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Original article

Effects of different blood flow restriction pressure levels on muscular hemodynamics

Efeito de diferentes níveis de restrição de fluxo sanguíneo sobre a hemodinâmica muscular

Ramon Franco Carvalho[1], Paulo Sergio Chagas Gomes[1], Márcio Lopes Fernandes Júnior[2], Claudia Mello Meirelles[3]

1. Instituto de Educação Física e Desportos, Universidade do Estado do Rio de Janeiro, RJ, Brazil
2. Universidade Estácio de Sá, Campus Duque de Caxias 2, Duque de Caxias, RJ, Brazil
3. Seção de Pesquisa e Extensão, Escola de Educação Física do Exército, Rio de Janeiro, RJ, Brazil

ABSTRACT

Introduction: Resistance exercise with blood flow restriction (BFR) is an effective method to promote muscle strength gains and hypertrophy. However, little is known about the effects of different BFR levels on hemodynamic responses. **Objective:** To verify whether the different blood flow restriction pressures applied to the upper limb cause acute changes in vascular microcirculation in young, healthy male adults. **Methods:** Ten young male visited the laboratory on four occasions. In the first visit, after 10-min rest in supine position, the brachial artery occlusion pressure (AOP) was identified with a Doppler ultrasound. Thereafter, the participants were submitted to a protocol consisting of 1 min for baseline measurements, 2 min of BFR, and 2 min after cuff deflation. It was used a cuff placed on the proximal portion of the forearm and inflated with pressures equivalents to 30% (30BFR), 50% (50BFR) 80% (80BFR), or 100% (100BFR) of the AOP in a random order in separate days. Measurements of tissue saturation index (TSI), oxyhemoglobin, deoxyhemoglobin, and total hemoglobin were collected continuously using near-infrared spectrometry. **Results:** A two-way ANOVA with repeated measures demonstrated: 1) a significant decrease in TSI in all conditions, with higher decay in 100BFR; 2) a significant increase in oxyhemoglobin in all conditions, but 100BFR; 3) a similar increase in deoxyhemoglobin in all conditions; 4) a significant increase in total hemoglobin in all conditions, mainly in both 30BFR and 50BFR. **Conclusion:** The relative pressures adopted demonstrated that the hemodynamic changes do not occur linearly with the pressure level imposed by the inflated cuff.

Keywords: spectroscopy, near-infrared; vascular closure devices; resistance training.

RESUMO

Introdução: O exercício contrarresistência com restrição do fluxo sanguíneo (RFS) é um método eficaz para ganho de força e hipertrofia muscular. No entanto, pouco se sabe sobre os efeitos dos diferentes níveis de RFS nas respostas hemodinâmicas. **Objetivo:** Verificar se as diferentes pressões de restrição ao fluxo sanguíneo aplicadas no membro superior causam alterações na microcirculação vascular em adultos jovens saudáveis do sexo masculino. **Métodos:** Dez jovens do sexo masculino visitaram o laboratório em quatro ocasiões. Na primeira visita, após 10 min de repouso em decúbito dorsal, a pressão de oclusão da artéria braquial (POA) foi identificada através de ultrassom com Doppler. Posteriormente, os participantes foram submetidos a um protocolo que consistia de 1 min para as medidas basais, 2 min de RFS e 2 min após a liberação da restrição sanguínea. Foi utilizado um manguito colocado na porção proximal do antebraço e inflado com pressões equivalentes a 30% (30RFS), 50% (50RFS) 80% (80RFS) ou 100% (100RFS) do POA em ordem aleatória em dias separados. As medições do índice de saturação do tecido (IST), oxiemoglobina, desoxihemoglobina e hemoglobina total foram coletadas continuamente usando espectrometria de infravermelho próximo. **Resultados:** Uma ANOVA de duas vias com medidas repetidas demonstrou 1) uma diminuição significativa no IST em todas as condições, com maior queda em 100RFS; 2) um aumento significativo na oxihemoglobina em todas as condições, exceto 100RFS; 3) um aumento semelhante na desoxihemoglobina em todas as condições; 4) um aumento significativo na hemoglobina total em todas as condições, principalmente em 30RFS e 50RFS. **Conclusão:** As pressões relativas adotadas demonstraram que as alterações hemodinâmicas não ocorrem linearmente com o nível de pressão imposto pelo manguito insuflado.

Palavras-chave: espectroscopia de luz próxima ao infravermelho; dispositivos de oclusão vascular; treinamento de força.

