurance training blunts the exercise pressor reflex (EPR) (Somers et al. 1992; Mostoufi-Moab et al. 1998; Ray 1999) and that endurance-trained subjects have a lower EPR than untrained and strength-trained subjects (Kolegard et al. 2013).

The aim of the present study was to investigate whether aerobic training with and without relative ischemia would act to modify the EPR. The experimental model used was single leg exercise in which one leg exercise was done with restricted blood flow, inducing a higher metabolic stress and activation of the muscle chemoreflex (Eiken and Bjurstedt 1987), and the other leg being trained without any blood flow restriction but with identical workload.

Our main hypothesis was that flow restriction during training at a given workload (R-training), would act to decrease the EPR when comparing with regular training on the same workload (NR-training). It was further hypothesized that the difference in EPR would be shown towards the end of the isometric contraction, when the accumulation of metabolites and fatigue occurs. A significant inter-individual variability was expected with regards to the training effect on peak performance.

Methods

Ten healthy male subjects participated in the study. Their ages, weights, heights and VO_2-max were [mean (range)] 24 (20–27) years, 78 (68–95) kg, 181 (173–191) cm, and 50 (45–57) ml/min/kg. Because of the strenuous nature of the training program, some familiarity with physical training was considered important, whereas recruiting subjects that were too well-trained (> 60 ml/min/kg) was avoided, as this might diminish the possibility of detecting significant training effects. The subjects were informed about the experimental procedures and the nature of the training program before consenting to participate. The study was performed in accordance with the Declaration of Helsinki and the ethics committee at Karolinska Institutet, Stockholm, approved the protocol and experimental procedures. Results from this study, related to gene analysis and skeletal muscle tissue adaptations, have been used in other publications (Rullman et al. 2009; Gustafsson et al. 2007).

Exercise model

A method first described by Eiken and Bjurstedt (1987) was used for induction of restricted blood flow during exercise. Local application of increased pressure around the working leg was used to reduce exercise blood flow in a controlled fashion. The subject was positioned supine in the opening of a pressure chamber with the upper body outside and both legs inside the chamber and one leg strapped to a pad on the lower leg. The dynamic constant-load one-legged knee-extension exercise was done in a similar fashion as earlier described (Andersen et al. 1985). Each voluntary contraction extended the leg from 70° to 150° knee angle. Flexion was performed passively using the ergometer flywheel momentum to move the leg for the next extension. The chamber opening was sealed off at the level of the crotch by a rubber diaphragm with holes and self-sealing sleeves for the legs. Shoulder supports were used to prevent displacement of the body as the chamber pressure was increased. For exercise under restricted blood flow, the chamber pressure acting on the working leg was elevated to 50 mm Hg above atmospheric pressure. This has been shown to reduce leg blood flow during one-legged cycle exercise by 15–20%, reduce 10–12% units of venous oxygen saturation and a greater depletion of ATP in the working muscle and release of lactate (Sundberg 1994). Exercise under non-restricted blood flow condition was done using the same experimental arrangements but at current atmospheric pressure.

Training protocol

One-leg training was performed four times a week for 5-weeks, giving a total of 20 training sessions. One leg trained under restricted blood flow condition (R-leg), providing relative ischemia during exercise, while the other leg trained with non-restricted blood flow (NR-leg). The subjects were randomized into two groups; one group trained their right leg and the other group their left leg during restricted blood flow. Each training session began by 45 min of training under restricted blood flow condition. The subjects were instructed to perform knee extensions at a constant rate of 60/min at the highest tolerable workload for 45 min, taking in to account that the whole 45-min session must be accomplished. The workload was changing during the training session; hence the load was adjusted to maintain high intensity while still ensuring that the bout could be completed without interruptions. After 10 min of rest, the same workload protocol was performed by the other leg, but with normal leg blood flow. Accordingly, the two legs developed the same power and amount of work in each session. The subjects were encouraged to achieve a subjective rating of perceived leg effort (Borg scale 6–20) during training with flow restriction above 15 (hard) for the last 5 min in all training sessions. The Borg scale was designed for rating of perceived exertion during general exercise with large muscle groups, but has also been validated for use in exercise with smaller muscle groups (Garcin et al. 1998). The R-leg training was invariably experienced as extremely strenuous with periods of ischemic muscle pain occurring frequently during the training session, whereas training of the NR-leg was experienced as very light. The cumulative (total) work load per bout (W*min) was calculated (Table 1).

Table 1 Training intensity, perceived exertion and heart rate during training

Total work load, first training bout (W × min)	503 ± 181
Total work load, last (20th) training bout (W × min)	848 ± 100[a]
Perceived exertion (units), R-training	17 (15–18)
Perceived exertion (units), NR-training	12 (9–13)[b]
Heart rate (bpm), R-training	98 ± 11
Heart rate (bpm), NR-training	89 ± 7[b]

Mean ± SD, Median (range)

[a]Significant difference between first and last exercise bout

[b]Significant difference compared to R-trained leg. Perceived exertion and heart rate were recorded at the first and last exercise session and the presented values denotes the average in the R and NR condition, respectively

Performance test

After familiarization with the experimental model, one-legged step-wise incremental exercise tests were performed during the week before the training period and the week after the training period. Each leg was tested during non-restricted blood flow conditions. The subject was instructed to maintain 60 knee extensions per min, starting with 2 min at 5 W. The workload was then increased by 5 W every min up to 20 W and then by 2.5 W until the kicking rate could no longer be maintained. When the rate fell below 55 rpm for more than 5 s, time and peak load were recorded and the experiment terminated.

Exercise pressor response test

Isometric torque (maximal voluntary contraction; MVC) was determined during knee-extension contractions in both legs using a dynamometer (Cybex II, Lumex Inc. N.Y., USA). During the knee extension, the subject sat in a chair with a vertical back support. The thighs and pelvis were secured with Velcro straps. The dynamometer lever arm was attached proximal to the ankle with the knee joint at an angle of 90°. The subject was instructed to produce maximal force as rapidly as possible without kicking, and to sustain maximal force for 5 s. The procedure was repeated three times with a 30-s rest period between contractions. Torque was recorded with a computer-based data acquisition and analysis system (BioPac Systems, Santa Barbara, USA). Using the same experimental arrangements as for the MVC measurements, the subject performed a 90-s sustained isometric knee-extensor contraction at 35% MVC. The subject was given visual feedback from a torque-gauge display to keep the pre-set torque level. To avoid straining (Valsalva) maneuvers during the contractions, the subject was also instructed to continuously count out loud during the contractions. The procedure was done once in each leg before and after the 5-week training period. The 35% MVC defined before the training period was used also after training, e.g., the same absolute torque during isometric contraction before and after. Heart rate (HR) and arterial pressure (AP) were measured beat-by-beat with a volume clamp technique (Finapres 2300, Ohmeda, Englewood, OH, USA) with a cuff placed around the mid phalanx of the third finger of the right arm. The arm was supported by a mitella and the finger was kept at heart level. The Finapres method is widely used and known to reliably follow changes in MAP in various conditions, the absolute values might differ compared to invasive measurements (Azabji Kenfack et al. 2004), but these changes are typically systematic. ECG was recorded with the electrodes positioned in a 5-lead precordial arrangement using a cardiomonitor (Physio-Control Lifepak 8, Cardiomonitor, Physio-Control Corp, Redmond, USA). AP, ECG, and torque were recorded with the same acquisition systems as mentioned above. Offline mean AP (MAP) was computed as the arithmetic mean between systolic peaks and stored as a level during that interval. The cardiovascular response to the isometric contraction was characterized by measurements of the increase in HR and MAP at four different times during the isometric contraction. HR and MAP were averaged over 5 s at 20, 40, 60, and 80 s after the initiation of contraction. These measurements were done on both legs before and after the training period. The responses from both legs before training were averaged in each subject and this average was used as the control when comparing to the responses after R- and NR-training respectively.

