

Research article

Effect of heat preconditioning by microwave hyperthermia on human skeletal muscle after eccentric exercise

Norio Saga, Shizuo Katamoto and Hisashi Naito ✉

Department of Exercise Physiology, Graduate School of Health and Sports Science, Juntendo University, Chiba, Japan

Abstract

The purpose of this study was to clarify whether heat preconditioning results in less eccentric exercise-induced muscle damage and muscle soreness, and whether the repeated bout effect is enhanced by heat preconditioning prior to eccentric exercise. Nine untrained male volunteers aged 23 ± 3 years participated in this study. Heat preconditioning included treatment with a microwave hyperthermia unit (150 W, 20 min) that was randomly applied to one of the subject's arms (MW); the other arm was used as a control (CON). One day after heat preconditioning, the subjects performed 24 maximal isokinetic eccentric contractions of the elbow flexors at $30^\circ \cdot s^{-1}$ (ECC1). One week after ECC1, the subjects repeated the procedure (ECC2). After each bout of exercise, maximal voluntary contraction (MVC), range of motion (ROM) of the elbow joint, upper arm circumference, blood creatine kinase (CK) activity and muscle soreness were measured. The subjects experienced both conditions at an interval of 3 weeks. MVC and ROM in the MW were significantly higher than those in the CON ($p < 0.05$) for ECC1; however, the heat preconditioning had no significant effect on upper arm circumference, blood CK activity, or muscle soreness following ECC1 and ECC2. Heat preconditioning may protect human skeletal muscle from eccentric exercise-induced muscle damage after a single bout of eccentric exercise but does not appear to promote the repeated bout effect after a second bout of eccentric exercise.

Key words: delayed-onset muscle soreness, muscle damage, repeated bout effect, heat shock proteins.

Introduction

It is well known that unaccustomed eccentric exercise induces muscle damage and muscle soreness. This phenomenon, known as delayed-onset muscle soreness (DOMS) (Clarkson and Hubal, 2002; Clarkson et al., 1992; Fridén and Lieber, 2001; Warren et al., 1999; 2002), is characterized by swelling and prolonged decreases in the maximal voluntary contraction (MVC) and range of motion (ROM) (Howell et al., 1993; Warren et al., 2002). Given the decrease in muscular performance that accompanies DOMS (Warren et al., 2002), reducing muscle damage and muscle soreness is of great concern in the sports medicine and athletic fields. Therefore, various means of controlling and reducing DOMS have been studied (Connolly et al., 2003), including pre- and post-exercise stretching, icing, warm-up, and massaging, as well as treatment with ultrasound and topical analgesics (Connolly et al., 2003; Nosaka et al., 2004).

Exercise itself has also been shown to reduce eccentric exercise-induced muscle damage. It is well estab-

lished that repeating similar eccentric exercises over a period of several weeks results in significantly less muscle damage and muscle soreness (Chen and Hsieh, 2000, 2001; Nosaka and Clarkson, 1995; Paddon-Jones et al., 2000) via a phenomenon known as the repeated bout effect (McHugh, 2003; McHugh et al., 1999; Nosaka and Newton, 2002; Nosaka et al., 2001).

Although the mechanism underlying the repeated bout effect is unclear, several neural, mechanical, and muscle cell-related mechanisms have been postulated (McHugh, 2003; McHugh et al., 1999; Stauber and Smith, 1998). For example, Thompson et al. (2002, 2001) reported that eccentric contractions resulted in the induction of heat shock proteins (HSPs) in skeletal muscle. HSPs are induced in response to various stresses, and a rapid increase in HSP transcription and translation helps to protect cells from injury and death (Koh, 2002). Therefore, Thompson et al. (2002, 2001) suggested that adaptations by the HSPs in muscle cells may reduce muscle damage and muscle soreness following repeated bouts of exercise.

It has been proposed that the induction of HSPs by certain kinds of stress actually protects cells from other types of stressors. This phenomenon, known as cross tolerance (Koh, 2002), suggests that if muscles were treated by heat preconditioning, the induced HSPs might protect the muscle cells from eccentric exercise-induced muscle damage. Thus in this study, we tested whether heat preconditioning prior to eccentric exercise would result in less eccentric exercise-induced muscle damage and muscle soreness. We also examined whether heat-preconditioned muscles would suffer less eccentric exercise-induced muscle damage after repeated bouts of exercise, to test the hypothesis that the repeated bout effect is enhanced by heat preconditioning prior to eccentric exercise.

Methods

Subjects

Nine untrained male volunteers (means \pm SD: age, 22.9 ± 2.6 yr; height, 1.75 ± 0.06 m; weight, 69.7 ± 11.6 kg) participated in this study. During the experimental period, the subjects were prohibited from using such interventions as icing, heating, massage, and exercise. All subjects were fully informed of the purpose, procedures, and possible risks of the study, and then gave written informed consent. This study was approved by the Juntendo University Human Ethics Committee and was conducted in accordance with the Helsinki Declaration.

