South African Journal for Research in Sport, Physical Education and Recreation, 2010, 32(1):99-106.
Suid-Afrikaanse Tydskrif vir Navorsing in Sport, Liggaamlike Opvoedkunde en Ontspanning, 2010, 32(1):99-106.
ISSN: 0379-9069

DOES AEROBIC EXERCISES INDUCE mtDNA MUTATION IN HUMAN BLOOD LEUCOCYTES?

Bahman MIRZAEI*, Fatemeh SALAMI**, Farhad RAHMANI-NIA*, Afshar JAFARI***, Massoud HOUSHMAND****, Mehdi SHAFA SHARIAT PANAHI**** & Rahman RAHIMI*****

** Department of Exercise Physiology, University of Guilan, Rasht, Iran*
***Department of Exercise Physiology, Tarbiat Moallem University, Tehran, Iran*
****Department of Physical Education, University of Tabriz, Tabriz, Iran*
*****Department of Medical Genetics, National Institute for Genetic Engineering and Biotechnology, Tehran, Iran*
******Department of Physical Education & Sport Science, Islamic Azad University branch of Mahabad, Mahabad, Iran*

ABSTRACT

The aim of this study was to determine the effect of eight weeks aerobic training on mitochondrial DNA (mtDNA) mutation in human blood leucocytes. Twenty untrained healthy students (training group: n =10, age = 20.7±1.5 yrs, weight = 67.7±10 kg, BF% = 17.5±7.35 & control group: n =10, age = 21±1.3 yrs, weight = 78.5±18.5 kg, BF% = 18.2±6.4) were randomly assigned to two groups and participated in an eight weeks aerobic training program. Blood samples were collected before and after the eight weeks aerobic training. mtDNA mutation were analyzed using a multiplex polymerase chain reaction (PCR) method. Although, all subjects were fairly young and the possibility of mtDNA mutation at normal condition was unlikely, just to be certain, all blood samples were also analyzed for possible mutation. Those subjects who had mutant mtDNA prior to the study, were excluded. The findings show that there were no changes in mtDNA mutation in human leucocytes in both groups before and after eight weeks aerobic training (P>0.05). These results demonstrate that eight week aerobic training dose not causes mtDNA mutation in human blood leucocytes.

Key words: mtDNA; Oxidative stress; Common deletion; Aerobic training.

INTRODUCTION

Human mitochondrial DNA (mtDNA) is a 16,569-bp circular double-stranded molecule that encodes 37 genes including 13 critical subunits of the respiratory chain complexes, 2 ribosomal RNAs and 22 transfer RNAs. Each nucleated human cell contains a few thousand copies of mtDNA. Because each mitochondrion contains multiple copies of genome (Wallace, 1992), injury results in heteroplasmy, in which each cell contains mtDNA molecules with varied sequences (Grossmann, 1990; Wallace, 1992).

Mitochondria are composed of gene products originating from both nuclear and mitochondrial DNA (nDNA and mtDNA, respectively). Mutations within mtDNA occur at a

significantly higher frequency than in nDNA because mtDNA lack a protective histone sheath, has minimal DNA repair activity, and is located in close proximity to reactive oxygen species (ROS) produced via the electron transport chain (Bohr *et al.,* 2002; Adhihetty *et al*., 2003). Also, this mutation in the regulatory regions of mtDNA could interfere with replication, transcription, or processing of mitochondrial transcripts (Larsson & Clyton, 1995). Increased mtDNA mutation frequently occurs in humans with aging (Fayet *et al.,* 2002; Liu *et al*., 1998) and has been associated with a number of age related degenerative diseases (Berneburg *et al*., 2006).

In addition, oxygen metabolism within the mitochondria cause the production of ROS such as the superoxide radical, the hydroxyl radical and hydrogen peroxide (Chance *et al*., 1979). The production of such potentially harmful substances increases during aerobic exercise where oxygen consumption within the mitochondria increases 10-40 times the resting level which could damage mtDNA (Ji, 1995; Liu *et al.*, 2000; Leeuwenburgh & Heinecke, 2001).

