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Original Contribution

Lifelong training preserves some redox-regulated adaptive responses after an acute exercise stimulus in aged human skeletal muscle

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ABSTRACT

Several redox-regulated responses to an acute exercise bout fail in aged animal skeletal muscle, including the ability to upregulate the expression of antioxidant defense enzymes and heat shock proteins (HSPs). These findings are generally derived from studies on sedentary rodent models and thus may be related to reduced physical activity and/or intraspecies differences as opposed to aging per se. This study, therefore, aimed to determine the influence of age and training status on the expression of HSPs, antioxidant enzymes, and NO synthase isoenzymes in quiescent and exercised human skeletal muscle. Muscle biopsy samples were obtained from the vastus lateralis before and 3 days after an acute high-intensity-interval exercise bout in young trained, young untrained, old trained, and old untrained subjects. Levels of HSP72, PRX5, and eNOS were significantly higher in quiescent muscle of older compared with younger subjects, irrespective of training status. 3-NT levels were elevated in muscles of the old untrained but not the old trained state, suggesting that lifelong training may reduce age-related macromolecule damage. SOD1, CAT, and HSP27 levels were not significantly different between groups. HSP27 content was upregulated in all groups studied postexercise. HSP72 content was upregulated to a greater extent in muscle of trained compared with untrained subjects postexercise, irrespective of age. In contrast to every other group, old untrained subjects failed to upregulate CAT postexercise. Aging was associated with a failure to upregulate SOD2 and a downregulation of PRX5 in muscle postexercise, irrespective of training status. In conclusion, lifelong training is unable to fully prevent the progression toward a more stressed muscular state as evidenced by increased HSP72, PRX5, and eNOS protein levels in quiescent muscle. Moreover, lifelong training preserves some (e.g., CAT) but *not all* (e.g., SOD2, HSP72, PRX5) of the adaptive redox-regulated responses after an acute exercise bout. Collectively, these data support many but not all of the findings from previous animal studies and suggest parallel aging effects in humans and mice at rest and after exercise that are not modulated by training status in human skeletal muscle.

A myriad of exercise-induced perturbations disrupt skeletal muscle homeostasis and activate signaling cascades that mediate exercise adaptations with repeated activation [1]. It appears that transient exercise-induced increases in the production of reactive oxygen and nitrogen species (RONS) are important in transducing certain exercise adaptations [2–4], and the fact that antioxidants may blunt exercise training adaptations supports this notion [5]. It is, however, emphasized that some of these findings have not been replicated [6]. Nevertheless, RONS are involved in the activation of redox-sensitive transcription factors, notably activating protein 1 (AP-1), nuclear factor κB (NF-κB), and heat shock factor 1 (HSF-1), that promote cytoprotective adaptations [7]. For example, a hallmark

Abbreviations: CAT, catalase; eNOS, endothelial nitric oxide synthase; HSP27, heat shock protein 27; HSP72, heat shock protein 72; nNOS, neuronal nitric oxide synthase; PRX5, peroxiredoxin 5; RONS, reactive oxygen and nitrogen species; SOD1, superoxide dismutase 1; SOD2, superoxide dismutase 2; 3-NT, 3-nitrotyrosine.

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of exercise-induced redox signaling is an increase in the abundance and activity of antioxidant defense enzymes and heat shock proteins (HSPs) that help protect against the potential cytotoxic outcomes of increased RONS generation [8–11]. An increase in the levels of antioxidant defense enzymes and HSPs is often evident after a single bout of exercise [8–10], highlighting the extraordinary malleability of skeletal muscle.

In an aging setting, a residual elevation in RONS production is associated with an increase in oxidative macromolecule damage in skeletal muscle [12–14]. A series of animal studies have identified that this phenomenon has important implications for exercise-induced redox signaling [14–18]. It seems that an increasingly stressed muscular redox environment results in constitutive AP-1, HSF-1, and NF-κB activation with concomitant antioxidant enzyme and HSP upregulation in quiescent aged muscle [15,17]. This response to basal stress in aged skeletal muscle is reminiscent of those induced by acute exercise stress in adult skeletal muscle [15,17]. As a consequence of basal stress, muscles from old animals demonstrate a significantly attenuated ability to *further* increase RONS production [18] and hence fail to upregulate HSPs and antioxidant defense proteins after an acute exercise challenge [15–17]. From a mechanistic perspective, these findings seem intrinsically connected to resting adaptations that obviate the need to further adapt to exercise stress coupled to failed transmission of an exercise-induced redox signal, possibly owing to depressed exercise-induced RONS generation in skeletal muscle of old individuals [17–19].

