Overexpression of HSP70 in mouse skeletal muscle protects against muscle damage and age-related muscle dysfunction

ANNE McARDLE,*,[2] WOLFGANG H. DILLMANN,[†] RUBEN MESTRIL,[‡] JOHN A. FAULKNER,[§] AND MALCOLM J. JACKSON*

*Department of Medicine, University of Liverpool, Liverpool, UK; [†]Department of Medicine, University of California, San Diego, California, USA; [‡]Department of Physiology, Loyola University, Chicago, Illinois, USA; and [§]Institute of Gerontology, University of Michigan, Ann Arbor, Michigan, USA

SPECIFIC AIMS

Skeletal muscle aging is characterized by atrophy, a deficit in the generation of specific force, increased susceptibility to contraction-induced injury, and a prolonged force deficit after severe injury. The ability of muscles of old mice to produce HSPs in response to stress is severely diminished. The aim of this study was to examine the effect of overexpression of HSP70 on these functional deficits after a severe and damaging protocol of lengthening contractions in adult and old mice.

PRINCIPAL FINDINGS

1. Transgenic overexpression of HSP70 in skeletal muscle protects against lengthening contraction-induced damage and facilitates rapid and successful recovery after damage

The investigation involved adult (10–12 months) and old (26–28 months) male and female wild-type (WT) B6XSJL mice and transgenic mice that had a chimeric transgene that consisted of an inducible HSP70 gene of a rat under a β-actin promoter. This resulted in a 10- to 20-fold increase in HSP70 content of EDL muscles of adult and old HSP70 transgenic mice compared with that of age-matched WT mice. The absolute maximum tetanic force (P_0) was determined at optimal length by analysis at increasing frequency of stimulation, prior to a severe lengthening contraction protocol and at 3 h, 3, 14 and 28 days after a severe and damaging lengthening contraction protocol. At 3 h after the contraction protocol, the muscles of both adult and old WT and HSP70 transgenic mice had a force deficit of 50 to 70%. Between 3 h and 3 days, the P_0 of adult and old WT mice showed a further decrease of ~20%. In contrast, no evidence of this secondary loss of force was observed for muscles of adult or old HSP70 transgenic mice at the 3 day time (**Fig. 1**). Three days after the contraction-induced injury, histological analysis of muscles of adult and old age groups of both WT and HSP70 transgenic mice showed widespread necrosis with the presence of phagocytic cells within fibers of the EDL muscles. Quantification of the percentage of intact muscle fibers that were present in muscles at 3 days after the contraction protocol demonstrated that a higher percentage of intact fibers remained in adult HSP70 transgenic mice than in adult WT mice.

2. Muscles of adult and old HSP70 transgenic mice demonstrate an enhanced recovery in comparison with muscles of age-matched WT mice

The force deficit was eliminated by 28 days after the lengthening contraction protocol in the EDL muscles of adult WT mice. In contrast, the muscles of old WT mice did not recover completely and still demonstrated a force deficit of 44% at 28 days following the protocol (Fig. 1). A dramatic improvement occurred in the capacity to develop maximum force in EDL muscles of adult and old HSP70 transgenic mice such that no significant force deficit was evident by 14 days after the contraction protocol (Fig. 1).

3. Increased muscle HSP70 content protects against the age-related fall in specific force

The maximum force generation per unit cross-sectional area (specific P_0) for EDL muscles of old WT mice was 26% less than that of the adult WT mice (**Fig. 2**). In contrast, no differences were observed for the specific P_0 for the old compared with adult HSP70 transgenic mice. This occurred even though the decrease in absolute P_0 of EDL muscles with age was similar in WT and HSP70 transgenic mice, with the value for the old WT mice 28% less than the adult value and 23% less for the HSP70 transgenic mice. No differences were observed between the absolute P_0 of EDL muscles of HSP70 and WT mice of the same age.

[1] To read the full text of this article, go to http://www.fasebj.org/cgi/doi/1096/fj.03-0395fje; doi: 10.1096/fj.03-0395fje

[2] Correspondence: Department of Medicine, University of Liverpool, Liverpool L69 3GA, UK. E-mail: mdcr02@liv.ac.uk

