DIFFERENT COOLING STRATEGIES BETWEEN SETS FOR 30 SECONDS DOES NOT HAVE BENEFICIAL EFFECT ON RESISTANCE EXERCISE WITH BLOOD FLOW RESTRICTION

DIFERENTES ESTRATÉGIAS DE RESFRIAMENTO ENTRE SÉRIES POR 30 SEGUNDOS NÃO TÊM EFEITO BENÉFICO NO TREINAMENTO RESISTIDO COM RESTRIÇÃO DE FLUXO SANGUÍNEO

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RESUMO

A aplicação do gelo (resfriamento) tem sido uma estratégia popular durante as atividades físicas para aumentar o desempenho. O objetivo deste estudo foi testar se diferentes locais de resfriamento podem aumentar o número de repetições (volume) durante o treinamento resistido com restrição do fluxo sanguíneo (RFS). Dez mulheres se voluntariaram para este estudo. A caracterização da amostra é apresentada em média e desvio padrão: idade: $28,5 \pm 8,6$ anos; estatura: $164,6 \pm 8,3$ cm; massa corporal total: $61,5 \pm 7,1$; teste de força muscular dinâmica (1RM): $236,5 \pm 54,8$ kg; 30% 1RM: $71,6 \pm 16,5$; PAS: $124,7 \pm 7,7$ mm Hg; 1,3 x PAS: $161,8 \pm 10,4$ mmHg, participaram do estudo. Os sujeitos realizaram cinco sessões de exercício resistido com RFS. Três séries foram realizadas em cada sessão, com a intensidade de 30% de 1RM até falha muscular e 30 segundos de descanso entre as séries. Os locais de resfriamento foram: mãos, pescoço e temperatura do túnel. Uma sessão sem resfriamento foi realizada e considerada como grupo controle. Não houve diferença no número total de repetições entre as intervenções, nem diferença entre as intervenções para percepção subjetiva de esforço (P = 0,49). Portanto, nós não recomendamos o resfriamento para manter um alto número de repetições durante o treinamento de força com restrição do fluxo sanguíneo.

Palavras-chave: Fadiga. Kaatsu training. Treinamento de força.

ABSTRACT

The ice application (cooling) has become popular during physical activities to improve performance. This study aimed to test whether different cooling places could increase the number of repetitions (volume) during resistance training with blood flow restriction (BFR). Ten women volunteered for this study. The sample characterization is presented in mean and standard deviation: age: 28.5 ± 8.6 years; height: 164.6 ± 8.3 cm; total body mass: 61.5 ± 7.1 maximal dynamic strength test (1RM): 236.5 ± 54.8 kg; 30% 1RM: 71.6 ± 16.5 ; SBP: 124.7 ± 7.7 mm Hg; $1.3 \times$ SBP: 161.8 ± 10.4 mm Hg. The subjects performed five sessions of resistance exercise with BFR. Three sets were held in each session, with the intensity of 30% of 1RM until muscle failure; and 30-second rest period between sets. The cooling sites were: hands, neck, and tunnel temperature. One session without cooling was done and considered as a control group. There was neither difference in the total number of repetitions of repetitions among interventions, nor a significant difference among interventions for RPE (P = 0.49). Therefore, we do not recommend cooling to maintain a high number of repetitions during strength training with BFR. Keywords: Fatigue. Kaatsu training. Strength training.

Introduction

The strength training with blood flow restriction (BFR) is composed of light load (20 to 50% of 1RM), short recovery period between sets (30 to 60 seconds), and sets performed until muscle failure^{1,2}. This method is used to induce muscle hypertrophy and strength³.

The total number of repetitions (volume) during resistance training is important to improve muscle hypertrophy⁴. However, during a session of strength training with BFR muscle fatigue is observed, decreasing the number of repetitions and, consequently time under

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tension⁵. Thus, we are interested in strategies that could sustain a high number of repetitions during a strength training session.

