ENVIRONMENTAL DETERMINANTS OF MITOCHONDRIAL DNA COPY NUMBER

By

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A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, Maryland

May 2022

Abstract

This dissertation was designed to examine environmental determinants of mitochondrial DNA copy number (mtDNA-CN), a biomarker for mitochondrial dysfunction, and the association of mtDNA-CN with a health outcome. Low levels of mitochondrial DNA copy number (mtDNA-CN) are a biomarker of mitochondrial dysfunction often induced by oxidative stress. We sought to examine whether environmental factors, such as air pollution, season, and temperature, are associated with mtDNA-CN using the UK Biobank Study. We also evaluated the association of mtDNA-CN with incident heart failure (HF) using the Atherosclerosis Risk in Communities (ARIC) Study.

First, we reviewed the current literature on environmental exposures associated with mtDNA damage or mtDNA-CN. Mitochondria is a common target of environmental toxins, such as air pollution, smoking, chemicals, and metals. Because mitochondrial DNA is particularly vulnerable these environmental stressors because they lack the repair mechanisms present in the nucleus, mtDNA-CN is highly reactive to various environmental stressors. We found that there is limited epidemiologic evidence on the association between these stressors and mtDNA-CN. In particular, the existing studies are limited by cross-sectional study design, small sample size, inconsistent methods of measuring the exposures, and mtDNA-CN, and inadequate control for potential confounders.

Second, we evaluated the association between long-term exposure to air pollutants (PM ≤ 10 μm diameter [PM_{10}], $PM_{2.5}$, $PM_{2.5-10}$, nitrogen dioxide [NO_2], and nitric oxide [NO]) and mtDNA-CN in the UK Biobank. An increase of 10 $\mu g/m^3$ in annual average PM_{10} and NO_2 exposure was associated with an adjusted difference in mtDNA-CN of -0.089 (95% confidence

interval [CI]; -0.090, -0.087) and -0.018 (-0.018, -0.017), respectively. The associations persisted for lags of up to 3 years. $PM_{2.5-10}$ was also inversely associated with mtDNA-CN.

Third, we evaluated the seasonal trends of mtDNA-CN and the association between short-term exposure to ambient temperature and mtDNA-CN. We also evaluated the proportion explained by metabolites in the association of temperature and mtDNA-CN in the UK Biobank. mtDNA-CN, on average, was lower in the warmer months and higher in the colder months. The average difference in mtDNA-CN by 10°C increase in daily maximum temperature (T_{max}) was -0.07 (95% CI -0.08, -0.06) and the results were similar across different temperature metrics. The inverse association between ambient temperature and mtDNA-CN persisted over 7 days with the strongest association at lag 0. The association of T_{max} with mtDNA-CN was most explained by metabolites involved in glucose and lipid metabolism, including pyruvate, lactate, and ketone bodies.

Lastly, we examined the association between mtDNA-CN and the risk of incident HF among 10,802 participants free of HF at baseline from the ARIC study, a large bi-racial population-based cohort. We found that the hazard ratios (95% CI) for HF comparing the 2nd through 5th quintiles of mtDNA-CN to the 1st quintile were 0.91 (0.80–1.04), 0.82 (0.72–0.93), 0.81 (0.71–0.92), and 0.74 (0.65–0.85), respectively (P for trend < 0.001). In stratified analyses, the associations between mtDNA-CN and HF were similar across examined subgroups. The inverse association between mtDNA-CN and incident HF was stronger in HF with reduced ejection fraction (HFrEF) than in HF with preserved ejection fraction (HFpEF).

These findings suggest that mitochondria and mitochondrial DNA are common targets of environmental stressors, as reflected by low mtDNA-CN, and mitochondrial dysfunction may

explain the potential mechanism of various adverse health effects associated with environmental exposures.

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Acknowledgements

This dissertation would not have been possible without the support of my advisor and mentors, family, and friends. I would first like to thank my advisor, Dr. Eliseo Guallar, who has provided invaluable guidance and wisdom throughout the masters and doctoral programs. From him, I have learned so much about epidemiology and research in general, starting from formulating research questions to putting them into an appropriate research design, to applying statistical methods, and to writing and reviewing manuscripts. I have also learned many qualities that a researcher or any person needs - patience, to challenge myself, to learn from experience, and the resilience to spring back, and to try again. I am grateful for all his support and trust which allowed me to complete this thesis and to explore other areas of research.

I also had the pleasure of working closely with Dr. Dan E. Arking who has introduced me to this field of mitochondria, genetics, and omics. I am grateful for all the opportunities that expanded my knowledge and understanding of genetics and biological mechanisms. I have benefited greatly from the many meetings and discussions we had about this thesis and from his insightful comments and feedback. I would also like to thank the readers of thesis, Drs. Carlos Castillo-Salgado and Christopher D. Heaney, for their support. Dr. Carlos Castillo has been supportive throughout my PhD program as well as the master's program and has provided more global perspectives and reminded me the public health implications of my work. The enthusiasm and insights for environmental epidemiology that Dr. Christopher Heaney has shown have been a great motivation and inspiration throughout the program.

I also would like to thank all the co-authors on the papers included in this dissertation and the faculty members who served on my examination committees, including Drs. Edgar R. (Pete) Miller, Wendy Post, Noel Mueller, Miranda Jones, Aisha Dickerson, Jung-Im Shin, Jessie Buckley, and Kirsten Koehler. I have also had the pleasure of working with the wonderful group

of AMDACS, Drs. Alvaro Muñoz, Christopher Cox, Derek Ng, Christopher Pierce, and Michael Schneider.

I am grateful to the Department of Epidemiology for all the support and the welcoming environment. I would also like to thank my friends and colleagues both inside and outside of Johns Hopkins for providing such a wonderful experience. I was very fortunate to meet Dr. Juhee Cho, an alumnus of the School of Public Health, when I was struggling with clinical research as a physician for introducing me to this area of work and her encouragement to study epidemiology.

I really would not have been able to complete this long journey without my family. My dad taught me to think like a public health researcher even before I was admitted to medical school and has been a role model – both as a doctor and as a human being – all my life. He has always taught me to think and dream big and has been an inspiration to all my work. My mom is the wisest person I know, someone I can always turn to seek for advice. She knew exactly what I needed to hear every time I sought for advice during the most difficult times. My sister was always there to listen and to support. She has been the best travel buddy and go-to person whenever I needed a break. Thank you to my mom, dad, and my sister for believing in me and all the support. Finally, I want to dedicate this presentation and thesis to my grandparents, who are true survivors and have shown me what strength is in every aspect.

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List of Acronyms

ARIC	Atherosclerosis Risk in Communities
ATP	Adenosine triphosphate
BMI	Body mass index
CKD	Chronic kidney disease
CVD	Cardiovascular disease
HF	Heart failure
mtDNA-CN	Mitochondrial DNA copy number
PM	Particulate matter
ROS	Reactive oxygen species
SNP	Single nucleotide polymorphism
UK	United Kingdom
US	United States
WES	Whole exome sequencing
WGS	Whole genome sequencing

Chapter 1

Introduction

Exposure to environmental pollutants, such as air pollution, smoking, chemicals, pesticides, and metals, is associated with various adverse health outcomes.[1] These environmental stressors may act through diverse pathways, one of which includes disturbances in the redox balance. Mitochondria play a central role in the generation and regulation of cellular reactive oxygen species (ROS) and ROS participate in the regulation of cell signaling. Mitochondria, therefore, are considered as a common target of environmental stressors and mitochondrial dysfunction as a hallmark of environmental insults.[2,3] One useful biomarker of mitochondrial dysfunction is mitochondrial DNA copy number.[4,5] Compared to nuclear DNA, mitochondrial DNA (mtDNA), is susceptible to these stressors because of its inefficient repair system and damage caused by excessive ROS.[6] Levels of mitochondrial DNA copy number change in response to energy demand, oxidative stress, and external stressors.[4,5] However, experimental and epidemiologic studies on mtDNA damage or changes in mtDNA-CN caused by environmental exposures are scarce.

Current evidence on mtDNA damage and levels of mtDNA-CN associated with environmental exposures will be reviewed.

MITOCHONDRIA, MITOCHONDRIAL FUNCTION, AND MITOCHONDRIAL DNA COPY NUMBER

Mitochondrion is a double membraned organelle that generate over 90% of cellular energy in the form of adenosine triphosphate (ATP) via oxidative phosphorylation.[4] Mitochondria are also essential in the production of biosynthetic intermediates, and signaling cellular stress responses, such as autophage and apoptosis.[4,7] Division and fusion of mitochondria, or mitochondrial membrane dynamics, is also tightly regulated to maintain

homeostasis in the presence of mitochondrial stress and impairment of division or fusion results in mitochondrial dysfunction.[8] Mitochondrial function is integrated with other cellular compartments and regulate the communication within a cell as well as between cells and tissues.[7] As a result, mitochondrial dysfunction is associated with various chronic diseases and is a hallmark of aging.[9]

Each cell contains 10s to 1000s of mitochondria, and each mitochondrion contains 2–10 copies of mtDNA.[10] mtDNA is a circular, double-stranded, haploid DNA, which encodes 37 genes, 13 of which are involved in oxidative phosphorylation.[4] mtDNA replication is regulated by the D-loop, a non-coding region within mtDNA, and occurs independently of nuclear DNA replication.[11] The number of mtDNA vary depending on cell type and tissues that have greater energy demand generally have a higher mtDNA content. For instance, heart and skeletal muscle cells have 1000~5000 mtDNA copies per cell, while spleen and liver cells that have low energy requirements have as low as ~100 copies per cell.[12,13]

mtDNA-CN changes in response to energy demands, metabolic changes, and environmental stressors.[4,14,15] mtDNA-CN levels are closely linked to key functions of mitochondria and are directly related to energy demand, oxidative stress, and mitochondrial membrane potential.[16] They also depend on the stability of mitochondrial genome through intact mitochondrial translation and proper mitochondrial membrane dynamics.[17,18] mtDNA, however, is susceptible to oxidative damage caused by excessive ROS because of its proximity to the inner mitochondrial membrane where oxidation products are generated (primarily in the complexes I and III of the electron transport chain [ETC]) and because of its inefficient protection and repair mechanisms.[6] Moreover, damaged mtDNA induces excess ROS production by altering the expression of the ETC components or by generating dysfunctional ETC components, which may

further aggravate and accumulate mtDNA damage.[19,20] Such damage initially triggers mtDNA replication and increases in mtDNA-CN as a compensatory mechanism to enhance energy supply and remove damage. Under prolonged exposure and chronic stress, however, mutations accumulate beyond the capacity to repair, mtDNA copy number decreases, and mitochondria become dysfunctional, leading to decreased ATP production, dysregulation of cell signaling, and, eventually, to cell death.[5,19] Indeed, lower levels of mtDNA-CN is associated with various chronic diseases,[4,7] including overall mortality,[21,22] cardiovascular disease,[23-25] and chronic kidney disease.[26]

ENVIRONMENTAL EXPOSURES ON MITOCHONDRIAL AND MTDNA-CN

Overview

Environmental contaminants, including air pollutants, heavy metals, pesticides, chemicals, and drugs cause numerous health effects.[1] Many of these are known to induce damage and mutations to the DNA and lead to cancer, metabolic disorders, aging, and death.[27,28] However, most of the studies focused on nuclear DNA damage and mutations. Although mtDNA is more vulnerable to damage than nuclear DNA, due to its inefficient impair system and tendency to accumulate lipophilic, positively charged molecules,[29] studies on mtDNA damage or change in mtDNA-CN caused by environmental exposure are scarce. Thus, we review the existing evidence on environmental exposures on mitochondrial function or mtDNA-CN in the following sections.

Air pollution

Ambient air pollution is primarily generated by industry and motor vehicle exhaust, and by household air pollution. The composition of ambient air pollution varies with the source of exposure, time of day, time of year, weather conditions, local geography, and geographical region.[30] The main toxicants with air pollution include particulate matter (PM), ozone, carbon monoxide (CO), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and lead.[31] In particular, exposure to PM is the 5th leading risk factor for mortality worldwide, responsible for over 7 million deaths each year.[32] The association between air pollution and mitochondrial toxicity has been focused mostly on PM.

PM is a major component of both household and ambient air pollution. PM is a mixture of pollutants made up mostly of ammonium, elemental carbon, organic carbon matter, nitrate, silicon, sodium, and sulfate. The composition of PM changes dynamically by its source, region, and season.[33] Several studies have identified that PM is associated with mitochondrial toxicity and mtDNA damage.[34-38] Iron-derived free radicals in ambient PM can alter mtDNA and disrupt mitochondrial membrane potential, leading to apoptosis of alveolar epithelial cells in a dose-dependent and time-dependent manner.[35] Moreover, PM size is an important factor in the pathogenesis of mitochondrial toxicity due to air pollution. Larger PM (PM_{10} or $PM_{2.5}$) are either cleared upon entering the respiratory tract or sequestered in vacuoles and less likely to cause mitochondrial damage.[34] $PM_{0.1}$, on the other hand, can cross the blood-brain barrier and enter the systemic circulation, accumulate in the mitochondria, and damage mitochondrial cristae and membrane.[34] Moreover, in epidemiologic studies, long-term exposure to $PM_{2.5}$ was inversely associated with mtDNA-CN in women[39] and in two elderly populations.[40,41] The results, however, were inconsistent for short-term exposure.[42-44 40,45]

Ozone is created by chemical reactions between oxides of volatile organic compounds (VOCs) and nitrogen oxides in the presence of sunlight. Ozone may be associated with mitochondrial toxicity through various mechanisms, including increased oxidative stress, mtDNA damage, and decreased mitochondrial membrane potential.[46,47]

Plausible mechanisms have been suggested to explain air pollution, particularly PM, as a source of mitochondrial damage. There are few epidemiologic studies, however, evaluating the associations of short-term and long-term PM exposure with mtDNA-CN and the results are inconsistent. The evidence for non-PM air pollutants, including ozone, NO_2, are even more scarce and both mechanistic and epidemiologic studies are needed to better understand the role of mitochondria in health outcomes related to air pollution.

Smoking

Tobacco smoke is estimated to contain thousands of chemicals, including CO, polycyclic aromatic hydrocarbons (PAHs), tobacco-specific nitrosamines, nitrogen oxides, aldehydes, VOCs, nicotine, fine PM, and oxidants.[48,49] First-hand and second-hand smokers are exposed to similar composition of tobacco smoke but to different concentrations of toxicants. Both first-hand smoking and second-hand smoking is the leading cause of mortality and premature mortality globally.[50]

Active and passive cigarette smoke cause mitochondrial toxicity. First, cigarette smoke decreases mitochondrial respiration and membrane potential, and as a result, decrease ATP production and increase ROS production in a dose- and time-dependent manner.[51-54] More specifically, mitochondria are rich in enzymes that are easily modified and inactivated by ROS, such as mitochondrial isoform of superoxide dismutase, manganese SOD or SOD2.[55] In addition,

ROS impairs the oxidative phosphorylation complexes and reduces the proton pumping efficiency leading to decreased mitochondrial membrane potential and ATP production.[56] Mitochondrial toxicity induced by smoking is reversible and cessation of both active and passive smoking may improve various measures of mitochondrial function.[57,58] Second, cigarette smoke can changes mitochondrial morphology.[53,54]

In contrast with our understanding of mitochondrial damage due to smoking, in a meta-analysis, cigarette smoking was positively associated with mtDNA-CN.[59] On the other hand, a cross-sectional study of participants selected from VITamin D and OmegA-3 TriaL-Depression Endpoint Prevention (VITAL-DEP) study, which was not included in the meta-analysis, the amount of smoking was inversely associated with mtDNA-CN.[60] A large epidemiologic study with detailed information on smoking history, including duration, intensity, and type, may help elucidate the association between smoking and mtDNA-CN.

Chemicals, pesticides, and drugs

Dioxins are persistent organic pollutants (POP) produced from industrial activities, incineration or burning fuels, chlorine bleaching of paper and pulp, and smoking, and are known carginogens.[61] Dioxins include polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, polychlorinated, biphenyls (PCBs), and other related compounds.[62] An in vitro study, treatment with 2,4,7,8-tetrachlorodibenzodioxin (TCDD) induced mitochondrial dysfunction, ROS, and stress signaling similar to that in mtDNA-depleted cells.[63] TCDD also decreased the mitochondrial membrane potential markedly and induced ROS production and mitochondrial dysfunction.[1] In the JAR cells derived from a human trophoblastic

tumor of the placenta, treatment with TCDD reduced ATP content, mitochondrial membrane potential, and mtDNA copy number.

PCBs has several properties, such as insulation, chemical stability, and relative inflammability, which lead to a widespread use of PCBs in electrical equipment, adhesives, coolants and lubricants, and plastics, oil-based paint, and many others.[64] Due to evidence that they persist and accumulate in the environment and can cause toxic effects, the manufacture of PCBs has been banned and phased out most PCB uses in 1979. However, it remains in the environment and continues to be an important health concern. In vitro and in vivo studies had shown that PCBs affects mitochondrial function through an increase ROS levels, a decrease mitochondrial membrane potential, and induction of apoptosis.[65-67]

Several pesticides are known to cause adverse health effects through ROS generation and induction of mitochondrial membrane depolarization, and inhibition of complexes I, II and/or III.[68,69] For instance, rotenone, which may be a risk factor for Parkinson's disease, is lipophilic, allowing it to cross biological membranes, and induces mtDNA damage.[70] Organophosphate compounds, such as tri-ortho tolyl phosphate, triphenyl phosphite, and parathion, may cause changes in mitochondrial transmembrane potential, the key driver of oxidative phosphorylation and maintaining homeostasis.[71] In a study of occupational exposure to off-gassing haloalkane pesticides in storage facilities and home environment, the exposure was associated with elevated mtDNA-CN, which increased over time.[72]

Benzene, a known risk factor for leukemia, is formed from both naturally and anthropogenically. Benzene is commonly used in industries to manufacture plastics, synthetic fibers, lubricants, rubbers, dyes, and pesticides. In two studies, benzene exposure was associated with an increase in mtDNA-CN.[73,74] In a study of benzene-exposed workers in 3 Italian cities,

airborne benzene exposure was associated with an increase in mtDNA-CN was determined in blood cells associated with airborne benzene exposure.[73] In an in vitro study, treatment with benzene increased mtDNA-CN, mitochondrial contents, and mitochondrial membrane potential.[74]

Polycyclic aromatic hydrocarbons, or PAHs, are a class of numerous chemicals that are composed of two or more fused aromatic rings of carbon and hydrogen atoms. They occur naturally by burning coal, oil, gas, wood, and tobacco, and by anthropogenic activities.[75] Many PAH compounds have toxic properties, including mutagenicity, carcinogenicity, teratogenicity, and immunotoxicity, but each individual PAH compounds may not exhibit the same health effects. Benzo(a)pyrene, for instance, has been associated with cancer in animal studies and is classified as carcinogenic to humans by the International Agency for Research on Cancer (IARC, group 1). The association between PAHs exposure and mtDNA-CN was inconsistent. In two studies of coke-oven workers, chronic exposure to PAH was inversely associated with mtDNA-CN in one study[76] and positively associated with mtDNA-CN in another study.[77] However, because PAHs are a heterogeneous group of chemicals, the associations of may depend on the individual components.

Bisphenol A is an industrial chemical primarily used in the manufacturing of polycarbonate plastics. The major source of exposure is through ingestion from food or from plastic containers or tableware. Its structure is similar to estrogen, allowing it to interact with estrogen receptors and act as an endocrine disruptor.[78] It has been associated with blood pressure, diabetes, and cardiovascular disease in adults and may have neurodevelopmental, endocrine, and reproductive effects in children.[79-81] In an in vitro study, long-term exposure to low-dose bisphenol A decreased mitochondrial DNA copy number.[82] In addition, prenatal exposure in the

first trimester to bisphenol S, a substitute for bisphenol A, was associated with lower mtDNA-CN in male newborns but not in female newborns.[83] There was also no associations for bisphenol S measured in second and third trimesters and mtDNA-CN in newborns. Previous studies have suggested bisphenol-induced oxidative stress and inflammation as a potential mechanism for the relationship between bisphenol A and mitochondrial function.[81]

The evidence suggests potential mechanisms linking these chemicals to the impairment of mitochondrial functions and possibly health outcomes. However, to date, there are few epidemiological studies evaluating the association between chemical exposures and mitochondrial function, especially in the low-exposure, general population settings. Moreover, the results are not consistent across studies, possibly due to differences in the duration and intensity of exposure, lack of adjustment for potential confounders, and method of mtDNA-CN measurement.

Metals and metalloids

Heavy metals, such as lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg), can cause various health effects that are mediated through several different mechanisms involved in the generation and regulation of oxidative stress.[2,84] First, heavy metals and metalloids may interfere with the cell's ability to detoxify ROS. Metals, including Pb, Cd, As, and Hg, have high affinity to thiol or selenol residues in enzymes involved in ROS detoxification, such as glutathione peroxidase and peroxiredoxin isoenzymes.[2] Binding of these metals to active site thiol or selenol inhibits the interaction of these antioxidants with hydrogen peroxide (H_2O_2) and other peroxides, leading to the accumulation of ROS. Moreover, due to their high affinity, the metals do not readily dissociate from the enzymes, resulting in prolonged inhibition of

antioxidant activities. In addition, heavy metals and metalloids limit the availability of cofactors, such as glutathione and thioredoxin, that necessary for glutathione peroxidase and peroxiredoxin isoenzymes to function.[85] They also decrease the activity of enzymes involved in recycling the cofactors and enzymes, thereby further reducing the capacity to regulate oxidative stress.[2]

Second, some metals, such as iron, copper, and chromium, take part in redox cycling ("redox-active metals") and directly hydroxyl radicals ($HO\cdot$), superoxide radicals ($O_2\cdot^-$), or H_2O_2.[84] Redox-inactive metals, such as Pb, Cd, and Hg, may promote the ROS formation by inhibiting the intrinsic antioxidative mechanisms directly (e.g. glutathione peroxidase) or indirectly (e.g. Sirtuin 3).[2,86,87] Third, exposure to heavy metals and metalloids induces inflammation, which activates and amplifies formation.[2] Occupational Pb exposure, for instance, was positively associated with inflammatory markers, including interleukin (IL)-6 and tumor necrosis factor (TNF)-α, in a meta-analysis.[88] Cd exposure has also been associated with changes in innate and adaptive immunity, and inflammation.[89]

The associations of metals / metalloids and mtDNA-CN or mitochondrial function have not been well explored. In an epidemiologic study of early pregnancy metal mixtures (As, barium [Ba], Cd, cesium [Cs], Pb, Hg, magnesium [Mg], manganese [Mn], selenium [Se], and zinc [Zn]) and mtDNA-CN in maternal and cord blood, concentrations of Pb in the first trimester was positively associated with maternal mtDNA-CN but not with cord blood mtDNA-CN.[90] No other heavy metal was independently associated with mtDNA-CN. However, nonessential metals (As, Ba, Cd, Cs, Pb, and Hg), in combination, was positively associated with maternal mtDNA-CN. In an experimental study using mouse oocytes, even relatively low concentrations, within the environmental pollution levels, of arsenic trioxide (As_2O_3), which is used in pesticides or wood preservatives, decreased mtDNA-CN in a dose-dependence relationship and caused

deletion in mtDNA.[91] Inorganic arsenic (iAs/As(III)) alters the activity of enzymes involved in electron transfer, redox reactions, protein synthesis, and DNA repair.[2] Exposure to inorganic arsenic compounds, therefore, leads to ROS production[2] and may reduce mtDNA-CN.

In a study of children between 6 and 15 years in Mexico, blood lead levels were inversely associated with mtDNA-CN but there was no association between urinary arsenic levels and mtDNA-CN.[92] On the other hand, blood lead levels were positively associated with mtDNA-CN in a study of 10 men with high levels of occupational exposure to Pb compared to 10 age-matched men with lower blood lead levels. However, both studies were limited by small sample size and inadequate control of potential confounders.

In the Seychelles Child Development Study Nutrition Cohort 2 of 1488 mother-child pairs, they used maternal hair and blood samples and cord blood samples for Hg and maternal and fetal blood samples for mtDNA-CN to evaluate the association between Hg exposure and mtDNA-CN.[93] Maternal blood Hg levels were positively associated with maternal blood mtDNA-CN, whereas fetal cord blood Hg levels were inversely associated with fetal mtDNA-CN. In a small study of rural Peruvian population, there was no associations of total Hg measured in hair samples and mtDNA-CN or mtDNA damage, measured as lesions per 10 kilobase pairs.[94] In addition, low-dose methylmercury exposure increased the number of specific point mutations in the D-Loop of human embryonic neural progenitor cells mtDNA, leading to ATP depletion, decreased membrane potential, and apoptosis.[95]

Cadmium can induces oxidative damage associated with mitochondrial dysfunction, deregulation of intracellular antioxidants and apoptosis.[96] However, epidemiologic evidence for the association between cadmium and mtDNA-CN is currently lacking.

SUMMARY OF CURRENT EVIDENCE

mtDNA-CN, a biomarker of mitochondrial function, is often inversely associated with adverse health events, including mortality and cardiovascular disease. Mitochondria is a common target of environmental toxins, such as air pollution, smoking, chemicals, and metals. mitochondrial DNA is particularly vulnerable these environmental stressors because they lack the repair mechanisms present in the nucleus, and thus, mtDNA-CN is highly reactive to various environmental stressors. However, there is limited epidemiologic evidence on the association between these stressors and mtDNA-CN. In particular, the existing studies are limited by cross-sectional study design, small sample size, inconsistent methods of measuring the exposures, and mtDNA-CN, and inadequate control for potential confounders. Therefore, further investigation is needs to better understand the potential role of mitochondrial function in the biological mechanisms between environmental stressors and health outcomes.

To increase understanding of the potential involvement of mitochondrial function and mtDNA-CN in the health effects of common environmental exposures (air pollution and climate) and to examine the role of mtDNA-CN as a biomarker for HF, we leveraged existing data from two large cohorts.

STUDY AIMS

This dissertation was intended to address key issues related to environmental determinants of mtDNA-CN and to elucidate the role of mtDNA-CN as a biomarker of heart failure.

Aim 1. To investigate the associations of long-term air pollution exposure and mtDNA-CN in the UK Biobank.

Aim 2. To investigate the seasonal trends of mtDNA-CN and the associations of short-term ambient temperature and mtDNA-CN in the UK Biobank.

Aim 3. To investigate the relationship between mtDNA-CN and incident heart failure in the Atherosclerosis Risk in Communities (ARIC) Study.

DISSERTATION DATA SOURCES

Aims 1 and 2 use data from the UK Biobank, a prospective cohort study of approximately 500,000 adults 40 to 69 years of age recruited across the United Kingdom (UK) from 2006 to 2010. Demographics, lifestyle factors, and medical history were collected through questionnaire. Participants underwent anthropometric measurements and health exams and provided blood samples. mtDNA-CN metrics were generated from the blood samples collected from this visit. Annual average air pollution concentrations were developed as separate project, the Small Area Health Statistics Unit of the BioSHaRE-EU Environmental Determinants of Health project, but were incorporated into the UK Biobank for each participant. Daily maximum and minimum ambient temperatures across the UK from 2006 to 2010 were obtained from the HadUK-Grid provided by the Met Office Hadley Center for Climate Science and Services (https://www.metoffice.gov.uk/). HadUK-Grid is a publicly available data set of gridded land surface climate variables which interpolates in situ land surface station data to a regular grid using inverse-distance weighted averaging to various temporal (daily, monthly, seasonal, and yearly) and spatial (1 km, 5 km, 12 km, and 60 km) resolutions.

Aim 3 uses data from the Atherosclerosis Risk in Communities (ARIC) Study, a population-based prospective cohort of 15,792 individuals 45–65 years of age at the time of recruitment (1987–1989; Visit 1). ARIC participants were recruited from 4 US communities: Forsyth County, North Carolina; Jackson, Mississippi; suburban Minneapolis, Minnesota; and Washington County, Maryland. Since the first study visit, there have been 6 subsequent in-person visits (visits 2–7, with visit 7 currently underway) and regular telephone interviews (annually and then semiannually since 2012). Our analysis was restricted to 11,453 White or Black participants who had DNA collected in one of the visits to generate mtDNA-CN measurements. Incident HF was defined as the first hospitalization for HF or death related to HF after the visit in which DNA was obtained for mtDNA-CN assays. Hospitalizations and deaths related to HF were identified as International Classification of Disease, 9th Revision code 428, and International Classification of Diseases, 10th Revision code I50 in discharge codes or in underlying cause of death, respectively. Since 2005, ARIC began adjudication of HF events by the ARIC HF Classification Committee.