Statistics

The difference in the cardiovascular exercise response before and after training with and without flow restriction was tested using a 2-way ANOVA with repeated measurements. If the ANOVA indicated a significant difference ($p < 0.05$), a post hoc analysis, planned comparison, was used to locate at what times during the isometric contraction that the difference occurred. 2-way ANOVA was also used for testing the difference in the peak load test and maximum isometric torque before and after training. Student's *t* test was used to analyze the change in training load early and late during the training period, and also for testing the difference in perceived exertion and HR during R- and NR-training respectively.

Results

All 10 subjects successfully completed the 20 exercise sessions and the related experiment procedures. The average total workload during an exercise session increased from 503 ± 181 W × min at the first exercise bout to 848 ± 100 W

× min during the last bout ($p < 0.01$) (Table 1). Overall, the perceived exertion and heart rate (HR) were higher during R-training vs NR-training (Table 1).

Performance tests

Peak load at the performance test increased after both R and NR training, from 43 ± 3 to 56 ± 2 W and from 41 ± 3 to 51 ± 2 W, respectively (main effect $p < 0.05$). There were no significant differences between the R- and NR-trained legs (Table 2). There was a large inter-individual variability regarding the change in peak performance, where subjects with larger training effect in the R-trained leg also had larger training response in the NR-trained leg (Fig. 1). Isometric knee-extension torque increased after training in both the R- and NR-trained legs, from 278 ± 20 to 283 ± 22 Nm and from 279 ± 17 vs 293 ± 20 N m, respectively (main effect $p < 0.05$), whereas no significant difference was noted between the R- and NR-trained leg (Table 2).

Exercise pressor test

Figures 2 and 3 show mean responses to 90 s isometric knee extensions at 35% of MVC, before training (control) and after training with and without ischemia, N and NR condition, respectively. Absolute values of MAP and HR are denoted in Table 3. There was in general a similar increase up to 45 s in all conditions, both in MAP and HR responses. After 60 s, the pressor response in the R-leg diverged from the control and the NR-leg, displaying less increase in both MAP and HR. There was no significant difference between control and NR condition at any time point.

Hence after 80–85 s of isometric contraction, the increase in MAP in response to isometric contraction in the NR-leg and in the control condition were 41 ± 4 and 38 ± 4 mmHg, respectively, whereas the increase in the R leg was 30 ± 4 mmHg, corresponding to a decrease of about 25%. A similar patter was observed with respect to response in HR at the last time interval, where the increase was 28 ± 3 and 28 ± 3 bpm in the NR and control condition, and 22 ± 4 in the R condition. The response at 80–85 s in the R condition was significantly lower than the responses in the NR and control conditions, both for MAP and HR ($p > 0.05$). At the 60–65 s period, the R condition was significantly different

Table 2 Peak load test and maximum isometric torque

	R-trained leg	NR-trained leg
Peak load, control (W)	43 ± 3	41 ± 3
Peak load, after training (W)	56 ± 2[a]	51 ± 2[a]
Isometric torque, control (Nm)	278 ± 20	279 ± 17
Isometric torque, after training (Nm)	283 ± 22[a]	293 ± 20[a]

Mean ± SE

[a]Significant difference compared to control in the same leg ($p < 0.05$), main effect of training, no difference between, R- and NR-trained leg

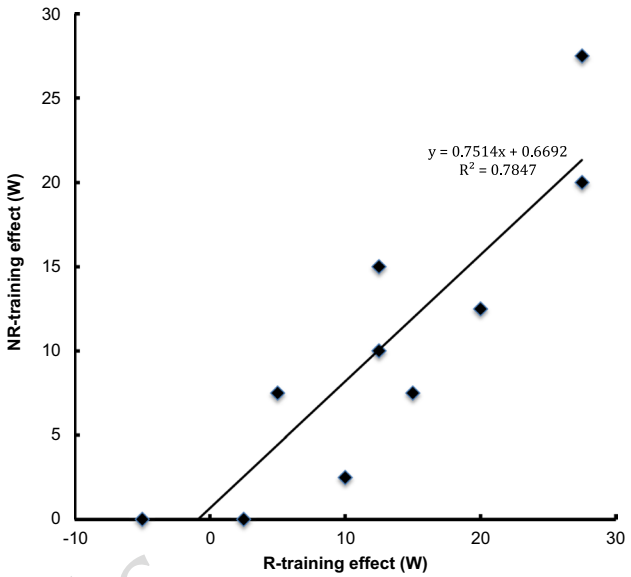


Fig. 1 Change in the peak work load during the performance test after 5 weeks of training, in the R- and NR-trained leg of each subject

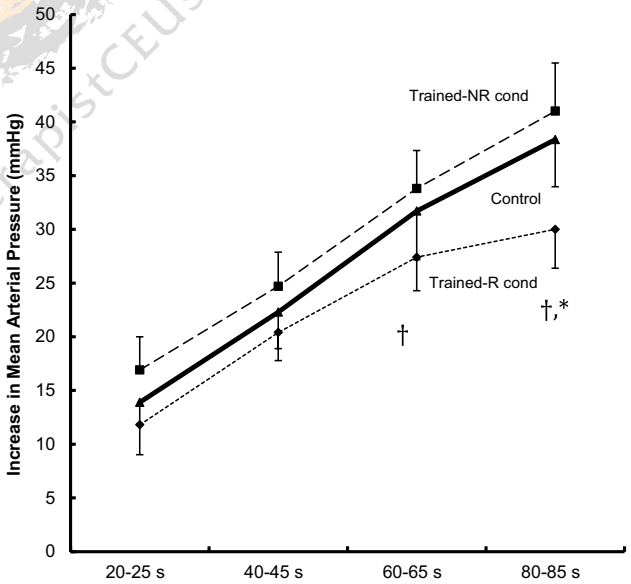


Fig. 2 Mean arterial pressure responses to 90 s isometric unilateral knee extension at 35% of maximal voluntary contraction. Control; mean response from both legs before training, Trained-NR cond; response while contracting the leg that had received non-ischemic training, and Trained-R cond; response in the leg that had undergone ischemic training. $n = 10$, values are mean ± SE. †Significant difference between NR and R condition at a given time interval, *significant difference between control and R condition at a given time interval ($p < 0.05$)

