HEALTH SCIENCES

Strength training with and without arteriovenous blood flow restriction improves performance, regardless of changes in muscle hypertrophy, in Wistar rats

NÁDIA F. GARCIA, CAMILA DE MORAES, MACÁRIO A. REBELO, SAVANAH MARIA
G.R. PETERS, FÁBIO M.P. DE CASTRO & ENRICO F. PUGGINA

Abstract: Strength training (ST) with blood flow restriction (BFR) is known to promote increases in hypertrophy and strength sometimes similar to traditional ST despite the effects of the arterial BFR on muscle adaptations and safety are not well established. The aim of this study was to assess whether ST with arterial BFR is able to improve muscular adaptations, performance and its safety in Wistar rats. Animals aging 8 weeks were divided in four groups: sedentary sham (S/S), sedentary with arterial BFR (S/BFR), trained sham (T/S), and trained with arterial BFR (T/BFR). Training protocol consisted of four weeks of ST composed by six sets of 10 ladder climbing with 50% of 1 maximal voluntary contraction. Body weight, epididymal fat, maximum loaded weight, manual grip strength, muscular hypertrophy index, systolic blood pressure, enzyme activity of superoxide dismutase, nitrite/nitrate concentration and tumor necrosis factor alpha were analyzed. The BFR rate was between 36% and 38%. T/BRF was effective to promote strength and hypertrophy. T/S is an alternative to improve strength, but it did not promote hypertrophy. Furthermore, we found no significant cardiac and metabolic changes. Thus, T/BFR is able to improve muscle adaptations and performance in rats, without causing cardiovascular and metabolic damage.

Key words: blood flow restriction, strength, hypertrophy, rats, strength training, safety.

INTRODUCTION

Blood flow restriction (BFR) exercise is a method which has been studied exhaustively in the last 20 years and consists of applying pressure through an external device, such as a tourniquet or blood pressure cuff around the limbs, to promote blood pooling distally from the occlusion point while performing exercise (Slysz et al. 2016). Although BFR alone can attenuate disuse atrophy, it is normally employed with some exercise stimulus for enhanced muscular development (Scott et al. 2015). The advantage of this training method is the use of strength exercises performed at relatively low loads

(20% - 50% of 1 maximal voluntary contraction (MVC)) (Grønfeldt et al. 2020, Lixandrão et al. 2018, Wernbom & Aagard 2020). It has been demonstrated that low intensity exercise with BFR exhibit greater increases in muscle mass and strength than the low intensity exercise alone (Slysz et al. 2016) but controversial results about strength when compared to high load strength training (ST) have been reported with similar (Grønfeldt et al. 2020) or lower increases in strength despite similar hypertrophy (Lixandrão et al. 2018). Nevertheless, because high load ST is contraindicated for certain individuals, such as the elderly, persons with chronic disease,

or rehabilitating and recovering athletes, low load ST with BFR is suggested as an interesting possibility (Slysz et al. 2016).

It is known that the adaptations from BFR training are dependent on both BFR stimulus itself, which is accepted to be high enough to occlude venous return from the muscles but low enough to maintain arterial inflow into the muscle, and the exercise protocol performed (Scott et al. 2015). Recently, it has been suggested that the pressure employed in the BFR training be based on the arterial occlusion pressure (AOP) (Scott et al. 2015, Patterson et al. 2019). However, there is evidence that the blood flow response between upper (Mouser et al. 2017) and lower limbs (Mouser et al. 2018) to various relative pressures is different. While the blood flow in posterior tibial artery decreases in a linear manner across increasing pressures with many participants still having blood flow through that artery at 100% of their pretesting AOP (Mouser et al. 2018), the application of different pressures decreased the brachial blood flow in a nonlinear, stepped fashion with the blood flow decreased at 10% AOP and remained constant until decreasing again at 40%, where it remained until 90% AOP (Mouser et al. 2017). Thus, this different response between lower and upper limbs may influence the adaptations from training.

A study comparing muscular adaptations between low (40% AOP) and high (90% AOP) pressure did not find differences between conditions in elbow flexors (Counts et al. 2016). These results are totally in accordance and can be easily explained by the data mentioned above. Nevertheless, and in the authors´ knowledge, no similar study has been conducted on lower limbs and the effects of a high degree of arterial occlusion on adaptations from training is unknown.

It is recommended that limbs with larger circumferences be occluded using wide cuffs because the larger the limb circumference, the higher is the pressure required (Scott et al. 2015). However, wide cuffs may produce discomfort or pain as well as reduced exercise performance (number of repetitions) compared to narrow cuffs for the same relative (Spitz et al. 2019) or absolute pressure (Rossow et al. 2012). Likewise, the pressure employed affect both the feelings from BFR exercise (Mattocks et al. 2017, Soligon et al. 2018, Spitz et al. 2019) and performed repetitions (Mattocks et al. 2017, Spitz et al. 2019) with high pressures showing the worse outcomes.

Although there is no conclusive dose-response relationship between volume and adaptations for BFR training, it is suggested that 75 repetitions, across four sets (30, 15, 15, 15) or 3-5 sets to concentric failure promotes an efficient volume to lead to adaptations in most people (Patterson et al. 2019). Taken together, because of the requirement of a high pressure or wide cuffs to cause arterial BFR on lower limbs and the negative impacts of these factors on exercise performance, the total exercise volume cannot be reached, as shown by Rossow et al. (2012) and in some trials carried in our lab with 50% AOP (data not published). Because these difficulties, we opted for an arterial BFR in animal model, which was employed previously (Lloyd et al. 2001) to study the effects of arterial ischemic training on muscle adaptations. Thus, the aim of the present study was to assess whether ST with arterial BFR is able to improve muscular adaptations in the hindlimbs and the performance in strength tests in Wistar rats.