The application of ice (cooling) has become popular during physical activities⁶ because this strategy could enable the increase of the total work performed. For strength training, the cooling strategy could maintain a high number of repetitions and the time under tension, favouring the muscle hypertrophy. Cooling techniques have improved performance in resistance training in men⁷ and women⁸ when applied to their hands, with a rest interval between sets of 2.5 and 3 minutes. The cooling procedure might change afferent signals from the body to the central nervous system (CNS), according to the Central Governor Model⁹. However, the cooling method, location, size of the region, duration, type of exercise, and protocol might influence the magnitude of the effect¹⁰. Here we were not able to apply the cooling in the active muscle because of the sphygmomanometer covering the muscle.

Then, we cooled three different places (palm of hands; neck; tunnel of temperature), in different days, aiming to improve performance. The choice of the palm was related to positive results found in previous research on cooling and performance in strength training^{7,8}. The cooling of the neck was because it is widely used in endurance sports^{11,12}. The temperature tunnel through the vein and superior ophthalmic artery located in the cantal projection eyelid is directly related to hypothalamic temperature¹³, and this area is the key region that controls metabolism¹⁴, therefore, cooling this place could promote a refresh sensation and influence the performance positively. Therefore, this study aimed to test whether different cooling places could increase the number of repetitions (volume) in resistance exercise with BFR.

Methods

Participants

Ten women volunteers provided written informed consent to participate in this study, which was approved by the Ethics Committee of the Federal University of São Paulo under the number 1174/2016. The subjects volunteered after being fully informed of the requirements and risks associated with the research. Subject characteristics (mean and standard deviation) were: age, 28.5 ± 8.6 years; height, 164.6 ± 8.3 cm; total body mass, 61.5 ± 7.1 ; maximal dynamic strength test (1RM), 236.5 ± 54.8 kg; 30% 1RM, 71.6 ± 16.5 ; systolic blood pressure (SBP), 124.7 ± 7.7 mm Hg; $1.3 \times$ SBP, 161.8 ± 10.4 mm Hg; and time experience in the resistance training, 6.4 ± 5.8 years.

Experimental design

A crossover design was used, with each volunteer serving as their control. The subjects performed five sessions of resistance exercise with BFR. On the first session, we measured volunteers' body weight, height, systolic blood pressure at rest and maximal dynamic strength test (1RM) in the leg press 45° exercise. From 2^{nd} to 5^{th} sessions, the interventions were randomized, but unbalanced because of the number of participants. We gave them 48-hour of rest interval between sessions to avoid performance decrement¹⁵. Three sets were held in each session, with the intensity of 30% of 1RM¹⁶ until muscle failure; and 30-second rest period between sets because previous studies recommended a rest period between 30 and 60 seconds when applying BFR on resistance exercise¹⁷. The BFR was performed in both thighs with a sphygmomanometer and placed on the proximal thigh portion; the pressure applied was according to the protocol described by Takano (2005) (i.e., resting systolic blood pressure x 1.3). The same BFR was kept during the rest intervals between sets. The cooling places were: hands, neck, and tunnel temperature. One session without cooling was done and considered as a control group. The cooling length had the same

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duration of rest interval between sets. We used a metronome to monitor the movement cadence, with 2-seconds on the concentric phase and 2-seconds for eccentric. Immediately after each set, the rating of perceived effort was obtained by adapted Borg scale $(CR-10)^{18}$.

Maximal Dynamic Strength Test (1RM)

The 1RM tests were performed on a Leg Press 45° machine, according to Brown and Weir protocol¹⁹. Initially, the volunteers performed the leg press exercise with the metronome to familiarize themselves with the interventions. The interval between trials during the 1RM test was 3 minutes ⁸. We determined the load of 30% of the maximum load for the accomplishment of the BFR protocol after the 1RM test²⁰.

Protocol of Blood Flow Restriction

Initially, we measured the systolic blood pressure at rest (SBPR) on the left arm through the electronic blood pressure monitor (Intellisense mark, JZK -002 models). The value of the SBPR time 1.3 was used to determine the pressure used during a resistance training session with BFR²⁰. We used the sphygmomanometer Premium Artery clamp with a large range (35 to 51cm) to perform the partial occlusion. We placed a sphygmomanometer on each of the volunteers' thighs, followed by pressure adjustment to each set. After adjusting the cuff pressure to each set, the volunteers did the maximum number of repetitions. We stopped the set when subjects had a failure during concentric action or a mismatch with the metronome. Subjects did not receive any verbal encouragement during protocols.

