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Brief Communication

MUSCLE SORENESS-INDUCED REDUCTION IN FORCE GENERATION IS ACCOMPANIED BY INCREASED NITRIC OXIDE CONTENT AND DNA DAMAGE IN HUMAN SKELETAL MUSCLE

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Abstract—We examined the effect of exercise-induced muscle soreness on maximal force generation, tissue nitric oxide (NO) and 8-hydroxydeoxyguanosine (8-OHdG) content in human skeletal muscle. Female volunteers were assigned to control (C) and muscle soreness (MS) groups ($n = 6$ in each). MS group performed 200 eccentric muscle actions of the rectus femoris to induce muscle soreness. Maximal force generation was measured 24 h before and after exercise in both groups. Needle biopsy samples were assayed for NO content with electron spin resonance spectroscopy after ex vivo spin trapping, and 8-OHdG content were measured with an enzyme-linked immuno assay. Maximal force decreased by 11 ± 5.4% ($p < .05$) 24 h after exercise in MS group. Muscle soreness increased NO and 8-OHdG contents from their control values of 0.39 ± 0.08 arbitrary units and 0.035 ± 0.004 pmol/μmol DNA to 0.96 ± 0.05 ($p < .05$) arbitrary units and 0.044 ± 0.005 ($p < .05$) pmol/μmol DNA, respectively. This is the first demonstration that muscle soreness-induced decrease in maximal force generation is a result of an increase in muscular NO content and associated with enhanced formation of 8-OHdG in human skeletal muscle. © 1999 Elsevier Science Inc.

Keywords—Human muscle, Muscle soreness, Nitric oxide, 8-Hydroxydeoxyguanosine, Maximal force, Exercise, Free radicals

INTRODUCTION

The beneficial effects of regular physical exercise are well known, it improves cardiovascular function, muscular strength, endurance, and it increases the activity of antioxidant systems. Many of us experienced the discomfort of muscle soreness one or two days following unaccustomed exercise. Unaccustomed exercise might cause mechanical, ischemic, and oxidative stress leading to muscle damage [1]. It was proposed that eccentric muscle action which produces high muscle tension leads to a significant increase in extracellular fluid pressure, inflammation and mechanical damage in muscle cells [2–4]. In addition, muscle soreness is associated with a marked decrease in maximal muscle force [2–4]. The biochemical background of these phenomena are poorly understood. Exercise enhances cytokine production and activates macrophages and neutrophils, thereby increasing the production of nitric oxide (NO) [5,6]. Mammalian skeletal muscle is equipped with both neural and endothelial types of NO synthases (NOS), which enzymes play a physiological role in controlling muscle contractions [7–9]. NO was shown to reduce contractile force of diaphragm [9] and ventricular myocytes [10]. Therefore, we hypothesized that the reduction of maximal peak force observed during muscle soreness might be a result of increased NO production. The first objective of the current investigation was to test this hypothesis.

It was shown by Maughan and associates [11] that downhill running, which was used to evoke muscle soreness, results in an accumulation of lipid peroxidation products in the blood. Thus, it is highly plausible that the muscle damage associated with muscle soreness causes oxidative damage by enhancement of free radical generation. The cytotoxic effects of free radicals include the oxidative damage of cellular DNA. Alessio [12] reported

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that marathon running increased the 8-hydroxydeoxyguanosine (8-OHdG)/creatinine ratio in urine samples of runners after the race suggesting exercised-induced damage and repair of DNA. On the other hand, there was no increase in 8-OHdG level in tissue samples of dogs, excluding colon, after a single bout of exercise [13]. It is not known whether muscle soreness induced by exercise causes oxidative damage to DNA in human skeletal muscle. Hence, the second objective of this study was to apprise the level of DNA damage by the measurement of 8-OHdG content by immunoreactive antibodies in skeletal muscle of human subjects.

METHODS

Twelve female physical education students (age: 20–23) volunteered for the study after being informed of the purpose, methods and possible complications of the procedures. Verbal and written informed consent were obtained for the study that was reviewed and approved by the local ethics committee. In order to minimize the number of biopsy sampling the subjects were randomly separated into control (C) and exercised (MS) groups. Both groups went through medical examinations and the subjects were found healthy before the test.