As an additional goal of this study, cardiovascular (blood pressure (BP) and nitrite/nitrate concentration) and inflammatory (superoxide dismutase (SOD) and tumor necrosis factor alpha (TNF-α) parameters were analyzed.

Despite evidences suggesting that BFR training is safe (Patterson et al. 2019), doubts about its safety, mainly on vascular health, remain (da Cunha Nascimento et al. 2020).

MATERIALS AND METHODS

Animals

All procedures were reviewed and approved by the Ethics Committee on Animal Use in Research (CEUA/EEFERP protocol number 2016.5.80.90.4) in compliance with the "Principles of laboratory animal care" (NIH publication Nº 86-23, revised 1985) and the national law (CONCEA publication Nº 11.794, 2008).

Male wistar Hannover rats (8 weeks old, weighting 250 - 270g) were divided into four groups: sedentary sham (S/S (16)), sedentary with BFR (S/BFR (12)), trained sham (T/S(12)), and trained with BFR (T/BFR(12)). The animals were housed in collective cages (3 or 4 animals/cage), and kept under a 12h light/dark cycle and controlled temperature (22°C to 24°C). All animals had free access to standard chow (Nuvilab, Quimtia) and drinking water (tap water). The body weight (BW) was measured weekly, from the beginning to the end of the study.

Arteriovenous restriction surgery

Surgery for arteriovenous occlusion was performed as previously described (Lloyd et al. 2001) in animals in the S/BFR and T/BFR groups. The group of sham animals (S/S and T/S) underwent the same procedure, but was not occluded. The purpose of the surgery was to restrict arterial flow and venous return in the segment (right rear leg). Therefore, the animals were fasted for 12 hours, had the inguinal region sterilized with iodine and shaved to remove hair. A pre-surgery measurement of blood flow in the right rear leg was also performed using a non-invasive blood flow meter (Laser Doppler Blood FlowMeter, ADInstruments) coupled with a signal amplifier (PowerLab 400™, ADInstruments, Australia). Subsequently, the animals were anesthetized with 2,2,2 - 97% tribromoethanol (Sigma-Aldrich Co. LLC, USA), 1 mg/kg of animal intraperitoneally.

The superficial right femoral artery and the right femoral vein were isolated and blood flow was restricted approximately 5 mm distally to the inguinal ligament using a 4-0 nylon thread. The skin was closed with an intradermal suture with 4-0 nylon thread and the effectiveness of arteriovenous restriction surgery was confirmed by measuring blood flow. The rate of BFR was between 36% and 38%. The area was then cleaned with iodopolividone (1%). After surgery, animals were maintained in heated cages until the anesthesia effect was abolished. A post-surgery treatment was carried out using antiinflammatory (ketoprofen 3 mg/kg, subcutaneously) every 12 hours for 5 days and antibiotic (pentabiotic Fort Dodge, 40,000 IU/kg, intramuscularly) every 48 hours for 3 days.

Familiarization with the strength training protocol, maximum loaded weight test and Manual Grip Strength Test

After two weeks of the surgical procedure, all animals were adapted to the ST protocol, which consists of climbing a vertical ladder (110 cm high, 18 cm wide, 2 cm grid, 80° inclination) that has a housing (20 x 20 x 20 cm) located at the top used as a shelter during the rest period (figure 1). During two days (Monday and Wednesday) of the first week, they were familiarized with the equipment and the task they should perform in the exercise sessions. Thus, the familiarization consisted of climbing the ladder with a load equipment (conic tube), without weight, tied to the animal's tail (adapted from Neves et al. 2016). The number of sets on

Figure 1. Vertical ladder used for strength training protocol.

the first day of familiarization (Monday) was 3 and of repetitions, 10. On the second day of familiarization (Wednesday), the number of sets and repetitions was 6 and 10, respectively.

On Friday, the maximum loaded weight (MLW) was determined for all groups of animals. For the initial climb, they were loaded with 75% of the animal's BW. Then, a load of 30g was added until reaching the maximum load, considered when the animal fails to climb the ladder after 3 consecutive stimuli on the tail (using tweezers). The largest load that the animal carries along the entire length of the ladder, added to the animal's BW on the day of the test was defined as the MLW (adapted from Neves et al. 2016).

This test was performed at the beginning of the training protocol, at the end of the second and fourth weeks of the experimental protocol

to adjust the training load and determine the effectiveness of the ST protocol, respectively. At the end of the second week (training load adjustment), only this reassessment was made, that is, there was no training. Similarly, the last evaluation was carried out replacing the last training day.

The Manual grip strength test (MGS) was performed at the beginning and at the end fourth weeks of the experimental protocol to determine the effectiveness of physical training. The last evaluation was also carried out replacing the last training day. The MGS was performed four hours after the end of the MLW test, a guaranteed time for full recovery of the animal, using a gripping force system (model: DFE-002, San Diego Instruments, San Diego, Cal, USA). Three attempts were made to grasp with the front and rear legs without the values being considered. Each attempt consisted of placing the animal on the grid and, as soon as it is stable, the researcher carried out a traction by the tail so that both paws passed through all the wires of the grid and the rodent disengaged from it (figure 2). Subsequently, valid attempts were made and the three grip values applied to the grids were recorded (Anderson et al. 2004). The test result was determined by calculating the arithmetic mean of the three attempts. It is important to note that for both tests (MLW and MGS), only the pre and post values were considered for analysis.