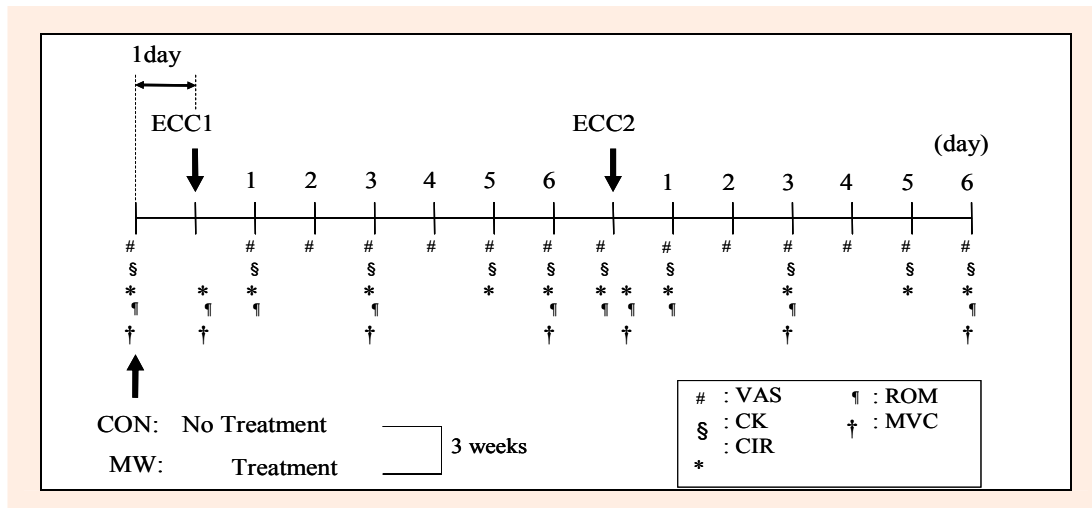


Figure 1. Experimental design and time course of the measurements.

The experimental protocol included two bouts of eccentric exercise, ECC1 and ECC2. The subjects performed the exercises at an interval of 3 weeks with (Microwave condition: MW) and without (Control condition: CON) heat preconditioning by microwave exposure.

Time point for the measurement of muscle soreness using a visual analog scale (VAS). § Time point for the measurement of blood creatine kinase activity (CK). * Time point for the measurement of upper arm circumference (CIR). ¶ Time point for the measurement of range of motion (ROM). † Time point for the measurement of maximal voluntary contraction (MVC).

Experimental protocol

Experimental design and time course of the measurements were summarized in Figure 1. A computer-interfaced dynamometer (BIODEX System 3, Biodex Medical Systems, Inc., Shirley, NY, USA) was used in the passive mode to induce muscle damage. Each subject performed 24 maximal isokinetic eccentric contractions of the elbow flexors at $30^{\circ}\cdot\text{s}^{-1}$ (ECC1) starting with the elbow flexed to 50° and ending at an angle of 170° . The subjects rested for 12 s between each contraction. During the rest period, the dynamometer arm was returned passively to the starting position. One week later, the procedure was repeated (ECC2).

Heat preconditioning (MW) was performed 1 day before the first bout of exercise, with the subjects in a sitting position. One of each subject's upper arms was randomly selected for exposure to a microwave hyperthermia unit (Microtizer, MT-SDi, Minato Medical Co. Ltd., Osaka, Japan) set at 150 W for 20 min (Nosaka et al., 2007; Ogura et al., 2007). This study used an arm-to-arm comparison model, in which one arm was used for the MW condition, while the same subject's other arm was used to test the control condition (CON). Each arm performed ECC1 separated by 5 weeks. The testing order (CON vs. MW) was randomly assigned.

Assessment of muscle damage and muscle soreness

Muscle damage was evaluated based on physiological, biochemical, and subjective markers using a crossover design. MVC, ROM, and upper arm circumference were measured as physiological markers of muscle damage, and blood creatine kinase (CK) activity was used as a biochemical marker. Muscle soreness was evaluated subjectively.

MVC: After the subject was seated in the test position with the elbow flexed at an angle of 90° , the subject was asked to perform two 5-s maximal isometric contractions of the elbow flexors, with a 1-min rest between the two trials. Using an isokinetic dynamometer, the amount

of muscle torque generated over 3 s at the steady state was averaged for each of the two trials, and the higher value was taken as the MVC for subsequent analysis. The MVC was measured before ECC1, and immediately, 3, and 6 days after ECC1 and ECC2, respectively.

ROM: ROM was assessed based on the angular displacement of the relaxed and flexed elbow joint, using a flexometer (Leighton Flexometer Inc., Spokane, WA, USA) placed at the wrist. Before taking the baseline measurement, the measurement point on the wrist was marked with semi-permanent ink; the same point was used throughout the experimental period. ROM was measured before and immediately, 1, 3, and 6 days after ECC1 and ECC2, respectively.

Upper arm circumference: With the arm in a relaxed position (i.e., the arm was allowed to hang down to the side), the upper arm circumference was measured at 3, 5, 7, 9, and 11 cm above the elbow joint using a tape measure (Nosaka and Clarkson, 1996). Five measurement points were marked using semi-permanent ink, and all measurements were taken by the same investigator. Upper arm circumference was measured before and immediately, 1, 3, 5, and 6 days after ECC1 and ECC2, respectively. The average of the five measurement points was used in our analysis.

Blood CK activity: Approximately 32 μl of blood were collected from the fingertip of each subject for analysis of blood CK activity using a dry chemistry strip analyzer (Reflotron Plus, Roche Diagnostics Inc., Basel, Switzerland). The blood was collected before and 1, 3, 5, and 6 days after ECC1 and ECC2, respectively.

