Time course of responses of human skeletal muscle to oxidative stress induced by nondamaging exercise

MUNA KHASSAF,1 ROBERT B. CHILD,1 ANNE McARDLE,1 DAVID A. BRODIE,2 CRISTIAN ESANU,1 AND MALCOLM J. JACKSON1
Departments of 1Medicine and 2Movement Science and Physical Education, University of Liverpool, Liverpool L69 3GA, United Kingdom

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A SUBSTANTIAL AMOUNT OF DATA indicates that exercise is associated with an increase in the production of free radicals and other reactive oxygen species by skeletal muscle (6, 12). This increase appears to occur because a proportion of the molecular oxygen used in normal respiration undergoes one-electron reduction to generate superoxide radicals (3). It has been estimated that exercise can increase oxygen utilization 200-fold above resting levels in active muscle fibers (18), and superoxide production appears to increase with this large increase in oxygen flux through muscle mitochondria during exercise. This has been claimed to lead to release of superoxide (31) and hydrogen peroxide by the muscle cell and the local formation of hydroxyl radicals (27). Skeletal muscle also contains nitric oxide synthases and a number of other potential sources for the generation of reactive oxygen species (30). A number of studies have examined the potential consequences of the exercise-induced increase in oxidative stress, and data have been presented suggesting that oxidative stress plays an important role in the muscle fatigue or damage (2) that accompanies some forms of exercise. However, these roles remain contentious (see Ref. 11 for a review).

Evidence from animal studies indicates that muscle cells adapt to this increased free radical activity to reduce the risk of free radical damage to the tissue. Thus some studies report that an acute bout of exercise increases the activities of superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase in skeletal muscle of rats (15). Longer term exercise training also appears to increase the activity of several antioxidant enzymes, such as superoxide dismutase, catalase (10), or glutathione peroxidase (15) in muscle, although these are not consistent findings (1). In humans, exercise training has been reported to increase skeletal muscle superoxide dismutase activities (14) and the activities of various protective enzymes in blood (32). Oxidative and other stresses to cells are also known to induce the formation of heat shock proteins (HSPs) as an important component of the cellular protective response (5), and recent data also indicate that an increase in muscle HSP content occurs after exercise in rats (9, 17, 33). These proteins are thought to facilitate successful repair from injury and to aid adaptation and remodeling of the cell to prevent the damage from recurring after a repeat of the same stress (7). HSPs are named according to their molecular weight and include HSP25, HSP60, HSP70, HSP90, and HSP110 families of proteins in mammalian cells (8). HSPs are molecular chaperones that are necessary for facilitating the correct folding of newly synthesized cellular proteins and correct translocation to cellular compartments (25, 36), without which cellular proteins would degrade or aggregate and the cell would die (24).
Few data have been reported concerning expression of HSPs in human skeletal muscle after exercise, although Liu et al. (19) have reported an increase in the HSP70 content of human vastus lateralis muscle after 1–4 wk of rowing training. In contrast, Putschart and co-workers (29) examined the effect of an acute bout of exercise on muscle HSP70 expression and found that, although the content of HSP70 mRNA increased, there was no change in the muscle HSP70 protein content.

We hypothesized that a single bout of sustained submaximal exercise to a single muscle group would lead to adaptive responses of both the activity of muscle antioxidant enzymes and muscle HSP content. The responses of muscle superoxide dismutase and catalase activities and muscle HSP60 and HSP70 content were therefore examined in biopsies from a group of subjects before and after a bout of one-legged cycle ergometry. HSP60 was selected for analysis because this protein is primarily mitochondrial in cellular location and has a key role in the intracellular protein translocation and cytoprotection (34), and HSP70 (also known as HSP72 or HSP70i) was selected because this protein typically demonstrates very large increases after various forms of cellular stress (37).

METHODS

Subjects. Seven sedentary male volunteers participated in the study. Their heights, masses, and ages were 1.79 ± 0.03 m, 81.6 ± 4.2 kg, and 28 ± 2 yr (means ± SE), respectively. None of the subjects consumed antioxidant supplements during or in the month before the experimental procedures. The protocol was approved by the Research Ethical Committee of the Royal Liverpool and Broadgreen University Hospital Trust, and all subjects gave informed, written consent.

Incremental exercise protocol. Each subject exercised at a cadence of 70 rpm, using a single leg, on a friction-loaded cycle ergometer (Monark 864) that was modified specifically for this purpose. An incremental work test was performed to determine peak oxygen uptake, during which the workload was increased by 35 W every 4 min to volitional exhaustion. Expired air was monitored continuously during exercise using an on-line gas analysis system (Sensormedics Vmax 20 Pulmonary Spirometry Instrument). Average oxygen uptake was determined over the last 45 s of each workload and in the 45 s before test termination. Because the subjects were unacclimated to this exercise protocol, a second incremental work test was performed within a week of the first test to assess the reliability of the measurements. These were used to calculate the workload to elicit an oxygen uptake of 70% maximum oxygen uptake.