Strength training protocol

After the adaptation and testing protocols, animals from T/S and T/BFR groups underwent ST during 4 weeks, once a day, and three days a week. The training session consisted of six sets of 10 ladder climbing, 1-minute rest between sets, carrying a load fixed to the tail (50% of 1 MVC obtained through the MLW test more the BW of each animal) to mimicking a muscular
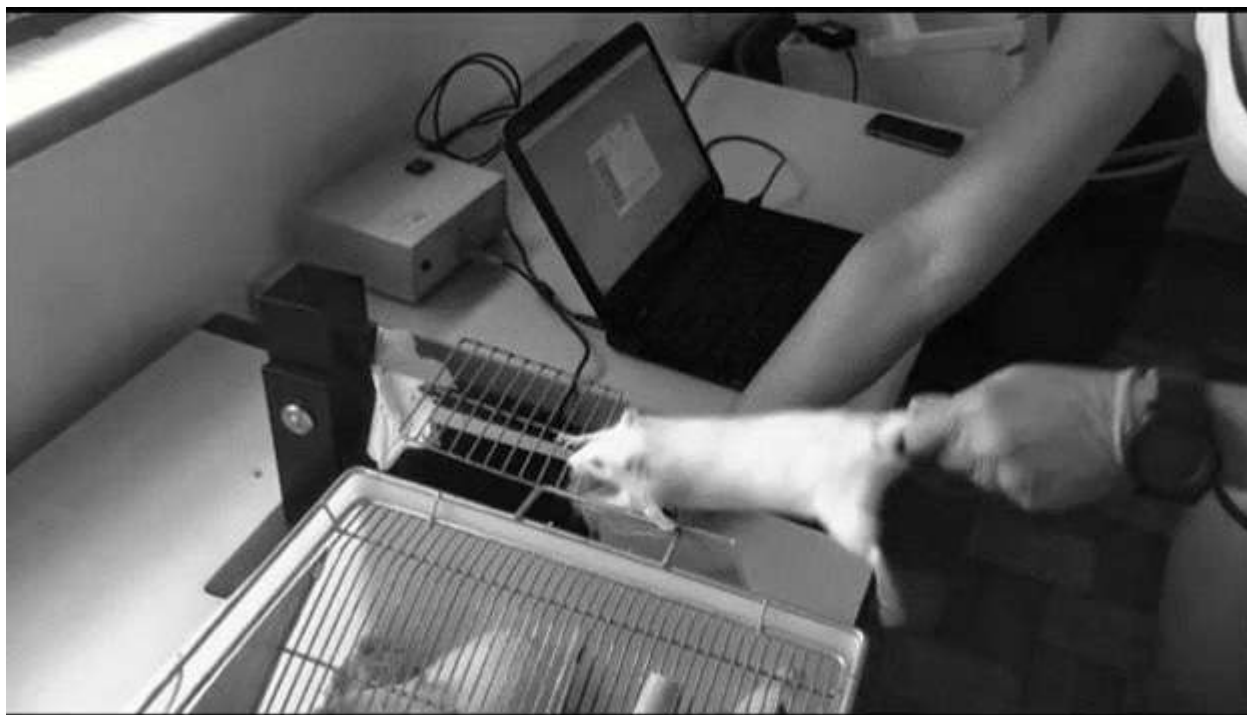
Figure 2. Manual grip strength test.

hypertrophy training (adapted from Neves et al. 2016). The average session time obtained in a pilot study was 20 to 30 minutes as well as the time under tension measured as the sum of the rise times in each series was 60 seconds.

The load was prepared using zinc fishing weights inside conical tubes that are fixed to the proximal part of the animal's tail using Scotch rubber tape (Scotch 3M, São Paulo, Brazil) (adapted from Neves et al. 2016).

Euthanasia

Forty-eight hours after the last exercise session animals were decapitated under anesthesia with ketamine/xylazine (40-2 mg/kg intraperitoneally). The animals remained 12 hours fasting before the euthanasia. After this procedure, the skin of the animal's abdominal region was removed and samples of white adipose tissue from the epididymal region were collected and weighed immediately. Skeletal muscles (soleus, plantar and gastrocnemius) were collected and weighed immediately for analysis. The animal's tibia was dissected and its length measured with a tape measure.

Muscular hypertrophy

Muscular hypertrophy was determined as previously described (Do Carmo et al. 2011). Briefly, a ratio between muscle weight and the length of the tibia was calculated. This ratio was calculated for soleus, plantar and gastrocnemius muscles separately and presented as mg/mm.

Hemodynamic measurements

BP measurement of the animals was performed before surgery, in the second week of recovery from surgery and one week before the animals were euthanized, using the tail cuff technique. For this, awake animals were placed in a container and a cuff was placed at the base of the animal's tail, as well as the pressure sensors. After this procedure, the cuff was automatically inflated and deflated and the systolic BP (SBP)

signal was captured by the sensors that send the information to the signal acquisition system amplifier (PowerLab 400™, ADInstruments, Australia).

Mensurement activity of the superoxide dismutase antioxidant enzyme

Serum samples were collected without using an anticoagulant and clotted for 30 minutes. Then it was centrifuged at 2,000 x g for 15 minutes at 4°C. The test was performed following the manufacturer's recommendations (Cayman Chemical Company, Ann Arbor, MI, EUA).

Mensurement nitrite/nitrate concentration

Plasma samples were collected and nitrate/nitrite levels (index of nitric oxide (NO)) were measured calorimetrically by commercially available kit according to the manufacturer's instructions (Cayman Chemical Company, Ann Arbor, MI, EUA).