Muscle soreness: The subjects were asked to rate their levels of muscle soreness based on a 100-mm visual analog scale (VAS), with the far-left end point representing no pain (0 mm), the mid-point representing pain, and the far-right end point representing extreme pain (100 mm). The subjects placed a mark on the VAS that represented the muscle soreness they experienced in the elbow flexor region when the elbow was flexed or extended.

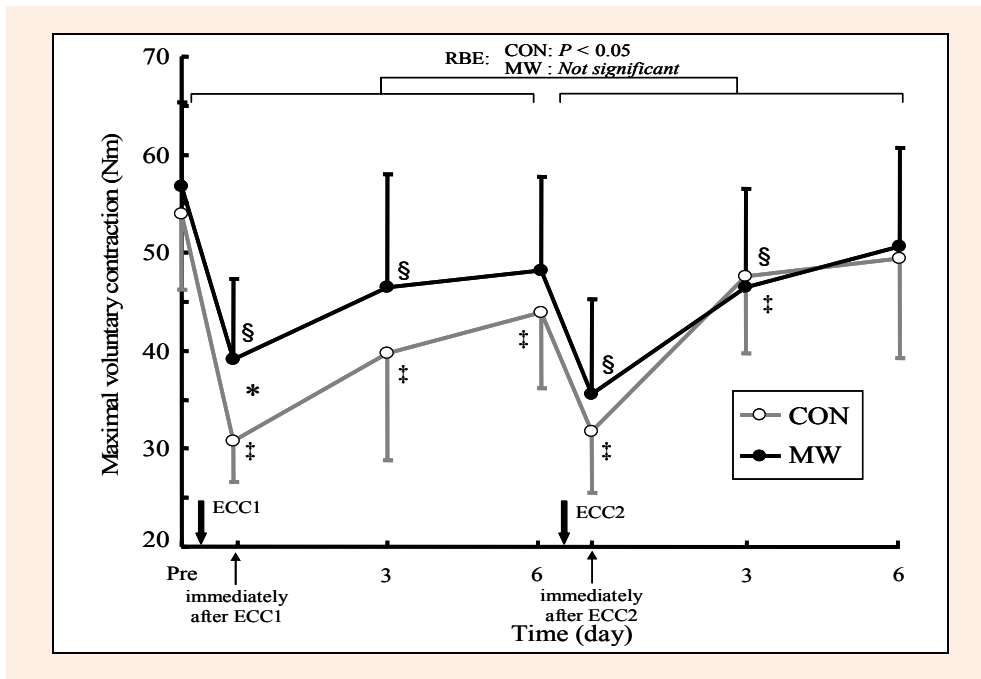


Figure 2. Changes in maximal voluntary contraction (MVC) in the CON and MW after ECC1 and ECC2. Open circles denote the CON results. Closed circles denote the MW results. The values are the means \pm SD. RBE: repeated bout effect. RBE was analyzed by the Bout \times Time interaction for the CON and MW. The p-values for each condition are indicated. * $p < 0.05$ significantly different between the CON and MW. ‡ $p < 0.05$ significantly different from the pre-level in the CON. § $p < 0.05$ significantly different from the pre-level in the MW.

Muscle soreness was evaluated before and 1, 2, 3, 4, 5, and 6 days after ECC1 and ECC2, respectively.

Statistical analysis

The data are presented as means \pm SD. A two-way repeated-measures ANOVA was performed using Treat-

ment (CON vs. MW) by Time as the conditions for ECC1 and ECC2, and Bout (ECC1 vs. ECC2) by Time as the conditions for the CON and MW. Changes from the pre-levels in all markers were analyzed using a one-way ANOVA with Scheffe's *post hoc* comparisons for the CON and MW. Statistical significance was set at $p < 0.05$.