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Correspondence: Paulo Sergio Chagas Gomes, PhD, Universidade do Estado do Rio de Janeiro, Instituto de Educação Física e Desportos, Rua São Francisco Xavier, 524, 8o Andar, Bloco F, Sala 8104, Maracanã, 20550-900 Rio de Janeiro RJ. paulo.gomes@uerj.br

Introduction

Resistance exercise (RE) with restricted blood flow (BFR) is an effective method to promote strength gains [1-3] as well as muscle hypertrophy [2,4,5]. This method consists of using an inflated cuff at the proximal extremity of the limbs during the performance of an activity with relatively low resistance overload, ranging from 10 to 50% of 1RM [6-8].

The purpose of restricting the influx of arterial blood to the limb is to cause a more significant metabolic stress and stimulate the mechanisms of muscle hypertrophy, such as additional recruitment of motor units, cell swelling, the release of anabolic hormones, altered production of myokines, and reactive oxygen species [9-11]. Although the magnitude of the responses to strength gains are lower than the ones obtained with traditional high resistive loads strength training routines, resistance training with blood flow restriction (BFR) may be a more appropriate strategy in populations that are unable to mobilize high overload, such as the elderly [12], and people recovering from musculoskeletal injury or surgery [13].

When inflating a cuff in the proximal region of the upper or lower limb, venous blood is easily occluded, generating blood storage in the venules and thus preventing the removal of metabolites from muscle contraction. This procedure can prevent venous blood return to the limb but still allow the entry of arterial blood, even if in a limited way [14].

Understanding the impact of different percentages of blood flow restriction on muscle hemodynamics can help clarify the best relationship between metabolic stress and the lowest health risk associated with blood flow restriction [14]. Also, the scientific literature indicates that high-pressure loads promote a higher level of discomfort [15].

Previous studies [16,17] carried out in healthy young subjects at rest observed that the reduction in blood flow occurs in a staggered and non-linear manner due to increased pressure load. Using ultrasound in the Doppler mode, Mouser *et al.* [17] observed that 10% of the pressure at the artery occlusion pressure (AOP) applied by a cuff is enough to significantly reduce absolute and relative blood flow speed in the brachial artery when compared to resting condition. This flow reduction remained similar until 40% of the artery occlusion pressure (AOP) when a further significant drop in blood flow was observed and remained up to 80% (absolute blood flow) or 90% (relative blood flow) when the last phase of fall occurred. Despite the importance of this finding in blood flow, the study, as mentioned earlier, did not observe the impact on hypoxia. The literature has shown that the intracellular deviation of blood plasma and cellular hypoxia generated by flow restriction significantly influences the mechanisms associated with increased muscle strength and hypertrophy [18].

Muscle hemodynamics measurements can also be performed by near-infrared spectroscopy (NIRS), which is widely used in research to monitor acute and chronic muscle perfusion changes under different settings [19].

Kilgas *et al.* [20] showed that 30 seconds under BFR did not change muscle hemodynamics at pressures lower than 60% AOP, assessed by a NIRS probe placed on the forearm of ten healthy men. Less is known about higher periods of BFR, as employed in typical resistance exercise protocols.

Blood flow reduction seems to occur staggered and not linear or parallel by increased pressure levels exerted externally by a cuff. With this shortcoming in mind, it is necessary to identify the impact of different pressure level ranges on local hemodynamic responses, especially in cell hypoxia. This knowledge may contribute to a better understanding of the physiological responses, allowing a safer and more efficient prescription method.

Thus, the present study aimed to verify whether the different blood flow restriction pressures applied to the upper limb cause acute changes in vascular microcirculation in young, healthy male adults.