Measurement tumor necrosis factor alpha concentration

Serum samples were collected all reagents, samples and standards were prepared as instructed. Then, 100 μl of the standard or sample was added to each well of the plate 2.5 hours were incubated at room temperature. Thereafter, an additional 100 μl of biotin antibody prepared in each well was added. Incubate for 1 hour at room temperature 100 μl of prepared streptavidin solution was added and incubated again for 45 minutes at room temperature. Another 100 μl TMB One-Step Substrate Reagent to each well and another 30 minutes of incubation at room temperature. Finally, 50 μl Stop Solution to each well was added. Reading at 450 nm immediately (R&D System, Inc., Minneapolis, USA).

Statistical analysis

Data were expressed as mean ± standard deviation (S.D) for n experiments. Shapiro-Wilk and Levene tests were used to check normality and homogeneity of variances in our data respectively. When the normality and/or homogeneity assumptions were not met, a data transformation technique (Log) was applied in an attempt to get around this problem. One-way ANOVA was used for hypertrophy, epididymal fat, TNFα, nitrite/nitrate and SOD while for the data of BW, MLW, MGS and BP the mixed ANOVA tests were used and, when not indicated a parametric test, the Kruskal-Wallis test. The post-hocs were Bonferroni for parametric and Mann-Whitney tests corrected by Bonferroni's correction for non-parametric. The Graph Pad Prism 5.0 program (GraphPad Software, CA, USA) was used for figures and the SPSS (Statistical Package for Social Sciences) 17.0 was used to run the statistical analysis. Statistical significance was considered at 5% (p<0.05).

RESULTS

Body weight and epididymal fat

Table I shows the parameters related to BW and the amount of epididymal fat of animals in the S/S, S/BFR, T/S and T/BFR groups. There was a time-group interaction [$F_{(3, 48)}$ = 5,960; p = 0,002] for the BW. At the end of the experimental protocol, the T/S group showed lower BW compared to the other groups (p = 0,001). All groups differed from pre to post (p < 0,001). No statistical difference was found for epididymal fat [$F_{(3, 47)}$ = 1,117; p = 0,352].

Physical performance: Maximum loaded weight test and Manual Grip

Strength Test

The physical performance at the beginning was not different among groups [$\chi^2(3)$ = 6,079; p = 0,108 for MGS and; $\chi^2(3)$ = 6,126; p = 0,106 for MLW]. However, there was an effect of groups on both

Table I. Initial weight, final weight and epididymal fat of rats from the sedentary (S/S) groups, sedentary with blood flow restriction (S/BFR), trained (T/S), and trained with blood flow restriction (T/BFR).

	S/S (n =16)	S/BFR (n=12)	T/S (n=12)	T/BFR (n=12)
Initial Weight (g)	273,31±5,85	262,91±3,98	257,16±8,54	260,5±6,46
Final Weight (g)	378,56±6,42	380,83±7,21	339,91±6,32[*, +, &]	381,66±6,32
Epididymal Fat (g)	7± 0,72	5,63±0,43	5,35±0,41	5,78±0,68

*different to S/S, +different to S/BFR and &different to T/BFR. *p* = 0,001.

MLW [$\chi^2(3)$ = 36,416; *p* < 0,001] and MGS [$\chi^2(3)$ = 38,302; *p* < 0,001] final values. BFR did not impair physical performance of S/BFR compared to S/S. On the other hand, the ST protocol was effective to improve both physical performance tests in either T/S or T/BFR rats. T/S had increased their MLW in 31% and T/BFR in 34% when compared to their initial values (figure 3a and 3b) and they were greater than S/S and S/BFR (*p* < 0,001). Regarding MGS, T/S increased MGS in 28% that was greater than S/S and S/BFR (*p* < 0,001) and T/BFR in 37% that was greater than all groups (*p* < 0,001 to S/S, S/BFR and T/S) (figure 3c and 3d).

Muscular Hypertrophy Index

Effects of group on muscular hypertrophy index of both plantar [$F(3, 48)$ = 3,836; *p* = 0,015] and gastrocnemius [$F(3, 48)$ = 2,940; *p* = 0,042] were found. There was no effect of group on muscular hypertrophy index of soleus [$F(3, 48)$ = 2,322; *p* = 0,087]. T/BFR showed an increase in the plantar muscle hypertrophy index in relation to the groups S/S (*p* = 0,027) and T/S (*p* = 0,028) while S/BFR showed an increase in the gastrocnemius muscle hypertrophy index in relation to T/S (*p* = 0,043) (figure 4).

Blood pressure

There was no time main effect [$F(2, 94)$ = 1,452; p = 0,239] as well as no effect of groups [$F(3, 47)$ = 1,470; p = 0,235] for the BP. Also, an interaction effect between time and groups was not found [$F(6, 94)$ = 0,887; p = 0,507]. Data not show (S/S = 138mmHg±1.5; S/BFR = 135mmHg±1.6; T/F = 137mmHg±1.5; T/BFR = 133mmHg±1,1)

