ELSEVIER

Journal of Thermal Biology 29 (2004) 839–843

Journal of
THERMAL BIOLOGY

www.elsevier.com/locate/jtherbio

Adaptive changes in muscular performance and circulation by resistance training with regular cold application

N. Ohnishi[a,*], M. Yamane[b], N. Uchiyama[a], S. Shirasawa[a], M. Kosaka[b], H. Shiono[c], T. Okada[c]

[a]*Department of Human Sciences, Aichi Mizuho College, Hiratobashi, Toyota 470-0394, Japan*
[b]*Laboratory for Exercise Physiology and Biomechanics, School of Health and Sport Sciences, Chukyo University, Kaizu-cho, Toyota 470-0393, Japan*
[c]*Department of Physiology, Aichi Medical University, Nagakute, Aichi 480-1195, Japan*

Abstract

(1) Sixteen male subjects participated in resistance training comprising three sets of 8-handgrip exercises at a workload that could be performed no more than eight times, three times a week for 6 weeks. Eight subjects immersed their experimental forearm in cold water ($10 \pm 1\,^{\circ}C$) for 20 min following each training period, while the remaining eight served as controls. (2) Muscular endurance with rhythmic handgrips significantly ($p < 0.01$) increased in both groups after the training period with a non-significant difference between groups. The relative diameter of ultrasonography-evaluated brachial artery failed to increase in the immersion group despite a significant increase ($p < 0.05$) of that in the control group after training. (3) Regular post-exercise cold application might attenuate the improvement in muscular endurance, possibly in association with reduced vascular remodeling.

Keywords: Ice treatment; Cryotherapy; Cold; Exercise; Training; Vascular remodeling

1. Introduction

Cold application (ice treatment or cryotherapy) is an effective treatment for acute sports injuries and soft tissue bruising, acting by decreasing tissue temperature, blood flow and inflammatory cell invasion of muscle and thereby inhibiting excessive local inflammatory reactions (Knight, 1995; Thorlacius et al., 1998). Such treatment has also been applied to heal exercise-induced damage following vigorous exercise among athletes. Recently, cold application has been regularly used to facilitate recovery from physical and mental fatigue following exercise in which minimal muscular damage occurs. Knowledge of the physiological basis for the application of this treatment to uninjured muscles would be assumed necessary in order to ensure the effectiveness of athletic conditioning. However, few studies have evaluated the effects of regular post-exercise cold application to uninjured muscles. The authors previously observed that at 4 weeks of resistance training, improvements in local muscular endurance were less pronounced with regular post-exercise application of cold (Teruya et al., 2002).

Although physiological mechanisms to explain our previous results are unclear, local muscular endurance might depend on the metabolic capacity of working muscle cells and/or blood perfusion to working muscles (Andersen and Henriksson, 1977). It is reported that not only aerobic exercise training (Baynard et al., 2003;

*Corresponding author. Tel.: +565-43-0111; fax +565-46-5220.
E-mail address: ohnishi@mizuho-c.ac.jp (N. Ohnishi).

0306-4565/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jtherbio.2004.08.069

Gavin and Wagner, 2001; Gavin et al., 2004; Laughlin et al., 2004; Maeda et al., 2001; Miyachi et al., 2001) but also resistance training (Franke et al., 1998; Rus et al., 2003), produces vascular growth and increases vasodilatory responsiveness. Increased flow-related shear stress within the vessels and metabolic requirements of skeletal muscles, including those resulting from tissue hypoxia, are considered to be the primary factors leading to these vascular adaptations by inducing the upregulation of angiogenic growth factors. Moreover, mechanical loading to skeletal muscles is reported to increase local inflammatory cell infiltration in the presence or absence of overt injury (McLoughlin et al., 2003; Pizza et al., 2002). Inflammatory cells are capable of producing reactive oxygen species, cytokines, and growth factors that are known to influence skeletal muscle adaptation. On the other hand, cold application decreases tissue temperature, blood flow, and metabolic rate. It is considered that these cold-induced events could decrease flow-mediated shear stress within the vessels, metabolic requirements of skeletal muscles and/or neutrophil invasion of muscle during the recovery period following exercise, which would affect vascular adaptation in response to resistance training.

