ORIGINAL ARTICLE

Effect of aerobic exercise training on mtDNA deletion in soleus muscle of trained and untrained Wistar rats

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Background: According to the theory of mitochondrial aging, oxidative stress plays a major role in aging and age related degenerative diseases. Since oxygen consumption and reactive oxygen species rate increase during aerobic exercise, we hypothesised that heavy aerobic training could lead to enhanced mitochondrial DNA (mtDNA) deletion in postmitotic tissues, leading in turn to premature aging and degenerative diseases.

Methods: Sixty adult male 2 month old Wistar$_{14848}$ rats were divided into six equal groups. Two groups were trained for 3 months by running on a treadmill (5 days/week, incline 6°; group 1: 40 m/min, 20 min/day; group 2: 20 m/min, 40 min/day), while two sedentary groups participated in aerobic exercise only at the end of the study (incline 6°; group 3: 40 m/min; group 4: 20 m/min). To control for physical and physiological parameters, two groups of untrained animals were killed at the beginning (group 6) and end (group 5) of the study. Expand long PCR was used to investigate mtDNA deletion in soleus muscle and a sequencing method was used to confirm the mtDNA deletion break point.

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.

M itochondria are unique organelles in that they possess DNA.[1] Mitochondrial DNA (mtDNA) is the only extrachromosomal DNA in mammalian cells.[2] The mtDNA contains 37 genes, encoding 13 proteins (all of which are respiratory chain subunits), 22 tRNAs, and two rRNAs.[3]

Mutation in the regulatory regions of mtDNA could interfere with replication, transcription, or processing of mitochondrial transcripts.[4] Deletion or mutation of mtDNA has been found to be responsible for dysfunction of energy production or increase in necrosis.[5]

According to the theory of mitochondrial aging, mitochondrial respiration as a source of reactive oxygen species (ROS)[6] plays a major role in mtDNA damage.[7 8] 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.[7–15] Muscle and nerve are particularly prone to oxidative damage because they consist mostly of postmitotic cells liable to accumulate oxidatively modified molecules.[7 10 13]

Several studies have discussed the effect of exercise on the production of oxygen radicals.[16] 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.[17 18] Overall, the accumulation of oxygen radicals is influenced by the mode, intensity, and duration of contraction.[18] Under certain circumstances, aerobic metabolism might also cause more harm than good.[18]

Most studies have identified the consequence of DNA damage in urine and blood samples, primarily changes in leukocytes.[19–22] However, Sakai and colleagues demonstrated that acute overload exercise leads to mtDNA deletion in the soleus muscle.[5] 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.[23] 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$_{14848}$ 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

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Table 1 Characteristics of trained and untrained rats

	Trained		Untrained		Control	
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Age (months)	5.00	5.00	5.00	5.00	5.00	2.00
BW (wt, mg)	336 (44)	335.2 (48)	321.6 (26)	304 (44)	315 (60)	191.1 (33)
SW (wt, mg)	205 (26.91)	209.4 (20.77)	196.2 (15.98)	185.4 (27.24)	192.2 (36.95)	110 (10.33)
HW (wt, g)	1.29 (0.21)	1.35 (0.16)	1.11 (0.09)	1.14 (0.13)	1.11 (0.28)	0.68 (0.06)
HV (ml)	2.13 (0.20)	2.22 (0.22)	1.13 (0.15)	1.15 (0.19)	1.12 (0.22)	0.76 (0.13)
ET (min)	20*	40†	4.73 (1.67)*	35.57 (3.73)†	–	–
Vo_2 (ml/kg/min)	23.71 (3.11)	18.98 (2.77)	22.70 (1.85)	17.22 (2.53)	–	–
WR (kg/m/min)	28.09 (3.68)	28.03 (4.09)	6.42 (2.55)	22.55 (3.85)	–	–

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.
*Heavy exercise (40 m/min); †moderate exercise (20 m/min).

treadmill running was expressed as kg/m/min and defined as [speed (m/min)] [Sin6°] [time (s)] [body wt (kg)]. Vo_2 was calculated using the equation [Y = 42.685+0.697X (speed m/min)] derived from the studies of Armstrong *et al*[3] and Lawler *et al*.[24] At the end of the study, groups 3 and 4 were participating in heavy (40 m/min) and moderate (20 m/min) exercise, respectively.