Constant-load protocol. During the experimental protocol, the subjects each cycled at 70% peak maximum oxygen uptake for 45 min using the leg contralateral to that used for the preliminary incremental tests. Pyrexercise, resting oxygen uptake was determined from respiratory data collected during the 10 min before exercise, and oxygen uptake was measured over the last minute of each 5-min period during exercise. Heart rate was recorded before and throughout the exercise test.

Muscle biopsies. Muscle biopsies were taken from the vastus lateralis muscle of the exercised leg under local anesthesia (2% lignocaine), using a Bergstrom-type needle (6.5-mm diameter).

Preliminary investigations were performed on two sedentary male subjects to assess whether repeated biopsies influenced muscle HSP70 content. A total of four biopsies was collected from sites in the vastus lateralis muscle that were 2.5–3.0 cm apart. Two studies were undertaken in which biopsies were either taken linearly along the muscle at 0, 24, 48, and 72 h or from sites surrounding a first biopsy site at 3, 5, and 6 days later. No elevation in HSP70 content was observed in subsequent biopsies from either subject, indicating that serial muscle biopsies could be taken without inducing the production of stress proteins in the remaining tissue (results not shown in detail).

Muscle biopsies were taken from randomized sites in the vastus lateralis muscle of the experimental leg at 7 days before the exercise protocol and at 1, 2, 3, and 6 days after the exercise. Samples (~100 mg) were obtained and immediately frozen in liquid nitrogen and stored at −70°C until analyzed.

Biochemical methods. Muscle samples were homogenized in 100 mM phosphate buffer, pH 7.0, and the homogenate was analyzed for catalase activity by the kinetic decomposition of hydrogen peroxide followed spectrophotometrically at 240 nm, using a method derived from Claiborne (4). Total superoxide dismutase (SOD) activity was measured using the SOD-525 kit (R & D Systems Europe), based on the method of Nebot et al. (26). For analysis of HSPs, muscle samples were homogenized in 1% SDS containing 1 mM iodoacetamide, 1 mM benzenthionium chloride, 5.7 mM p-hydroxybenzylsulfonyl fluoride, and 5 mM EGTA. Samples were then centrifuged at 4°C, and the supernatant was analyzed for total protein content by using the bicinchoninic acid method (Sigma Chemical, Poole, UK). Fifty micromolars of total protein were separated on SDS-PAGE followed by Western blotting. The content of HSP60 and HSP70 was analyzed by using monoclonal antibodies obtained from Bioquote and Amersham Life Sciences (21). Bands were visualized on X-ray film using the ECL chemiluminescent detection system (Amersham Life Sciences). Membranes were exposed to film for three to four different exposure times to ensure that saturation of film had not occurred. Samples from each subject were applied to the same gel, the intensity of staining for known bands was determined from the imaging system (Imaging Station, Imaging), and the content of HSPs was expressed as a percentage of the preexercise content for each subject.

Peripheral blood samples were obtained from the subjects before the muscle biopsy at 7 days before exercise and at 1 and 3 days postexercise. Serum was separated and analyzed for total creatine kinase activity as previously described (16).

Statistical analysis. Data are presented as means ± SE. Comparisons between means was undertaken by repeated analysis of variance followed by paired Student’s t-test where appropriate.

RESULTS

Peak oxygen uptake for the first and second preliminary incremental work tests were 3.0 ± 0.4 and 3.1 ± 1.1 l/min, respectively. Data from seven subjects are presented; six were able to complete the endurance test, but one subject was unable to continue after 28 min because of perceived exhaustion. The oxygen uptake, exercise intensity, and heart rate of the subjects during the experimental protocol are given in Table 1.
Exercise more than doubled resting heart rate (P < 0.001) and resulted in a fivefold increase in whole body oxygen utilization (P < 0.001). Mean serum creatine kinase activities were not significantly changed by the exercise (preexercise: 91 ± 8 U/l; 1 day postexercise: 99 ± 8 U/l; 3 day postexercise: 124 ± 19 U/l).

The activity of superoxide dismutase in muscle biopsies was found to increase significantly within 24 h of completing the exercise protocol (Fig. 1). This increase was maintained until at least 3 days postexercise. In contrast, catalase activities in the muscle were unchanged by the exercise protocol and remained relatively constant throughout the postexercise period (Fig. 2).