DISSERTATION STRUCTURE

This dissertation includes 4 additional chapters, one dedicated to each study aim and a conclusion. Each of the chapters is formatted as a publishable manuscript. Chapter 2 examines the association between long-term air pollution exposure and mtDNA-CN. Chapter 3 assesses seasonal patterns of mtDNA-CN and the association between ambient temperature and mtDNA-CN. Chapter 4 examines the association between mtDNA-CN and incident HF. Chapter 5, the Conclusion, synthesizes the findings of the dissertation and proposes next steps for this research.

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Chapter 2

Air pollution exposure and mitochondrial DNA copy number in the UK Biobank

ABSTRACT

Background: Low levels of mitochondrial DNA copy number (mtDNA-CN) are a biomarker of mitochondrial dysfunction often induced by oxidative stress. Air pollution is a pervasive source of oxidative stress, but the association between air pollution exposure and mtDNA-CN is inconclusive.

Objective: We evaluated the association between long-term exposure to PM ≤10 μm diameter (PM_{10}) and nitrogen dioxide (NO_2) with mtDNA-CN in 195,196 adults in the UK Biobank study.

Methods: Annual average PM_{10} and NO_2 concentrations were estimated using separate land-use regression models for years 2007 and 2010. mtDNA-CN was measured in blood samples collected between 2007 and 2010 and was calculated as the ratio of mitochondrial coverage to the nuclear genome coverage derived from whole-genome sequencing in 195,196 UK Biobank participants. We used standardized values (Z-scores) after log-transformation as the mtDNA-CN metric.

Results: The median (interquartile range) annual average concentrations of PM_{10} and NO_2 were 21.8 (20.3–23.6) and 28.9 (23.7–35.1) $\mu g/m^3$ in 2007, and 16.2 (15.5–17.1) and 27.8 (22.7–32.4) $\mu g/m^3$ in 2010. An increase of 10 $\mu g/m^3$ in annual average PM_{10} and NO_2 exposure was associated with an adjusted difference in mtDNA-CN of -0.089 (95% confidence interval; -0.090, -0.087) and -0.018 (-0.018, -0.017), respectively. The associations persisted for lags of up to 3 years. $PM_{2.5-10}$ was also inversely associated with mtDNA-CN.

Discussion: In this large-scale study, long-term exposure to PM_{10} and NO_2 were inversely associated with mtDNA-CN. These findings suggest that oxidative stress-induced mitochondrial

dysfunction, reflected by reduced mtDNA-CN, may be an additional mechanism mediating the health effects of air pollution.

INTRODUCTION

Ambient air pollution is associated with a wide range of acute and chronic health outcomes, including all-cause mortality, cardiovascular and pulmonary morbidity and mortality, cognitive decline, and increased hospital and emergency department visits.[1-8] Exposure to ambient particulate matter (PM) is the 5th leading risk factor for mortality worldwide, causing an estimated 4.2 million deaths and a loss of over 103 million disability-adjusted life years in 2015.[2] The adverse health effects of air pollution are mediated through oxidative stress, alterations in innate and adaptive immunity, inflammation, and epigenetic changes.[9-12]

Mitochondria are essential to energy production, generating over 90% of the cell's energy in the form of adenosine triphosphate (ATP).[13] Mitochondria also play a major role in the regulation of metabolism and multiple homeostatic, inflammatory, and apoptotic signaling pathways.[14] Each mitochondrion has 2–10 copies of mitochondrial DNA (mtDNA), a circular, double-stranded, haploid DNA strand that encodes 37 genes involved in oxidative phosphorylation or in assembling amino acids into functional proteins and an individual cells may have 100 to 1000 copies of mitochondria.[15] mtDNA is susceptible to oxidative stress caused by excessive reactive oxygen species (ROS) because of its proximity to the mitochondrial membrane where oxidation products are generated and because of its inefficient protection and repair mechanisms.[16] Moreover, damaged mtDNA induces excess ROS production, which may further aggravate and accumulate mtDNA damage.[17,18] Decreased mtDNA copy number is a marker of mitochondrial dysfunction that can be measured in peripheral blood and is associated with increased all-cause mortality,[19,20] cardiovascular disease,[21-24] and chronic kidney disease (CKD).[25]

While air pollution is a pervasive source of oxidative stress, the association between exposure to air pollution and mtDNA copy number has been inconclusive.[26-35] Among 2,758

healthy women in the Nurses' Health Study, higher 12-month average particulate matter ≤2.5 μm in diameter ($PM_{2.5}$) was associated with lower mtDNA copy number.[26] On the other hand, occupational exposures to PM and respirable dust were associated with higher mtDNA copy number.[31,32] Previous studies are difficult to compare because of small sample sizes,[27,29,33] inadequate control of potential confounders,[28,29] wide variations in exposure concentration and composition,[32,34] possible differences between short- and long-term effects of air pollution, and heterogeneity in exposure assessment and mtDNA copy number measurement.

We therefore evaluated the association between long-term exposure to air pollution (PM_{10}, $PM_{2.5}$, $PM_{2.5-10}$, NO_2, and NO) with mtDNA copy number in over 190,000 adult men and women from the UK Biobank study.

METHODS

Study population

The UK Biobank is a prospective cohort study of approximately 500,000 adults 40 to 69 years of age recruited across the United Kingdom (UK) from 2006 to 2010 via mail based on the UK National Health Services registration.[36] Among 199,945 UK Biobank participants with whole genome sequencing (WGS) data available, we excluded individuals who had their blood samples collected in year 2006 (n = 170) or who had abnormal blood cell counts (n = 377; **Supplementary Figure 1**). We further excluded participants with missing values on body mass index (BMI; n = 824). The final study population was 198,573 (89,210 men and 109,363 women). Analyses for each specific pollutant were further restricted to participants with information on the concentration of each air pollutant (**Supplementary Table 1**).

All participants provided written informed consent prior to participation. The scientific protocol and operational procedures of the UK Biobank study were approved by the North West – Haydock Research Ethics Committee in the UK.

Measurement of air pollutant concentrations

We used data on air pollution estimates provided by the Small Area Health Statistics Unit as part of the BioSHaRE-EU Environmental Determinants of Health project (http://www.sahsu.org/). The data were available for 2007 and 2010. For each participant, we used their residential address at the time of the UK Biobank visit to estimate annual average concentrations of each air pollutant.

The estimates of PM_{10} and NO_2 for 2007 were derived from European Union (EU)-wide air pollution maps based on a land-use regression (LUR) model for Europe, which incorporated satellite-derived air pollution estimates, with a resolution of 100 × 100 m.[37] The adjusted R^2 across the UK for PM_{10} and NO_2 were 0.57 and 0.64, respectively.

The estimates of air pollutants for 2010 were modeled using a LUR model developed as part of the European Study of Cohorts for Air Pollution Effects (ESCAPE).[38,39] The R^2 of training models for $PM_{2.5}$, $PM_{2.5\text{-}10}$, and NO in the UK were 0.35–0.60, 0.68–0.79, and 0.83–0.89, respectively. The accuracy of particulate matter estimates was unclear for areas beyond the range of 400 km from Greater London and, therefore, air pollution concentrations were coded as missing for all addresses beyond this range.

Measurement of mitochondrial DNA copy number

To estimate mtDNA copy number in UK Biobank participants, we used WGS data downloaded from the UKB data repository which contains WGS information from 199,945 individuals. We first obtained the number of mapped reads through Samtools (`idxstats` command) which we used to calculate the nuclear genome and mitochondrial genome coverage. Nuclear genome coverage was calculated as the product of total number of aligned reads and read length divided by the estimated human genome size (3.03 G bp). Mitochondrial genome coverage was calculated as the product of number of reads aligned to mitochondria and read length divided by mitochondrial DNA size (16,569 bp). Read length was 151 bp. A raw mtDNA copy number was then calculated as the ratio of (2 × mitochondrial coverage) to the nuclear genome coverage. For the analysis, the mtDNA copy number was log-transformed and standardized by subtracting the mean and dividing by the standard deviation (SD). The mtDNA copy number metric in this study thus represents SD units.

mtDNA copy number measured from WGS performs substantially better than the gold standard using qPCR or whole exome sequencing (WES).[40] However, as the WGS was available only in a subset of the UK Biobank population, we performed sensitivity analysis using a separate mtDNA copy number metric generated using the WES and mitochondrial single nucleotide polymorphisms (SNP) probe intensities in the full UK Biobank cohort.[41] In brief, with the WES data from 49,997 individuals, we generated residuals from a linear regression model of mitochondrial chromosome read counts adjusted for total DNA and potential technical artifacts. We then used rank-transformed SNP probe intensities and also generated 250 principal components (PCs) from autosomal nuclear probes by randomly sampling 5% of probes (n ~ 19,500 probes) from odd chromosomes that were on both UKBelieve and Axiom arrays. For each array type, all mitochondrial SNP probes (UKBelieve, n = 181; Axiom, n = 244) and 250

PCs were regressed on the residuals derived from the linear regression model using the WES results. Coefficients from each model were subsequently used to generate the predicted values in all UK Biobank participants. The estimates were also standardized by subtracting the mean and dividing by the SD. After applying the same exclusion criteria as the main analysis, 378,607 participants (174,055 men and 204,552 women) were included in the sensitivity analysis.

Measurement of other covariates

Study participants visited one of 22 assessment centers where they completed a touch-screen questionnaire and a computer-assisted interview and provided blood, urine, and saliva samples. Physical measures, including height, weight, and blood pressure, were also taken. All assessments were administered according to a standardized procedure. Participants provided information on age, sex, race / ethnicity, average total household income before tax (<£18,000, £18,000–£30,999, £31,000–£51,999, £52,000–£100,000, and >£100,000), and education (categorized as less than college, college or university degree, professional degree, or none of the above). Smoking status was categorized as never, former, and current smokers. Alcohol intake was categorized as never, former, and current drinkers. Physical activity was measured by self-report using the adapted International Physical Activity Questionnaire (IPAQ) Short Form,[42,43] which was converted into total physical activity in metabolic equivalent task (MET) minutes per week by intensity (walking, moderate, and vigorous physical activity).[44] Total METs were then categorized as low, moderate, and high physical activity based on the IPAQ guideline.[42]

Body mass index (BMI) was calculated as weight in kg divided by height in m squared. Blood pressure was measured using an automated blood pressure monitor (Omron 705 IT, OMRON Healthcare Europe BV). Hypertension was defined as a self-reported physician's

diagnosis of hypertension, a self-reported use of antihypertensive medication, or a measured systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg. Prevalence of myocardial infarction (MI)[45] and stroke were defined by an algorithm developed by the UK Biobank.[46] Cardiovascular disease was defined as the presence of either MI or stroke.

Complete blood count, differential white blood cell (WBC) counts (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), and reticulocytes were measured on fresh samples using an a Beckman automated hematology analyzer.[47] Serum glucose levels were measured by hexokinase analysis, total cholesterol levels by CHO-POD method, low-density lipoprotein (LDL) cholesterol levels by enzymatic protective selection analysis, and triglyceride levels by GPO-POD method (Beckman Coulter AU5800, UK). Hemoglobin A1c (HbA1c) was measured in frozen packed red blood cells using high-performance liquid chromatography (Bio-Rad Variant II Turbo analyzer, Bio-Rad Laboratories, US). Details of the laboratory measurements can be found in the UK Biobank online showcase (http://ukbiobank.ac.uk). Diabetes was defined as a self-reported physician's diagnosis of diabetes, a self-reported use of antidiabetic medication, or a measured HbA1c ≥6.5%. Hyperlipidemia was defined as a self-reported use of lipid-lowering medication, or a measured total cholesterol ≥200 mg/dl or triglycerides ≥150 mg/dl.

Statistical analysis

For the main analysis, we used the average concentrations of PM_{10} and NO_2 in 2007 and 2010 as the exposure and mtDNA copy number derived from blood samples collected in 2007–2010 as the outcome. That is, air pollution measured in 2007 was used as exposure for mtDNA copy number derived from samples collected in 2007–2010 (lags of 0, 1, 2, and 3 years), and air

pollution measured in 2010 was used as exposure for mtDNA copy number derived from samples collected in 2010 (lag 0) to ensure that the exposure was measured either in the same year or before the outcome (**Supplementary Table 1**). All measurements were combined into a single dataset for the main analyses, but we also conducted separate analyses by year of air-pollution exposure and blood sample collection. For $PM_{2.5}$, $PM_{2.5-10}$, and NO concentrations with mtDNA copy number measures, we used only air pollution and mtDNA copy number measures from 2010.

We used linear regression models with progressive degrees of adjustment to estimate the association between a 10 $\mu g/m^3$ increase in air pollutant exposure and mtDNA copy number using covariates measured at the time of the UK Biobank visit. Model 1 was adjusted for age, sex, race, year of air pollution measurement, and year of blood collection. Model 2 was further adjusted for average annual income, education level, smoking, alcohol intake, physical activity, BMI, and history of hypertension, diabetes, hyperlipidemia, and cardiovascular disease. Finally, Model 3 was further adjusted for blood cell counts. Because participants who had blood samples collected in 2010 were included twice in the analysis (using air pollution exposure levels in 2007 and in 2010), we used sandwich covariance matrix estimation (sandwich package in R) using a degrees of freedom-based correction to estimate the standard errors. Standard errors were also adjusted to account for genetically related individuals in the study using sandwich covariance matrix estimation.

In addition to modeling air pollution as a linear term, we compared the differences in mtDNA copy number by quintiles of air pollutant concentrations using the lowest quintile as the reference, and we modeled air pollutant concentrations as restricted cubic splines with knots at

the 5th, 27.5th, 50th, 72.5th, and 95th percentiles to provide a smooth and flexible description of the dose-response relationship between air pollution and mtDNA copy number.

We performed stratified analyses to identify subgroups that may potentially be more susceptible using pre-specified subgroups: age (<60 or ≥ 60), sex, race (White, Black, Asian, other), BMI (underweight, normal, overweight, or obese), smoking status (never, former, or current), alcohol intake (never, former, or current), physical activity (low, moderate, or high), and presence of hypertension and diabetes, separately. Finally, we performed sensitivity analysis using the mtDNA copy number metric derived from the WES and mitochondrial SNP probe intensities in the full UK Biobank cohort. For this analysis, because the metric was validated in non-related White individual, we excluded non-White individuals and who had genetically related individuals in the study. All statistical analyses were performed using R software version 4.1.2.

RESULTS

The mean age (SD) of the 198,573 study participants (89,210 men and 109,363 women) was 57.5 (8.1) years (**Table 1**). Compared to participants in the lower quintiles of mtDNA copy number, those in the higher quintiles of mtDNA copy number were more likely to be younger and female, to have higher average annual income, to be never or former smokers and physically active, and to have lower body mass index, systolic blood pressure, serum glucose, LDL cholesterol, triglyceride levels, and lower prevalence of hypertension, diabetes, and cardiovascular disease. The median (interquartile range) annual average concentrations of PM_{10} and NO_2 were 21.8 (20.3–23.6) and 28.9 (23.7–35.1) $\mu g/m^3$, respectively, in 2007, and 16.2

(15.5–17.1) and 27.8 (22.7–32.4) μg/m^3, respectively, in 2010 (**Supplementary Table 1**). The characteristics of participants included in the PM_{10} and the NO_2 analyses were similar.

In the fully-adjusted model, an increase in PM_{10} of 10 μg/m^3 was associated with a difference in mtDNA copy number of -0.089 (95% confidence interval [CI] -0.090, -0.087; **Table 2**). For NO_2, the corresponding difference in mtDNA copy number was -0.018 (-0.018, -0.017). Compared to participants in the lowest quintile of air pollutant exposure, the difference in mtDNA copy number among those in the highest quintile of PM_{10} exposure was -0.090 (-0.092, -0.088; *P* for trend < 0.001) and among those in the highest quintile of NO_2 exposure it was -0.033 (-0.047, -0.019; *P* for trend < 0.001). The inverse trend for PM_{10} with mtDNA copy number was also evident in spline regression analyses (**Figure 1**). For NO_2, there was an inverse association between NO_2 and mtDNA copy number at concentrations above 30 μg/m^3. The associations did not materially change after adjusting for seasonality and additional hematologic parameters (not shown). The associations were also similar when we used the mtDNA copy number metric generated using WES and mitochondrial SNP probe intensities (**Supplementary Table 2**).

In the analysis using air pollutant concentrations and mtDNA copy numbers measured in the concurrent year (Lag 0), an increase of 10 μg/m^3 in PM_{10} and NO_2 was associated with a difference in mtDNA copy number of -0.358 (-0.416, -0.301) and -0.060 (-0.075, -0.046), respectively, in 2007, and with a difference of -0.053 (-0.101, -0.006) and -0.007 (-0.019, 0.005), respectively, in 2010 (**Table 3**). For PM_{10} and NO_2 concentrations from 2007 and mtDNA copy numbers measured in 2008–2010, analyses conducted separately by year of measurement of mtDNA copy number consistently showed an inverse association but did not show a clear trend with increasing lag (**Table 3**). For air $PM_{2.5}$, $PM_{2.5-10}$, and NO concentrations and mtDNA copy

number measured only in 2010, the fully-adjusted differences in mtDNA copy number associated with an increase of 10 $\mu g/m^3$ were -0.038 (-0.133, 0.057), -0.127 (-0.226, -0.027), and -0.004 (-0.009, 0.002), respectively (**Table 4**).

In stratified analysis, the associations between PM_{10} and mtDNA copy number were more pronounced in the higher BMI categories (*P* for interaction = 0.002), in current smokers (*P* for interaction = 0.008), and in participants with diabetes (*P* for interaction = 0.05; **Figure 2**). The associations were similar across other subgroups examined. The subgroup analysis for NO_2 showed similar results.

DISCUSSION

In this large-scale population-based study, we found that higher concentrations of PM_{10} and NO_2 were inversely associated with mtDNA copy number concentrations. These findings support the hypothesis that oxidative stress-induced mitochondrial dysfunction, reflected by reduced mtDNA copy number, is a potential mechanism linking air pollution with various adverse health outcomes.

Air pollution is a major cause of mortality and global burden of disease.[1,2] Its health effects include increased mortality, hospital admissions and emergency department visits, and higher risk of pulmonary, cardiovascular, neurodegenerative, and other diseases.[3-8] Exposure to air pollutants causes local inflammation and oxidative stress in the lung and generation of ROS, triggering the release of cytokines, a systemic inflammatory response, and higher levels of stress hormones due to sympathetic activation. Chronic exposure to air pollution can alter innate and adaptive immunity and cause epigenetic changes.[9,10,48-50]

mtDNA copy number fluctuates with changes in energy demands, oxidative stress, and various physiological and environmental conditions.[15,51] mtDNA is a particularly susceptible target of ROS-induced damage and mutation as it lacks protective histones and has limited repair capacity.[16] mtDNA damage and mutation result in inefficient mitochondrial function, which initially triggers mtDNA replication and increases in mtDNA copy number to enhance energy supply and remove damage. Under prolonged exposure and chronic stress, however, mutations accumulate, mtDNA copy number decreases, and mitochondria become dysfunctional.[17] Mitochondrial dysfunction is characterized by impaired oxidative phosphorylation and energy synthesis, increased ROS generation, disruption of calcium homeostasis and calcium signaling pathways, and alterations in metabolic, inflammatory, and apoptotic signaling pathways due to changes in the expression of associated nuclear genes.[10,15] mtDNA copy number measured in the peripheral blood is a useful biomarker reflecting oxidative stress-mediated mitochondrial dysfunction, and it has been associated with adverse health outcomes,[15] including all-cause mortality,[19,20] cardiovascular disease,[21-24] and CKD.[25]

Air pollutants can have adverse effects on mitochondrial structure and function.[10] First, PM can impair the mitochondrial respiratory chain, calcium regulation, and mitochondrial membrane potential, resulting in reduced ATP production.[52-55] For example, exposure to diesel exhaust PM decreased the mitochondrial respiratory activity and reduced ATP production in rats.[52] In addition, PM increased mitochondrial permeability transition in other animal models[53,54] and decreased mitochondrial membrane potential in endothelial cells.[55] Second, PM can promote mitochondrial ROS formation via downregulation or loss of function of antioxidant enzymes[56] and activation of inflammatory and inhibition of anti-inflammatory pathways.[57] Third, PM may induce changes in the expression of genes involved in the fission and fusion of

mitochondria,[10] leading to both an inhibition of mitochondrial fusion[55] and an increase in the production of giant mitochondria by the fusion of multiple mitochondria.[58] Lastly, PM may damage the integrity and the function of mtDNA through increased levels of ROS within mitochondria or DNA methylation.[59] However, further studies are needed to understand the link between other air pollutants, such as NO_2, and mitochondrial dysfunction.

Long-term exposure to air pollution was inversely associated with mtDNA copy number in other studies. In a study of 2,758 healthy women from the Nurses' Health Study, an interquartile range (5.5 $\mu g/m^3$) increase in annual average $PM_{2.5}$ was associated with a difference of -0.07 (95% CI -0.13, -0.01) units in mtDNA copy number (z-scores).[26] In addition, PM exposure in the past year was inversely associated with mtDNA copy number in two elderly populations[30,34] and in three small other studies evaluating the short-term effects of air pollution.[27,29,32] However, $PM_{2.5}$ exposure in the past month and the past week were positively associated with mtDNA copy number in an elderly population,[30] and 5-day to 28-day average exposures of black carbon were associated with higher mtDNA copy number.[35]

The results of previous studies are difficult to compare because many studies were small, restricted to occupational exposures or to a highly selective group of population, and with limited adjustment for potential confounders. Moreover, the durations and concentrations of exposure and the methods of mtDNA copy number assessment varied substantially across studies. Despite these limitations, and consistent with our findings, long-term (≥1 year) exposure to air pollutants was mostly associated with lower mtDNA copy number.

Evidence on the relationship between non-PM air pollutants and mtDNA copy number is relatively scarce. In our study, NO_2 was inversely associated with mtDNA copy number at higher levels. Similarly, higher concentrations of nitrate (NO_3^-) as a component of $PM_{2.5}$ were

associated with lower mtDNA copy number in a study of elderly men.[34] Prenatal exposure of NO_2 in the last month and third trimester of pregnancy was also inversely associated with placental mtDNA copy number.[60] We, however, did not find any association between NO and mtDNA copy number. Oxidation of atmospheric nitrogen (N_2) forms NO at high temperatures, which, due to its unstable property, reacts rapidly with oxygen to form NO_2.[61] Therefore, NO_2 or nitrogen oxides (NO_x), which includes both NO and NO_2, may be a better metric to represent long-term outdoor exposures and associated health effects.

We have also evaluated the associations of PM_{10} and NO_2 with mtDNA copy number measured with different time intervals (Lags 0–3 years). Regardless of the time interval, each air pollutant was inversely associated with mtDNA copy number but we did not see a clear trend by time interval. These results, however, are difficult to interpret because different lags include different people, making it difficult to know if the differences are due to the lags or to the participants included in each lag. Longitudinal studies with multiple measurements of air pollution levels or mtDNA copy number are needed for identification and evaluation of the most critical exposure window.

Several limitations need to be considered in the interpretation of our findings. First, mtDNA copy number was measured on a single occasion which limited our ability to evaluate the change in mtDNA copy number over time by exposure concentration and the most critical window of exposure. Second, there are inherent limitations of using air pollution concentrations from prediction models. Only ambient concentrations of air pollutants were available and we were not able to consider personal exposure levels that may vary by activity patterns, workplace exposures, and indoor air pollution. The measurement error in the air pollution levels, however, is likely to be unrelated to mtDNA copy number and would have attenuated the association.

Different modeling methods were used for 2007 and 2010, which may explain the differences in the estimates of Lag 0 models when the analysis was performed separately for the two years. Third, as an observational study, there may be unmeasured confounders that we could not account for. Fourth, the mtDNA copy number metric was validated only among White individuals and restricted to our study population. Therefore, further studies including a diverse population are needed to evaluate the generalizability of the findings.

CONCLUSIONS

In this large-scale population-based study, prior and concurrent exposure to PM_{10} and NO_2 were inversely associated with mtDNA copy number. These findings suggest oxidative stress-induced mitochondrial dysfunction, reflected by reduced mtDNA copy number, as a potential mechanism between air pollution and various adverse health outcomes.

ACKNOWLEDGEMENTS

None.

SOURCES OF FUNDING

This work was supported by the US National Institutes of Health grants R01HL131573 and R01HL144569. This research was also conducted using the UK Biobank Resource under Application Number 17731.

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FIGURE LEGENDS

Figure 1. Average mtDNA copy numbers by PM_{10} and NO_2 concentrations.

The curves represent estimated mtDNA copy numbers (solid line) and their 95% confidence intervals (gray area) by PM_{10} and NO_2 concentrations based on fully adjusted regression models using restricted cubic splines with knots at the 5^{th}, 27.5^{th}, 50^{th}, 72.5^{th}, and 95^{th} percentiles of its distribution. The spline regression model was adjusted for age, sex, race, year of air pollution measurement, year of blood collection, average annual income, education level, smoking, alcohol intake, physical activity, body mass index, history of hypertension, diabetes, hyperlipidemia, and cardiovascular disease, and blood cell counts (red blood cell, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and platelets).

Figure 2. Association between 10 $\mu g/m^3$ increase in PM_{10} (A) and NO_2 (B) and mtDNA copy number by subgroups.

The figure displays the difference in mitochondrial DNA copy number associated with a 10 $\mu g/m^3$ increase in PM_{10} (A) and NO_2 (B) in models adjusted for age, sex, race, year of air pollution measurement, year of blood collection, average annual income, education level, smoking, alcohol intake, physical activity, body mass index, history of hypertension, diabetes, hyperlipidemia, and cardiovascular disease, and blood cell counts (red blood cell, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and platelets). Pre-specified subgroups were age (<60 or ≥ 60), sex, race (White, Black, Asian, or Other), body mass index (underweight, normal, overweight, or obese), smoking status (never, former, or current), alcohol intake (never, former, or current), physical activity (low, moderate, or high), and presence of hypertension and diabetes, separately.

Table 1. Participant characteristics by quintiles of mitochondrial DNA copy number.

	Overall (n=198,573)	Quintile 1 (n=43,991)	Quintile 2 (n=39,623)	Quintile 3 (n=38,556)	Quintile 4 (n=38,816)	Quintile 5 (n=37,587)
mtDNA-CN*	-0.05 (-4.84, 7.02)	-1.20 (-4.84, -0.78)	-0.48 (-0.78, -0.26)	0.01 (-0.26, 0.21)	0.51 (0.21, 0.80)	1.32 (0.80, 7.02)
Age (years)	56.5 (8.1)	57.5 (8.1)	56.8 (8.1)	56.5 (8.1)	56.0 (8.1)	55.4 (8.0)
Men (%)	44.9	49.3	46.2	44.4	42.8	41.2
Race (%)						
White	94.1	94.5	94.4	94.5	94.2	92.8
Black	1.5	0.9	1.0	1.3	1.6	2.8
Asian	2.4	2.6	2.5	2.2	2.3	2.2
Other	1.6	1.5	1.7	1.5	1.5	1.7
Average income (%)						
< £18,000	18.9	21.8	19.4	18.8	17.7	16.3
£18,000 – £30,999	21.6	22.4	21.9	21.4	21.4	20.6
£31,000 – £51,999	22.6	21.0	22.3	22.9	23.2	23.7
£52,000 – £100,000	17.8	15.4	17.2	17.8	18.7	20.2
> £100,000	4.7	4.0	4.6	4.8	5.0	5.5
Education (%)						
Less than college	37.8	36.8	37.8	38.2	38.1	38.4
College or university degree	33.1	30.6	32.5	32.9	34.1	35.8
Professional degree	11.6	12.1	11.5	11.7	11.4	11.2
Smoking (%)						
Never	55.1	52.7	54.6	55.1	56.0	57.2
Former	34.8	35.4	34.5	35.0	34.4	34.6
Current	9.7	11.3	10.4	9.5	9.1	7.8
Alcohol intake (%)						
Never	4.3	4.6	4.3	4.3	4.1	4.1
Former	3.4	4.0	3.6	3.3	3.2	2.9
Current	92.0	91.0	91.8	92.2	92.5	92.8
Physical activity (%)						

Low	15.0	15.9	15.2	14.8	14.9	14.2
Moderate	33.0	32.8	33.4	33.2	32.9	32.9
High	32.9	31.2	32.3	33.2	33.5	34.4
BMI (kg/m^2)	27.4 (4.7)	28.0 (5.1)	27.7 (4.8)	27.4 (4.6)	27.1 (4.5)	26.6 (4.4)
SBP (mmHg)	137.6 (18.4)	139.3 (18.7)	138.2 (18.5)	137.5 (18.4)	136.9 (18.3)	136.0 (18.1)
Glucose (mg/dL)	92.2 (22.0)	93.8 (24.8)	92.7 (22.8)	92.0 (21.0)	91.5 (20.9)	90.7 (19.2)
Total cholesterol (mg/dL)	220.3 (43.9)	219.9 (45.3)	220.9 (44.1)	220.8 (43.7)	220.5 (43.2)	219.2 (43.0)
LDL cholesterol (mg/dL)	137.5 (33.4)	137.7 (34.4)	138.2 (33.5)	138.0 (33.3)	137.5 (32.9)	136.2 (32.7)
Triglyceride (mg/dL)[§]	130.9 (96.5)	139.6 (103.0)	136.0 (99.2)	132.0 (96.4)	127.2 (93.8)	118.5 (86.8)
Hypertension (%)	52.9	58.3	54.6	52.7	50.5	47.6
Diabetes (%)	5.8	7.6	6.0	5.6	5.0	4.3
Hyperlipidemia (%)	80.6	82.5	81.7	81.1	79.9	77.3
Prevalent MI (%)	1.6	2.1	1.7	1.5	1.4	1.2
Prevalent stroke (%)	1.4	1.8	1.4	1.4	1.2	1.2
Prevalent CVD (%)	2.9	3.8	2.9	2.8	2.5	2.3

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; LDL, low-density lipoprotein; MI, myocardial infarction; mtDNA-CN, mitochondrial DNA copy number; NO_2, nitrogen dioxide; PM, particulate matter; SBP, systolic blood pressure.