Exercise and muscle soreness

After warming up, both the C and MS groups were asked to perform maximal isometric contraction in a sitting position with 90° of knee angle, and the maximal force values were recorded. Then the MS group was asked to perform 200 eccentric muscle action to induce muscle soreness. The intensity of the exercise was set at 60% of maximum isometric contraction. Ten repetitions were done in one set, and 20 sets were performed by all subjects. The time to complete the requested 200 muscle actions was between 60–90 min. Eccentric exercise was used, because it causes greater muscle tension than concentric contraction [14] and this high tension was proposed to cause mechanical damage, swelling and muscle soreness [15]. Our pilot study demonstrated that the given exercise induces muscle soreness 24 h later. The degree of muscle soreness was evaluated by a perceptive scale numbered from 0 (lack of muscle soreness) to 10 (extremely strong muscle soreness) as described earlier [16–18]. Twenty four h after the exercise, maximal force generation was measured again, and muscle biopsies were taken from the quadriceps femoris muscles by needle biopsy technique described by Evans et al. [19] in both C and MS groups.

Biochemichal assays

Electron spin resonance spectroscopy (ESR) was used to measure NO content of the biopsies after ex vivo spin trapping as follows. After sampling, tissue samples were immediately cut into small species and immersed into spin-trapping solution and incubated for 5 min at 20°C degree. Then, the samples were placed into quartz tubes and frozen in liquid nitrogen until assayed for ESR spectra of NO-Fe^{2+}-$(MGD)_2$ complex. The spin trapping solution contained 55 mM concentration of the spin-trap *N*-methyl-glucosamine-dithiocarbamate (MGD) and 12 mM $FeSO_4$ dissolved in distilled water (pH set to 7.4). To avoid oxidation of NO and Fe^{2+}, all stock solutions were bubbled with Argon for 30 min. Spectra of Fe^{2+}-$(MGD)_2$ complex is superimposed on the dominant background spectra of Cu^{2+}-$(MGD)_2$. Background spectra of Cu^{2+}-$(MGD)_2$ was obtained using a spin-trapping solution which did not contain $FeSO_4$. The detection limit of NO by this ESR method is 0.05 nM/g tissue [20]. ESR spectra were recorded with Bruker ECS 106 (Rheinstetten, Germany) spectrometer operating at X band with 100 kHz modulation frequency at a temperature of 160 K, using 10 mW microwave power to avoid saturation. Scans were traced with 2.85 G modulation amplitude, 340 G sweep width, and 3356 G central field as described [21]. After subtraction of the background signal Cu^{2+}-$(MGD)_2$, analysis of NO content was performed with double integration of all spectra.

For the measurement of 8-OHdG the obtained biopsy samples were immediately immersed in liquid nitrogen and then stored at −80°C until the analysis. At the first step the DNA was isolated according to the method of Gubta [22]. In brief, the cytosolic fraction of muscle homogenate was removed by centrifugation. Protein was digested by incubation for 1 h at 37°C with proteinase K (Merck, Germany) and SDS in 1 mM EDTA at pH 8.0. From mixture, nuclear DNA was extracted with phenol under nitrogen atmosphere. The isolated DNA was treated with ribonucleases T1 and A (Boehringer Mannheim, USA) to remove RNA. The purity of the DNA isolated was confirmed spectrophotometrically. The measurement of 8-OHdG was done by ELISA immuno kit as described by the supplier (Genox Corp. Baltimore, USA). The downside of this measurement is that the result is given in pmol/μmol DNA which is not as reliable as giving the 8-OHdG content in guanosine base (8-OHdG/10^5dG).

The Wilcoxon Mann–Whitney U-test was applied to determine the differences in variables between C and MS groups. Significance was set at $p < .05$.

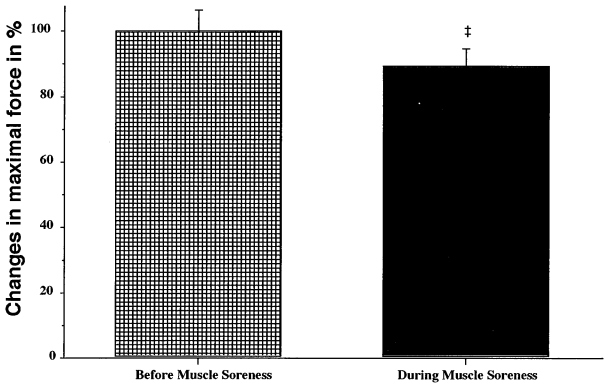

Fig. 1. The maximal isometric force was measured before and 24 h after 200 eccentric muscle actions which resulted in significant muscle soreness. The subjects were not able to reproduce the same maximal isometric force during muscle soreness, the drop was 11 ± 5.4% compared with pre-exercise value. The data are expressed as mean ±SD of 6 subjects. ‡$p < .05$.

