Am J Physiol Regul Integr Comp Physiol 289: R134–R139, 2005.
First published March 10, 2005; doi:10.1152/ajpregu.00497.2004.

Heat treatment reduces oxidative stress and protects muscle mass during immobilization

Joshua T. Selsby and Stephen L. Dodd

Department of Applied Physiology and Kinesiology, University of Florida, Gainesville, Florida

Submitted 26 July 2004; accepted in final form 8 March 2005

Selsby, Joshua T., and Stephen L. Dodd. Heat treatment reduces oxidative stress and protects muscle mass during immobilization. *Am J Physiol Regul Integr Comp Physiol* 289: R134–R139, 2005. First published March 10, 2005 doi:10.1152/ajpregu.00497.2004.—This study examined the role of heating on oxidative stress and muscle mass in immobilized limbs. Rats were divided into three groups ($n = 9$/group): a control group (Con), an immobilized group (Im), and an immobilized and heated group (ImH). Rats were immobilized in the plantarflexed position for 8 days. The core temperature of the ImH group was elevated to 41–41.5°C on alternating days and maintained for 30 min before cooling. On *day 8*, both heat shock protein 25 (HSP25) and HSP72 were markedly elevated in the ImH compared with the Im group, whereas results in the Im group were not different from Con. Most notably, the ImH group had significantly larger solei compared with the Im group, which were less than those shown in the Con group. Furthermore, immobilization alone caused a significant increase in oxidative damage, and the addition of heating to immobilization significantly reduced oxidative damage. In an effort to further identify the cause of this protective effect, antioxidant enzyme activities were assessed. CuZnSOD was sharply elevated in Im compared ($P < 0.025$) with that in the Con and reduced in the ImH group compared with that in the Im group ($P < 0.025$). Catalase was elevated 8% ($P < 0.025$) in the Im group compared with the Con group and was similar to the ImH group. Glutathione peroxidase, glutathione reductase, and MnSOD did not differ between groups. These data indicate that heating provides protection against oxidative stress and preserves muscle mass during disuse atrophy. These data also suggest that antioxidant protection is not conferred via antioxidant enzymes, and HSPs may play an important role.

free radicals; antioxidant; rat; soleus

SKELETAL MUSCLE IS A TISSUE that will readily adapt in response to changes in loading pattern. In the case of increased load, the response is hypertrophy. In contrast, atrophy occurs with a reduction in load. The most obvious indication of atrophy is a reduction in muscle mass and cross-sectional area (4, 11, 16, 28). Despite the well-known characteristics of an atrophying fiber, little is known about the basic mechanisms of atrophy.

Two models commonly used to induce atrophy are cast immobilization and hindlimb unweighting (HLU) (for review, see Refs. 1, 30). With HLU, there is an initial reduction in recruitment, which is nearly absent by 1 wk. However, with immobilization, there is a reduction in recruitment that persists for the duration of immobilization (3, 17). Despite the fact that the unweighted soleus is capable of contracting, atrophy still occurs because the muscle is only capable of very low-force contractions as the only load is provided by the mass/inertia of the foot. During immobilization, although the muscle is capable of producing force through an isometric contraction, EMG activity is drastically reduced; therefore, few fibers are recruited. Furthermore, when the soleus is immobilized in a shortened position, any fiber recruitment will result in very low-force contractions because the actomyosin complexes are in a near-maximally shortened position. Although differing in their nature, HLU and immobilization both result in dramatic losses of skeletal muscle mass. This loss in mass appears to be primarily of type I fibers, with a minor contribution from type II fibers during HLU and both types I and II during immobilization (8, 46). Nevertheless, both models result in a soleus that has undergone a type II shift and that has developed contractile properties reminiscent of this fiber type (15, 36, 47, 49).

Several reports have strongly implicated oxidative stress as partially causative of disuse atrophy as both damage to lipids and proteins have been detected (4, 21–26, 28). In addition to oxidative damage present during muscle disuse, antioxidant enzymes respond in a manner that suggests an elevated free radical content (22, 28). Indeed, Kondo et al. (26) were able to show elevated hydroxyl radicals in immobilized tissue. This group also showed that xanthine oxidase and free iron were contributing sources of free radical production during immobilization (23–25).

Several studies have furthered these observations and utilized antioxidant supplementation in an effort to reduce oxidative damage and preserve muscle mass and muscle cross-sectional area with varying degrees of success during disuse atrophy (4, 19, 21, 24). Kondo et al. (21, 24) supplemented immobilized animals with vitamin E and reduced oxidative damage and increased muscle mass in immobilized animals. In addition, Appell et al. (4) found larger muscle cross-sectional area in vitamin E-supplemented animals following immobilization. In contrast, Koesterer et al. (19) found that an antioxidant cocktail containing vitamin E failed to preserve muscle mass during unweighting.