* Median (range); [§]Median (interquartile range).

Table 2. Average difference in mtDNA copy number (95% confidence interval) association with a 10 μg/m^3 increase in PM_{10} and NO_2 and by quintile of air pollutant.

	Linear	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P trend
PM_{10}		11.8, 17.8 (n = 48,167)	17.8, 20.4 (n = 48,255)	20.4, 21.9 (n = 47,900)	21.9, 23.7 (n = 48,124)	23.7, 36.6 (n = 47,942)	
Model 1	-0.107 (-0.108, -0.105)	Reference	-0.039 (-0.049, -0.028)	-0.071 (-0.090, -0.053)	-0.082 (-0.099, -0.065)	-0.110 (-0.124, -0.095)	< 0.001
Model 2	-0.103 (-0.104, -0.101)	Reference	-0.035 (-0.046, -0.025)	-0.059 (-0.074, -0.044)	-0.067 (-0.081, -0.053)	-0.100 (-0.111, -0.089)	< 0.001
Model 3	-0.089 (-0.090, -0.087)	Reference	-0.038 (-0.041, -0.034)	-0.054 (-0.060, -0.049)	-0.059 (-0.064, -0.054)	-0.090 (-0.092, -0.088)	< 0.001
NO_2		7.3, 22.2 (n = 48,237)	22.2, 26.7 (n = 48,126)	26.7, 30.8 (n = 48,226)	30.8, 36.0 (n = 48,099)	36.0, 128.0 (n = 48,131)	
Model 1	-0.023 (-0.023, -0.023)	Reference	-0.009 (-0.018, 0.000)	-0.021 (-0.032, -0.011)	-0.023 (-0.027, -0.020)	-0.058 (-0.073, -0.043)	< 0.001
Model 2	-0.021 (-0.021, -0.020)	Reference	0.007 (-0.003, 0.016)	0.000 (-0.008, 0.008)	-0.002 (-0.007, 0.003)	-0.043 (-0.060, -0.026)	< 0.001
Model 3	-0.018 (-0.018, -0.017)	Reference	0.012 (0.010, 0.014)	0.014 (0.011, 0.017)	0.010 (0.007, 0.014)	-0.033 (-0.047, -0.019)	< 0.001

Abbreviations: mtDNA, mitochondrial DNA; NO_2, nitrogen dioxide; PM, particulate matter.

Model 1: Adjusted for age, sex, race, year of air pollution measurement, and year of blood collection; Model 2: Model 1 + average annual income, education level, smoking, alcohol intake, physical activity, body mass index, and history of hypertension, diabetes, hyperlipidemia, and cardiovascular disease; Model 3: Model 2 + cell counts (red blood cells, neutrophil, lymphocytes, basophils, eosinophils, monocytes, and platelets).

Table 3. Average difference in mtDNA copy number association with a 10 μg/m^3 increase in PM_{10} and NO_2 by lag of measurement between air pollution and mtDNA copy number (0, 1, 2, and 3 years).

	Air pollutant measured in 2007				Air pollutant measured in 2010
mtDNA copy number measured in	**2007 (Lag 0)**	**2008 (Lag 1)**	**2009 (Lag 2)**	**2010 (Lag 3)**	**2010 (Lag 0)**
PM_{10}	Range: 13.0, 32.7 (n = 18,723)	Range: 12.2, 36.5 (n = 67,550)	Range: 11.8, 36.6 (n = 63,980)	Range: 12.8, 36.6 (n = 45,047)	Range: 11.8, 26.2 (n = 45,088)
Model 1	-0.366 (-0.424, -0.307)	-0.086 (-0.115, -0.056)	-0.102 (-0.130, -0.075)	-0.081 (-0.108, -0.053)	-0.077 (-0.124, -0.029)
Model 2	-0.332 (-0.395, -0.269)	-0.073 (-0.105, -0.041)	-0.078 (-0.108, -0.048)	-0.100 (-0.131, -0.070)	-0.078 (-0.129, -0.027)
Model 3	-0.358 (-0.416, -0.301)	-0.069 (-0.098, -0.039)	-0.062 (-0.089, -0.034)	-0.080 (-0.107, -0.052)	-0.053 (-0.101, -0.006)
NO_2	Range: 7.3, 102.0 (n = 18,726)	Range: 7.3, 111.3 (n = 67,671)	Range: 8.3, 128.3 (n = 64,206)	Range: 8.4, 117.9 (n = 45,108)	Range: 12.9, 97.7 (n = 45,108)
Model 1	-0.074 (-0.089, -0.059)	-0.017 (-0.026, -0.008)	-0.027 (-0.034, -0.020)	-0.014 (-0.021, -0.006)	-0.011 (-0.023, 0.001)
Model 2	-0.055 (-0.071, -0.039)	-0.005 (-0.015, 0.004)	-0.023 (-0.031, -0.015)	-0.019 (-0.027, -0.011)	-0.011 (-0.024, 0.002)
Model 3	-0.060 (-0.075, -0.046)	-0.006 (-0.015, 0.003)	-0.019 (-0.026, -0.012)	-0.014 (-0.022, -0.007)	-0.007 (-0.019, 0.005)

Abbreviations: mtDNA, mitochondrial DNA; NO_2, nitrogen dioxide; PM, particulate matter.

Model 1: Adjusted for age, sex, and race; Model 2: Model 1 + average annual income, education level, smoking, alcohol intake, physical activity, body mass index, and history of hypertension, diabetes, hyperlipidemia, and cardiovascular disease; Model 3: Model 2 + cell counts (red blood cells, neutrophil, lymphocytes, basophils, eosinophils, monocytes, and platelets).

Table 4. Average difference in mtDNA copy number associated with a 10 μg/m^3 increase in $PM_{2.5}$, $PM_{2.5-10}$, and NO measured concurrently in 2010 (Lag 0).

$PM_{2.5}$	Range: 8.2, 18.4 (n = 45,088)
Model 1	-0.072 (-0.167, 0.023)
Model 2	-0.050 (-0.153, 0.053)
Model 3	-0.038 (-0.133, 0.057)
$PM_{2.5-10}$	Range: 5.6, 12.2 (n = 45,088)
Model 1	-0.119 (-0.220, -0.019)
Model 2	-0.155 (-0.263, -0.046)
Model 3	-0.127 (-0.226, -0.027)
NO	Range: 19.7, 252.0 (n = 45,108)
Model 1	-0.004 (-0.010, 0.001)
Model 2	-0.003 (-0.010, 0.003)
Model 3	-0.004 (-0.009, 0.002)

Abbreviations: mtDNA, mitochondrial DNA; NO, nitric oxide; PM, particulate matter.
Model 1: Adjusted for age, sex, and race; Model 2: Model 1 + average annual income, education level, smoking, alcohol intake, physical activity, body mass index, and history of hypertension, diabetes, hyperlipidemia, and cardiovascular disease; Model 3: Model 2 + cell counts (red blood cells, neutrophil, lymphocytes, basophils, eosinophils, monocytes, and platelets).

Figure 1.

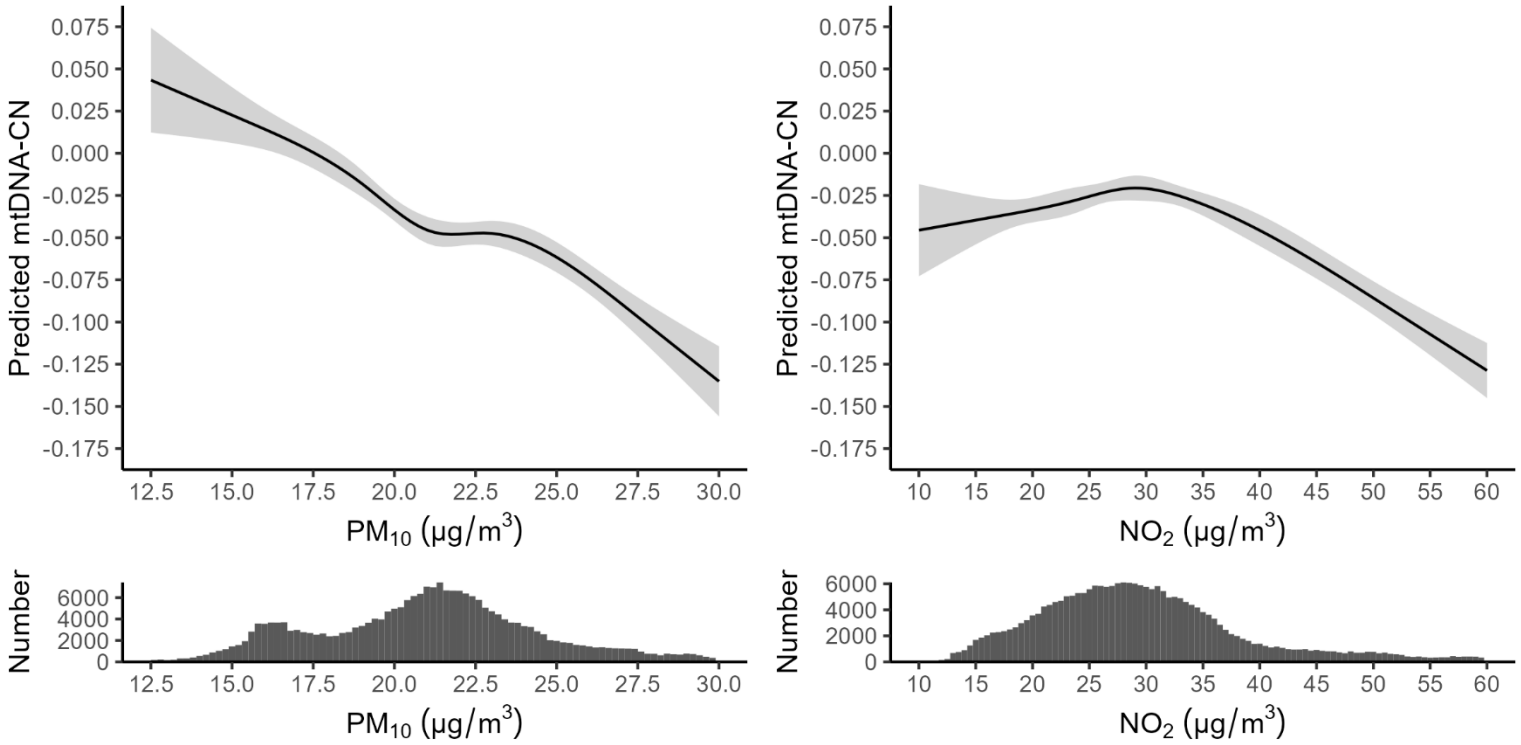
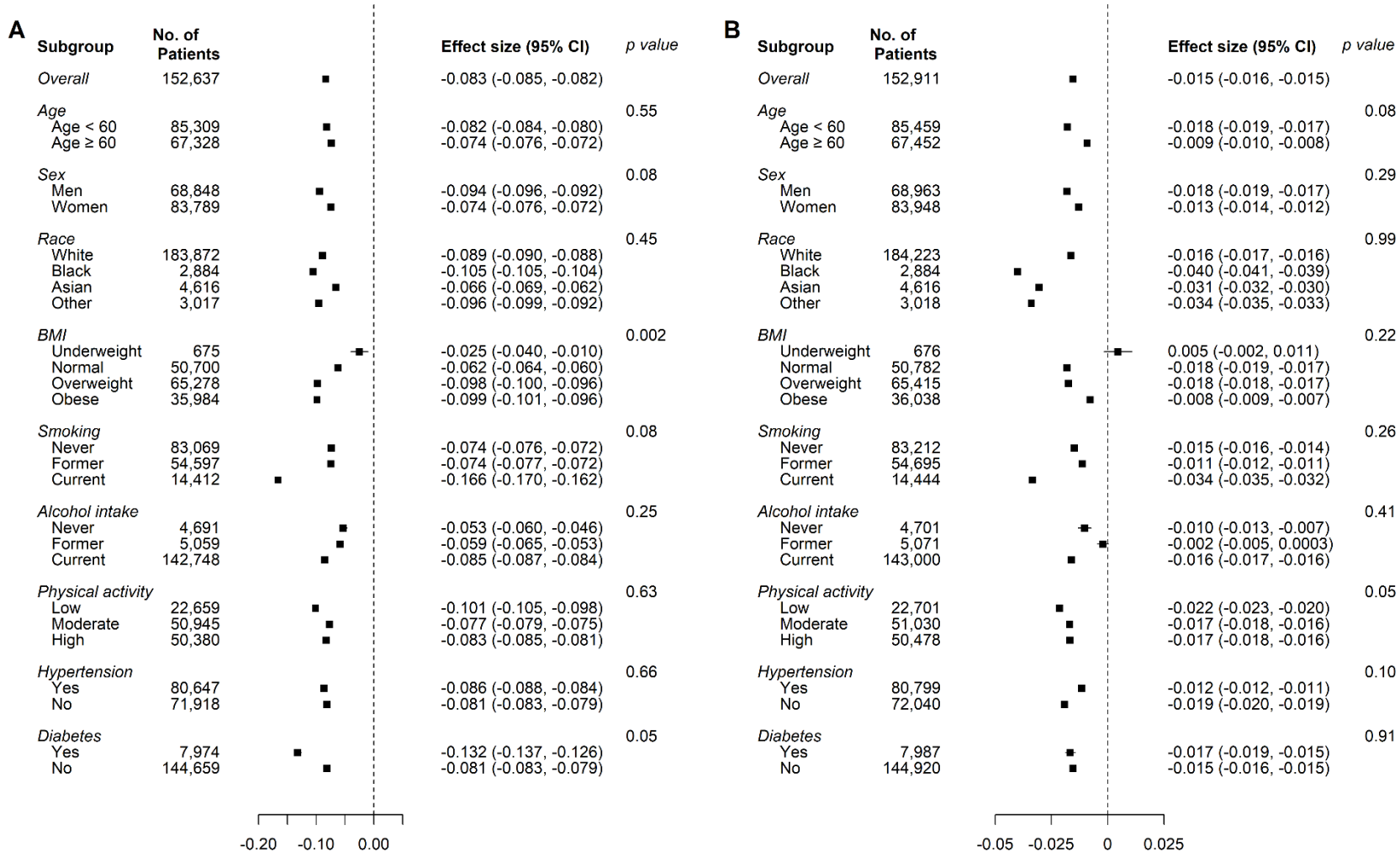

Predicted mtDNA-CN
0.075
0.050
0.025
0.000
-0.025
-0.050
-0.075
-0.100
-0.125
-0.150
-0.175
12.5 15.0 17.5 20.0 22.5 25.0 27.5 30.0
PM10 (μg/m3)
Number
6000
4000
2000
0
12.5 15.0 17.5 20.0 22.5 25.0 27.5 30.0
PM10 (μg/m3)
Predicted mtDNA-CN
0.075
0.050
0.025
0.000
-0.025
-0.050
-0.075
-0.100
-0.125
-0.150
-0.175
10 15 20 25 30 35 40 45 50 55 60
NO2 (μg/m3)
Number
6000
4000
2000
0
10 15 20 25 30 35 40 45 50 55 60
NO2 (μg/m3)

Figure 2.


A
Subgroup
No. of Patients
Effect size (95% CI)
p value
Overall 152,637 -0.083 (-0.085, -0.082)
Age 0.55
Age < 60 85,309 -0.082 (-0.084, -0.080)
Age ≥ 60 67,328 -0.074 (-0.076, -0.072)
Sex 0.08
Men 68,848 -0.094 (-0.096, -0.092)
Women 83,789 -0.074 (-0.076, -0.072)
Race 0.45
White 183,872 -0.089 (-0.090, -0.088)
Black 2,884 -0.105 (-0.105, -0.104)
Asian 4,616 -0.066 (-0.069, -0.062)
Other 3,017 -0.096 (-0.099, -0.092)
BMI 0.002
Underweight 675 -0.025 (-0.040, -0.010)
Normal 50,700 -0.062 (-0.064, -0.060)
Overweight 65,278 -0.098 (-0.100, -0.096)
Obese 35,984 -0.099 (-0.101, -0.096)
Smoking 0.08
Never 83,069 -0.074 (-0.076, -0.072)
Former 54,597 -0.074 (-0.077, -0.072)
Current 14,412 -0.166 (-0.170, -0.162)
Alcohol intake 0.25
Never 4,691 -0.053 (-0.060, -0.046)
Former 5,059 -0.059 (-0.065, -0.053)
Current 142,748 -0.085 (-0.087, -0.084)
Physical activity 0.63
Low 22,659 -0.101 (-0.105, -0.098)
Moderate 50,945 -0.077 (-0.079, -0.075)
High 50,380 -0.083 (-0.085, -0.081)
Hypertension 0.66
Yes 80,647 -0.086 (-0.088, -0.084)
No 71,918 -0.081 (-0.083, -0.079)
Diabetes 0.05
Yes 7,974 -0.132 (-0.137, -0.126)
No 144,659 -0.081 (-0.083, -0.079)
-0.20 -0.10 0.00
B
Subgroup
No. of Patients
Effect size (95% CI)
p value
Overall 152,911 -0.015 (-0.016, -0.015)
Age 0.08
Age < 60 85,459 -0.018 (-0.019, -0.017)
Age ≥ 60 67,452 -0.009 (-0.010, -0.008)
Sex 0.29
Men 68,963 -0.018 (-0.019, -0.017)
Women 83,948 -0.013 (-0.014, -0.012)
Race 0.99
White 184,223 -0.016 (-0.017, -0.016)
Black 2,884 -0.040 (-0.041, -0.039)
Asian 4,616 -0.031 (-0.032, -0.030)
Other 3,018 -0.034 (-0.035, -0.033)
BMI 0.22
Underweight 676 0.005 (-0.002, 0.011)
Normal 50,782 -0.018 (-0.019, -0.017)
Overweight 65,415 -0.018 (-0.018, -0.017)
Obese 36,038 -0.008 (-0.009, -0.007)
Smoking 0.26
Never 83,212 -0.015 (-0.016, -0.014)
Former 54,695 -0.011 (-0.012, -0.011)
Current 14,444 -0.034 (-0.035, -0.032)
Alcohol intake 0.41
Never 4,701 -0.010 (-0.013, -0.007)
Former 5,071 -0.002 (-0.005, 0.0003)
Current 143,000 -0.016 (-0.017, -0.016)
Physical activity 0.05
Low 22,701 -0.022 (-0.023, -0.020)
Moderate 51,030 -0.017 (-0.018, -0.016)
High 50,478 -0.017 (-0.018, -0.016)
Hypertension 0.10
Yes 80,799 -0.012 (-0.012, -0.011)
No 72,040 -0.019 (-0.020, -0.019)
Diabetes 0.91
Yes 7,987 -0.017 (-0.019, -0.015)
No 144,920 -0.015 (-0.016, -0.015)
-0.05 -0.025 0 0.025

SUPPLEMENTARY MATERIALS

Supplementary Table 1. Description of data available on air pollutants and mtDNA copy number.
Supplementary Table 2. Average difference in mtDNA copy number (95% confidence interval) association with a 10 $\mu g/m^3$ increase in PM_{10} and NO_2 and by quintile of air pollutant in the full UK Biobank.

Supplementary Figure 1. Flowchart of study participants.

Supplementary Table 1. Description of data available on air pollutants and mtDNA copy number.

	Years of air pollutants	Year of blood collection	Number of participants	Median (IRQ) of air pollutants ($\mu g/m^3$)
PM_{10}	2007	2007	18,723	
		2008	67,550	
		2009	63,980	
		2010	45,047	
		All years	195,300	21.8 (20.3–23.6)
	2010	2010	45,088	16.2 (15.5–17.1)
	2007, 2010	All years	195,359	21.2 (18.7–23.1)
NO_2	2007	2007	18,726	
		2008	67,671	
		2009	64,206	
		2010	45,108	
		All years	195,711	28.9 (23.7–35.1)
	2010	2010	45,108	27.8 (22.7–32.4)
	2007, 2010	All years	195,711	28.7 (23.4–34.3)
$PM_{2.5}$	2010	2010	45,088	9.9 (9.3–10.4)
$PM_{2.5-10}$	2010	2010	45,088	6.2 (5.9–6.7)
NO	2010	2010	45,108	43 (35.8–50.4)

Supplementary Table 2. Average difference in mtDNA copy number (95% confidence interval) associated with a 10 μg/m^3 increase in PM_{10} and NO_2 and by quintile of air pollutant in the full UK Biobank.

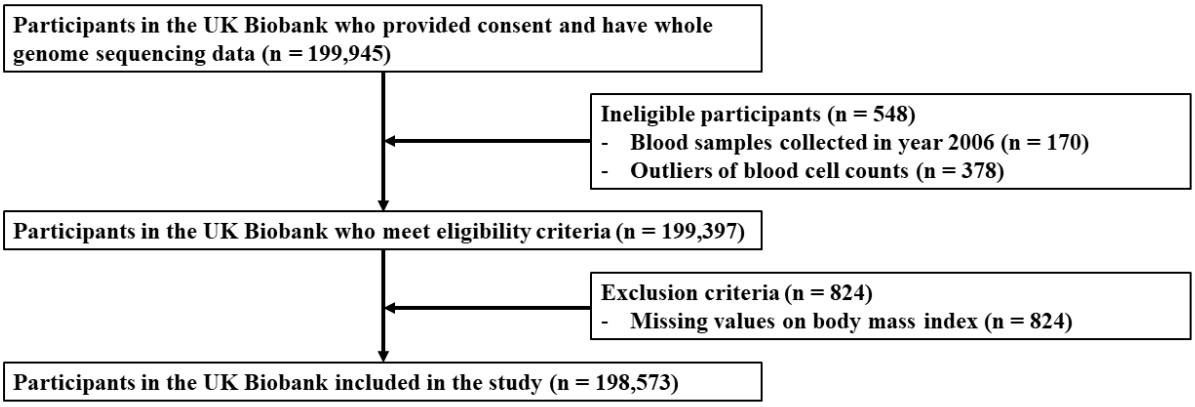
	Linear	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P trend
PM_{10}		11.8, 18.3 (n = 87,306)	18.3, 20.5 (n = 87,437)	20.5, 21.9 (n = 87,259)	21.9, 23.6 (n = 86,956)	23.6, 36.6 (n = 86,766)	
Model 1	-0.084 (-0.085, -0.083)	Reference	-0.026 (-0.038, -0.015)	-0.054 (-0.065, -0.042)	-0.054 (-0.066, -0.042)	-0.071 (-0.083, -0.059)	< 0.001
Model 2	-0.079 (-0.080, -0.078)	Reference	-0.024 (-0.036, -0.011)	-0.048 (-0.060, -0.035)	-0.045 (-0.058, -0.032)	-0.064 (-0.077, -0.051)	< 0.001
Model 3	-0.068 (-0.069, -0.067)	Reference	-0.024 (-0.036, -0.012)	-0.039 (-0.051, -0.027)	-0.035 (-0.047, -0.023)	-0.053 (-0.066, -0.041)	< 0.001
NO_2		7.0, 21.9 (n = 87,336)	21.9, 26.4 (n = 87,488)	26.4, 30.3 (n = 87,192)	30.3, 35.4 (n = 87,385)	35.4, 138.0 (n = 87,207)	
Model 1	-0.024 (-0.024, -0.023)	Reference	-0.013 (-0.022, -0.004)	-0.028 (-0.037, -0.018)	-0.015 (-0.024, -0.005)	-0.058 (-0.068, -0.048)	< 0.001
Model 2	-0.021 (-0.021, -0.020)	Reference	-0.006 (-0.016, 0.005)	-0.014 (-0.024, -0.004)	0.004 (-0.006, 0.014)	-0.046 (-0.057, -0.035)	< 0.001
Model 3	-0.019 (-0.019, -0.018)	Reference	0.000 (-0.009, 0.010)	-0.003 (-0.013, 0.006)	0.013 (0.004, 0.023)	-0.036 (-0.041, -0.026)	< 0.001

Abbreviations: mtDNA, mitochondrial DNA; NO_2, nitrogen dioxide; PM, particulate matter.

In this analysis, mtDNA copy number was derived using whole exome sequencing and mitochondrial probe intensities to the full UK Biobank cohort.

Model 1: Adjusted for age, sex, race, year of air pollution measurement, and year of blood collection; Model 2: Model 1 + average annual income, education level, smoking, alcohol intake, physical activity, body mass index, and history of hypertension, diabetes, hyperlipidemia, and cardiovascular disease; Model 3: Model 2 + cell counts (red blood cells, neutrophil, lymphocytes, basophils, eosinophils, monocytes, and platelets).

Supplementary Figure 1. Flowchart of study participants.



Chapter 3

Short-term effects of ambient temperature and seasonality on mitochondrial DNA copy number

ABSTRACT

Background: Ambient temperature is associated with a wide range of health outcomes. Mitochondria is essential in regulating body temperature, however, the association of temperature and mtDNA copy number (mtDNA-CN), particularly in relation to metabolites, has not been well studied.

Objective: We evaluated the relationship between short-term exposure to ambient temperature with mtDNA-CN in association with metabolites in 374,397 adult men and women from the UK Biobank study.

Methods: Daily maximum (Tmax) and minimum (Tmin) ambient temperatures of 1 km resolution across the UK from 2006 to 2010 were linked to participants' residential address. mtDNA-CN was generated using mitochondrial single nucleotide polymorphisms probe intensities based on the residuals derived from whole exome sequencing and standardized (mean 0 and standard deviation [SD] 1). We used linear regression models and distributed non-linear lag models to estimate the cross-sectional and cumulative associations of different ambient temperature metrics and mtDNA-CN across 7 days. We also evaluated the proportion in the association of temperature and mtDNA-CN explained by metabolites.

Results: The mean (SD) Tmax and Tmin were 14.2 (5.8)°C and 6.6 (4.9)°C, respectively. The average difference in mtDNA-CN by 10°C increase in $Tmax_0$ was -0.07 (95% confidence interval [CI] -0.08, -0.06) and the results were similar across different temperature metrics. The inverse association between ambient temperature and mtDNA-CN persisted over 7 days with the strongest association at lag 0. The associations of Tmax0 and $Tmax_0 – Tmin_0$ with mtDNA-CN was most explained by metabolites produced during glucose and lipid metabolism, including pyruvate, lactate, and ketone bodies.

Conclusions: In this population-based study, short-term exposure to ambient temperature up to 7 days was inversely associated with mtDNA-CN, and it was mostly explained by metabolites involved in glucose and free fatty acid metabolism. These findings suggest an active involvement of mitochondria in thermoregulation and a potential mechanism of various adverse health effects associated with ambient temperature.

