Does Regular Post-exercise Cold Application Attenuate Trained Muscle Adaptation?

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Abstract

This study examined the effects of regular post-exercise cold application on muscular and vascular adaptations induced by moderate-intensity resistance training. 14 male subjects participated in resistance training: 5 sets of 8 wrist-flexion exercises at workload of 70–80% of the single repetition maximum, 3 times a week for 6 weeks. 7 subjects immersed their experimental forearms in cold water (10 ± 1°C) for 20 min after wrist-flexion exercises (cooled group), and the other 7 served as control subjects (noncooled group). Measurements were taken before and after the training period; wrist-flexor thickness, brachial-artery diameter, maximal muscle strength, and local muscle endurance were measured in upper extremities. Wrist-flexor thicknesses of the experimental arms increased after training in both groups, but the extent of each increase was significantly less in the cooled group compared with the noncooled group. Maximal muscle strength and brachial-artery diameter did not increase in the cooled group, while they increased in the noncooled group. Local muscle endurance increased in both groups, but the increase in the cooled group tended to be lower compared to the noncooled group. Regular post-exercise cold application to muscles might attenuate muscular and vascular adaptations to resistance training.

Introduction

Cold application in the form of ice treatment or cryotherapy is one component of the well-established rest, ice, compression and elevation (RICE) treatment for acute sports injuries such as muscle contusions, dislocations and sprains [28]. This treatment attenuates secondary hypoxic injury, swelling, pain and muscle spasm by decreasing tissue temperature, metabolism, blood flow and vascular permeability [21, 35]. The efficacy of cold application has also been investigated for its usefulness against microscopic damage such as sarcomere length instability and disruption associated with delayed-onset muscle soreness (DOMS). Systematic reviews have reported that cold-water immersion is an effective strategy to decrease post-exercise DOMS [2,3,24]. Under the premise that inflammatory processes inhibit muscle reconstitution, cryotherapy, which was introduced early in sports medicine to treat muscle and ligament strains and lesions [36], may also be adequate as a muscle treatment after strenuous exercise. However, the protective mechanism specific to muscle tissue has not been elucidated. On the other hand, some researchers have expressed doubt regarding exercise-induced myofiber microdamage as a pathological state [29]. Different from trans-fiber and myotendinous ruptures where repair may involve growth of scar tissue, the responses of the myofibers, which are multinuclear syncytial structures, to microdamage are essentially regenerative, proceeding from the proliferation and incorporation of satellite cells, such as stem cells, into the myofibers to produce new sarcomeres [5]. Recently, cold application has been regularly used to facilitate recovery from physical and mental fatigue following exercise in which minimal muscle damage occurs. Thus trained muscles with presumed myofiber microdamage may react differently to cryotherapy than muscles with macroscopic damage due to muscle strain.

Understanding the physiological effects of cold treatment in muscle is necessary to understand its potential effects on athletic conditioning. However, few studies have addressed this issue.
Previous humans study conducted in our laboratory indicate that improvement in aerobic capacity, local muscle endurance and vascular adaptation to moderate-intensity resistance and endurance training was less pronounced with regular post-exercise cold application [41]. This indicates that regular post-exercise cold application of uninjured tissue during a long-term training period for athletes could diminish the anticipated improvement in physical performance, even though post-exercise cold application may transiently facilitate recovery from physical and mental fatigue after exercise. Accordingly, to ensure that athletes effectively and safely use post-exercise cold application, it is necessary to first investigate the effects of regular post-exercise cold application to uninjured muscles.

Decreasing tissue metabolism, blood flow and inflammatory responses by cold application would not be considered favorable during rehabilitation because the combined effect could impair tissue regeneration [22]. Specifically, inflammatory cells, such as neutrophils and macrophages, can mediate impaired tissue regeneration by the release of a number of growth-promoting and cytokines [4,20]. It has been suggested that these factors also contribute to exercise-training adaptations, such as skeletal muscle hypertrophy and angiogenesis [4,17]. McLaughlin et al. [30] reported that mechanical loading to skeletal muscles increased local inflammatory cell infiltration in the absence of overt injury, and these cells might influence skeletal muscle. Deal et al. [8] indicated that cold-induced reduction in vascular permeability could attenuate inflammatory responses by decreasing leukocyte transmigration. Thus regular post-exercise cold applications to muscles may attenuate muscular and vascular adaptations in response to exercise training. However, previous study conducted in our laboratory identified only attenuation of vascular adaptation in response to resistance training and not muscular adaptation [41]. The lack of muscle hypertrophy in that study could have resulted from using the low-intensity handgrip exercise.

