RESEARCH ARTICLE

Association of eating habits with mitochondrial DNA copy number in eveningness chronotypes: Origin research

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Abstract

Objective: To determine the relationship between eating habits and mitochondrial deoxyribonucleic acid copy number in adult cases of eveningness chronotypes.

Method: The cross-sectional, analytical study was conducted from September 2022 to June 2023 at the Physiology Department of the Islamic International Medical College, Rawalpindi, in collaboration with the Genetic Resource Centre, Rawalpindi, Pakistan, and comprised adult subjects who were assessed using the Morningness-Eveningness Questionnaire. The participants' eating habits were assessed using the Healthy Eating Assessment Questionnaire, and on they were divided into those with healthy eating habits in group A and those with unhealthy eating habits in group B. Deoxyribonucleic acid was extracted using the Chelex method, the mitochondrial deoxyribonucleic acid copy number of all participants was quantified using quantitative polymerase chain reaction. Data was analysed using SPSS 27.

Results: Of the 80 subjects, 30(37.5%) were males and 50(62.5%) were females. The overall mean age was 24.27±6.91 years (range: 18-45 years). There were 40(50%) subjects in each group. The mean mitochondrial deoxyribonucleic acid copy number in group A was 2.74±0.14 compared to 2.26±0.25 in group B ($p<0.001$).

Conclusion: Subjects with healthy eating habits exhibited higher mitochondrial deoxyribonucleic acid copy numbers, indicating reduced damage to mitochondrial deoxyribonucleic acid.

Keywords: Mitochondria, Mitochondrial DNA, Eating habits, Chronotype. (JPMA 74: 1099; 2024)

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Introduction

Mitochondria are double-membrane organelles that serve critical roles in a variety of biological processes, such as adenosine triphosphate (ATP) generation, metabolism, apoptosis and inflammation. They have their own genetic material called mitochondrial deoxyribonucleic acid (mtDNA), which may be found in various amounts inside the cells. This difference in the quantity 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 possible biomarker of ageing and a reliable indicator of stress that takes into account both physiological and environmental variables.[2,3] Studies have shown that mitochondrial dysfunction leads to abnormal cellular energy production, nuclear gene expression and excessive reactive oxygen species (ROS) production, which all contribute to chronic diseases, like cardiovascular diseases (CVDs) and type 2 diabetes mellitus (T2DM).[2] Multiple factors render mtDNA more sensitive to damage than nuclear DNA (nDNA). Due to its closeness to elevated quantities of ROS, lack of protective histones, and restricted repair mechanisms, it leads to increase incidence of mtDNA damage. Metabolic stresses, such as an imbalance in redox homeostasis caused by excessive fat consumption, might lead to mtDNA damage.[4]

Diet is critical to sustaining metabolic health and general wellbeing. The global burden of non-communicable illnesses is increasing, posing a major public health concern, a large portion of which is preventable. These diseases have been significantly linked to harmful lifestyle choices, especially unhealthy eating habits.[5] Eating habits include the patterns and decisions that people make about their food consumption, which include food types and quantities consumed, meal frequency, meal timing, food preferences and dietary practises.[6] Eating habits have a major effect on general health and wellbeing since they directly affect nutritional intake and can contribute to the development of a variety of health issues.[7] Adopting healthy eating habits often include eating a well-balanced diet rich in nutrient-dense foods, such as fruits, vegetables, whole grains, lean meats and healthy fats.[6] Appropriate portion control is critical for maintaining a healthy calorie intake. Furthermore, following regular meal times while limiting excessive snacking might help to regulate hunger and satiety signals.[8] Irregular meal habits, such as skipping meals or overeating frequently, can be harmful to one's

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health and wellbeing.[9,10] Mitochondrial dysfunction caused by poor dietary habits can upset the balance of lipid homeostasis in the liver, leading to an excess of ROS. Non-alcoholic fatty liver disease (NAFLD) can be exacerbated by oxidative stress (OS).[11]