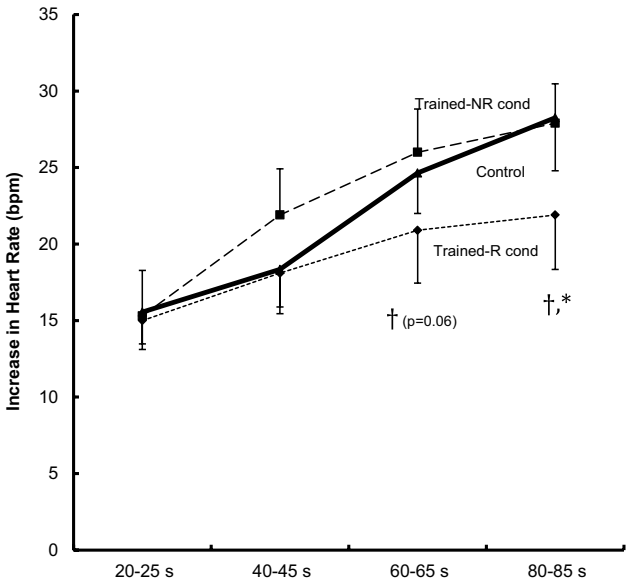


Fig. 3 Heart rate responses to 90 s isometric unilateral knee extension at 35% of maximal voluntary contraction. Control; mean response from both legs before training, Trained-NR cond; response while contracting the leg that had received non-ischemic training, and Trained-R cond; response in the leg that had undergone ischemic training. $n = 10$, values are mean ± SE. †Significant difference between NR and R condition at a given time interval, *significant difference between Control and R condition at a given time interval ($p < 0.05$)

Table 3 Mean arterial pressure (MAP) and heart rate (HR) before and after EPR

	Control	NR-trained leg	R-trained leg
MAP (mmHg)			
Baseline	94 ± 7	97 ± 7	96 ± 8
End EPR	133 ± 17	138 ± 17	126 ± 13
HR (bpm)			
Baseline	78 ± 11	80 ± 12	80 ± 12
End EPR	106 ± 12	108 ± 15	101 ± 17

Mean ± SD

to the NR condition but not to the control condition, both concerning the responses in MAP and HR.

Discussion

This study indicates that relative ischemia induced by flow restriction during exercise appear to decrease the exercise pressor reflex. Furthermore, the fact that the difference in responses occurred only during the second half of an isometric contraction suggest that the altered exercise pressor response depends on an altered afferent signaling originating from the muscle chemoreflex or a blunted increase in central command due decreased muscle fatigue.

It has previously been shown that endurance training of the forearm reduces the increase in muscle sympathetic nerve activity (MSNA), but not MAP or HR, during isometric handgrip (Somers et al. 1992). Mostoufi-Moab et al. (1998) indicated that endurance training of the forearm, in this case reduced the increase in MAP during flow-restricted dynamic exercise, and also decreased lactate accumulation and blunted the decrease in pH. A similar adaptation was demonstrated during one-legged training, where MSNA and MAP were decreased during dynamic knee extension of 40 W after 6 weeks of training (Ray 1999). In the present study, both legs were trained with the same workload, with one leg being under stronger metabolic stress i.e. with increased lactate release and reduced pH due to an imposed restriction of blood flow (Sundberg and Kaijser 1992). The advantage of such a model is exclusion of inter-individual genetical differences in the comparison of the two conditions and the utilization of small muscle mass with minor hemodynamic effects makes it possible to relates any obtained findings to the peripheral tissue. The present results indicate that the metabolic disturbance *per se* induces peripheral adaptations that reduce the EPR. There were no observed changes in EPR after NR-training, despite about 20% increase in peak performance (Table 2). This could be coupled to the relative low intensity and slight metabolic disturbance in this condition compared to R-training as also reflected in the perceived exertion (Table 1). An increased intensity during NR-training might have had an effect on the EPR; however, the current findings indicate that any such effect would likely then be coupled to relative hypoxia with partly anaerobic metabolism.

The difference between the R- and NR-trained leg, in both the HR and MAP exercise pressure responses, occur in the 2nd half of the isometric contraction (Figs. 2, 3). The cardiovascular response of an isometric contraction comprises of effects from central command, peripheral chemo- and mechano-reflexes (Rowell and O'Leary 1990). The chemoreflex comes into effect after some time, because of its dependence of the gradual accumulation of metabolites and decrease in pH (Victor et al. 1988; Seals et al. 1988; Pryor et al. 1990). Hence, one conclusion could be that R-training primarily alters the chemoreflex, either by less accumulation of substances, due to an augmented muscle adaptation, that triggers the reflex or by a blunted afferent signal. An alternative mechanism, partly related to reduced accumulation of metabolites, could be that R-training decreases the muscle fatigue (Eiken et al. 1991) in a way that acts to damp the increase in central command over time during the isometric contraction (Fisher and White 1999; Schibye et al. 1981).

Regardless of mechanism, it has been shown that fibers with aerobic rather than glycolytic profile, have a smaller

exercise pressor response (Petrofsky and Lind 1980; Wilson et al. 1995) and a better endurance during isometric contractions (Hulten et al. 1975). Previous studies have indeed shown that R-training, performed as in the present study, increases the aerobic capacity (Kaijser et al. 1990; Gustafsson et al. 2007; Esbjornsson et al. 1993), and acts to alter the metabolic capacity towards a more aerobic profile with an increased capillary density (Eiken et al. 1991; Esbjornsson et al. 1993). In line with this reasoning, it has also been shown that endurance-trained individuals have a lower exercise pressor response during isometric contraction of the quadriceps muscle, compared to untrained subjects (Kolegard et al. 2013). In addition to the effects of differences in metabolic pathways on metabolic end products there are several additional mechanisms that might affect receptors coupled to group III and IV muscle afferent fibers differently (Kaufman and Forster 1996). In fact, receptor density might differ between types of muscle, buffering capacity has been shown to be different (Sahlin and Henriksson 1984), and afferent neurons in oxidative fibers appear to respond different compared to those in more glycolytic fibers (Xing et al. 2008).

There was large variation between subjects related to the magnitude of the training effect (Fig. 1), which is commonly seen in training studies (Lortie et al. 1984; Prud'homme et al. 1984; Bouchard et al. 2011). The inherent nature of adaptive responses to physical exercise was further supported by the observation that subjects with large training effects in the NR-trained leg also had large effects in the R-trained leg (Fig. 1).

Methodological consideration

The EPR test done before and after training was performed with the same absolute power defined as 35% of maximal knee torque (MVC) assessed before training. Hence since the MVC increased after training (Table 2), with approximately 2% in the R-trained leg and 5% in the NR-trained leg, it could be argued this load corresponds to less than 35% and thus might explain a lower magnitude of the EPR. However, since a decrease in EPR was only apparent in the R-leg, to an extent that provides significant difference compared to the NR condition, despite a numerically larger increase in MVC in the NR condition, any such effect would have no effect on the current conclusions.