In addition to conventional antioxidants, heating has been used as a countermeasure to disuse atrophy (32). At the completion of one bout of whole body heating, Naito et al. (32) unweighted the hindlimbs of rats for 8 days. After the unweighting period, heat-treated animals had higher heat shock protein 72 (HSP72) levels as well as an attenuated soleus muscle mass loss compared with suspended rats not receiving heat treatment. Naito et al. (32) proposed that expression of HSP72 was necessary for maintenance of protein synthesis rates in the heated hindlimbs, whereas a nonheated suspended group would be characterized by a decline in protein synthesis. It has been hypothesized that increased expression of HSP72 enhances protein synthesis by maintaining the elongation phase

Address for reprint requests and other correspondence: S. L. Dodd, PO Box 118205, Univ. of Florida, Gainesville, FL 32611 (E-mail: sdodd@hhp.ufl.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

of protein synthesis. Furthermore, it has been hypothesized that HSP72 protects proteins from proteolysis by refolding damaged proteins.

It is now well accepted that heating animals increases not only HSP72 but many other HSPs as well; among them is HSP25 (HSP27 in humans). The most recent evidence suggests that these proteins can assist in the maintenance of protein synthesis rates, refold damaged proteins, scavenge free radicals, and inhibit apoptosis (6, 13, 27, 29, 40, 41). By chaperoning nascent polypeptides, the rate of protein synthesis may remain elevated, whereas the reduction in synthesis rates may be prevented. By refolding damaged proteins, they are not degraded and hence remain functional. If free radicals are removed, they cannot contribute to protein damage, leading to their subsequent degradation. Finally, preserving myonuclei may help to preserve the protein synthesis potential of a muscle. Therefore, the purpose of this investigation was to determine the effect of heating on oxidative stress and muscle mass during immobilization. We hypothesized that heating during immobilization would decrease oxidative damage and result in a larger muscle mass compared with a group exposed to immobilization alone.

METHODS

Experimental design. All procedures and experiments were conducted with the approval of the Institutional Animal Care and Use Committee at the University of Florida. Animals were housed in a 12:12-h light-dark photoperiod in an environmentally controlled room. Upon arrival in the facility, animals were handled daily for 1 wk before the initiation of experiments in an effort to minimize contact stress. Male Sprague-Dawley rats were randomly divided into three groups: a control group (Con; $n = 9$), an immobilized group (Im; $n = 9$), and an immobilized and heated group (ImH; $n = 9$). The hindlimbs of animals were casted in the plantarflexed position for a period of 8 days. Animals given the heat therapy were heated 24 h before immobilization and then on alternating days during the immobilization period. The Im and ImH animals were pair fed, whereas the Con animals were fed ad libitum. After completion of the 8-day immobilization period, a surgical plane of anesthesia was induced via intraperitoneal pentobarbital injection and the cast was removed. Then, the soleus was removed, trimmed of excess fat, tendon, and nerve, blotted dry, weighed, and immediately frozen in liquid nitrogen for subsequent analysis. The final heat treatment was given 48 h before death.

Immobilization. Anesthesia was induced with a 5% isoflurane gas-oxygen mixture and maintained with a 1.5–2% isoflurane gas-oxygen mixture administered through a calibrated air flowmeter (Veterinary Equipment and Technical Service, Gainesville, FL). Animals were immobilized bilaterally in the plantarflexed position as to cause maximal atrophy in the triceps surae muscle group in accordance with the model described by Booth and Kelso (9) with modifications. Briefly, animals were wrapped in a protective adhesive (Medipore Dress-it, 3M, St. Paul, MN) so that the animals would not come in contact with the plaster. The wrap began in the supra-abdominal area, below the level of the ribs, and continued down the abdomen of the animal and stopped in the infra-abdominal area such that urination would not be affected and continued down the hindlimbs. A quick-drying plaster was then applied and allowed to dry (Specialist, Johnson and Johnson, New Brunswick, NJ). Finally a Plexiglas wrap was applied so that rats could not chew through the cast (Scotchcast Plus, 3M).

Heat treatment. Animals were anesthetized using isoflurane, as detailed above. A rectal probe was inserted and secured to the tail to ensure that it would not become displaced (YSI, Yellow Springs, OH). The animal was then wrapped in a prewarmed thermal blanket (Kaz, Hudson, NY), keeping the tail and head exposed. The tail was exposed because it was the anchor for the rectal probe. The head was visible to ensure that the nose cone remained in place to prevent accidental recovery from anesthesia.

Core temperature was continuously monitored and recorded every 2 min. Heating time started as soon as the core temperature of the animal breached 41°C, and temperature was maintained at 41–41.5°C for 30 min. At 30 min, the animal's core temperature was lowered via convection cooling and continually monitored until the core temperature dropped below 39.9°C. Our preliminary work has shown that this produces an elevation in HSP expression that peaks between 24 and 48 h before the unheated control levels are reached by 48 h (data not shown). This is in contrast to local heating, which produces a peak in HSP levels that is resolved by 8 h postheating (34). Immobilized animals not receiving a heat treatment were given the same treatment except that core temperature was maintained at ~37°C for the 30-min period.