Borg Scale

All subjects were provided with scaling instructions and anchoring procedures for the RPE scale (CR- 10) 10 points¹⁸. The scaling instructions define the perceived exertion as the subjective intensity of effort, strain, discomfort, or fatigue experienced during the exercise in the active muscle. Immediately after 30 seconds of recovery between sets, with or without cooling, the subjects answered the question "How hard was your set?" while looking at the RPE scale to assess the level of effort of each set.

Cooling Techniques

The application of cooling was during the rest period between the 1^{st} and 2^{nd} sets, and the 2^{nd} and 3^{rd} sets of the completion of the leg press 45° exercise, lasting 30 seconds each cooling. Participants were submitted to the following trials for cooling: hands, neck, and temperature tunnel. Cooling was done by two gel ice packs called "Keep Pack," with a size of 13 x 12 cm. An evaluator applied the ice packages to cool the specifics places.

Statistical Analysis

We used the number of repetitions as a main output for the sample calculation, obtaining power of 0.79. The data of repetitions and RPE of the first sets were discarded from the analysis since the interventions with cooling were performed after the first sets. Data are presented as a mean and standard deviation. We applied the Shapiro-Wilk test, assuming data normality for repetitions. We applied the Mauchly's test to verify the data sphericity and Greenhouse – Geiser for correction when sphericity was violated. Moreover, we applied ANOVA for repeated measures. For RPE, we applied the Levene test, assuming homogeneity. The Friedman test was used to verify if there was any difference between the interventions. For each intervention, a coefficient of variation (CV) of total volume was determined. It was accepted level $\alpha \leq 0.05$. The effect size (ES) was calculated for total volume and RPE (control group x interventions) using Hedge's g, with their results classified according to Rhea²¹. The ES was calculated by subtracting the mean of the control group performance from the mean of

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the experimental group and divided by the pooled weighted standard deviation (Square root of the weighted pooled variance)²². We used Magnitude-Based Inference (MBI) to determine practical significance. The threshold values for Cohen's d statistical power were considered as >0.2 (small), >0.5 (moderate), and >0.8 (large)²³. Cohen's unit of 0.2 was used to determine the smallest worthwhile value of change. Using Microsoft Excel® spreadsheet designed for sports science research²⁴, mean effects and the 90% confidence limits were estimated to establish the percentage likelihood of each experimental condition having a positive/trivial/negative effect on performance.

Results

Figures 1 and 2 are shown in the mean and standard deviation of the total number of repetitions and RPE, respectively.

Although the coefficient of variation (CV) is too high in comparison with traditional resistance exercise, the value is similar among interventions with hands 48.9% (4.6 - 7.2), neck 49.2% (4.6 - 7.2), temperature tunnel 56.3% (4.7 - 7.7) and control 65.3% (4.6 - 8.3), and with other studies of resistance exercise with BFR.

There was neither difference in the total volume of repetitions among interventions (P=1.00), nor a significant difference among interventions for RPE (P=0.49).











Source: Authors

The criteria to interpret the magnitude of the ES were 0.0-0.35 trivial, 0.36-0.80

small, moderate 0.81-1.50, and large >1.50 (19). The effect size was classified as trivial for total volume and RPE when compared with control. The ES for total volume was: hands 0.15 (IC: -1.4/1.7), neck 0.14 (IC: -1.6/1.8) and temperature tunnel 0.06 (IC: -1.4/1.6). The effect size for RPE was: hands 0.00 (IC: -0.4/0.4), neck -0.23 (IC: -0.6/0.1) and temperature tunnel -0.23 (IC: -0.6/0.1).

The MBI analyses revealed an unclear output for all treatments. Therefore, the cooling strategies used here did not have an ergogenic effect.