Antioxidant activity of superoxide dismutase

There was no effect of groups for SOD antioxidant activity [$F(3, 28)$ = 2,443; p = 0,085]. Data not show (S/S = 1.8 U/mL±0.2; S/BFR = 2.1 U/mL ±0.1; T/S = 1.6 U/mL ± 0.1; T/BFR = 1.5 U/mL ±0.2)
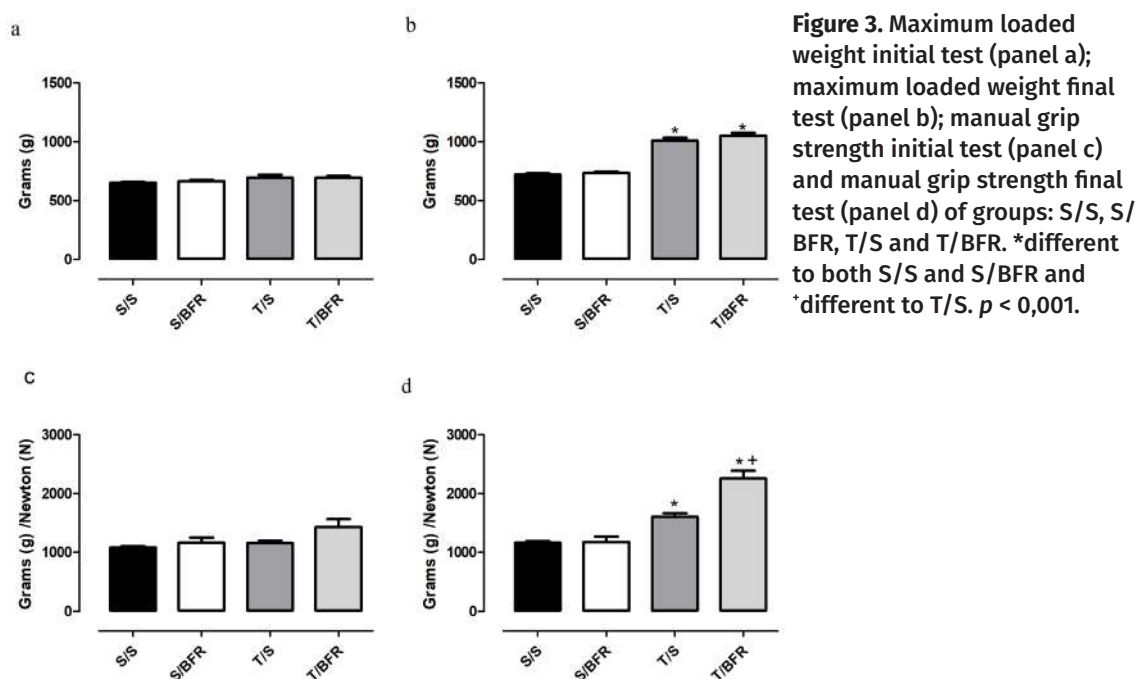
Concentration of Nitrite/Nitrate

For the data of nitrite and nitrate no effect of groups was found [$F(3, 28)$ = 1,883; p = 0,164]. Data not show (S/S = 35.2 n/M ± 2.2; S/BFR = 48.7 n/M ± 4.7; T/S = 38.5 n/M ± 3.2; T/BFR = 39.4 n/M ± 1.5).

Tumor necrosis factor alfa

Effect of groups was found for TNF-α values [$F(3, 24)$ = 13,026; p <0,001] with S/S showing lower values compared to the values obtained in the S/BFR (p = 0,005) and T/BFR groups (p <0,001) as well as lower values in the T/S group compared to the T/BFR group (p = 0,002) (Figure 5).

DISCUSSION

The main results of this study showed that the ST protocol was able to improve physical performance, as demonstrated by the increased strength in the MLW and MGS tests for T/S or T/BFR and that the hypertrophy index was mainly influenced by the arteriovenous BFR with the greater hypertrophy index of the plantar muscles in response to T/BFR than S/S and T/S while

Figure 3. Maximum loaded weight initial test (panel a); maximum loaded weight final test (panel b); manual grip strength initial test (panel c) and manual grip strength final test (panel d) of groups: S/S, S/BFR, T/S and T/BFR. *different to both S/S and S/BFR and †different to T/S. *p* < 0,001.

significant difference in the hypertrophy index of the gastrocnemius muscle being observed in response to S/BFR compared to T/S. Also, arteriovenous BFR with and without ST seems to have no significant effect on cardiovascular health since no changes were found in SBP, as well as in the blood levels of SOD and nitrite/nitrate compared to S/S group. On the other hand, serum level of TNFα was increased in response to BFR and enhanced by associated ST.

All the animals independent of the group increased the BW during the protocol. Because the animals were young, the effects of maturation on BW were evident, as seen in S/S group, and BFR, as observed in S/BFR group, did not interfere with animals' growth (Kawada & Ishii 2008).

It is known that physical exercise increases the energy expenditure and it is an important factor that can affect the weight gain (Coqueiro et al. 2017, 2018). Interestingly, male rats, but not female, undergone forced exercise program usually decrease or not change energy intake different from voluntary exercise (running

wheels) (Titchenal 1988). In this way, when rats were subjected to ST reduced food intake was found (Coqueiro et al. 2017). Moreover, optimized lipid oxidation can contribute for lower BW and improved body composition (Coqueiro et al. 2017, Gollisch et al. 2009). However, in spite of epididymal fat assessment in the present study, it does not translate accurately the body fat mass despite being part of the total body fat content (Coqueiro et al. 2017). Thus, it is possible that the reduction on body fat has occurred although no significant changes in epididymal fat mass have been verified. These facts can explain the lower final BW in T/S group despite no assessment of these parameters had been conducted in the present study and, this fat loss may have been made up for a possible hypertrophy in the T/BFR group (see below).

