Free Radical Biology and Medicine 73 (2014) 166–173

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

Roles of sedentary aging and lifelong physical activity in exchange of glutathione across exercising human skeletal muscle

Michael Nyberg [a,b,*], Stefan P. Mortensen [b,c], Helena Cabo [d], Mari-Carmen Gomez-Cabrera [d], Jose Viña [d], Ylva Hellsten [a,b]

[a] Department of Nutrition, Exercise, and Sports, University of Copenhagen, DK-2100 Copenhagen Ø, Denmark
[b] Copenhagen Muscle Research Centre and University of Southern Denmark, Copenhagen, Denmark
[c] Department of Cardiovascular and Renal Research, University of Southern Denmark, Copenhagen, Denmark
[d] Department of Physiology, Faculty of Medicine, University of Valencia, and Fundación Investigacion Hospital Clinico Universitario/INCLIVA, Valencia, Spain

ARTICLE INFO

Article history:
Received 5 February 2014
Received in revised form
10 April 2014
Accepted 14 May 2014
Available online 22 May 2014

Keywords:
Agingra
Skeletal muscle
Reactive oxygen species
Glutathione
Antioxidants
Free radicals

ABSTRACT

Reactive oxygen species (ROS) are important signaling molecules with regulatory functions, and in young and adult organisms, the formation of ROS is increased during skeletal muscle contractions. However, ROS can be deleterious to cells when not sufficiently counterbalanced by the antioxidant system. Aging is associated with accumulation of oxidative damage to lipids, DNA, and proteins. Given the pro-oxidant effect of skeletal muscle contractions, this effect of age could be a result of excessive ROS formation. We evaluated the effect of acute exercise on changes in blood redox state across the leg of young (23 ± 1 years) and older (66 ± 2 years) sedentary humans by measuring the whole blood concentration of the reduced (GSH) and oxidized (GSSG) forms of the antioxidant glutathione. To assess the role of physical activity, lifelong physically active older subjects (62 ± 2 years) were included. Exercise increased the venous concentration of GSSG in an intensity-dependent manner in young sedentary subjects, suggesting an exercise-induced increase in ROS formation. In contrast, venous GSSG levels remained unaltered during exercise in the older sedentary and active groups despite a higher skeletal muscle expression of the superoxide-generating enzyme NADPH oxidase. Arterial concentration of GSH and expression of antioxidant enzymes in skeletal muscle of older active subjects were increased. The potential impairment in exercise-induced ROS formation may be an important mechanism underlying skeletal muscle and vascular dysfunction with sedentary aging. Lifelong physical activity upregulates antioxidant systems, which may be one of the mechanisms underlying the lack of exercise-induced increase in GSSG.

Skeletal muscle generates superoxide and hydrogen peroxide at rest and this formation is increased during contractile activity along with an increase in superoxide and hydrogen peroxide in skeletal muscle interstitial fluid of animal models [1–3]. Accumulation of indicators of oxidative damage to lipids, DNA, and proteins has been observed in tissues of aged organisms, which may contribute to loss of tissue homoeostasis. In accordance, there is accumulating evidence that reactive oxygen species (ROS) can be deleterious to cells under conditions where the formation of these substances is not sufficiently counterbalanced by the antioxidant system and this imbalance may be one of the mechanisms underlying skeletal muscle and vascular dysfunction in various disease states and in aging [4–6]. However, ROS are also important signaling molecules with regulatory functions that modulate changes in cell and tissue homeostasis and gene expression [7]. In young and adult organisms, production of ROS stimulates redox-sensitive signaling pathways that modify the cellular content of cytoprotective regulatory proteins such as superoxide dismutases (SODs) and catalase that prevent oxidative damage to tissues [8]. Aging is associated with increased levels of ROS in resting skeletal muscle [4,9], but the ability of skeletal muscle to respond to an increase in ROS formation by activation of redox-sensitive transcriptions factors is severely attenuated [8]. These effects of age on ROS generation and handling suggest that skeletal muscle formation of ROS in response to exercise could be excessive, which potentially causes cellular damage in the aged state [6]. Such an effect of age is in accordance with one study demonstrating that sedentary aging increases biomarkers of oxidative stress at rest and after maximal knee-extensor exercise [10].

Exercise training effectively improves antioxidant capacity in skeletal muscle, as evidenced by increased activity of glutathione

* Corresponding author at: University of Copenhagen, Department of Nutrition, Exercise and Sports, Section of Human Physiology, Universitetsparken 13, The August Krogh Building, 2100 Copenhagen Ø, DK, Denmark.
E-mail address: mnyberg@ifi.ku.dk (M. Nyberg).

http://dx.doi.org/10.1016/j.freeradbiomed.2014.05.008
0891-5849/

peroxidase (GPx), SOD1, and SOD2 after training [6]. However, evidence of increased skeletal muscle antioxidant capacity after exercise training in older humans is sparse. In a recent study on the effects of 8 weeks of exercise training an increase in skeletal muscle SOD2 protein expression was detected [11]. To what extent a more prolonged period of exercise training affects the antioxidant system and the generation of ROS in skeletal muscle in aged humans remains to be elucidated.

Detection of ROS during exercise is technically challenging because of their high level of reactivity and low steady-state concentration [12] and, as a consequence, most studies have determined indicators of oxidative damage to lipids, proteins, and DNA formed downstream of the primary production pathway. Reduced glutathione (GSH), a potent antioxidant and major component of the antioxidant defense, is, in the presence of ROS, converted to its oxidized form (GSSG), leading to an alteration in the GSSG/GSH ratio. Measurement of changes in GSH and GSSG are, therefore, indicative of changes in the presence of ROS. In this study, assessments of GSH and GSSG were made across the exercising leg to provide an indication of the rate of exercise-induced ROS formation in an isolated skeletal muscle group. To further assess redox status, the measurements of GSH and GSSG were also performed with infusion of the antioxidant compound *N*-acetylcysteine (NAC). When infused, NAC is rapidly deacetylated to cysteine, which is a precursor and the rate-limiting substrate for glutathione resynthesis [13]. Infusion of NAC could, therefore, also potentially unmask any age- and physical activity-related differences in cysteine availability.