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Treatments	Control (A)	Hands (B)	Neck (C)	Tunnel (D)
Repetitions (mean ± SD)	13 ± 5.1	11.9 ± 4.6	12.4 ± 5.3	11.9 ± 3.3
Substantially positive Trivial	Chances (%)	<i>Tt B - Tr A</i> 13 37	<i>Tt C - Tr A</i> 18 43	<i>Tt D - Tr A</i> 6 45
Substantially negative		50	39	50
Classification		Unclear	Unclear	Unclear

Table 1. The Magnitude Based Inference for a number of repetitions across cooling strategies

Source: Authors

Discussion

This study is the first that tested whether the cooling under conditions of resistance exercise with BFR could improve performance (number of repetitions). In this study, the interventions with cooling between sets during a resistance exercise with BFR neither the improved number of repetitions nor decreased the rating of perceived exertion (RPE) when compared to the control group, as well as among them. These data are the opposite of our initial hypotheses. Therefore, for this method applied during resistance exercise with BFR, the cooling strategy does not have any advantage to maintain the number of repetitions and consequently the time under tension.

The results of this study contrast with previous studies that demonstrated improved performance with cooling between sets during resistance training^{7,8}, and decreased RPE⁷. This ergogenic effect is justified by "The Gate Control Theory." This theory consists of a "Close the Gate" for pain transmission, where peripheral stimuli such as cooling can close this "gate" by raising the pain threshold¹⁰. However, in our study, the cooling strategy did not improve performance. Perhaps the short time of recovery is insufficient to "close the gate."

To our knowledge, this is the first study with cooling in resistance training combined with BFR. In this study, we searched for interventions based on strategies adopted in sports, where cooling is not applied to the exercised muscle. This strategy aims at greater excitability and muscle activity for possible performance enhancement^{25,26}. However, we showed that the cooling strategies did not present any ergogenic effect when resistance training was associated with BFR.

A subjective way to monitor the changes in peripheral (afferent) and central (efferent) signals is through the rating of perceived exertion (RPE), expressing the intensity of effort for a particular activity quantitatively²⁷. In our results, there was no decrease in the RPE (P = 0.49) in any of cooling techniques compared to control or among interventions, nor improvement in the total volume of repetitions (P = 1.00). Thus, through conventional inferential statistics, all the cooling interventions were not able to decrease the central fatigue and, consequently, did not alter the performance.

Thereby, we calculated the CV, ES²⁸, and MBI²⁹ to assess the accuracy of the results providing the researcher with a better interpretive analysis for the magnitude of a treatment

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effect and the relevance for intervention practices. The CV of the repetitions on the present study was higher than other studies under both cooling and non-cooling conditions, respectively, being 35.6% and 46.8%⁷, and 28.2% and 26.9%⁸. The higher CV showed here might be due to a greater individual pain sensation caused by BFR³⁰. The treatments had no positive effect as we can see through effect size analyses. The disappointing result was found in Kwon⁸ study with an ES of 0.29, thus reinforcing no influence of cooling on the RPE. However, for performance, higher ES was shown (0.78) and $(0.60)^{10}$ compared to our study, where the cooling interventions did not cause any ergogenic manifestation, obtaining a trivial ES in hands (0.15), temperature tunnel (0.14) and neck (0.06). The idea of the MBI concept is to verify the true effect of treatment or training programs. So, our results through MBI were presented as unclear. The high coefficient of variation could explain the unclear output because of large confidence intervals. The MBI also shows that 30 seconds of cooling between sets on the resistance exercise with BFR is not an effective strategy to increase the number of repetitions.

The duration, protocol, and type of cooling can influence performance. We did not find studies with cooling time in resistance training with less than 2.5 minutes. We hypothesized that the time of 30 seconds for recovery between sets might not have been enough to result in an ergogenic effect because of BFR, once it is known that vascular occlusion may overestimate the feeling of RPE and consequently decrease performance^{30,31}. This is due to possible mechanical deformation in the afferent nerves caused by the sphygmomanometer used for vascular occlusion, changing the afferent signals from the peripheral to the CNS and negatively interfering with performance³⁰. Therefore, we can hypothesize that one of the hypotheses for the ergogenic effect in the Kwon⁸ study called The Gate Control Theory may not occur in 30 seconds of cooling with BFR. The cooling time was adopted in this study according to the Kaatsu training methodology with time around 20 to 30 seconds^{1,32}, being shorter than the studies Kwon^{7,8} which was 3 minutes.