Both MLW and MGS increased in trained animals with higher values for T/BFR group in MGS test. According to the authors knowledge, there is no study that assessed the effects of ST with BFR in rats. Nevertheless, a lot of studies have shown the positive effects of ST on different
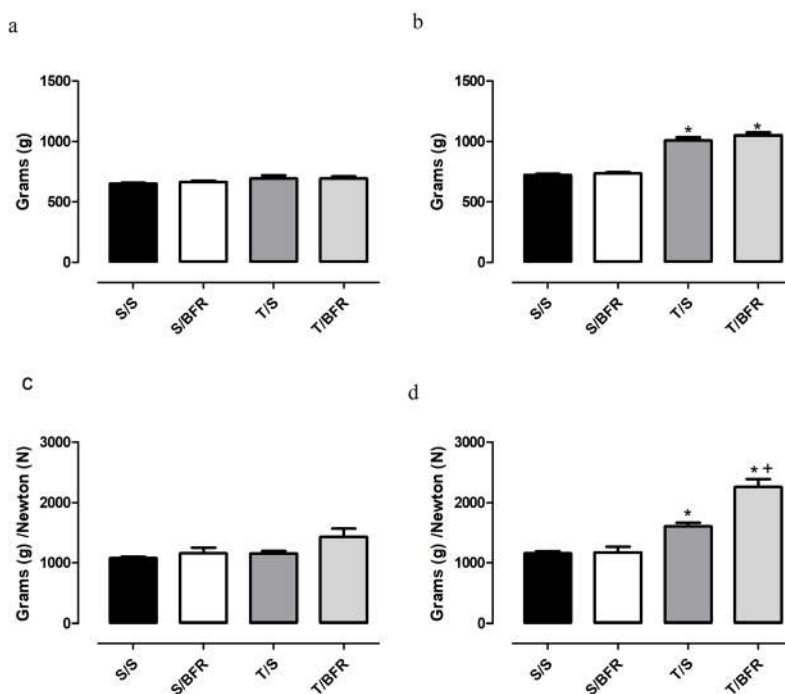
Figure 4. Skeletal muscle hypertrophy index of the paw with restriction surgery (right) soleus (panel A), plantar (panel B) and gastrocnemius muscles (panel C), corrected by the length of the tibia of groups: S/S, S/BFR, T/S and T/BFR. *different to S/S; #different to T/S. $p < 0,05$.

strength tests in rodents (Do Carmo et al. 2011, Cassilhas et al. 2012, Hornberger & Farrar 2004, Neves et al. 2016, Coqueiro et al. 2018, Philippe et al. 2019). Loads of 45–50% of 1 MVC (and less) have shown to increase dynamic muscular strength in untrained individuals (American College of Sports Medicine 2009). Because the animals evaluated were naive to ST, it is possible that the loads used were sufficient to cause gains in strength. This fact can be explained, at least partially, by the learning process in rats which is similar to neural adaptations that occurs in early strength gains in humans (Hornberger & Farrar 2004). However, for T/BFR group, the impact of hypertrophy on strength gain can be huge (Loenneke et al. 2012) and it is likely that the hypertrophy was the advantage obtained by T/BFR over T/S, mainly in MGS test, since the hypertrophy may occur in the muscles which are not under direct influence of BFR but that was minimally stimulated (Madarame et al. 2008) such as the muscles involved in the grabbing ability in the forelimbs.

We must recognize that the MGS test used in our study has some limitations. First, the MGS used to assess the grip strength is dependent on examiner manipulation (Hays et al. 2013). For example, the traction force magnitude employed by researcher in the tail of the animal may be different from one trial to another one. In the same way, the angle of force traction application may be different between two trials. These aspects related to examiner handling can affect the grab capacity of the animals and can interfere with the test results. However, despite this, it is a validated, simple, cheap and widely used test in studies with rats.

No evidence for muscle hypertrophy following ST alone was found in the present investigation contradicting other studies (Hornberger & Farrar 2004, Do Carmo et al. 2011, Casilhas et al. 2012, Coqueiro et al. 2017, Philippe et al. 2019). These differences between our data and previous results may be explained by methodological aspects. The ST protocol used can offer an efficient stimulus for a muscle group but not for another one. For
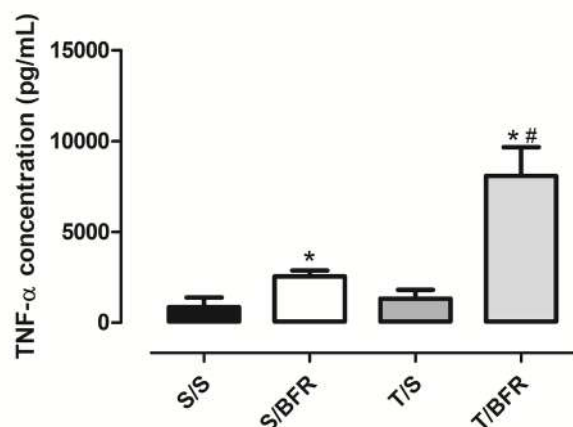
Figure 5. Measurement of the concentration of TNF-α in serum. Sedentary groups (S/S), sedentary with blood flow restriction (S/BFR), strength trained (T/S) and strength trained with blood flow restriction (T/BFR). Data are expressed as mean ± SEM. Experimental *n* is 12 to 16. *different to S/S, $p < 0.001$; #different to T/S, $p = 0.002$.

example, ladder climbing can be an effective stimulus for flexor hallucis longus (FHL) or flexor digitorum longus (FDL), highly involved in the grabbing ability which is suggested to limit the climbing ability of the animals. However, rats undergone the squat in a special apparatus obtained hypertrophy in plantar flexors muscles (plantar and gastrocnemius) (Do Carmo et al. 2011). Hypertrophy has been observed in FDL (Cassilhas et al. 2012), deltoid and flexor digitorum profundus (FDP) (Philippe et al. 2019) and tibialis muscle (Coqueiro et al. 2017) but not in plantar flexors (Neves et al. 2016) following ST composed by ladder climbing. Because we evaluated plantar, soleus and gastrocnemius muscles, it is possible that the hypertrophy which has occurred in another muscle group that was hard involved in the ladder climbing movement, such as FDL or FHL, was not evidenced. Therefore, it does not permit us to discard totally the occurrence of muscle hypertrophy and the effects of that as a factor contributing to the increased strength in T/S group.

