SHORT REPORT

ABSTRACT: The present study was conducted to determine the effects of acute overload exercise on mitochondrial DNA and the structure of skeletal muscles. Rats were forced to run for 20 min until reaching complete exhaustion. We detected the large-scale deletion (7052 bp) of mitochondrial DNA by the nested polymerase chain reaction, and also observed mitochondrial ultrastructural changes in the soleus muscle.
 Muscle Nerve **22:** 258–261, 1999

ACUTE EXERCISE CAUSES MITOCHONDRIAL DNA DELETION IN RAT SKELETAL MUSCLE

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Accepted 15 September 1998

It has been suggested that acute overload exercise increases oxidative stress.[2] However, the mechanism of tissue damage caused by oxidative stress has not been determined. It has been widely established that alterations in mitochondrial DNA (mtDNA) are closely related to various diseases or conditions including degenerative diseases,[7,13] aging,[4,8,9,11,14,17,19–21] and the influence of radiation.[10] In these conditions, the deletion or mutation of mtDNA has been found to be responsible for dysfunction of energy generation or necrosis in tissues. These findings suggest that damage in muscle tissue due to overload exercise may be initiated by mtDNA alterations. To determine whether overload exercise causes mtDNA alterations and mitochondrial degeneration, we examined rats that were forced to run until reaching complete exhaustion.

Abbreviations: AE, acute exercise; mtDNA, mitochondrial DNA; nt, nucleotides; PCR, polymerase chain reaction; ROS, reactive oxygen species; SO, soleus muscle; TA, tibial anterior muscle
Key words: acute exercise; mitochondrial DNA deletion; mitochondrial swelling; blood lactate; rat soleus
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CCC 0148-639X/99/020258-04

MATERIALS AND METHODS

Wistar rats (8-week-old males) were divided into an acute exercise (AE) group (n = 4) and a control group (n = 5). In the AE group, rats were forced to sprint on a motorized rodent treadmill at a running speed of 40 m/min for 20 min until they were completely exhausted. Animals were not trained prior to the experiment. Control group rats did not exercise. Under anesthesia, blood samples were drawn from the left ventricle, and the soleus muscles (SO) and tibial anterior muscles (TA) were dissected out from rats 30 min after exercise. Total genomic DNA was isolated from muscles by the sodium iodide method (DNA Extractor WB Kit, Wako, Japan) for the nested polymerase chain reaction (PCR). Primers for the initial PCR amplification corresponded to rat mtDNA nucleotides (nt) 7484–7508 and 15,078–15,056.[3,5] Second PCR primers corresponded to nt 7517–7541 and 14,923–14,899. Amplification was performed with 50 ng of target DNA in a 10-μL volume containing 0.5 μmol/L of primers and 1.25 U EX-taq polymerase (Takara, Japan). After a 150-s incubation at 94°C, PCR was performed for 30 cycles of 94°C for 30 s, 60°C for 25 s, and 72°C for 90 s in a DNA thermal cycler (Thermal Sequencer TRS-300,

Iwaki Glass, Japan). A second PCR using the inner pair of primers was performed for 30 additional cycles. After amplification, the reaction mixtures were subjected to electrophoresis on 2.0% agarose gels. The amplified fragments were detected by ethidium bromide staining and subjected to mtDNA sequence analysis using a DNA autosequencer. Samples of muscles were fixed with 2.5% glutaraldehyde and 4.0% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4) for 24 h. The samples were postfixed in 1% osmium tetroxide, dehydrated through a graded ethanol series, and then embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and examined with an electron microscope. For deproteinization, blood was mixed with 8% perchloric acid and centrifuged for 10 min at 1500 *g*. Lactate concentration was measured using an 826 Lactate kit (Sigma, USA).

RESULTS

We used nested PCR to examine mtDNA deletion in the muscles after acute exercise. This set of inner primers would produce a 7407-nt product from the wild-type mitochondrial genome. A 0.38-kb mtDNA fragment was detected in the SO from the AE group (Fig. 1a), but not from the control SO or from the TA in either group. To ensure that the PCR product represented true mtDNA deletion and was not the result of primer misannealing, we performed sequence analysis after acute exercise. DNA sequencing identified breakpoints at nucleotides 7847 and 14,897, confirming that PCR-amplified mtDNA products represented true 7052-bp deletions (data not shown). These findings were commonly observed in muscle samples from all rats from the AE group. On electron micrographs, the cristae of mitochondria in SO from the AE group were blurred due to swelling of the matrix (Fig. 1b), and electron density dropped due to the exercise. On the other hand, the cristae of mitochondria from the TA in both groups revealed a normal matrix. These alterations were only observed in the white muscle. Most mitochondria were swollen in the white muscle fibril area. In comparison with the con-

