Sports Science

Local muscle oxygenation during different cuff-pressures intervention: a punctual near-infrared spectroscopy measurement

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Abstract - Aim: To verify the response of tissue saturation index (TSI) during ischemia-reperfusion (IR) interventions with different cuff-pressures. **Methods:** Twenty-nine healthy men experienced in resistance training were recruited. Each one has undergone a control condition (no cuff) and one of the three IR interventions: 1) 190 mmHg (CP-190, 22.7 ± 3.0 years; 176.6 ± 3.9 cm; 77.3 ± 9.5 kg; 2) 100 mmHg (CP-100, 22.9 ± 6.3 years; 180.5 ± 4.0 cm; 85.2 ± 14.1 kg) and 3) 20 mmHg (CP-20, 20.3 ± 2.4 years; 171.8 ± 5.2 cm; 72.4 ± 6.0 kg). Cuffs were placed on the proximal region of the thighs. IR interventions consisted of three cycles of 2-min occlusion-reperfusion. TSI was measured using near-infrared spectroscopy (NIRS), positioned on the middle portion of the vastus lateralis of the dominant leg. The oxygenation was measured at the control conditions (no cuff) and during cuff interventions. **Results:** While TSI values of CP-20 did not change compared to control ($p > 0.05$), the TSI in CP-190 was lower in the ischemia ($p < 0.05$), and CP-100 was lower in the second and third ischemia ($p < 0.05$). However, the TSI value increased during reperfusion but did not return to control levels ($p < 0.05$). **Conclusion:** TSI of the CP-190 significantly decreased during ischemia. However, these values increased by about 16% in the reperfusion period. Thus, our results show that the RI intervention may have caused an increase in metabolic demand, as even with the release of blood flow, the TSI values were below those of the other interventions.

Keywords: local muscle oxygenation, ischemia, reperfusion, near-infrared spectroscopy.

Introduction

Brief cycles of ischemia and reperfusion usually performed before or immediately after exercise (Ischemic-reperfusion interventions - IR) have been shown positive ergogenic effects for enhancements in sports performance and muscle recovery[1,2]. The IR is characterized as an easy low-cost and non-invasive ergogenic strategy, which has aroused the interest of sports science researchers[3]. The bouts of ischemia and reperfusion are performed by a cuff placed on the proximal region of the lower or upper limbs[2].

Although the term IR was investigated by Murry and colleagues in 1986, in a clinical trial that investigated the influence of brief cycles of IR in the heart of dogs, showing a protective effect by reducing the infarct area[4]. However, contrary to what is believed, episodes of hyperemia were reported in the 50s by Nukada[5]. This study investigated the ability of muscle performance in reactive hyperemia of muscles. The search for strategies that promote increasing sports performance is something frequently in the field of performance. In this context, in 1998 the IR migrated to the area of sports performance, investigating the influence of the maneuver on muscle performance[6]. The main result showed that muscle performance increased when the IR was applied before exercise. In the last seven years, the number of studies on IR and physical performance increased ~85%.

Whilst studies have reported beneficial IR effects related to sports or exercise performance, physiological mechanisms remain unclear. Some hypotheses are due to changes in blood flow during the reperfusion phase and releasing of endothelial components such as nitric oxide, adenosine, and other dilating factors[7]. This physiological event caused by IR induces a more supply of energy substrates, increased local oxygenation, and clearance of metabolites in the blood, resulting in more energy production during exercise, thus contributing to more performance and acceleration of recovery of muscle fatigue[8].

In this way, it is possible to verify whether the increase in muscle oxygenation is following the hypothesis suggested in the literature during the reperfusion of IR

interventions, as it is believed that the maneuver increases blood flow and promotes vasodilation during, improving exercise conditions. in this way, it can enrich the literature once one of the mechanisms caused by the strategy is confirmed, thus being able to help in the construction of future studies in the area[9].

Thus, this study aimed to verify the response of TSI during ischemia-reperfusion interventions with different cuff-pressures. We hypothesize that during the reperfusion phase TSI would increase.

Methods

Subjects

Using the G*Power software (version 3.1.9.6), the number of participants was estimated a priori, based on a statistical power of 0.97, assuming: $\alpha = 0.05$, effect size (ES) = 0.05, f = 0 .4, Test family = F tests, statistical test = ANOVA: repeated measures, within- between interaction, the total sample size was twenty-one subjects. Thus, twenty-nine healthy men with experience in resistance training volunteered in this study (Table 1). Participants should have been training resistance exercises for at least six months.