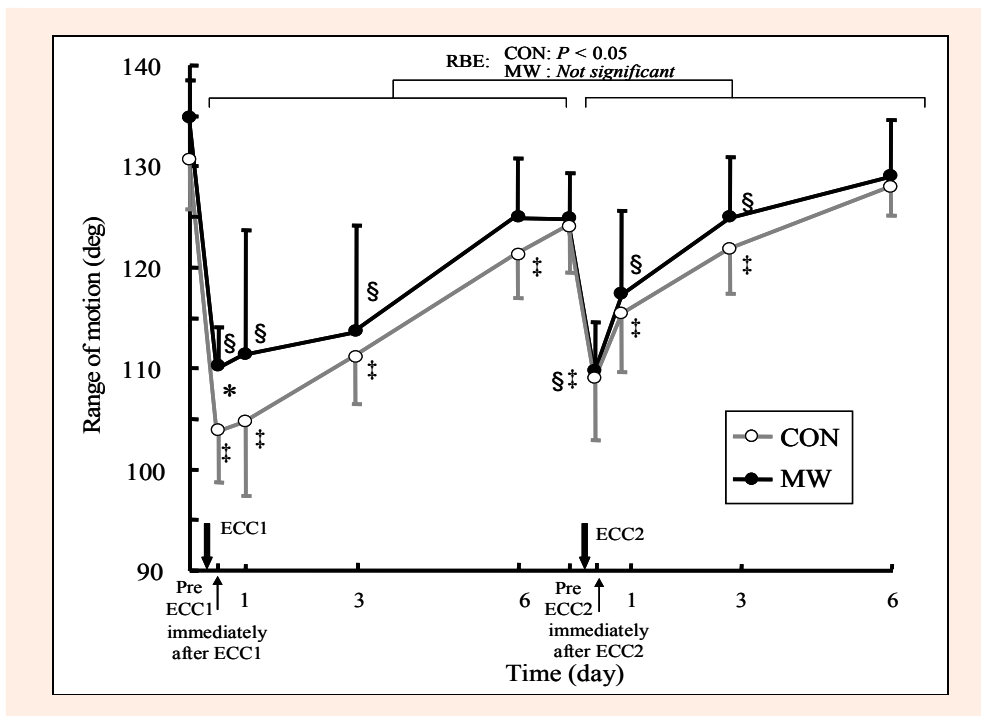


Figure 3. Changes in range of motion (ROM) in the CON and MW after ECC1 and ECC2. Open circles denote the CON results. Closed circles denote the MW results. The values are the means \pm SD. RBE: repeated bout effect. RBE was analyzed by the Bout \times Time interaction for the CON and MW. The p-values for each condition are indicated. * $p < 0.05$ significantly different between the CON and MW. ‡ $p < 0.05$ significantly different from the pre-level in the CON. § $p < 0.05$ significantly different from the pre-level in the MW.

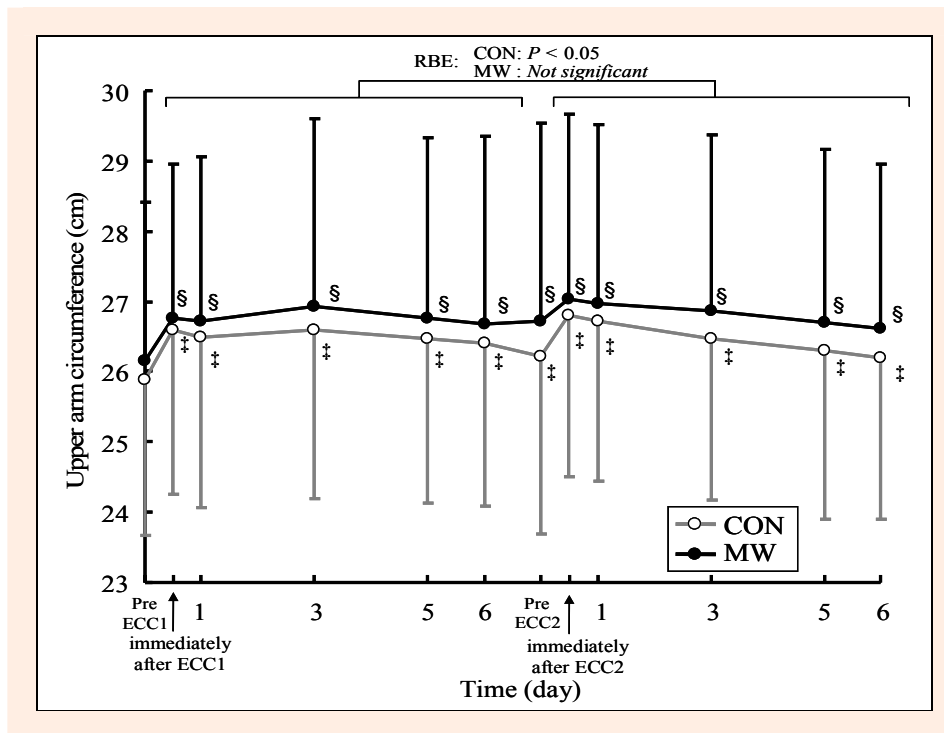


Figure 4. Changes in upper arm circumference in the CON and MW after ECC1 and ECC2.

Open circles denote the CON results. Closed circles denote the MW results. The values are the means \pm SD. RBE: repeated bout effect. RBE was analyzed by the Bout \times Time interaction for the CON and MW. The p-values for each condition are indicated.

‡ $p < 0.05$ significantly different from the pre-level in the CON. § $p < 0.05$ significantly different from the pre-level in the MW.

Results

MVC: ECC1 and ECC2 induced significant decreases in MVC under the CON and MW conditions ($p < 0.05$). A significant effect for Treatment on MVC was demonstrated for ECC1 only ($p < 0.05$), and there was no significant Treatment by Time interaction for ECC1 and ECC2 (Figure 2). A significant Bout by Time interaction (i.e., the repeated bout effect) in MVC was demonstrated for the CON only ($p < 0.05$) (Figure 2).

ROM: ECC1 and ECC2 induced significant decreases in ROM under the CON and MW conditions ($p < 0.05$). The effect of Treatment on ROM was significant only for ECC1 ($p < 0.05$), and there was no significant Treatment by Time interaction effect for ECC1 or ECC2 (Figure 3). A significant Bout by Time interaction (i.e., the repeated bout effect) was demonstrated for ROM only in the CON ($p < 0.05$) (Figure 3).

Upper arm circumference: ECC1 and ECC2 induced significant increases in upper arm circumference under the CON and MW conditions ($p < 0.05$). Upper arm circumference was not significantly affected by Treatment, nor was there a significant Treatment by Time interaction in upper arm circumference for ECC1 or ECC2. A significant Bout by Time interaction (i.e., the repeated bout effect) in upper arm circumference was demonstrated only for the CON ($p < 0.05$) (Figure 4).

Blood CK activity: ECC1 and ECC2 induced significant increases in blood CK activity under the CON and MW conditions ($p < 0.05$). Blood CK activity was not significantly affected by Treatment, nor was there a significant Treatment by Time interaction in blood CK activity for ECC1 or ECC2. A significant Bout by Time

interaction (i.e., the repeated bout effect) was demonstrated for blood CK activity in the CON only ($p < 0.05$) (Figure 5).

