Mitochondria are unique organelles in that they possess DNA. Mitochondrial DNA (mtDNA) is the only extrachromosomal DNA in mammalian cells. The mtDNA contains 37 genes, encoding 13 proteins (all of which are respiratory chain subunits), 22 tRNAs, and two rRNAs.

Mutation in the regulatory regions of mtDNA could interfere with replication, transcription, or processing of mitochondrial transcripts. Deletion or mutation of mtDNA has been found to be responsible for dysfunction of energy production or increase in necrosis.

According to the theory of mitochondrial aging, mitochondrial respiration as a source of reactive oxygen species (ROS) plays a major role in mtDNA damage. The mtDNA damage caused by ROS has been proposed to be an important factor in both aging and a number of age-related degenerative diseases. Muscle and nerve are particularly prone to oxidative damage because they consist mostly of post-mitotic cells liable to accumulate oxidatively modified molecules.

Several studies have discussed the effect of exercise on the production of oxygen radicals. Indeed, the production of ROS increases during aerobic exercise where oxygen consumption within the skeletal muscle mitochondria increases to 10–40 times the resting level. Overall, the accumulation of oxygen radicals is influenced by the mode, intensity, and duration of contraction. Under certain circumstances, aerobic metabolism might also cause more harm than good.

Most studies have identified the consequence of DNA damage in urine and blood samples, primarily changes in leukocytes. However, Sakai and colleagues demonstrated that acute overload exercise leads to mtDNA deletion in the soleus muscle. Subsequently, Haller’s group examined the effects of aerobic exercise on 10 people with mtDNA diseases. They found that mutant mtDNA was increased in six participants. However, there have been few studies on mtDNA damage induced by exercise in skeletal muscle. We therefore examined the effect of aerobic exercise training on mtDNA deletion in the skeletal muscle of trained and untrained Wistar rats.

**METHODS**

**Animal care and exercise training**

Sixty male Wistar rats (Rattus norwegicus) were obtained from the Pasteur Institute in Tehran, Iran. The rats were housed in an animal room at 22°C with lights on from 12:00 to 24:00 h and with free access to food (commercial rat chow) and water. At 8 weeks old the animals were randomly divided into the following six groups: heavy aerobic training (group 1, n = 10), moderate aerobic training (group 2, n = 10), heavy aerobic exercise (group 3, n = 10), moderate aerobic exercise (group 4, n = 10), control 1 (group 5, n = 10), and control 2 (group 6, n = 10).

Groups 5 and 6 did not exercise and were killed at the beginning (group 6, aged 2 months) and the end (group 5, aged 5 months) of the program to establish physical and physiological parameters. Two groups (groups 1 and 2) were trained by running 5 days/week for 12 weeks on a motor driven treadmill designed for rats (made by the Sports Science Research Centre of Iran, Tehran). Exercise started at 09:00 h. The intensity of aerobic exercise was gradually increased during the initial 3 weeks of the program from a speed of 10–20 m/min at 6° incline for 20 min. Each group was trained using a specific program. The intensity of exercise in group 1 was increased gradually by 2 m/min/week from 20 to 40 m/min, while the duration of exercise in group 2 was increased gradually by 2 min/week from 20 to 40 min but the intensity was fixed. Work rate during

**RESULTS**

Our results did not show any mtDNA deletion in untrained rats or in those that underwent moderate training (group 2). We only found mtDNA deletion (4.6 kb) in the soleus muscle of heavily trained rats (group 1).