Conclusions

The present study indicates that the ischemic component during exercise changes the EPR during isometric exercise.

The differences in EPR appear towards the end of the contraction indicating an effect of an altered muscle chemoreflex response and/or decreased muscle fatigue cou-pled to a decrease in central command. Understanding the plasticity of the exercise pressor reflex has relevance not only for healthy individuals, but also for patient groups such as heart failure patients if this reflex is exaggerated and acts to decrease exercise capacity (Amann et al. 2014).

Conflict of interest The authors declare that they have no conflict of interest.

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Anaerobic metabolism induces greater total energy expenditure during exercise with blood flow restriction

Abstract

Purpose

We investigated the energy system contributions and total energy expenditure during low intensity endurance exercise associated with blood flow restriction (LIE-BFR) and without blood flow restriction (LIE).

Methods

Twelve males participated in a contra-balanced, cross-over design in which subjects completed a bout of low-intensity endurance exercise (30min cycling at 40% of $\dot{V}O_{2max}$) with or without BFR, separated by at least 72 hours of recovery. Blood lactate accumulation and oxygen uptake during and after exercise were used to estimate the anaerobic lactic metabolism, aerobic metabolism, and anaerobic alactic metabolism contributions, respectively.

Results

There were significant increases in the anaerobic lactic metabolism ($P = 0.008$), aerobic metabolism ($P = 0.020$), and total energy expenditure ($P = 0.008$) in the LIE-BFR. No significant differences between conditions for the anaerobic alactic metabolism were found ($P = 0.582$). Plasma lactate concentration was significantly higher in the LIE-BFR at 15min and peak post-exercise (all $P \leq 0.008$). Heart rate was significantly higher in the LIE-BFR at 10, 15, 20, 25, and 30min during exercise, and 5, 10, and 15min after exercise (all $P \leq 0.03$). Ventilation was significantly higher in the LIE-BFR at 10, 15, and 20min during exercise (all $P \leq 0.003$).

Conclusion

Low-intensity endurance exercise performed with blood flow restriction increases the anaerobic lactic and aerobic metabolisms, total energy expenditure, and cardiorespiratory responses.

Competing interests: The authors have declared that no competing interests exist.

Introduction

It is well known that moderate to high intensity endurance training (i.e., 60–90% of maximum oxygen uptake—$\dot{V}O_{2max}$) is the principal exercise protocol to induce an increase in $\dot{V}O_{2max}$ [1, 2]. However, a novel endurance exercise protocol, using lower limb blood flow restriction (BFR) during low-intensity (~40% of $\dot{V}O_{2max}$) endurance exercise (LIE-BFR), has also been shown to significantly improve $\dot{V}O_{2max}$ (6.4%) [3, 4], suggesting that performing endurance training at low intensity, when associated with blood flow restriction, can induce cardiorespiratory fitness, providing a great advantage. For instance, Abe and co-workers [3] reported increased $\dot{V}O_{2max}$ following 24 training sessions of low-intensity cycle exercise (15min at 40% $\dot{V}O_{2max}$) performed with BFR compared to the same exercise intensity without BFR. These findings suggested that peripheral perturbation induced by BFR in arterial and venous leg blood flow, including local hypoxia [5] and reduce venous return [6], produces elevated metabolic demand. Accordingly, the BFR may be related to an enhanced anaerobic metabolism during muscle contraction, resulting in metabolite accumulation, and thus stimulating cardiorespiratory (i.e., via metaboreflex and reduced venous return) [7] and muscular mechanisms [8] to possibly increase $\dot{V}O_{2max}$.

Accordingly, some studies have suggested that during exercise performed under local hypoxia the aerobic metabolism is decreased, while the anaerobic metabolism (i.e., alactic and lactic energy systems) is increased [9, 10]. It is believed that increased anaerobic energy production may evoke higher metaboreflex activity and, consequently enhance heart rate (HR) response, cardiac output, and ventilation ($\dot{V}_E$) to adequately supply O_2 to exercising muscle and remove the metabolites generated from the anaerobic metabolism [11–13]. As a result, this compensatory mechanism produced by local hypoxia, together with a reduced venous return characteristic of BFR training, may overload central components of the cardiorespiratory system and lead to improvement in $\dot{V}O_{2max}$ [14, 15]. Furthermore, Egan et al. [8] showed that higher blood lactate ($[La^-]_{net}$) accumulation, indicating greater contribution of the anaerobic metabolism, can result in significantly higher metabolic stress and consequently enhance mitochondrial biogenesis synthesis, a local factor which can also induce greater $\dot{V}O_{2max}$. Although these are plausible hypotheses; both present the anaerobic metabolism as a trigger factor that still needs to be identified during LIE-BFR.

Therefore, the aim of the present study was to compare the energy system contributions (i.e., aerobic, anaerobic alactic, and lactic metabolisms) and total energy expenditure following low intensity endurance exercise associated with blood flow restriction (LIE-BFR) and low intensity endurance exercise without BFR (LIE). We hypothesized that LIE-BFR would increase energy production by the anaerobic lactic metabolism due to reduction in O_2 delivery to thigh muscles induced by cuff pressure, and thus increase total energy expenditure.

Methods

Subjects

Twelve healthy sedentary male subjects voluntarily participated in this study (subject characteristics are described in Table 1). Participants were recruited via fliers and posters at the University. As inclusion criteria, participants should be young (18 to 30 years old) men, sedentary at most, without contraindications to the practice of bicycle exercise and with availability of schedule to comply with all visits of the project. As exclusion criteria, participants could not have any cardiovascular or metabolic disease or musculoskeletal injuries in the lower limbs that compromise their ability to perform the exercise protocol. In addition, no participant

Table 1. Characteristics of the participants.

	n = 12
Age (years)	24.5 ± 4.0
Body mass (kg)	82.8 ± 12.6
Height (m)	1.80 ± 0.05
$\dot{V}O_{2max}$ (mL/kg/min)	33.4 ± 4.6
PO_{max} (W)	222.3 ± 47.6
RCP (mL/kg/min)	26.6 ± 4.7
RCP (%$\dot{V}O_{2max}$)	79.7 ± 9.8
RCP (W)	182 ± 51.8
VT (mL/kg/min)	20.4 ± 4.3
VT (%$\dot{V}O_{2max}$)	61.1 ± 10.4
VT (W)	127.9 ± 53

Values are presented as mean ± SD. $\dot{V}O_{2max}$: maximum oxygen uptake; PO_{max}: maximal power output; RCP: respiratory compensation point; VT, ventilatory threshold.