Methods

Study sample

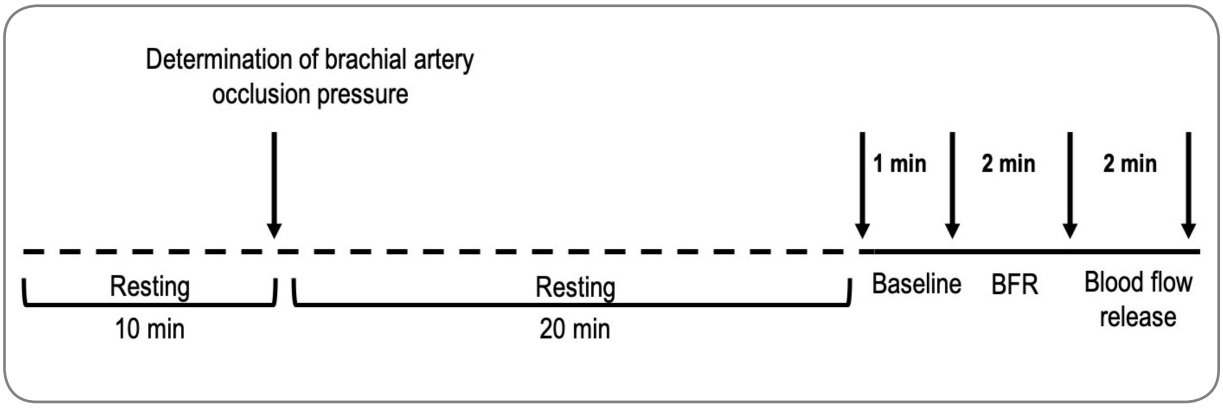
Ten young college male students volunteered for the present study (age: 26 ± 5 years, biceps skinfold: 3.4 ± 1.1 mm, systolic blood pressure: 122.9 ± 7.1 mmHg, diastolic blood pressure: 81, 4 ± 7.5 mmHg, resting heart rate: 69.3 ± 5.7 bpm; body mass index: 24.7 ± 1.1 kg/m²). All participants were normotensive and healthy based on the Physical Activity Readiness Questionnaire (Par-Q) evaluation, and nobody was involved in any systematic physical training practice in the last six months. All of them signed the informed consent form before starting the tests. The Research Ethics Committee of the President Antônio Carlos University approved this study (CAAE: 83463517.7.0000.5156), based on the principles of the Declaration of Helsinki.

Study design

The study was characterized by a randomized controlled trial model, and participants attended the research laboratory on four separate occasions with two to seven-day between trial intervals. All participants were instructed not to consume any drink or food, like caffeine and alcohol, that would affect hemodynamic responses and not to practice any physical activity 24 hours before the test. Also, all visits took place within the same time of day, with a maximum variation of one hour more or less to avoid the effect of the circadian cycle on blood pressure responses.

At each visit, participants were tested under one of the four experimental treatments. The subjects were submitted to different percentages of AOP: 30%, 50%, 80%, and 100% (30BFR, 50BFR, 80BFR, and 100BFR, respectively).

Upon reaching the laboratory, the volunteers rested on a stretcher in the supine position for 10 minutes. At the end of this period, the pressure level representing the AOP was identified using ultrasound equipment in Doppler mode. This procedure took between 40 to 60 seconds. After 20 minutes of recovery in the supine position, the subjects were submitted, in random order, to one of the experimental treatments, in order to have the hemodynamic variables monitored for five minutes, as follows: one minute to obtain baseline measurements, two minutes with the cuff inflated in the proximal portion of the right upper limb and two minutes of observation with the cuff deflated. The NIRS measurements O_2Hb, HHb, tHb, and TSI, were collected continuously during the five min test procedure. Figure 1 shows the procedures performed.



Caption = BFR - blood flow restriction; the arrows indicate the start and/or end of each phase (Baseline, BFR and flow released)
Figure 1 - Experimental design. Treatment conditions (30%, 50%, 80% and 100% of brachial artery blood flow occlusion pressure) were randomly assigned for each subject

Determination of brachial artery blood flow occlusion pressure (AOP)

The AOP was determined with an ultrasound scanner (*Logic e, General Electric - GE Healthcare, Milwaukee, WI, USA*) equipped with Doppler. A 10 cm-wide cuff was positioned at the most proximal portion of the right arm. A 40 mm-ultrasound transducer was placed on the anteromedial face of the right arm. The transducer was positioned perpendicular to its axis, 5 to 10 cm above the antecubital fold. The cuff pressure was progressively slowly released until the first sign of flow was observed in the brachial artery procedure was repeated two or three more times to confirm the pressure level of the cuff, operationally defined as the AOP. This procedure was performed in all visits and lasted approximately one minute.

