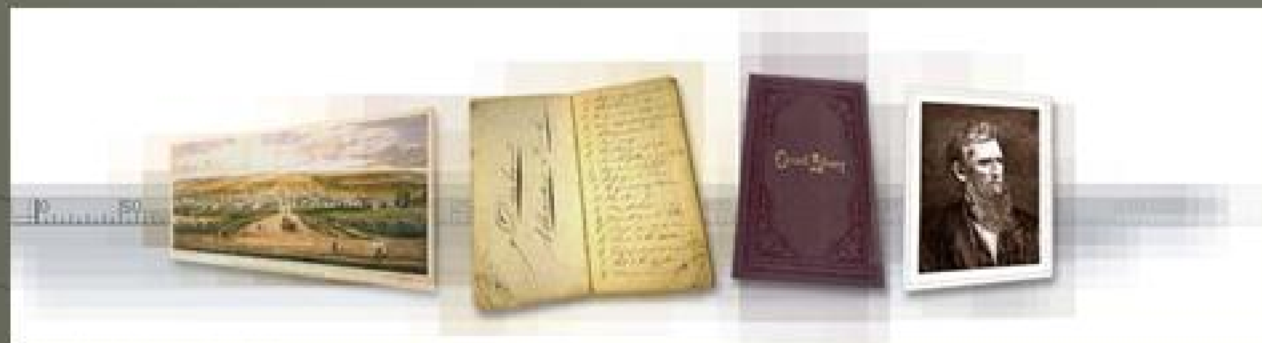

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Heat stress produces an early phase of protection against oxidative damage in human muscle

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Background: It has been rarely reported that heat stress induces an early phase of protection against oxidative damage, whereas a delayed phase of protection is shown in heat stress. To explore the early effect of heat stress against oxidative damage, we evaluated the changes in contractility, lipid peroxidation, and ultrastructure induced by hydrogen peroxide (H_2O_2) with or without heat stress (HS) in human skeleton muscle.
Methods: Thirty-two muscle samples were obtained from the vastus lateralis muscle of 7 subjects. These specimens were divided into three groups based on form of treatment: HS (n=13), non HS (n=14), and control group (n=5). The control group was performed under identical conditions without H_2O_2. Specimens in the HS group were incubated at 42°C for 20 min, while those in the non-HS and control groups were maintained at 37°C.
Results: The control group showed no significant change in contractile force. Although contractile force significantly decreased 30 min after H_2O_2 administration in both the HS and non-HS groups, only the HS group showed apparent recovery of contractile force 60 min after H_2O_2 administration. Lipid peroxidation was lower in the HS group than in the non-HS group. Ultrastructural examination revealed less mitochondrial damage in the HS group compared with the non-HS group.
Conclusion: We found that human skeleton muscle escaped cellular damage induced by H_2O_2 in the early phase after heat stress. These data suggest evidence for an early effect of heat stress against ischemia/reperfusion injury in human muscle.

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Key words: Heat stress; oxidative damage; skeleton muscle; human; electron microscopy.

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A LARGE NUMBER of elements for cellular preservation have been proposed in animal and clinical research. In recent studies, it has been noticed that oxidants mainly produce ischemia/reperfusion injury, and that heat stress reduces the oxidative damage. Most reports have demonstrated that the protection against cellular damage becomes evident more than 24 h after heat stress (1–3). It has been rarely suggested that heat stress elicits an early phase of protection, whereas a biphasic (early and delayed) pattern is shown in ischemic preconditioning (4, 5).

The purpose of this study is to clarify the early effect of heat stress against oxidative damage, by assessing the changes in contractility, lipid peroxidation, and microstructure induced by hydrogen peroxide (H_2O_2) with or without the application of heat stress in human skeleton muscle.

Methods

Thirty-two muscle samples were obtained from the vastus lateralis muscle of 7 subjects with informed consent (2 men and 5 women, mean age 30±2 years), who underwent biopsy for the detection of malignant hyperthermia. The subjects were diagnosed as negative for malignant hyperthermia according to the European protocol (6). The muscle specimens were immediately suspended in Krebs–Ringer-solution (pH 7.4) at 37°C after biopsy. The solution was bubbled continuously with 95% O_2 and 5% CO_2. Of the 32 muscle samples, 27 specimens were divided into two groups based on form of treatment: heat stress and non-heat stress, and 5 (control group) were performed under identical conditions without H_2O_2. Specimens in the HS group (n=13) were incubated at 42°C for 20 min, while those in the non-HS (n=14) and control groups were maintained at 37°C.