The non-inclusion criteria were a) any cardiovascular or metabolic diseases; b) use of exogenous drugs, such as anabolic androgen steroids or any potential substance that could influence vascular functions (self-report). All subjects abstained from alcohol and caffeine consumption 24 h prior to data collection. This study was previously approved by the research ethics local committee for experiments on humans of Universidade Federal de Juiz de Fora (n. 4.120.625), written consent was obtained, and procedures were followed in accordance with the Declaration of Helsinki.

Experimental design

This study is an experimental, counterbalanced, placebo-controlled and single-blind trial (i.e., the researcher did not know which intervention each subject would receive). Each participant visited the laboratory three times (minimum interval of 48 h). On the 1st visit: the volunteers answered some questions about their physical fitness and anthropometric measurements were taken (i.e., weight, height, skinfold thickness), identification of anatomical points for placement of the NIRS device, verification of arterial occlusion pressure, familiarization of all equipment with the device and an isometric exercise protocol was performed in an extensor chair of 8x maximum voluntary isometric contraction 20 s duration alternating with 10 s rest. The purpose of this isometric exercise protocol was to allocate individuals into three different groups by relative force (force/body mass). The groups were cuff-pressures 190 mmHg (CP-190), cuff-pressures 100 mmHg (CP-100), and cuff-pressures 20 mmHg (CP-20) (Figure 1).

On 2nd visit, the TSI was measured during the maneuvers or the control condition (non-cuff) the order was randomized. At the laboratory, the individual remained at rest for 5 min. Following this, the NIRS device was placed in the vastus lateralis for measuring local oxygenation during the maneuver or control condition. The order of interventions was defined by randomization. The 3rd visit was conducted in the same way as the second, alternating the order of intervention received on the second visit. All tests were performed at the same time to try to reduce some influences on the circadian cycle.

Table 1 - Sample characterization.

Characteristics	CP-20 (n = 9)	CP-100 (n = 10)	CP-190 (n = 10)	P value
Age (years)	22.7±3.0	22.9±6.3	20.3±2.4	0.085
Height (cm)	176.6±3.9	180.5±4.0	171.8±5.2	0.001*
Body mass (kg)	77.3±9.5	85.2±14.1	72.4±5.2	0.054
IOP (mmHg)	167.2±20.1	177.0±33.7	169.5±22.7	0.641
ST (mm)	10.7±5.1	14.5±6.6	12.6±4.6	0.412

Legend: CP-20: cuff-pressures of 20 mmHg; CP-100: cuff-pressures of 100 mmHg; CP-190: cuff-pressures of 190 mmHg; IOP, Individual occlusion pressure; ST: Skinfold thickness. Values are expressed as mean ± standard deviation.

Cuff maneuvers and control condition

The maneuvers were performed using a pneumatic cuff (Riester®, Germany – 96 cm length x 13 cm width) placed in the sub-inguinal region of both thighs. The protocol adopted for each group was three cycles of 2 min of ischemia for 2 min of reperfusion[10], varying the occlusion pressure for each group as described above. The CP-190 groups used a cuff-pressures of 190 mmHg (20 mmHg above the individual occlusion pressure)[11], the CP-20 used a cuff-pressures of only 20 mmHg (pseudo-maneuver)[12], and the CP-100 used a cuff-pressures of 100 mmHg. The occlusion pressure was checked using Doppler (MedPeg® DV-2001, Ribeirão Preto, Brazil). In the control condition (non-cuff), the participant remained at rest in the supine position for the same time as the sums of the IR intervention (12 min).

Near-infrared spectroscopy (NIRS)

TSI was assessed by near-infrared spectroscopy (NIRS; Moxy, Fortiori Design LLC, Hutchinson, USA). The device was placed on the skin according to the adapted protocol[13]. To mark anatomical points on the skin a specific pen was used (Freehand Skin Marker Pen®). The volunteers were instructed to preserve the marked points to maintain a pattern of device placement at the three visits. Before the NIRS fixation, was make tricometry, and cleaned the area using 70% alcohol and cotton. The NIRS device was placed exactly on the vastus lateralis muscle of