INTRODUCTION

Ambient temperature is a major determinant of health outcomes, including all-cause mortality, cardiovascular and respiratory mortality and morbidity, hospitalization, and emergency department visits.[1-7] Variations in temperature are responsible for over 5 million deaths annually, corresponding to 9·4% of all deaths.[1] Moreover, climate change is projected to increase global temperature and to result in more frequent extreme weather events and temperatures,[8] with a subsequent increase in temperature-related excess mortality.[9,10]

Mitochondria are essential in energy production and regulation of body temperature through heat generation (thermogenesis).[11] Cold exposure activates the sympathetic nervous system which triggers non-shivering and shivering thermogenesis.[12] Non-shivering thermogenesis occurs in brown or beige adipose tissue, mainly through heat generation in mitochondria.[13] When this is not sufficient, shivering occurs, which involves involuntary bursts of or continuous movement of proximal skeletal muscles. Heat stress, however, induces the generation of reactive oxygen species, mitochondrial protein aggregation, and mitochondrial damage.[14,15]

Each cell contains 10s to 1000s of mitochondria, and each mitochondrion contains 2–10 copies of mitochondrial DNA (mtDNA) encoding 13 genes involved in oxidative phosphorylation.[11,16] mtDNA copy number (mtDNA-CN) changes in response to energy demands, metabolic changes, and environmental stressors.[16-18] mtDNA, however, is susceptible to oxidative damage, which results in mitochondrial dysfunction and reduced levels of mtDNA-CN,[19] a biomarker that can be readily measured in peripheral blood and is associated with increased all-cause mortality,[20] cardiovascular disease,[21,22] and respiratory disease.[23]

Despite the importance of mitochondria in maintaining core body temperature, the association of ambient temperature and mtDNA-CN has been explored only in two studies.[24,25] A study in China found that the annual average temperature was inversely associated with population-level average mtDNA-CN, but the association was not evaluated on an individual level and the results could be attributed to differences across ethnic groups.[24] In contrast, the VA Normative Aging Study found no association between temperature and mtDNA-CN in elderly men.[25]

We therefore evaluated the association between short-term exposure to ambient temperature with mtDNA-CN in over 370,000 adult men and women from the UK Biobank study. In addition, we used metabolomics data in a subsample of approximately 90,000 participants in the UK Biobank to evaluate if the association between ambient temperature and mtDNA-CN could be related to changes in metabolite levels.

METHODS

Study population

The UK biobank is a prospective cohort study of approximately 500,000 adults 40 to 69 years of age recruited across the United Kingdom (UK) from 2006 to 2010.[26] Among 502,413 participants who provided consent to the study, we excluded individuals with genetically related participants in the study (n = 81,222), who reported their race/ethnicity as non-White (n = 29,797), who had blood samples collected in 2006 (as metabolomic profiles were not measured in that year; n = 8,951), and who had abnormal blood cell counts (n = 1,119; **Supplementary Figure 1**). We further excluded participants with missing information on mtDNA-CN (n = 5,521), latitude and longitude of residence (n = 3,702), or daily temperature (n = 5,554). The final study population was 374,397 (172,005 men and 202,392 women). Analyses for

temperature-metabolite associations were further restricted to participants with information on the relevant metabolites (**Supplementary Table 1**).

All participants provided written informed consent prior to the study. The scientific protocol and operational procedures of the UK Biobank study was approved by the North West – Haydock Research Ethics Committee in the UK.

Measurement of ambient temperature

Daily maximum (Tmax) and minimum (Tmin) ambient temperatures across the UK from 2006 to 2010 were obtained from the HadUK-Grid provided by the Met Office Hadley Center for Climate Science and Services (https://www.metoffice.gov.uk/). HadUK-Grid is a publicly available data set of gridded land surface climate variables which interpolates in situ land surface station data to a regular grid using inverse-distance weighted averaging to various temporal (daily, monthly, seasonal, and yearly) and spatial (1 km, 5 km, 12 km, and 60 km) resolutions.[27] To reflect individual exposure to ambient temperature, we used daily temperature variables from the 1 km resolution grid in HadUK-Grid. For each participant, we used the temperature values closest in distance to their residential address at the time of the UK Biobank blood sampling, geocoded rounded to a 1 km distance.

We obtained daily Tmax and Tmin from the day of the blood collection visit and up to 6 days prior to the day of blood collection (lag 0 to lag 6 days). For each day, we also calculated the daily average temperature (Tavg) as the average between Tmax and Tmin and the within-day temperature range (Tmax–Tmin) on the same day to reflect temperature variability.

Measurement of mitochondrial DNA copy number

Details on the generation of mtDNA-CN are described in the **Supplementary Material**.[28] In brief, we downloaded the initially released whole exome sequencing (WES) data on 49,997 individuals from the UK Biobank repository and generated residuals from a linear regression model of the mitochondrial chromosome read counts adjusted for total DNA, potential WES technical artifacts, age, and sex. We then used these residuals and the mitochondrial single nucleotide polymorphisms (SNP) probe intensities to estimate mtDNA-CN in all UK Biobank participants. First, all probe intensities were rank-transformed to reduce the impact of outliers. Then, to correct for potential artifacts and/or batch effects, we generated 250 principal components (PCs) from autosomal nuclear probes by randomly sampling 5% of probes (n ~ 19,500 probes) from odd chromosomes that were on both UKBelieve and Axiom arrays (rsvd package in R, version 1.0.3). For each array type, all mitochondrial SNP probes (UKBelieve, n = 181; Axiom, n = 244) and 250 PCs were regressed on the residuals of the linear regression model using the WES results. Coefficients from each model were subsequently used for prediction to the rest of the UK Biobank population. Participants who reported their ethnicity as non-White, participants with related individuals in the study, and participants who had abnormal cell counts were excluded from this process. The predicted values were then adjusted for age and sex and were standardized by subtracting the mean and dividing by the standard deviation (SD).

Measurement of leukocyte telomere length

We used leukocyte telomere length as a negative control outcome,[29] as telomere length is unlikely to change rapidly by short-term changes in ambient temperature. Leukocyte telomere length was measured in 473,999 UK Biobank participants using the multiplex quantitative polymerase chain reaction (PCR) method from peripheral blood leukocytes at the University of

Leicester, blinded to phenotype information. Leukocyte telomere length was derived by comparing the amplification of a telomere length PCR product (T) against a PCR product of a reference single copy gene (S, Hgb) to produce a T/S ratio.[30] T and S were calculated relative to a calibrator sample (pooled DNA from 20 individuals) that was included in every run using the Rotor-Gene comparative quantification software (Qiagen). T/S was adjusted for technical variation in three stages. For analysis, the adjusted T/S ratios were log-transformed and standardized (UK Biobank data field 22192).

Measurement of metabolites

In the UK Biobank, 249 metabolic biomarkers spanning multiple metabolic pathways were measured in a randomly selected sample of approximately 120,000 participants. Metabolomic biomarkers were measured from EDTA plasma samples using a high-throughput nuclear magnetic resonance (NMR)-based metabolic biomarkers generated by Nightingale Health Ltd. Details of the measurements can be found in the UK Biobank online showcase (http://ukbiobank.ac.uk). The measurements included 165 absolute concentration measures of low molecular weight metabolites, such as amino acids, ketone bodies, and glycolysis metabolites, and biomarkers of lipid metabolism, 81 ratio measures, and 3 average diameters (nm) of lipids.

Of the 374,397 study participants with mtDNA-CN measurements, there were 90,191 participants with information on metabolites (**Supplementary Table 1**). For analysis, we selected the 165 metabolites measured in absolute concentrations (**Supplementary Table 2**) and generated a ratio of lactate to pyruvate (lactate / pyruvate) as a biomarker of mitochondrial

function.[31] We $\log_2$-transformed metabolite values. For 4 metabolites with zero values (glycine, beta-hydroxybutyrate, acetate, acetoacetate), we added 1 before $\log_2$-transformation.

Measurement of other covariates

Study participants completed a touchscreen questionnaire and a computer-assisted interview and provided blood samples at one of the 22 assessment centers located throughout the UK. Age, sex, ethnicity (White, Asian/Asian British, Black/Black British, Mixed, Chinese, or other ethnic group), average total household income, education, smoking status (never, former, and current smokers), and alcohol intake (never, former, and current drinkers) were self-reported. Physical activity was measured using the adapted International Physical Activity Questionnaire (IPAQ) Short Form and categorized as low, moderate, and high physical activity based on the IPAQ guideline.[32]

Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Hypertension was defined as a self-reported physician's diagnosis of hypertension, a self-reported use of antihypertensive medication, or a measured blood pressure ≥140/90 mmHg.

Details of laboratory measurements can be found in the UK Biobank online showcase (http://ukbiobank.ac.uk). Diabetes was defined as a self-reported physician's diagnosis of diabetes, a self-reported use of antidiabetic medication, or a measured HbA1c ≥6.5%. Hyperlipidemia was defined as a self-reported use of cholesterol-lowering medication, or a measured total cholesterol ≥200 mg/dl or triglycerides ≥150 mg/dl. Estimated glomerular filtration rate (GFR) was calculated using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[33]

Statistical analysis

Daily ambient temperature, seasonal trends, and mtDNA-CN

We evaluated the association between ambient temperature on the day of blood sampling (lag 0) and mtDNA-CN using linear regression models, first with temperature as a linear term, and then modeled as restricted cubic splines with knots at the 5th, 27.5th, 50th, 72.5th, and 95th percentiles of each temperature metric to evaluate non-linear associations. For each temperature metric, we used models adjusted for age, sex, year of blood collection, center, seasonality using periodic restricted cubic splines with 5 knots,[34] and hour of blood collection using restricted cubic splines with 3 knots. When within-day temperature range was used, Tavg was added to this model, and we evaluated the interaction of the within-day temperature range and Tavg. We further evaluated these associations by age (<50, 50–60, and ≥60 years) and sex subgroups.

To evaluate seasonal trends, we also used linear regression models first with month of blood sample collection as an indicator variable, and then using periodic restricted cubic splines (peRiodicCS package in R) to simultaneously account for non-linear and cyclic trends of mtDNA-CN throughout the year.[34] Each model was adjusted for age, sex, center, year and hour of blood collection, and average temperature of the day of blood collection ($Tavg_0$).

Distributed lag non-linear models for temperature and mtDNA-CN

In addition to evaluating the effect of temperature on the day of blood collection, we evaluated the potential effect of temperature during the 6 days prior to blood collection on mtDNA-CN using a distributed lag non-linear model (dlnm package in R).[35,36] Distributed lag non-linear models allow for simultaneous description of the temperature-mtDNA-CN relationship and the lag-mtDNA-CN relationship, that is, the effects occurring after a given

exposure. Specifically, we estimated the overall association between temperature and mtDNA-CN cumulating over 7 days (lag 0 – 6 days) using a linear regression model with a cross-basis function using a restricted cubic spline function of temperature with 5 knots placed at equally spaced values to model the temperature-mtDNA-CN relationship, and an indicator variable for each lag day to model lag-response relationship. All models were adjusted for age, sex, center, year, seasonality, and hour of blood collection, and the models for within-day temperature range were also adjusted for daily average temperature. We, then, estimated the difference in mtDNA cumulated over 7 days comparing the 97.5^{th} to the 2.5^{th} percentiles of each temperature metric.

Daily ambient temperature, metabolites, and mtDNA-CN association

We evaluated if differences in metabolite levels were related to the association between temperature and mtDNA-CN. We initially evaluated the proportion of the association between temperature and mtDNA-CN explained by individual metabolites (proportion explained). To do so, we selected the metabolites that were associated with temperature in models adjusted for age, sex, center, year, seasonality, and hour of blood collection with a Benjamini-Hochberg False discovery rate (FDR)-adjusted $P < 0.1$ for further analysis. Then, we estimated the overall effect of temperature metrics ($\beta_{overall}$) in models adjusted for age, sex, center, year, seasonality, and hour of blood collection, and the adjusted effects ($\beta_{adjusted}$) in models further adjusted for the selected individual metabolites. For each metabolite, the proportion explained was calculated as the difference between the overall and adjusted effects divided by the overall effect ($\beta_{overall} - \beta_{adjusted} / \beta_{overall}$) using the mediation package in R.[37] 95% confidence intervals were obtained by bootstrapping (1,000 resamples) and P values were corrected for multiple testing by applying

Benjamini-Hochberg correction with a false positive threshold set at 5%.[38] All analyses using $Tmax_0$–$Tmin_0$ were further adjusted for $Tavg_0$.

In addition to evaluating individual metabolites, we evaluated metabolomic profiles obtained by independent component analysis (ICA)[39,40] to reduce the dimension of the metabolite data into 10 independent components (ICs). ICA maximizes the independence between components of non-Gaussian variables and each metabolite is assigned a weight of its contribution to each ICA component. We then estimated the proportion of the association between temperature and mtDNA-CN explained by each of the ICs as well as the proportion explained by all 10 ICs simultaneously.

Finally, we evaluated whether the temperature-mtDNA-CN association could explain the association between temperature and metabolite levels. We first estimated the overall association between temperature and individual metabolites using the limma method ("limma" package in R), which provides an empirical Bayes estimator of moderated residual variances from individual metabolite-wise linear models, adjusting for age, sex, center, year, seasonality, and hour of blood collection.[41,42] We adjusted the P values for multiple testing using the Benjamini-Hochberg procedure with an FDR of 5% and selected metabolites with an adjusted $P < 0.1$. We then run the limma model again further adjusted for mtDNA-CN and calculated the proportion explained as $(\beta_{overall} - \beta_{adjusted} / \beta_{overall})$ and derived 95% confidence intervals by bootstrap.

RESULTS

The mean age (standard deviation [SD]) of 347,397 study participants (172,005 men and 202,392 women) was 56.8 (8.0) years (**Table 1**). Compared to participants in the lower quintiles of mtDNA-CN, those in the higher quintiles of mtDNA-CN were more likely to have higher average annual income, to be never or former smokers and physically active, and to have lower

BMI, systolic blood pressure, serum glucose, total cholesterol, LDL cholesterol, and triglyceride levels, higher estimated GFR levels, and lower prevalence of hypertension, diabetes, and hyperlipidemia. The mean daily maximum and minimum temperatures were 14.2 (5.8) °C and 6.6 (4.9) °C, respectively (**Table 1, Supplementary Figures 2–4**).

In the fully adjusted model, a 10°C increase in $Tmax_0$ was associated with a difference in mtDNA-CN of -0.07 (95% CI -0.08, -0.06, **Table 2**). The associations were similar for $Tmin_0$, $Tavg_0$, and $Tmax_0$–$Tmin_0$. The inverse trend for daily temperature with mtDNA-CN was also evident in spline regression analysis (**Figure 1**). The association between $Tmax_0$–$Tmin_0$ and mtDNA-CN was attenuated at a higher daily average temperature (P for interaction = 0.004; **Supplementary Figure 5**). In addition, mtDNA-CN was lower in warmer months and higher in colder months (**Supplementary Table 3, Figure 1**). This seasonal trend persisted even after adjusting for $Tavg_0$.

In subgroup analyses, the association of temperature with mtDNA-CN was stronger in younger participants. The adjusted difference in mtDNA-CN associated with a 10°C increase in $Tmax_0$ were -0.09 (-0.10, -0.07), -0.07 (-0.08, -0.06), -0.06 (-0.07, -0.05) in participants <50, 50–60, and ≥60 years, respectively (**Figure 2, Supplementary Table 4**). The inverse association between temperature and mtDNA-CN was attenuated in participants ≥60 years across all ambient temperature metrics. There were, however, no differences in the association by sex.

The inverse association between ambient temperature and mtDNA-CN was the strongest on the day of blood collection, appeared to be compensated on lag 1, and disappeared by lag 4 or 5 (**Supplementary Figure 7**). Comparing the 97.5^{th} percentile to the 2.5^{th} percentile of each temperature distribution, the adjusted differences in mtDNA-CN cumulated over 7 days of exposure were -0.21 (-0.24, -0.18), -0.15 (-0.18, -0.12), -0.18 (-0.21, -0.15), and -0.14 (-0.18, -

0.10) for Tmax, Tmin, Tavg, and Tmax–Tmin, respectively (**Figure 2** and **Table 3**). There was no difference in leukocyte telomere length between the 97.5th and 2.5th percentiles of each temperature metric (**Supplementary Table 5**).

There were 40 and 35 metabolites associated with $Tmax_0$ and $Tmax_0$–$Tmin_0$, respectively, but no metabolites were associated with $Tmin_0$ and $Tavg_0$. The association of temperature and mtDNA-CN was explained primarily by metabolites involved in glucose and lipid metabolism (**Table 4, Figure 4**). For instance, pyruvate, lactate, and lactate/pyruvate ratio explained 67.52% (43.63, 144.79), 65.77% (42.02, 139.99), and 8.09% (4.79, 17.67) of the association between $Tmax_0$ and mtDNA-CN. Similarly, IC4, which has the largest weights coming from lactate, pyruvate, and lactate/pyruvate ratio, explained 60.18% (38.82, 128.85) of the association between $Tmax_0$ and mtDNA-CN (**Supplementary Table 6**). The association between $Tmax_0$–$Tmin_0$ and mtDNA-CN was primarily explained by pyruvate, lactate, and HDL-related metabolites (**Figure 4, Supplementary Tables 7 and 8**). None of the metabolites explored explained the associations of $Tmin_0$ and $Tavg_0$ with mtDNA-CN.

The proportion of the association between $Tmax_0$ and individual metabolites explained by mtDNA-CN was generally small (**Supplementary Table 9, Supplementary Figure 8**). mtDNA-CN explained the associations between $Tmax_0$ and metabolites related to IDL, LDL, VLDL, and HDL cholesterol, lactate, pyruvate, lactate/pyruvate ratio, and ketone bodies, and the associations between $Tmax_0$–$Tmin_0$ and free fatty acids, HDL-related metabolites, lactate, pyruvate, and lactate/pyruvate ratio (**Supplementary Table 10, Supplementary Figure 8**). mtDNA-CN, however, did not explain the associations of $Tmin_0$ and $Tavg_0$ with metabolites.

DISCUSSION

In this study, we found that short-term exposure to higher ambient temperature up to 7 days was inversely associated with mtDNA-CN. The association between temperature and mtDNA-CN was largely explained by metabolites produced during glucose and lipid metabolism. These findings may explain the potential mechanism of various adverse health effects associated with ambient temperature.

In our study, short-term ambient temperature was inversely associated with mtDNA-CN, with the lowest average mtDNA-CN in warmer months and the highest in colder months. Unfortunately, there are few epidemiologic studies evaluating the association between temperature and mtDNA-CN. In a study of 27 ethnic populations across different regions in China, populations exposed to lower annual average temperature were had higher mtDNA-CN.[24] However, they were evaluating the adaptation of mitochondria to long-term temperature exposure and the differences in mtDNA-CN may be due to inherent differences in population characteristics by region or ethnicity. In addition, they did not account for potential confounders such as age and sex. In another study of elderly men, they evaluated the role of mtDNA-CN in the association between ambient temperature and cognitive function and found no relationship between temperature and mtDNA-CN.[25] However, participants were from a single region which may have reduced variability in temperature. Moreover, the association may not have been observed because the study used ambient temperature from the prior day (lag 1) for analysis when temperature at lag 0 has the strongest association and study population was of elderly men with a mean age of 75.5 years and their biological response may be less adaptive to changes in temperature due to aging.[43,44]

On the other hand, in an experimental study in the brown adipose tissue of mice, cold exposure increased more outer mitochondrial membrane proteins relative to mitochondrial

matrix proteins, suggesting increase in mitochondrial number.[45] In addition, administration of L-carnitine, which potentially regulates thermogenesis in brown adipose tissue, to goat brown adipocyte increased mtDNA content in a dose-response manner.[46]

Mitochondria actively reacts to change in ambient temperature as it is the central organelle for energy production, regulating metabolism, and maintaining body temperature.[11,12,16] Sympathetic nervous system stimulation upon cold exposure triggers shivering and non-shivering thermogenesis to maintain body temperature.[12] Shivering thermogenesis involves the involuntary movement of proximal skeletal muscles to generate energy and can generate 4–5 times heat than normal condition.[47,48] Skeletal muscle cells are particularly rich in mitochondria (~1000–3000 copies) even in thermoneutral condition.[19] In experimental studies, exposure to cold induced expression of peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α), a key regulator of mitochondrial biogenesis, in the skeletal muscle.[49,50] An increase in measured mtDNA-CN in the presence of mitochondrial biogenesis implies adequate adaptation to increased energy demand.[19]

Non-shivering thermogenesis prompts the activation of mitochondria-rich brown and beige adipose tissue through β_3-adrenergic stimulation and generates 10–15% of heat.[12,48] Uncoupling protein 1 (UCP1) is uniquely localized in the mitochondrial inner membrane of brown adipose tissue and is key mechanism of heat generation by uncoupling the mitochondrial respiration from ATP synthesis.[12] UCP1 expression and PGC-1α in the brown adipose tissue increase by dramatically after cold exposure underscoring the role of mitochondria in increasing energy expenditure to maintain body temperature.[13]

Heat exposure, on the other hand, stimulates the reactive oxygen species (ROS) production.[14] mtDNA is particularly vulnerable to damage by oxidative stress due to limited

repair capacity and absence of protective histones.[51] Moreover, increases in metabolic demand, vasodilation to dissipate heat, increased cardiac output, and visceral hypoperfusion lead to systemic oxidative stress, further leading to mtDNA damage.[52,53] As such, heat stress was associated with higher mtDNA lesions and methylation.[52,54] Lower mtDNA-CN in the presence of DNA damage suggests increased ROS production and antioxidant defenses.[19]

In subgroup analysis, the association between temperature and mtDNA-CN was attenuated with older age. Adequate responses to cold, such as vasoconstriction and reduction in metabolic heat production, and brown and beige adipose tissues diminish with age.[43,55] In addition, reduction in sweating, skin blood flow, capacity to increase cardiac output, and redistribution of heat from deep visceral circulation, and increase in stored body heat makes older adults more vulnerable to heat.[43,44] On the other hand, there was no difference in the temperature and mtDNA associations by sex. Other studies have also shown that shivering sensitivity and brown adipose distribution does not differ by men and women of similar anthropometric and body composition.[56,57]

We found the ambient temperature and mtDNA-CN association to be consistent across daily maximum, minimum, and average temperature metrics. Although slightly attenuated, we observed a similar association between within-day temperature range and mtDNA-CN. Within-day (diurnal) temperature range is associated with increased all-cause mortality, cardiovascular and respiratory mortality, and hospital admissions and emergency room visits.[58,59] Similar to our results, however, studies on mortality have generally reported smaller associations for within-day temperature range than absolute temperature.[60,61] The potential mechanisms of adverse health effects due to within-day temperature range is not well understood but an increase in blood pressure and heart rate, which reduces cardiovascular stability, systemic inflammation, and

delayed physiologic and behavioral responses to sudden temperature changes have been suggested.[62-64]

In our study, exposure to higher temperatures was associated with lower mtDNA-CN over a 7-day period. Furthermore, the difference in mtDNA-CN per 10°C increase in temperature was greater for the overall association than when using only the temperature of blood collection (lag 0), suggesting cumulative effect across several days. We, also, found that the associations were strongest at lag 0, reversed at lag 1, and progressively declined with increasing lag with no associations by the following week (lag 7). It is plausible that any change in mtDNA-CN by temperature was compensated on the following day. However, further studies are needed to better understand the dynamic nature of the mtDNA-CN in response to ambient temperature. Moreover, although studies on ambient temperature and mortality have reported distinct critical time windows for heat (an acute effect up to 1 day) and cold (a delayed effect, up to 2–3 weeks),[65-67] we did not find these differences with mtDNA-CN, which also reflects its rapid and dynamic thermoregulatory response to a changing temperature environment. In fact, the Ucp1 mRNA expression in brown adipose tissue increases by 100-fold within 12 hours after cold exposure and regresses to the basal level within a day after warming.[68] UCP1 protein expression changes more gradually, achieving a comparable degree of change in 5–7 days.[68]

Interestingly, we found a range of temperatures of 8–10°C around the median values of each temperature metric where there was no difference in the mtDNA-CN. This range also corresponded to the non-linear range in the association of temperature at lag 0 with mtDNA-CN. These findings support the presence of long-term adaptation to temperature and the geographical and population differences in minimum mortality temperature, an indicator of climate adaptation.[69]

We also found that metabolites involved in glucose and free fatty acid metabolism explained much of the temperature– mtDNA-CN association. In a cohort of elderly men, temperature exposure 30 days ago altered metabolites involved in glycolysis/gluconeogenesis, pyruvate, pyrimidine, and propanoate metabolisms.[70] This study, however, did not find any associations for short-term (24 hours or 1 week) exposure to temperature, which may be due to relatively small sample size and a study population mainly of older aged men. In another study of two European cohorts, higher temperature 1 week ago was associated with elevated VLDL, intermediate-density lipoprotein (IDL), and HDL cholesterols, and amino acids (leucine, phenylalanine, and tyrosine), and lower unsaturated fatty acids. The metabolites identified in this study were distinct from the metabolites that were found to be associated with temperature in our study, in which pyruvate, lactate, ketone bodies, LDL cholesterol and its components were positively associated and HDL and VDLD cholesterols and their components were inversely associated with temperature. Currently, there is no epidemiological study linking the associations of temperature and mtDNA-CN with metabolites, and therefore, further epidemiological studies are needed to replicate these findings.

Nonetheless, there are biological mechanisms to explain our findings. First, during shivering thermogenesis, skeletal muscles use lipid in the form of fatty acids as their main source of energy production at low intensity of shivering and glucose derived from muscle glycogen and plasma glucose at increased intensity.[47] Second, during non-shivering thermogenesis, brown adipose tissue accelerates the uptake of glucose and lipid and initiates free fatty acid oxidation for heat production.[12,71] In one study, swimming in ice-cold river (2.6°C) for 10–15 minutes activated amino acid metabolism pathways and also increased pyruvate and lactate levels in plasma.[72] In another study, non-shivering cold exposure (20°C) increased plasma free fatty acid

and glycerol increased shortly after the exposure but decreased triglycerides and VLDL cholesterol 1 day after the exposure.[71] These findings suggest the importance of glucose and fatty acid metabolism in thermoregulation and that metabolites involved in thermoregulation could be highly dynamic by degree and duration of cold exposure and at different periods after the exposure. On the other hand, the associations of temperature and metabolites were explained by mtDNA-CN to a smaller extent. Taken together, it is possible that cold exposure induces both mitochondrial biogenesis[49,50] and mtDNA replication simultaneously to generate heat efficiently and maintain core body temperature.

This is the first large-scale population study to evaluate the association between ambient temperature and mtDNA-CN across a wide range of temperature distribution. In addition, we used temperature measurement of a high temporal and spatial resolution which allowed the evaluation of short-term and lagged relationship between temperature and mtDNA-CN with precision. Moreover, the incorporation of metabolomics data and evaluating the proportion explained by each metabolites helps us better understand the physiological response of mitochondria to ambient temperature.

This study also has several limitations. First, it is a cross-sectional study with a single measurement of mtDNA-CN and, therefore, we were unable to evaluate the change in mtDNA-CN after exposure. A longitudinal study with repeated measurement of mtDNA-CN at a daily or sub-daily intervals would allow us to identify the most critical time window of temperature exposure on the change of mtDNA-CN. Second, the study was done in a single country from a single climate zone and included only White individuals and may have limited generalizability. Studies including a diverse geographic region, climate zone, and populations are needed to further support our findings. Third, although we had temperature data of high temporal and

spatial resolution, the values came from prediction models, Therefore, we were unable to account for personal exposure levels or behavioral patterns to accommodate ambient temperature. The measurement error in temperature exposure, however, is unrelated to mtDNA-CN levels and would have attenuated the association. Lastly, plasma levels of metabolites do not reflect cellular or subcellular levels metabolic response. A more complete list of metabolites involved in carbohydrate, lipid, and amino acid metabolism, temperature regulation and mitochondrial function will, however, provide a more comprehensive understanding of the complex physiological response to ambient temperature.

CONCLUSIONS

In this large-scale population-based study, short-term exposure to ambient temperature up to 7 days was inversely associated with mtDNA-CN and the association was attenuated in older age. In addition, metabolites involved in glucose and free fatty acid metabolism explained much of the temperature– mtDNA-CN association. This study supports the active involvement of mitochondria in thermoregulation and may explain the potential mechanism of various adverse health effects associated with ambient temperature.

ACKNOWLEDGEMENTS

None.

SOURCES OF FUNDING

This work was supported by the US National Institutes of Health grants R01HL131573 and R01HL144569. This research was also conducted using the UK Biobank Resource under Application Number 17731.

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FIGURE LEGENDS

Figure 1. Average mtDNA copy numbers by ambient temperature.