RESULTS

The discomfort of muscle soreness ranged between 6–9 on a 10 grade scale in MS group and muscle soreness was not reported by C group. Maximal force was significantly decreased by 11 ± 5.4% ($p < .05$) during muscle soreness compared with pre-exercised data (Fig. 1) in MS group. No significant change was recorded in C group. Exercise induced approximately a three-fold increase in NO content from the control value of 0.39 ± 0.08 arbitrary units to 0.96 ± 0.05 arbitrary units (Fig. 2). In the MS group, muscular 8-OHdG content was significantly increased to 0.044 ± 0.005 pmol/μmol DNA ($p < .05$), as compared to its baseline level of 0.035 ± 0.004 pmol/μmol DNA in C group (Fig. 3).

DISCUSSION

This is the first demonstration that muscle soreness-induced decrease in maximal force generation is associated with an increase in muscular NO content and formation of 8-OHdG, a product of DNA oxidation, in human skeletal muscle. It is well known that the working muscle produces increased amount of NO [5,6]. However, the facts that the muscle biopsies were taken 24 h after the exercise protocol and that NO has a short half-life [23] exclude the possibility that the increase in muscular NO content was a result of the acute NO production of the muscle during exercise. Our present results suggest that the enhanced NO production 24 h after exercise may be due to a late effect of exercise, possibly mediated by "secondary free radical sources" that are active after eccentric exercise [1,24], therefore related to the development of muscle soreness. The involvement of NO in the regulation of the force generation of skeletal muscle might include a variety of mechanisms. NO was reported to decrease contractile force by inhibition of Ca^{2+}-ATPase activity in the sarcoplasmic reticulum [25] and via a cyclic guanosine 3′, 5′-cyclic-monophospate [7] dependent pathway. NO might evoke hyperpolarization of membrane potential, thereby leading to reduced force generation, as observed in smooth muscle cells [26]. NO may directly inhibit the force-generating proteins in skeletal muscle [8]. In addition, formation of NO-derived oxidant species such as peroxynitrite may result in nitration of proteins thereby leading to enhanced degradation of nitrated proteins [27]. It cannot be excluded that NO-induced decrease in maximal force generation is a part of a protective mechanism