**CONCLUSIONS**

These results demonstrate that one session of aerobic exercise does not cause mtDNA deletion in skeletal muscle. The difference in results between heavy and moderate aerobic training may be due to low work rate or up-regulation of inducible antioxidant systems in moderate training.
treadmill running was expressed as kg/m/min and defined as \( [\text{speed (m/min)}] [\text{Sin6}°] [\text{time (s)}] [\text{body wt (kg)}] \). \( V_o_2 \) was calculated using the equation \( Y = 42.685 + 0.697X \) (speed m/min) derived from the studies of Armstrong et al.\textsuperscript{25} and the concentrations were spectrophotometrically determined.\textsuperscript{26}

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Trained</th>
<th>Group</th>
<th>Untrained</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>BW (wt, mg)</td>
<td>336 (44)</td>
<td>335 (2)</td>
<td>321 (6)</td>
<td>324 (4)</td>
</tr>
<tr>
<td>SW (wt, mg)</td>
<td>205 (9.26)</td>
<td>209 (2.70)</td>
<td>196 (2.15)</td>
<td>185 (2.74)</td>
</tr>
<tr>
<td>HW (wt, g)</td>
<td>1.29 (2.01)</td>
<td>1.35 (1.16)</td>
<td>1.11 (0.09)</td>
<td>1.14 (0.13)</td>
</tr>
<tr>
<td>HV (ml)</td>
<td>2.13 (0.20)</td>
<td>2.22 (0.22)</td>
<td>1.13 (0.15)</td>
<td>1.13 (0.19)</td>
</tr>
<tr>
<td>ET (min)</td>
<td>20\textsuperscript{a}</td>
<td>40\textsuperscript{b}</td>
<td>20\textsuperscript{a}</td>
<td>40\textsuperscript{b}</td>
</tr>
<tr>
<td>( V_o_2 ) (ml/kg/min)</td>
<td>23.71 (3.11)</td>
<td>18.98 (2.77)</td>
<td>22.70 (1.85)</td>
<td>17.22 (2.53)</td>
</tr>
<tr>
<td>WR (kg/m/min)</td>
<td>28.09 (3.68)</td>
<td>28.03 (4.09)</td>
<td>6.42 (2.55)</td>
<td>22.55 (3.85)</td>
</tr>
</tbody>
</table>

Values are means (SE) for each group. BW, body weight; ET, exercise time; HV, heart volume; HW, heart weight; SW, soleus muscle weight; WR, work rate.

### Extraction of DNA from muscle samples

The frozen muscles were powdered with a mortar and pestle cooled in liquid nitrogen. Total DNA extraction was performed using proteinase K, SDS, and phenol-chloroform method,\textsuperscript{25} and the concentrations were spectrophotometrically determined.\textsuperscript{26}

### Oligonucleotide primers

Two oligonucleotide primers (L7\textsuperscript{f} and H12\textsuperscript{R}) were used for PCR (polymerase chain reaction) amplification and sequencing. These oligonucleotide primers were designed by Gene Runner software and obtained from MWG Biotech (Ebersberg, Germany). The sequence and position of these oligonucleotide primers on rat mtDNA genome are as follow: L7\textsuperscript{f} (5’-CATC CGA AGA CGT CCT GCA CTC-3’) 12946–12925 on the heavy strand.

### Long PCR

mtDNA deletion in skeletal muscle was screened for using the Expand Long Template PCR System kit according to the manufacturer’s instructions (Roche Applied Science, Indianapolis, IN) using a thermal cycler (MWG Biotech). DNA template (75 ng) was amplified in 25 \( \mu \)l reaction mixture containing 10 mM of each dNTP, 10 \( \mu \)mol of each primer, 2 U of Taq/Taq DNA polymerase, 0.2 \( \mu \)l DMSO, and 2.5 \( \mu \)l of PCR buffer (10X with 1.75 mM MgCl\textsubscript{2}). The PCR profile was as follows: 95°C for 2 min; 10 cycles of 95°C for 20 s, 61°C for 40 s, 68°C for 3 min; 20 cycles of 95°C for 20 s, 61°C for 40 s, 68°C for 3 min plus 5 s increments/cycle; 68°C for 10 min. Amplified fragments were separated by electrophoresis (at a constant voltage of 90 V for approximately 30 min) on 0.7% agarose gel. The gel was stained with ethidium bromide and visualised on a transilluminator and photographed with a camera (Ultraviolet Benchtop Transilluminator M-15; UVP, Upland, CA, USA). The wild-type and deleted mtDNA could be detected with this method in one reaction.