Therefore the aim of the present study was to examine the effects of regular post-exercise cold application on muscle hypertrophy and maximal muscle strength, and to confirm vascular adaptation and local muscle endurance in response to aerobic capacity, local muscle endurance and vascular adaptation to moderate-intensity resistance and endurance training. The other 7 subjects who were recreationally active but had no formal weight training experience within the past year. 13 declared their dominant arms as right and one as left. Subjects were randomly assigned to one of the 2 groups. Approval was obtained from the ethics committee of the Chukyo University for these experiments. Moreover, this study met the ethical standards of the International Journal of Sports Medicine [15].

Experimental protocol
All subjects performed a resistance-training program for the unilateral wrist flexors 3 times a week for 6 weeks. One training session consisted of 5 sets of 8 wrist-flexion exercises with the non-dominant arm (experimental arm) using a custom-built, weight-loaded wrist flexion ergometer. During the 6 weeks of training, the subjects trained with loads of 70–80% of a single repetition maximum (1 RM). Loads of 70% RM were used during the first 3 weeks, and loads of 80% RM were used during the last 3 weeks. Before the onset of the resistance-training program, subjects completed an intensity-matched (70–80% of 1 RM) acute bout of resistance exercise. The acute exercise consisted of 4 sets and 8 repetitions of wrist-flexion exercises. The final set was performed to volitional failure. Every 6 training sessions, RM was reassessed and the training weights adapted. Subjects’ dominant arms served as controls (control arm).

The forearm was fixed in the armrest of the wrist flexion ergometer in a supinated position and the elbow angle was kept at 90° so that only wrist-joint motion was allowed. The subject held the handle of the wrist-flexion ergometer, which was aligned such that the pivot of the handle centered on the axis of the wrist joint. A metal wire from the handle was attached to a weight by a pulley. The subject flexed the wrist from 40° extension to 60° flexion in 1 s (concentric phase), and returned to the starting position in 2 s (eccentric phase). This custom-made wrist-flexion ergometer applied a load during both the 1-s concentric phase and the 2-s eccentric phase. A metronome was used to ensure precise pacing. A 2-min rest period was provided between sets. 3 min after the fifth set, 7 of 14 subjects immersed their experimental arms from the wrist to just above the elbow joint in cold water for 20 min (cooled group). The cold water was stirred and its temperature was maintained at 10°C ± 1°C using a constant temperature water bath unit (Thermal Robo, AS ONE Corporation, Osaka, Japan) and cold-application unit (Coolpipe 300-L, TAIITEC Co. Ltd., Tokyo, Japan). The other 7 subjects who served as controls remained in a sitting position for 20 min after training without cold-water immersion (noncooled group). In both the groups, plasma IL-6 and VEGF levels were measured before and after the training session on the first day of the training period.

Before and 3 days following the 6-week training period, wrist-flexor thicknesses, forearm circumference, brachial-artery diameter, maximal strength of wrist flexors and local muscle endurance were measured in the experimental and control arms of each subject. Changes of these measurements were compared between the 2 groups.
All experiments were performed in a laboratory maintained at 25°C ± 1°C air temperature and approximately 50% relative humidity.

Measurements

Wrist-flexor thicknesses and brachial-artery diameter were measured using a B-Mode ultrasonograph (EUB-565, Hitachi Medical Corp., Tokyo, Japan) in the resting supine position with the arm in full extension and 90° of abduction. An X-Y positioning device with an accuracy of 1 mm was placed 30 cm above the subject’s forearm. To reproduce the measuring position in tests before and after training period, the medial epicondyle of the humerus and the extreme ends of the positions of wrist’s minimum circumference were adjusted to the same positions using the thin beam of a laser pointer mounted to the device for each subjects. The wrist flexors were imaged at one-third of the distance distal from the medial epicondyle to the styloid process. Muscle thicknesses were measured as the distance from the inner border of the subcutaneous adipose layer to the outer surface of the ulna measured while the subjects tightly grasped their hands. The brachial artery was imaged at a position of the upper arm’s maximum circumference, between the biceps and triceps muscles, with the diameter determined as the distance between the lumen-intima interfaces of anterior and posterior walls. Variations of these imaging positions were also minimized using the X-Y positioning device. All images were printed out and analyzed using computerized image-processing software. These analyses were performed by the same investigator, who was blinded to the assignment of the noncooled and cooled groups.