The purpose of this study was to examine the effect of regular post-exercise cold application on local muscular endurance and vascular adaptation in response to resistance training. In addition, plasma interleukin-6 (IL-6, a proinflammatory cytokine) and vascular endothelial growth factor (VEGF, a very potent angiogenic agent that acts as a specific mitogen for vascular endothelial cells) were monitored as markers of inflammation and vascular adaptation in response to a bout of exercise during the training period.

2. Methods

2.1. Subjects

Sixteen healthy young male volunteers [age 20.7 ± 2.3 years, weight 66.2 ± 8.8 kg and height 173.3 ± 5.6 cm (mean $\pm$ SD)] participated in this study, after providing written informed consent. Subjects were randomly assigned to one of two groups.

2.2. Experimental protocol

The subjects performed three sets of eight-handgrip exercises with their left, non-dominant, hand using a weight-loaded handgrip ergometer at a that workload could be performed no more than eight times. Subjects' right arms served as controls. Participants repeated this resistance training protocol three times a week for a total of 6 weeks. Eight of the 16 subjects immersed their left forearm in cold water for 20 min following the last set of handgrip exercises during each protocol (immersion group). The cold water was stirred and its temperature was maintained at $10 \pm 1\,^{\circ}C$ using a constant temperature water bath unit (Thermal Robo, AS ONE Corporation, Osaka, Japan) and cooling unit (Coolpipe 300-L, TAITEC Co. Ltd., Tokyo, Japan). The other eight subjects served as controls, remaining in a sitting position for 20 min following training without immersing their forearm in cold water (control group).

Before and after the 6-week training period, forearm circumference, maximal handgrip strength, muscular endurance, and brachial artery diameter in a resting condition were measured in both arms. Changes of these measurements in the immersion group were compared against those in the control group.

IL-6 and VEGF have been known to stimulate inflammation process and vascular adaptation, respectively. Exercise has been shown to increase Plasma IL-6 and VEGF. In order to examine the possibility that the cold application might affect exercise-induced adaptive changes via these processes, plasma IL-6 and VEGF concentrations were measured before and after the training protocol at the end of fourth week of the training period in six subjects in the control group and seven subjects in the immersion group.

2.3. Measurements

Forearm circumference was measured at the largest part of the forearm. Maximal handgrip strength was measured using a digital grip strength meter (ED100N, Yagami Inc., Nagoya, Japan). Local muscular endurance was evaluated by counting the number of handgrip repetitions performed every 2 s until the subject could not tolerate any further contraction at the precise pace using a weight-loaded handgrip ergometer. A metronome was used to precisely pace the handgrip. Subjects performed the handgrip exercise in a supine position at a workload of 30% of maximal pre-training handgrip strength. Brachial artery diameter was measured by ultrasonography (EUB-565, Hitachi Medical Corp., Tokyo, Japan) in the supine position with the arm in 90° of abduction. The brachial artery was imaged above the antecubital fossa in the longitudinal plane, with diameter determined as the distance between the lumen–intima interfaces of the anterior and posterior walls.

Plasma IL-6 and VEGF concentrations were measured by analyzing blood samples taken from the left antecubital vein before, 30, 60, 90, and 120 min after the handgrip training protocol. Blood samples were withdrawn into EDTA-containing tubes and centrifuged, and plasma was stored at $-80\,^{\circ}C$ until analysis. Plasma concentrations of IL-6 and VEGF were determined by quantitative sandwich ELISA, using a 96-well microtiter plate (BioSource International Inc., Camarillo, CA

USA). All samples and provided standards were analyzed in duplicate. An ultra-sensitive kit was used for IL-6 detection.

2.4. Statistical analysis

All values are reported as mean ± SD. Differences across the testing conditions were determined by two-way analysis of variance. Significant differences were accepted at $p < 0.05$.

3. Results

No subjects experienced muscle soreness or injury during or after the training period. The circumference of the trained forearm tended to increase after training in both groups (from 25.0 ± 1.5 to 25.4 ± 1.7 cm in the control group and from 25.3 ± 1.7 to 25.7 ± 1.8 cm in the immersion group) with no significant differences detected. The maximal handgrip strength in the trained arm did not change significantly in either group over the training period (from 45.1 ± 7.9 to 45.9 ± 9.1 kg in the control group and from 44.3 ± 6.4 to 44.6 ± 6.5 kg in the immersion group). Exhaustion time for repeated handgrip exercise in both arms increased significantly ($p < 0.01$) in the control group and the immersion group after training (Fig. 1A and B). Increase in that of the immersion group tended to be smaller as compared with the control group (nonsignificant difference) after training. Brachial artery diameter in the trained arm showed no significant differences before and after training in both groups (Fig. 2). Left/right (experimental arm/control arm) ratio for brachial artery diameter in the control group significantly ($p < 0.05$) increased, while that of the immersion group did not change (Fig. 3).