The impact of eating habits on mitochondrial function is profound and multifaceted. The quality and quantity of nutrients consumed play a pivotal role, with essential vitamins, minerals and antioxidants, like B vitamins, magnesium, vitamin C, vitamin E and coenzyme Q10, being crucial for mitochondrial health. Striking a balance between nutrient intake is essential. Moreover, caloric intake holds sway, as both overeating and severe calorie restriction can disrupt mitochondrial function.[12,13]

Chronotype is the term used to describe a person's innate propensity to sleep and wake up at particular times of the day or night, which is impacted by their biological clock as well as external variables, including heredity and environment. Work performance, mood and general health are just a few areas of daily living that chronotype can affect. Chronotype can be classified into three categories; morningness chronotype (MC) that prefer early bed and wake time, eveningness chronotype (EC) that prefer a later bedtime and later wake time, and intermediate chronotype (IC) that lie in between the two.[14]

Numerous health-related variables and chronotypes have been linked in studies. Compared to MC, EC tends to have more health issues, such as psychological illnesses, CVDs, T2DM and metabolic syndrome (MS).[15] However, data on the effects of healthy and unhealthy eating habits on mtDNA-CN in EC is sparse. The current study was planned to determine association of eating habits with mtDNA-CN in EC.

Subjects and Methods

The cross-sectional, analytical study was conducted from September 2022 to June 2023 at the Physiology Department of the Islamic International Medical College (IIMC), Rawalpindi, in collaboration with the Genetic Resource Centre (GRC), Rawalpindi, Pakistan. After approval from the IIMC ethics review committee, the sample was raised from among IIMC students, faculty members and staff who were recruited by circulating the Morningness Eveningness Questionnaire (MEQ), which is a widely used questionnaire that assesses an individual's chronotype preference.[16] The participants were aged 18-50 years in order to avoid any age-related changes in mtDNA-CN.[17] Smokers and individuals with any comorbidity were excluded. Written informed consent was taken from all the participants. The sample size was calculated using the formula $Z=pq/e^2$ while taking EC prevalence 5.3%.[18] Non-probability convenient sampling technique was used.

The eating habits of the participants were assessed using the 10-item Healthy Eating Assessment Questionnaire (HEAQ)[19] that covers different food groups, like fruits, vegetables, dairy products, snacks, desserts, soda, fast food, meat, beans and fish.

The participants were then divided into those with healthy eating habits in group A and those with unhealthy eating habits in group B. Those scoring in the 'Good' and 'Excellent' categories of HEAQ were in group A, while those in the 'Needs Improvement' and 'Fair' categories were in group B.

DNA extraction was performed on 2-3ml of peripheral blood using the Chelex method,[20] which is a popular choice for DNA extraction due to its simplicity, cost-effectiveness and efficacy in producing high-quality DNA.[21] The DNA extraction from whole blood using the Chelex method involved collecting 200 microliters of venous blood in sterile vacutainer tubes. Distilled water was added, and after vortexing, the solution was subjected to centrifugation to pellet white cells. Following multiple repetitions of the process, 200 microliters of 7% Chelex solution were added to the white cell pellet, followed by incubation at 95°C.[20] After centrifugation, the supernatant was transferred, labelled and stored at -20°C. Samples with residual haemoglobin (Hb) underwent re-extraction to ensure clear DNA. The extracted DNA was then preserved for subsequent quantitative polymerase chain reaction (qPCR) amplification. An assay based on qPCR using HOT FIREPol EvaGreen qPCR Mix Plus (ROX) 5x was adapted as a measure of the amount of mtDNA-CN. HOT FIREPol EvaGreen qPCR Mix Plus (ROX) 5x is an optimized ready to use solution for real time quantitative PCR assays by Solis BioDyne. It comprises all the components necessary to perform qPCR: HOT FIREPol DNA Polymerase, ultrapure dNTPs, $MgCl_2$, EvaGreen dye and ROX dye. To quantify mtDNA-CN of mitochondrial gene nicotinamide adenine dinucleotide (NAD) + hydrogen (NADH) dehydrogenase subunit 1 (ND1), qPCR was used. Cystic fibrosis transmembrane conductance regulator (CFTR) gene was used as the reference for nDNA-CN. Real-time PCRs were carried out using Rotor-Gene Q by Qiagen to acquire the respective cycle threshold (Ct) values for CNs of ND1 and CFTR (control) genes. The Rotor-Gene Q 5plex by Qiagen (headquartered in Hilden, Germany) is a real-time PCR instrument enabling up to 5-plex reactions with high sensitivity and a wide dynamic range.