The curves represent estimated mtDNA copy numbers (solid line) and their 95% confidence intervals (area in light blue) by each ambient temperature metric based on fully adjusted regression models using restricted cubic splines with knots at the 5^{th}, 27.5^{th}, 50^{th}, 72.5^{th}, and 95^{th} percentiles of its distribution. The spline regression model was adjusted for age, sex, year of blood collection, center, seasonality using periodic restricted cubic splines with 5 knots, and hour of blood collection using restricted cubic splines with 3 knots. For within-day temperature range, daily average temperature was also adjusted.

Figure 2. Cumulative overall association between ambient temperature and mtDNA copy number over 7 days.

The curves represent the cumulative overall association between ambient temperature and mtDNA copy number over 7 days (from the day of to 6 days prior to blood collection) using a distributed lag non-linear model. Specifically, we used a linear regression model with a cross-basis function using a restricted cubic spline function of temperature with 5 knots placed at equally spaced values to model the temperature-mtDNA copy number relationship, and an indicator variable for each lag day to model lag-response relationship. The median temperature for each metric was used as the reference. All models were adjusted for age, sex, center, year, seasonality, and hour of blood collection. Daily average temperature was further adjusted for in the association of $Tmax_0$–$Tmin_0$ and mtDNA copy number.

Figure 3. Proportion explained by metabolites in the association of daily maximum temperature (A) and within-day temperature (B) with mtDNA copy number.

Each line represents the proportion of the association between temperature and mtDNA copy number explained by individual metabolites ("proportion explained", [$\beta_{overall} - \beta_{adjusted} / \beta_{overall}$]) by calculating the difference in the overall and adjusted effect divided by the overall effect. We first tested for the association between temperature and each metabolite adjusted for age, sex, center, year, seasonality, and hour of blood collection. We, then, selected metabolites with the Benjamini-Hochberg False discovery rate (FDR)-adjusted $P < 0.1$ for further analysis. The overall effect was estimated in a model adjusted for age, sex, center, year, seasonality, and hour of blood collection. The adjusted effect was estimated by further adjusting for individual metabolites. 95% confidence intervals were obtained by bootstrapping the sample 1,000 times and P dvalues were corrected for multiple testing by applying Benjamini-Hochberg FDR with a false positive threshold set at 5%. All analysis using $Tmax_0$–$Tmin_0$ was further adjusted for daily average temperature.

Table 1. Participant characteristics by mtDNA copy number categories.

	Overall (n=347,397)	**Quintile 1 (n=74,880)**	**Quintile 2 (n=74,879)**	**Quintile 3 (n=74,879)**	**Quintile 4 (n=74,879)**	**Quintile 5 (n=74,879)**
mtDNA-CN		-4.86, -0.82	-0.82, -0.29	-0.29, 0.19	0.19, 0.77	0.77, 6.58
Age (years)	56.8 (8.0)	56.7 (8.0)	56.7 (8.0)	56.8 (8.0)	56.8 (8.0)	56.7 (8.0)
Men (%)	45.9	45.5	46.1	46.1	46.2	45.8
Average income (%)						
< £18,000	18.7	20.0	19.1	18.9	18.2	17.5
£18,000 – £30,999	21.8	21.7	22.0	22.1	21.6	21.4
£31,000 – £51,999	22.7	22.3	22.4	22.5	23.0	23.3
£52,000 – £100,000	18.1	17.2	17.9	18.0	18.4	19.0
> £100,000	4.9	4.7	4.7	4.7	5.0	5.3
Education (%)						
Less than college	37.9	37.9	38.2	38.0	38.0	37.5
College or university degree	32.9	31.9	32.4	32.7	33.1	34.3
Professional degree	11.6	11.8	11.6	11.6	11.5	11.4
Smoking (%)						
Never	53.8	52.6	53.3	53.7	54.2	55.2
Former	35.4	34.5	35.1	35.7	35.7	36.3
Current	10.4	12.6	11.2	10.2	9.7	8.2
Alcohol intake (%)						
Never	3.2	3.3	3.3	3.2	3.1	3.0
Former	3.5	3.9	3.5	3.4	3.4	3.1
Current	93.2	92.7	93.2	93.2	93.4	93.7
Physical activity (%)						
Low	15.1	15.6	15.5	15.1	14.9	14.5
Moderate	33.3	33.1	33.2	33.4	33.2	33.4
High	32.8	31.8	32.2	32.9	33.2	34.0
BMI (kg/m^2)	27.4 (4.8)	27.7 (5.1)	27.6 (4.9)	27.4 (4.7)	27.3 (4.6)	26.9 (4.5)
SBP (mmHg)	137.9 (18.6)	138.5 (18.9)	138.2 (18.7)	138.0 (18.6)	137.7 (18.4)	137.0 (18.3)
Glucose (mg/dL)	92.2 (21.7)	93.3 (23.8)	92.6 (22.4)	92.1 (21.2)	91.8 (21.2)	91.1 (19.8)

Total cholesterol (mg/dL)	220.5 (44.1)	222.9 (44.9)	221.3 (44.2)	220.4 (44.0)	219.7 (43.7)	218.5 (43.5)
LDL cholesterol (mg/dL)	137.7 (33.5)	139.3 (34.1)	138.3 (33.7)	137.7 (33.4)	137.2 (33.2)	136.2 (33.1)
Triglyceride (mg/dL)*	131.6 (97.4)	136.3 (100.8)	134.3 (99.9)	132.7 (98.0)	131.0 (96.5)	123.9 (91.6)
Estimated GFR	90.7 (13.3)	90.5 (13.8)	90.6 (13.3)	90.6 (13.2)	90.8 (13.0)	91.1 (12.9)
Hypertension (%)	53.2	55.1	54.2	53.7	52.6	50.6
Diabetes (%)	5.5	6.3	5.7	5.6	5.2	4.7
Hyperlipidemia (%)	81.1	82.2	81.7	81.4	81.0	79.1
Cholesterol-lowering medication (%)	17.2	17.3	17.6	17.4	17.4	16.5

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; mtDNA-CN, mitochondrial DNA copy number; SBP, systolic blood pressure.

* Median (interquartile range).

Table 2. Average difference in mtDNA copy number (95% confidence interval) with a 10°C increase in ambient temperature.

	Model 1	Model 2	Model 3
Tmax	-0.08 (-0.08, -0.07)	-0.07 (-0.08, -0.06)	-0.07 (-0.08, -0.06)
Tmin	-0.07 (-0.08, -0.07)	-0.04 (-0.05, -0.03)	-0.07 (-0.08, -0.06)
Tavg	-0.08 (-0.09, -0.08)	-0.07 (-0.08, -0.06)	-0.07 (-0.08, -0.06)
Tmax – Tmin*	-0.05 (-0.06, -0.04)	-0.04 (-0.05, -0.03)	-0.04 (-0.05, -0.03)

Model 1: adjusted for age, sex, year of blood collection, and center; Model 2: further adjusted for seasonality using periodic restricted cubic splines with 5 knots; Model 3: further adjusted for hour of blood collection using restricted cubic splines with 3 knots.
*For Tmax–Tmin, daily average temperature was added to Model 1.

Table 3. Difference in the cumulative overall association of mtDNA copy number comparing 97.5th to 2.5th percentiles of temperature over 7 days.

	97.5th vs. 2.5th	Model 1	Model 2	Model 3
Tmax	25.0°C vs. 3.5°C	-0.21 (-0.24, -0.19)	-0.20 (-0.24, -0.17)	-0.21 (-0.24, -0.18)
Tmin	15.0°C vs. -3.0°C	-0.18 (-0.21, -0.15)	-0.15 (-0.18, -0.12)	-0.15 (-0.18, -0.12)
Tavg	19.5°C vs. 1.0°C	-0.18 (-0.21, -0.16)	-0.18 (-0.21, -0.15)	-0.18 (-0.21, -0.15)
Tmax – Tmin*	14.5°C vs. 2.0°C	-0.17 (-0.21, -0.13)	-0.14 (-0.18, -0.10)	-0.14 (-0.18, -0.10)

Model 1: adjusted for age, sex, year of blood collection, and center; Model 2: further adjusted for seasonality using periodic restricted cubic splines with 5 knots; Model 3: further adjusted for hour of blood collection using restricted cubic splines with 3 knots.
*For Tmax–Tmin, daily average temperature was added to Model 1.

Table 4. Proportion explained by metabolites in the association of daily maximum temperature ($Tmax_0$) and mtDNA copy number.

Metabolites	Temperature – metabolites	Metabolites – mtDNA copy number	Proportion explained, mean (95% bootstrapped CI)
Pyruvate	1.124 (1.116, 1.133)	-0.153 (-1.166, -0.140)	67.523 (43.632, 144.794)
Lactate	1.090 (1.084, 1.096)	-0.203 (-2.219, -0.185)	65.770 (42.022, 139.985)
Acetone	1.037 (1.030, 1.043)	-0.073 (-0.090, -0.056)	9.808 (5.761, 22.624)
Beta-hydroxybutyrate	1.003 (1.002, 1.005)	-0.642 (-0.736, -0.547)	8.311 (4.569, 19.001)
Lactate/pyruvate	0.969 (0.963, 0.976)	0.069 (0.054, 0.084)	8.089 (4.791, 17.673)
Phenylalanine	1.016 (1.011, 1.021)	-0.078 (-0.100, -0.056)	4.661 (2.466, 9.805)
Creatinine	1.011 (1.007, 1.014)	-0.102 (-0.132, -0.072)	4.155 (2.100, 9.752)
Total lipids in small LDL	1.007 (1.002, 1.012)	-0.066 (-0.086, -0.045)	1.709 (0.365, 4.562)
Phospholipids in medium LDL	1.008 (1.003, 1.014)	-0.053 (-0.071, -0.035)	1.672 (0.405, 4.398)
Phospholipids in small LDL	1.008 (1.004, 1.013)	-0.053 (-0.075, -0.031)	1.646 (0.529, 4.539)
Acetate	1.000 (0.999, 1.000)	0.973 (0.474, 1.471)	1.609 (0.602, 4.179)
Total lipids in LDL	1.007 (1.002, 1.012)	-0.058 (-0.078, -0.039)	1.500 (0.290, 4.076)
Large LDL particles	1.007 (1.002, 1.013)	-0.052 (-0.072, -0.033)	1.467 (0.348, 4.288)
Phospholipids in LDL	1.007 (1.002, 1.012)	-0.056 (-0.076, -0.036)	1.442 (0.296, 3.989)
Total lipids in large LDL	1.007 (1.002, 1.012)	-0.054 (-0.073, -0.034)	1.400 (0.292, 3.968)
Cholesterol in small LDL	1.007 (1.001, 1.012)	-0.052 (-0.070, -0.033)	1.296 (0.181, 3.622)
Cholesteryl esters in large LDL	1.008 (1.002, 1.013)	-0.043 (-0.060, -0.025)	1.216 (0.279, 3.397)
LDL cholesterol	1.007 (1.001, 1.013)	-0.045 (-0.063, -0.026)	1.170 (0.188, 3.278)
Cholesterol in large LDL	1.008 (1.002, 1.013)	-0.037 (-0.054, -0.019)	1.046 (0.204, 3.004)
Cholesteryl esters in medium HDL	0.992 (0.987, 0.997)	-0.023 (-0.043, -0.003)	-0.713 (-2.064, -0.101)
Cholesterol in medium HDL	0.992 (0.987, 0.997)	-0.030 (-0.050, -0.010)	-0.933 (-2.525, -0.214)
Total lipids in HDL	0.994 (0.990, 0.998)	-0.070 (-0.095, -0.044)	-1.624 (-4.234, -0.471)
Free cholesterol in medium HDL	0.991 (0.986, 0.997)	-0.052 (-0.070, -0.035)	-1.722 (-4.487, -0.441)
Apolipoprotein A1	0.995 (0.992, 0.999)	-0.101 (-0.133, -0.069)	-1.801 (-4.725, -0.489)
Medium HDL particles	0.991 (0.987, 0.996)	-0.060 (-0.081, -0.039)	-1.962 (-4.976, -0.688)
Total lipids in medium HDL	0.992 (0.988, 0.996)	-0.087 (-0.111, -0.063)	-2.662 (-6.690, -1.030)
Phospholipids in HDL	0.993 (0.989, 0.997)	-0.097 (-0.123, -0.071)	-2.698 (-6.726, -1.064)
Total lipids in small HDL	0.997 (0.994, 0.999)	-0.209 (-0.244, -0.173)	-2.773 (-7.731, -0.639)
Phospholipids in chylomicrons and extremely large VLDL	0.956 (0.921, 0.992)	-0.019 (-0.022, -0.017)	-3.293 (-9.033, -0.555)
Cholesterol in chylomicrons and extremely large VLDL	0.978 (0.959, 0.998)	-0.042 (-0.047, -0.037)	-3.534 (-9.820, -0.327)

Histidine	1.004 (1.001, 1.008)	0.215 (0.185, 0.246)	-3.557 (-9.800, -0.722)
Phospholipids in medium HDL	0.991 (0.987, 0.995)	-0.111 (-0.136, -0.086)	-3.882 (-9.420, -1.740)
Chylomicrons and extremely large VLDL particles	0.959 (0.932, 0.986)	-0.028 (-0.032, -0.024)	-4.509 (-11.588, -1.479)
Phospholipids in small HDL	0.995 (0.992, 0.998)	-0.228 (-0.263, -0.194)	-4.535 (-11.633, -1.817)
Free cholesterol in chylomicrons and extremely large VLDL	0.970 (0.950, 0.990)	-0.040 (-0.045, -0.035)	-4.675 (-11.803, -1.396)
Triglycerides in chylomicrons and extremely large VLDL	0.917 (0.881, 0.954)	-0.014 (-0.017, -0.012)	-4.756 (-11.566, -2.166)
Triglycerides in medium HDL	0.989 (0.982, 0.997)	-0.120 (-0.134, -0.106)	-4.931 (-13.870, -1.436)
Total lipids in chylomicrons and extremely large VLDL	0.960 (0.967, 0.983)	-0.033 (-0.037, -0.029)	-5.196 (-13.267, -2.033)
Glycoprotein acetyls	0.996 (0.993, 0.999)	-0.395 (-0.428, -0.362)	-5.815 (-17.625, -1.408)
Glycine	1.003 (1.002, 1.004)	0.966 (0.869, 1.063)	-12.383 (-28.818, -7.006)

Abbreviations: HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

The association between $Tmax_0$ and mtDNA copy number per 10°C increase in $Tmax_0$ was -0.038 (-0.059, -0.017). Temperature–metabolites are reported also for 10°C increase in temperature. Metabolite-mtDNA copy number associations are reported for doubling of each metabolite. Only the estimates of proportion explained with FDR-adjusted p values < 0.1 are reported.

Figure 1.

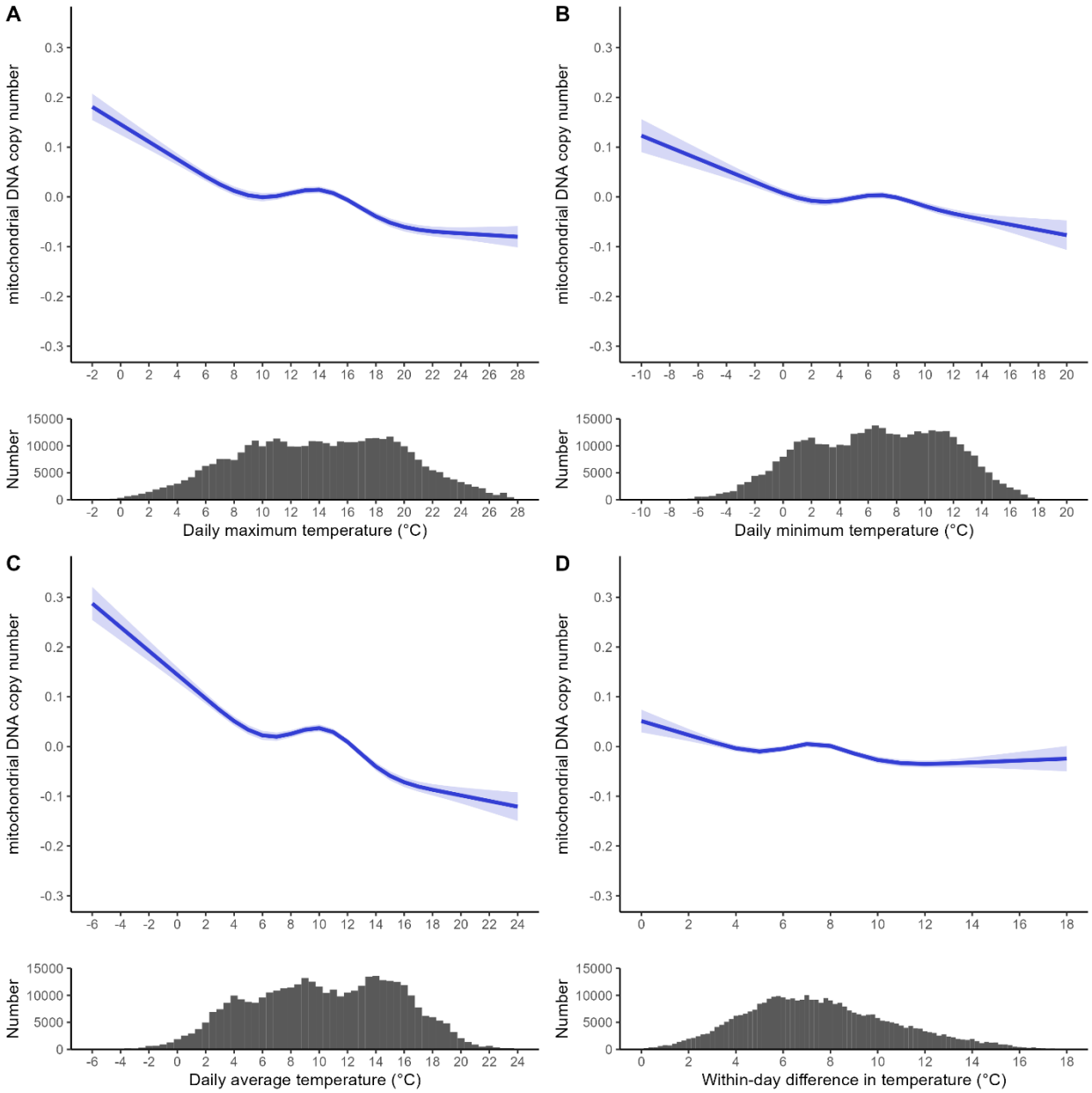

A
B
C
D
mitochondrial DNA copy number
Number
Daily maximum temperature (°C)
Daily minimum temperature (°C)
Daily average temperature (°C)
Within-day difference in temperature (°C)

Figure 2.

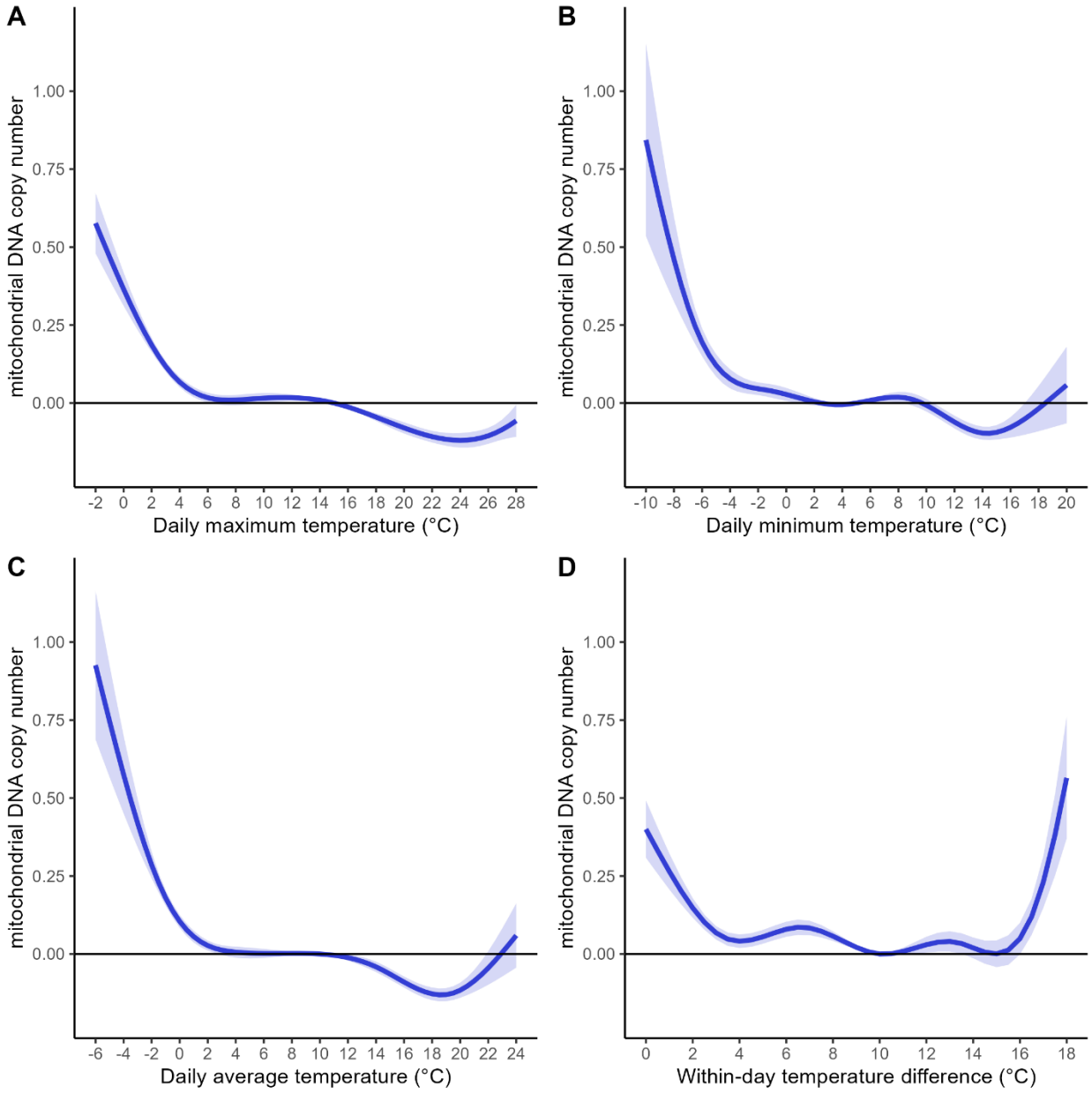
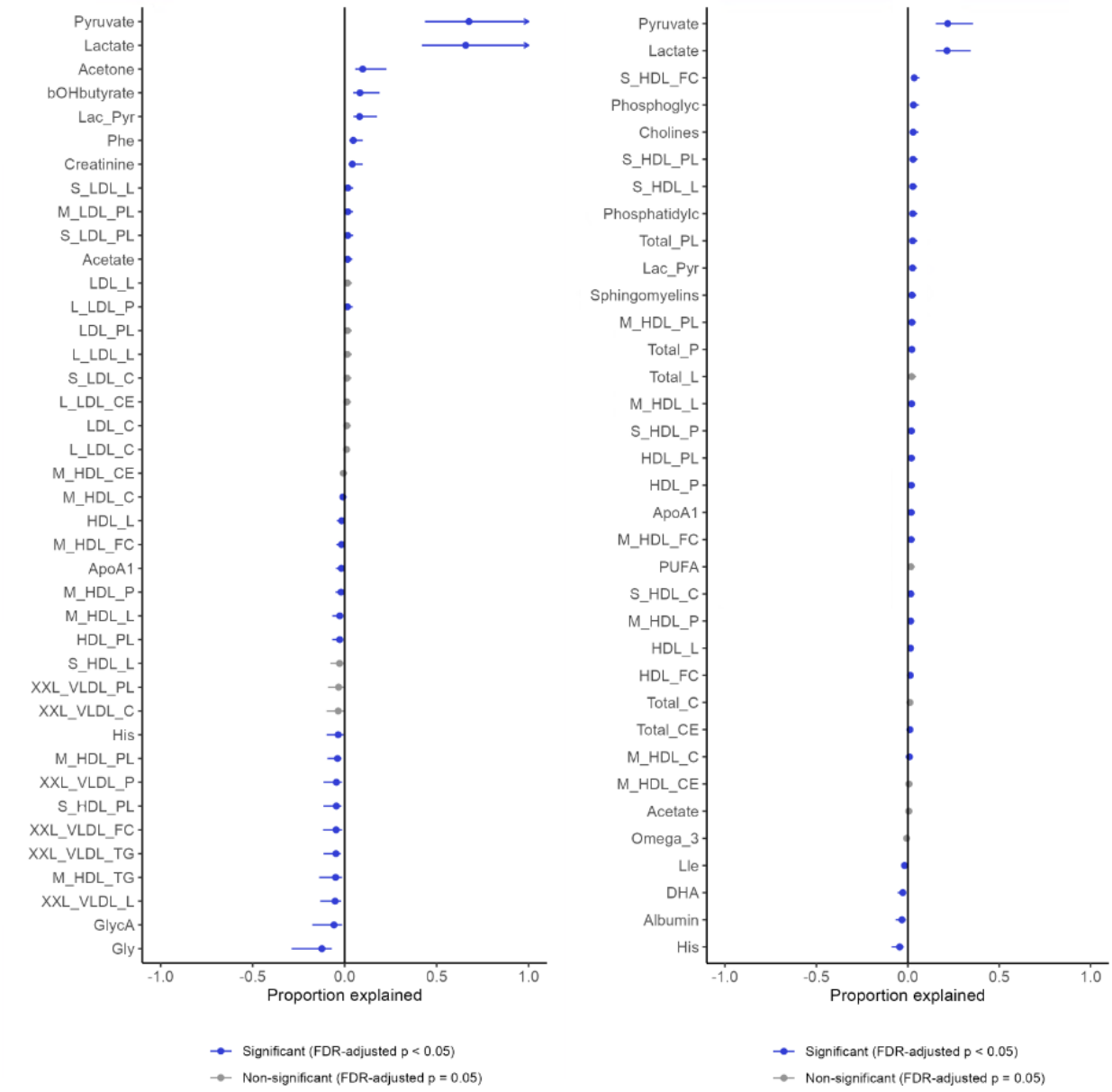

A
mitochondrial DNA copy number
1.00
0.75
0.50
0.25
0.00
-2 0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
Daily maximum temperature (°C)
B
mitochondrial DNA copy number
1.00
0.75
0.50
0.25
0.00
-10 -8 -6 -4 -2 0 2 4 6 8 10 12 14 16 18 20
Daily minimum temperature (°C)
C
mitochondrial DNA copy number
1.00
0.75
0.50
0.25
0.00
-6 -4 -2 0 2 4 6 8 10 12 14 16 18 20 22 24
Daily average temperature (°C)
D
mitochondrial DNA copy number
1.00
0.75
0.50
0.25
0.00
0 2 4 6 8 10 12 14 16 18
Within-day temperature difference (°C)

Figure 3.


Pyruvate
Lactate
Acetone
bOHbutyrate
Lac_Pyr
Phe
Creatinine
S_LDL_L
M_LDL_PL
S_LDL_PL
Acetate
LDL_L
L_LDL_P
LDL_PL
L_LDL_L
S_LDL_C
L_LDL_CE
LDL_C
L_LDL_C
M_HDL_CE
M_HDL_C
HDL_L
M_HDL_FC
ApoA1
M_HDL_P
M_HDL_L
HDL_PL
S_HDL_L
XXL_VLDL_PL
XXL_VLDL_C
His
M_HDL_PL
XXL_VLDL_P
S_HDL_PL
XXL_VLDL_FC
XXL_VLDL_TG
M_HDL_TG
XXL_VLDL_L
GlycA
Gly
-1.0
-0.5
0.0
0.5
1.0
Proportion explained
Significant (FDR-adjusted p < 0.05)
Non-significant (FDR-adjusted p = 0.05)
Pyruvate
Lactate
S_HDL_FC
Phosphoglyc
Cholines
S_HDL_PL
S_HDL_L
Phosphatidylc
Total_PL
Lac_Pyr
Sphingomyelins
M_HDL_PL
Total_P
Total_L
M_HDL_L
S_HDL_P
HDL_PL
HDL_P
ApoA1
M_HDL_FC
PUFA
S_HDL_C
M_HDL_P
HDL_L
HDL_FC
Total_C
Total_CE
M_HDL_C
M_HDL_CE
Acetate
Omega_3
Lle
DHA
Albumin
His
-1.0
-0.5
0.0
0.5
1.0
Proportion explained
Significant (FDR-adjusted p < 0.05)
Non-significant (FDR-adjusted p = 0.05)

SUPPLEMENTARY MATERIALS

Supplementary Table 1. Study population used for each analysis.
Supplementary Table 2. List of metabolites and abbreviations.
Supplementary Table 3. Predicted average mtDNA copy number by month.
Supplementary Table 4. Average difference in the association between temperature and mtDNA copy number association per 10°C increase in ambient temperature by age and sex.
Supplementary Table 5. Difference in the cumulative overall effect of temperature and leukocyte telomere length comparing 97.5^{th} to 2.5^{th} percentile.
Supplementary Table 6. Proportion explained by independent components of metabolites in the association of daily maximum temperature ($Tmax_0$) and mtDNA copy number.
Supplementary Table 7. Proportion explained by metabolites in the association of within-day difference in temperature ($Tmax_0$–$Tmin_0$) and mtDNA copy number.
Supplementary Table 8. Proportion explained by independent components of metabolites in the association of within-day difference in temperature ($Tmax_0$–$Tmin_0$) and mtDNA copy number.
Supplementary Table 9. Daily maximum temperature ($Tmax_0$) – mtDNA copy number – metabolites associations.
Supplementary Table 10. Within-day difference in temperature ($Tmax_0$–$Tmin_0$) – mtDNA copy number – metabolites associations.