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could have been engaged in resistance and/or endurance training at least six months prior to the study and they were advised to do not consume alcohol and drugs or any kind of artificial substance (e.g., supplements) at least one week before the study commencement. At last, participants were instructed to refrain from consuming caffeine and other energetic substances in the 24 hours preceding all visits. The experimental procedures and possible risks associated with the study were explained to all subjects, who provided written consent prior to participation. The study was approved by the Ethics Committee of the University of Campinas (process number: 848.145) and conducted in accordance with the policy statement regarding the use of human subjects conforming to the latest revision of the *Declaration of Helsinki*.

Experimental design

The study employed a counter-balanced, cross-over design in which all subjects completed a session of low intensity endurance exercise associated with blood flow restriction (LIE-BFR) and one session of low intensity endurance exercise (LIE) without BFR on the cycle ergometer. Prior to the first exercise session, participants underwent $\dot{V}O_{2max}$ testing and one exercise familiarization session. Exercise trials were separated by a period of between 72 hours and one-week, during which subjects were instructed to maintain their habitual diet and physical activity patterns.

Cardiorespiratory testing ($\dot{V}O_{2max}$). Participants performed a maximum graded exercise test on a cycle ergometer with electromagnetic braking (Quinton model: Corival 400, Lode BV, Groningen, Netherlands). After resting on the bike for 5 min to establish cardiorespiratory parameters, participants commenced the incremental test protocol. Briefly, subjects commenced cycling at an initial load of 50 W for 1min and the workload was increased by 15 W/min until a workload of 200 W was reached, after which further increases were in 10 W/min increments until voluntary fatigue [3, 16]. Participants were instructed to maintain pedal frequency between 60 to 70 rpm. Gas exchange and HR data were collected continuously using an automated breath-by-breath metabolic system (CPX, Medical Graphics, St. Paul, Minnesota, USA) and HR transmitter (Polar Electro Oy, Kempele, Finland) connected to the gas analyzer. A test was considered maximal when two or more of the following criteria were met: a plateau in oxygen uptake, a respiratory exchange ratio greater than 1.1, heart rate >90% of the predicted maximal heart rate (i.e., 220-age), and a score > 17 in 6–20 perceived exertion scale

[17, 18]. The maximum oxygen uptake was defined as the mean oxygen consumption values ($\dot{V}O_{2max}$) over the final 30 s of the test. Ventilatory threshold (VT) was determined at the point of a non-linear increase in the $\dot{V}_E/\dot{V}O_2$ relationship. Respiratory compensation point (RCP) was determined at the point of a non-linear increase in $\dot{V}_E/\dot{V}CO_2$ and the first decrease in the expiratory fraction of CO_2 [19]. Two independent investigators determined these thresholds; when the investigators disagreed, a third independent investigator was consulted. Typical error coefficient of variation is following: VO_{2max} = 3.0%, power = 1.9%, respiratory exchange ratio (RER) = 5.6%, time to exhaustion = 1.6%. In addition, test-retest typical error and coefficient of variation of lactate concentration ([La^-]) measurements were 0.07 mmol/L and 5.2%.

Experimental testing sessions. Participants returned to the laboratory a minimum of one week after performing the $\dot{V}O_{2max}$ test and familiarization to undertake the first of two randomly assigned exercise sessions (described below). After resting in the sitting position for ~5min they started the exercise. During both exercise sessions (resting, exercise, and recovery) gas exchange and HR data were collected continuously using the same automated breath-by-breath metabolic system described above. The HR and $\dot{V}_E$ data were averaged every 5 min of resting, exercise, and recovery to compare the LIE and LIE-BFR cardiorespiratory patterns. Blood samples to analyze [La^-] were collected (earlobe) before each exercise session, 15 min during the exercise, immediately post- and 3, 5, and 7 min post-exercise and 15 min of recovery. Peak post-exercise [La^-] was used for further analysis. Blood samples (25 μL) were immediately placed in microtubes containing 25 μL of 1g% sodium fluoride and then centrifuged at 3000 rpm for 5 min to separate the plasma before being aliquoted and frozen / stored at -80˚C. Subsequently, plasma lactate concentration was measured using a spectrophotometer (ELx800, Biotek, Winooski, USA) using a commercial kit (Biotecnica, Varginha, Brazil).

The exercise protocols consisted of 30 min continuous cycling with a pedal frequency between 60 and 70 rpm. The LIE-BFR and LIE protocols were carried out at power output corresponding to 40% of $\dot{V}O_{2max}$ (70 ± 9.8 W), determined at preliminary testing. The LIE-BFR protocol was performed with a cuff strapped over the thigh. Immediately before trials, subjects rested on a stretcher while the arterial occlusion pressure was measured. An 18-cm wide cuff was placed on the proximal portion of the thigh (inguinal fold region) and once in position, inflated until an absence of auditory blood pulse in the tibial artery was detected through auscultation with a vascular Doppler probe (DV-600; Marted, São Paulo, Brazil)[20]. Pressure was then slowly released until the first arterial pulse was detected, considered the systolic pressure in the tibial artery. Cuff pressure was set at 80% of the maximum tibial arterial pressure [20], the cuff was inflated throughout the exercise session and deflated immediately after exercise. The average of maximum occlusion pressure was 136±22 mmHg and average of 80% occlusion pressure was 109±18 mmHg.

Diet/Exercise control. Before each experimental trial, subjects were instructed to refrain from exercise training and vigorous physical activity, and alcohol and caffeine consumption for a minimum of 48h. In addition, subjects were asked to record dietary intake 24h before the first trial. In the posterior trials, subjects were asked to ingest a similar diet to the first trial.

Energy system contributions and energy expenditure. Blood lactate accumulation and oxygen uptake during and after exercise were used to estimate the anaerobic lactic metabolism, aerobic metabolism, and anaerobic alactic metabolism contributions, respectively [21]. The aerobic metabolism contribution was determined by the area under the curve of exercise $\dot{V}O_2$ and subtracting the baseline amount of $\dot{V}O_2$ corresponding to total exercise time (i.e., 30 min). To estimate the anaerobic alactic metabolism contribution, a mono-exponential model (Eq 1) was used to fit oxygen uptake recovery data (10 min after exercise) [21], and the amplitude multiplied by the time constant (Eq 2). To estimate the anaerobic lactic metabolism a value of

1 mmol·l^{-1} [La^{-}]$_{net}$ was considered to be equivalent to 3 ml O_2·kg^{-1} body mass [22]. Total energy expenditure was calculated as the sum of the three energy systems. These estimates were performed using free software specifically developed to calculate energy system contributions (GEDAE-LaB, São Paulo, Brazil), available at http://www.gedaelab.org [21].

$$\dot{V}O_{2(t)} = \dot{V}O_{2baseline} + A_1[e_1^{-(t-td)/t_1}] \quad (1)$$

$$AL_{MET} = A_1 \cdot \tau_1 \quad (2)$$

Where $\dot{V}O_{2(t)}$ is the oxygen uptake at time t, $\dot{V}O_{2rest}$ is the oxygen uptake at baseline, A is the amplitude, *td* is the time delay, *t* is the time constant, and $_1$ denotes the fast component, respectively.