Near infrared spectroscopy (NIRS) measurements

Monitoring of the muscle hemodynamics was performed using near-infrared spectroscopy (NIRS). This is a non-invasive optical technique that measures changes in the relative concentration of oxyhemoglobin (O_2Hb) and deoxyhemoglobin (HHb) in arterioles, venules, and capillaries [19]. The electrons of the hemoglobin chromophores can absorb light near the infrared region at different peaks according to the presence or absence of oxygen bound to the hemoglobin molecule [21]. In this way, using the Lambert-Beer law, it is possible to calculate changes in the concentration of the chromophores of interest, such as O_2Hb or HHb. Changes in the concentrations of oxygenated hemoglobin (O_2Hb), deoxygenated (HHb), total hemoglobin (tHb =

O_2Hb + HHb), and the tissue saturation index (TSI) were measured continuously, in random order, for 5 min in all experimental conditions (30BFR, 50BFR, 80BFR and 100BFR), using a near-infrared continuous-wave spectrometer (*NIRS; PortaMon, Artinis Medical Systems BV, Zetten, Netherlands*). The total hemoglobin concentration (tHb) was obtained by adding the concentration of O_2Hb with HHb and is an indirect indicator of blood volume. The TSI is a direct indication of the percentage of oxygenated hemoglobin and was obtained through the following equation: TSI (%) = (O_2Hb/tHb) x 100.

The sensor was positioned in the most distal position on the belly of the biceps brachii muscle. The sensor was surrounded by a plastic film, attached to the skin by tape, and covered with a dark towel to avoid distortion of the signal caused by sweat and ambient light. Data were collected using dedicated OxySoft software version (*OxySoft Ver. 2.1.1-2.1.6 Artinis Medical Systems BV, Zetten, Netherlands*) with a sampling frequency of 10 Hz.

Statistical analysis

NIRS variables values at baseline were obtained by averaging the 15 s before blood flow restriction. Measurements were obtained at the end of a 2-min period of blood flow restriction and 30 s after deflation of the cuff. All measurements obtained during and after blood flow restriction were normalized by the baseline obtained on the same day to reduce the influence of the measurements collected on different days.

After testing the assumptions of normality and sphericity using Shapiro-Wilk and Mauchly tests, respectively, a two-way ANOVA with repeated measures was used to determine a significant interaction difference between treatments and time conditions. Where significant F was observed, Sidak's post hoc test was applied to analyze possible differences in the dependent variables among conditions (30BFR, 50BFR, 80BFR, and 100BFR) within each phase (BFR and blood flow release). The level of significance adopted in this study was 0.05. Also, the effect size (ES) was used to identify the clinical effect through the magnitude of the difference [22,23].

Except for the variable O_2Hb in groups 30BFR, 50BFR, and 100BFR during the blood flow restriction phase, TSI in group 30BFR, and tHB in 100BFR, all other variables showed normal distribution. However, the ANOVA test was used in all analyses because it is robust enough to be used even when normality is not observed [24]. The Greenhouse-Geisser correction was used to compare TSI between the conditions due to the violation of sphericity.

All analysis were performed using commercially available SPSS statistical software (*IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 21.0. Armonk*, NY: *IBM Corp.*).

Results

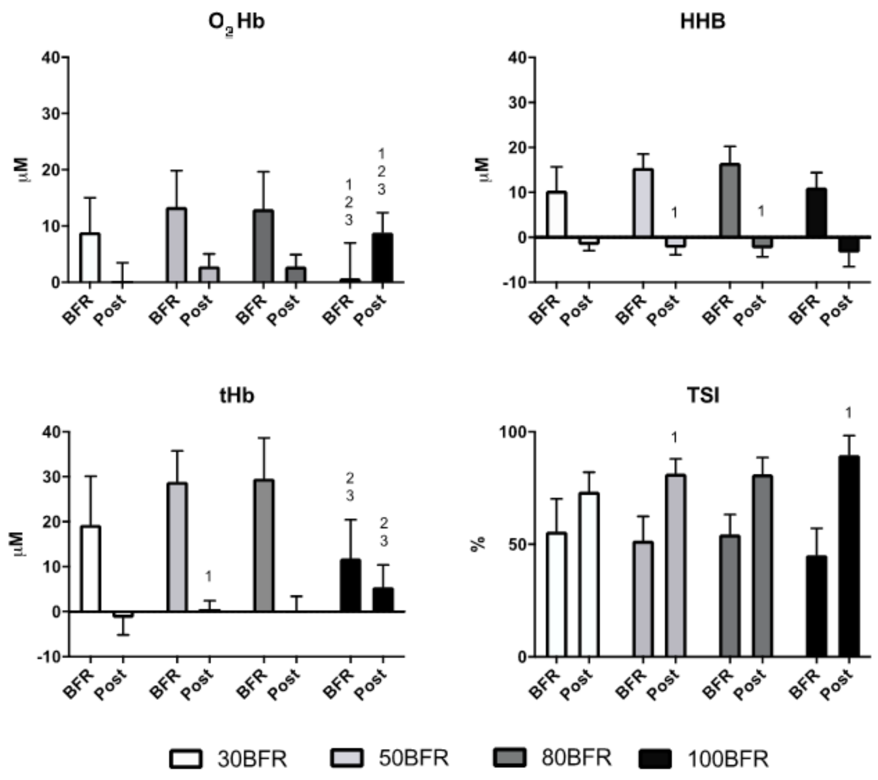
Post-hoc analysis identified the study's power at 0.83. For this result, an effect size of 0.40 was considered, an error α: 0.05, for a sample size of 10 participants, in four conditions (of blood flow restriction), three measures repeated over time (baseline, restriction of blood flow and after the release of arterial flow), a correlation between repeated measures of 0.8 and non-sphericity correction of 1.