Supplementary Figure 1. Flowchart of study participants.
Supplementary Figure 2. Distribution of temperature.
Supplementary Figure 3. Distribution of temperature by year.
Supplementary Figure 4. Distribution of temperature by center.
Supplementary Figure 5. Within-day difference in temperature and average mtDNA copy numbers by daily average temperature.
Supplementary Figure 6. Ambient temperature and average mtDNA copy numbers by age and sex.
Supplementary Figure 7. Difference in mtDNA copy number with 10°C increase in ambient temperature by lag.
Supplementary Figure 8. Proportion explained by mtDNA copy number in the association of daily maximum temperature (A) and within-day temperature (B) with individual metabolites.

Supplementary Table 1. Study population used for each analysis.

Exposure	Outcome	Additional variable for proportion explained	Number of participants
Temperature	mtDNA copy number	-	374,397
Seasonal trends	mtDNA copy number	-	374,397
Temperature	mtDNA copy number	Metabolites	90,191
Temperature	Metabolites	mtDNA copy number	85,221
Temperature	Leukocyte telomere length	-	362,612

Supplementary Table 2. List of metabolites and abbreviations.

Category	Metabolite	Abbreviation
Cholesterol	Total cholesterol	Total_C
	Total cholesterol minus HDL-C	Non_HDL_C
	Remnant cholesterol (Non-HDL, Non-LDL-cholesterol)	Remnant_C
	VDLD cholesterol	VLDL_C
	Clinical LDL cholesterol	Clinical_LDL_C
	LDL cholesterol	LDL_C
	HDL cholesterol	HDL_C
Triglycerides	Total triglycerides	Total_TG
	Triglycerides in VLDL	VLDL_TG
	Triglycerides in LDL	LDL_TG
	Triglycerides in HDL	HDL_TG
Phospholipids	Total phospholipids in lipoprotein particles	Total_PL
	Phospholipids in VLDL	VLDL_PL
	Phospholipids in LDL	LDL_PL
	Phospholipids in HDL	HDL_PL
Cholesteryl esters	Total esterified cholesterol	Total_CE
	Cholesteryl esters in VLDL	VLDL_CE
	Cholesteryl esters in LDL	LDL_CE
	Cholesteryl esters in HDL	HDL_CE
Free cholesterol	Total free cholesterol	Total_FC
	Free cholesterol in VLDL	VLDL_FC
	Free cholesterol in LDL	LDL_FC
	Free cholesterol in HDL	HDL_FC
Total lipids	Total lipids in lipoprotein particles	Total_L
	Total lipids in VLDL	VLDL_L
	Total lipids in LDL	LDL_L
	Total lipids in HDL	HDL_L
Lipoprotein particle concentrations	Total concentration of lipoprotein particles	Total_P
	Concentration of VLDL particles	VLDL_P
	Concentration of LDL particles	LDL_P
	Concentration of HDL particles	HDL_P
Lipoprotein subclasses	Concentration of chylomicrons and extremely large VLDL particles	XXL_VLDL_P
	Total lipids in chylomicrons and extremely large VLDL	XXL_VLDL_L
	Phospholipids in chylomicrons and extremely large VLDL	XXL_VLDL_PL
	Cholesterol in chylomicrons and extremely large VLDL	XXL_VLDL_C
	Cholesteryl esters in chylomicrons and extremely large VLDL	XXL_VLDL_CE
	Free cholesterol in chylomicrons and extremely large VLDL	XXL_VLDL_FC
	Triglycerides in chylomicrons and extremely large VLDL	XXL_VLDL_TG

Concentration of very large VLDL particles	XL_VLDL_P
Total lipids in very large VLDL	XL_VLDL_L
Phospholipids in very large VLDL	XL_VLDL_PL
Cholesterol in very large VLDL	XL_VLDL_C
Cholesteryl esters in very large VLDL	XL_VLDL_CE
Free cholesterol in very large VLDL	XL_VLDL_FC
Triglycerides in very large VLDL	XL_VLDL_TG
Concentration of large VLDL particles	L_VLDL_P
Total lipids in large VLDL	L_VLDL_L
Phospholipids in large VLDL	L_VLDL_PL
Cholesterol in large VLDL	L_VLDL_C
Cholesteryl esters in large VLDL	L_VLDL_CE
Free cholesterol in large VLDL	L_VLDL_FC
Triglycerides in large VLDL	L_VLDL_TG
Concentration of medium VLDL particles	M_VLDL_P
Total lipids in medium VLDL	M_VLDL_L
Phospholipids in medium VLDL	M_VLDL_PL
Cholesterol in medium VLDL	M_VLDL_C
Cholesteryl esters in medium VLDL	M_VLDL_CE
Free cholesterol in medium VLDL	M_VLDL_FC
Triglycerides in medium VLDL	M_VLDL_TG
Concentration of small VLDL particles	S_VLDL_P
Total lipids in small VLDL	S_VLDL_L
Phospholipids in small VLDL	S_VLDL_PL
Cholesterol in small VLDL	S_VLDL_C
Cholesteryl esters in small VLDL	S_VLDL_CE
Free cholesterol in small VLDL	S_VLDL_FC
Triglycerides in small VLDL	S_VLDL_TG
Concentration of very small VLDL particles	XS_VLDL_P
Total lipids in very small VLDL	XS_VLDL_L
Phospholipids in very small VLDL	XS_VLDL_PL
Cholesterol in very small VLDL	XS_VLDL_C
Cholesteryl esters in very small VLDL	XS_VLDL_CE
Free cholesterol in very small VLDL	XS_VLDL_FC
Triglycerides in very small VLDL	XS_VLDL_TG
Concentration of IDL particles	IDL_P
Total lipids in IDL	IDL_L
Phospholipids in IDL	IDL_PL
Cholesterol in IDL	IDL_C
Cholesteryl esters in IDL	IDL_CE
Free cholesterol in IDL	IDL_FC
Triglycerides in IDL	IDL_TG
Concentration of large LDL particles	L_LDL_P
Total lipids in large LDL	L_LDL_L
Phospholipids in large LDL	L_LDL_PL
Cholesterol in large LDL	L_LDL_C
Cholesteryl esters in large LDL	L_LDL_CE
Free cholesterol in large LDL	L_LDL_FC
Triglycerides in large LDL	L_LDL_TG
Concentration of medium LDL particles	M_LDL_P
Total lipids in medium LDL	M_LDL_L

	Phospholipids in medium LDL	M_LDL_PL
	Cholesterol in medium LDL	M_LDL_C
	Cholesteryl esters in medium LDL	M_LDL_CE
	Free cholesterol in medium LDL	M_LDL_FC
	Triglycerides in medium LDL	M_LDL_TG
	Concentration of small LDL particles	S_LDL_P
	Total lipids in small LDL	S_LDL_L
	Phospholipids in small LDL	S_LDL_PL
	Cholesterol in small LDL	S_LDL_C
	Cholesteryl esters in small LDL	S_LDL_CE
	Free cholesterol in small LDL	S_LDL_FC
	Triglycerides in small LDL	S_LDL_TG
	Concentration of very large HDL particles	XL_HDL_P
	Total lipids in very large HDL	XL_HDL_L
	Phospholipids in very large HDL	XL_HDL_PL
	Cholesterol in very large HDL	XL_HDL_C
	Cholesteryl esters in very large HDL	XL_HDL_CE
	Free cholesterol in very large HDL	XL_HDL_FC
	Triglycerides in very large HDL	XL_HDL_TG
	Concentration of large HDL particles	L_HDL_P
	Total lipids in large HDL	L_HDL_L
	Phospholipids in large HDL	L_HDL_PL
	Cholesterol in large HDL	L_HDL_C
	Cholesteryl esters in large HDL	L_HDL_CE
	Free cholesterol in large HDL	L_HDL_FC
	Triglycerides in large HDL	L_HDL_TG
	Concentration of medium HDL particles	M_HDL_P
	Total lipids in medium HDL	M_HDL_L
	Phospholipids in medium HDL	M_HDL_PL
	Cholesterol in medium HDL	M_HDL_C
	Cholesteryl esters in medium HDL	M_HDL_CE
	Free cholesterol in medium HDL	M_HDL_FC
	Triglycerides in medium HDL	M_HDL_TG
	Concentration of small HDL particles	S_HDL_P
	Total lipids in small HDL	S_HDL_L
	Phospholipids in small HDL	S_HDL_PL
	Cholesterol in small HDL	S_HDL_C
	Cholesteryl esters in small HDL	S_HDL_CE
	Free cholesterol in small HDL	S_HDL_FC
	Triglycerides in small HDL	S_HDL_TG
Other lipids	Phosphoglycerides	Phosphoglyc
	Total cholines	Cholines
	Phosphatidylcholines	Phosphatidylc
	Sphingomyelins	Sphingomyelins
Apolipoproteins	Apolipoprotein B	ApoB
	Apolipoprotein A1	ApoA1
Fatty acids	Total fatty acids	Total_FA
	Degree of unsaturation	Unsaturation
	Omega-3 fatty acids	Omega_3
	Omega-6 fatty acids	Omega_6
	Polyunsaturated fatty acids	PUFA

	Monounsaturated fatty acids	MUFA
	Saturated fatty acids	SFA
	Linoleic acid	LA
	Docosahexaenoic acid	DHA
Amino acids	Alanine	Ala
	Glutamine	Gln
	Glycine	Gly
	Histidine	His
	Total concentration of branched-chain amino acids (leucine, isoleucine, valine)	Total_BCAA
	Isoleucine	Lle
	Leucine	Leu
	Valine	Val
	Phenylalanine	Phe
	Tyrosine	Try
Glycolysis related metabolites	Glucose	Glucose
	Lactate	Lactate
	Pyruvate	Pyruvate
	Citrate	Citrate
Ketone bodies	3-Hydroxybutyrate	bOHbutyrate
	Acetate	Acetate
	Acetoacetate	Acetoacetate
	Acetone	Acetone
Fluid balance	Creatinine	Creatinine
	Albumin	Albumin
Inflammation	Glycoprotein acetyls	GlycA

Abbreviations: HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

Supplementary Table 3. Predicted average mtDNA copy number by month.

	Categorical		Periodic RCS	
	Model 1	**Model 2**	**Model 1**	**Model 2**
January	0.057 (0.044, 0.069)	0.027 (0.012, 0.042)	0.034 (0.029, 0.040)	0.017 (0.011, 0.023)
February	0.028 (0.016, 0.039)	0.001 (-0.013, 0.014)	0.030 (0.026, 0.035)	0.004 (-0.002, 0.010)
March	-0.003 (-0.014, 0.007)	-0.021 (-0.033, -0.010)	0.025 (0.019, 0.030)	-0.009 (-0.017, -0.002)
April	-0.036 (-0.047, -0.025)	-0.042 (-0.053, -0.030)	0.010 (0.004, 0.016)	-0.021 (-0.029, -0.014)
May	-0.057 (-0.067, -0.046)	-0.048 (-0.058, -0.037)	-0.018 (-0.024, -0.013)	-0.031 (-0.037, -0.025)
June	-0.037 (-0.047, -0.027)	-0.014 (-0.026, -0.002)	-0.046 (-0.051, -0.041)	-0.032 (-0.038, -0.027)
July	-0.062 (-0.073, -0.051)	-0.031 (-0.045, -0.018)	-0.050 (-0.054, -0.045)	-0.019 (-0.025, -0.012)
August	-0.092 (-0.010, -0.080)	-0.062 (-0.076, -0.048)	-0.023 (-0.030, -0.016)	0.009 (0.000, 0.017)
September	-0.029 (-0.041, -0.016)	-0.011 (-0.025, 0.002)	0.012 (0.002, 0.022)	0.035 (0.024, 0.046)
October	0.047 (0.035, 0.058)	0.047 (0.036, 0.058)	0.034 (0.023, 0.045)	0.047 (0.035, 0.058)
November	0.067 (0.055, 0.079)	0.054 (0.042, 0.066)	0.040 (0.030, 0.050)	0.043 (0.033, 0.053)
December	0.038 (0.023, 0.052)	0.007 (-0.009, 0.024)	0.038 (0.030, 0.046)	0.031 (0.023, 0.039)

Model 1: adjusted for age, sex, center, year of blood collection, and hour of blood collection using restricted cubic splines with 3 knots; Model 2: further adjusted for daily average temperature.

Supplementary Table 4. Average difference in mtDNA copy number with a 10°C increase in ambient temperature by age and sex.

	By Age			By Sex	
	<50 years	**50–60 years**	**≥60 years**	**Men**	**Women**
Tmax	Reference	0.01 (-0.00, 0.03)	0.02 (0.01, 0.04)	Reference	-0.00 (-0.01, 0.01)
Tmin	Reference	0.01 (-0.01, 0.03)	0.02 (0.00, 0.04)	Reference	0.00 (-0.01, 0.01)
Tavg	Reference	0.01 (-0.00, 0.03)	0.02 (0.01, 0.04)	Reference	-0.00 (-0.01, 0.01)
Tmax – Tmin*	Reference	0.03 (-0.00, 0.05)	0.03 (0.00, 0.05)	Reference	-0.01 (-0.03, 0.01)

Models are adjusted for age, sex, center, year of blood collection, seasonality using periodic restricted cubic splines with 5 knots, and hour of blood collection using restricted cubic splines with 3 knots.

* For Tmax–Tmin, daily average temperature was added to the model.

Supplementary Table 5. Overall effect of temperature and leukocyte telomere length comparing 97.5th to 2.5th percentile.

	97.5th vs. 2.5th	Model 1	Model 2	Model 3
Tmax	25.0°C vs. 3.5°C	-0.01 (-0.04, 0.01)	-0.02 (-0.05, 0.01)	-0.02 (-0.05, 0.01)
Tmin	15.0°C vs. -3.0°C	0.01 (-0.02, 0.04)	0.01 (-0.03, 0.04)	0.01 (-0.03, 0.04)
Tavg	19.5°C vs. 1.0°C	0.00 (-0.02, 0.03)	-0.00 (-0.03, 0.03)	-0.00 (-0.03, 0.03)
Tmax – Tmin*	14.5°C vs. 2.0°C	-0.01 (-0.05, 0.04)	-0.01 (-0.05, 0.03)	-0.01 (-0.05, 0.03)

Model 1: adjusted for age, sex, year of blood collection, and center; Model 2: further adjusted for seasonality using periodic restricted cubic splines with 5 knots; Model 3: further adjusted for hour of blood collection using restricted cubic splines with 3 knots.
*For Tmax–Tmin, daily average temperature was added to Model 1.

Supplementary Table 6. Proportion explained by independent components of metabolites in the association of daily maximum temperature ($Tmax_0$) and mtDNA copy number.

Metabolites	Temperature – independent components	Independent components – mtDNA copy number	Proportion explained, mean (95% bootstrapped CI)
IC4	-0.267 (-0.288, -0.247)	0.086 (0.079, 0.093)	60.181 (38.824, 128.849)
IC8	-0.031 (-0.051, -0.010)	0.034 (0.026, 0.041)	2.682 (0.871, 6.888)
IC5	-0.010 (-0.032, 0.011)	0.004 (-0.003, 0.011)	0.104 (-0.211, 0.677)
IC6	0.022 (0.001, 0.043)	0.011 (0.004, 0.018)	-0.627 (-2.040, 0.022)
IC2	0.022 (0.001, 0.043)	0.011 (0.004, 0.018)	-0.652 (-1.916, -0.416)
IC3	0.039 (0.017, 0.060)	0.009 (0.002, 0.016)	-0.908 (-2.559, -0.176)
IC9	0.028 (0.006, 0.049)	0.019 (0.012, 0.026)	-1.135 (-3.753, -0.282)
IC7	-0.024 (-0.045, -0.003)	-0.033 (-0.040, -0.026)	-2.074 (-5.872, -0.328)
IC10	-0.021 (-0.042, -0.001)	-0.042 (-0.049, -0.034)	-2.324 (-6.964, -0.154)
IC1	-0.060 (-0.051, -0.009)	-0.031 (-0.038, -0.025)	-2.501 (-6.878, -0.680)

Abbreviations: CI, confidence interval; IC, independent component.

The association between $Tmax_0$ and mtDNA copy number per 10°C increase in $Tmax_0$ was -0.038 (-0.059, -0.017). Temperature–independent component associations are also reported for 10°C increase in temperature.

Supplementary Table 7. Proportion explained by metabolites in the association of within-day difference in temperature ($Tmax_0$–$Tmin_0$) and mtDNA copy number.

Metabolites	Temperature – metabolites	Metabolites – mtDNA copy number	Proportion explained, mean (95% bootstrapped CI)
Pyruvate	1.063 (1.053, 1.072)	-0.153 (-1.166, -0.140)	21.822 (15.284, 35.605)
Lactate	1.046 (1.040, 1.052)	-0.204 (-2.222, -0.185)	21.419 (15.259, 34.380)
Free cholesterol in small HDL	1.008 (1.004, 1.011)	-0.195 (-0.230, -0.160)	3.485 (1.810, 6.356)
Phosphoglycerides	1.007 (1.003, 1.011)	-0.193 (-0.221, -0.164)	3.064 (1.243, 6.048)
Cholines	1.007 (1.003, 1.010)	-0.189 (-0.221, -0.158)	2.899 (1.200, 5.701)
Phospholipids in small HDL	1.005 (1.002, 1.009)	-0.229 (-0.264, -0.194)	2.821 (1.029, 5.426)
Total lipids in small HDL	1.006 (1.002, 1.009)	-0.209 (-0.245, -0.174)	2.760 (1.111, 5.149)
Phosphatidylcholines	1.007 (1.003, 1.011)	-0.164 (-0.192, -0.136)	2.693 (1.078, 5.380)
Total phospholipids	1.006 (1.002, 1.009)	-0.199 (-0.230, -0.168)	2.629 (0.867, 5.364)
Lactate/pyruvate	0.984 (0.977, 0.992)	0.069 (0.053, 0.084)	2.559 (1.229, 4.837)
Sphingomyelins	1.007 (1.003, 1.011)	-0.143 (-0.175, -0.112)	2.267 (0.903, 4.552)
Phospholipids in medium HDL	1.008 (1.004, 1.013)	-0.111 (-0.136, -0.087)	2.202 (0.814, 4.429)
Total lipoprotein particles	1.008 (1.004, 1.011)	-0.119 (-0.153, -0.085)	2.196 (1.022, 4.147)
Total lipids in lipoprotein particles	1.005 (1.001, 1.010)	-0.162 (-0.187, -0.136)	2.085 (0.334, 4.529)
Total lipids in medium HDL	1.010 (1.005, 1.015)	-0.087 (-0.111, -0.064)	2.074 (0.954, 4.018)
Small HDL particles	1.006 (1.003, 1.010)	-0.135 (-0.170, -0.099)	1.956 (0.797, 3.600)
Phospholipids in HDL	1.009 (1.004, 1.013)	-0.097 (-0.124, -0.071)	1.937 (0.820, 3.897)
HDL particles	1.009 (1.005, 1.012)	-0.096 (-0.128, -0.064)	1.914 (0.876, 3.648)
Apolipoprotein A1	1.008 (1.004, 1.012)	-0.102 (-0.133, -0.070)	1.859 (0.797, 3.616)
Free cholesterol in medium HDL	1.015 (1.008, 1.022)	-0.053 (-0.070, -0.035)	1.812 (0.766, 3.546)
Polyunsaturated fatty acids	1.005 (1.001, 1.009)	-0.151 (-0.181, -0.120)	1.675 (0.034, 3.668)
Cholesterol in small HDL	1.007 (1.003, 1.010)	-0.105 (-0.140, -0.070)	1.656 (0.069, 3.099)
Medium HDL particles	1.011 (1.006, 1.017)	-0.060 (-0.081, -0.040)	1.612 (0.066, 3.180)
Total lipids in HDL	1.009 (1.005, 1.014)	-0.070 (-0.096, -0.045)	1.514 (0.060, 3.089)
Free cholesterol in HDL	1.011 (1.006, 1.016)	-0.054 (-0.076, -0.032)	1.377 (0.050, 2.852)
Total cholesterol	1.006 (1.001, 1.011)	-0.081 (-0.105, -0.058)	1.144 (0.018, 2.616)
Total cholesteryl esters	1.006 (1.002, 1.011)	-0.076 (-0.099, -0.052)	1.142 (0.024, 2.519)
Cholesterol in medium HDL	1.012 (1.006, 1.018)	-0.030 (-0.050, -0.011)	0.852 (0.020, 2.008)
Cholesteryl esters in medium HDL	1.012 (1.006, 1.018)	-0.023 (-0.043, -0.003)	0.622 (0.005, 1.618)
Acetate	1.000 (1.000, 1.000)	0.968 (0.469, 1.466)	0.556 (0.006, 1.358)
Omega-3 fatty acids	1.012 (1.002, 1.022)	0.022 (0.010, 0.034)	-0.627 (-1.533, -0.107)

Isoleucine	0.987 (0.980, 0.995)	-0.059 (-0.075, -0.044)	-1.793 (-3.529, -0.719)
Docosahexanoic acid	1.016 (1.007, 1.024)	0.074 (0.060, 0.088)	-2.737 (-5.553, -1.226)
Albumin	1.003 (1.001, 1.005)	0.442 (0.386, 0.497)	-3.206 (-6.686, -0.981)
Histidine	1.009 (1.005, 1.013)	0.215 (0.185, 0.246)	-4.447 (-8.869, -2.211)

Abbreviations: HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

The association between $Tmax_0$–$Tmin_0$ and mtDNA copy number per 10°C increase in $Tmax_0$–$Tmin_0$ was -0.061 (-0.085, -0.037). Temperature–metabolites associations are also reported for 10°C increase in temperature. Metabolite-mtDNA copy number associations are reported for doubling of each metabolite. Only the estimates of proportion explained with FDR-adjusted p values < 0.1 are reported.

Supplementary Table 8. Proportion explained by independent components of metabolites in the association of within-day difference in temperature ($Tmax_0$–$Tmin_0$) and mtDNA copy number.

Metabolites	Temperature – independent components	Independent components – mtDNA copy number	Proportion explained, mean (95% bootstrapped CI)
IC4	-0.115 (-0.138, -0.091)	0.086 (0.079, 0.093)	18.797 (13.043, 30.126)
IC10	0.049 (0.026, 0.073)	-0.042 (-0.049, -0.034)	3.024 (1.375, 5.921)
IC1	0.018 (-0.007, 0.042)	-0.032 (-0.038, -0.025)	0.702 (-0.461, 2.146)
IC6	-0.028 (-0.052, -0.004)	0.011 (0.004, 0.018)	0.429 (-0.034, 1.136)
IC2	-0.008 (-0.032, 0.016)	0.011 (0.004, 0.018)	0.091 (-0.362, 0.669)
IC5	0.005 (-0.019, 0.030)	0.004 (-0.003, 0.012)	-0.026 (-0.281, 0.188)
IC8	0.004 (-0.019, 0.027)	0.034 (0.026, 0.041)	-0.036 (-1.497, 1.397)
IC3	0.018 (-0.032, 0.016)	0.009 (0.002, 0.016)	-0.308 (-0.972, 0.052)
IC9	0.017 (-0.007, 0.041)	0.019 (0.012, 0.026)	-0.582 (-1.601, 0.162)
IC7	-0.035 (-0.059, -0.010)	-0.031 (-0.038, -0.025)	-1.914 (-3.990, -0.529)

Abbreviations: CI, confidence interval; IC, independent component.

The association between $Tmax_0$–$Tmin_0$ and mtDNA copy number per 10°C increase in $Tmax_0$–$Tmin_0$ was -0.061 (-0.085, -0.037). Temperature–independent component associations are also reported for 10°C increase in temperature.

Supplementary Table 9. Daily maximum temperature ($Tmax_0$) – mtDNA copy number – metabolites associations.