Statistical analyses

Data normality and variance equality were assessed through the Shapiro-Wilk and Levene's tests. The paired T-test was used to perform comparisons between conditions for study mean dependent variables: total energy expenditure, aerobic metabolism, anaerobic alactic metabolism, and anaerobic lactic metabolism. A two-way (*time* vs. *condition*) ANOVA for repeated measures was used to compare the [La^{-}], HR and $\dot{V}_E$ responses. When a significant F value was found, the Bonferroni Post Hoc was performed to localize differences. Data are presented as Mean ± Standard Deviation (SD). The significance level was set at $P \leq 0.05$. Statistical analysis was performed using SAS version 9.3 for Windows (SAS Institute Inc., Cary, NC, USA). In addition, the statistic power was calculated using G*Power 3.2.1 software, with a type I (α) error rate of 5%, sample size of 12, and the specific correlation and Cohen's effect size among the repeated measures of each main dependent variables [23].

Results

Energy system contributions and total energy expenditure

Fig 1 presents the energy system contributions and total energy expenditure during exercise. There were significant increases in the aerobic metabolism (Fig 1A, P = 0.020; statistic power = 0.89), lactic metabolism (Fig 1C, P = 0.008; statistic power = 0.99), and total energy expenditure (Fig 1D, P = 0.008; statistic power = 0.96) with the LIE-BFR. No significant differences between conditions were found for the anaerobic alactic metabolism (Fig 1B, P = 0.582; statistic power = 0.34).

Lactate concentration [LA-]

Table 2 shows plasma lactate concentration before, during, and after exercise. A significantly higher [La^{-}] was found in the LIE-BFR compared with LIE at 15min (P = 0.008) during exercise, and peak post-exercise (P = 0.001). Plasma lactate concentration increased over time in the LIE-BFR (15min P = 0.004, peak post-exercise P = 0.04) and LIE (15min P = 0.003, peak post-exercise P = 0.03), returning to the pre-exercise levels after 15 min recovery for both protocols.

Cardiorespiratory responses

Fig 2 shows the heart rate (A) and ventilation (B) response to LIE and LIE-BFR. There were no significant differences between conditions for the HR and $\dot{V}_E$ at baseline. Heart rate was significantly higher in the LIE-BFR at 10 (P<0.0001), 15 (P = 0.0004), 20 (P = 0.0001), 25

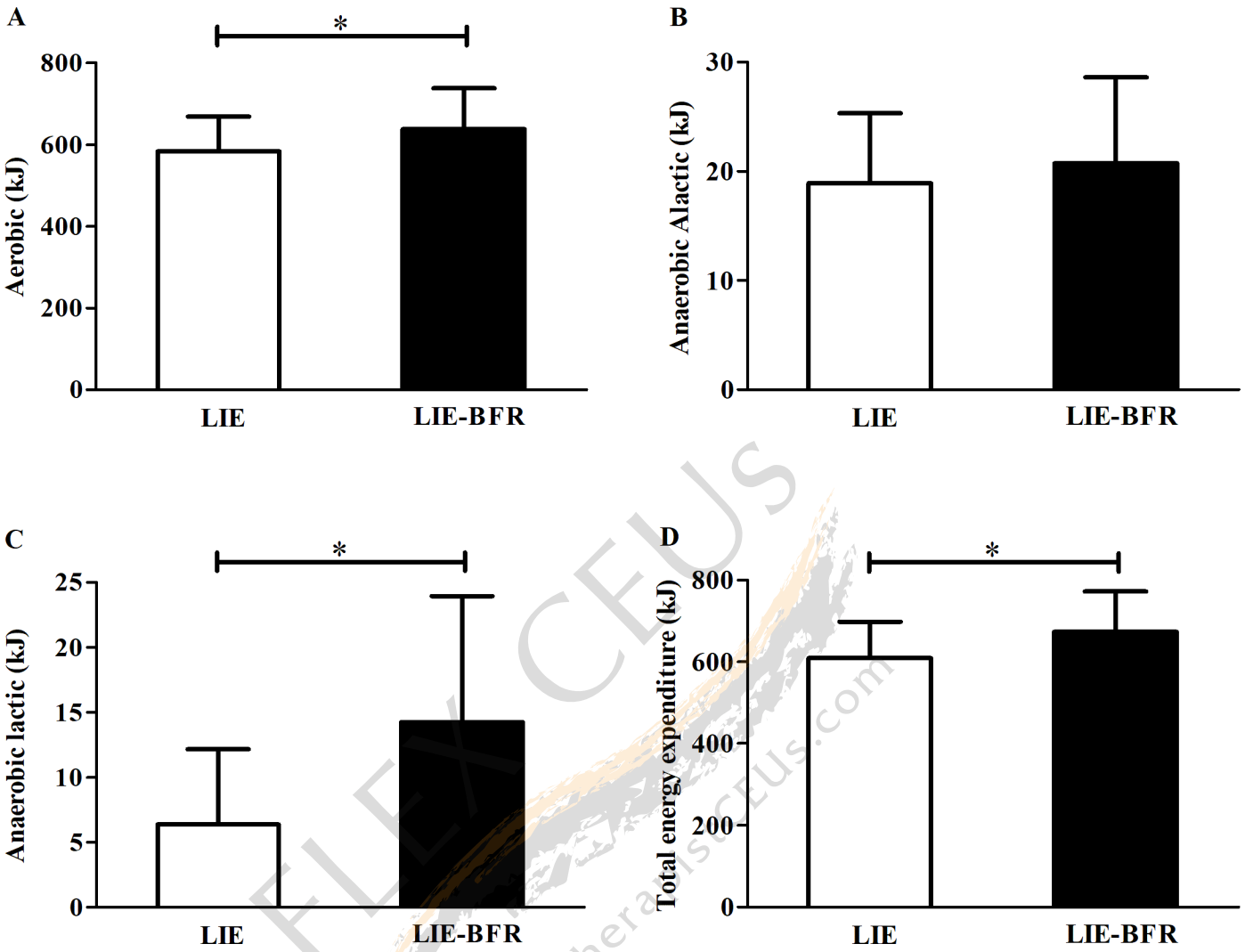

Fig 1. Aerobic metabolism (A), anaerobic alactic metabolism (B), anaerobic lactic metabolism (C), and total energy expenditure (D) during low intensity endurance exercise (LIE) and low intensity endurance exercise with blood flow restriction (LIE-BFR). Values are mean ± SD (n = 12). * Significant difference between groups ($P \leq 0.05$).

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(P<0.0001), and 30 min (P<0.0001) during exercise, and at 5 (P = 0.03), 10 (P<0.0001) and 15 min (P<0.0001) of recovery. Heart rate increased over time during exercise and recovery in

Table 2. Plasma lactate concentration responses to LIE and LIE-BFR.