Western blot. Muscle was homogenized utilizing the technique of Solaro et al. (42). Briefly, frozen tissue was homogenized in sucrose buffer at a mass-to-buffer ratio of 20:1. The resulting homogenate was then centrifuged at 1,000 *g* for 10 min, and the supernatant was removed and the pellet discarded. Protein concentration was then determined by use of the biuret technique of Watters (48). Samples were diluted to 1 mg/ml in sample buffer containing 62.5 mM Tris (pH 6.8), 1.0% SDS, 0.01% bromphenol blue, 15.0% glycerol, and 5% β-mercaptoethanol. Samples were denatured via heating to 60°C for 15 min in a glass bead heater.

Precisely 10 μg of protein were loaded into 4–20% vertical precast gels (Cambrex, Rockland, ME). Samples were then electrophoresed at room temperature for 30 min at 50 V followed by 90 min at 120 V (Bio-Rad, Hercules, CA). Gels were removed from the electrophoresis apparatus and allowed to condition for 15 min in transfer buffer containing 25 mM Tris, 192 mM glycine, 0.02% SDS, and 20% methanol (pH 8.3). After the conditioning period, horizontal electrophoresis (100 V, 60 min, 4°C) was performed, and proteins were transferred to a nitrocellulose membrane with a pore diameter of 0.2 μm (Bio-Rad). Membranes were then stored in Tris-buffered saline containing 0.1% Tween 20 (TTBS). Membranes were blocked by exposure to a 5% dehydrated milk TTBS solution for 60 min. Membranes were washed for 10 min three times and exposed to the appropriate primary antibody as follows: HSP25 (SPA 801, Stressgen, Victoria, British Columbia; primary: 1:10,000; secondary: 1:2,000), HSP72 (SPA 810, Stressgen; primary: 1:1,000; secondary: 1:1,000), 4-hydroxy-2-nonenol (HNE; HNE11-S, Alpha Diagnostic International, San Antonio, TX; primary: 1:500; secondary: 1:1,000), and nitrotyrosine (no. 9691, Cell Signaling Technology, Beverly, MA; primary: 1:1,000; secondary: 1:2,000) for 90 min. The membranes were washed three times at 10 min each and exposed to the appropriate secondary antibody for 60 min (Amersham, Little Chalfont, Buckinghamshire, UK). HNE is a highly cytotoxic compound formed by free radical attack of fatty acids. Nitrotyrosine is a product of reactive nitrogen species acting on tyrosine residues. These are included as markers of oxidative stress.

The secondary antibody was diluted in TTBS containing 1.5–2% milk protein. Membranes were then washed three times at 10 min each and exposed to enhanced chemiluminescence (Amersham) for 2 min. Finally, the membranes were placed in a Kodak Image Station 440 CF developer, and the emitted signal was captured. The signal was analyzed using Kodak ID image analysis software (Eastman Kodak Scientific Imaging Systems, Rochester, NY).

Enzymatic assays. All enzymatic assays were performed in triplicate in microplates using a Spectramax 190 microplate reader (Molecular Devices, Downingtown, PA) in whole homogenate diluted 1:100 in PBS buffer. Glutathione peroxidase (GPX) activity was determined by the method of Flohe and Gunzler (12); glutathione reductase (GR) activity was determined by the method of Carlberg

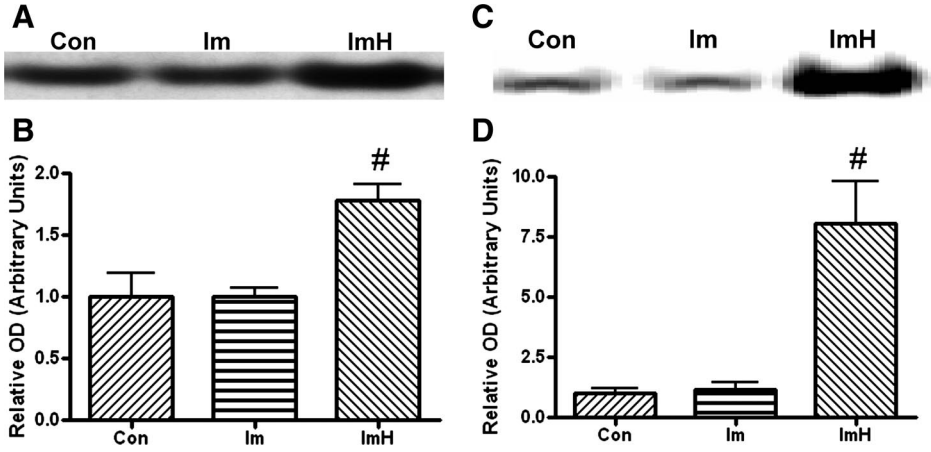

Fig. 1. HSP25 representative blot (*A*) and relative expression (*B*) and HSP72 representative blot (*C*) and relative expression (*D*) after heating on alternating days for 8 days during hindlimb immobilization. There is no difference between control (Con) and immobilized (Im) animals. Animals receiving the heat treatment (ImH) had significantly more HSP25 and HSP72 than Im animals. OD, optical density. #Significantly different from Im ($P < 0.025$).

and Mannervik (10); catalase activity was determined by the method of Aebi (2); and superoxide dismutase (MnSOD and CuZnSOD) activities were determined simultaneously by the method of McCord and Fridovich (31).