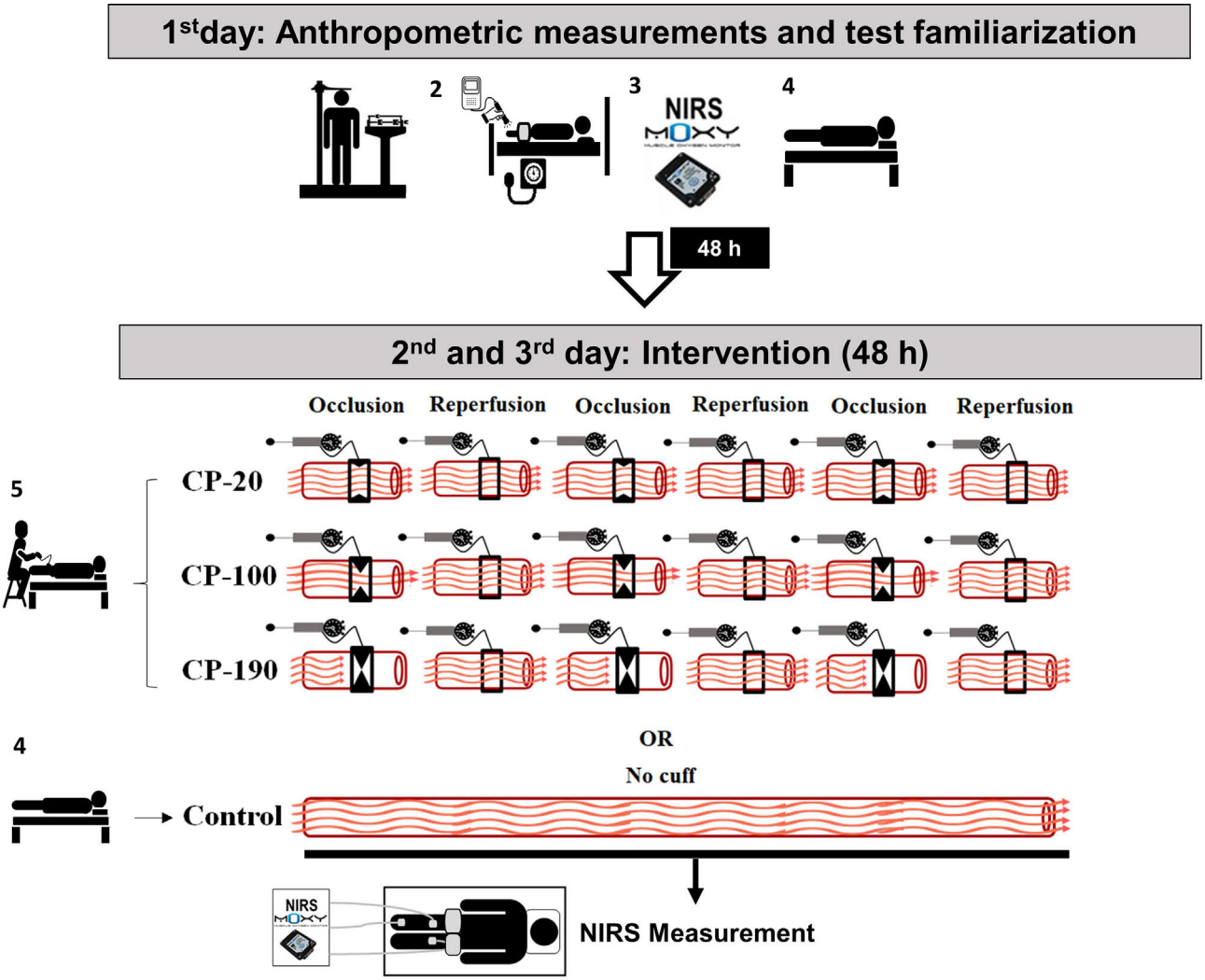

Figure 1 - Experimental Design. CP-190, cuff-pressures of 190 mmHg; CP-100, cuff-pressures of 100 mmHg; CP-20, cuff-pressures of 20 mmHg; 1, Anthropometric measurements; 2, Arterial occlusion pressure check; 3, NIRS device placement; 4, Control condition (no cuff); 5, Maneuvers.

the dominant leg between the midpoint of the Trochanter Major with Lateral Epicondyle, Inguinal Fold with the Patella identified through palpatory anatomy (Figure 2).

The device unit was inserted in a specific black silicone cover capable of isolating interference from external light (i.e., light ambient), to attach the cover to the skin, we used adhesive tape. In addition to the standardized location, to ensure reproducibility, before collecting data, the average of the values recorded for one minute was used so that we would have a value always close to the three interventions since the variations in the measures are frequent. The sample frequency acquisition of the NIRS device is 0.5 Hz[14].

