Original Article

Association of physical activity with Mitochondrial DNA Copy Number in Eveningness Chronotypes

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Objective: To determine the association of physical activity with mitochondrial DNA copy number (mtDNA-CN) in eveningness chronotype.
Methodology: In this cross-sectional analytical study, 80 eveningness chronotypes were recruited by distributing the morningness-eveningness questionnaire (MEQ). Physical activity levels of participants were assessed by using International Physical Activity Questionnaire (IPAQ), and 2 groups were made. Group A included active (n=40) and Group B included inactive participants (n=40). Mitochondrial DNA copy number of all the participants was determined by quantitative polymerase chain reaction (PCR). Data were analyzed using SPSS 27.
Results: A total of 80 participants (Male=30, Female=50) with mean age of 24.27±6.91 years (range 18-45 years) were included in the study. Mean mitochondrial DNA copy number (log-transformed) was significantly higher in active participants as compared to inactive ones ($p<0.001$).
Conclusion: Participants who were physically active had higher mitochondrial DNA copy number indicating less mitochondrial DNA damage and improved mitochondrial function despite being eveningness chronotype.
Keywords: Mitochondria, mitochondrial DNA, oxidative stress, chronotype.

INTRODUCTION

Mitochondria are double membranous organelles that are involved in a wide range of biological processes such as ATP synthesis, metabolism, apoptosis, and inflammation. Mitochondria have their own genomes, mitochondrial DNA (mtDNA), that can have tens to thousands of copies per cell. This quantity variation is known as mitochondrial DNA copy number (mtDNA-CN), and it varies greatly between cell types and individuals.[1] The mtDNA-CN has been postulated as a viable biomarker of aging and an effective focus of stress including physiological and environmental stress.[2,3]

Mitochondrial dysfunction leads to abnormal cellular energy production, nuclear gene expression and excessive reactive oxygen species production, which all contribute to chronic diseases like cardiovascular diseases and type 2 diabetes mellitus (T2DM).[2] Physical activity (PA) is believed to slow down biological aging, by lowering oxidative stress and inflammation. However, knowledge about the frequency, total quantity of PA or the type of PA (low vs vigorous) affecting mtDNA-CN is deficient.[4]

Regular aerobic PA may boost the production of reactive oxygen species and C-reactive protein or interleukin-6 by body, which could raise resistance to adverse effects of environmental variables.[5] Individuals who are physically active have shown reduced insulin resistance.[6] This suggests that PA may have a beneficial effect on metabolic health and mitochondrial function. Studies have shown that moderate and vigorous-intensity physical activity has led to increased mtDNA-CN both in human and animal models.[7,8]

Chronotype is the term used to describe a person's innate inclination to sleep and wake up at times of the day or night, which is impacted by their biological clock. Work performance, mood, and general health are just a few areas of daily living that chronotype can affect. Chronotypes can be classified into three categories; morningness chronotypes who prefer an early bed and wake time, eveningness chronotypes who prefer a later bedtime and later wake time and intermediate who lie in between the two.[9]

Compared to morningness chronotype, eveningness chronotype tends to have more health issues, such as psychological illnesses, cardiovascular diseases, and higher mortality. Additionally, it has been noted that eveningess chronotype is more likely to acquire metabolic illnesses such as type 2 diabetes and metabolic syndrome.[10] However, data on effects of PA and inactivity on mtDNA-CN in eveningness chronotype is sparse. Therefore, the current study aimed to determine association of PA with mtDNA-CN in eveningness chronotypes.

METHODOLOGY

This cross-sectional analytical study was conducted at

Physiology Department of Islamic International Medical College (IIMC), Rawalpindi, in collaboration with Genetic Resource Center Rawalpindi, Pakistan. The study received ethical approval from Institutional Review Committee (IRC) of IIMC (Ref number Riphah/IIMC/IRC/22/2068). The study was carried out from September 2022 to May 2023. A written informed consent was taken from all participants.

A total of 80 healthy individuals with an eveningness chronotype were recruited for the study. Participants were selected by circulating the Morningness Eveningness Questionnaire (MEQ) among students, faculty, and staff members of IIMC.[11] A self-assessment questionnaire consisting of 19 questions to determine morning, intermediate or evening chronotype. Scores can range from 16-86. Scores of 41 and below indicate "evening types." All participants were between 18 to 50 years of age to avoid any age-related changes in mtDNA-CN.[12] Smokers and individuals with any comorbidity (heart, liver and kidney diseases) were also excluded from the study.

The levels of PA were assessed using the International Physical Activity Questionnaire (IPAQ). The IPAQ is a validated tool widely used to measure physical activity levels in various populations.[13] This is a self-reported physical activity assessment tool for individual aged 15 to 69. The outcomes might be presented as a categorical variable (low, moderate, or high activity levels) or as a continuous variable (MET minutes per week).

Those who score high physical activity on the IPAQ engage in vigorous-intensity activity on at least 3 days and accumulating at least 1500 MET minutes/week Or 7 days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum of at least 3000 MET-minutes/week.

Those who score moderate PA on the IPAQ engage in 3 or more days of vigorous activity of at least 20 minutes per day Or 5 or more days of moderate-intensity activity or walking of at least 30 minutes per day Or 5 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum of at least 600 MET-min/week. On the IPAQ, a low level of PA indicates that you do not fulfill any of the criteria for moderate or high levels of PA.

WHO provides recommendations for PA levels in different age groups. For adults aged between 18 and 64 years, WHO recommends engaging in moderate to high levels of PA.[14] Participants were then categorized into two groups: Group A (Active) and Group B (Inactive). Participants who reported high or moderate levels of physical activity, according to the IPAQ criteria, were classified as active. On the other hand, participants who did not meet the criteria for high or moderate physical activity levels were categorized as inactive.