Only both BFR groups exhibited significant differences in hypertrophy index in the assessed muscles. Initially, the greater hypertrophy index for gastrocnemius in S/BFR group compared to T/S group, can be attributed to a trend of lower hypertrophy index in T/S group than greater hypertrophy in S/BFR group. As mentioned earlier, rats subjected to ST can reduce the food intake (Coqueiro et al. 2017) and it is known that energy deficit results in loss of skeletal muscle even when higher protein diet is consumed (Philippe et al. 2019). Thus, hypothetically speaking, the low stimulation of gastrocnemius muscle in the exercise protocol together with an energy deficit created by reduced food intake and higher energy expenditure could have attenuated the muscle development in T/S group.

Moreover, both gastrocnemius (Kawada & Ishii 2005) and plantar muscles (Kawada & Ishii 2005, 2008) have been shown to respond positively to BFR alone in rats. However, difference in occlusion protocols might affect these adaptations from BFR. The occlusion model in the studies cited above (vena saphena magna, vena saphena accessoria medialis, vena circumflexa ilium superficialis, and vena epigastrica superficialis) could have obtained a more severe BFR than our model (femoral artery and vein) although the blood flow in the limbs occluded was not checked by the authors of these studies. If it was the case, greater hypoxia was generated in the muscles triggering a more intense activity of the mechanisms related to muscle adaptations (De Castro et al. 2017). Also, because plantar muscle is smaller than gastrocnemius muscle, maybe the minimum stimulation promoted by exercise with BFR was efficient to trigger the hypertrophy in this muscle while no hypertrophy was observed for gastrocnemius muscle. In practice, bodybuilders generally employ greater workloads through

higher volumes and intensities in large muscle groups compared to small muscle groups what may indicate that larger muscle groups require higher stimulation level.

Soleus hypertrophy index was not different among groups in the present study. Evidences reported in the literature showed no significant difference between rats undergone BFR or sham operation (Kawada & Ishii 2005, Pour et al. 2017, Bahreinipour et al. 2018) but when low intensity endurance exercise with BFR is analyzed increased soleus hypertrophy index were found (Pour et al. 2017, Bahreinipour et al. 2018). The difference between our results and those previously mentioned may reflect the training protocol employed. Strength exercise differ from endurance exercise with the former being intermittent efforts interspersed with rests between the sets while the latter is characterized by long continuous periods of exercise that is dependent on oxidative muscle fibers which are resistant to fatigue. Soleus is a muscle group with high percentage type I fibers which may became this muscle group less responsive to the occlusion suggesting that the effects of occlusion may depend on the muscle-fiber composition (Kawada & Ishii 2005). Therefore, neither occlusion alone nor ST with and without BFR may be an adequate stimulation to promote muscle adaptation in the soleus muscle.

Regarding cardiovascular assessments, our results showed that there was a maintenance of SBP levels during experimental protocol in all groups. It is known that SBP is not altered in both hypertensive and normotensive rats after a ST protocol compared to their respective sedentary peers (Gomes et al. 2017). This fact indicate that ST is safe even in hypertensive condition. In fact, a systematic review carried out in humans (Cardoso et al. 2010) recommends ST as an important strategic tool in maintaining cardiovascular health because active individuals are less likely to develop high BP.

As mentioned earlier, no studies have been found in the literature on animals submitted to ST with BFR. However, in humans, Sardeli et al. (2017) demonstrated that all groups (high load, low load, low load with BFR and control) exhibited no different values of SBP before, after and 30 minutes after 45° leg press exercise in healthy elderly people what led the authors to conclude that ST with BFR seems to be a safe method. In addition, a systematic review by Neto et al. (2016), in humans, also corroborates these results and recommends ST with BFR as a safe and viable method for special populations, such as the elderly and cardiac patients, among others, because it promotes increased muscle strength and hypertrophy without negatively altering the hemodynamic response.

Regarding the concentration of SOD, an important antioxidant enzyme that can prevent muscle stress and increase tolerance to intense or ischemic exercise, we did not find any changes between the groups. Corroborating this result, Cumming et al. (2016) found that SOD concentration remain unchanged at the end of the protocol in both leg trained in high load ST, and the contralateral limb trained in low load ST with BFR. However, more studies should be concerned with analyzing the SOD concentrations in the exercised muscle.

Another important biomarker involved in cardiovascular health is nitrite/nitrate, an indirect marker of the production of NO. In our study, the results did not differ between groups. Acutely, a study by Ozaki et al. (2013), in healthy young people, also found no difference in nitrite/nitrate concentration between groups (high load ST, low load ST and low load ST with BFR). Likewise, a study by Ramis et al. (2020) found no differences between high load ST and low load ST with BFR after 8 weeks, but comparing the pre

and post intragroup moments, they found an increased bioavailability of NO which indicates a similar effect of flow mediated dilatation in both groups. Thus, our results indicate that T/BFR had neither beneficial nor negative effects on endothelial health.

This study was also the pioneer in evaluating the concentration of TNF-α in animals submitted to ST with BFR. It is known that aerobic training with adequate intensity and volume, is capable of producing anti-inflammatory substances, improving systemic inflammation, which in turn can protect against problems associated with metabolic syndrome, such as arterial hypertension, diabetes, among others, and consequently preventing the development of cardiovascular diseases (Franklin et al. 2015, Dorneles et al. 2019, Mcleod et al. 2019). With regard to ST, the results found in the literature also indicate such beneficial effects (Mcleod et al. 2019, Da Silva et al. 2020).