Conclusion

Therefore, we concluded that the 30 seconds of cooling between sets in resistance exercise with BFR was neither able to promote the increase in the number of repetitions, nor to reduce the RPE. For this reason, we do not encourage the use of the same cooling strategies to increase the number of repetitions during resistance training with BFR.

References

- Takarada Y, Nakamura Y, Aruga S, Onda T, Miyazaki S, Ishii N. Rapid increase in plasma growth hormone after low-intensity resistance exercise with vascular occlusion. J Appl Physiol 2000;88(1):61-5. Doi: 10.1152/jappl.2000.88.1.61.
- 2. Slysz J, Stultz J, Burr JF. The efficacy of blood flow restricted exercise: A systematic review & metaanalysis. J Sci Med Sport 2016;19(8):669-75. Doi: 10.1016/j.jsams.2015.09.005.
- Takarada Y, Takazawa H, Sato Y, Takebayashi S, Tanaka Y, Ishii N. Effects of resistance exercise combined with moderate vascular occlusion on muscular function in humans. J Appl Physiol 2000;88(6):2097-106. Doi: 10.1152/jappl.2000.88.6.2097.
- Morton RW, Oikawa SY, Wavell CG, Mazara N, McGlory C, Quadrilatero J, et al. Neither load nor systemic hormones determine resistance training-mediated hypertrophy or strength gains in resistance-trained young men. J Appl Physiol 2016;121(1):129-38. Doi: 10.1152/japplphysiol.00154.2016.
- 5. Fujita S, Mikesky A, Sato Y, Abe T. Fatigue characteristics during maximal concentric leg extension exercise with blood flow restriction. IJKTR 2008;3(2):27-31. Doi: 10.3806/ijktr.3.27.
- 6. Verducci FM. Interval cryotherapy and fatigue in university baseball pitchers. Res Q exercise sport 2001;72(3):280-7. Doi 10.1080/02701367.2001.10608961