After the blood flow was released, a significant difference was observed in TSI between the 30BFR and 50BFR ($p = 0.012$) and 30BFR and 100BFR ($p = 0.006$). The tHb showed significant difference between the 30BFR and 50BFR ($p = 0.037$), between 50BFR and 100BFR ($p = 0.002$), and between 80BFR and 100BFR ($p = 0.007$). In addition, a significant difference was observed in O_2Hb between the 30BFR and 100BFR ($p = 0.000$), 50BFR and 100BFR ($p = 0.000$) and between 80BFR and 100BFR ($p = 0.007$) conditions. Finally, HHb showed significant difference between 30BFR and 50BFR ($p = 0.032$), as well as between 30BFR and 80BFR ($p = 0.007$). Figure 2 shows the results of each dependent variable evaluated in this study. For comparisons within groups, there was a difference in O_2Hb between all conditions (baseline, blood flow restriction, and blood flow released), except for 100BFR (baseline vs. blood flow restriction; $p = 0.999$) and 30BFR (baseline vs. blood flow released; $p = 0.699$). For tHB there was a difference for all combinations, except 50BFR ($p = 0.991$) and 80BFR ($p = 0.995$) between baseline and blood flow released. In HHb conditions, only there was no difference between baseline and blood flow released to 30BFR ($p = 0.258$) and 100BFR ($p = 0.225$). Finally, there was a significant difference in all conditions over time to TSI.

The effect size varied from very small to huge in the most diverse combinations between groups, according to Sawilowsky's classification [25]. The following Tables I to III show the results of all effect sizes related to TSI, tHb, O_2Hb, and HHb. In Table I, it was possible to observe that the most significant clinical impacts between the measurements obtained during and after blood flow restriction occurred in the TSI and HHb measurements in all restriction conditions. In the tHB variable, the 100BFR condition had the lowest clinical impact, while in the O_2Hb variable, the 50BFR and 80BFR conditions had a huge effect.

Table II shows the clinical impact of the difference between groups during the period of blood flow restriction. The 100BFR condition had larger effect sizes than all other conditions for the variables. O_2Hb, tHb, and TSI, indicating that this condition is the one that generated the most significant impact on tHb and muscle oxygenation while the cuff was inflated. On the other hand, the impact of changes in tHb and muscle oxygenation between 30BFR and 50BFR were the smallest.

Table III shows the clinical impact of the difference between treatments after the period of blood flow restriction. All conditions of blood flow restrictions showed a large effect size between the TSI variable, indicating that each change in the restriction range causes a great clinical impact on muscle oxygenation. Muscle volume measured indirectly by tHb indicated a little clinical impact on the change observed between 50BFR and 80BFR, but the other changes at each restriction range change occurred with greater impact.



All values during BFR were statistically different from baseline in each pressure level. All values post-BFR were statistically different from during BFR in each pressure level. 1 = different from 30BFR; 2 = different from 50BFR; 3 = different from 80BFR. All differences for $p < 0.05$

Figure 2 - Oxyhemoglobin (O_2Hb), desoxyhemoglobin (HHb), total hemoglobin (tHB) and tissue saturation index (TSI) modifications from baseline at the different body flow restriction pressure levels (30%, 50% 80% and 100% BFR) during blood flow restriction (BFR) and after flow release (Post)

Table I - Effect size (ES) of the dependent variables TSI, O_2Hb, HHb e tHb for repeated measures (BFR vs Post-BFR) between treatments, based on the criteria proposed by Sawilowsky [25]