Metabolites	Temperature – metabolites	mtDNA copy number – metabolites	Proportion explained, median (95% bootstrapped CI)
Total lipids in IDL	1.004 (1.000, 1.009)	0.993 (0.991, 0.994)	5.839 (1.509, 38.159)
Cholesteryl esters in IDL	1.005 (1.000, 1.010)	0.993 (0.992, 0.994)	5.149 (1.434, 30.413)
Cholesterol in IDL	1.005 (1.000, 1.010)	0.993 (0.991, 0.995)	5.093 (1.377, 33.628)
IDL particles	1.005 (1.001, 1.010)	0.994 (0.993, 0.996)	3.917 (1.304, 19.553)
Non-HDL cholesterol	1.006 (1.001. 1.011)	0.994 (0.992, 0.996)	3.806 (1.235, 18.391)
Cholesteryl esters in medium LDL	1.007 (1.000, 1.013)	0.994 (0.992, 0.996)	3.092 (0.879, 21.346)
Apolipoprotein B	1.006 (1.001, 1.010)	0.996 (0.994, 0.997)	3.003 (0.957, 12.636)
Cholesteryl esters in small LDL	1.009 (1.001, 1.013)	0.995 (0.993, 0.997)	2.780 (0.926, 14.630)
Beta-hydroxybutyrate	1.003 (1.002, 1.004)	0.998 (0.997, 0.998)	2.525 (1.158, 4.662)
Cholesteryl esters in LDL	1.009 (1.003, 1.015)	0.994 (0.992, 0.996)	2.471 (0.878, 7.716)
Phospholipids in large LDL	1.007 (1.002, 1.011)	0.996 (0.994, 0.997)	2.381 (0.899, 7.453)
Cholesterol in medium LDL	1.008 (1.002, 1.015)	0.995 (0.993, 0.997)	2.291 (0.723, 9.742)
Total lipids in large LDL	1.008 (1.003, 1.013)	0.995 (0.994, 0.997)	2.279 (0.890, 6.157)
LDL particles	1.006 (1.002, 1.011)	0.996 (0.995, 0.998)	2.250 (0.722, 8.285)
Total lipids in LDL	1.008 (1.003, 1.012)	0.995 (0.994, 0.997)	2.209 (0.841, 6.520)
Total lipids in medium LDL	1.007 (1.002, 1.013)	0.996 (0.994, 0.998)	2.130 (0.709, 8.133)
LDL cholesterol	1.009 (1.003, 1.015)	0.995 (0.993, 0.997)	2.104 (0.775, 5.895)
Cholesteryl esters in large LDL	1.008 (1.003, 1.014)	0.995 (0.994, 0.997)	2.071 (0.791, 5.593)
Cholesterol in large LDL	1.009 (1.003, 1.014)	0.995 (0.994, 0.997)	1.950 (0.715, 5.196)
Phospholipids in LDL	1.008 (1.003, 1.012)	0.996 (0.995, 0.998)	1.853 (0.674, 5.209)
Cholesterol in small LDL	1.009 (1.003, 1.014)	0.996 (0.994, 0.998)	1.743 (0.602, 5.281)
Large LDL particles	1.007 (1.003, 1.012)	0.997 (0.995, 0.998)	1.742 (0.579, 5.034)
Total lipids in small LDL	1.007 (1.003, 1.012)	0.997 (0.995, 0.998)	1.727 (0.586, 5.215)
Clinical LDL cholesterol	1.009 (1.003, 1.015)	0.996 (0.994, 0.998)	1.722 (0.533, 5.364)
Free cholesterol in large LDL	1.010 (1.004, 1.016)	0.995 (0.993, 0.997)	1.640 (0.574, 3.996)
Leucine	1.006 (1.001, 1.011)	0.998 (0.996, 0.999)	1.449 (0.261, 5.885)
Phospholipids in medium LDL	1.010 (1.004, 1.015)	0.996 (0.995, 0.998)	1.361 (0.445, 3.624)
Free cholesterol in LDL	1.011 (1.005, 1.017)	0.996 (0.994, 0.998)	1.301 (0.407, 3.425)
Lactate/pyruvate	0.969 (0.963, 0.975)	1.010 (1.008, 1.012)	1.132 (0.504, 1.950)
Phospholipids in small LDL	1.008 (1.004, 1.013)	0.998 (0.996, 0.999)	1.031 (0.317, 2.680)
Acetone	1.036 (1.030, 1.042)	0.991 (0.989, 0.992)	0.975 (0.458, 1.648)
Tyrosine	1.005 (1.001, 1.009)	0.999 (0.997, 1.000)	0.903 (-0.296, 4.775)

Pyruvate	1.125 (1.116, 1.133)	0.973 (0.970, 0.975)	0.847 (0.403, 1.135)
Free cholesterol in medium LDL	1.012 (1.006, 1.019)	0.997 (0.995, 0.999)	0.832 (0.126, 2.332)
Cholesteryl esters in medium VLDL	1.014 (1.003, 1.025)	0.997 (0.993, 1.000)	0.829 (-0.115, 4.941)
Lactate	1.090 (1.084, 1.096)	0.983 (0.981, 0.984)	0.715 (0.213, 1.126)
Phenylalanine	1.015 (1.011, 1.020)	0.997 (0.996, 0.998)	0.696 (0.256, 1.391)
Free cholesterol in small LDL	1.012 (1.006, 1.019)	0.999 (0.997, 1.001)	0.210 (-0.546, 1.220)
Glucose	0.970 (0.965, 0.975)	1.002 (1.000, 1.003)	0.182 (0.002, 0.468)
Acetate	0.999 (0.999, 1.000)	1.000 (1.000, 1.000)	-0.475 (-3.971, 1.442)
Creatinine	1.006 (1.004, 1.009)	1.001 (1.000, 1.002)	-0.581 (-1.466, -0.103)
Triglycerides in chylomicrons and extremely large VLDL	0.915 (0.881, 0.980)	0.957 (0.946, 0.970)	-1.781 (-3.805, -0.792)
Docosahexanoic acid	1.008 (1.001, 1.014)	1.006 (1.004, 1.008)	-2.753 (-19.197, -0.834)
Total lipids in chylomicrons and extremely large VLDL	0.956 (0.935, 0.977)	0.967 (0.959, 0.973)	-2.835 (-6.345, -1.250)
Triglycerides in very large VLDL	0.978 (0.960, 0.996)	0.982 (0.976, 0.988)	-2.938 (-12.885, -1.085)
Glycine	1.004 (1.003, 1.005)	1.003 (1.003, 1.003)	-3.030 (-5.486, -1.325)
Chylomicrons and extremely large VLDL particles	0.954 (0.930, 0.978)	0.961 (0.953, 0.969)	-3.129 (-7.774, -1.301)
Free cholesterol in chylomicrons and extremely large VLDL	0.967 (0.949, 0.985)	0.970 (0.964, 0.976)	-3.312 (-7.905, -1.380)
Total lipids in very large VLDL	0.983 (0.968, 0.998)	0.983 (0.978, 0.988)	-3.537 (-17.331, -1.054)
Triglycerides in VLDL	0.987 (0.978, 0.997)	0.987 (0.984, 0.990)	-3.761 (-13.850, -1.295)
Phospholipids in chylomicrons and extremely large VLDL	0.951 (0.919, 0.985)	0.951 (0.940, 0.962)	-3.769 (-12.232, -1.368)
Phospholipids in very large HDL	0.984 (0.971, 0.998)	0.983 (0.979, 0.988)	-3.953 (-18.669, -1.175)
Alanine	1.006 (1.001, 1.011)	1.006 (1.005, 1.008)	-3.998 (-19.078, -1.401)
Phospholipids in medium HDL	0.993 (0.989, 0.997)	0.992 (0.990, 0.993)	-4.219 (-11.090, -1.704)
Histidine	1.007 (1.003, 1.010)	1.007 (1.006, 1.008)	-4.244 (-8.897, -1.724)
Cholesteryl esters in medium HDL	0.995 (0.990, 0.999)	0.993 (0.992, 0.995)	-4.404 (-21.566, -1.254)
Cholesterol in chylomicrons and extremely large VLDL	0.974 (0.957, 0.992)	0.969 (0.963, 0.975)	-4.492 (-13.131, -1.721)
Phospholipids in small HDL	0.996 (0.993, 0.999)	0.995 (0.994, 0.996)	-4.786 (-15.818, -1.833)
Triglycerides in medium HDL	0.988 (0.981, 0.995)	0.985 (0.983, 0.987)	-4.786 (-12.359, -1.932)
Cholesterol in medium HDL	0.995 (0.990, 0.999)	0.993 (0.991, 0.994)	-4.826 (-23.473, -1.441)
Total triglycerides	0.991 (0.983, 0.999)	0.987 (0.985, 0.990)	-4.884 (-21.717, -1.563)
Total lipids in medium HDL	0.994 (0.990, 0.998)	0.992 (0.991, 0.993)	-4.948 (-16.219, -1.946)

Medium HDL particles	0.994 (0.989, 0.998)	0.992 (0.990, 0.993)	-4.975 (-20.004, -1.868)
Phospholipids in HDL	0.994 (0.991, 0.998)	0.992 (0.991, 0.993)	-5.180 (-15.050, -2.055)
Cholesteryl esters in chylomicrons and extremely large VLDL	0.979 (0.961, 0.998)	0.967 (0.961, 0.973)	-5.771 (-29.955, -1.457)
Triglycerides in HDL	0.991 (0.985, 0.998)	0.986 (0.984, 0.989)	-5.811 (-19.697, -2.138)
Total lipids in HDL	0.996 (0.992, 0.999)	0.992 (0.991, 0.994)	-6.266 (-27.538, -2.153)
Free cholesterol in medium HDL	0.994 (0.989, 1.000)	0.989 (0.988, 0.991)	-6.479 (-33.149, -1.917)
Apolipoprotein A1	0.997 (0.994, 1.000)	0.994 (0.993, 0.995)	-6.659 (-40.021, -2.113)
Triglycerides in large HDL	0.991 (0.983, 0.998)	0.982 (0.979, 0.984)	-6.876 (-28.571, -2.212)
Glycoprotein acetyls	0.996 (0.993, 0.999)	0.993 (0.992, 0.993)	-7.075 (-21.798, -2.501)
Saturated fatty acids	0.995 (0.991, 1.000)	0.988 (0.987, 0.990)	-9.041 (-62.759, -2.344)

Abbreviations: HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

The association between $Tmax_0$ and mtDNA copy number per 10°C increase in $Tmax_0$ was -0.037 (-0.057, -0.017). Temperature–metabolites associations are also reported for 10°C increase in temperature. Metabolite-mtDNA copy number associations are reported for doubling of each metabolite.

Supplementary Table 10. Within-day difference in temperature ($Tmax_0$–$Tmin_0$) – mtDNA copy number – metabolites associations.

Metabolites	Temperature – metabolites	mtDNA copy number – metabolites	Proportion explained, median (95% bootstrapped CI)
Saturated fatty acids	1.006 (1.001, 1.011)	0.988 (0.987, 0.990)	11.275 (5.017, 46.977)
Beta-hydroxybutyrate	1.001 (1.000, 1.003)	0.998 (0.997, 0.998)	10.724 (4.318, 49.276)
Total fatty acids	1.005 (1.001, 1.010)	0.991 (0.989, 0.992)	10.009 (4.412, 39.705)
Omega-6 fatty acids	1.005 (1.001, 1.008)	0.994 (0.993, 0.995)	8.065 (3.858, 25.773)
Linoleic acid	1.006 (1.001, 1.010)	0.993 (0.991, 0.994)	7.944 (3.584, 28.154)
Total phospholipids in lipoprotein particles	1.006 (1.003, 1.010)	0.993 (0.992, 0.994)	7.042 (3.640, 16.644)
Total lipids	1.006 (1.002, 1.011)	0.993 (0.991, 0.994)	6.810 (3.283, 19.803)
Phosphoglycerides	1.007 (1.003, 1.011)	0.992 (0.991, 0.993)	6.789 (3.579, 15.681)
Phosphatidylcholines	1.007 (1.003, 1.011)	0.992 (0.991, 0.993)	6.570 (3.473, 15.250)
Total free cholesterol	1.006 (1.001, 1.011)	0.994 (0.991, 0.995)	6.495 (2.736, 28.395)
Phospholipids in large HDL	1.014 (1.005, 1.024)	0.985 (0.982, 0.988)	6.411 (3.139, 17.930)
Free cholesterol in large HDL	1.017 (1.005, 1.030)	0.982 (0.978, 0.986)	6.216 (2.870, 20.635)
Total cholines	1.007 (1.003, 1.010)	0.993 (0.992, 0.994)	6.170 (3.291, 13.208)
Phospholipids in HDL	1.008 (1.004, 1.012)	0.992 (0.991, 0.993)	6.071 (3.148, 12.826)
Total lipids in large HDL	1.014 (1.005, 1.024)	0.986 (0.983, 0.988)	6.071 (2.911, 17.615)
Polyunsaturated fatty acids	1.005 (1.002, 1.009)	0.995 (0.994, 0.996)	6.023 (2.920, 19.234)
Phospholipids in medium HDL	1.008 (1.004, 1.013)	0.992 (0.990, 0.993)	5.988 (3.087, 14.282)
Lage HDL particles	1.016 (1.005, 1.028)	0.984 (0.981, 0.987)	5.799 (2.737, 18.087)
Total lipids in HDL	1.009 (1.004, 1.013)	0.992 (0.991, 0.994)	5.229 (2.713, 10.810)
Free cholesterol in HDL	1.010 (1.005, 1.015)	0.991 (0.990, 0.993)	5.181 (2.763, 10.230)
Phospholipids in small HDL	1.006 (1.003, 1.009)	0.995 (0.994, 0.996)	5.170 (2.706, 11.758)
Total cholesterol	1.007 (1.003, 1.011)	0.994 (0.993, 0.995)	5.170 (2.472, 16.034)
Cholesterol in large HDL	1.016 (1.005, 1.029)	0.986 (0.983, 0.990)	4.997 (2.252, 18.313)
Total lipids in medium HDL	1.010 (1.005, 1.014)	0.992 (0.991, 0.993)	4.868 (2.619, 9.407)
Total esterified cholesterol	1.007 (1.003, 1.012)	0.994 (0.993, 0.995)	4.785 (2.329, 13.267)
Sphingomyelins	1.007 (1.004, 1.011)	0.994 (0.993, 0.995)	4.780 (2.579, 9.971)
Apolipoprotein A1	1.008 (1.004, 1.011)	0.994 (0.993, 0.995)	4.689 (2.563, 9.332)
Cholesteryl esters in large HDL	1.016 (1.004, 1.028)	0.988 (0.984, 0.991)	4.607 (1.928, 18.300)
Medium HDL particles	1.011 (1.006, 1.016)	0.992 (0.990, 0.993)	4.553 (2.398, 9.666)
Free cholesterol in medium HDL	1.014 (1.008, 1.020)	0.989 (0.988, 0.991)	4.531 (2.476, 9.446)
Phospholipids in large LDL	1.006 (1.001, 1.011)	0.996 (0.994, 0.997)	4.242 (1.547, 24.332)

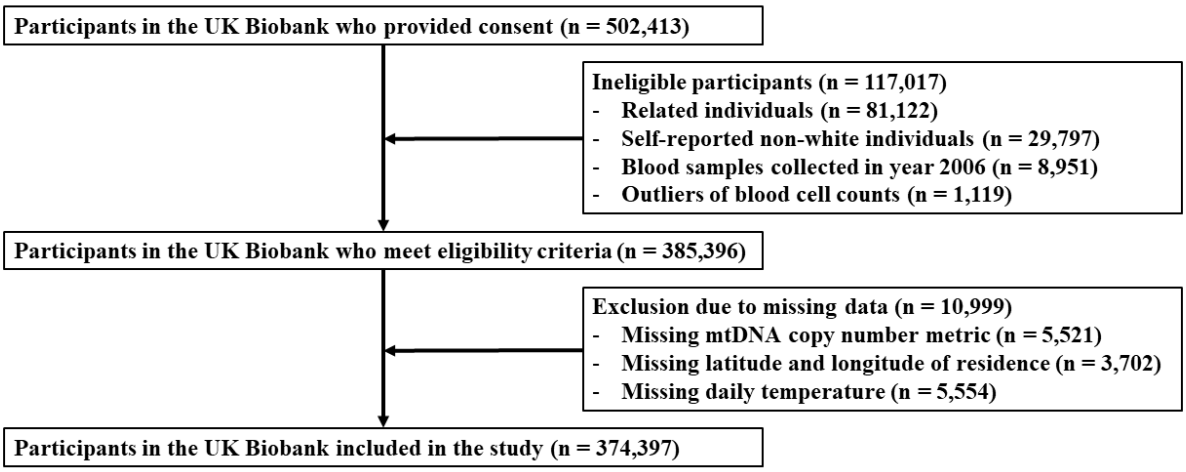
Total lipids in small HDL	1.006 (1.003, 1.010)	0.996 (0.995, 0.996)	4.008 (2.137, 8.392)
Total lipids in large LDL	1.008 (1.002, 1.013)	0.995 (0.994, 0.997)	3.779 (1.559, 14.060)
HDL cholesterol	1.010 (1.005, 1.015)	0.994 (0.992, 0.995)	3.724 (1.957, 7.704)
Free cholesterol in small HDL	1.008 (1.005, 1.011)	0.995 (0.994, 0.996)	3.708 (2.051, 6.797)
Total lipids in LDL	1.007 (1.002, 1.013)	0.995 (0.994, 0.997)	3.699 (1.449, 15.009)
Total lipoprotein particles	1.008 (1.005, 1.011)	0.995 (0.994, 0.996)	3.697 (2.012, 6.647)
Cholesterol in medium HDL	1.012 (1.006, 1.017)	0.993 (0.991, 0.994)	3.687 (1.946, 8.179)
Phospholipids in LDL	1.006 (1.001, 1.011)	0.996 (0.994, 0.998)	3.579 (1.247, 20.025)
Total lipids in small LDL	1.005 (1.000, 1.011)	0.997 (0.995, 0.998)	3.522 (0.947, 22.573)
HDL particles	1.009 (1.005, 1.012)	0.995 (0.994, 0.996)	3.513 (1.933, 6.408)
Cholesteryl esters in medium HDL	1.011 (1.006, 1.017)	0.993 (0.992, 0.995)	3.471 (1.791, 8.139)
Total lipids in medium LDL	1.007 (1.001, 1.013)	0.996 (0.994, 0.998)	3.445 (1.014, 22.323)
Lactate/pyruvate	0.983 (0.976, 0.990)	1.010 (1.008, 1.012)	3.351 (1.781, 6.605)
Cholesteryl esters in HDL	1.010 (1.005, 1.015)	0.994 (0.993, 0.996)	3.313 (1.678, 6.953)
Cholesteryl esters in large LDL	1.008 (1.003, 1.014)	0.995 (0.994, 0.997)	3.268 (1.273, 12.264)
Cholesterol in large LDL	1.008 (1.002, 1.014)	0.995 (0.994, 0.997)	3.248 (1.195, 12.803)
Phospholipids in medium LDL	1.007 (1.001. 1.013)	0.996 (0.995, 0.998)	2.878 (0.756, 18.204)
Phospholipids in small LDL	1.005 (1.000, 1.010)	0.998 (0.996, 0.999)	2.706 (0.632, 17.256)
Pyruvate	1.063 (1.054, 1.072)	0.973 (0.970, 0.975)	2.638 (1.623, 3.703)
Small HDL particles	1.007 (1.004, 1.010)	0.997 (0.996, 0.998)	2.268 (1.171, 4.343)
Lactate	1.046 (1.040, 1.052)	0.983 (0.981, 0.984)	2.198 (1.354, 3.141)
Cholesterol in small HDL	1.008 (1.004, 1.011)	0.997 (0.997, 0.998)	1.936 (0.950, 3.699)
Cholesteryl esters in small HDL	1.006 (1.003, 1.010)	0.998 (0.997, 0.999)	1.405 (0.379, 4.842)
Tyrosine	1.006 (1.001, 1.011)	0.999 (0.997, 1.000)	1.217 (-0.373, 6.648)
Glucose	0.984 (0.978, 0.097)	1.002 (1.000, 1.003)	0.584 (0.042, 1.440)
Omega-3 fatty acids	1.012 (1.002, 1.021)	1.003 (1.000, 1.005)	-1.321 (-8.305, 0.122)
Isoleucine	0.988 (0.981, 0.995)	0.995 (0.993, 0.997)	-2.332 (-6.782, -0.959)
Docosahexanoic acid	1.014 (1.006, 1.022)	1.006 (1.004, 1.008)	-2.482 (-6.373, -1.102)
Histidine	1.010 (1.006, 1.014)	1.007 (1.006, 1.008)	-4.340 (-7.480, -2.406)
Albumin	1.003 (1.001, 1.005)	1.004 (1.003, 1.004)	-8.808 (-35.023, -3.750)

Abbreviations: HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein.

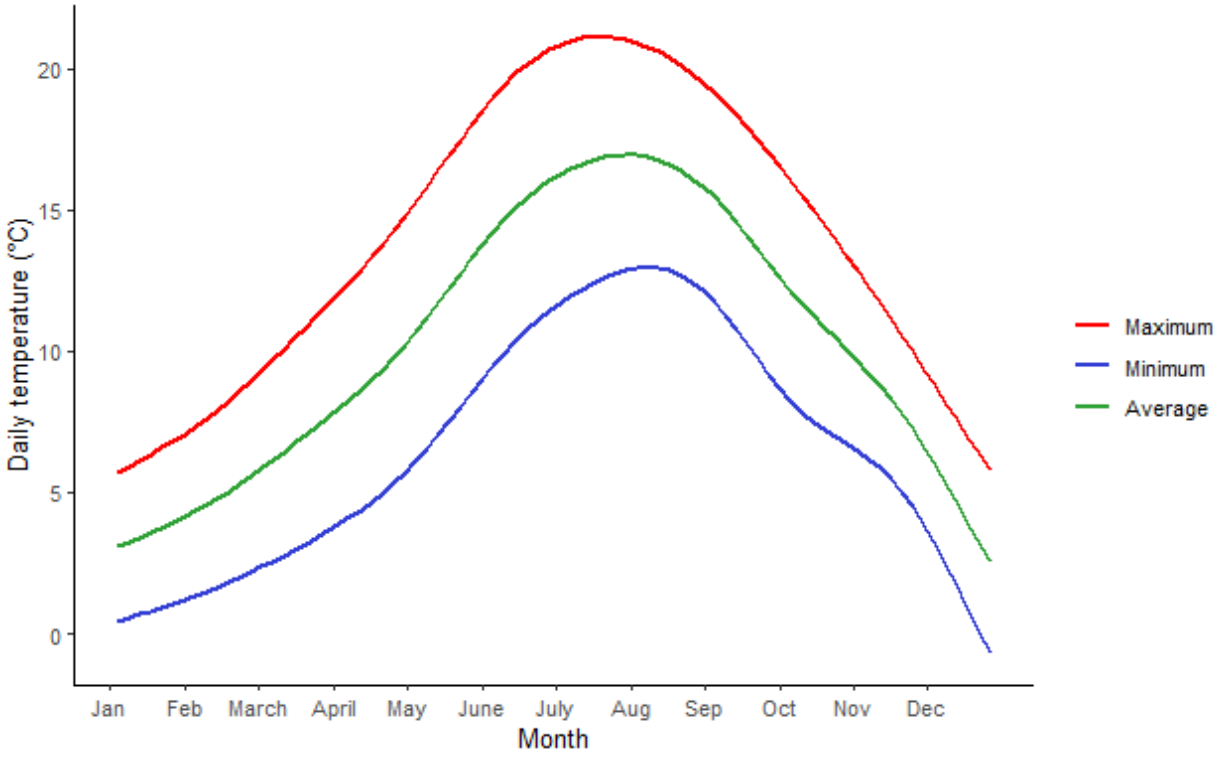
The association between $Tmax_0$–$Tmin_0$ and mtDNA copy number per 10°C increase in $Tmax_0$–$Tmin_0$ was -0.058 (-0.081, -0.036).

Temperature–metabolites associations are also reported for 10°C increase in temperature. Metabolite-mtDNA copy number associations are reported for doubling of each metabolite.

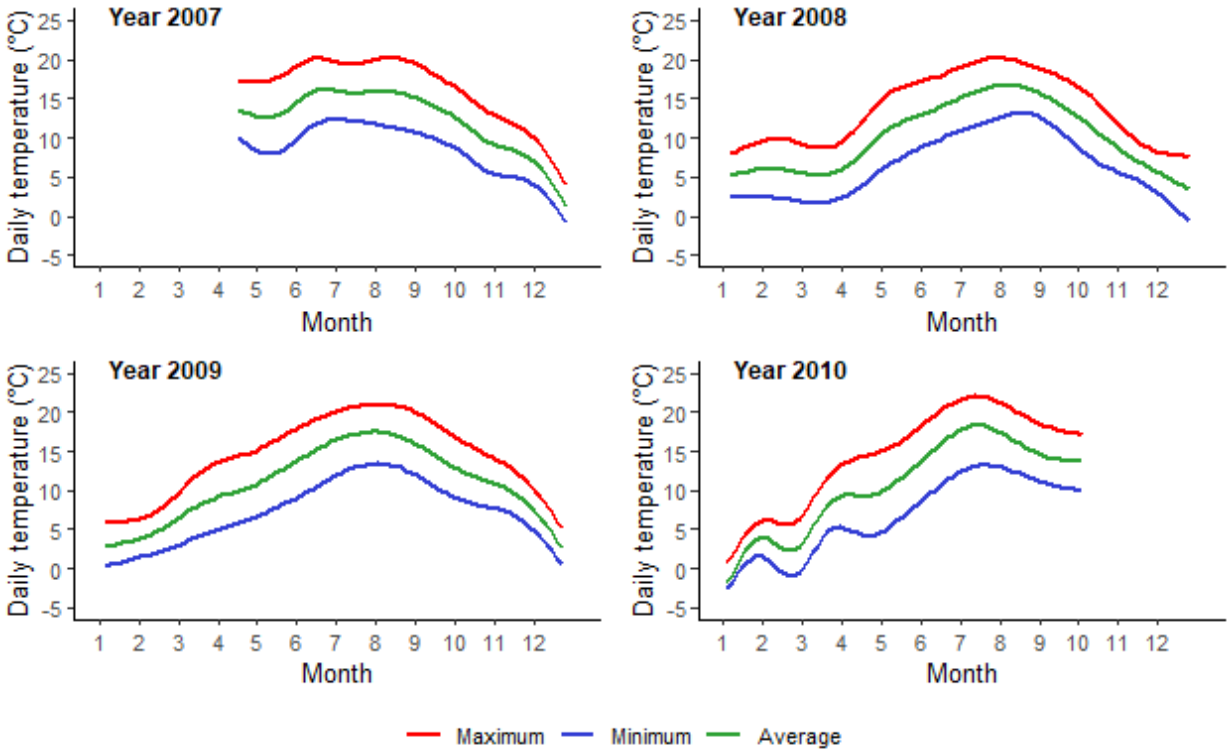
Supplementary Figure 1. Flowchart of study participants.



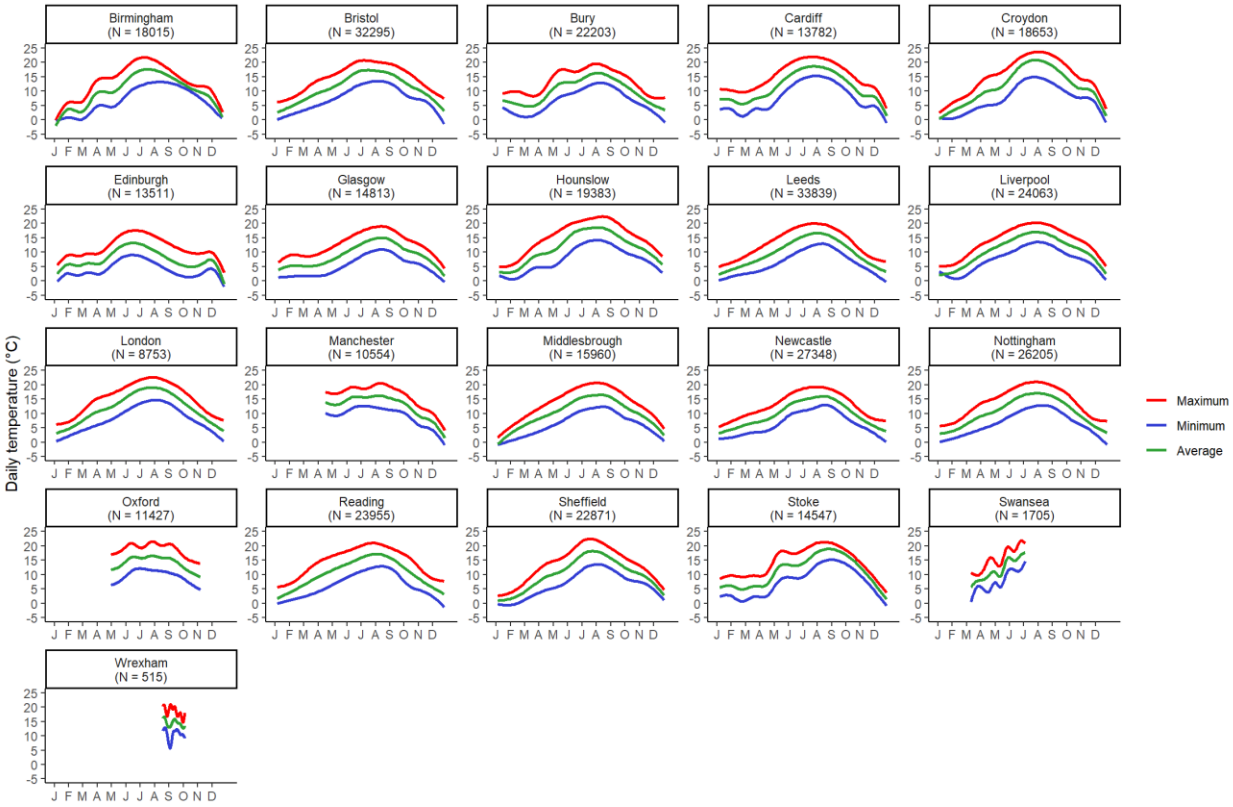
Supplementary Figure 2. Distribution of temperature.



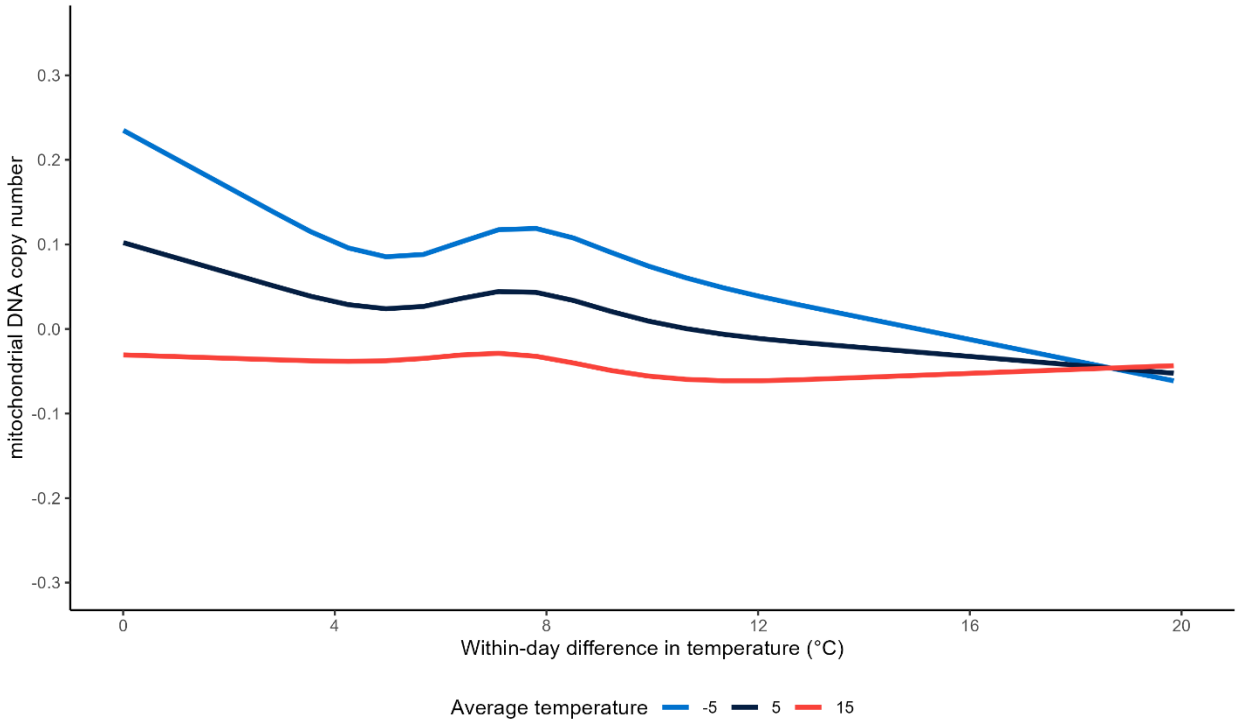
Supplementary Figure 3. Distribution of temperature by year.