Unfortunately existing literature in this area is limited by two important caveats: (1) a lack of translational human data and (2) the use of sedentary animal models. In particular, the use of sedentary animals renders separating out the effect of biological aging from the confounding effect of physical inactivity problematic. We and others have recently shown that habitual physical activity levels are important in governing the magnitude of physiological decline in the elderly [20–26]. We therefore sought to directly address these two caveats with a translational human study that accounted for habitual activity levels. This study aimed to determine protein levels of antioxidant defense enzymes, HSPs, and NO synthase isoforms, the principle end-point of redox signaling, in both quiescent and exercised young and old, trained and untrained human skeletal muscle. To this end, muscle biopsies were obtained from the vastus lateralis of young trained (YT), young untrained (YU), old trained (OT), and old untrained (OU) subjects before and 3 days after an acute exercise bout. We hypothesized that (1) levels of antioxidant defense enzymes and HSPs would be increased in muscles from OU subjects compared with the other three groups and (2) muscles from OT subjects would retain the ability to respond to an acute exercise challenge in a manner comparable to the young groups, whereas muscles from OU would not.

Methods

Subjects

Ethical approval was granted from the Liverpool John Moores Ethics Committee and the study adhered to the Declaration of Helsinki. Using Minitab version 15.0 (Minitab, Inc., USA) it was estimated that a sample size of 6 would enable the detection of a significant 0.5-fold upregulation of HSP72 at 3 days postexercise with a statistical power of 80%. After providing written informed consent, 12 younger (18–30 years) and 12 older ($\geq$55 years) Caucasian males participated. Younger and older subjects were further segregated according to training status (endurance trained vs untrained) to yield four subject groups: young trained (n=6), young untrained (n=6), old trained (n=6), and old untrained (n=6). Trained subjects were all competitive amateur cyclists that had habitually completed at least five endurance exercise sessions per week (all $\geq$45 min) as part of a systematic training regime. Old trained subjects had adopted such an exercise regime throughout adulthood. Untrained subjects completed $\leq$3 non-endurance-based exercise sessions per week (all $\leq$30 min). In addition, no subjects were engaged in systematic resistance training. Verbal reports and physiological assessments were utilized to verify the training history of our cohorts. All subjects completed a medical history questionnaire and undertook blood tests to ensure that they had a normal kidney, liver, and blood lipid profile. Every older subject performed a graded exercise echocardiogram (ECG) under the supervision of a consultant cardiologist to ensure there was no underlying cardiac pathology. Subjects were excluded if they presented with a history of chronic illness, were taking medication or supplements that might interfere with the present study's findings, or were smokers or if any cardiac abnormalities were highlighted on the exercise ECG test. Baseline physical and physiological characteristics are shown in Table 1.

Physiological assessment

Maximal oxygen uptake (VO_{2max}) and peak power output (PPO) were determined approximately 1 week before the main experimental trial using an incremental exercise test performed until volitional exhaustion on a bicycle ergometer (Daum Electronic Ergo Bike, Daum, Germany). Oxygen uptake (Online Systems, Metamax Cortex, Germany) and heart rate (Polar S610i, Finland) were measured throughout the test. After a 5-min warm-up at 50 W, participants completed successive 1-min exercise bouts with wattage being increased by 30 W every min until volitional exhaustion. VO_{2max} was deemed to have been attained if the following criteria were met: (1) heart rate within 10 beats min^{-1} of age-predicted maximum, (2) respiratory exchange ratio $>$1.1, and (3) plateau of oxygen consumption despite increased workload. All subjects fulfilled these criteria.

Intermittent exercise protocol

Subjects reported to the laboratory after an overnight fast on the morning of the exercise trial after abstaining from exercise, alcohol, and caffeine for 48 h. After a 5-min warm-up at 50% PPO, a 20-min high-intensity interval (HIT) session was completed on a bicycle ergometer (Daum Electronic Ergo Bike, Daum). The HIT session consisted of a 2-min bout at 40% PPO followed by a 2-min bout at 80% PPO. This work–rest ratio was repeated five times. This model of HIT exercise was chosen because it has been shown to be a tolerable, enjoyable, time-efficient, and effective method of inducing metabolic adaptations in skeletal muscle [27–29]. In addition, HIT cycling exercise is low impact and activates both type I and type II fibers, which is an important consideration when assaying a mixed fiber-type muscle such as the vastus lateralis [30]. Oxygen uptake was recorded continuously using an online system (Metamax Cortex), whereas both heart rate (Polar S610i) and ratings of perceived exertion (Borg 6–20 scale) were recorded at 2-min intervals.

Muscle biopsies

After the administration of a local anesthetic (0.5% marcaine), muscle biopsies were obtained from the vastus lateralis muscle using a Bard Monopty disposable biopsy instrument (12 $\times$ 10-cm gauge, Bard Monopty Systems, USA). Muscle biopsies were obtained at baseline and 3 days after the exercise trial. Previous work from our group has shown that the muscle content of HSPs is increased 3 days post-exercise in human skeletal muscle [10,11,31,32]. The same leg was utilized for both biopsies, and biopsy sites were separated by at least