	TSI		O_2Hb		HHb		tHb	
	ES	Classif	ES	Classif	ES	Classif	ES	Classif
30BFR	-2.02	Huge	1.86	Very large	3.72	Huge	2.85	Huge
50BFR	-3.58	Huge	2.44	Huge	7.85	Huge	6.49	Huge
80BFR	-5.09	Huge	2.35	Huge	6.93	Huge	4.85	Huge
100BFR	-7.76	Huge	-1.67	Very large	4.56	Huge	0.94	Large

TSI = Tissue Saturation Index; O_2Hb = Oxyhemoglobin; HHb = Deoxyhemoglobin; tHb = Total Hemoglobin; Classif: classification

Table II - Effect size (ES) of the dependent variables TSI, O_2Hb, HHb and tHb for treatment comparisons during blood flow restriction, based on the criteria proposed by Sawilowsky [25]

	TSI		O_2Hb		HHb		tHb	
	ES	Classif	ES	Classif	ES	Classif	ES	Classif
30BFR-50BFR	0.26	Medium	0.06	Very small	-0.95	Large	-0.40	Small
30BFR-80BFR	0.36	Medium	-0.01	Very small	-0.99	Large	-0.46	Small
30BFR-100BFR	1.55	Very large	1.99	Very large	-0.30	Small	1.15	Large
50BFR-80BFR	0.01	Very small	-0.07	Very small	-0.14	Very small	-0.12	Very small
50BFR-100BFR	1.25	Very large	1.88	Very large	0.84	Large	1.90	Very large
80BFR-100BFR	2.05	Huge	1.93	Very large	0.91	Large	1.77	Very large

TSI = Tissue Saturation Index; O_2Hb = Oxyhemoglobin; HHb = Deoxyhemoglobin; tHb = Total Hemoglobin; Classif: classification

Table III - Effect size (ES) of the dependent variables TSI, O_2Hb, HHb and tHb for condition comparisons, after blood flow release, based on the criteria proposed by Sawilowsky [25

	TSI		O_2Hb		HHb		tHb	
	ES	Classif	ES	Classif	ES	Classif	ES	Classif
30BFR-50BFR	-1.85	Very large	0.86	Large	1.28	Very large	1.44	Large
30BFR-80BFR	-1.18	Large	0.57	Medium	1.62	Very large	1.18	Large
30BFR-100BFR	-2.26	Huge	-1.32	Very large	0.53	Medium	-0.75	Medium
50BFR-80BFR	0.65	Large	-0.37	Small	0.45	Small	-0.02	Very small
50BFR-100BFR	-0.79	Large	-2.37	Huge	-0.27	Small	-2.15	Huge
80BFR-100BFR	-1.31	Very large	-2.12	Huge	-0.55	Medium	-1.83	Very large

TSI = Tissue Saturation Index; O_2Hb = Oxyhemoglobin; HHb = Deoxyhemoglobin; tHb = Total Hemoglobin; Classif: classification

Discussion

This study showed that different levels of blood flow restriction in the upper limb do not promote linear changes in the percentage of tissue oxygenation and total hemoglobin. This finding agrees with previous studies that also identified that total hemoglobin reduction is not linear with pressure load.

The differential of the current study was, in addition to observing the behavior of total hemoglobin (indirectly), having followed the impact on cellular hypoxia.

Identifying non-linear behavior in cellular hypoxia is important because this seems to be a stimulus condition for muscle hypertrophy mechanisms [18].

The current results demonstrate that it is unnecessary to exert high-pressure loads to significantly impact hypoxia, allowing the participant to reach a possible hypertrophic stimulation with loads between 30 and 50% of total occlusion without experiencing the significant discomfort generated by heavier loads.

The main findings were as follows. Tissue saturation index (TSI) decreased under all conditions, significant for 100BFR compared to 80BFR during the blood flow restriction phase. After the release of blood flow, a significant increase was observed in all conditions, indicating a rebound effect, and for loads of 100BFR and 50BFR, those showed more significant effect than 30BFR. The tHb value is higher in moderate blood flow restriction loads, mainly 50BFR and 80BFR compared to more extreme pressure level (100BFR). The oxygenated hemoglobin increased significantly with submaximal pressure load. However, after the release of blood flow, O_2Hb augmented for 100BFR conditions while the other groups decreased. Furthermore, the muscle oxygenation returned to baseline condition for 30BFR. Finally, the deoxygenated hemoglobin was higher in medium and high blood pressure loads (50BFR and 80BFR) when compared to lower blood flow restriction loads (30BFR) after the release of the blood flow.