Muscle HSP content is presented relative to the content in the initial biopsy sample from each subject. The content of HSPs in muscle samples showed a variable increase between subjects. HSP60 content tended to increase after exercise, although this increase only became statistically significantly different from baseline (P < 0.05) at 3 days postexercise (Fig. 3). The mean muscle HSP70 content increased dramatically by 6 days postexercise. The variability between subject responses was remarkably large, and the changes reached statistical significance compared with baseline values (P < 0.05) only at 6 days postexercise (Fig. 4). Examples of the variability in response between individual subjects are also shown in Fig. 4.

**DISCUSSION**

The data presented clearly show that human skeletal muscle responds to the stress of a single period of nontraumatic aerobic exercise by upregulating expression of superoxide dismutase and HSP60 and HSP70.

The one-legged cycling exercise model was chosen for these studies to minimize effects due to muscle trauma and subsequent inflammation, while maximizing the increase in blood flow and oxygen uptake by the muscle. We reasoned that exercise predominantly involving a single muscle group would help ensure that the muscle mitochondria maintained a high rate of respiration throughout the exercise. Our previous experience also indicated that this type of activity was one that untrained individuals could readily adhere to.

The transient rise in skeletal muscle SOD activity (Fig. 1) after a single acute exercise period has been reported in experimental animals by Ji (15) but does not appear to have been previously described in humans. This was not accompanied by a rise in muscle catalase activity (Fig. 2), a pattern similar to most reports from animal studies (28). The rise in SOD activity implies that exercise increases the exposure of human muscle tissue to superoxide radicals, leading to an adaptive response. Lack of tissue prevented analysis of the two forms of SOD (i.e., MnSOD and Cu/Zn SOD), although by analogy with animal studies it seems likely that both may have been elevated by the protocol used (28). Reid and co-workers (31) have previously reported an increase in the release of superoxide radicals from isolated strips of rat diaphragm in vitro, but similar data do not appear to have been reported for human skeletal muscle.

![Fig. 1. Activity of superoxide dismutase (SOD) in the vastus lateralis muscle of control subjects before and after exercise on a cycle ergometer. *P < 0.05 vs. preexercise values.](http://jap.physiology.org/)

![Fig. 2. Activity of catalase in the vastus lateralis muscle of control subjects before and after exercise on a cycle ergometer.](http://jap.physiology.org/)
These data also appear to represent the first report of an increase in the content of HSPs in human skeletal muscle after a single bout of exercise, although similar data have been presented from animal studies (22, 33), and muscle HSP70 content was increased in rowers after long-term exercise training (19). Additionally, this appears to be the first report of changes in HSP content in response to nontraumatic exercise in any species. In the present study, no rise in serum creatine kinase activity was seen, indicating that the induction appeared to have occurred in the absence of overt damage to the muscle resulting from the exercise. The signal responsible for activation of the stress response after this form of exercise is unclear, although the synthesis of HSPs in skeletal muscle also increases after a variety of other stresses, most notably hyperthermia. Our data from animal studies indicate that a period of contractile activity of muscle results in increased production of superoxide radicals and a transient, nondamaging oxidation of muscle protein thiol groups (23). This oxidative stress may activate the stress response, either by direct interaction with the transcription factor involved, heat shock factor-1, or by causing some minor damage to proteins that is detected by the cell with a consequent increase in HSP expression (13, 35).

The variability in the response of HSP70 between subjects was large (Fig. 4). This appeared to be in part due to a smaller proportionate response in those subjects in whom baseline levels were relatively high. An example of this can be seen in the illustrative Western blots in Fig. 4. The time course of changes in HSP60 was compatible with those seen in animal studies, but HSP70 did not show a significant rise until 6 days postexercise. Evaluation of the individual data illustrated that some subjects showed a clear rise in muscle HSP70 content within 24–48 h (e.g., see the lower series of blots on Fig. 4), whereas others showed a slower and reduced proportionate change in content. Thus the lack of a significant change until 6 days postexercise is, at least in part, due to the variability of the interindividual responses.

These data indicate that skeletal muscle contains an elevated content of HSPs during the period from 3 to, at least, 6 days postexercise. Because such proteins are cytoprotective in other systems (20), it is feasible that these proteins facilitate protection of skeletal muscle from exercise-induced damage during subsequent exercise after the initial bout. In addition, an increased content of HSPs will facilitate any cellular remodeling, which is known to occur after unaccustomed exercise. Preliminary data indicate that this situation occurs in mouse muscle after exposure to a period of nondamaging exercise (22). A further understanding of the role of these proteins in protection of skeletal muscle against exercise-induced damage and of the time course of their production after different types of exercise may facilitate the logical planning of optimal exercise and training regimens to minimize muscle damage and maximize adaptation to the contractile activity.

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REFERENCES