### Statistical analysis

Data were expressed as means (SE). The characteristics and observed band differences between all groups were analysed by ANOVA and Kruskal-Wallis one way analysis of variance, respectively. The significance level was set at \( p<0.05 \).

### RESULTS

Body weight and soleus muscle weight were slightly greater in the trained than in the untrained rats, but the difference was not significant (table 1). However, the heart weight and volume differences between the groups were significant, the heart volume of trained animals being larger than that of untrained animals.

Both trained groups were able to undertake intense exercise at end of the study, but the sedentary groups were not. Exercise time and work rate in trained animals were significantly higher than in the other groups (table 1).

mtDNA deletion was found only in the soleus muscle of group 1 which had participated in heavy exercise training (fig 1). The other groups had no mtDNA deletion in skeletal muscle. Deletion size on 0.7% agarose gel (46 kbp) was determined by size marker (Ladder II, Roche Applied Science, Mannheim, Germany) and the difference between the two observed bands (wildtype and deletion bands were 5.5 kbp and 820 bp, respectively). Automatic sequencing of mtDNA confirmed the mtDNA deletion in rats who had
participated in heavy exercise training. The sequence of the deleted region in soleus muscle is shown in Table 2. The deletion spanned from position 7879 to position 12550. These results indicate that heavy aerobic training caused mtDNA deletion in skeletal muscle (soleus).

**DISCUSSION**

The uncontrolled generation of ROS during physical activity might result in oxidative damage in mammals. Increasing information is available about the effect of exercise on DNA damage. However, there are few studies on mtDNA damage induced by exercise training. The first report demonstrated that acute overload exercise leads to mtDNA deletion (7 kb) in the soleus muscle of rats. However, neither heavy nor moderate aerobic exercise led to mtDNA deletion. In addition, in our study, moderate aerobic exercise (20 m/min, 40 min) did not induce mtDNA deletion in soleus muscle of rats, and the duration of heavy aerobic exercise (4.73 min) was not sufficient to cause mtDNA deletion. The differences between our study and another investigation may be due to the work rate or exercise intensity.

In addition, we found that heavy, but not moderate, aerobic training caused mtDNA deletion (4.6 kb) in rat skeletal muscle (soleus). This finding is in agreement with a recent report which showed that mutant mtDNA was increased after aerobic training in people with mtDNA disease. The difference between heavy and moderate aerobic training may be due to the low work rate or up-regulation of inducible antioxidant systems in moderate training (not measured): inducible antioxidants, including antioxidant enzymes and the GSH systems, have demonstrated prominent adaptive responses to chronic exercise (training) at least in skeletal muscle. However, heavy aerobic training may deplete non-enzymatic antioxidant reserves.

In a recent study, Iwai and colleagues proposed that there are dynamic changes of deleted mtDNA (4977 bp) in human leukocytes after endurance exercise. These researchers reported that consideration must be given to the difference between mtDNA in tissues and in leukocytes because oxidative damage during exercise is greater in skeletal muscle than the other tissues. However, our results do not confirm their findings. This disagreement may be due to differences in subject, tissue, workload, or length of training.

Overall, much remains to be learned about the effects of single session and long term exercise on mtDNA damage. Therefore, it will be interesting to examine in the future the effects of different types of acute and chronic exercise together with pathophysiological and pharmacological factors on mtDNA damage in different human tissues.