Muscle soreness: ECC1 and ECC2 induced significant decreases in muscle soreness under the CON and MW conditions ($p < 0.05$). Muscle soreness was not significantly affected by Treatment, nor was there a significant Treatment by Time interaction for ECC1 or ECC2 (Figure 6). A significant Bout by Time interaction (i.e., the repeated bout effect) was demonstrated for muscle soreness in the CON and MW, respectively ($p < 0.05$) (Figure 6).

Discussion

One main finding of this study is that decreases in MVC and ROM after ECC1 were attenuated by heat preconditioning with microwave hyperthermia, supporting the hypothesis that heat preconditioning prior to ECC1 results in less eccentric exercise-induced muscle damage and muscle soreness. On the other hand, heat preconditioning did not affect any markers related to muscle damage after ECC2. Thus, the idea that heat preconditioning prior to ECC1 might enhance the repeated bout effect may be rejected. The explanations for our findings are as follows.

Effect of microwave preconditioning on muscle damage and muscle soreness

It is believed that eccentric exercise-induced muscle damage and muscle soreness develop through different mechanisms. Eccentric exercise induces ultrastructural muscle damage within the sarcomere, leading to membrane damage and failure of the excitation-contraction

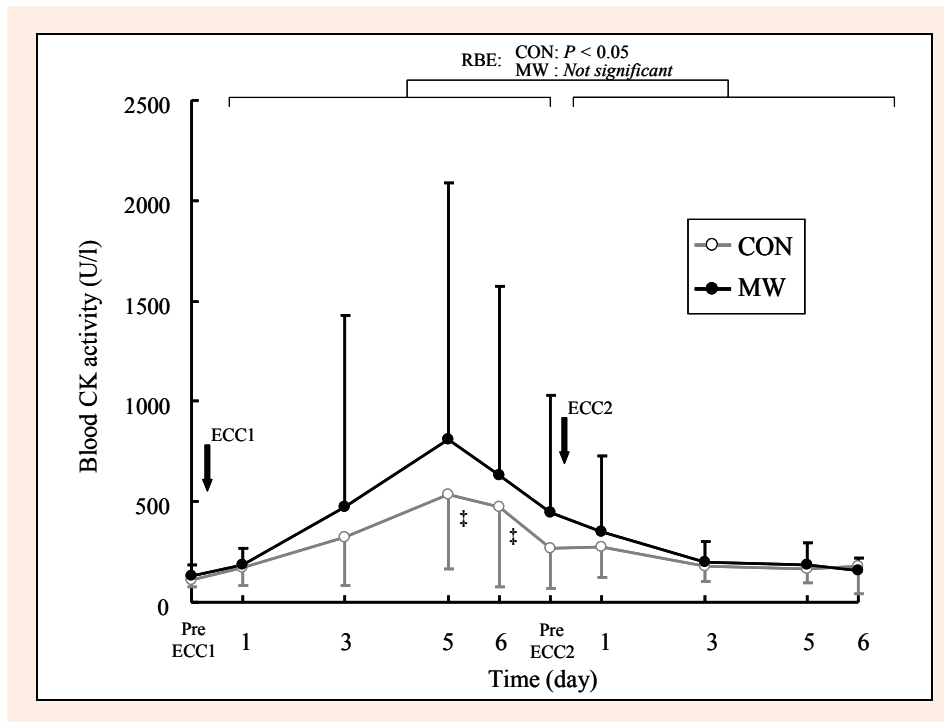


Figure 5. Changes in blood CK activity in the CON and MW after ECC1 and ECC2.

Open circles denote the CON results. Closed circles denote the MW results. The values are the means \pm SD. RBE: repeated bout effect. RBE was analyzed by the Bout \times Time interaction for the CON and MW. The p-values for each condition are indicated.

\ddagger $p < 0.05$ significantly different from the pre-level in the CON. \S $p < 0.05$ significantly different from the pre-level in the MW.

coupling pathway (Koh, 2002; Warren et al., 2002). Next, as a result of muscle injury, calcium homeostasis is altered, raising the intracellular calcium concentration. In addition, it is reported that nitric oxide (NO) production during muscle soreness is significantly increased in human skeletal muscle and that this could result in reduced muscle force generation (Radák et al., 1999). Together, these events induce inflammation and muscle soreness and decrease muscle function. Therefore, to prevent and/or attenuate muscle damage, muscle soreness, and decreases in muscle function, the processes related to this mechanism must be controlled. We used heat preconditioning to prevent eccentric exercise-induced muscle damage and muscle soreness. The reason behind this approach is that heat preconditioning induces HSP expression, which is thought to protect cells by promoting the synthesis of and controlling the degradation of muscle proteins (Koh, 2002; Naito et al., 2000), although each class of HSP has a unique function. HSP72 may play a major role in preventing muscle damage, given that it is the most commonly induced HSP. In addition, HSP72 has the ability to act as a chaperone by binding partially denatured proteins, thereby preventing improper intra- and inter-protein interactions, and guiding these proteins into refolding or degradation pathways. It has also been suggested that heat-induced HSP72 protects skeletal muscle against impairments of excitation-contraction coupling (Febbraio et al., 2002) and that HSP72 can reduce the toxicity of NO (Bellman et al. 1996). Moreover, small HSPs are responsible for stabilizing actin and intermediate filaments against stress (Koh, 2002). Therefore, we hypothesized that the heat preconditioning-induced HSPs in muscle cells play a protective role in reducing muscle damage and muscle soreness. As shown by our results,

heat preconditioning successfully suppressed the decreases in MVC and ROM observed after ECC1.