Many studies have identified mutations or deletions of mtDNA as responsible for dysfunction of energy production or an increase in necrosis in tissues (Sakai *et al.,* 1999). Also, acute overload exercise has been demonstrated to cause a mtDNA deletion in the soleus muscle and the tibial anterior muscle of Wistar rats (Sakai *et al.,* 1999). One of the deletions appears in regions of the same alignment and is known as a common deletion (Schon *et al.,* 1989; Corral-Debrinski *et al.,* 1992; Chung *et al.,* 1994). This mtDNA deletion is 4977 bp in length ($mtDNA^{4977}$) in human mtDNA and is often seen with increasing age (Hayakawa *et al*., 1992).

Iwai *et al.* (2003) examined dynamic changes of deleted mtDNA in human leucocytes after endurance exercise. They demonstrated that a common deletion in leucocytes disappears over a period of several days after endurance exercise and reappears a number of days thereafter. Sakai *et al.* (1999) showed that acute exercise loading causes a deletion in mtDNA in rat skeletal muscle, and concluded that the oxidative stress induced by acute exercise modifies mtDNA. Jafari *et al.* (2005) demonstrated that one session of aerobic exercise dose not cause mtDNA deletion in rat skeletal muscle.

However, there is not consensus about aerobic training induced mtDNA deletion in human blood leucocytes. In addition, in previous studies mtDNA has been extracted from tissues. These studies have been limited to the biochemical and histochemical aspects. This method can, however, be painful and distressing for the human subjects. Also, there is a strong correlation between the degree of heteroplasmy in blood leukocyte and oral mucosa DNA (Van Essen *et al.,* 2000). In order to determine whether mtDNA mutations are notable in easily accessible tissue, we examined the effect of aerobic exercise training on mtDNA deletion in blood leucocytes of untrained healthy young men.

MATERIAL AND METHODS

Subjects

Twenty untrained male students voluntarily participated in this study and were randomly assigned to either aerobic training (mean ± SD: age = 20.7±1.5 yrs, weight = 67.7±10 kg,

BF% = 17.5±7.35, n =10) or a control group (mean ± SD: age = 21±1.3 yrs, weight = 78.5±18.5 kg, BF% = 18.2±6.4, n = 10). Subjects were informed of the experimental risks and signed an informed consent document prior to the investigation. Exclusion criteria included: evidence of coronary heart disease; congestive heart failure; uncontrolled hypertension; chronic obstructive pulmonary disease; renal failure; major orthopedic disability; smoking and supplementing by vitamins A, C and E. Subjects were asked to follow their normal routine diet during the study and not to perform any strenuous exercise two days before attending the laboratory. The study protocol was reviewed and approved by the research committee at the University of Guilan. Physical examination prior to the test revealed no barriers to endurance exercise in any of the subjects.

Exercise and blood sampling

Subjects exercised three times a week at 60 rpm for 30 min in a session for eight weeks on a cycle ergometer. Training load was increased gradually during the study from 50 to 75% of heart rate reserve (HRR) on each subject. Peripheral blood samples were obtained from the subjects' antecubital vein before and after eight weeks of aerobic training.

Extraction of mtDNA and multiplex PCR

DNA was extracted from blood samples after lyses of white blood cells using a DNA extraction kit (Diatom DNA Extraction Kit, Gen fanavaran, Iran). Multiplex polymerase chain reaction (PCR) (table 1) was carried out using two sets of primers: ONP89/ONP86 and ONP74/ONP25. The primers were used to amplify at 279-bp fragment in the deleted region as an internal control in each sample. PCR products were separated on 2% agarose gels, run in 0.5X TBE at 110 V for 50 min, stained in 0.02 µg/mL ethidium bromide and visualized by means of UV light (figure 1). Genomic DNA (50 ng) was added to Multiplex PCR mixture and amplified as follows: 94°C for 5 minutes, followed by 35 cycles of 94°C for 60 seconds, 55°C for 60 seconds, and 72°C for 60 seconds, with a final extension at 70°C for 10 minutes. Position and oligonucleotide sequences of primers used in PCR amplification are presented in table 2.