Statistical analysis

The Shapiro-Wilk test was used to verify data normality. To verify the characteristics of the participants, Kruskal-Wallis was conducted on non-normal data, and One Way Anova was performed on normal data. To analyze the TSI between maneuvers, and within groups, was used a mixed two-way ANOVA, followed by Bonferroni's post hoc test. The significance level of $p < 0.05$ was adopted. The GraphPad program (PRISM®, 6.0, San Diego, CA, USA) was used for data analysis. Data were presented as mean ± standard deviation.

To verify the accuracy of the protocol used to measure TSI has calculated the coefficient of variation (CV) of oxygenation was collected during familiarization and control (no cuff on both days). The CV was calculated with the equation (SD/mean*100)[13,14]. According to Tew et al., CV of < 10%, 10-25%, and > 25% were considered good, moderate, and poor, respectively[15]. The TSI presented the following results: CP-190, CV = 7.2%; CP-20, CV = 6.0% and CP-100, CV = 9.8%.

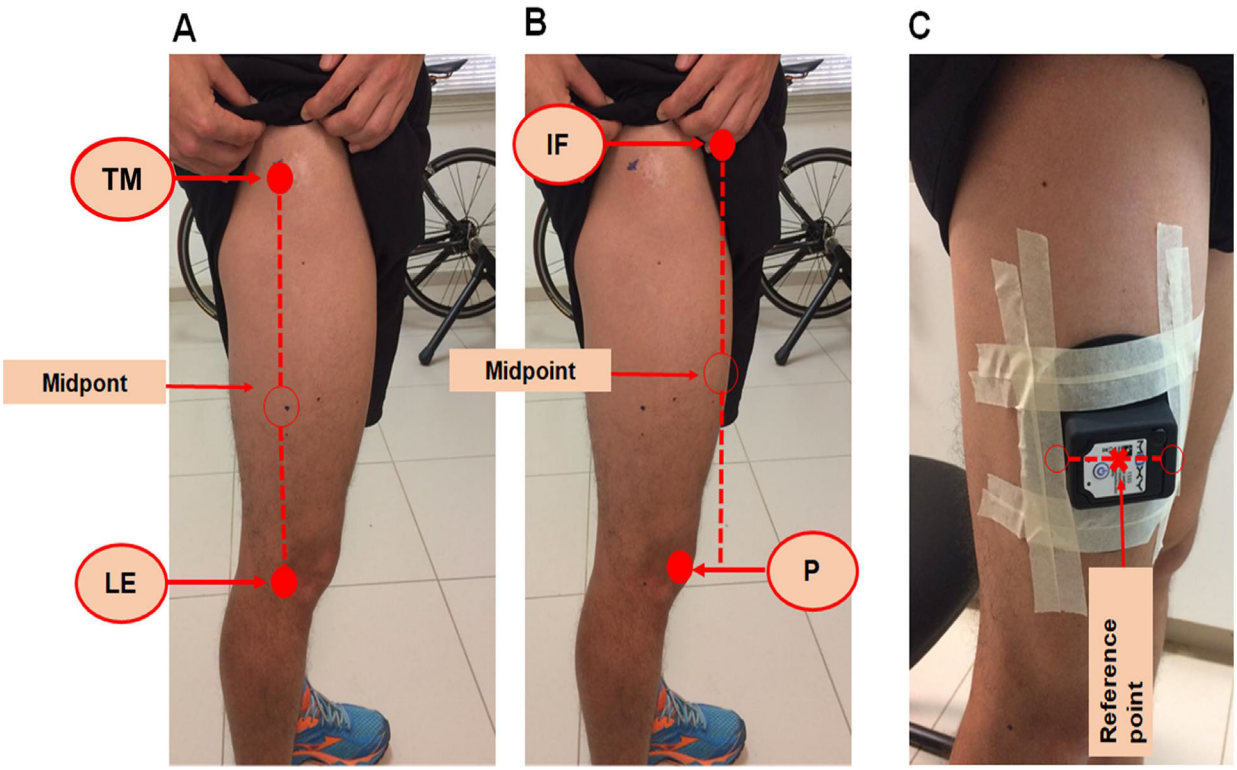

Figure 2 - Place where NIRS was insertions. TM, Trochanter Major; LE, Lateral Epicondyle; IF, Inguinal Fold; P, patella.