The purpose of this study was to examine the effects of acute exercise on the exchange of GSH and GSSG across the leg in young healthy subjects and in older sedentary and older lifelong physically active humans. We hypothesized that an exercise-induced increase in ROS production would be detectable in young sedentary subjects and that the increase in formation of ROS would be more pronounced in older sedentary subjects but similar to young in older lifelong physically active subjects.

Methods and materials

Eight healthy young sedentary (less than 2 h of moderate intensity exercise per week during the past 3 years), eight healthy lifelong older sedentary (less than 2 h of moderate intensity exercise per week during the past 30 years), and eight healthy lifelong older endurance-trained (more than 5 h of high-intensity exercise per week during the past 30 years) male subjects were studied (Table 1). All subjects were nonsmokers and none of the subjects had been diagnosed with cardiovascular disease, renal dysfunction, insulin resistance, diabetes, or hypercholesterolemia. Five of the older trained subjects had extrasystoles at rest, whereas the remaining subjects had no arrhythmias at rest and none of the subjects had arrhythmias during exercise (ECG). Subject characteristics and hemodynamic data from rest and exercise have previously been published [4,14].

The study was approved by the Ethics Committee of the Copenhagen and Frederiksberg communities (H-3–2009-090) and conducted in accordance with the guidelines of the Declaration of Helsinki. Oral and written informed consent was obtained from all subjects before enrollment into the study.

Initial testing

Before the experimental day the subjects visited the laboratory to become accustomed to the one-leg knee-extensor model [15] and to perform an incremental bicycle ergometer exercise test in which pulmonary maximal oxygen uptake (L min^{-1}, VO_{2max}) was determined (Quark CPET system, Cosmed; Table 1). An incremental test was also performed in a one-leg knee-extensor ergometer to determine maximal workload.

Table 1
Baseline characteristics.

	Young sedentary ($n=8$)	Older sedentary ($n=8$)	Older active ($n=8$)
Age, years	23 ± 1	66 ± 2***	62 ± 2***
Height, cm	183 ± 2	175 ± 3*	178 ± 2
Body weight, kg	79.4 ± 4.3	79.2 ± 1.8	75.7 ± 3.1
Body fat, %	17.7 ± 2.6	26.5 ± 1.2*	15.2 ± 1.5†
VO_{2max}, L/min	3.6 ± 0.1	2.1 ± 0.1***	3.7 ± 0.2†††
VO_{2max}, ml/min/kg	45.5 ± 2.3	25.7 ± 1.3***	49.2 ± 2.4†††
45% knee extensor, W_{max}	31 ± 2	21 ± 2*	35 ± 2†††
Experimental leg mass, kg	12.5 ± 0.7	11.1 ± 0.4	11.8 ± 0.5
Experimental fat-free leg mass, kg	9.9 ± 0.4	8.5 ± 0.3*	10.1 ± 0.4†
Systolic blood pressure, mm Hg	122 ± 2	150 ± 5*	152 ± 6*
Diastolic blood pressure, mm Hg	66 ± 3	70 ± 3	69 ± 3
Mean arterial pressure, mm Hg	85 ± 2	98 ± 4	95 ± 3†
Total cholesterol, mmol/L	3.8 ± 0.4	5.3 ± 0.4*	4.9 ± 0.2*
HDL, mmol/L	1.4 ± 0.1	1.4 ± 0.2	1.5 ± 0.2
LDL, mmol/L	1.9 ± 0.3	3.3 ± 0.4*	2.9 ± 0.2
Triglycerides, mmol/L	0.83 ± 0.11	1.55 ± 0.32*	1.00 ± 0.08

Values are means ± SEM.

* $P < 0.05$.
*** $P < 0.001$, significantly different from young sedentary.
† $P < 0.05$.
††† $P < 0.001$, significantly different from older sedentary.

Experimental protocol

Subjects refrained from caffeine, alcohol, and exercise for 24 h before the experimental day. On the day of the experiment the subjects arrived at the laboratory after a light breakfast. After local anesthesia, catheters were placed in the femoral artery and vein of the experimental leg and in the femoral artery of the nonexperimental leg, and a muscle biopsy was obtained from the m. vastus lateralis of the nonexperimental leg.

The subjects completed 10 min of one-leg knee-extensor exercise at an absolute workload of 12 W and at a relative workload corresponding to 45% of the maximal workload (45% W_{max}) obtained in the incremental test (separated by 10 min of rest). After 70 min, intravenous infusion of the antioxidant compound NAC was started and after an additional 35 min the first of the two exercise bouts (12 W and then 45% W_{max}) was performed (separated by 10 min of rest) during constant infusion of NAC. Blood samples for analysis of GSH and GSSG were collected at rest and after 9 min of exercise.

N-acetylcysteine

Intravenous infusion of NAC consisted of a loading dose of 125 mg kg^{-1} h^{-1} for 15 min to increase the plasma concentration of NAC, followed by a constant infusion of 25 mg kg^{-1} h^{-1} to achieve a plateau in NAC concentration, with exercise commencing after 20 min of constant infusion [16]. NAC infusion was continued throughout the exercise. The pharmacokinetics of NAC using this infusion protocol is reported elsewhere [17].

Measurements and calculations

Femoral arterial blood flow was measured with ultrasound Doppler (Logic E9, GE Healthcare) as previously described, and