Plasma concentration of IL-6 from samples collected at the fourth week of training tended to increase with time after the 3 sets of handgrip exercise in both groups (Fig. 4A). In the immersion group, plasma IL-6 concentration was significantly increased at 120 min after exercise compared to pre-exercise levels ($p < 0.05$). No significant changes in plasma VEGF concentration were observed in response to the handgrip exercise in either group (Fig. 4B). No significant differences in plasma IL-6 or VEGF levels were observed between groups.

4. Discussion

The 6-week program of resistance training with regular post-exercise cold application tended to be shown to inhibit remodeling of the brachial artery. This coincided with the attenuation of improvement in local muscular endurance in response to resistance training

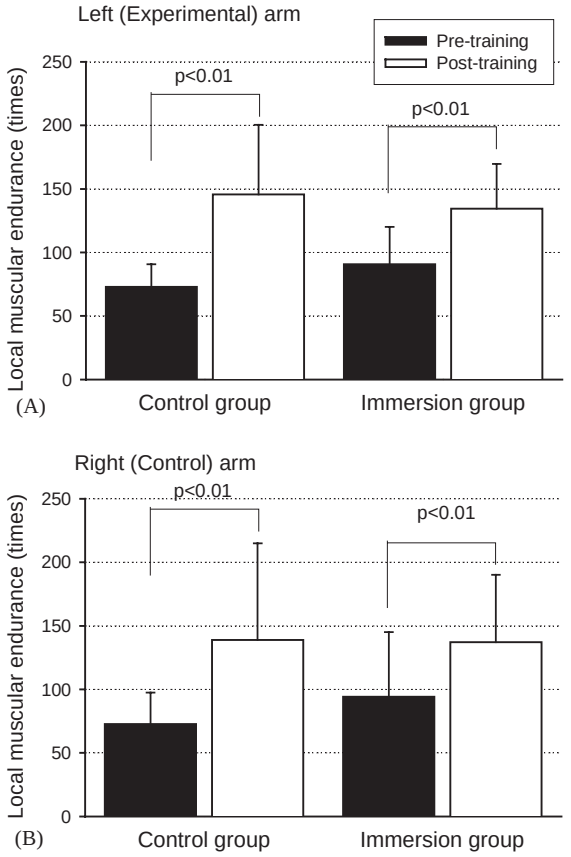

Fig. 1. Local muscular endurance of the left (experimental) arm (A) and the right (control) arm (B) evaluated by counting repetitions of rhythmic handgrips performed until exhaustion using a weight-loaded handgrip ergometer before and after 6 weeks of training in the control and the immersion group. Mean + SD.

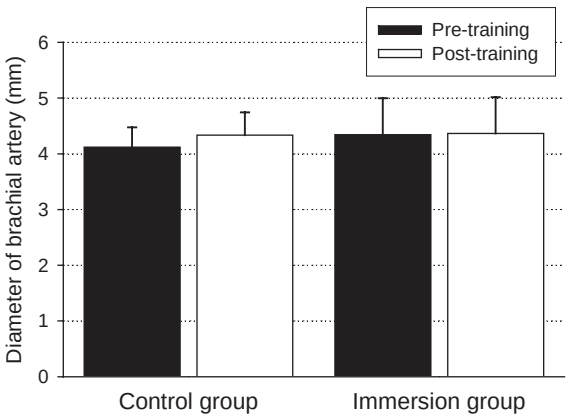

Fig. 2. Diameter of the brachial artery in a resting condition of the trained arm measured by ultrasonography before and after the training period in the control and the immersion group. Mean + SD.