Results

TSI during the maneuvers

Within groups, CP-190 showed lower values in all three ischemia (I) compared to reperfusion (R) cycles (1st I versus R = ~ 18.2%; 50.7 ± 14.9 versus 68, 9 ± 5.1, p = 0.001; 2nd I versus R = ~ 13.6%, 54.2 ± 15.5 versus 67.8 ± 7.0, p = 0.01; and 3rd I versus R = ~17 .2%, 57.1 ± 13.9 versus 67.3 ± 4.9, p = 0.015). No differences were found for CP-20 and CP-100 for the same parameter (p > 0.005; Figure 3B).

Regarding interventions, TSI showed also lower values in CP-190 compared to CP-20 during I cycles (1st I: ~ 28.3%, 47.0 ± 8.9 versus 75.3 ± 12.3, p = 0.001; 2nd I: ~ 21.6%, 55.8 ± 10.1 versus 77.4 ± 9.6, p = 0.001; 3rd I: ~ 18.7%, 57.6 ± 12.6 versus 76.3 ± 10.1, p = 0.002) and R (1st R: ~ 11.3%, 66.9 ± 7.1 versus 78.2 ± 9.4, p = 0.003; 2nd R: ~ 9.8%, 67.5 ± 8.0 versus 77.3 ± 9.6, p = 0.024; 3rd R: ~ 13.2%, 65.0 ± 6.7 versus 78.2 ± 7.9, p = 0.002). CP-20 versus CP-100 showed a significant difference in TSI only in the 2nd I (~ 12.5%, 77.4 ± 9.6 versus 64.9 ± 13.6, p = 0.026), with no difference in the other ischemia and reperfusion cycles (Figure 3B).

TSI during control condition versus maneuvers

The TSI values in the control condition of the CP-190 group remained higher in relation to the TSI values when applied to the maneuver with 190 mmHg (1st I: ~ 33.7%, 80.7 ± 5.4 versus 47, 0 ± 8.9, p < 0.001; 1st R = ~ 14.7%, 81.6 ± 6.1 versus 66.9 ± 7.1, p < 0.001; 2nd I = ~ 27.6%, 81, 4 ± 6.4 versus 53.8 ± 10.1, p = 0.001; 2nd R = ~ 12.6%, 80.1 ± 7.3 versus 67.5 ± 8.0, p = 0.018; 3rd I = ~ 19.8%, 77.4 ± 9.8 versus 57.6 ± 12.6, p = 0.004 and 3rd R = ~ 13.5%, 78.5 ± 9.0 versus 65.0 ± 6.7, p = 0.005; Figure 4C).

TSI values in the control condition of the CP-100 group remained higher from the second ischemia and reperfusion concerning the values when applied in the maneuver: (2nd I = ~ 19.2%, 84.1 ± 9.5 versus 64.9 ± 13.6, p = 0.001; 2nd R = ~ 11.7%, 84.0 ± 10.2 versus 72.3 ± 9.5, p = 0.018, 3rd I = ~ 16.8%, 83.6 ± 10.3 versus 66.8 ± 12.4, p = 0.004; and 3rd R = ~11.6, 83.4 ± 9.3 versus 71.8 ± 10.7, p = 0.005; Figure 4B). The CP-20 showed no difference in TSI in relation to the control and maneuver condition in the ischemia and reperfusion cycles p > 0.05 (Figure 4A).

Discussion

The present study aimed to evaluate the influence of CP-190, CP-100, and CP-20 on the response of TSI during ischemia and reperfusion interventions. Previous studies have shown that IR increased muscle performance[6] in different modalities: swimming[16], cycling[17], running[18] and resistance training[19], in addition