DNA extraction was performed on 2-3 ml of peripheral blood using Chelex method.[15] An assay based on quantitative polymerase chain reaction using *HOT FIREPol® EvaGreen® qPCR Mix Plus (ROX), 5*x was adapted as a measure of the amount of mtDNA-CN. qPCR was used to quantify copy number of the mitochondrial gene NADH dehydrogenase, subunit 1 (ND1). The Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene was used as a reference for nuclear DNA copy number. The real-time PCRs were carried out using Rotor-Gene Q by Qiagen to acquire respective cycle thresholds (Ct) values for copy numbers of ND1 and CFTR (control) gene.

Following primers were used to amplify nuclear and mitochondrial genes of interest:

Mt DNA Primers: (153 bp).[12]

MtDNA-F 5’-AACATACCCATGGCCAACCT-3’
MtDNA-R 5’-AGCGAAGGGTTGTAGTAGCCC-3’

Control (CFTR Gene) Primers: (97 bp)

CFTR-F 5'-GTTTTCCTGGATTATGCCTGGCAC-3’
CFTR-R 5'-GTTGGCATGCTTTGATGACGCTTC-3’

The PCR was run in a tube containing forward and reverse primers specific to the gene. The final total volume of the PCR reaction was 20 micro-liters. PCR was run for both nuclear and mitochondrial DNA of each sample simultaneously. PCR was done by initial DNA denaturation at 95°C (2 min) accompanied by 35 cycles of 95°C for 20 seconds (denaturation), 60°C for 60 seconds (annealing, extension, and image acquisition). Relative Mitochondrial DNA copy number was calculated by first calculating delta Ct through following formula: Delta Ct = Ct nuclear gene – Ct mitochondrial DNA. After calculation of delta Ct following formula was applied to get mitochondrial DNA copy number:[16]

Mitochondrial DNA copy number = $2^{\Delta Ct}$

Statistical Analysis: Data were analyzed using SPSS version 27. Independent samples t-test was applied to determine the association of PA with mtDNA-CN. $p \leq 0.05$ was considered statistically significant.

RESULTS

Out of 80 participants, 30 (37.5%) were male and 50 (62.5%) females. Mean age was 24.27±6.91 years (range 18-45). The determination of mtDNA-CN in all groups was normally distributed when transformed to log values. In group A, out of 40 participants, 25 were female (62.5%) and 15 (37.5%) were male. In group B, there were 26 (65%) females and 14 (35%) males. A comparison between males and females across both

Group A and Group B revealed no significant difference in mitochondrial DNA copy number (mt DNA-CN) (p=0.19).

Comparison of MtDNA-CN in peripheral blood between genders is shown in Table 1. Comparison of MtDNA-CN in peripheral blood by professional status is shown in Table 2. We found significantly higher mtDNA-CN levels in the Group A (active) mean 2.74±0.14 as compared to Group B (inactive) mean 2.26±0.25 (p<0.001) (Table 3).

Table 1: Comparison of MtDNA-CN in peripheral blood between genders.

Gender	Number	Mean ± SD	Range	p value
Male	30	2.56 ± 0.27	2.29-2.83	0.19
Female	50	2.46 ± 0.34	2.12-2.8	

Table 2: Comparison of MtDNA-CN in peripheral blood by professional status.

Status	Number	Mean ± SD	Range	p value
Students	49	2.51 ± 0.29	2.22-2.80	0.613
Faculty	31	2.48 ± 0.35	2.13-2.83	

Table 3: Comparison of MtDNA-CN in peripheral blood of Group A and B.

Group	Number	Mean ± SD	Range	p value
Group-A (active)	40	2.74 ± 0.14	2.44-2.93	<0.001
Group-B (inactive)	40	2.26 ± 0.25	1.36-2.67	

DISCUSSION

Our study confirmed positive effects of PA on MtDNA-CN in eveningness chronotype. MtDNA-CN of physically active eveningness chronotype was significantly higher as compared to inactive ones. Our results are similar to a study by Chang et al.[17] Chang et al exclusively examined postmenopausal women.

Cao et al investigated effects of exercise intensity on mtDNA and mtDNA-CN in mice and found that moderate PA decreased mutations in mice's mitochondrial D-loop regions, indicating a beneficial effect on mtDNA integrity and mtDNA-CN.[7] They focused on mice and gastrocnemius muscles, but our work goes a step further by looking at physical activity and mtDNA-CN in human population with an eveningness chronotype.[7]

Mury et al examined effects of PA on oxidative stress and inflammation and concluded that regular PA reduced oxidative damage by increasing secretion of anti-inflammatory markers as well as increasing oxidative capacities of mitochondria.[18] These findings provide a potential explanation for increase in mtDNA-CN observed in current study. It is plausible that reduction in oxidative damage and inflammation associated with regular PA may promote mitochondrial integrity and contribute to an increase in mtDNA-CN. Additionally, a study found that swimmers who were highly trained had greater mtDNA-CN as compared to less trained swimmers, which may be related to higher blood ATP levels.[8]

A study by Cancello et al on obese individuals with a BMI ≥ 30 kg/m2 found a notable rise in mtDNA copy numbers following a one-year lifestyle intervention, encompassing both dietary improvements and increased PA.[19] Another cross-sectional study on a diverse sample of 391 community-dwelling mid-life and older adults, reported that there was no statistically significant association between PA and mtDNA-CN.[4] This however is contrary to the findings of current study.

CONCLUSION

Physical activity enhances mitochondrial function and promotes better health outcomes in populations characterized by an eveningness preference as people with active lifestyle have a higher mtDNA-CN than those with inactive physical activity levels. Change in the activity level may counteract the negative effects of increased oxidative stress and aging brought on by eveningness behavior.

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