TABLE 1. **THE MULTIPLEX POLYMERASE CHAIN REACTION (PCR)**

Taq 0.5u	ONP_{86} 10ρmol/µl	ONP_{89} 10ρmol/µl	ONP_{74} 10ρmol/µl	ONP_{25} 10ρmol/µl	DNA 50ng/µl	dNTP 10mmol	PCR $buffer_{10}$ x + Mgcl2	H_2O
1 µl	1 µl	1µl	1µl	1µl	1 µl	0.7 µl	2.5 µl	15.8 µl

TABLE 2. **POSITION AND OLIGONUCLEOTIDE SEQUENCES OF PRIMERS USED IN PCR AMPLIFICATION**

Gen Sequencing		Position of oligonucleotide	Primer
ND_2 CCCTTACCACGCTACTCCTA-3'	5'-	5461 – 5480	ONP_{86}
OL GGCGGGAGAAGTAGATTGAA-3'	5'-	5740 – 5721	ONP_{89}
CO_{II} CTACGGTCAATGCTCTGAAA-3'	5'-	8161 – 8180	ONP_{25}
ND_5 GGTTGACCTGTTAGGGTGAG-3'	5'-	13640 – 13621	ONP_{74}

Statistical analysis

All values were expressed as mean ±SD. The sign test was used to compare mtDNA mutation before and after the eight weeks aerobic training.

RESULTS

Blood samples were screened for $mtDNA^{4977}$ site using the multiplex PCR. No mtDNA mutation was found in human blood leucocytes samples before and after the eight weeks aerobic training. Also, the results of sign test revealed no significant difference in mtDNA mutation between pre- and post test in both groups ($P>0.05$).

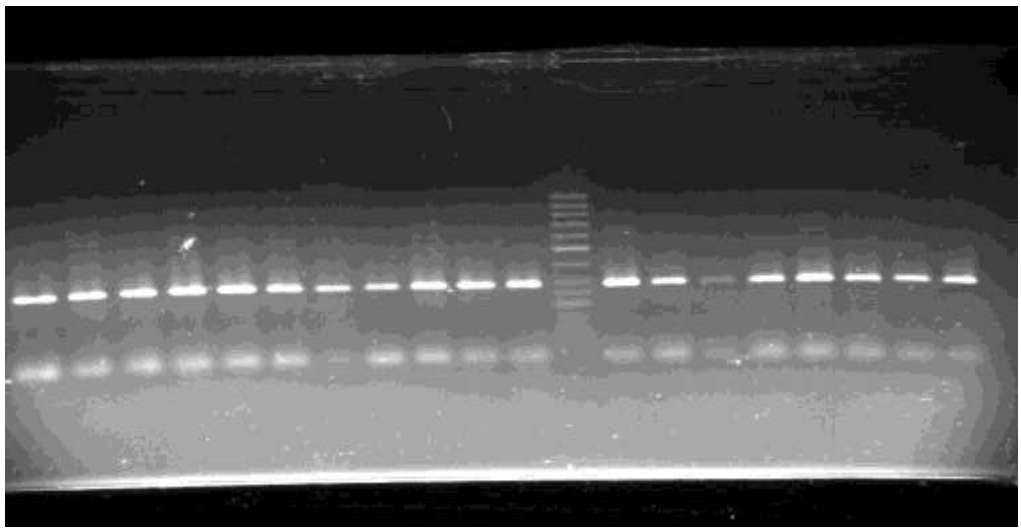

FIGURE 1. **IMAGE OF AGAROSE GEL WITH THE CONTROL BANDS AMPLIFIED. THE FRAGMENTS OF MTDNA WERE SEPARATED ON 2% AGAROSE GEL AND STAINED WITH ETHIDIUM BROMIDE AND VISUALIZED BY MEANS OF UV LIGHT**