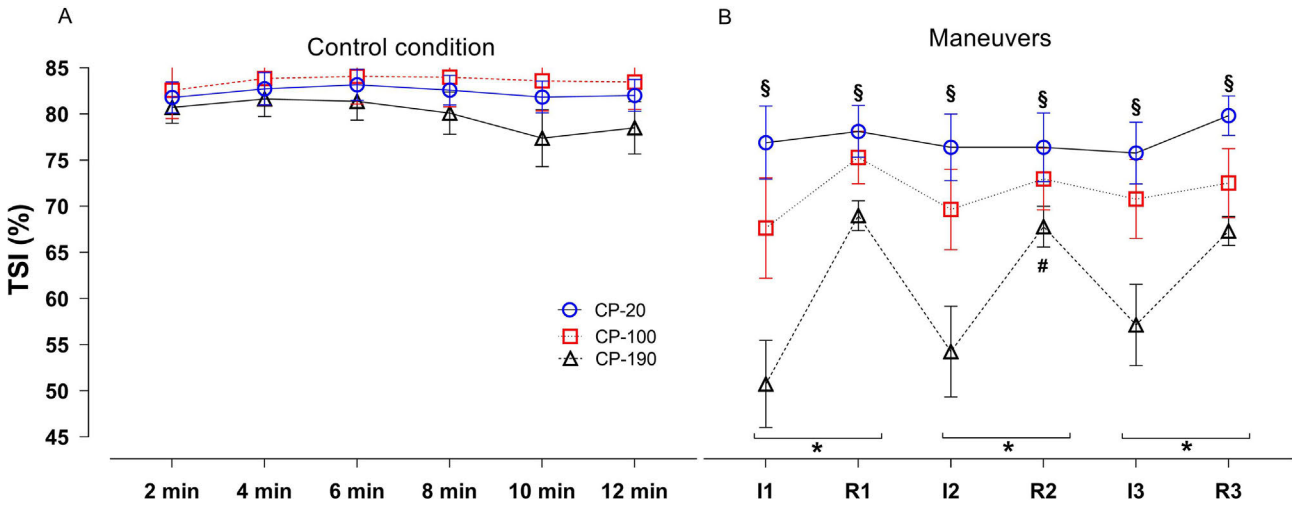

Figure 3 - (A) Graphic of TSI in the control condition (no cuff). (B) Graphic of TSI during the ischemia and reperfusion cycles of the maneuvers and TSI, tissue saturation index; CP-190, cuff-pressures of 190 mmHg; CP-100, cuff-pressures of 100 mmHg; CP-20, cuff-pressures of 20 mmHg; I, Ischemia; R, Reperfusion. Data express in mean ± SD. Difference in comparison between I and R within CP-190 represent by⋆. Difference in comparison between CP-190 and CP-20 represent by §. Difference in comparison between CP-20 and CP-100 represent by #.

to evidence concerning recovery optimization[20]. Under the premise of humoral mechanisms, the reestablishment of blood flow induces the vasodilation of the vessels[9]. Thus, the IR would stimulate a greater delivery of energy substrates and oxygen to the muscles[9]. Therefore, this vascular effect is associated with performance optimization[3].

The expectation was that during blood flow release, the release of nitric oxide caused by shear stress would cause sufficient vasodilation to increase local blood volume and, therefore, oxygen[7]. Our findings showed that TSI decreased considerably in ischemia and increased in the period of reperfusion for CP-20 and CP-10 However, for the high-pressure cuff intervention CP-190, TSI values remained lower, even during the reperfusion phase. A possible explanation could be due to less oxygen support during ischemia, reflecting in the increase in mitochondrial metabolic activity consuming more oxygen (i.e., higher metabolic demand)[21], which justifies the lower values in reperfusion concerning the control conditions. Such alterations caused by the maneuver imply in preparation of the organism for the exercise since the maneuver increases the metabolic demand.

The choice of a protocol using 100 mmHg was due to the hypothesis that this pressure reduces arterial blood flow and blocks venous return, thus allowing blood to enter the muscle and not exit, and when deflating the tourniquet, there is also greater vasodilation during reperfusion[22]. Being able, perhaps, to demonstrate different O_2 responses in the occlusion period concerning the reperfusion period. From another perspective, offering less pressure on the cuff can decrease the pain caused by higher pressures. However, it was observed that except for the first ischemia and reperfusion, the group that received the CP-100 presented lower values of O_2 in comparing the control situation. This result may also suggest a greater mitochondrial activity as well as in CP-190.

A study evaluated the oxygenation during the IR protocol as well as in the exercise, to found that during ischemia the local oxygen level decreased significantly, and during five minutes of reperfusion the values were back to normal levels[23]. Such findings partially confirm our results. However, the study used a longer reperfusion time compared to the present study, which may have influenced the return of oxygenation to normal values. While the aim was not to compare oxygen levels between occlusion and reperfusion, another study evaluated TSI during IR intervention finding that the application of strategy did not influence oxygenation levels during exercise[24]. However, Patterson et al. showed[17] that TSI decreases significantly during the period of occlusion, corroborating our findings and the oxygenation remained higher during exercise concerning placebo, this study does not use a control condition (without a tourniquet), which perhaps cannot isolate a placebo effect.