The animals were anesthetised with ether and dissected after drawing blood from the left ventricle. The soleus muscles of both legs were immediately removed, freeze clamped at liquid nitrogen temperature, and stored at −70°C until use. In addition, body weight, soleus muscle weight, and heart weight together with heart volume were measured in both groups using conventional methods (table 1).

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,[25] and the concentrations were spectrophotometrically determined.[26]

Oligonucleotide primers
Two oligonucleotide primers ($L7_f$ and $H12_R$) 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: $L7_f$ (5′-CATC CGA AGA CGT CCT GCA CTC-3′) 7454–7475 on the light strand and $H12_R$ (5′-AGG GCT CAG GCG TTG GTG TTA C-3′) 12946–12925 on the heavy strand.
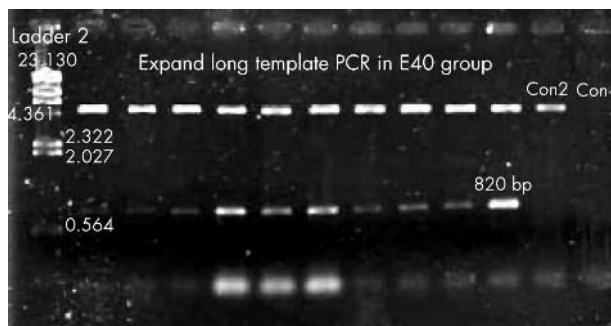

Figure 1 mtDNA deletion in rats who had participated in heavy exercise training (group 1 is referred to as E40 on the figure). The fragments of mtDNA were separated on 0.7% agarose gel and stained with ethidium bromide. The size marker (Ladder II, Roche Applied Science) is shown as ''Ladder 2'' on the photograph. The negative controls (Con2 and Con--) obtained from group 5 are shown with and without DNA template.

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 μl reaction mixture containing 10 mM of each dNTP, 10 μmol of each primer, 2 U of Taq/Taq DNA polymerase, 0.2 μl DMSO, and 2.5 μl of PCR buffer (10× with 1.75 mM $MgCl_2$). 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.

Sequencing
Automatic sequencing of mtDNA was performed using a MWG Biotech robotics DNA sequencer (Ebersberg, Germany) and data were analysed using Blast search and Chromas2 software.

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 kp) 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 kb and 820 bp, respectively). Automatic sequencing of mtDNA confirmed the mtDNA deletion in rats who had

Table 2 Deleted mtDNA region sequence

Deleted domain	Gene
7879–7946	ATPase 8
7904–9363	ATPase 6
8584–9367	Cytochrome c oxidase III
9368–9435	tRNA-Gly
9436–9783	NADH-subunit-3
9785–9852	tRNA-arg
9855–10151	NADH-subunit-L4
10145–11522	NADH-subunit-4
11523–11590	tRNA-His
11591–11650	tRNA-Ser
11650–11720	tRNA-Leu
11721–12550	NADH-subunit-5

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.[16 18 21] Increasing information is available about the effect of exercise on DNA damage.[27] 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.[5] 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[5] 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[23] 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.[28–33] However, heavy aerobic training may deplete non-enzymatic antioxidant reserves.[28]

In a recent study, Iwai and colleagues proposed that there are dynamic changes of deleted mtDNA (4977 bp) in human leucocytes after endurance exercise.[17] These researchers reported that consideration must be given to the difference between mtDNA in tissues and in leucocytes because oxidative stress during exercise is greater in skeletal muscle than the other tissues.[30] 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.

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.

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