Lactate ($mmol \cdot l^{-1}$)	LIE	LIE-BFR
Pre	1.32 ± 0.55	1.17 ± 0.44
15min exercise	2.14 ± 1.30	2.94 ± 1.10*
Peak post-exercise	1.97 ± 1.18	2.89 ± 1.49*
15min after	1.41 ± 0.67	1.55 ± 0.75

Values are presented as mean ± SD, n = 12. LIE: low intensity endurance exercise; LIE-BFR: low intensity endurance exercise with blood flow restriction.

* Significant difference between groups ($P \leq 0.05$). Differences over time were omitted to improve clarity.

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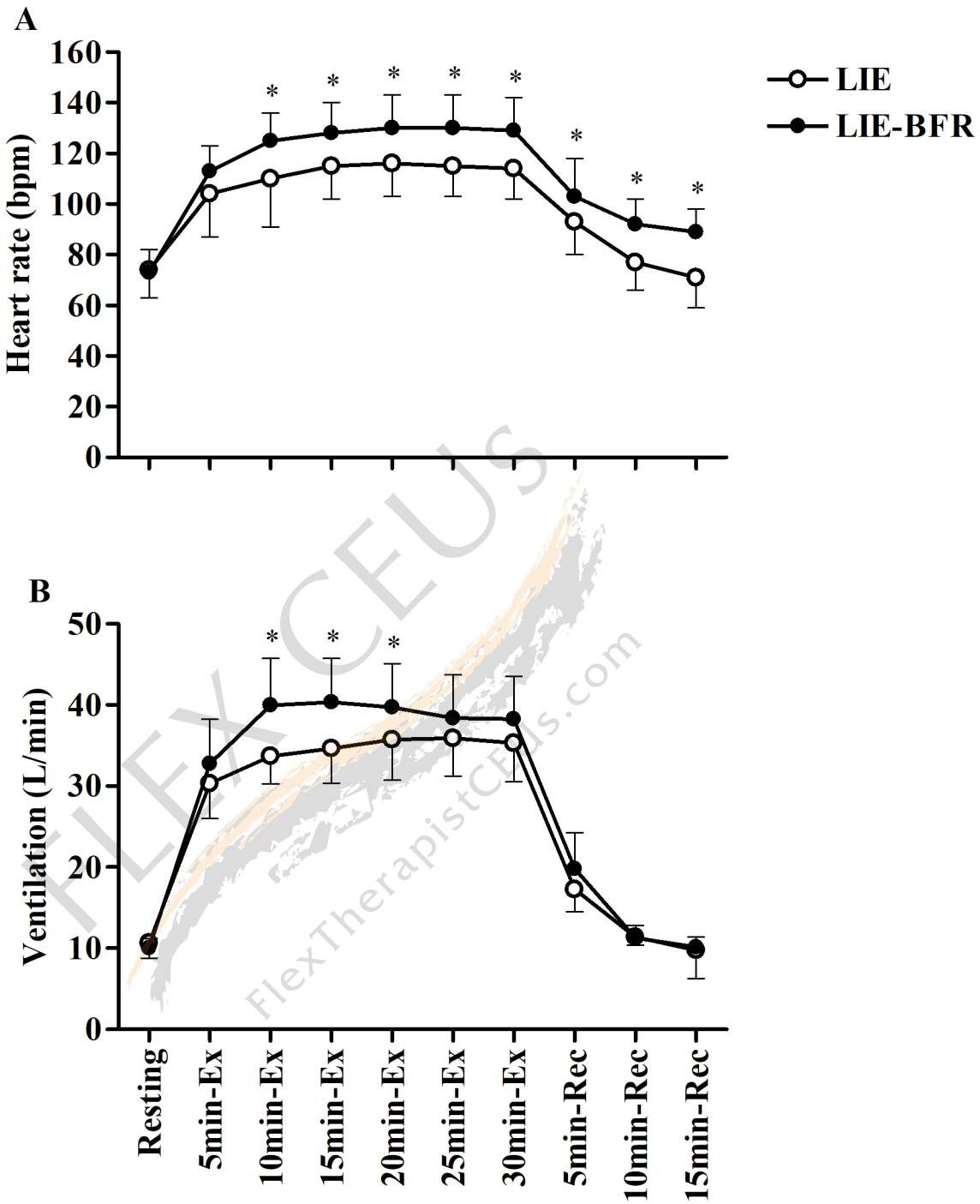

Fig 2. Heart rate and ventilation during and after low intensity endurance exercise (LIE) and low intensity endurance exercise with blood flow restriction (LIE-BFR). Ex: exercise; Rec: recovery. Values are mean ± SD (n = 12). * Significant difference between groups ($P \leq 0.05$). Differences over time were omitted to improve clarity.

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both protocols (all $P<0.0001$), however, only in the LIE, returned to the pre-exercise level at 10 and 15min of recovery. Ventilation was significantly higher in the LIE-BFR at 10 ($P<0.0001$), 15 ($P<0.0001$), and 20 min ($P<0.0001$) during exercise. Ventilation increased

over time during exercise and 5 min of recovery (all $P<0.0001$) returning to the pre-exercise levels after 10 min recovery for both protocols.

Discussion

Low intensity exercise with blood flow restriction has been shown to promote increases in cardiorespiratory fitness [3, 4]. Accordingly, we hypothesized that local hypoxia induced by BFR would produce elevated muscular metabolic demand and could increase whole-body energy expenditure and cardiorespiratory responses. We report that low intensity endurance exercise performed with blood flow restriction increased the aerobic and anaerobic lactic metabolisms resulting in augmented total energy expenditure. Additionally, we found greater [La^-], and HR and $\dot{V}_E$ responses in the LIE-BFR compared to LIE. Taken collectively, our findings suggest that cycling exercise undertaken with blood flow restriction is able to provoke additional perturbations to homeostasis necessary to induce improvements in $\dot{V}O_{2max}$, which normally take place during moderate-vigorous intensity endurance exercise without blood flow restriction.

A growing body of evidence suggests exercise undertaken with blood flow restriction can enhance exercise adaptation [3, 4, 24, 25]. Some previous studies have reported that endurance walking/cycling exercise performed with blood flow restriction can increase cardiorespiratory fitness, although with smaller gains compared to high intensity endurance exercise alone [3, 4]. However, little is known about the mechanisms mediating these responses when low intensity endurance exercise is undertaken with BFR. As such, we recently reported that, despite great lactate response after LIE-BFR, there are no significant responses of genes and proteins related to mitochondrial biogenesis and angiogenesis after LIE-BFR [16]. In spite of the lack of significant results for these local markers we still believe that there is a link between local metabolic perturbations and cardiorespiratory adaptation induced by LIE-BFR.

It has been shown that BFR on leg muscles during exercise acutely changed cardiovascular responses compared to normal blood flow conditions [26, 27]. Although it is plausible to suppose that these changes are related to the effects of cuff-pressing the thigh musculature on changes of movement pattern, it was showed that decreases in locomotion economy with BFR were caused by the increased ventilation, which is likely matched to the rate of CO_2 output [28]. Additionally, Ozaki et al., (2010) [27] showed that during cycle exercise at 20, 40, and 60% of $\dot{V}O_{2max}$ with BFR can significantly increase HR and trend to increase $\dot{V}O_{2max}$ while there was no significant change to the same exercise without BFR. Accordingly, Sakamaki-Sunaga et al., (2012) [29] compared cardiorespiratory and lactate responses to a graded walking test with and without BFR and showed an increased HR and $\dot{V}O_2$ at a given submaximal workload in BFR condition. Thus, it is suggested that the elevated cardiovascular response is due to the local hypoxia induced by BFR [5].

