Near-infrared spectroscopy during low-intensity blood flow restriction resistance exercise

Espectroscopia no infravermelho próximo durante exercício contrarresistência de baixa intensidade com restrição de fluxo sanguíneo

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ABSTRACT

Introduction: Low-intensity resistance exercises with blood flow restriction are known to be effective in promoting muscular strength and hypertrophy; however, there is a paucity of evidence on their acute hemodynamic responses. Objective: To compare the changes in muscular oxyhemoglobin (O2Hb), deoxyhemoglobin (HHb) concentrations, and O2 saturation (StO2) during low load exercise under free blood flow (FreeBF) and blood flow restriction (BFR). Methods: Fifteen healthy males were subjected to bilateral knee extension tests under FreeBF and BFR conditions, in a random order. The knee extension exercise included four sets of 15 repetitions at 20% of one-repetition maximum, with 30s interval between the sets. In the BFR condition, subjects exercised with a cuff positioned on the proximal thigh and inflated to 50% of total occlusion pressure. Changes in the O2Hb, HHb, total hemoglobin (tHb), and StO2 in vastus lateralis muscle were monitored using near-infrared spectroscopy. Results: A two-way repeated-measures ANOVA revealed significant main effects for sets for all variables (P < 0.05). Moreover, the values in StO2 during sets 2, 3, and 4 in BFR conditions were significantly lower than those in FreeBF. Significant differences were also seen between the exercise conditions during rest intervals for HHb (rest intervals 2, 3, and 4) and tHb (rest interval 3; P < 0.05). There were no significant interactions between conditions and sets or conditions and intervals for O2Hb. Conclusion: Low-intensity resistance exercise performed with BFR significantly decreased the acute muscle StO2 and increased total muscle hemoglobin.

Keywords: resistance training; muscle, skeletal; muscle strength; near-infrared spectroscopy.
Introduction

Resistance exercise with blood flow restriction (BFR-RE) has gained greater attention in recent years since it has shown significant gains in muscle strength and hypertrophy in athletes [1] and non-athletes [2].

Blood flow is generally restricted by applying inflatable cuffs in the proximal region of limbs. Blood flow restriction pressure is commonly used with 50% to 80% of the required pressure for total occlusion of the venous blood flow, yet low enough to maintain arterial inflow into the muscle. When combined with restricted blood flow, resistance training is performed with 20% to 50% of an individual’s maximum repetition (1 RM) [3].

Available evidence advocates the feasibility and safety of BFR-RE [4]; however, the underlying mechanisms responsible for the observed results remain unclear. Potential proposed mechanisms are still under scrutiny, but researchers have suggested that muscle growth stimulated by BFR-RE might be related to the induced increase in post-exercise hyperemia and cell swelling, as well as the greater metabolic stress associated to the relative hypoxia caused by blood flow restriction [5].

Monitoring of the acute muscle hemodynamic responses during and after the BFR-RE might be an opportunity to shed some light on understanding the mechanisms involved in BFR-RE-induced hypertrophy, particularly those related to the modifications of muscle blood volume and oxygenation. Near infrared spectroscopy (NIRS) is widely used in the research for acute and chronic effects of resistance exercise under various conditions [6-10].

To the best of our knowledge, changes in the muscle oxygen saturation during BFR resistance exercise remained less explored. Previous studies have adopted protocols involving only one set [11], sets to volitional fatigue [12] or isokinetic equipment [13, 14], which limits the extrapolation of their findings to the real world.

As the knowledge of hemodynamic responses can be useful in understanding the phenomena behind the BFR-RE effects, this study aimed to compare the changes in muscular oxyhemoglobin (O$_2$Hb) and deoxyhemoglobin (HHb) and O$_2$ saturation (StO$_2$) during low-load exercise under BFR and freeBF.

Methods

Subjects

Fifteen healthy males (age: 18.7 ± 0.5 years; body mass: 66.8 ± 8 kg; height: 172 ± 9 cm; body fat: 9.4 ± 4.6%) who were inexperienced in resistance training, were included in this study. The criterion for exclusion included current musculoskeletal injuries of lower limbs. The subjects were advised to maintain their physical activity throughout the study period. All participants received a detailed verbal explanation of the study procedures and risks involved in the experimental procedures, and they signed a written informed consent form prior to participation in the study. The study
was approved by the Naval Hospital Marcílio Dias Ethical Review Board (#718.602),
with the procedures following the principles of the Declaration of Helsinki.