The forward (F) and reverse (R) primers used to amplify nuclear and mitochondrial genes of interest were mtDNA primers 153bp:[17]

MtDNA-F 5'-AACATACCCATGGCCAACCT-3'

MtDNA-R 5'-AGCGAAGGGTTGTAGTAGCCC-3',

And control CFTR gene primers (97bp:

CFTR-F 5'-GTTTTCCTGGATTATGCCTGGCAC-3'

CFTR-R 5'-GTTGGCATGCTTTGATGACGCTTC-3'.

The PCR reaction was run in a tube containing F and R primers specific to the gene. The final total volume of the PCR reaction was 20 microlitres. PCR was run for both nDNA and mtDNA of each sample simultaneously. PCR was done by initial DNA denaturation at 95°C for 2min accompanied by 35 cycles of 95°C for 20s (denaturation), and 60°C for 60s (annealing, extension and image acquisition). Relative mtDNA-CN was calculated by first calculating delta Ct by subtracting Ct mtDNA from Ct nDNA, and mtDNA-CN equalled 2ΔCt.[22]

Data was analysed using SPSS 27. Mean and standard deviations were used to report continuous quantitative variables, while frequencies and percentages were used for categorical variables. Independent samples t-test was applied to determine the association of eating habits with mtDNA-CN. P≤0.05 was considered statistically significant.

Results

Of the 80 subjects, 30(37.5%) were males and 50(62.5%) were females. The overall mean age was 24.27±6.91 years. There were 40(50%) subjects in each group. The mean mtDNA-CN in group A was 2.74±0.14 compared to 2.26±0.25 in group B (p<0.001) (Table).

In both the groups, there were 25(62.5%) females and 15(37.5%) males, and mtDNA-CN was not significant with respect to gender (p=0.19).

There were 15(37.5%) faculty or staff members and 25(62.5%) students in group A, while the corresponding values in group B were 16(40%) and 24(60%), and mtDNA-CN was not significantly associated with professional status in either group (p=0.613).

Table: Comparison of MtDNA-CN in peripheral blood of the study group.

Group	Number	Mean± SD	Range
Group-A			
(Healthy Eating Habits)	40	2.74±0.14	2.44-2.93
Group-B			
(Unhealthy Eating Habits)	40	2.26±0.25	1.36–2.67
p-value	<0.001		

MtDNA-CN: Mitochondrial deoxyribonucleic acid copy number.

Discussion

The current study confirmed positive effects of healthy eating habits on mtDNA-CN in EC. The findings were in line with Ma et al., who in 2021 claimed that their study was the first of its kind to evaluate the effects of diet quality on mtDNA-CN. They identified a link between a high diet score and a higher mtDNA-CN in whole blood samples.[23] That study had 90% individuals aged >40 years23 compared to the current study's age range of 18-45 years. The distinction was made since age-related mitochondrial alterations become more evident beyond the age of 50.[17]