All of the specimens were then fixed by sutures in the test chamber and at the force transducer (Lectromed, Welwyn Garden City, Great Britain) (7). They were electrically stimulated (frequency: 0.2/s) and stretched to optimal length, as measured by twitch response. The contractions were continuously recorded. For an

equilibrium period, the definition of the European protocol (6) was strictly used (there is no change of >2 mN over 10 min); hence, the contractions were evaluated in isometric condition. The electrical stimulation was stopped 60 min after an equilibrium period of 30 min. H_2O_2 was administered in both the HS and non-HS groups after the equilibrium period. The incubation was conducted for 15 min at H_2O_2 concentration of 1×10^{-5} mol/L. Contractile force (%contractility) was expressed as a percent of the value measured just after the equilibrium period (0 min: 100%).

Analysis of lipid peroxidation

Eleven samples (6 HS and 5 non-HS) were selected for analyzing the extent of lipid peroxidation, which is a parameter for evaluating metabolic damage in cell membrane. The muscle samples were blotted and weighed at the end of the incubations. The specimens were homogenized in nitrogen at −80°C. Lipid peroxidation was monitored by measuring the amount of malondialdehyde (MDA) according to the thiobarbituric acid assay (8). The absorbance of the sample was determined at 535 nm against a blank that contained all the reagents minus the lipid. MDA concentration was calculated by using an extinction coefficient of 1.56×10^5/mol $L^{-1}\cdot$ cm. Amounts of MDA were adjusted for wet weight of sample tissue.

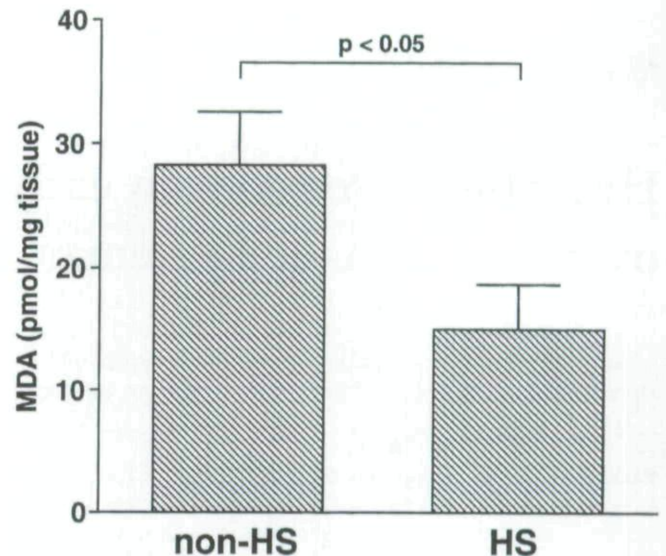

Fig. 2. Comparison of malondialdehyde (MDA) in the heat stress (HS) and non-heat stress (non-HS) groups. A significant decrease of MDA was shown in the HS group compared with the non-HS group.