**Study design**

The study comprised three testing sessions, with an interval of one week. The first session determined the demographic and anthropometric data, maximal dynamic strength, and occlusion pressure. The subject’s familiarization with the exercise protocol using the inflated cuff was also part of the first visit.

The second and third sessions comprised bilateral knee extension tests under freeBF and BFR conditions in random order. In BFR, two sphygmomanometers were fitted individually to both thighs. The subjects had muscle hemodynamics changes monitored in the right limb before, during, and after exercises in both experimental conditions.

**Determination of occlusion pressure**

Blood flow occlusion pressure was determined by ultrasound equipment with Doppler (GE LogiqE, GE Healthcare, EUA). Subjects were instructed to lie in a prone position while the cuff positioned in the proximal thigh was inflated up to no pulse, which was detected with the Doppler probe positioned over the popliteal artery. The pressure employed during the exercise visits was equal to 50% of the total arterial occlusion pressure.

**Resistance exercise testing**

All subjects were submitted to bilateral knee extension test using isotonic equipment (Technogym, Rehabilitation Device, Gambettola, Italy).

To minimize any extraneous movement during the strength tests, participants were strapped over the hips for immobilization of the hip joint. All individual settings accomplished during that visit were recorded and replicated in the subsequent experiment.

Maximum dynamic strength was determined with the one-repetition maximal test (1 RM) in the same isotonic equipment using in the experimental visits. The protocol comprised ten submaximal repetition warm-ups followed by the 1 RM test attempts (maximum 6). Loads were added progressively, with a minimum of 3-min rest period between the attempts. The highest load successfully lifted was recorded as the subject’s 1 RM.

Experimental tests comprised four sets of 15 repetitions at 20% 1 RM, with 30 s intervals between sets. Only repetitions executed over the complete range of 90° motion were considered valid. Subjects performed the movement at their convenience while receiving verbal stimulus from the testers towards the end of the pre-determined exercise. For BFR sessions, a commercial nylon-cuffed thigh-sized aneroid sphygmomanometer (70 cm x 16 cm tourniquet) (B.Well Swiss AG, Switzerland) was placed around the proximal portion of both thighs individually. Inflation pressure
applied was equivalent to 50% of the arterial occlusion pressure determined previou-
sly. Inflation started just before the exercise began and was released shortly after the
last repetition.

Muscle hemodynamics monitoring with NIRS

Muscle hemodynamics were monitored continuously in real-time by near-in-
frared spectroscopy (Oxiplex TS, ISS, Champaign, IL, USA). Total muscle microvascu-
lar concentrations of hemoglobin and myoglobin was measured for 1-min rest during
the strength test, and the 30-s rest period. The instrument uses near-infrared light at
two wavelengths (690 and 830 nm) with an optical fiber-based light and detector
source, providing absolute values of tissue hemoglobin saturation, and individual
concentration of O$_2$Hb and HHb. Throughout the entire exercise protocol tHb (sum
of O$_2$Hb and HHb) and in StO$_2$ were monitored in real-time.

The NIRS probe was initially covered with a plastic film to avoid humidity
from the skin. The probe was placed on the skin over the vastus lateralis muscle of
the subject’s right thigh, while seated, 2 to 3 cm from the outer limit of the cuff. To
secure it on the skin and minimize movement during exercise, an elastic bandage
was wrapped around the subject’s thigh and covered with a black towel, reducing
the possibility of extraneous light influencing the signal. The adipose thickness at
the subject’s thigh did not impair light penetration because the measured thickness
(Harpenden Skinfold Caliper, Baty International, West Sussex, United Kingdom) was
less than 25 mm, the depth of penetration of the NIRS. The NIRS device was calibra-
ted before every test as recommended by the manufacturer. All data were collected
online at a frequency of 1 Hz, using specific dedicated software.