### Table 2 Deleted mtDNA region sequence

<table>
<thead>
<tr>
<th>Deleted domain</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>7879–7946</td>
<td>ATPase 8</td>
</tr>
<tr>
<td>7904–9363</td>
<td>ATPase 6</td>
</tr>
<tr>
<td>8584–9367</td>
<td>Cytochrome c oxidase III</td>
</tr>
<tr>
<td>9368–9435</td>
<td>tRNA-Gly</td>
</tr>
<tr>
<td>9436–9783</td>
<td>NADH-subunit-3</td>
</tr>
<tr>
<td>9785–9852</td>
<td>tRNA-arg</td>
</tr>
<tr>
<td>9855–10151</td>
<td>NADH-subunit-L4</td>
</tr>
<tr>
<td>10145–1152</td>
<td>NADH-subunit-4</td>
</tr>
<tr>
<td>11523–11590</td>
<td>tRNA-His</td>
</tr>
<tr>
<td>11591–11650</td>
<td>tRNA-Ser</td>
</tr>
<tr>
<td>11650–11720</td>
<td>tRNA-Leu</td>
</tr>
<tr>
<td>11721–12550</td>
<td>NADH-subunit-5</td>
</tr>
</tbody>
</table>

### What is already known on this topic

According to the theory of mitochondrial aging, oxidative stress plays a major role in aging and age related degenerative diseases.

### What this study adds

Heavy aerobic training causes mtDNA deletion (4.6 kb) in the rat soleus muscle.

### Authors' affiliations

A Jafari, M A Hosseinpourfazl, A A Ravasi, Tabriz University, Tabriz, Iran

M Houshandi, National Research Center for Genetic Engineering and Biotechnology, Tehran, Iran

Competing interests: none declared

### REFERENCES

Clinical Evidence—Call for contributors

Clinical Evidence is a regularly updated evidence-based journal available worldwide both as a paper version and on the internet. Clinical Evidence needs to recruit a new number of contributors. Contributors are healthcare professionals or epidemiologists with experience in evidence-based medicine and the ability to write in a concise and structured way.

Areas for which we are currently seeking authors:
- Child health: nocturnal enuresis
- Eye disorders: bacterial conjunctivitis
- Male health: prostate cancer (metastatic)
- Women’s health: pre-menstrual syndrome; pyelonephritis in non-pregnant women

However, we are always looking for others, so do not let this list discourage you.

Being a contributor involves:
- Selecting from a validated, screened search (performed by in-house Information Specialists) epidemiologically sound studies for inclusion.
- Documenting your decisions about which studies to include on an inclusion and exclusion form, which we keep on file.
- Writing the text to a highly structured template (about 1500–3000 words), using evidence from the final studies chosen, within 8–10 weeks of receiving the literature search.
- Working with Clinical Evidence editors to ensure that the final text meets epidemiological and style standards.
- Updating the text every six months using any new, sound evidence that becomes available. The Clinical Evidence in-house team will conduct the searches for contributors; your task is simply to filter out high quality studies and incorporate them in the existing text.
- To expand the topic to include a new question about once every 12–18 months.

If you would like to become a contributor for Clinical Evidence or require more information about what this involves please send your contact details and a copy of your CV, clearly stating the clinical area you are interested in, to Klara Brunnhuber (kbrunnhuber@bmjgroup.com).

Call for peer reviewers

Clinical Evidence also needs to recruit a number of new peer reviewers specifically with an interest in the clinical areas stated above, and also others related to general practice. Peer reviewers are healthcare professionals or epidemiologists with experience in evidence-based medicine. As a peer reviewer you would be asked for your views on the clinical relevance, validity, and accessibility of specific topics within the journal, and their usefulness to the intended audience (international generalists and healthcare professionals, possibly with limited statistical knowledge). Topics are usually 1500–3000 words in length and we would ask you to review between 2–5 topics per year. The peer review process takes place throughout the year, and our turnaround time for each review is ideally 10–14 days.

If you are interested in becoming a peer reviewer for Clinical Evidence, please complete the peer review questionnaire at www.clinicaledvidence.com or contact Klara Brunnhuber (kbrunnhuber@bmjgroup.com).
Effect of aerobic exercise training on mtDNA deletion in soleus muscle of trained and untrained Wistar rats
A Jafari, M A Hosseinpourfaizi, M Houshmand and A A Ravasi

doi: 10.1136/bjsm.2004.014068