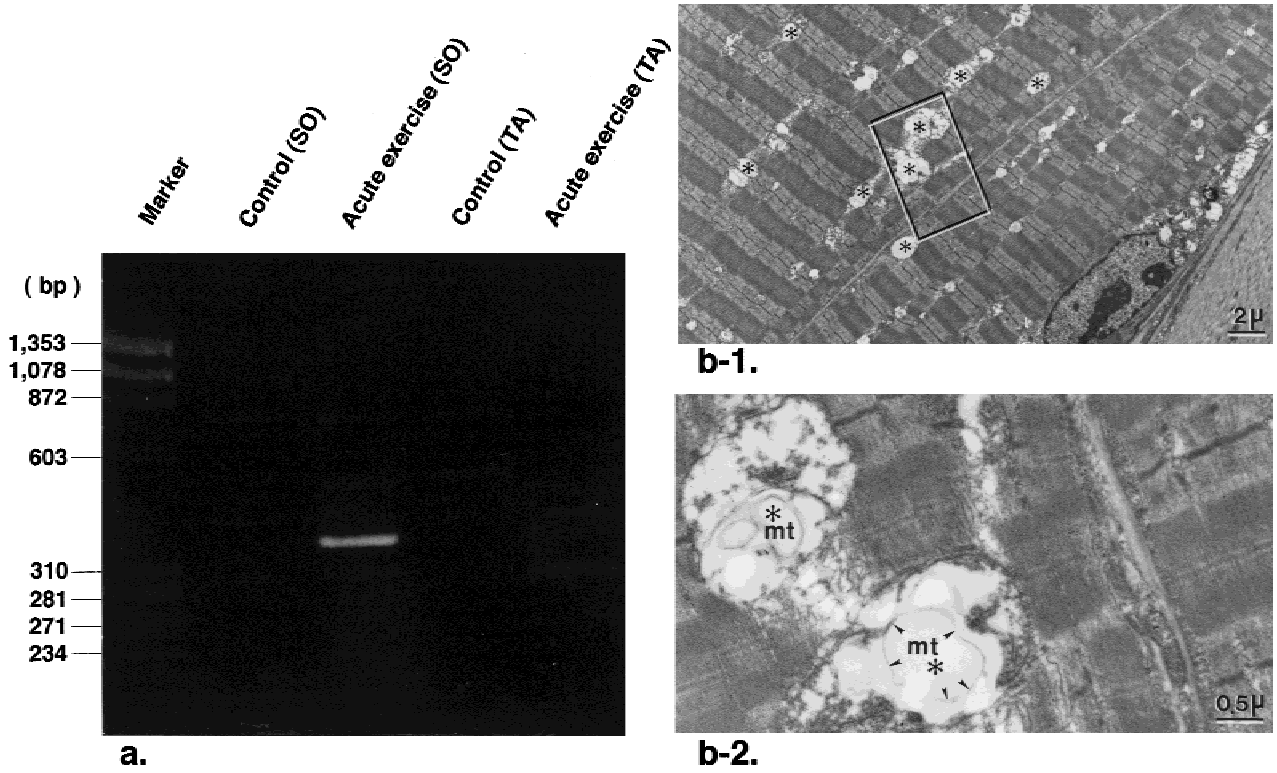

FIGURE 1. Detection of mtDNA deletion by PCR. Representative samples among the AE group and the control group are shown. **(a)** Electrophoresis in agarose gel with ethidium bromide. This set of inner primers produces a 7407-nt produce from a wild-type mitochondrial genome. The size markers (bp) are shown on the left. Acute exercise resulted in amplification of a 380-bp fragment in SO. SO and TA in the control group and TA in the acute exercise group showed no amplified fragments. **(b)** Electron micrographs showing mitochondrial swelling (*) in rat soleus muscle with acute exercise. **(b-2)** Magnified view of the area outlined by the square in photo **b-1.** Arrows indicate swelling matrix of mitochondria (mt) **(b-2).**

trol group (3.88 ± 1.17 mmol/L), the AE group (18.81 ± 5.64 mmol/L) showed a significantly elevated blood lactate concentration (unpaired Student's *t*-test, $P < 0.01$). This indicated that the exercise loading was heavy enough to induce exhaustion in the AE group.

DISCUSSION

There have been a few studies regarding cellular damage of skeletal muscle in acute overload exercise focusing on oxidative stress.[1,6] However, these have been limited to the biochemical and histochemical aspects. The experiment presented have demonstrated that acute running overload exercise leads to mtDNA deletion in the soleus muscle. Because we lacked information on the deletion site in the mtDNA caused by acute exercise, we chose primers that were previously reported as useful in detecting multiple age-associated mtDNA deletion in skeletal muscle.[3] We found that the 3′ end of the deleted sequence was only 2 base pairs upstream of the second primers. Moreover, we observed abnormal mitochondria in the soleus muscle by electron microscopy. We believe that the difference in the effect of acute exercise between the soleus and tibial anterior muscles was due to the different physiological characteristics of the muscles; the soleus muscle is mainly comprised of type I fibers, while the tibial anterior is a mixture of type I and II fibers. mtDNA plays an important role in the function of respiratory chain enzymes, which generate energy. Therefore, mtDNA deletion or mutation possibly causes an energy crisis and leads to tissue damage directly.[12,14] However, there have been no previous reports of mtDNA damage caused by overload exercise.

Reactive oxygen species (ROS) are physiological metabolites, and some pathological states, including ischemia-reperfusion, increase ROS generation. Of inhaled oxygen, 1–5% is believed to be converted to ROS within cells by mitochondria.[15] Overload exercise with high oxygen consumption increases ROS generation. Moreover, the ROS from the respiratory chain may trigger mtDNA deletion and cause ROS to accumulate. The deletion site includes the coded region of respiratory chain enzymes. Therefore, in muscle during overload exercise, energy production in mitochondria is disturbed by the impairment of oxidative phosphorylation due to depression of respiratory chain enzymes coded by mtDNA. We hypothesized that these changes may contribute to muscle tissue damage and mitochondria morphological changes due to overload exercise. This hypothesis of a vicious circle has recently been confirmed by measurement 8-hydroxydeoxyguanosine as a marker for mtDNA oxidation.[15,16,18] Oxidative stress has been suggested to cause mtDNA alterations during acute exercise, and energy-generating systems may be affected as a consequence of such mutation of mtDNA.[11] Thus, we concluded that acute overload exercise causes mtDNA deletion and may alter the energy-generating system due to oxidative stress in muscles.

This study was supported in part by the Project Research Grant and Research Promotion Program at Ibaraki Prefectural University of Health Sciences.

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