The NIRS data were recorded throughout the exercise period, including in-
tervals between sets. For analysis purposes, the lowest values of tHb, O$_2$Hb, and StO$_2$
were recorded, as well as the highest values of HHb obtained during the series. Du-
ring the rest intervals, the highest values of tHb, O$_2$Hb, and StO$_2$, and the lowest
values of HHb were also recorded, as well as the last 10 s of baseline.

Unpublished data from our laboratory have demonstrated the test-retest
intraclass correlation coefficients for muscle oxygenation (minimum or amplitude
values, either on the same day or on two separate testing days), ranging from R =
0.724 to 0.989.

Statistical analysis

All data are presented as mean and standard deviation. Two-way repeated me-
asures ANOVA was used to identify the differences in NIRS variables, obtained du-
ring the exercise stages (sets and rest intervals separately) between FreeBF and BFR
conditions. All statistical analyses were performed using a commercially available
software (SPSS for Mac, Ver. 20.0, Armonk, NY: IBM Corp). All statistical analyses
were tested at 95% probability.
Results

None of the subjects reported any side effects during or after the exercise protocols. One repetition maximum for the bilateral leg extension exercise was $122 \pm 17$ kg, and the load applied in tests was $24 \pm 3$ kg (20% 1 RM). The mean inflation cuff pressure for total popliteal artery occlusion was $168 \pm 19$ mmHg, and the inflation pressure applied during BFR exercise condition was $84 \pm 10$ mm Hg (50% total occlusion). The $O_2$Hb, HHb, StO$_2$, and tHb trends in a typical subject on freeBF and BFR conditions are illustrated in Figure 1.

![Figure 1](image_url)

**Figure 1** - Muscle NIRS variables trend during four sets of knee extension in free blood flow (upper panel) and blood flow restriction (lower panel) conditions

Figure 2 presents the mean values of NIRS variables in each exercise condition and stage. For all NIRS variables there were significant main effects of sets.
Asterisks indicate significant interactions between conditions

Figure 2 - Mean changes in NIRS variables during four sets of knee extension in free blood flow (circles) and blood flow restriction (squares) conditions.

No statistically significant interactions (condition × sets or condition × rest intervals) were observed for O$_2$Hb. Significant lower values were observed for StO$_2$ during sets 2, 3, and 4 in BFR condition as compared to those in freeBF. Moreover, significant differences were seen between the exercise conditions during rest intervals for HHb (rest intervals 2, 3, and 4) and tHb (rest interval 3).

Discussion

This study assessed the NIRS variable responses with and without BFR to understand the effects of blood restriction on muscle O$_2$Hb and HHb concentrations and StO$_2$ during knee extension. Our results contributed mainly to a better understanding of the hemodynamic responses during resistance exercise performed in isotonic equipment, comprising a predetermined repetition count instead of a fatigue protocol. Another important aspect is the use of a restriction pressure related to the individual’s estimated arterial occlusion, strengthening the external validity of the results. Besides, the application of 50% arterial occlusion was appropriate to ensure comfort to the participant with accurate blood flow restriction, as reported in a study by Mouser et al. [15], wherein the blood flow was relatively unchanged between 50% and 90% of arterial occlusion pressure. Furthermore, low to moderate relative pressure (40% to 50% estimated arterial occlusion) is sufficient to maximize the acute response to BFR exercise.
The NIRS data collected during the knee extension exercise revealed that from the third resting exercise interval, muscle tHb was higher for the BFR condition. A significant increase was observed in HHb concentration in the BFR compared to Fre-eBF during rest intervals. Traditionally, this represents an increase in oxygen consumption by the muscle [16]. Nevertheless, the overall findings (similar O$_2$Hb, higher tHb, and lower StO$_2$ in BFR condition) revealed that presumably, the venous pooling during the rest intervals led to this event, rather than an actual increase in the muscular oxygen consumption.

Venous blood accumulation can favor blood influx in the sarcoplasm, causing cell swelling and accumulation of metabolites. One of the possible components explaining muscle hypertrophy is acute cell edema since it can stimulate protein synthesis and suppress proteolysis [17]. In this study, possibly the higher muscle tHb observed during the knee extensions could have helped augment the intracellular swelling due to osmotic water shifts into the cell, thus collaborating to the understanding of the hypertrophic effect of low-intensity resistance exercises with BFR. This issue needs to be investigated further to confirm this hypothesis.