DISCUSSION

The most frequent and best characterized mutation of mtDNA is a 4977 bp deletion, designated as the common deletion. This deletion includes genes for the NADH dehydrogenase complex, cytochrome-c oxidase and ATP-synthetase, all of them functionally important enzymes in the respiratory chain. The common deletion has been detected in suprabasal levels in hereditary diseases (Boles *et al*., 1998), developmental disorders, aging tissues and following UV radiation (Berneburg *et al.,* 2000). Furthermore, it is closely linked to ROS which the common deletion is almost absent when ROS quenching agents (antioxidants) were added (Bernburg *et al.,* 1999).

Alternatively, reports indicate that prolonged and intense exercise is associated with increases in skeletal muscle oxidative stress and ROS production, which have the potential to damage mtDNA and increase susceptibility of mitochondrial membrane proton conductance to oxidative stress (Di Meo & Venditti, 2001; Ji, 1999).

There are a few studies on mtDNA damage induced by aerobic training. Our findings however showed that eight week aerobic training (three times a week at 60 rpm for 30 min at 50 to 75% HRR) did not induced mtDNA deletion which is in accordance with Jafari *et al.,* (2005), demonstrating that one session of aerobic exercise does not cause mtDNA deletion in rat skeletal muscle. However, our results are inconsistent to the findings of Sakai *et al.* (1999) that showed that acute exercise loading causes a deletion in mtDNA in rat skeletal muscle, and concluded that the oxidative stress induced by acute exercise modifies mtDNA. This difference may be due to difference in subjects, training intensity, volume, training period (acute versus chronic) and the difference between mtDNA in tissues and blood leucocytes.

Iwai *et al.* (2003) examined the dynamic changes of the deleted mitochondrial DNA in human blood leucocytes after endurance exercise. In this study, the $mtDNA^{4977}$ deletion in leucocytes was detected prior to the exercise regime in all five subjects, who were all living normal, exercise-free lifestyles. They demonstrated that the common deletion in leucocytes disappear over a period of several days after endurance exercise and reappears a number of days thereafter. In our study, the $mtDNA^{4977}$ deletion in leucocytes was not observed prior to the exercise regime and aerobic exercises did not induce mtDNA deletion in human blood leucocytes.

To date, there have been no investigations examining the effect of long-term aerobic exercise on mtDNA deletions in blood leucocytes of young healthy men. However, mtDNA deletions have been quantified before and after resistance training in patients with mitochondrial disease (Taivassalo *et al.,* 1999). Parise *et al.* (2005) reported that fourteen weeks of progressive resistance exercise training resulted in a significant reduction in oxidative damage to DNA, and there was no change in the number of mtDNA deletions.

Importantly, the findings from our study suggest that aerobic training does not augment the number of mtDNA deletions, and we hypothesis that positive adaptation, may serve to help reduce the rate at which mtDNA aberrations accumulate with age. Although the precise mechanisms underlying this adaptation requires further investigation, we postulate that prolonged aerobic exercise can lead to up-regulation of inducible antioxidant systems

including antioxidant enzymes and the GSH systems (not measured) which have demonstrated prominent adaptive responses to chronic aerobic training (Ji, 1997; Hollander *et al.,* 2000; Radak *et al.,* 2001). It is proposed that ROS production decreases due to antioxidant system adaptations subsequently attenuating mtDNA damage. In addition, it appears that oxidative stress-induced adaptations therefore play an important role in the beneficial effects of regular exercise.

In conclusion, eight week aerobic training did not induce mtDNA deletion in human blood leucocytes. The lack of any effect on mtDNA in the present study may be due to the relatively short period of exercise training in this study precluding our ability to detect changes in mtDNA. Also, small sample size may affect on our results. Further work is needed to elucidate the mechanism responsible for the positive effect of aerobic exercise on mtDNA damage. Also, further work is needed to examine the effect of different types of aerobic training on mtDNA deletion, oxidative DNA damage biomarkers, and mtDNA content.