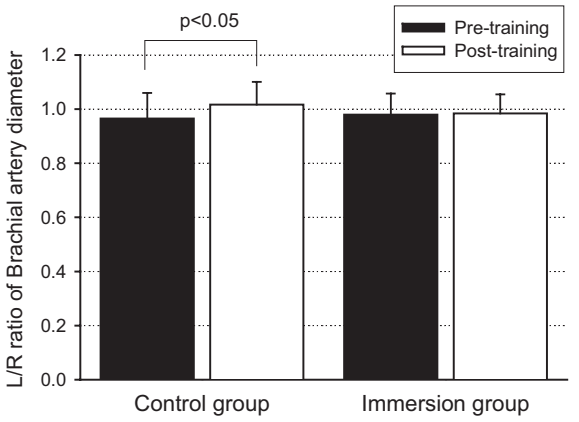

Fig. 3. Left/right (experimental arm/control arm) ratio of the diameter of the brachial artery measured by ultrasonography before and after the training period in the control and the immersion group. Mean + SD.

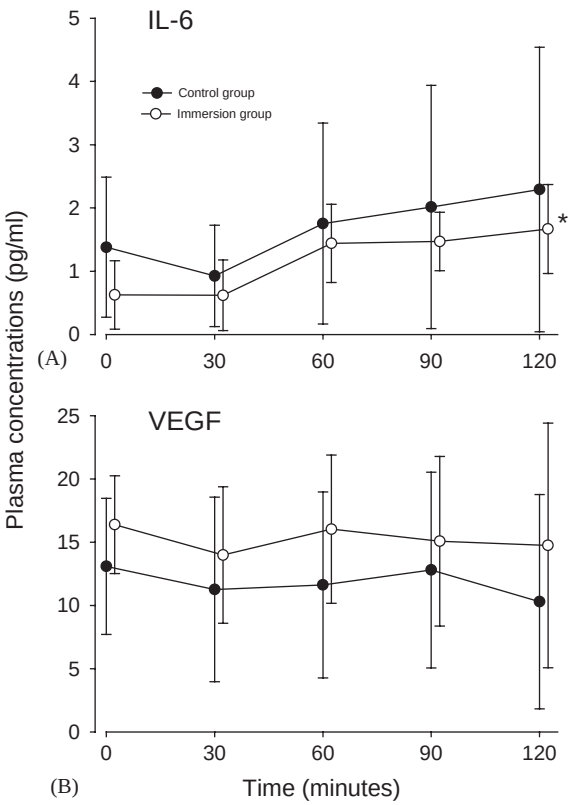

Fig. 4. Time-related changes in plasma concentration of IL-6 (A) and VEGF (B) determined by quantitative sandwich ELISA at the end of the fourth week of training in the control and the immersion group. Mean ± SD. *Significantly different from pre-exercise state ($p < 0.05$).

observed in our previous study (Teruya et al., 2002) and in the present study (nonsignificant tendency). A possible mechanism for these observed changes would be reduced vascular adaptation due to regular post-exercise application of cold, leading to attenuated improvement in muscular endurance. However, the difference in local muscular endurance between groups was less pronounced when compared with that seen in the previous study. In the present study, local muscular endurance showed a similar tendency to improve even in control arms that did not undergo training. Because the subjects in this study were physically untrained, it is possible that a training-induced increase in centrally derived motor activity might have contributed to elevated muscular activity and also obscured the local effect with cold application.

It is possible that attenuated modification of the brachial artery in response to training results in cold-induced vasoconstriction, a decrease in metabolic rate and/or limitation of neutrophil invasion during the recovery period. Reduced blood flow due to the application of cold might decrease biomechanical stress to vascular endothelial cells during the recovery period, which might in turn lessen the stimulus for vascular adaptation. If neutrophil invasion of muscle tissue decreased with cold application, inflammatory reactions promoting vascular adaptation might also be inhibited. However, no physiological evidence was available to support these speculations, as no significant differences were observed in plasma IL-6 or VEGF levels in response to cold application. IL-6 and VEGF levels have been examined in response to systemic exercise in many previous studies. As muscle mass influences the performance of handgrip exercise, it is possible that the workload employed in the present study was insufficient to elicit significant change in plasma IL-6 and VEGF levels. Moreover, it has been reported that neutrophil invasion (Pizza et al., 2002) and VEGF mRNA expression (Gavin and Wagner, 2001; Richardson et al., 2000) in response to exercise, decrease with advancing exercise training. Further studies under different experimental conditions, including increased work load and duration, are needed to ascertain the behavior of these markers in response to cold application during training.