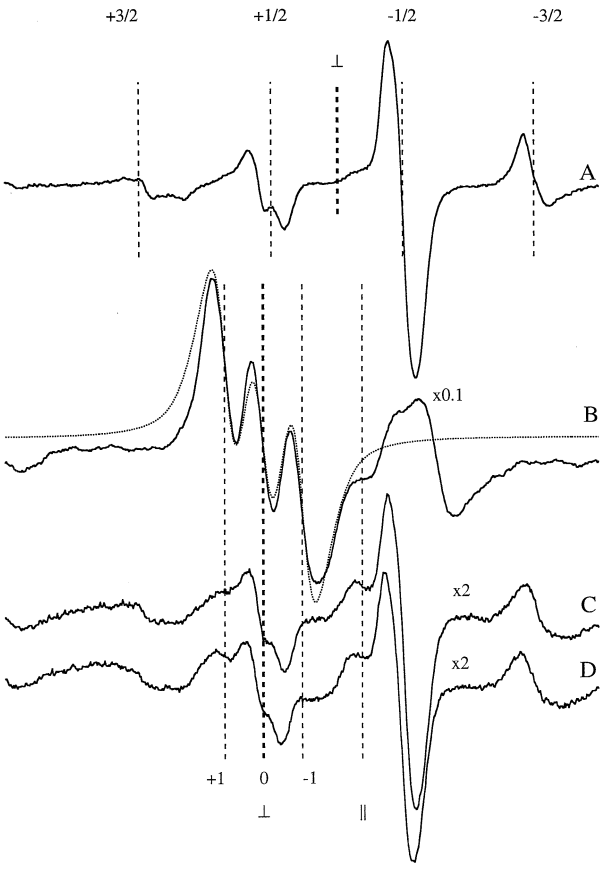

Fig. 2. Original electron spin resonance spectra of NO-Fe^{2+}-[*N*-methyl-glucoseamine-dithiocarbamate (MGD)]^2 complex in human biopsies obtained from rectus femoris muscles show nearly a three-fold increase in NO content in the muscle soreness group. Curve A: background spectrum of Cu(MGD)^2. Curve B: solid line, positive control obtained with incubation with 10^{-5} M Na-nitroprusside; dotted line, fitted curve; ×0.1, reduced gain compared to curve A. Curve C: control biopsy. Curve D: biopsy taken 24 h after exercise (muscle soreness); ×2, increased gain compared to curve A. +1 0 −1: hyperfine splitting of NO-Fe^{2+}-(DETC)^2 triplet; +3/2 +1/2 −1/2 −3/2: hyperfine splitting of Cu(DETC)^2 ESR parameters: X band, 100 kHz modulation frequency, 160 K, 10 mW microwave power, 2.85 G modulation amplitude, 340 G sweep width, and 3356 G central field.

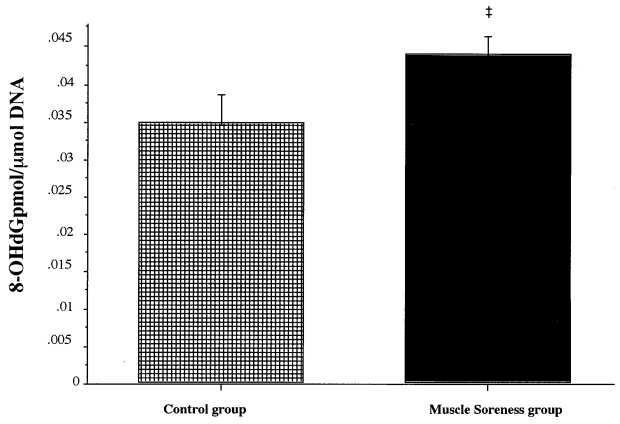

Fig. 3. Muscle soreness is associated with an increase of 8-OHdG content measured by ELISA as described in the Methods section. The data are expressed as mean ±SD of 6 subjects. ‡$p < .05$.

by which skeletal muscle protects itself from further peak force-generated damage. The source of NO in human skeletal muscle may be due to activation of iNOS in the muscle cells or in activated macrophages due to the damage of cells by muscle soreness. These hypotheses need further verification.

Our present finding shows that muscle soreness is accompanied with an increase in tissue 8-OHdG content which indicates oxidative damage of DNA. The mechanism of this oxidative damage cannot be answered by our present study in humans. Increased 8-OHdG content might be either a result of acute increase in free radical generation during exercise [28], or it may be due to a late effect of exercise associated with muscle soreness [1]. 8-OHdG is repaired rapidly and efficiently due to its mutagenetic potential [29]. However, we observed the increased 8-OHdG level 24 h after the exercise in our study. This suggests that the DNA damage occured at the time of the development of muscle soreness. Our finding is in accordance with a recent report of Asami et al. [30], who demonstrated that forced exercise increased 8-OHdG content in different organs of the rat compared with a group that performed spontaneous exercise. The increased production of NO during muscle soreness may favour the formation of peroxynitrite, which, among a variety of other free radicals, may lead to increased 8-OHdG formation [31].

The limitation of our results is that the causative relation between muscle soreness and maximal force generation, NO, and 8-OHdG contents can not be proven in this human study, where the use of specific NOS inhibitors or antioxidants are excluded. Because the discomfort of muscle soreness, reduction of maximal force and the increases in NO and 8-OHdG content were registered only in the MS group, it seems to be reliable to suggest that these changes are due to muscle soreness.

We conclude that muscle soreness-induced reduction in force generation, after unaccustomed exercise in human skeletal muscle, is a result of increased NO content and is accompanied by DNA damage.

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ABBREVIATIONS

C—control group
ESR—electron spin resonance spectroscopy
8-OHdG—8-hydroxydeoxyguanosine
MS—exercised group
NO—nitric oxide
NOS—nitric oxide synthase