Statistical analysis. Data were analyzed using a *t*-test comparing Con to Im and Im to ImH. At no time was Con compared with ImH. Inflation of α was corrected via the Bonferroni correction; α was set a priori at $P < 0.05$; however, the correction requires $P < 0.025$ for significance to be achieved. Data are reported as means ± SE.

RESULTS

Body weights before the study began were not different between the groups (Con = 321 ± 6 g, Im = 318 ± 3 g, and ImH = 319 ± 7 g). After the 8-day period of immobilization, body weight in the Im group was significantly less than that for the Con group (Im = 269 ± 3 g; Con = 347 ± 4 g). Application of the heating protocol did not correct this reduction (ImH = 265 ± 4 g).

There was no difference in HSP25 (Fig. 1, *A* and *B*) or HSP72 (Fig. 1, *C* and *D*) expression between the Im and Con groups. However, there was a 75% increase in HSP25 and a sevenfold increase in HSP72 in the ImH group compared with the Im group.

In regard to the soleus muscle mass, there was an ~40% reduction in soleus mass in the Im group compared with the Con group. The reduction in mass was attenuated by nearly 20% in the ImH group compared with the Im group, resulting in a significantly larger muscle (Fig. 2*A*). Furthermore, when expressed relative to body weight, muscle mass was nearly 20% smaller in the Im group compared with the Con group. Heat treatment was able to attenuate this reduction by 50% (Fig. 2*B*).

Oxidant damage was determined by detection of HNE compounds as well as nitrosylated tyrosine residues. HNE was increased 33% in the Im group compared with the Con group, and heat treatment significantly reduced this elevation (Fig. 3, *A* and *B*). In a similar pattern, nitrotyrosine was increased 35% in the Im group compared with the Con group, and heat treatment significantly reduced this elevation (Fig. 3, *C* and *D*). Thus heat treatment does reduce the oxidative stress encountered during immobilization.

Finally, we wanted to determine whether HSPs provided the antioxidant effect observed. By eliminating the antioxidant enzymes as contributors to the overall antioxidant effect observed in the ImH, we proposed that evidence for the protective effects of HSPs would be gained. To determine this, we measured the activities of MnSOD, CuZnSOD, catalase, and the glutathione-handling enzymes GPX and GR (Table 1). There was no change in the activities of MnSOD, GPX, or GR. Catalase was significantly elevated in the Im group by ~10% compared with that shown in the Con group, and results for the ImH group were similar to those found in the Im group. CuZnSOD was significantly elevated with immobilization by 50% compared with that shown in the Con group, but heating eliminated this increase. These changes are supportive of the

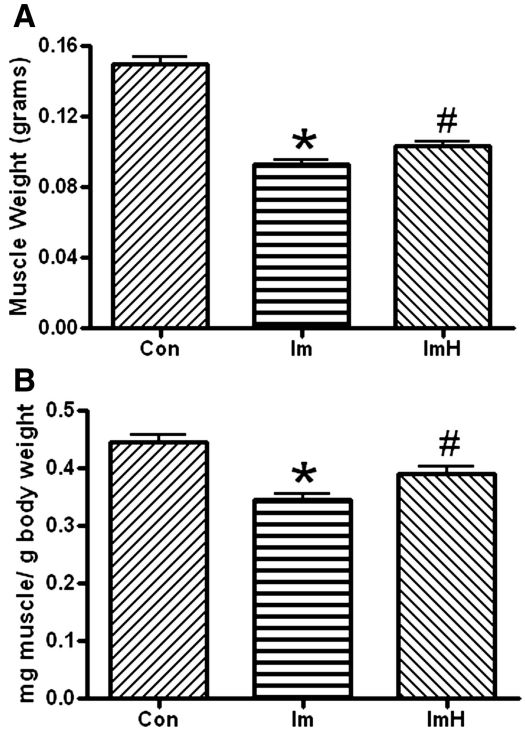

Fig. 2. After the immobilization period, the soleus muscle was removed, cleaned, and weighed (*A*). Immobilization caused a significant reduction in soleus muscle weight, whereas heating reduced the atrophy experienced by the muscle. Soleus muscle weight was then expressed relative to the body weight in grams (*B*). Immobilization resulted in a significant reduction in muscle-to-body weight ratio, whereas heating attenuated this loss. *Significantly different from Con and #significantly different from Im ($P < 0.025$).