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- Kwon YS, Robergs RA, Kravitz LR, Gurney BA, Mermier CM, Schneider SM. Palm cooling delays fatigue during high-intensity bench press exercise. Med Sci Sports Exerc 2010;42:1557-65. Doi: 10.1249/MSS.0b013e3181d34a53.
- Kwon YS, Robergs RA, Mermier CM, Schneider SM, Gurney AB. Palm cooling and heating delays fatigue during resistance exercise in women. J Strength Cond Res 2010;29(8):2261-9. Doi: 10.1519/JSC.0b013e31829cef4e.
- 9. Noakes TD. Fatigue is a brain-derived emotion that regulates the exercise behavior to ensure the protection of whole body homeostasis. Front Physiol 2012;3(82):1-13. Doi: 10.3389/fphys.2012.00082.
- Kwon YS, Robergs RA, Schneider SM. Effect of local cooling on short-term, intense exercise. J Strength Cond Res 2013;27(7):2046-54. Doi: 10.1519/JSC.0b013e3182773259.
- 11. Gordon N, Bogdanffy G, Wilkinson J. Effect of a practical neck cooling device on core temperature during exercise. Med Sci Sports Exerc 1990;22(2):245-9.
- 12. Tyler CJ, Wild P, Sunderland C. Practical neck cooling and time-trial running performance in a hot environment. Eur J Appl Physiol 2010;110(5):1063-74. Doi: 10.1007/s00421-010-1567-7.
- 13. Haddadin AS, Abreu MM, Silverman DG, Luther M, Hines RL. Noninvasive assessment of intracranial temperature via the medial canthal-brain temperature tunnel. Am Soc Anesthesiology Annual Meeting; 2005.
- 14. Schwartz MW, Woods SC, Seeley RJ, Barsh GS, Baskin DG, Leibel RL. Is the energy homeostasis system inherently biased toward weight gain? Diabetes 2003;52(2):232-8. Doi: 10.2337/diabetes.52.2.232.
- Umbel JD, Hoffman RL, Dearth DJ, Chleboun GS, Manini TM, Clark BC. Delayed-onset muscle soreness induced by low-load blood flow-restricted exercise. Eur J Appl Physiol 2009;107(6):687. Doi: 10.1007/s00421-009-1175-6
- 16. Madarame H, Neya M, Ochi E, Nakazato K, Sato Y, Ishii N. Cross-transfer effects of resistance training with blood flow restriction. Med Sci Sports Exerc 2008;40(2):258-63. Doi: 10.1249/mss.0b013e31815c6d7.
- 17. Takarada Y, Sato Y, Ishii N. Effects of resistance exercise combined with vascular occlusion on muscle function in athletes. Eur J Appl Physiol 2002;86(4):308-14. Doi: 10.1007/s00421-001-0561-5.
- 18. Foster C, Florhaug JA, Franklin J, Gottschall L, Hrovatin LA, Parker S, et al. A new approach to monitoring exercise training. J Strength Cond Res 2001;15(1):109-15.
- 19. Brown LE, Weir JP. ASEP procedures recommendation I: Accurate assessment of muscular strength and power. J Exerc Physiol Online 2001;4(3):1-21.
- Takano H, Morita T, Iida H, Asada K-i, Kato M, Uno K, et al. Hemodynamic and hormonal responses to a short-term low-intensity resistance exercise with the reduction of muscle blood flow. Eur J Appl Physiol 2005;95(1):65-73. Doi: 10.1007/s00421-005-1389-1.
- 21. Rhea MR. Determining the magnitude of treatment effects in strength training research through the use of the effect size. J Strength Cond Res 2004;18(4):918-20.
- 22. Thomas JR, French KE. The use of meta-analysis in exercise and sport: A tutorial. Res Q Exercise Sport 1986;57(3):196-204. Doi: 10.1080/02701367.1986.10605397.
- 23. Cohen J. Statistical power analysis for the behavioral sciences. Hillsdale: Laurence Erlbaum Associates;1988.
- 24. Batterham AM, Cox AJ. Spreadsheets for analysis of controlled trials, with adjustment for a subject characteristic. Sportscience 2006;10:46-51.
- Hopkins JT, Ingersoll CD, Edwards J, Klootwyk TE. Cryotherapy and transcutaneous electric neuromuscular stimulation decrease arthrogenic muscle inhibition of the vastus medialis after knee joint effusion. J Athlet Train 2002;37(1):25-31.
- Palmieri-Smith RM, Leonard-Frye JL, Garrison CJ, Weltman A, Ingersoll CD. Peripheral joint cooling increases spinal reflex excitability and serum norepinephrine. Int J Neurosci 2007;117(2):229-42. Doi: 10.1080/00207450600582702.
- Tucker R. The anticipatory regulation of performance: the physiological basis for pacing strategies and the development of a perception-based model for exercise performance. Br. J. Sports Med 2009;43(6):392-400. Doi: 10.1136/bjsm.2008.050799.
- Fröhlich M, Emrich E, Pieter A, Stark R. Outcome effects and effects sizes in sport sciences. IJSSE 2009;3(3):175-9.
- 29. Hopkins W, Marshall S, Batterham A, Hanin J. Progressive statistics for studies in sports medicine and exercise science. Med Sci Sports Exerc 2009;41(1):3-13. Doi: 10.1249/MSS.0b013e31818cb278.
- McCloskey D. Corollary discharges: motor commands and perception. Comprehensive Physiology; 1981. Doi: 10.1002/cphy.cp010232.
- Takarada Y, Nozaki D, Taira M. Force overestimation during tourniquet-induced transient occlusion of the brachial artery and possible underlying neural mechanisms. J Neurosci 2006;54(1):38-42. Doi: 10.1016/j.neures.2005.10.004.
- 32. Brandner CR, Warmington SA, Kidgell DJ. Corticomotor excitability is increased following an acute bout of blood flow restriction resistance exercise. Front Hum Neurosci 2015;9:652. Doi: 10.3389/fnhum.2015.00652

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