Maximal isometric strength of wrist flexors was measured at a neutral wrist angle in the same posture as the training protocol using a digital strength meter (KE-D300, Yagami Inc., Nagoya, Japan). Local muscle endurance was measured with the weight-loaded wrist-flexion ergometer by counting the number of wrist-flexion repetitions performed every 2 s until the subject could not maintain the precise metronome pace. Subjects performed the wrist-flexion exercise at a workload of 35% of baseline 1 RM. The performance tests were not blinded to the assignment of the noncooled and cooled groups. To maintain consistency among the research assistants involved in data collection, no verbal encouragement was given during the performance tests. Forearm circumference was measured by a tape measure at the largest part of the forearm. The presence or absence of symptoms such as muscle soreness were recorded as ‘yes’ (present) or ‘no’ (absent) before and after every training session.

Plasma IL-6 and VEGF levels were measured in blood samples taken from the antecubital vein of the experimental arm before and after wrist-flexion exercises. Blood sampled from that site reflects, in large part, drainage from the wrist flexors. Blood samples were drawn into EDTA tubes and, centrifuged, and the plasma was stored at −80°C until analysis. Plasma IL-6 and VEGF levels 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. IL-6 intra-assay coefficient of variation (CV) was 6.2% and the interassay CV was 4.4%. VEGF intra-assay CV was 7.9% and the interassay CV was 19.0%. The minimum detectable levels of IL-6 and VEGF were < 0.104 pg/ml and < 5 pg/mL, respectively.

Statistical analysis

Results were reported as mean ± SD. Two-way (time × group) analysis of variance (ANOVA) for repeated measures was used to determine the effect of post-exercise cold application on all variables. When ANOVA revealed significant interactions, a paired t-test was used to compare differences between values before and after training period in each group. Relative change after exercise was analyzed by an unpaired t-test. Statistical significance was set at P values of < 0.05 for all comparisons.

Results

All subjects completed the 6-week wrist-flexion exercise-training program. No subjects had symptoms of muscle soreness before and after any training session.

In the experimental arms, the wrist-flexor thicknesses (Fig. 1 left panel) and the forearm circumferences (Fig. 2 left panel) significantly increased after training in both the noncooled and cooled groups. The extents of increase in the wrist-flexor thicknesses (Fig. 1 right panel) and the forearm circumference (Fig. 2 right panel) were significantly less in the cooled group.
compared with the noncooled group (P<0.05, P<0.05, respectively). Maximal isometric strength of wrist flexors significantly increased in the noncooled group (P<0.05) but not in the cooled group (Fig. 3). Brachial-artery diameter significantly increased in the noncooled group (P<0.05) but not in the cooled group (Fig. 4). Time to exhaustion significantly increased after training in both the groups (main effect for time, P<0.05; Fig. 5 left panel). However, there was no difference between the 2 groups. The relative increase in this measure for the cooled group tended to be less than that for the noncooled group, but the difference was not significant (Fig. 5 right panel).

Plasma IL-6 and VEGF levels did not change significantly in response to wrist-flexion exercise in either group, and no differences were detected between the 2 groups (Fig. 6a, b). In the control arms, there were no changes after training in either groups, except for a significant decrease in the brachial-artery diameters within the noncooled group.

Discussion

The key findings of the present study are as follows. First, regular post-exercise cold application to muscles might attenuate skeletal muscle hypertrophy in response to resistance training. This parallels inhibition of increase in maximal isometric strength of the wrist flexors. Second, post-exercise cold application might inhibit vascular remodeling with subsequent improvement in local muscle endurance after resistance training, supporting our previous findings [41].

The main purpose of cold application among athletes is treatment for acute sports injuries, and there is no doubt of its effectiveness. However, cold application has also been used to facilitate recovery from physical and mental fatigue following exercise. If the inhibitory effects of cold application identified in the present study occur in athletes who regularly use ice treatments after exercise, they may not be able to attain expected improvements in physical performance. This seems disadvantageous for sports conditioning, in contrast to the benefit for treating acute sports injuries.