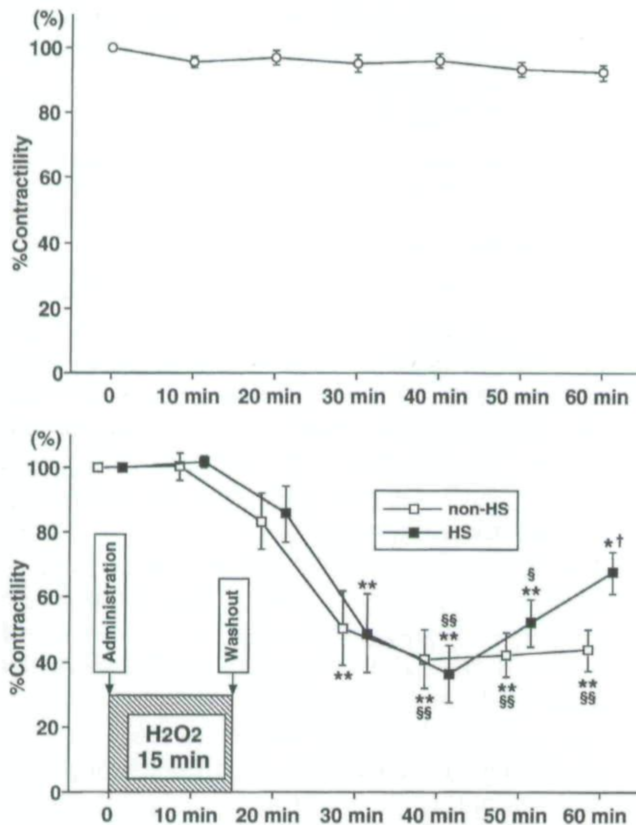

*Fig. 1. Comparison of changes in %contractility in the heat stress (HS), non-heat stress (non-HS), and control groups. There were no significant changes of contractile force in the control group (top). Only the HS group showed apparent recovery of contractile force 60 min after H_2O_2 administration, whereas contractile force significantly decreased 30 min after H_2O_2 administration in both the HS and non-HS groups (bottom). Values are expressed as mean±SEM. *=P<0.05 vs 0 min, **=P<0.01 vs 0 min, §=P<0.05 vs control group, §§=P0.01 vs control group, †=P<0.05 vs non-HS group.*

Ultrastructural studies

For ultrastructural studies, five to nine specimens in each group (5 control, 7 HS and 9 non-HS) were immediately fixed by immersion in glutaraldehyde (6%) for ultrastructural examination after the end of the incubations. Postfixation was done for 2 h with 1% OsO_4 plus 1.5% ferricyanide in 0.1 M cacodylate buffer (pH 7.2).

The samples were dehydrated in ethanol and contrasted in a saturated solution of uranyl acetate. The tissue blocks were embedded in a mixture of hard epoxy and araldite. Ultrathin sections of 100 nm were cut by ultra-microtome, cross-secting the muscle fiber bundles. Examinations of prepared tissue sections were performed with a Zeiss electron microscope (EM 902).

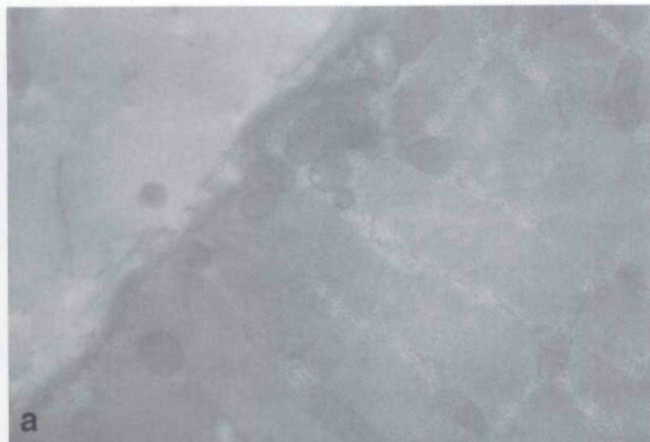

Fig. 3a. An electron photomicrograph in the control group. Mitochondria are normal. ×28000.

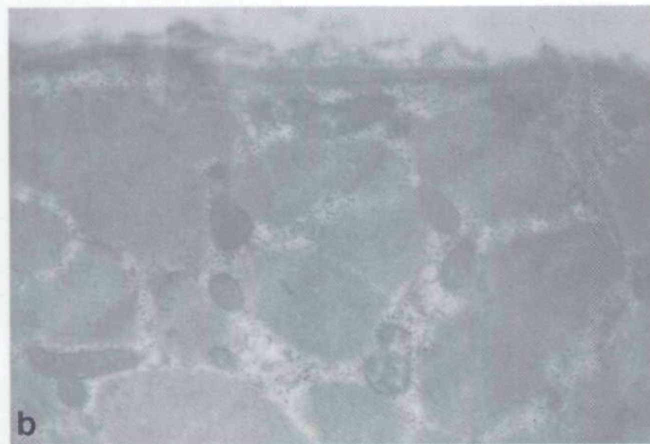

Fig. 3b. An electron photomicrograph in the heat stress group. Mitochondrial damage was less severe than that seen in the non-heat stress group. ×28000.