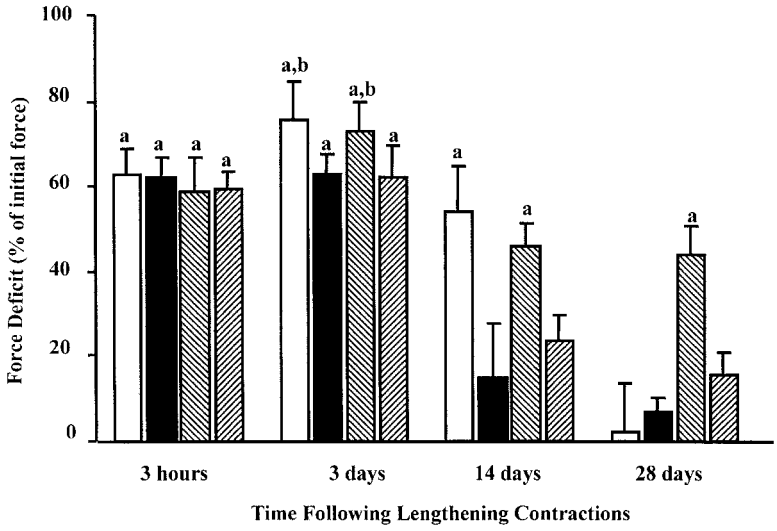

Figure 1. Force deficit of EDL muscles of adult (□) and old (▨) WT and adult (■) and old (▨) HSP70 transgenic mice at various times after the lengthening contraction protocol. a, $P < 0.05$ cf. pre-exercise value; b, $P < 0.05$ cf. data for the same group at the 3 h point. Extensor digitorum longus (EDL) muscles of adult and old male and female wild-type (WT) B6XSJL mice and HSP70 transgenic mice were administered a protocol of 450 lengthening contractions. Each lengthening contraction began with activation of a muscle at L_0 with a stimulation frequency of 150 Hz. The isometric contraction was held for 100 msec and the activated muscle was stretched through a strain of 20% of L_f at a velocity of 1.5 L_f/sec. The protocol of 450 lengthening contractions consisted of three 5 min periods with 150 contractions during each period and a 5 min rest period between each lengthening contraction period. The force deficit for each recovery time was calculated as the difference between a given recovery P_0 and the initial P_0 for the same muscle expressed as % of the initial P_0.

CONCLUSIONS AND SIGNIFICANCE

These data suggest that overexpression of HSP70 in skeletal muscle protects against the age-related fall in specific force, the increased susceptibility to damage and improves recovery from damage. These data are novel and indicate that preservation of the ability of muscles of old animals to produce HSP70 after stress may have significant consequences in prevention of the development of functional deficits by muscles of old animals. Data have demonstrated that EDL muscles of adult and old HSP70 transgenic mice showed a dramatic improvement in the capacity to develop maximum force after a severe lengthening contraction protocol. The rapid recovery by the EDL muscles of old HSP70 transgenic mice was remarkable, and was in sharp contrast to the persistence of a 44% force deficit at 28 days for the EDL muscles of old WT mice. Other workers have demonstrated that the deficit persists for up to 60 days after a period of lengthening contractions. The protection from the more severe secondary injury was evident in the 10% to 15% lower force deficits for the muscles of the HSP70 transgenic mice at 3 days after the contraction protocol. Previous studies have suggested that the underlying cause of the permanent force deficit in muscles of old WT mice at 28 days following the contraction protocol was a loss in muscle mass arising from a loss in the number of fibers. In the current study, no significant loss in total mass was observed in muscles of old WT mice at this time point but a 5–10% loss in the cross-sectional area composed of viable fibers was observed. This was not evident in muscles of adult WT or HSP70 transgenic mice. These data indicate that these age-related deficits in skeletal muscle are not inevitable.

The overexpression of HSP70 in the skeletal muscles of transgenic mice reduced body mass by ~10% and muscle mass by ~20%, compared with the body and muscle masses of adult and old WT mice. Furthermore, the old HSP70 transgenic mice were not protected from the age-related muscle atrophy of ~20%. Despite the lower muscle masses and the smaller cross-sectional areas of the HSP70 transgenic mice, the absolute P_0 of the EDL muscles of the age-matched HSP70 and WT mice were not different. The ~25–30% age-related loss in absolute P_0 of the two strains did not differ significantly from one another or from previous estimates. In the present study, the deficit in the absolute P_0 of 27% for EDL muscles of old vs. adult WT mice was accompanied by an 8% loss in total muscle cross-sectional area. This resulted in a 25% decline in specific force. This is in excellent agreement with previous estimates of the deficit attributable to age. In contrast, the specific P_0 of EDL muscles of old HSP70 transgenic mice was not different from that of the adult HSP70 transgenic mice. These data support the hypothesis that overexpression of HSP70 preserves the capability of muscle fibers to generate force per unit cross-sectional area.

Overexpression of HSP70 may have influenced damage and regeneration processes through a number of potential mechanisms. The secondary phase of damage after lengthening contractions is associated with increased production of free radicals. The increased

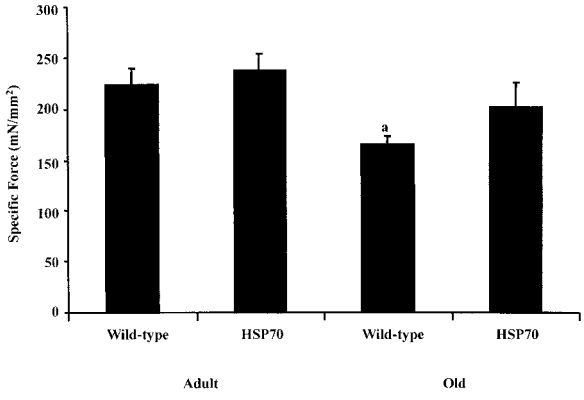

Figure 2. Specific force of EDL muscles of adult and old WT and adult and old HSP70 transgenic mice. a, $P < 0.05$ cf. value for adult WT mice. The specific P_o was calculated as the absolute P_o/total muscle CSA.