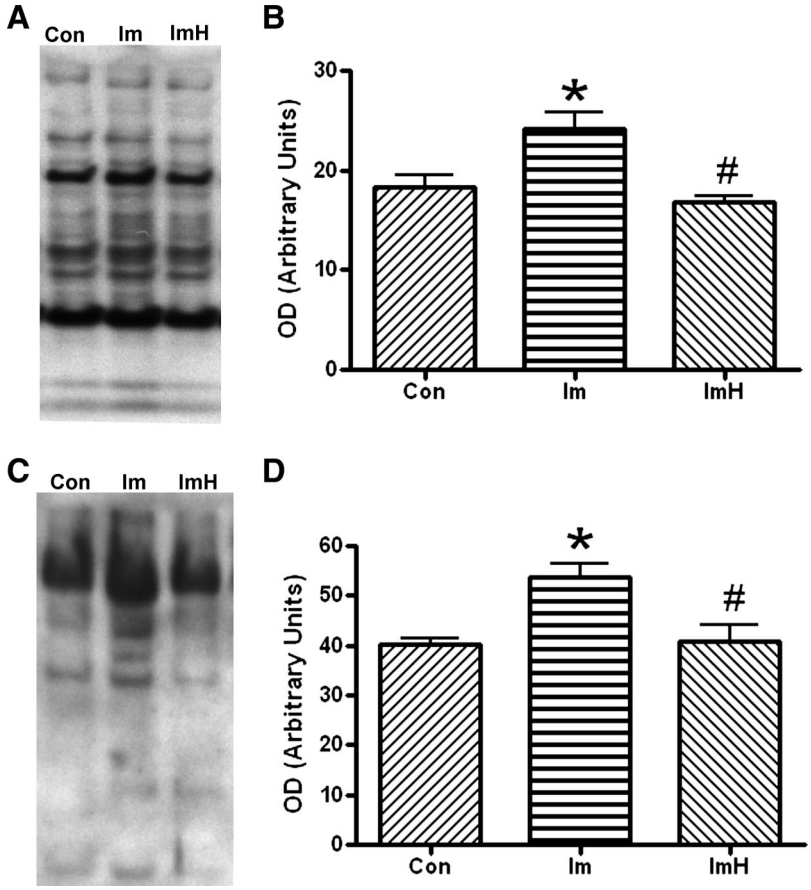

Fig. 3. Representative blot (*A*) and total OD (*B*) in 4-hydroxy-2-nonenol-treated membranes after 8 days of immobilization or immobilization with heat. Immobilization caused an elevation in these products, whereas heat attenuated the increase. Representative blot (*C*) and total OD (*D*) in nitrotyrosine-treated membranes after 9 days of immobilization or immobilization with heat. Immobilization caused a significant elevation in modified tyrosine products, whereas heat reduced this amount. *Significantly different from Con and #significantly different from Im ($P < 0.025$).

antioxidant effects of HSPs as none of the enzyme activities increased in response to heat treatment; in fact, CuZnSOD activity was lower, suggesting a reduced superoxide content.

DISCUSSION

This study investigated the possibility that heat treatment may attenuate oxidative stress and disuse atrophy induced via hindlimb immobilization. HSPs increased in the ImH group after heat treatment. Absolute muscle mass and muscle mass-to-body mass ratio were significantly higher in animals in the ImH group compared with that shown in the Im group. To our knowledge, this is the first study to show that heating significantly reduced oxidative stress in the soleus during immobilization. Finally, antioxidant enzyme activities in the ImH group changed in a manner consistent with reduced oxidant stress.

A loss of Ca^{2+} homeostasis within the cell may be responsible for the generation of free radicals (24, 26), and the associated oxidative stress may have contributed to the atrophy seen during immobilization. To assess this possibility, we measured two indexes of oxidative damage. Immobilization resulted in an increase in both HNE and nitrotyrosine compared with that shown in the Con group, indicating that there was an oxidant stress in immobilized muscle. This oxidative stress could be the result of an increased free radical production, a decreased antioxidant status, or a combination of the two. Both indexes were decreased in the ImH group compared with that shown in the Im group, indicating that heating was effective in reducing oxidant damage (Fig. 3). Because our heat treatment was clearly effective as an intervention to increase HSPs (Fig. 1), it is plausible, therefore, that the most important role of HSPs in this model may be their ability to buffer free radicals.

Given that HSPs have been shown repeatedly to decrease oxidative stress in a variety of models, it seems reasonable to suggest that they are providing protection against oxidant damage in this study. Specifically, transfected cells receiving an HSP25 or HSP72 expression gene demonstrated significantly higher survivability when exposed to H_2O_2 (20, 39) as well as reduced lactate dehydrogenase release (20). Additionally, transfected hearts overexpressing HSP72 suffered less mitochondrial dysfunction after ischemia reperfusion injury and had higher ventricular function compared with control hearts (18). Furthermore, gene array studies have shown that the only discernable trend is an increase in HSPs. Comparatively, these studies demonstrate inconsistent changes in other antioxidant substances, including MnSOD, CuZnSOD, cata-

Table 1. *Activities of native antioxidant enzymes*

Group	MnSOD	CuZnSOD	Catalase	GPX	GR
Con	304±55	334±42	3041±74	2488±104	1230±52
Im	216±43	506±37*	3286±56*	2719±119	1219±46
ImH	306±88	324±55†	3326±55	2553±156	1196±32

Values are means ± SE, expressed as U activity/g tissue. Animals were assigned to control (Con), 8 days of immobilization (Im), or 8 days of immobilization with a heat intervention (ImH). GPX, glutathione peroxidase; GR, glutathione reductase. *Significantly different from Con, $P < 0.025$. †Significantly different from Im, $P < 0.025$.

lase, and various glutathione handling enzymes (38, 43, 44, 50). For example, in one investigation, glutathione-*S*-transferase and MnSOD are reduced, whereas in another both are increased after heating (38, 50). Nevertheless, the possibility remains that proteins other than HSP72 or HSP25 are responsible for the observed protective effect.