TSI is a direct indicator of the percentage of oxygenated hemoglobin in the tissue directly below the sensor. The present study observed a reduction in TSI concentration during inflated cuff, which indicates that the oxygenated blood supply is less than muscle demand. The uptake of muscle oxygen can be influenced, among other factors, by the ability of the microcirculation to provide the necessary oxygen to the tissue [19]. The reduction in TSI has already been observed in other studies of blood flow restriction associated or not with the practice of physical exercise [25,26].

Kilgas *et al.* [20] observed a significant reduction in TSI compared to the control condition in four different pressure loads (60%, 80%, 100%, and 120% of the AOP) associated with the handgrip exercise. The authors identified a more significant reduction in TSI as the pressure level increased, but with no difference between 60 and 80% (submaximal loads) and between 100 and 120% of the AOP (maximum and supra-maximum, respectively). Although the present study did not associate blood flow restriction with exercise, there was also a tendency to reduce the TSI as the pressure level increased, with no significant difference between submaximal loads (baseline> 30BFR = 50BFR = 80BFR > 100BFR). Both studies used a 10 cm wide cuff.

A hyperemic rebound effect allowed the TSI indicators to remain higher than the resting condition even after 30 seconds of withdrawal of the cuff pressure. This result is reinforced by the clinical difference observed through the effect size obtained in the multiple comparisons between the conditions in the present study. The effect size was considered very small to medium between the 30BFR, 50BFR, and 80BFR conditions, but very large to huge when these intermediates were compared to 100BFR. Thus, the 100BFR had a more significant impact on the TSI compared to the other conditions.

In practical terms, the similar lower oxygen saturation between the 30BFR, 50BFR, and 80BFR experimental treatments indicates that this flow restriction margin appears to have a similar impact on cellular hypoxia. Disregarding exercise, a restriction between 30 and 80% of the AOP could have a similar impact on the hypertrophic mechanisms associated with the more metabolic environment. Previous studies have shown that simple exposure to blood flow restriction without exercise can promote hypertrophic stimuli that would reduce the impact of atrophy caused by an injury to the muscle-tendon structure [13]. Thus, the lower pressure level (30%) may be more comfortable and safer for most people, particularly older and untrained individuals, providing similar benefits to an 80% arterial pressure level restriction. On the other hand, higher pressures that allow total or close to AOP would probably promote a higher hypoxic ambient despite being more uncomfortable. Such a more favorable milieu would potentiate mechanisms such as cell swelling [10] and the release of growth hormone (GH) [11].

Although Hunt *et al.* [27] have observed that the deformation of the brachial artery occurs at approximately 110 mmHg of pressure with the use of an 11 cm wide cuff, the reduction in blood flow occurs early in order to change the arterial diameter. On average, men experienced a reduction in blood flow with 60 mmHg.

In another study by Mouser *et al.* [17], the venous system was impacted with pressure loads of 10 to 30% of AOP. Notwithstanding, the artery would only be impacted with pressure loads higher than 60%. This study was carried out with a 5 cm cuff, half the width of the cuff in the present study. The literature has shown that cuffs with a smaller width require a higher-pressure level to cause a similar impact to a broader cuff [28].

The present study observed an increase in tHB in all pressures used in relation to the baseline and being more significant in the conditions 50BFR and 80BFR compared to 100BFR (baseline> 30 BFR = 100BFR > 50BFR = 80BFR). When considering the results of these previous studies with the current observations, the 50BFR condition was performed with an average pressure level of 68 mmHg. It is possible to assume that 50BFR and 80BFR must have interfered equally in the blood flow, as noted by the small effect size between these conditions. This assumption is supported by another study by Mouser *et al.* [16]. The authors identified blood flow reduction up to 50% of arterial occlusion, followed by stabilizing the flow up to 90% before another sudden drop. This abrupt reduction in blood flow in the last 10% before reaching the point of AOP should explain why 100BFR had a lower tHb than intermediate pressure loads (50BFR and 80BFR). After the cuff deflated, blood flow observed by the tHb concentration returned to rest at 50BFR and 80BFR, but not at 30BFR and 100BFR.