REFERENCES

ADHIHETTY, P.J.; IRRCHER, I.; JOSEPH, A.M.; LJUBICIC, V. & HOOD, D.A. (2003). Plasticity of skeletal muscle mitochondria in response to contractile activity. *Experimental Physiology,* 88: 99-107.

BERNEBURG, M.; GRETHER-BECK, S.; KURTEN, V.; RUZICKA, T.; BRIVIBA, K.; SIES, H. & KRUTMANN, J. (1999). Singlet oxygen mediates the UVA-induced generation of the photoaging-associated mitochondrial common deletion, *Journal of Biological Chemistry,* 274: 15345-15349.

BERNEBURG, M.; PLETTENBERG, H. & KRUTMANN, J. (2000). Photoaging of human skin, *Photodermatology*, *Photoimmunology and Photomedicine*, 16: 239-244.

BERNEBURG, M.; KAMENISCH, Y. & KRUTMANN, J. (2006). Repair of mitochondrial DNA in aging and carcinogenesis. *Photochemical & Photobiological Science*, 5: 190-198.

BOHR, V.A.; STEVNSNER, T. & DE SOUZA-PINTO, N.C. (2002). Mitochondrial DNA repair of oxidative damage in mammalian cells. *Gene,* 286: 127-134.

BOLES, R.G.; ROE, T.; SENADHEERA, D.; MAHNOVSKI, V. & WONG, L.J.C. (1998). Mitochondrial DNA deletion with Kearns sayre syndrome in a child with Addison disease, *European Journal Pediatrics*, 157: 643-647.

CHANCE, B.; SIES, H. & BOVERIS, A. (1979). Hydroperoxide metabolism in mammalian organs. *Physiological Reviews*, 59: 527-605.

CHUNG, S.S.; WEINDRUCH, R.; SCHWARZE, S.R.; McKENZIE, D.I. & AIKEN, J.M. (1994). Multiple age-associated mitochondrial DNA deletions in skeletal muscle of mice. *Aging Clinical Experimental Research,* 6: 193-200.

CORRAL-DEBRINSKI, M.; HORTON, T.; LOTT, M.T.; SHOFFNER, J.M.; BEAL, M.F. & WALLACE, D.C. (1992). Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age. *Nature Genetics*, 2: 324-329.

DI MEO, S. & VENDITTI, P. (2001). Mitochondria in exercise-induced oxidative stress. *Biological Signals and Receptors*, 10: 125-140.

FAYET, G.; JANSSON, M.; STERNBERG, D.; MOSLEMI, A.R.; BLONDY, P.; LOMBES, A.; FARDEAU, M. & OLDFORS, A. (2002). Ageing muscle: clonal expansions of mitochondrial DNA point mutations and deletions cause focal impairment of mitochondrial function. *Neuromuscular Disorders,* 12: 484-493.

GROSSMANN, L.I. (1990). Mitochondrial DNA in sickness and in health. *American Journal* of *Human Genetics*, 46: 415-417

HAYAKAWA, M.; HATTORI, K.; SUGIYAMA, S. & OZAWA, T. (1992). Age-associated oxygen damage and mutations in mitochondrial DNA in human hearts. *Biochemistry & Biophysics,* 189: 979-985.

HOLLANDER, J.; BEJMA, J.; OOKAWARA, T.; OHNO, H. & JI, L.L. (2000). Superoxide dismutase gene expression in skeletal muscle: fiber-specific effect of age. *Mechanisms of Ageing and Development*, 116: 33-45.

IWAI, K.; MIYAO, M.; WADANO, Y. & IWAMURA, Y. (2003). Dynamic changes of deleted mitochondrial DNA in human leucocytes after endurance exercise. *European Journal Applied Physiology,* 88: 515-519.