To further implicate HSPs as providing the antioxidant effect observed in this study, several antioxidant enzyme activities were evaluated, including MnSOD, CuZnSOD, catalase, and the glutathione handling proteins GPX and GR (Table 1). MnSOD activity did not change as a result of immobilization, in agreement with previous work (22, 25), and may suggest that superoxide is not elevated in the mitochondria (5, 14). CuZnSOD was significantly increased with immobilization, supporting what has been found previously (22, 25, 28). This likely suggests an elevation of superoxide in the cytosol of immobilized skeletal muscle (14). Heating, however, caused a reduction in the CuZnSOD activity, suggesting a reduction in superoxide in the ImH group (5, 14). In accordance with the literature, GPX activity did not change after immobilization for 8 days (22, 25). The lack of change exhibited by GR activity in this investigation differs from what would be expected from the literature. In two investigations, Kondo et al. (22, 25) showed an increase in GR activity over the same time period. Although seemingly unlikely, it is possible that the difference in animal type could lead to these dissimilar results. Catalase activity is elevated with immobilization and is in good agreement with what has been observed before in immobilization studies of similar duration (22, 25). The changes in enzyme activity allow for the speculation that an increased activity of CuZnSOD resulted in the increased production of H_2O_2. Because there was no increase in GPX and only a slight increase in Cat, it seems likely that there was an increased production in OH· via fenton chemistry. Indeed, Kondo et al. detected both an increased level of iron, needed for fenton chemistry, as well as OH· after immobilization. Furthermore, we report an increase in oxidant stress after immobilization.

In addition to reduced oxidant damage, muscles in the ImH group were significantly larger than those in the Im group, indicating that heating protected muscle mass. Support for our findings comes from a study by Naito et al. (32), which used heating to protect muscle mass during hindlimb suspension. Naito et al. (32) demonstrated that a single bout of heat for 60 min was sufficient to detect an increase in HSP72 8 days later. Furthermore, muscle atrophy was reduced in the heated-suspended group by ~32%. Heating appears to be protective in both HLU and immobilization models, indicating a robust response to HSPs in disparate unloading models. Moreover, another indicator that oxidant stress plays a role in causing the loss of muscle mass is the observation that vitamin E has had some success as a countermeasure to disuse atrophy. It reduced the loss of cross-sectional areas by ~66% (4) in one investigation and attenuated losses in muscle mass by ~20% in another (21). However, in a third study, vitamin E did not protect against disuse atrophy (19).

Finally, it is noteworthy that HSP levels did not decrease during disuse in the Im group compared with the Con group. In several investigations, including those measuring mRNA and those measuring protein, reductions in HSP expression/content were detected (7, 32, 45). Furthermore, Ku et al. (27) detected a reduction in HSP70 associated with the polysomes following unloading. However, in this investigation and another (33), no such change is detected. Resolution to this disparity can be found when the gender of the animal used in each study is compared. Generally speaking, female animals tend to reduce HSP levels, whereas males do not. One notable exception to this trend was shown by Oishi et al. (35) who found a reduced HSP72 content following unloading in male rats.

In summary, we successfully reduced atrophy during 8 days of immobilization by application of a heat stress, which caused the elevation of both HSP25 and HSP72. Furthermore, this study is the first to show a reduction in oxidative stress in heated animals after immobilization. These data suggest that there may be an interaction between HSPs and oxidative stress under these conditions. The lack of an increase in antioxidant enzyme activity as well as data from gene array studies further supports the notion that HSPs provided protection from oxidative stress.

ACKNOWLEDGMENTS

This work was made possible by a grant provided by the National Football League Players Association Medical Charities.