The mechanism by which muscle damage and muscle soreness occur remains unclear, and we cannot address how heat preconditioning altered the process because we did not measure HSP expression or other biochemical parameters. However, we previously shown that the heat preconditioning protocol used in the present study (150 W, 20 min) increased the temperature of the vastus lateralis muscle to 41 °C and induced both HSP72 and HSP27 within 24 h (Ogura et al., 2007). Therefore, it is possible that the suppressed reductions in MVC and ROM are related to an increase in HSP expression following heat preconditioning.

Similar to our findings, Nosaka et al. (2007) reported that heat preconditioning prior to extension prevented decreases in MVC and ROM after ECC. However, suppression of the decreases in MVC and ROM was not observed immediately after ECC. On the other hand, in our study, the heat preconditioning effect was also demonstrated immediately after ECC1 (CON vs. MW, MVC: 30.8 ± 4.2 vs. 39.1 ± 8.2 Nm, ROM: $103 \pm 5.8^\circ$ vs. $110 \pm 3.8^\circ$, $p < 0.05$, respectively). Although the protocols used to induce muscle damage were not identical between the two studies, immediate decreases of about 50% in MVC following ECC from the pre-level were observed in each control condition and the magnitude of muscle damage induced by each ECC protocol was similar under the control conditions. Therefore, in the present study, heat preconditioning was effective not only for the recovery process but also for protection from muscle damage. However, it is difficult to explain the disparate results. One possible explanation for the difference might be the level of physical fitness among the subjects.

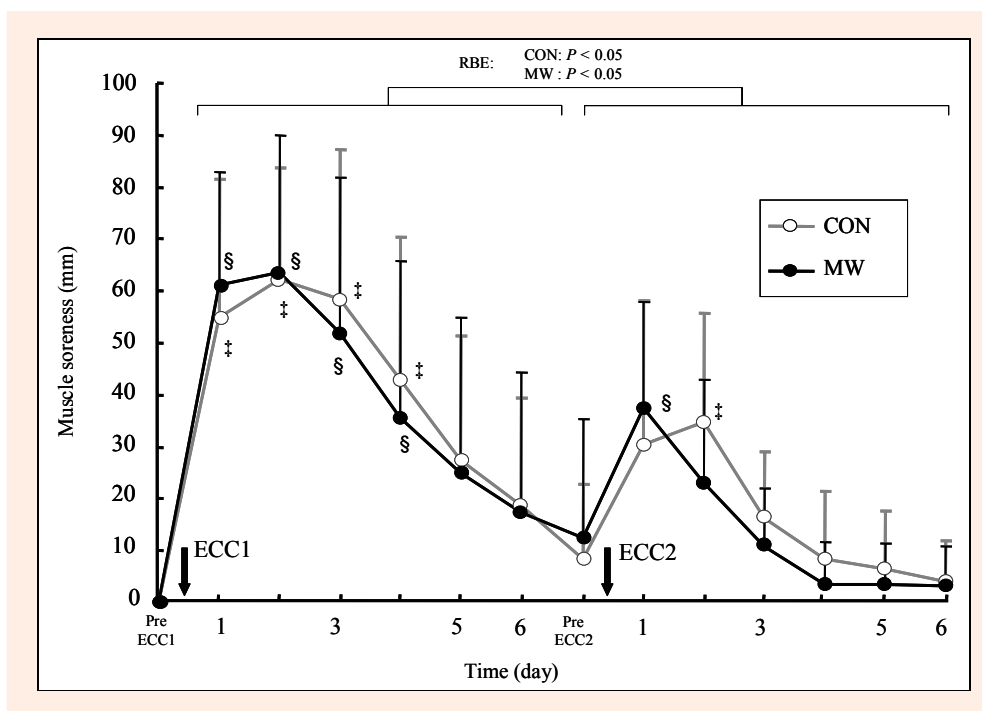


Figure 6. Changes in muscle soreness in the CON and MW after ECC1 and ECC2.

Measurement of muscle soreness was based on a 100-mm visual analog scale (VAS), with the far-left end point representing no pain (0 mm) and the far-right end point representing extreme pain (100 mm). Open circles denote the CON results. Closed circles denote the MW results. The values are the means \pm SD. RBE: repeated bout effect. RBE was analyzed by the Bout \times Time interaction for the CON and MW. The p-values for each condition are indicated.

‡ $p < 0.05$ significantly different from the pre-level in the CON. § $p < 0.05$ significantly different from the pre-level in the MW.

It was previously reported that trained subjects responded less to ECC, indicating that trained subjects could adapt to ECC (Prou et al., 1999). Our subjects were male students in a physical education course; thus, their daily physical activity level was high. In contrast, the subjects selected by Nosaka et al. (2007) were untrained male students who had not performed resistance training for at least 1 year prior to the study. In the future, it will be necessary to compare the effect of heat preconditioning between trained and untrained subjects.

With regard to a difference in the reported level of muscle soreness, Nosaka et al. (2007) showed that heat preconditioning resulted in significantly less muscle soreness following extension, compared with the control condition. In contrast, the subjects in our study complained of the same level of muscle soreness following ECC1 under the CON and MW conditions and heat preconditioning did not reduce muscle soreness. Although we do not have sufficient information to suggest a mechanism for our results, as mentioned above, our subjects were physically active and may have acquired tolerance to DOMS through the repeated bout effect, which lasts at least 6 months (Nosaka et al., 2001).