Although our study contradicts the hypothesis that the increase in TSI could be due to increased vasodilation caused by reperfusion, it is up to future trials to investigate the oxygenation responses from the perspective of other protocols since the ideal protocol is still unknown. Studies in the literature present different protocols with variation in the time of ischemia and reperfusion sessions (cycles of 1[25] to 5[26] times with a time of 2[25] to 5[26] min or occlusion time 2 min to 30 min), tourniquet size (how much the larger the contact area, the smaller the occlusion pressure), pressures used both in the occlusion group

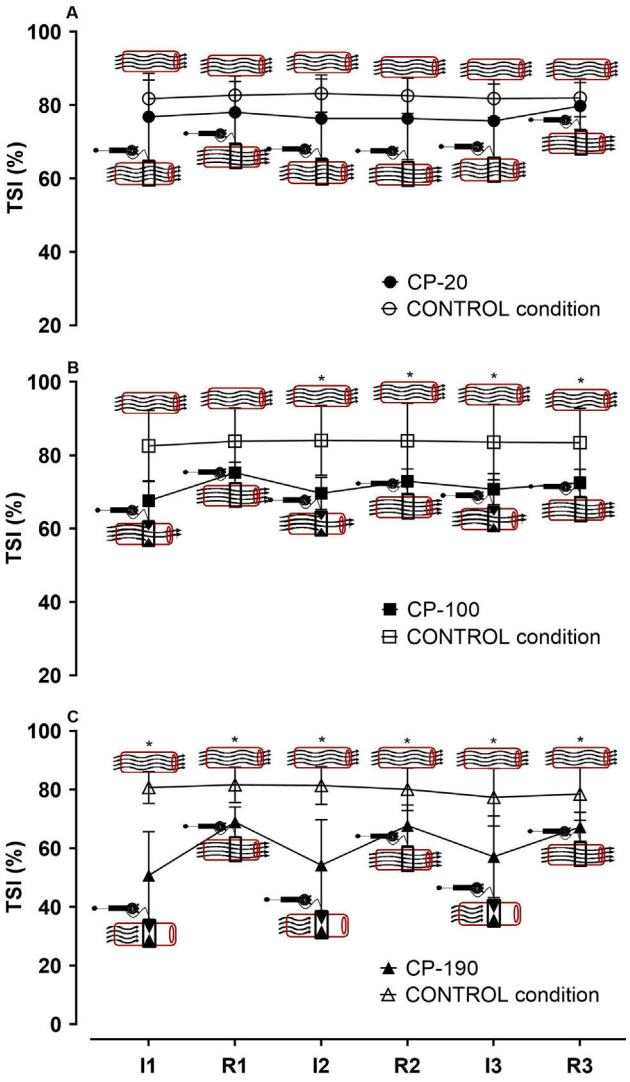

Figure 4 - (A) Graphic of TSI comparing control condition with the maneuver. TSI, local muscle oxygenation; CP-20, cuff-pressures of 20 mmHg; control condition, no cuff. (B) Graphic of TSI comparing control condition with the maneuver. TSI, tissue saturation index; CP-100. cuff-pressures of 100 mmHg; control condition, no cuff. (C) Graphic of TSI comparing control condition with the maneuver. TSI, local muscle oxygenation; CP-190. cuff-pressures of 190 mmHg; control condition, no cuff. Difference in comparison between the control condition and maneuver represent by⋆.

(individual occlusion pressure[27] to 300 mmHg[21]) and in the pseudo-maneuver - SHAM (10 mmHg[26] to 100 mmHg). Still, there is no evidence of better cuff-pressures used to cause increased blood flow and improved performance.

In this study, we allocated the subjects so that the groups were more homogeneous concerning the relative strength of the isometric chair. However, it is necessary to emphasize that even in homogeneous groups, could happen differences in the volunteer responses. However, it is worth mentioning that the measures presented a low percentage of variation

Conclusions

In conclusion, the TSI of the CP-190 group significantly decreased during ischemia, remaining lower during the reperfusion phase, which did not occur in other CP-20 and CP-100 interventions. Thus, our results show that the IR intervention may have caused an increase in metabolic demand, as even with the release of blood flow, the TSI values were below those of the other interventions.

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