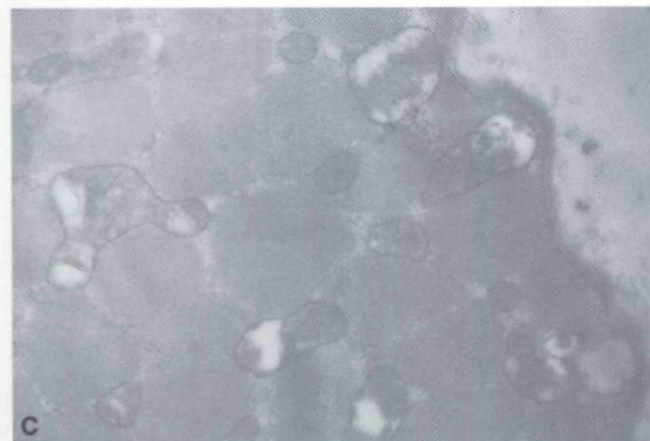

Fig. 3c. An electron photomicrograph in the non-heat stress group. Morphological findings showed severe damage of mitochondria. ×28000.

Statistical analysis

Data are presented as mean±SEM. Intergroup differences were tested for significance by ANOVA and for the subgroup analysis by Scheffe's *F* test.

Results

Contractility and lipid peroxidation

There was no significant difference in contractile force just after an equilibrium period of 30 min among the HS, non-HS, and control groups (HS=77.2±7.5 mN, non-HS=80.5±9.2 mN, control=73.2±7.6 mN, *P*=NS). In the control group, contractile force showed no significant change. Contractile force significantly decreased 30 min after H_2O_2 administration in both the HS and non-HS groups. However, only the HS group showed apparent recovery of contractile force 60 min after H_2O_2 administration (Fig. 1). MDA was significantly lower in the HS group than in the non-HS group (15.0±3.7 vs 28.2±4.4 pmol/mg tissue, *P*=0.046) (Fig. 2).

Ultrastructural findings

Mitochondria of the control group demonstrated normal morphology (Fig. 3a). In contrast, the non-HS group showed swelling of mitochondria and severe disruption of mitochondrial crests (Fig. 3b). Morphological findings in the HS group indicated less damage of mitochondria compared with the non-HS group (Fig. 3c).

Discussion

To clarify an early phase of protection against oxidative damage in heat stress, we assessed the changes in contractility, lipid peroxidation, and microstructure induced by H_2O_2 with or without heat stress in human skeleton muscle. Only the HS group showed apparent recovery of contractile force 60 min after H_2O_2 administration, whereas contractile force significantly decreased 30 min after H_2O_2 administration in both the HS and non-HS groups. Lipid peroxidation was lower in the HS group than in the non-HS group. Ultrastructural examination indicated less damage of mitochondria in the HS group compared with the non-HS group. These findings showed that human skeleton muscle escaped cellular injury induced by H_2O_2 in the early phase after heat stress, and hence, we suggest evidence for an early effect of heat stress against oxidative damage in human muscle.

Research has introduced many factors for cellular preservation. Especially, recent studies indicate that cellular damage is reduced by a family of proteins called stress protein. Heat shock protein is a part of stress proteins, and it is induced by hyperthermia. In most of the previous studies, it was demonstrated that the protection against tissue damage was induced by heat shock proteins more than 24 h after

heat stress, and that the significant increase of heat shock proteins was indicated more than 3 h after heat stress (1–3, 9, 10), while an animal study suggested that a brief period of heat stress applied only 5 min prior to 15 min of global ischemia may be sufficient to provide for enhanced myocardial function recovery in association with the accumulation of heat shock protein (11). In this study, we predicted that the protection against oxidative damage may begin at 30 min after heat stress. Therefore, our present study shows that except for heat stress proteins, other factors may induce adaptive protection of the muscle in the early phase after heat stress, although the mechanism is unclear. In a number of recent reports, it has been noticed that a brief period of ischemia followed by reperfusion produces myocardial tolerance to injury from subsequent longer ischemic episodes, which is commonly known as ischemic preconditioning (4, 5, 12–14). These studies have suggested that ischemic preconditioning produces early and delayed phases of protection against ischemia/reperfusion injury. Some reports have shown that the early phase of protection may be triggered by initial oxidative stress, such as superoxide or other species, that arise from free radicals during brief periods of anoxia (14–16). Recently, it has been commonly proposed that stress conditions, including heat stress, cause generation of free radicals within the cell (17–20). Accordingly, the early effect of heat stress in our study may be related to an early phase of protection in ischemic preconditioning, although the mechanism of early protection is not completely understood in ischemic preconditioning.

In conclusion, our present findings indicate that human skeleton muscle escapes H_2O_2-induced tissue damage in the early phase after heat stress. We suggest that heat stress produces an early phase of protection against oxidative damage in human muscle, and furthermore, our study may provide clinical implications for protection against ischemia/reperfusion injury.

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