The precise mechanism that regular post-exercise cold application attenuated training effects was not elucidated in the present study. Several stressors such as heat exposure induce production of heat-shock proteins (HSPs), also called stress proteins [27]. The roles of HSPs in skeletal muscle hypertrophy are considered important [13,40]. Stressors that result in exercise-induced HSPs include not only muscle hyperthermia [34] but also stretching of muscle fibers, muscular ischemia, glycogen depletion, and oxygen radicals release [9]. Large amounts of metabolic heat are generated in exercising skeletal muscles. In the present study, intramuscular temperature was not measured, but the development of moderate degrees of hyperthermia...
in the exercising muscle can be safely presumed despite removal of heat by the circulation of blood. In the case of eccentric exercise, transformation of external negative work into intramuscular heat is an additional factor contributing to muscular hyperthermia. There is at least indirect evidence from studies on myocardial adaptation that HSP induction in this particular striated muscle under exercise in rats did not occur when exercise-associated hyperthermia was suppressed by cool ambient conditions [16]. In vitro studies have shown that HSPs are among proteins that are induced not only by mechanical muscle stress and associated metabolic changes but also by temperature, suggesting that muscle mass may be increased by enhanced HSP formation under the influence of muscular hyperthermia [13]. Furthermore, mechanically induced skeletal muscle atrophy in vivo was shown to be attenuated in rats by heat stress [32]. Taken together, HSP induction under the combined influence of muscular stress and muscular hyperthermia may be an important contributor to beneficial training effects, such as myofiber regeneration and hypertrophy.

Myofiber microdamage and cellular and humoral events induced by resistance training within skeletal muscles must be considered as a physiological precondition not only for repair processes, such as myofiber regeneration but also for the adaptive processes leading to muscle hypertrophy arising from satellite cell proliferation and recruitment. Moreover, altered expression of myosin isoforms renders the trained muscles more capable of dealing with the imposed exercise [25]. Heat exposure may add to the stimulation of myogenic satellite cells under the influence of insulin-like growth factor 1 (IGF-1) [14]. Yoshihara et al. [42] reported that heat stress increased phosphorylation in a temperature-dependent manner in rat skeletal muscle and may itself be a key stimulator of Akt/mTOR signaling. Post-exercise cooling of a working muscle would at least remove the contribution of muscular hyperthermia to the training effects. Johnson et al. [19] reported that the gastrocnemius muscle temperature decreased by about 10°C after leg immersion in water of 10°C for 20 min. For the present study, muscle hyperthermia may be presumed for some duration after the end of exercise without cold application, but a state of distinctly nonphysiological muscle hypothermia is rapidly attained in the cooled arm. The metabolic response to resistance exercise is markedly influenced by the muscle environment, which may influence molecular and humoral processes for long-term adaptation during the recovery period [7]. Nemet et al. [33] reported that local ice therapy immediately following sprint-interval training was associated with greater decreases in both pro- and anti-inflammatory cytokines and anabolic hormones. Thus apart from partial suppression of HSP induction, the law of Arrhenius suggests that slowing of these processes associated with muscle regeneration and hypertrophy in the cooled muscles relative to noncooled muscles. Acute IL-6 release might occur from contracting muscle cells, where it contributes to numerous metabolic pathways.
Furthermore, alterations in IL-6 levels may be because of elevations in muscle temperature and metabolic status. However, plasma IL-6 levels in this study did not change significantly in response to wrist-flexion exercise in both the groups.

McLoughlin et al. [30] suggested that mechanical loading applied to skeletal muscles increased the infiltration of inflammatory cells, such as neutrophils and macrophages, in the presence or absence of overt injury. The role of neutrophils and macrophages in skeletal muscle is generally considered to be limited to phagocytosis of cellular debris following overt injury. However, inflammatory cells can also release cytokines and growth factors, such as fibroblast growth factor, IGF-1, and transforming growth factor-β1 [4, 17]. These inflammatory cell-derived products stimulate proliferation and differentiation of satellite cell-derived myogenic precursor cells, thereby contributing to muscle hypertrophy [4, 17, 20]. Deal et al. [8] reported that cold application inhibited leukocyte transmigration and inflammatory responses by decreasing microvascular permeability. The post-exercise cold application used in the present study might have influenced muscle adaptation as a result of inhibitory effects on inflammatory processes. In the present study, however, no evidence was provided to support these speculations, as significant increase in plasma IL-6 levels were not observed in either treatment group.