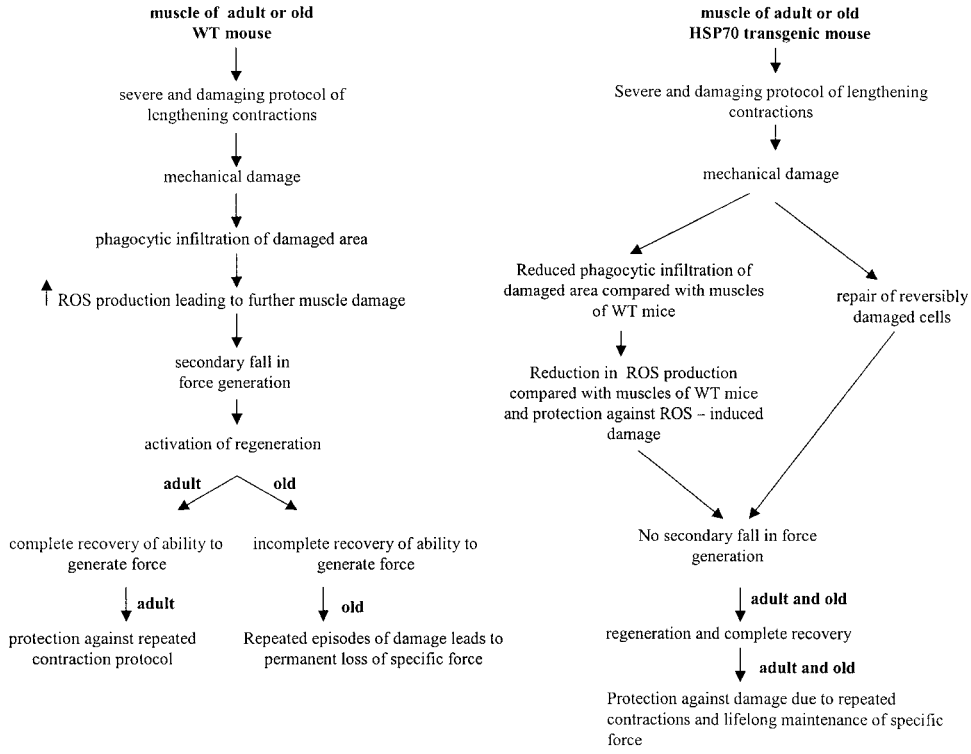

Figure 3. Schematic diagram of the potential role of lifelong HSP70 overexpression in muscles of mice. Data suggest that in adult and old WT mice subjected to a severe and damaging protocol of lengthening contractions, initial mechanical damage is rapidly followed by phagocytic infiltration, increased production of free radicals and further muscle damage. Muscles of adult WT mice recover from damage whereas muscles of old WT mice maintain a permanent force deficit. In contrast although muscles of adult and old HSP70 transgenic mice demonstrate a similar initial mechanical damage, the subsequent phagocytic infiltration is reduced compared with muscles of WT mice. We hypothesize that the increased efficiency of muscles of HSP70 transgenic mice to repair damaged cells and protect against damage induced by ROS production leads to no secondary fall in force generation. The rapid and complete recovery of damage in muscles of HSP70 transgenic mice of all ages allows for the maintenance of muscle force generation throughout life.

content of HSP70 may provide protection against this increased free radical activity at 3 days following the contraction protocol. This is supported by data that suggests that an increased cellular content of HSPs results in an increased resistance to some forms of free radical-mediated pathologies. Alternatively, HSP70 is a necessary component of the cellular repair machinery. Thus, a proportion of muscle fibers that were reversibly damaged following the contraction protocol, may be repaired more readily.

A decreased capacity for fiber regeneration in muscles of old WT mice agrees with previous reports after contraction-induced injury and whole muscle transplantation. The impaired regenerative capacity has been associated with the host environment. The factors in the environment of the old host are complex and likely vary with the underlying cause of the injury to fibers. We hypothesize that a critical issue in the failure of muscle to regenerate in an old host may be the inability of muscle of old rodents to produce HSPs in response to stress. The increased content of HSPs is crucial to structural and biochemical remodeling of muscle and so the inability to produce HSPs may play a direct role in failure of successful regeneration in muscles of old WT mice. Other workers have demonstrated that the phagocytic invasion is prolonged in muscles of old WT mice after contraction-induced injury and this prolongation may play an important role in the poor recovery of the muscles. Prolongation of phagocytic invasion may be directly related to the inability of muscles of old rodents to produce HSPs since these proteins participate in cytokine signaling, cytokine gene expression and enhance antigen presentation to T lymphocytes. The enhanced regeneration of muscles in HSP70 transgenic mice may be due to a resistance to cytokine-mediated toxicity and maintenance of efficient antigen presentation.

In summary, this study is the first to provide direct evidence of a beneficial effect of maintenance of muscle content of HSP70 in old age and is fundamental to our understanding of the consequences of the inability of muscles of old animals to produce these proteins. Data indicate that at least some of the age-related deficits in skeletal muscle are not inevitable and suggest that therapy designed at maintenance of the stress response during normal ageing could impact considerably on the quality of life of the elderly. FJ