JAFARI, A.; POURFAZI, M.A.H.; RAVASI, A.A. & HOUSHMAND, M. (2005). Effect of aerobic exercise training on mtDNA deletion in the skeletal muscle of trained and untrained Wistar Rats. *British Journal of Sports Medicine*, 39: 517-520.

JI, L.L. (1995). Oxidative stress during exercise: implication of antioxidant nutrients. *Free Radical Biology and Medicine*, 18: 1079-1086.

JI, L.L. (1997). Adaptations of glutathione antioxidant system to endurance training are tissue and muscle fiber specific. *American Journal Physiology*, 272: R363-R369.

JI, L.L. (1999). Antioxidants and oxidative stress in exercise. *Proceedings of the Society for Experimental Biology and Medicine*, 222: 283-292.

LARSSON, N.G. & CLYTON, D.A. (1995). Molocholar genetic aspects of human mitochondrial disorders. *Annual Review of Genetics*, 29: 151-178.

LEEUWENBURGH, C. & HEINECKE, J.W. (2001). Oxidative stress and antioxidants in exercise. *Current Medicinal Chemistry,* 8: 829-838.

LIU, V.W.; ZHANG, C. & NAGLEY, P. (1998). Mutations in mitochondrial DNA accumulate differentially in three different human tissues during ageing. *Nucleic Acids Research*, 26: 1268-1275.

LIU, J.; YEO, H.C.; OVERVIK-DOUKI, E.; STEPHANIE, J.T.; DANIEL D.W.; GEORGE C.; BROOKS, A. & AMES, B.N. (2000). Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. *Journal of Applied Physiology*, 89: 21-28.

PARISE, G.; BROSE, A.N. & TARNOPOLSKY, M.A. (2005). Resistance training decreases oxidative damage to DNA and increases cytochrome oxidase activity in older adults. *Experimental Gerontology*, 40: 173-180.

RADAK, Z.; TAYLOR, A.W.; OHNO, H. & GOTO, S. (2001) Adaptation to exercise-induced oxidative stress: from muscle to brain. *Exercise Immunology Review,* 7: 90-107.

SAKAI, Y.; IWAMURA, Y.; HAYASHI, J.; YAMAMOTO, N.; OHKOSHI, N. & NAGATA, H. (1999). Acute exercise causes mitochondrial DNA deletion in rat skeletal muscle. *Muscle & Nerve*, 22: 258-261.

SCHON, E.A.; RIZZUTOTO, C.T.; MORAES, C.T.; NAKASE, H.; ZEVIANI, M. & DIMAURO, S. (1989). A direct repeat is a hotspot for large-scale deletion of human mitochondrial DNA. *Science,* 244: 346-349.

TAIVASSALO, T.; FU, K.; JOHNS, T.; ARNOLD, D.; KARPATI, G. & SHOUBRIDGE, E.A. (1999). Gene shifting: a novel therapy for mitochondrial myopathy. *Human Molecular Genetics*, 8: 1047-1052.

VAN ESSEN, E.H.R.; ROEP, B.O.; 't HART, L.M.; JANSEN, J.J.; VAN DEN OUWELAND, J.M.W.; LEMKES, H.H.P.J. & MAASSEN, J.A. (2000). HLA-DQ polymorphism and degree of heteroplasmy of the A3243G mitochondrial DNA mutation in maternally inherited diabetes and deafness. *Diabetic Medicine*, 17: 841-847.

WALLACE, D.C. (1992). Disease of mitochondrial DNA. *Annual Review of Biochemistry*, 61: 1175-1212.

Dr. Bahman Mirzaei: Department of Exercise Physiology, Faculty of Physical Education & Sport Sciences, University of Guilan, P.O. Box 1438, Rasht, Iran. Cell.: (+98) 912 321 5778, Fax.: (+98) 1316690675/1316690815, E-mail: bmirzaei2000@Yahoo.com

(Subject editor: Prof. E. Terblanche)