In fact, the pressure held by the cuff on the upper portion of each thigh induces an accumulation of blood into the legs with reduction in femoral venous return [30, 31]. Likewise, some evidence endorses the idea that there is a limited capacity for delivering O_2 (e.g., reduced capacity of femoral arterial blood flow) to the exercising muscles and consequently decreased O_2 availability [9, 32, 33]. As the O_2 availability is decreased during exercise, the amount of energy provided by the anaerobic metabolism to maintain muscle contraction is increased [28, 34]. Confirming this rationale, we found herein that the aerobic and in particular anaerobic lactic metabolism was significantly higher during LIE-BFR compared to LIE (Fig 1A and 1C) which generates an increase in [La^-] (Table 1). It is possible to suggest that the increase of [La^-] identified in our study would be the result of the metabolite accumulation in the occluded vascular bed of the lower limbs, which would have restricted its circulation through the organism

and, consequently its use as substrate by the muscles and other tissues [35]. However, we believe that this fact had less influence on the [La^-] measurements, since with 15min of exercise, even with occluded blood flow, a higher [La^-] was observed in earlobe samples collected from the LIE-BFR condition. This indicate that even with BFR the lactate circulated throughout the body and was probably used as a substrate as well as in exercise without BFR. In this way, it is important to note that even though it has been shown that the aerobic metabolism is the main energy system during exercise performed below $\dot{V}O_{2max}$ intensity [36], it is plausible to consider that the anaerobic lactic metabolism is an important source of energy to sustain muscle contraction during LIE-BFR. Therefore, we suggest that the perturbation induced by BFR in arterial and venous leg blood flow increases the participation of the anaerobic lactic metabolism due to reduction in O_2 availability, possibly resulting in the increase of HR and $\dot{V}_E$ in an attempt to adequately provide O_2 to the exercising muscle.

By decreasing the availability of O_2 (reduced arterial and venous leg blood) to the exercising muscles and retaining the need for energy production to maintain muscle contraction, it is also plausible to suppose that cycling with BFR might increase cardiorespiratory response by metaboreflex [37] and/or by decreased venous return [6, 29]. During BFR exercise the metabolic stress (e.g., [La^-], Pi, pH) will be increased [38, 39] and stimulate metabolically sensitive group III and IV afferent nerve endings within the active muscle, eliciting a reflex increase in efferent sympathetic nerve activity and systemic arterial pressure, known as muscle metaboreflex [12, 40], a reflex that significantly contributes to the autonomic cardiorespiratory response to exercise, as well as, increasing $\dot{V}_E$ and HR [13, 41]. In fact, we have found that not only $\dot{V}_E$ was increased during exercise, but also the HR response during and after exercise was significantly higher in the LIE-BFR compared to LIE (Table 2), suggesting metaboreflex was activated and also cause a delay parasympathetic reactivation [42–44]. Another mechanism that potentially could contribute to increase HR response in this condition is the decreased venous return. Renzi et al., (2010) [6] investigated the effects of 2-minute treadmill walking at 2 miles/hour with 1-min interval either with or without BFR. They found that while exercise with BFR increase HR the venous return decrease, tanking in account that stroke volume is proportional to ventricular filling and thus the amount of blood returning to the heart by the venous vessels tree, it is possible to suggest that the HR increased as a compensatory maneuver to increase blood flow and O_2 availability to working muscles. Taken together, it is suggested that metaboreflex and blood venous return are involved in HR response during and after exercise with BFR. Additionally, our data supports the idea that HR responses during and after exercise depends of on exercise intensity performed.

The normal blood flow to exercising muscle during exercise at 40% of peak workload seems not to elicit cardiovascular responses, while exercise at 60% of peak workload can induce an important pressor effect [37]. Although both our exercise protocols were performed with mild intensity exercise (40% of power output at $\dot{V}O_{2max}$), it seems LIE-BFR induced a great combination between intensity and local hypoxia to increase $\dot{V}_E$ and HR, probably resulting from metaboreflex and reduced blood venous return. Altogether, we speculate that decreasing the availability of O_2 that culminates in the high participation of the anaerobic metabolism and further in the aerobic metabolism during exercise, accompanied by increased HR and $\dot{V}_E$, might increase cardiorespiratory stimulus and thus improve $\dot{V}O_{2max}$ after accumulated training sessions of LIE-BFR. Following this rationale, the link between metabolic perturbation and cardiorespiratory adaptation induced by LIE-BFR is the energy demand. Whilst this is an interesting hypothesis we suggest further chronic studies to confirm whether LIE-BFR can increase cardiorespiratory adaptation and studies testing different cuff pressure and/or

exercise intensities that verify the contribution of increased anaerobic metabolism to consequent cardiorespiratory overload.

A second hypothesis emerges from this find, since even LIE-BFR is performed at low intensity, the present data shows that LIE-BFR can induce significant energy expenditure which could consequently induce weight loss [45]. Regarding practical terms, it is attractive to suspect that the LIE-BFR could be a promising exercise strategy for people that are not able to perform exercise with moderate/high intensity and need to cardiorespiratory fitness and lose weight as well as elderly and obese people. Accordingly, Karabulut and Garcia (2017) [46] showed that obese subjects cycling with BFR increased energy expenditure and cardiovascular stress. However, it is important to highlight that endurance exercise performed with BFR shows lower cardiovascular stress, measured by heart rate variability and hemodynamic responses, to low load with BFR (40% $\dot{V}O_{2max}$) compared to high load without BFR (70% $\dot{V}O_{2max}$) in elderly [47], suggesting the safety of this physical exercise method. It is relevant to note that the features of the present study (sample characteristics and experimental design) does not allow us to draw definitive conclusion concerning the importance of the LIE-BFR for special populations as elderly and obese subjects, or even reflect in additional benefits to a period of training. Thus, long term studies with different populations could be conducted to test these hypotheses.

In summary, this is the first study to investigate the contribution of the energy systems (aerobic, anaerobic alactic, and lactic) during LIE-BFR. Herein we show that low intensity endurance exercise performed with blood flow restriction increases anaerobic lactic and aerobic system contributions, total energy expenditure, and cardiorespiratory responses. Longer training programs incorporating endurance exercise with BFR that correlate measurements of the contributions of the energy systems with adaptation responses such as changes in $\dot{V}O_{2max}$ and body composition will yield important information on the efficacy of this training method. If confirmed LIE-BFR training may become an important strategy to enhance training adaptation and improve health outcomes in populations that may be unable to perform prolonged/intense exercises, such as elderly and overweight/obese people.

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