The concentration of HHb is an important indicator of oxidative metabolism in muscles [29]. The increase in the concentration of HHb in all restriction conditions performed in the present study indicates the hypoxia generated by the mechanical restriction. This result agrees with what was observed in a previous study [30]. Although there was no difference between groups during the restriction phase, the effect size indicated that pressure levels between 50BFR and 80BFR had a more significant clinical impact concerning all conditions and with very little practical difference between them. Furthermore, all groups had a substantial clinical difference and statistically significantly higher HHb values than baseline.

The significance of this finding is that hypoxia is an important signal to stimulate some mechanisms of strength and muscle mass increase [11,31]. Thus, it is possible to assume that, at least at rest, the 30BFR level has the same impact on hypoxia and muscle oxidative metabolism as on higher pressure levels, thus reducing discomfort and cardiovascular risk. On the other hand, after blood flow release, HHb values reduced in all groups, but only at 30BFR and 100BFR did the levels return to baseline condition after 30 seconds.

Finally, a more pronounced increase in O_2Hb levels in the 30BFR, 50BFR and 80BFR than the 100BFR condition was observed during the cuff inflation phase. The very high clinical impact between the 100BFR and the other pressure levels confirmed this distinct behavior between maximum and submaximal pressure loads. On the other hand, observing a minimal effect size between submaximal loads demonstrated that the clinical implications generated with an arterial restriction level of 30 to 80% of the AOP at rest are practically insignificant.

The O_2Hb concentration during blood flow restriction in the 100BFR condition was similar to the baseline. Besides, a reduction in O_2Hb concentrations in the 30BFR, 50BFR, and 80BFR conditions was observed when the cuff pressure was released. Nevertheless, these values did not return to the baseline condition within 30 seconds of free blood flow. On the other hand, in the 100BFR condition, the O_2Hb concentration increased, indicating a possible rebound effect due to the action of some vasodilating substances, such as nitric oxide [32]. After the flow is released, blood moves more turbulently, increasing shear stress stimulating the production and release of nitric oxide, promoting local vasodilation [33,34]. Shear stress is influenced by blood flow speed, which is altered according to the pressure imposed by the cuff and blood flow release by removing the pressure exerted by the cuff [35]. A higher concentration of O_2Hb accompanies this increase in blood flow.

These results are opposite with those observed in previous studies. Such studies observed a reduction in O_2Hb during blood flow restriction [36], possibly due to the difference in the site of signal capture between the studies. The difference observed in the results during the blood restriction phase may be explained by the positioning of the NIRS probe about the site of compression exerted by the cuff. Bopp *et al.* [36] positioned the probe on the subject's forearm immediately after the cuff, restricting the blood, while in the present study, the NIRS probe was placed on the arm, and the cuff was placed on the forearm. This procedure was done to avoid interference in the vascular walls due to deformation by the inflated cuff.

The NIRS device captures hemoglobin concentrations (oxy and deoxygeated) to a depth of 1.5 cm below the transmitter/receiver. Thus, the relative O_2Hb concentration is measured in the small blood vessels (arterioles, venules, and capillaries) that cross this region captured by the equipment. In the 100BFR condition, the blood flow must have been interrupted or close to it, even in the deepest regions, and it must have kept the O_2Hb concentration in the arteries located before the inflated cuff. The equipment should not have picked up the blood in the most profound vessels.

Some limitations do apply to the present study. We implemented BFR during rest, and different muscle hemodynamic behaviors may be expected during resistance exercise. Besides, our findings are limited to the upper limbs and may not entirely represent blood flow restriction involving a larger muscle mass. Therefore, further studies are needed to confirm a possible relationship between acute muscle hemodynamics caused by different blood flow restriction pressure levels and hypertrophic markers secondary to resistance exercise with blood flow restriction.

Conclusion

In conclusion, this study revealed that pressure levels between 30 and 50% of the brachial artery blood flow occlusion are sufficient to cause hypoxia in the occluded muscle, in the same magnitude as higher pressure loads (up to 80%).

Conflict of interest
All authors declare that there is no conflict of interest regarding this study and manuscript.

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Author´s contributions
Conception of the study: Gomes PSC, Meirelles CM; **Study design:** Carvalho RF, Gomes PSC, Fernandes Junior ML, Meirelles CM; **Data collection:** Carvalho RF, Fernandes Junior ML; **Statistical analysis:** Carvalho RF, Gomes PSC; **Writing of the document:** Carvalho RF, Gomes PSC, Fernandes Junior ML, Meirelles CM; **Final review of the manuscript:** Meirelles CM; **Writing the English version of the manuscript:** Gomes PSC.

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