Wistar rats undergone a ST protocol for 8 weeks, performed 1 time every 3 days, with a minimum load of 50% of the BW demonstrated a decrease in serum TNF-α concentrations pointing that this type of training is an important intervention strategy for the prevention and treatment of the inflammatory state of obesity and associated chronic diseases (Speretta et al. 2012). In our study, ST did not alter the blood concentration of TNF-α but, when associated with BFR, there was an increase in its concentration compared to the control group. Although TNF-α has not caused any harm in increasing muscle strength and hypertrophy, it is directly related to the components of the metabolic syndrome and can predict cardiovascular risk.

On the other hand, TNF-α is also related to the activity of mTOR (Laplante & Sabatini 2012). Thus, this could help explain the fact that TNF-α increased in S/BFR and even more in T/BFR groups and that greater muscle hypertrophy

index was found in the BFR groups, more clearly in the T/BFR group. The neutrophils and macrophages responsible for tissue remodeling secrete both TNF-α and IL-6. However, the role of inflammatory activity in vascular occlusion is still unclear. Therefore, more studies are needed on this topic.

We found only one study that evaluated TNF-α concentration in high load ST (80% 1RM) and low load with BFR (30% 1RM) and the results corroborate ours (Da Silva et al. 2020). The BFR attenuated the beneficial adaptations of ST in overweight men, concentration of TNF-α, since high load ST group maintained the pre values, after 8 weeks of training. The authors argue that acute hypoxia suggests the migration of inflammatory cells to the exercised muscle. Thus, tissue inflammation and metabolic stress are linked to muscle adaptation to this training method (Dorneles et al. 2016, Da Silva et al. 2020). In this way, more studies are necessary to understand the adaptive mechanism of the inflammatory process, since the increase in this cytokine did not affect the increase in strength and muscle hypertrophy in the T/S and T/BFR groups. On the contrary, the T/BFR group had an even greater increase in manual pressure strength, and was the only group to increase plantar muscle hypertrophy values, when compared to its sedentary and trained counterparts.

Lastly, some considerations need to be made. In humans, BFR causes ischemia reperfusion during the restriction and release of the cuff, respectively. In this situation, an increase in arterial flow and venous return occur which, in turn, potentiate the shear stress and it can have physiological impacts such as in NO production (Horiuchi & Okita 2012). However, unlike the human model, flow restriction in animals is continuous (throughout the protocol) and there is a decrease in arterial flow and venous

return which could impact less NO production compared to the human model. Also, these methodological differences in occlusion protocol between humans and animals may take part in explaining why many studies (Kawada & Ishii 2005, 2008, Pour et al. 2017) not all (Bahreinipour et al. 2018) have shown that glycolytic muscles (plantar, gastrocnemius or extensor digitorum longus (EDL)) get hypertrophy following BFR alone in rats, but not in humans where BFR can attenuate the disuse atrophy but not cause hypertrophy (Takarada et al. 2000, Kubota et al. 2008).

In conclusion, the used chronic arteriovenous occlusion model was efficient to increase strength and hypertrophy in rats without negative impacts on cardiovascular health. However, some caution is necessary to extrapolate the results from the present investigation to humans due to methodological differences. Because of the discomfort and pain caused by BFR in lower limbs, it is necessary for the individuals have a high pain threshold. Finally, more research is needed to understand why and if the oxidative and glycolytic muscles share the same adaptations mechanism or not following BFR training.

CONCLUSIONS

Low load arteriovenous BFR ST is an effective and safety approach to improve strength and hypertrophy in untrained rats while low load ST without BFR is an alternative to improve strength but it does not promote hypertrophy, at least in short periods such as that used in the present study. This is similar what happens in untrained humans in the first weeks of training. From this study was not possible to detect how much of the strength gain was due to muscle hypertrophy what deserves attention in future studies together with comparative studies

between ST with either arterial occlusion and venous occlusion on muscle adaptions and adaptive mechanisms. In addition, we found no significant cardiac and metabolic changes. Thus, ST with arteriovenous BFR is able to improve muscle adaptations and performance in rats, without causing cardiovascular and metabolic damage.

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NÁDIA F. GARCIA[1]
https://orcid.org/0000-0003-3484-6605

CAMILA DE MORAES[2]
https://orcid.org/0000-0003-3735-1165

MACÁRIO A. REBELO[2]
https://orcid.org/0000-0003-4491-9660

SAVANAH MARIA G.R. PETERS[2]
https://orcid.org/0000-0002-7763-8147

FÁBIO M.P. DE CASTRO[1]
https://orcid.org/0000-0003-0134-8394

ENRICO F. PUGGINA[1,2]
https://orcid.org/0000-0002-8379-2247

[1]University of São Paulo (USP), School of Medicine, Department of Physiotherapy of Ribeirão Preto, Av. Bandeirantes, 3900, USP Campus, 14049-900 Ribeirão Preto, SP, Brazil

[2]University of São Paulo (USP), School of Physical Education and Sport of Ribeirão Preto, Av. Bandeirantes, 3900, USP Campus, 14040-907 Ribeirão Preto, SP, Brazil

Correspondence to: **Enrico Fuini Puggina**
E-mail: enrico@usp.br

Authors' Contributions

Nádia Fagundes Garcia - Conceived and designed the analysis, collected the data, contributed data of analysis tools, performed the analysis and wrote the paper. Camila de Moraes - Contributed data of analysis tools, performed the analysis and wrote the paper. Macário Arosti Rebelo - Collected the data and contributed data of analysis tools. Savanah Maria Gertrudis Rosa Peters - Collected the data and contributed data of analysis tools. Fábio Marzliak Pozzi de Castro - Collected the data and contributed data of analysis tools.
Enrico Fuini Puggina - Conceived and designed the analysis and wrote the paper.