Yasuda et al. [18] found that immediately after performing 75 repetitions of concentric biceps curls at 30% 1 RM, the elbow flexor muscle thickness acutely increased by 11.7%. Following a six-week training period, it was observed a significant 12% increase in MRI-measured muscle cross-sectional area of the elbow flexors. Authors suggested that the pronounced muscle cell swelling might crucially promote muscle hypertrophy.

Although no between-condition differences were observed in O$_2$Hb, since the applied blood flow restriction did not limit the arterial influx, a significant lower muscle StO$_2$ was observed from set 2 to set 4 performed under BFR, representing the hypoxic characteristic of BFR exercise. These results were consistent with those by previous studies adopting the exercise protocols involving repetitions to volitional fatigue [12] during isotonic [11,14] and isokinetic movement [13].

In accordance to Loenneke et al. [19], hypoxia can be adequate for enhancing the recruitment of high-threshold motor units, as well as provoking systemic elevation of hormonal and growth factors [20] and activation and proliferation of myogenic stem cells [3], thus promoting the hypertrophic response to perform the exercise.

During BFR exercise, due to the hypoxia conditions, seems to occur additional recruitment of more motor units to compensate for the oxygen deficit. Training can recruit more fast-twitch fibers than traditional resistance exercise does [21].

Another possible beneficial effect explaining the hypertrophic mechanisms unleashed by BFR is a higher anabolic hormonal response even with a lower training load, probably entailed by its inherent muscular hypoxia. Manini et al. [22] reported that low-load BFR resistance exercise was able to stimulate growth hormone (GH) secretion in an amount comparable to that produced by high-load resistance exercise without BFR.
As reported by Nielsen et al. [3] the exact mechanism underlying this phenomenon is not known in respect to increasing in myogenic stem cell proliferation. However, the authors believe that BFR-induced hypoxia and/or nitric oxide production may stimulate this process, which is required to cause substantial increases in muscle fiber cross-sectional area [23].

It could also be reasonable to speculate that low oxygen tension is one of the physiological signals related to the capacity of resistance exercise in inducing angiogenesis. Notably, during resistance exercise with BFR, the decrease in oxygen muscle saturation might stabilize hypoxia-inducible factor 1 alfa (HIF-1 alfa), activating vascular endothelial growth factor (VEGF) transcription, the main vascular growth factor. This cascade in angiogenesis would be involved in facilitating metabolic improvements in muscle cells helping in protein synthesis [24,25].

A limitation of the present study was the application of cuff occlusion immediately before the exercise commencement, which may have blunted the observed results. Cayot et al. [21] revealed that the occlusion pressure applied 5 min before isometric knee extension exercise elicited greater changes in HHb when compared to the immediately occluding pressure before exercise, suggesting that a higher occlusion time may be necessary to amplify the BFR-induced metabolic stress.

**Conclusion**

In conclusion, our findings indicate that exercise performed with blood flow restriction, based on 50% of total blood occlusion, promoted significant decreases in the acute muscle StO2 and an increase in muscle tHb during the exercise. As previously observed, significantly lower levels in StO2 represent greater metabolic stress when associated with the relative hypoxia caused by blood flow restriction. In contrast, the higher tHb at the end of each set suggests that the muscle growth stimulated by BFR-RE might be related to their induced increase in post-exercise hyperemia and cell swelling.

Further studies are warranted to monitor the influx of arterial blood flow during various degrees of occlusion, as well as to investigate the variables involved in the high individual variations in hemodynamic responses during the resistance exercise under blood flow restriction.

**Potential conflict of interest**
No potential conflicts of interest relevant to this article have been reported.

**Financing source**
There were no external funding sources for this study.

**Author contributions**
- **Conception and design of the research:** Meirelles CM; Data collection: Aguiar Jr CS, Meirelles CM; Data analysis and interpretation: Meirelles CM, Aguiar Jr CS and Gomes PSC; Static analysis: Meirelles CM and Gomes PSC; **Writing of the manuscript:** Meirelles CM and Gomes PSC; **Critical review of the manuscript regarding important intellectual content:** Meirelles CM and Gomes PSC; **All authors contributed substantially to the manuscript and approved the final submission.**
References


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