Cold-water immersion is associated to some degree with oxidative stress and a possible increase in free-radical-species formation [38]. When free-radical production exceeds the capacities of protection and repair mechanisms, oxidative stress occurs, resulting in damage to macromolecules such as proteins, lipids, and DNA [2]. However, no studies have evaluated more than one marker of oxidation in the quantification of oxidative stress. It is recommended that future studies include a more direct measurement of free-radical production, to quantify more accurately the level of potential oxidative damage resulting from cold immersion. There is evidence that shivering thermoregulation, induced by cold exposure, is associated with free-radical production [37]. The method of cold application used in the present study might not have resulted in shivering thermogenesis, as cold water was only applied to a part of the human body. Moreover, the long-term effects of cold-water immersion must also be fully elucidated.

Both aerobic exercise training [12, 31] and resistance training [11] produces vascular adaptations, such as vascular growth and increased vasodilatory responsiveness. Increased flow-related shear stress within the vessels [31] and metabolic requirements of skeletal muscles, including those resulting from tissue hypoxia [6, 18], are the primary factors leading to these vascular adaptations. These adaptations are induced by the upregulation of angiogenic growth factors, such as VEGF. Cold application decreases tissue temperature, blood flow and metabolic rate. These cold-induced events could decrease flow-mediated shear stress within the vessels and metabolic requirements of skeletal muscles, which might affect vascular adaptation in response to resistance training. In the present study, however, plasma VEGF levels after exercise in both the groups did not increase.

In the present study, no significant increases were observed in plasma IL-6 and VEGF levels after exercise in both the groups. One reason for this could be that the quantity of the training load and quantity of muscle mobilized by training were too small to effect plasma cytokine mobilized. Further research should also focus on other inflammatory markers and growth factors to elucidate the mechanisms resulting in the suppression of muscular and vascular adaptations.

The present study indicates the possibility that regular post-exercise cold application to muscles attenuates the muscular and vascular adaptations of resistance training in non-trained young men. However, it is unclear whether or not this is the case in athletes. Longer training session durations used by most athletes would provide longer muscle exposures to heat or other exercise stimuli than the wrist-flexion exercise utilized in the present study. This could diminish the possible inhibitory effects of cold application. Further research using training programs with various types, intensities and durations that simulate practical athletic conditioning is needed.

Athletes apply cold by various means (ice packs and frozen-gel packs) and protocols (duration and temperature). The cold application method used in the present study is considered to be effective for rapid cooling. A similar method is also used for refreshment and relieving fatigue after exercise among athletes. From our results, rapid cooling with ice used for acute sports injury might not be recommended for uninjured muscles, although a mild cold application might be recommended for refreshment purposes. Treatments for overuse and chronic pain might be better when cold is applied to a localized area. Effective, safe use of post-exercise cold application for conditioning in sports activities requires elucidation of the mechanisms resulting in the suppression of muscular and vascular adaptations.
Acknowledgements

We wish to thank all subjects who participated in this study for their cooperation. This study was supported, in part, by a Grant-in-Aid from the Descente and Iшимoto Memorial Foundation for the Promotion of Sports Science.

References

5 Chargé SBP, Rudnicky MA. Cellular and molecular regulation of muscle regeneration. Physiol Rev 2003; 84: 209–238
19 Johnson DJ, Moore S, Moore J, Oliver RA. Effect of cold submersion on intramuscular temperature of the gastrocnemius muscle. Phys Ther 1979; 59: 1238–1242
21 Knight KL. Effects of hypothermia on inflammation and swelling. Athl Train 1976; 11: 7–10
22 Knight KL. Cryotherapy in sport injury management. Champaign, IL: Human Kinetics; 1995: 77–84
30 McLoughlin TJ, Mylona E, Hornberger TA, Esser KA, Pizza FX. Inflammation: changes in satellite cells in rat skeletal muscle are elevated after electrically stimulated contractions. J Appl Physiol 2003; 94: 876–882