REFERENCES

1. **Adams GR, Caiozzo VJ, and Baldwin KM.** Skeletal muscle unweighting: spaceflight and ground-based models. *J Appl Physiol* 95: 2185–2201, 2003.
2. **Aebi H.** Catalase in vitro. *Methods Enzymol* 105: 121–126, 1984.
3. **Alford EK, Roy RR, Hodgson JA, and Edgerton VR.** Electromyography of rat soleus, medial gastrocnemius, and tibialis anterior during hind limb suspension. *Exp Neurol* 96: 635–649, 1987.
4. **Appell HJ, Duarte JA, and Soares JM.** Supplementation of vitamin E may attenuate skeletal muscle immobilization atrophy. *Int J Sports Med* 18: 157–160, 1997.
5. **Bai Z, Harvey LM, and McNeil B.** Physiological responses of chemostat cultures of *Aspergillus niger* (B1-D) to simulated and actual oxidative stress. *Biotechnol Bioeng* 82: 691–701, 2003.
6. **Beere HM, Wolf BB, Cain K, Mosser DD, Mahboubi A, Kuwana T, Tailor P, Morimoto RI, Cohen GM, and Green DR.** Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. *Nat Cell Biol* 2: 469–475, 2000.
7. **Bey L, Akunuri N, Zhao P, Hoffman EP, Hamilton DG, and Hamilton MT.** Patterns of global gene expression in rat skeletal muscle during unloading and low-intensity ambulatory activity. *Physiol Genomics* 13: 157–167, 2003.
8. **Booth FW.** Regrowth of atrophied skeletal muscle in adult rats after ending immobilization. *J Appl Physiol* 44: 225–230, 1978.
9. **Booth FW and Kelso JR.** Production of rat muscle atrophy by cast fixation. *J Appl Physiol* 34: 404–406, 1973.
10. **Carlberg I and Mannervik B.** Glutathione reductase. *Methods Enzymol* 113: 484–490, 1985.
11. **Dodd SL and Koesterer TJ.** Clenbuterol attenuates muscle atrophy and dysfunction in hindlimb-suspended rats. *Aviat Space Environ Med* 73: 635–639, 2002.
12. **Flohe L and Gunzler WA.** Assays of glutathione peroxidase. *Methods Enzymol* 105: 114–121, 1984.
13. **Garrido C.** Size matters: of the small HSP27 and its large oligomers. *Cell Death Differ* 9: 483–485, 2002.
14. **Halliwell B.** Antioxidant defence mechanisms: from the beginning to the end (of the beginning). *Free Radic Res* 31: 261–272, 1999.
15. **Herbert ME, Roy RR, and Edgerton VR.** Influence of one-week hindlimb suspension and intermittent high load exercise on rat muscles. *Exp Neurol* 102: 190–198, 1988.
16. **Herbison GJ, Jaweed MM, and Ditunno JF.** Muscle atrophy in rats following denervation, casting, inflammation, and tenotomy. *Arch Phys Med Rehabil* 60: 401–404, 1979.
17. **Hnik P, Vejsada R, Goldspink DF, Kasicki S, and Krekule I.** Quantitative evaluation of electromyogram activity in rat extensor and flexor muscles immobilized at different lengths. *Exp Neurol* 88: 515–528, 1985.
18. **Jayakumar J, Suzuki K, Sammut IA, Smolenski RT, Khan M, Latif N, Abunasra H, Murtuza B, Amrani M, and Yacoub MH.** Heat shock