A reduction in the mtDNA-CN in whole blood indicated mitochondrial malfunction and increased OS. Furthermore, the content of one's food has the ability to influence the level of oxidative damage as well as the effectiveness of antioxidant systems. This link between nutrition and a variety of chronic illnesses emphasises the significance of dietary changes as a strategy for avoiding chronic degenerative diseases and CVDs. As a result, improving daily eating habits can be a beneficial way to enhancing general health and lowering the risk of such illnesses.[24]

A recent systematic analysis that included both observational and interventional trials found evidence that a high-quality diet, such as the Dietary Approaches to Stop Hypertension (DASH) and Mediterranean diets, were associated with lower levels of OS.[25] The current study's findings were consistent with the findings that mtDNA-CN, mitochondrial function and OS were interlinked.

Wu et al. in 2019 found a positive association between fruit consumption and mtDNA-CN in blood.[26] In contrast, the current study looked at total dietary patterns rather than just fruit and vegetable consumption, although fruit and vegetable consumption are a component of healthy eating habits. In an ethanol-fed male mouse model, rice bran phenolic extract improved hepatic mitochondrial oxidative damage, increased mtDNA content, and reduced mitochondrial dysfunction. These findings emphasised the importance of a high-fibre diet.[27]

OS has emerged as a significant hallmark of metabolic diseases, which is frequently accompanied by persistent low-grade inflammation due to an imbalance between pro-oxidant and anti-oxidants, favouring the build-up of oxidised entities. The current study also reported that mtDNA-CN was an indicator of OS.[28] Despite a drop in daily energy requirements, there has been an alarming growth in the intake of high-energy meals, consisting of carbs, protein and fats, in modern lifestyle. Because of this eating pattern, OS has emerged as a significant hallmark of metabolic diseases. MS is intimately associated with OS, which results from an imbalance between pro-oxidant and anti-oxidants, favouring the build-up of oxidised entities. These patients typically show symptoms of OS-induced damage. A high-fat diet (HFD) and obesity, both of which

contribute to systemic OS, have been proven to have a direct influence on insulin sensitivity of metabolic organs and to induce inflammation.[28]

The current study acknowledged the research gap in understanding the link between various unhealthy eating habits and mitochondrial dysfunction, particularly in low- and middle-income countries (LMICs). Although inadequate data makes direct comparisons with LMICs difficult, the current study addressed the gap and gave valuable insights into the influence of eating habits on mitochondrial health. The findings, however, emphasised the need for future studies to explore these relationships in diverse settings.

The study looked at how eating habits affected mtDNA in ECs. By categorising the participants based on their eating habits and measuring mtDNA, the study threw light on how diet impacted those with eveningness preferences. This gave us fresh insights into how diet, body rhythms and mitochondrial health are connected. The findings might help create personalised diet recommendations for people with EC.

The current study has limitations as its cross-sectional design offered a limited snapshot of the relationship between eating habits and mitochondrial function at a single moment in time, rendering it impossible to establish causal connections. To unveil temporal patterns and establish causality, a longitudinal approach would be essential. Moreover, utilising self-reported data for the analysis of dietary behaviours and the intricate composition of meals, encompassing a variety of nutrients, could have introduced errors and oversimplifications. Additionally, the study neglected individual genetic variabilities and did not fully address potential confounding factors, like environmental influences, thereby constraining the broader applicability of the findings. Further investigation are necessary to gain a comprehensive understanding of the nuanced interplay between eating habits and mtDNACN.

Conclusion

Healthy eating habits enhanced mitochondrial function and promoted better health outcomes in populations characterised by an eveningness preference as people with healthy eating habits had a higher mtDNA-CN than those with unhealthy eating habits. Change in eating habits and dietary preferences may counteract the negative effects of increased OS and ageing brought on by the eveningness behaviour.

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Author Contribution:
SA: Concept, supervision of writing, conduction of experiment, reviewing and final approval.
MM: Design, drafting, acquisition, compilation, analysis, financial resource, writing.
SA: Facilitating the experimental procedure.
SF: Conceived the idea.