Effect of microwave preconditioning on the repeated bout effect

The second main focus of our study, which to our knowledge has not been addressed before, was to examine the effect of heat preconditioning on the repeated bout effect. Although the protective effect of heat preconditioning in ECC1 was demonstrated for MVC and ROM,

preconditioning prior to ECC1 did not affect any markers related to muscle damage after ECC2, and there was no significant Treatment (heat preconditioning) by Time interaction for ECC2. In addition, for MVC and ROM, there was a repeated bout effect in the CON but not the MW. The lack of the repeated bout effect in the MW can be attributed to the protection provided by heat preconditioning. That is, there was no additional protective effect following the second bout.

One reason why the repeated bout effect was not enhanced by heat preconditioning may be the time interval between heat preconditioning and ECC2. Conceivably, 1 week might be too long to maintain the high level of HSPs induced by heat preconditioning before ECC1, which reduced the level of muscle damage after ECC1. Alternatively, because exercise itself is strong enough to induce HSP expression (Milne and Noble, 2002; Thompson et al., 2002; Thompson et al., 2001), the effect of the heat-induced HSPs might be attenuated. For example, Ogura et al. (2007) showed that HSP72 and HSP27 were increased 72% and 40%, respectively, by microwave hyperthermia treatment, whereas Thompson et al. (2001) showed a 1,064% increase in HSC/HSP70 and a 234% increase in HSP27 following eccentric exercise. It is possible that the HSP level in both the CON and MW might have reached a similar level after 7 days but that the effect of the ECC-induced HSPs attenuated the effect of the heat-induced HSPs. Therefore, the heat preconditioning applied prior to ECC1 did not reduce the level of skeletal muscle damage after ECC2. Additional studies are necessary to confirm these results.

Conclusions

In the present study, heat preconditioning, applied 1 day prior to ECC1, suppressed the decreases in MVC and ROM, whereas heat preconditioning had no effect on the level of muscle damage caused by the second bout of eccentric exercise. These results suggest that muscle preconditioning with a microwave hyperthermia unit may increase resistance to eccentric exercise-induced muscle damage, although the effect may not extend to repeated bouts of eccentric exercise.

Acknowledgements

This study was supported in part by grants from Juntendo University and Grants-in-Aid for Scientific Research (16300212 and 16650135 to H.N.) from the Japanese Ministry of Education, Culture, Sports, Science and Technology and the Japan Society for the Promotion of Science.