protein 70 gene transfection protects mitochondrial and ventricular function against ischemia-reperfusion injury. *Circulation* 104: I303–307, 2001.
19. **Koesterer TJ, Dodd SL, and Powers S.** Increased antioxidant capacity does not attenuate muscle atrophy caused by unweighting. *J Appl Physiol* 93: 1959–1965, 2002.
20. **Komatsuda A, Wakui H, Oyama Y, Imai H, Miura AB, Itoh H, and Tashima Y.** Overexpression of the human 72 kDa heat shock protein in renal tubular cells confers resistance against oxidative injury and cisplatin toxicity. *Nephrol Dial Transplant* 14: 1385–1390, 1999.
21. **Kondo H, Kodama J, Kishibe T, and Itokawa Y.** Oxidative stress during recovery from muscle atrophy. *FEBS Lett* 326: 189–191, 1993.
22. **Kondo H, Miura M, and Itokawa Y.** Antioxidant enzyme systems in skeletal muscle atrophied by immobilization. *Pflügers Arch* 422: 404–406, 1993.
23. **Kondo H, Miura M, Kodama J, Ahmed SM, and Itokawa Y.** Role of iron in oxidative stress in skeletal muscle atrophied by immobilization. *Pflügers Arch* 421: 295–297, 1992.
24. **Kondo H, Miura M, Nakagaki I, Sasaki S, and Itokawa Y.** Trace element movement and oxidative stress in skeletal muscle atrophied by immobilization. *Am J Physiol Endocrinol Metab* 262: E583–E590, 1992.
25. **Kondo H, Nakagaki I, Sasaki S, Hori S, and Itokawa Y.** Mechanism of oxidative stress in skeletal muscle atrophied by immobilization. *Am J Physiol Endocrinol Metab* 265: E839–E844, 1993.
26. **Kondo H, Nishino K, and Itokawa Y.** Hydroxyl radical generation in skeletal muscle atrophied by immobilization. *FEBS Lett* 349: 169–172, 1994.
27. **Ku Z, Yang J, Menon V, and Thomason DB.** Decreased polysomal HSP-70 may slow polypeptide elongation during skeletal muscle atrophy. *Am J Physiol Cell Physiol* 268: C1369–C1374, 1995.
28. **Lawler JM, Song W, and Demaree SR.** Hindlimb unloading increases oxidative stress and disrupts antioxidant capacity in skeletal muscle. *Free Radic Biol Med* 35: 9–16, 2003.
29. **Locke M.** The cellular stress response to exercise: role of stress proteins. *Exerc Sport Sci Rev* 25: 105–136, 1997.
30. **Machida S and Booth FW.** Regrowth of skeletal muscle atrophied from inactivity. *Med Sci Sports Exerc* 36: 52–59, 2004.
31. **McCord JM and Fridovich I.** Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J Biol Chem* 244: 6049–6055, 1969.
32. **Naito H, Powers SK, Demirel HA, Sugiura T, Dodd SL, and Aoki J.** Heat stress attenuates skeletal muscle atrophy in hindlimb-unweighted rats. *J Appl Physiol* 88: 359–363, 2000.
33. **Oishi Y, Ishihara A, Talmadge RJ, Ohira Y, Taniguchi K, Matsumoto H, Roy RR, and Edgerton VR.** Expression of heat shock protein 72 in atrophied rat skeletal muscles. *Acta Physiol Scand* 172: 123–130, 2001.
34. **Oishi Y, Taniguchi K, Matsumoto H, Ishihara A, Ohira Y, and Roy RR.** Muscle type-specific response of HSP60, HSP72, and HSC73 during recovery after elevation of muscle temperature. *J Appl Physiol* 92: 1097–1103, 2002.
35. **Oishi Y, Taniguchi K, Matsumoto H, Kawano F, Ishihara A, and Ohira Y.** Upregulation of HSP72 in reloading rat soleus muscle after prolonged hindlimb unloading. *Jpn J Physiol* 53: 281–286, 2003.
36. **Pattison JS, Folk LC, Madsen RW, Childs TE, Spangenburg EE, and Booth FW.** Expression profiling identifies dysregulation of myosin heavy chains IIb and IIx during limb immobilization in the soleus muscles of old rats. *J Physiol* 553: 357–368, 2003.
37. **Powers SK, Quindry J, and Hamilton K.** Aging, exercise, and cardioprotection. *Ann NY Acad Sci* 1019: 462–470, 2004.
38. **Rockett JC, Mapp FL, Garges JB, Luft JC, Mori C, and Dix DJ.** Effects of hyperthermia on spermatogenesis, apoptosis, gene expression, and fertility in adult male mice. *Biol Reprod* 65: 229–239, 2001.
39. **Rogalla T, Ehrnsperger M, Preville X, Kotlyarov A, Lutsch G, Ducasse C, Paul C, Wieske M, Arrigo AP, Buchner J, and Gaestel M.** Regulation of Hsp27 oligomerization, chaperone function, and protective activity against oxidative stress/tumor necrosis factor alpha by phosphorylation. *J Biol Chem* 274: 18947–18956, 1999.
40. **Saleh A, Srinivasula SM, Balkir L, Robbins PD, and Alnemri ES.** Negative regulation of the Apaf-1 apoptosome by Hsp70. *Nat Cell Biol* 2: 476–483, 2000.
41. **Smolka MB, Zoppi CC, Alves AA, Silveira LR, Marangoni S, Pereira-Da-Silva L, Novello JC, and Macedo DV.** HSP72 as a complementary protection against oxidative stress induced by exercise in the soleus muscle of rats. *Am J Physiol Regul Integr Comp Physiol* 279: R1539–R1545, 2000.
42. **Solaro RJ, Pang DC, and Briggs FN.** The purification of cardiac myofibrils with Triton X-100. *Biochim Biophys Acta* 245: 259–262, 1971.
43. **Sonna LA, Gaffin SL, Pratt RE, Cullivan ML, Angel KC, and Lilly CM.** Effect of acute heat shock on gene expression by human peripheral blood mononuclear cells. *J Appl Physiol* 92: 2208–2220, 2002.
44. **Sonna LA, Wenger CB, Flinn S, Sheldon HK, Sawka MN, and Lilly CM.** Exertional heat injury and gene expression changes: a DNA microarray analysis study. *J Appl Physiol* 96: 1943–1953, 2004.
45. **Stevenson EJ, Giresi PG, Koncarevic A, and Kandarian SC.** Global analysis of gene expression patterns during disuse atrophy in rat skeletal muscle. *J Physiol* 551: 33–48, 2003.
46. **Thomason DB and Booth FW.** Atrophy of the soleus muscle by hindlimb unweighting. *J Appl Physiol* 68: 1–12, 1990.
47. **Thomason DB, Herrick RE, Surdyka D, and Baldwin KM.** Time course of soleus muscle myosin expression during hindlimb suspension and recovery. *J Appl Physiol* 63: 130–137, 1987.
48. **Watters C.** A one-step biuret assay for protein in the presence of detergent. *Anal Biochem* 88: 695–698, 1978.
49. **Witzmann FA, Kim DH, and Fitts RH.** Hindlimb immobilization: length-tension and contractile properties of skeletal muscle. *J Appl Physiol* 53: 335–345, 1982.
50. **Zhang HJ, Drake VJ, Morrison JP, Oberley LW, and Kregel KC.** Selected contribution: Differential expression of stress-related genes with aging and hyperthermia. *J Appl Physiol* 92: 1762–1769, 2002.