Ice treatment has obvious therapeutic benefits for tissue in which injury is apparent. However, the present results indicate the possibility that regular application of cold, ice treatment or cryotherapy, to uninjured tissue following exercise might diminish anticipated improvements in physical performance. However, because athletes who are already well trained might have smaller reserves for further adaptation, the influence of cold application might be minor. In addition, the type and intensity of exercise varies considerably, with exercise duration tending to be much longer in most training

programs. Decrease in the exercise-induced stimulus for adaptation due to application of cold might also be minor under conditions of longer exercise duration. In order to apply cryotherapy for conditioning in sports activities, it is valuable to ascertain any potentially disadvantageous effects of such therapy. Further studies are needed to elucidate the mechanisms behind the possible suppression of improved local muscular endurance and vascular adaptation in response to training that utilizes application of cold.

Acknowledgements

We sincerely thank Dr. Motohiko Miyachi (National Institute of Health and Nutrition, Japan) for valuable advice regarding determination of arterial diameter using ultrasonography. This study was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (13680065).

References

Andersen, P., Henriksson, J., 1977. Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise. J. Physiol. 270, 677–690.

Baynard, T., Miller, W.C., Fernhall, B., 2003. Effects of exercise on vasodilatory capacity in endurance- and resistance-trained men. Eur. J. Appl. Physiol. 89, 69–73.

Franke, W.D., Stephens, G.M., Schmid, P.G. III., 1998. Effects of intense exercise training on endothelium-dependent exercise-induced vasodilatation. Clin. Physiol. 18, 521–528.

Gavin, T.P., Wagner, P.W., 2001. Effect of short-term exercise training on angiogenic growth factor gene responses in rats. J. Appl. Physiol. 90, 1219–1226.

Gavin, T.P., Robinson, C.B., Yeager, R.C., England, J.A., Nifong, L.W., Hickner, R.C., 2004. Angiogenic growth factor response to acute systemic exercise in human skeletal muscle. J. Appl. Physiol. 96, 19–24.

Knight, K.L., 1995. Cryotherapy in Sport Injury Management. Human Kinetics.

Laughlin, M.H., Woodman, C.R., Schrage, W.G., Gute, D., Price, E.M., 2004. Interval sprint training enhances endothelial function and eNOS content in some arteries that perfuse white gastrocnemius muscle. J. Appl. Physiol. 96, 233–244.

Maeda, S., Miyauchi, T., Kakiyama, T., Sugawara, J., Iemitsu, M., Irukayama-Tomobe, Y., Murakami, H., Kumagai, Y., Kuno, S., Matsuda, M., 2001. Effects of exercise training of 8 weeks and detraining on plasma levels of endothelium-derived factors, endothelin-1 and nitric oxide, in healthy young humans. Life Sci. 69, 1005–1016.

McLoughlin, T.J., Mylona, E., Hornberger, T.A., Esser, K.A., Pizza, F.X., 2003. Inflammatory cells in rat skeletal muscle are elevated after electrically stimulated contractions. J. Appl. Physiol. 94, 876–882.

Miyachi, M., Tanaka, H., Yamamoto, K., Yoshioka, A., Takahashi, K., Onodera, S., 2001. Effects of one-legged endurance training on femoral arterial and venous size in healthy humans. J. Appl. Physiol. 90, 2439–2444.

Pizza, F.X., Koh, T.J., McGregor, S.J., Brooks, S.V., 2002. Muscle inflammatory cells after passive stretches, isometric contractions, and lengthening contractions. J. Appl. Physiol. 92, 1873–1878.

Richardson, R.S., Wagner, H., Mudaliar, S.R.D., Saucedo, E., Henry, R., Wagner, P.D., 2000. Exercise adaptation attenuates VEGF gene expression in human skeletal muscle. Am. J. Physiol. Heart Circ. Physiol. 279, H772–H778.

Rus, R.R., Ponikvar, R., Kenda, R.B., Buturovic-Ponikvar, J., 2003. Effect of local physical training on the forearm arteries and veins in patients with end-stage renal disease. Blood Purif. 21, 389–394.

Teruya, H., Ohnishi, N., Yamane, M., Kosaka, M., 2002. The effect of icing on the adaptive chnges in muscular performance resulting from the strength training. Jpn. J. Phys. Fitness Sports Med. 51, 572 (in Japanese, Abstract).

Thorlacius, H., Vollmar, B., Westermann, S., Torkvist, L., Menger, M.D., 1998. Effect of local cooling on microvascular hemodynamics and leukocyte adhesion in the striated muscle of hamsters. J. Traum. 45, 715–719.