References

- Bellmann, K., Jäättelä, M., Wissing, D., Burkart, V. and Kolb, H. (1996) Heat shock protein hsp70 overexpression confers resistance against nitric oxide. *FEBS Letters* **391**, 185-188.
- Chen, T.C. and Hsieh, S.S. (2000) The effects of repeated maximal voluntary isokinetic eccentric exercise on recovery from muscle damage. *Research Quarterly for Exercise and Sport* **71**, 260-266.
- Chen, T.C. and Hsieh, S.S. (2001) Effects of a 7-day eccentric training period on muscle damage and inflammation. *Medicine and Science in Sports and Exercise* **33**, 1732-1738.
- Clarkson, P.M. and Hubal, M.J. (2002) Exercise-induced muscle damage in humans. *American Journal of Physical Medicine and Rehabilitation* **81**, 52-69.
- Clarkson, P.M., Nosaka, K. and Braun, B. (1992) Muscle function after exercise-induced muscle damage and rapid adaptation. *Medicine and Science in Sports and Exercise* **24**, 512-520.
- Connolly, D.A., Sayers, S.P. and McHugh, M.P. (2003) Treatment and prevention of delayed onset muscle soreness. *Journal of Strength and Conditioning Research* **17**, 197-208.
- Febbraio, M.A., Steensberg, A., Fischer, C.P., Keller, C., Hiscock, N. and Pedersen, B.K. (2002) IL-6 activates HSP72 gene expression in human skeletal muscle. *Biochemical and Biophysical Research Communications* **296**, 1264-1266.
- Fridén, J. and Lieber, R.L. (2001) Eccentric exercise-induced injuries to contractile and cytoskeletal muscle fiber components. *Acta Physiologica Scandinavica* **171**, 321-326.
- Howell, J.N., Chleboun, G. and Conatser, R. (1993) Muscle stiffness, strength loss, swelling and soreness following exercise-induced injury in humans. *The Journal of Physiology* **464**, 183-196.
- Koh, T.J. (2002) Do small heat shock proteins protect skeletal muscle from injury? *Exercise and Sport Sciences Reviews* **30**, 117-121.
- McHugh, M.P. (2003) Recent advances in the understanding of the repeated bout effect: the protective effect against muscle damage from a single bout of eccentric exercise. *Scandinavian Journal of Medicine and Science in Sports* **13**, 88-97.
- McHugh, M.P., Connolly, D.A., Eston, R.G. and Gleim, G.W. (1999) Exercise-induced muscle damage and potential mechanisms for the repeated bout effect. *The Journal of Sports Medicine* **27**, 157-170.
- McHugh, M.P. and Tetro, D.T. (2003) Changes in the relationship between joint angle and torque production associated with the repeated bout effect. *Journal of Sports Sciences* **21**, 927-932.
- Milne, K.J. and Noble, E.G. (2002) Exercise-induced elevation of HSP70 is intensity dependent. *Journal of Applied Physiology* **93**, 561-568.
- Naito, H., Powers, S.K., Demirel, H.A., Sugiura, T., Dodd, S.L. and Aoki, J. (2000) Heat stress attenuates skeletal muscle atrophy in hindlimb-unweighted rats. *Journal of Applied Physiology* **88**, 359-363.
- Nosaka, K. and Clarkson, P.M. (1995) Muscle damage following repeated bouts of high force eccentric exercise. *Medicine and Science in Sports and Exercise* **27**, 1263-1269.
- Nosaka, K. and Clarkson, P.M. (1996) Changes in indicators of inflammation after eccentric exercise of the elbow flexors. *Medicine and Science in Sports and Exercise* **28**, 953-961.
- Nosaka, K., Muthalib, M., Lavender, A. and Laursen, P.B. (2007) Attenuation of muscle damage by preconditioning with muscle hyperthermia 1-day prior to eccentric exercise. *European Journal of Applied Physiology* **99**, 183-192.
- Nosaka, K. and Newton, M. (2002) Repeated eccentric exercise bouts do not exacerbate muscle damage and repair. *Journal of Strength and Conditioning Research* **16**, 117-122.
- Nosaka, K., Sakamoto, K., Newton, M. and Sacco, P. (2001) How long does the protective effect on eccentric exercise-induced muscle damage last? *Medicine and Science in Sports and Exercise* **33**, 1490-1495.
- Nosaka, K., Sakamoto, K., Newton, M. and Sacco, P. (2004) Influence of pre-exercise muscle temperature on responses to eccentric exercise. *Journal of Athletic Training* **39**, 132-137.
- Ogura, Y., Naito, H., Tsurukawa, T., Ichinoseki-Sekine, N., Saga, N., Sugiura, T. and Katamoto, S. (2007) Microwave hyperthermia treatment increases heat shock proteins in human skeletal muscle. *British Journal of Sports Medicine* **41**, 453-455.
- Paddon-Jones, D., Muthalib, M. and Jenkins, D. (2000) The effects of a repeated bout of eccentric exercise on indices of muscle damage and delayed onset muscle soreness. *Journal of Science and Medicine in Sport* **3**, 35-43.
- Prou, E., Guevel, A., Benezet, P. and Marini, J.F. (1999) Exercise-induced muscle damage: absence of adaptive effect after a single session of eccentric isokinetic heavy resistance exercise. *The Journal of Sports Medicine and Physical Fitness* **39**, 226-232.
- Radák, Z., Pucsek, J., Mecseki, S., Csont, T. and Ferdinandy, P. (1999) Muscle soreness-induced reduction in force generation is accompanied by increased nitric oxide content and DNA damage in human skeletal muscle. *Free Radical Biology & Medicine* **26**, 1059-1063.
- Stauber, W.T. and Smith, C.A. (1998) Cellular responses in exertion-induced skeletal muscle injury. *Molecular and Cellular Biochemistry* **179**, 189-196.
- Thompson, H.S., Clarkson, P.M. and Scordilis, S.P. (2002) The repeated bout effect and heat shock proteins: intramuscular HSP27 and HSP70 expression following two bouts of eccentric exercise in humans. *Acta Physiologica Scandinavica* **174**, 47-56.
- Thompson, H.S., Scordilis, S.P., Clarkson, P.M. and Lohrer, W.A. (2001) A single bout of eccentric exercise increases HSP27 and HSC/HSP70 in human skeletal muscle. *Acta Physiologica Scandinavica* **171**, 187-193.
- Warren, G.L., Ingalls, C.P., Lowe, D.A. and Armstrong, R.B. (2002) What mechanisms contribute to the strength loss that occurs during and in the recovery from skeletal muscle injury? *The Journal of Orthopaedic and Sports Physical Therapy* **32**, 58-64.
- Warren, G.L., Lowe, D.A. and Armstrong, R.B. (1999) Measurement tools used in the study of eccentric contraction-induced injury. *The Journal of Sports Medicine* **27**, 43-59.

Key points

- There have been few studies about the effects of heat preconditioning on muscle damage caused by eccentric exercise and the repeated bout effect after a second bout of eccentric exercise.
- Heat preconditioning with microwave hyperthermia may attenuate eccentric exercise-induced muscle damage.
- Heat preconditioning does not enhance the repeated bout effect.

AUTHORS BIOGRAPHY

Norio SAGA**Employment**

Ph.D. Student, Graduate School of Health and Sports Science,
Juntendo University, Japan

Degree

M.Sc.

Research interests

Heat treatment and muscle damage and soreness.

E-mail: sg8_nr1gk0@ybb.ne.jp

Shizuo KATAMOTO**Employment**

Professor, Graduate School of Health and Sports Science,
Juntendo University, Japan

Degree

Ph.D.

Research interests

Exercise training and aging.

E-mail: katamoto@sakura.juntendo.ac.jp

Hisashi NAITO**Employment**

Associate Professor, Graduate School of Health and Sports
Science, Juntendo University, Japan

Degree

Ph.D.

Research interests

Stress proteins and exercise.

E-mail: naitoh@sakura.juntendo.ac.jp

✉ Hisashi Naito

Department of Exercise Physiology, Graduate School of Health
and Sports Science, Juntendo University, 1-1 Hiragakuendai,
Inba, Chiba, 270-1695, Japan.