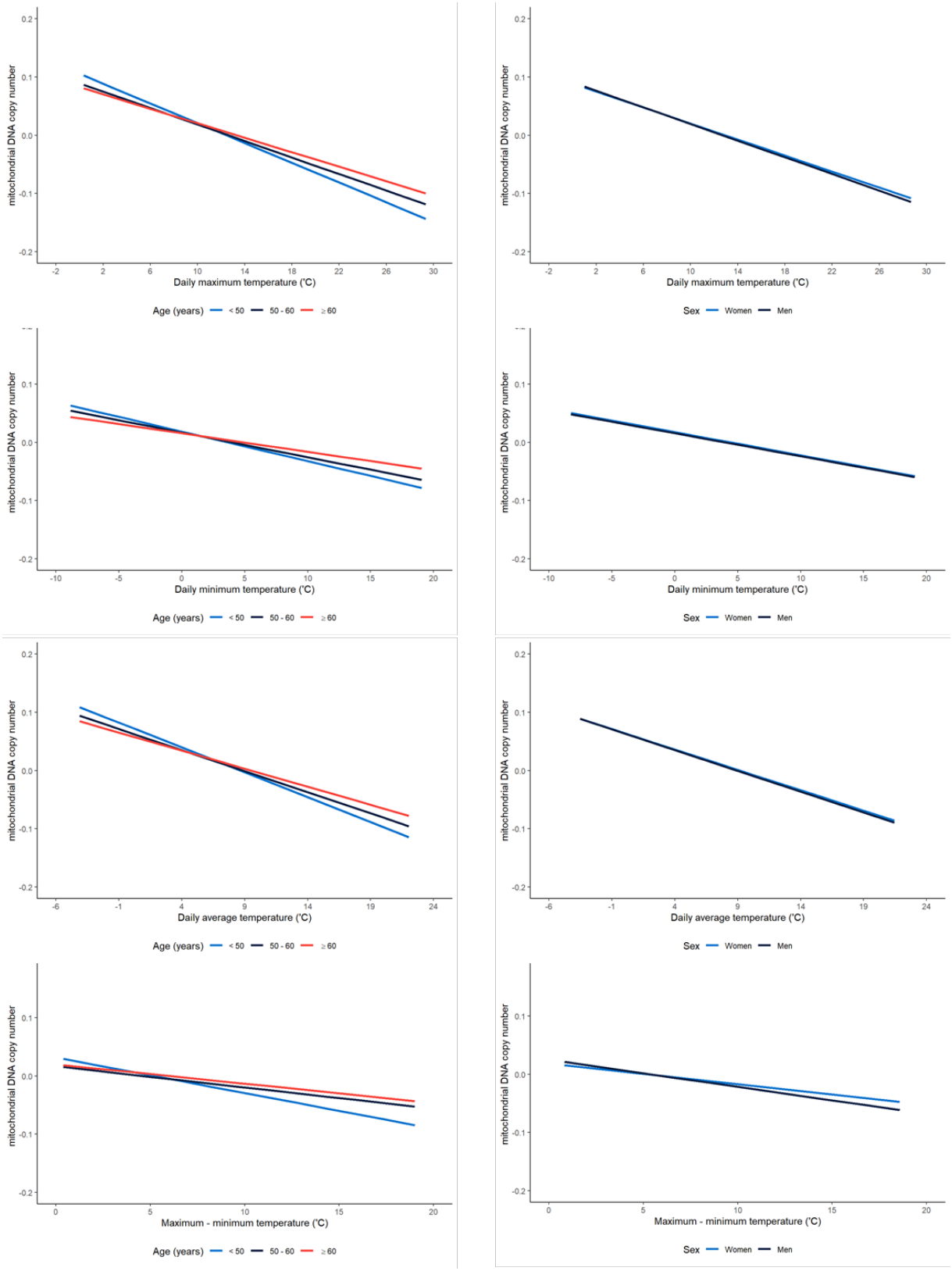
Supplementary Figure 4. Distribution of temperature by center.



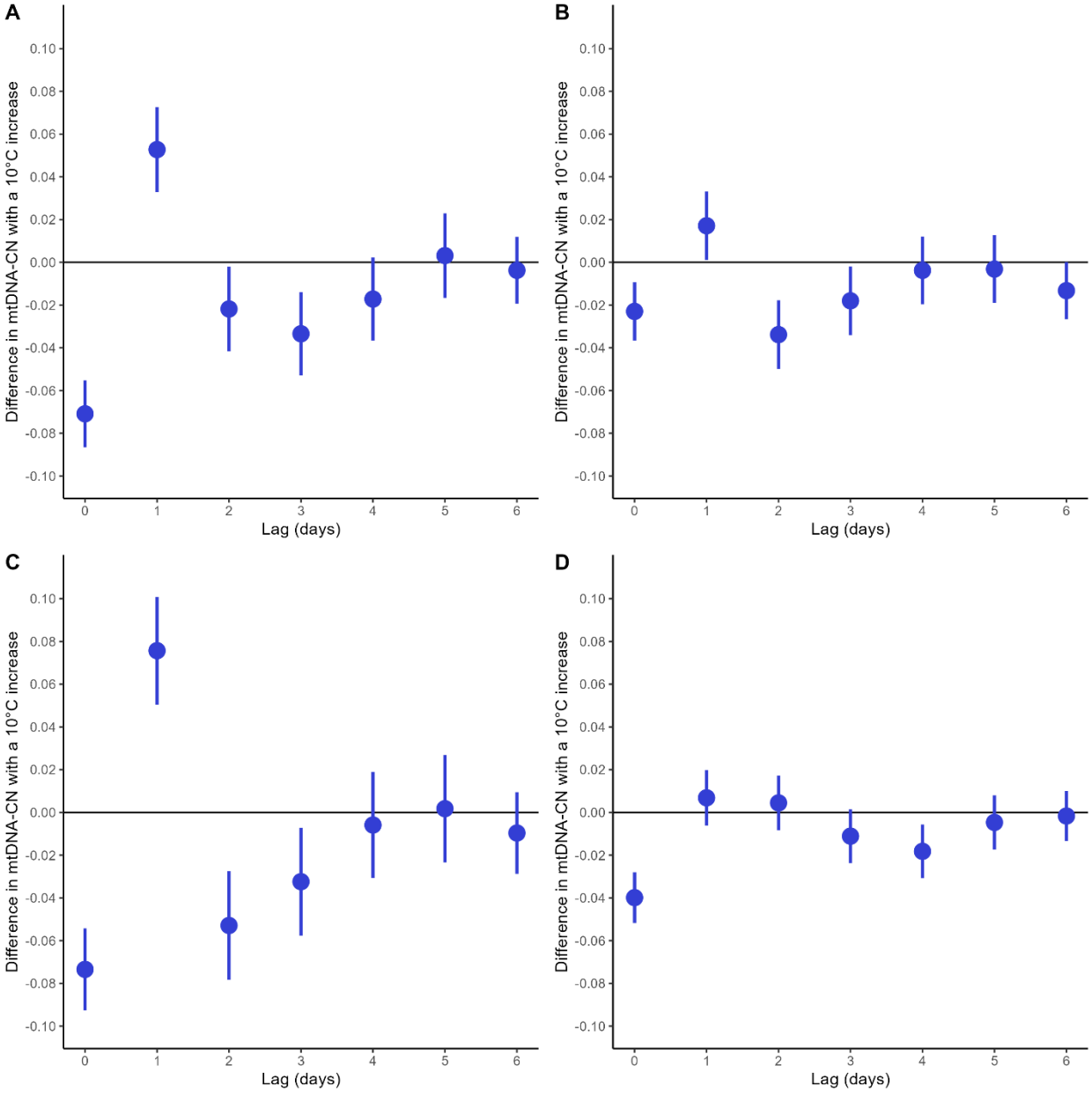
Supplementary Figure 5. Within-day difference in temperature and average mtDNA copy numbers by daily average temperature.



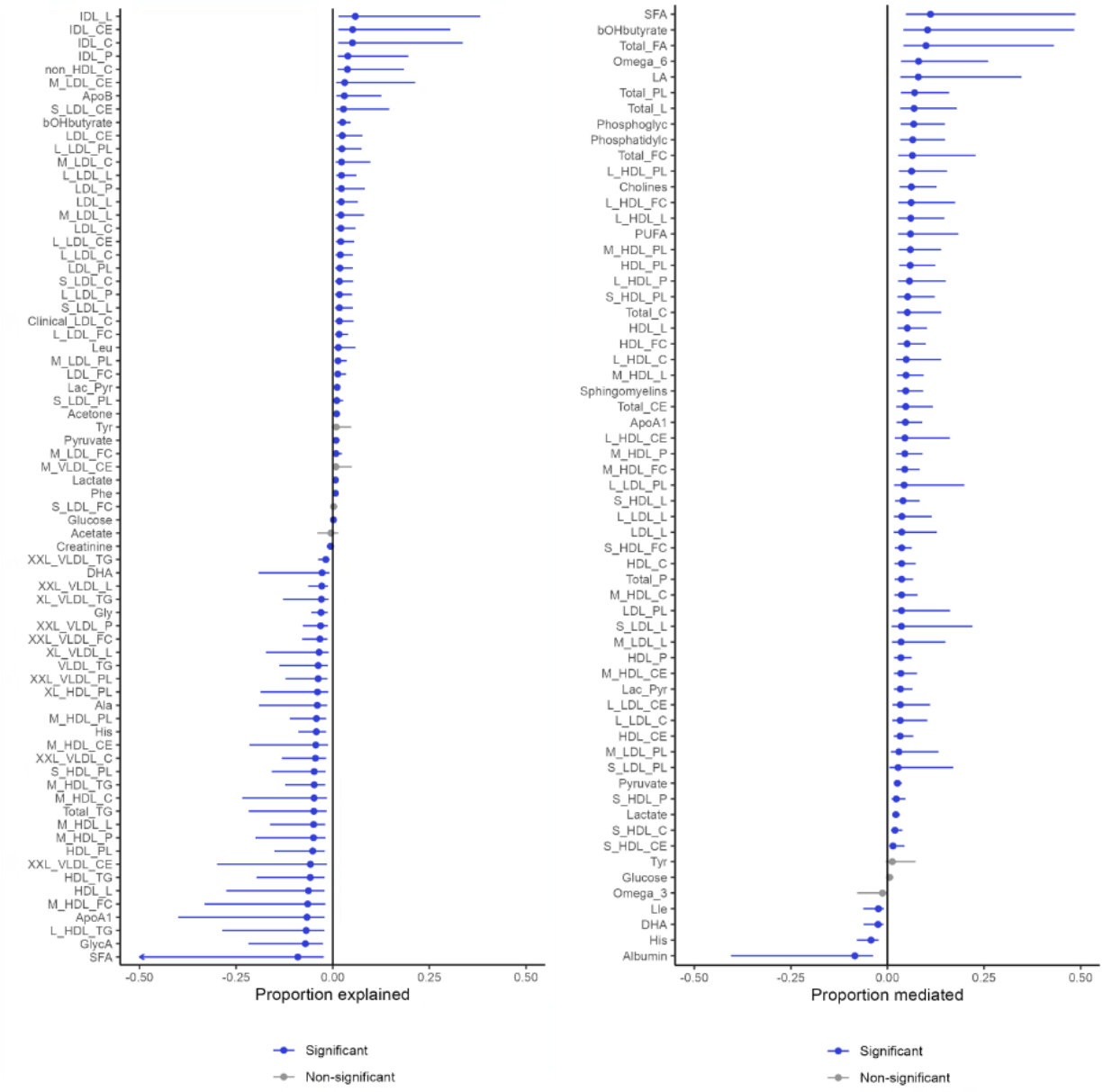
Supplementary Figure 6. Ambient temperature and average mtDNA copy numbers by age and sex.



Supplementary Figure 7. Difference in mtDNA copy number with 10°C increase in ambient temperature by lag.



Supplementary Figure 8. Proportion explained by mtDNA copy number in the association of daily maximum temperature (A) and within-day temperature (B) with individual metabolites.



Chapter 4

Mitochondrial DNA copy number and incident heart failure: The Atherosclerosis Risk in Communities (ARIC) study

ABSTRACT

Background: The association between mitochondrial DNA-copy number (mtDNA-CN) and incident heart failure (HF) in the general population is unclear.

Methods: We examined the association between mtDNA-CN and the risk of incident HF among 10,802 participants free of HF at baseline from the Atherosclerosis Risk in Communities (ARIC) study, a large bi-racial population-based cohort. mtDNA-CN was estimated using probe intensities on the Affymetrix Genome-Wide Human single nucleotide polymorphisms Array 6.0. Incident HF events were identified through hospital discharge codes from 1987 until 2005 and through adjudication by the ARIC HF Classification Committee since 2005.

Results: During a median follow-up of 23.1 years, there were 2,227 incident HF events (incidence rate 10.3 per 1000 person-years). In fully adjusted models, the hazard ratios (95% confidence intervals) for HF comparing the 2nd through 5th quintiles of mtDNA-CN to the 1st quintile were 0.91 (0.80–1.04), 0.82 (0.72–0.93), 0.81 (0.71–0.92), and 0.74 (0.65–0.85), respectively (P for trend < 0.001). In stratified analyses, the associations between mtDNA-CN and HF were similar across examined subgroups. The inverse association between mtDNA-CN and incident HF was stronger in HF with reduced ejection fraction (HFrEF) than in HF with preserved ejection fraction (HFpEF).

Conclusions: In this prospective cohort, mtDNA-CN was inversely associated with the risk of incident HF suggesting that reduced levels of mtDNA-CN, a biomarker of mitochondrial dysfunction, could reflect early susceptibility to HF.

INTRODUCTION

Heart failure (HF) is a leading clinical and public health concern affecting 23 million people globally and 6.2 million adults in the United States alone.[1,2] The lifetime risk of developing HF is 20% and the prevalence of HF in the US is expected to increase by nearly 50% by the year 2030.[3,4] Despite recent improvements, the prognosis of HF is still poor, with 5-year mortality >40%.[5-8] Thus, it is critical to identify novel pathways that can help design new preventive strategies and characterize subjects at high risk of developing HF.

Mitochondria generate nearly all energy used by the cell as adenosine triphosphate (ATP).[9] Each mitochondrion has 2 to 10 copies of mitochondrial DNA (mtDNA), for a total of 10^3 to 10^4 copies of mtDNA per cell. mtDNA copy number (mtDNA-CN) changes with energy demands and with oxidative stress, and has been established as an indirect biomarker of mitochondrial dysfunction.[10,11] Reduced mtDNA-CN measured in peripheral blood is associated with cardiovascular disease (CVD), all-cause mortality, hypertension, diabetes, chronic kidney disease, and sudden cardiac death.[12-18]

In a case-control study, hospitalized patients with HF had lower mtDNA-CN in peripheral blood compared to controls without HF, and when HF cases were followed up, those with lower mtDNA-CN had a higher risk of cardiovascular death and rehospitalization compared to those with higher mtDNA-CN.[19] In small case-control studies, depletion of mtDNA in heart tissue samples was associated with HF.[20,21] The association between mtDNA-CN and incident HF in the general population, however, is unknown. In the present study, we examined the association between baseline mtDNA-CN and the risk of incident HF in the Atherosclerosis Risk in Communities (ARIC) study, a large bi-racial population-based cohort.

METHODS

Study population

The ARIC study is a population-based prospective cohort of 15,792 individuals 45–65 years of age at the time of recruitment (1987–1989; Visit 1). ARIC participants were recruited from 4 US communities: Forsyth County, NC; Jackson, MS; suburban Minneapolis, MN; and Washington County, MD.[22] Since the first study visit, there have been 6 subsequent in-person visits (visits 2–7, with visit 7 currently underway) and regular telephone interviews (annually and then semiannually since 2012). Our analysis was restricted to 11,453 White or Black participants who had DNA collected in one of the visits to generate mtDNA-CN measurements (**Supplementary Figure 1**). We then excluded Black participants recruited from Minnesota or Maryland (n = 1), participants without follow-up information (n = 1), and participants with prevalent HF at the time of DNA collection (n = 596). We further excluded participants missing information on body mass index (n = 17) and high-density lipoprotein (HDL) cholesterol (n = 14). The final sample included 10,802 participants (4,918 men and 5,904 women without HF at the time of DNA sampling). All centers obtained approval from their respective institutional review boards and all participants provided written informed consent.

Measurements

ARIC participants underwent a comprehensive cardiovascular examination and interview by trained clinical staff members during each clinic visit. Age, sex, race/ethnicity, smoking status, alcohol intake, and medication history were self-reported. Smoking and alcohol intake were categorized as never, former, and current. Body mass index (BMI) was calculated from measured height and weight and categorized as underweight/normal (<25 kg/m^2), overweight

(≥25 to <30 kg/m^2), or obese (≥30 kg/m^2). Blood samples were collected for glucose, total cholesterol, and HDL-cholesterol measurements.

Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or current use of anti-hypertensive medication. Diabetes was defined as self-reported physician diagnosis of diabetes, fasting glucose ≥126 mg/dL, non-fasting glucose ≥200 mg/dL, or use of hypoglycemic medication. Prevalent coronary heart disease (CHD) was defined as the presence of a myocardial infarction based on self-report or electrocardiogram in visit 1, or the development of an adjudicated definite or probable myocardial infarction prior to the time of DNA collection for mtDNA-CN measurement.

Measurement of mtDNA copy number

The methods for measuring mtDNA-CN have been described previously.[17,23] Briefly, DNA was extracted using the Gentra Puregene Blood Kit (Qiagen N.V., Venlo, The Netherlands) from buffy coat of whole blood samples collected in visits 1–4. mtDNA-CN was calculated from probe intensities of mitochondrial single nucleotide polymorphisms (SNP) on the Affymetrix Genome-Wide Human SNP Array 6.0 using the Genvisis software package (www.genvisis.org), which uses the median mitochondrial probe intensity of 25 high-quality mitochondrial probes as initial raw measure of mtDNA-CN. Batch effects, DNA quality, and starting DNA quantity were corrected for by using surrogate variable analysis applied to probe intensities of 43,316 autosomal SNPs.[24] The mtDNA-CN metric used in this analysis was obtained as the standardized residuals (mean 0 and standard deviation 1) in a linear regression in which initial raw estimates of mtDNA-CN were regressed against age, sex, enrollment center, surrogate variables used in the surrogate variable analysis, and white blood cell count. White

blood cell count was not available in 14.9% of individuals and we imputed the mean for the missing values.[23] DNA for mtDNA-CN for this analysis was obtained from ARIC visit 1 (1987–1989) in 429 participants, visit 2 (1990–1992) in 8,655 participants, visit 3 (1993–1995) in 1,654 participants, and visit 4 (1996–1998) in 64 participants. For each participant, we used the visit in which DNA for mtDNA-CN assays was obtained as the baseline visit.

Outcome definition and adjudication

Incident HF was defined as the first hospitalization for HF or death related to HF after the visit in which DNA was obtained for mtDNA-CN assays. Hospitalizations and deaths related to HF were identified as International Classification of Disease, 9th Revision code 428, and International Classification of Diseases, 10th Revision code I50 in discharge codes or in underlying cause of death, respectively. Since 2005, ARIC began adjudication of HF events by the ARIC HF Classification Committee.[25] In addition, when available, adjudicated incident HF was further classified as HF with reduced ejection fraction (HFrEF, most recent left ventricular ejection fraction [LVEF] <50%), HF with preserved ejection fraction (HFpEF, LVEF ≥50%), or HF with unknown LVEF.

Statistical analysis

Study participants were followed from the visit of DNA collection for mtDNA-CN assay until the development of HF, death, loss to follow-up, or December 31, 2017, whichever came first. We used a Cox proportional hazards model to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the association between mtDNA-CN and incident HF. mtDNA-CN was first categorized into quintiles based on the overall sample distribution. In secondary

analysis, mtDNA-CN was modeled as a continuous variable to estimate the HR for incident HF comparing the 90th to the 10th percentile of mtDNA-CN. In addition, mtDNA-CN was modeled as restricted quadratic splines with knots at the 5th, 50th, and 95th percentiles to provide a smooth and flexible description of the dose-response relationship between mtDNA-CN and HF. We tested for the proportional hazards assumption using Schoenfeld residuals but the assumption was not met ($P < 0.001$). Therefore, to allow the effect of mtDNA-CN on incident HF to vary by time, we used a parametric survival models with spline variables created separately for baseline hazard and for time-dependent effects.[26]

To control for potential confounders, we used 4 models with progressive degrees of adjustment using covariates measured at the time of mtDNA-CN measurement: Model 1 was adjusted for age, sex, race, and enrollment center; Model 2 was further adjusted for BMI, smoking, and alcohol intake; Model 3 was further adjusted for total and HDL-cholesterol, cholesterol medication, hypertension, and diabetes; and Model 4 was further adjusted for prevalent CHD.

We performed stratified analyses by pre-specified subgroups defined by age (<60 or ≥60 years), sex, race (White or Black), smoking status (never, former, or current), alcohol intake (never, former, current), BMI (underweight/normal, overweight, or obese), and prevalent CHD status. We also performed several sensitivity analyses. First, we repeated the main analyses treating non-HF related deaths as a competing event using a proportional sub-distribution hazards model. Second, we used alternative definitions for incident HF: 1) discharge codes for HF for the entire follow-up period, regardless of adjudication; 2) HF events restricted to adjudicated cases that occurred since January 1, 2005, and 3) discharge codes for HF restricted to cases that occurred since January 1, 2005. For the two analyses restricted to events since 2005,

we used late entries to address the issue of immortal person time prior to 2005. Third, also using HF events adjudicated after 2005 and late entries, we estimated the HRs for HFrEF and HFpEF separately using a proportional sub-distribution hazards model with non-HF related deaths and unknown type of HF as competing events. All statistical analyses were performed using Stata version 15.0 (StataCorp LP, College Station, TX, USA).

RESULTS

The mean age (standard deviation) of study participants at baseline was 57.3 (5.9) years (**Table 1**). Participants with lower mtDNA-CN levels were more likely to be current smokers, to have a higher prevalence of hypertension, diabetes, and CHD, and to have lower HDL-cholesterol levels than those with higher mtDNA-CN levels.

During a median follow-up of 23.1 years, we identified 2,227 new cases of HF (incidence rate of 10.3 per 1000 person-years). In fully adjusted models, the HRs (95% CI) for HF comparing the 2nd through 5th quintiles of mtDNA-CN to the 1st quintile were 0.91 (0.80–1.04), 0.82 (0.72–0.93), 0.81 (0.71–0.92), and 0.74 (0.65–0.85), respectively (*P* for trend < 0.001; **Table 2**). The fully adjusted HR for HF comparing the 90th to the 10th percentile of mtDNA-CN was 0.76 (0.69–0.84). In spline regression analysis, mtDNA-CN was inversely associated with the risk of incident HF with an approximately linear dose-response relationship (P-value for non-linear spline terms 0.74; **Figure 1**). The results were similar when we used hospital discharge codes for HF, adjudicated HF cases since 2005, or hospital discharge codes for HF since 2005, or when non-HF related deaths were treated as a competing event (**Supplementary Tables 1–4**).

In stratified analyses, the associations between mtDNA-CN and HF were similar across subgroups defined by age, sex, race, smoking status, and history of CHD (**Figure 2**). The

associations were weaker in participants who currently drink alcohol compared to those who did not drink (*P* for interaction = 0.01), and in participants who were overweight compared to those who were underweight or normal weight (*P* for interaction = 0.05).

When we tested the proportional hazards assumption, the association between mtDNA-CN and HF was progressively attenuated from the time of mtDNA-CN measurement (**Table 3 and Figure 3**). The fully adjusted HRs for HF comparing the 90th to the 10th percentile of mtDNA-CN at 10, 20, and 30 years since mtDNA-CN measurement were 0.65 (0.54–0.79), 0.89 (0.76–1.04), and 0.99 (0.72–1.37), respectively.

Finally, when we separated HF events into HFpEF and HFrEF using data since 2005, the inverse association between mtDNA-CN and incident HF was stronger in HFrEF than in HFpEF (**Table 4)**, although the trend was not statistically significant in either type of HF (*P* for trend 0.73 and 0.12 in HFpEF and HFrEF, respectively).

DISCUSSION

In this large community-based prospective cohort, mtDNA-CN was inversely associated with the risk of incident HF. The association was approximately linear and consistent across population subgroups. The association, however, was strongest early after measurement of mtDNA-CN and was progressively attenuated over 30 years of follow-up. These findings suggest a potential role of mtDNA-CN as an early indicator for HF, particularly for events in the near future.

Decreased mtDNA-CN is associated with CVD events, including sudden cardiac death, all-cause mortality, hypertension, diabetes, and chronic kidney disease.[12-18] However, the association between mtDNA-CN and incident HF is largely unknown. In animal studies, mtDNA damage and depletion were associated with the development of dilated cardiomyopathy and

impaired left ventricular remodeling after ischemic injury.[27-30] In small case-control studies, patients with HF had decreased mtDNA-CN levels in heart tissue samples compared to controls without HF.[21,31] In another case-control study, hospitalized patients with HF had lower mtDNA-CN in peripheral blood compared to those without HF.[19] When patients with HF were followed up for a median of 17 months, those with peripheral blood mtDNA-CN levels below the median were more likely to experience cardiovascular death and rehospitalization compared to those with mtDNA-CN levels above the median.[19]

The mechanisms underlying the association between mtDNA-CN and HF are unclear. Established risk factors for HF include hypertension, diabetes, metabolic syndrome, ischemic heart disease, and non-ischemic cardiomyopathies,[32,33] and oxidative stress is assumed to play a major role in the development and progression of HF.[34-38] Endogenous mtDNA damage is mainly caused by reactive oxygen species (ROS) as a by-product of oxidative phosphorylation. High levels of ROS trigger further increases in ROS generation, a process known as mitochondrial ROS-induced ROS release.[39] Exogeneous agents, such as cigarette smoke, industrial by-products, ultraviolet and ionizing radiation, environmental toxins and chemicals, and chemotherapeutic drugs may also damage mtDNA.[40] mtDNA is particularly susceptible to damage and mutations due to its close proximity to mitochondrial ROS production sites, lack of protective histones, and limited repair activity.[34] Moreover, damage caused by ROS is more extensive and persists longer in mtDNA than in nuclear DNA.[41]

Mitochondrial ROS generation and mtDNA damage in cardiomyocytes lead to impaired electron chain transport and ATP synthesis, modifications to proteins involved in excitation-contraction coupling, and activation of hypertrophy signaling kinases and transcription factors, apoptotic pathways, inflammatory mediators, and matrix metalloproteinases.[34,42,43] This process

leads to a reduction in the number of mitochondria, hypertrophy, apoptosis, and necrosis of cardiomyocytes, impairment of contractile function, and fibrosis, which, in combination, lead to the initiation and progression of cardiac remodeling and ultimately to HF. Systemic oxidative stress is also closely linked to the development of vascular diseases, such as hypertension, which are major risk factors for HF.[34,38,44,45] Finally, treatment with chemotherapeutic agents that inhibit DNA replication, such as nucleoside reverse transcriptase inhibitors or anthracycline, is also associated with both mtDNA depletion and cardiomyopathy.[46-48]

In our study, mtDNA-CN measurements were derived from the buffy coat of peripheral blood and were not a direct measurement of mtDNA-CN in cardiomyocytes. Leukocyte mtDNA-CN was correlated with mtDNA-CN in cardiomyocytes in one study.[19] In another study of non-ischemic cardiomyopathy patients, there was a moderate correlation between whole blood mtDNA-CN and myocardial mtDNA-CN.[49] Additional research is needed to understand the association between mtDNA-CN in peripheral blood and in target tissues, and to elucidate the mechanisms linking mtDNA-CN in peripheral blood with incident HF.

Other limitations also need to be considered in the interpretation of our findings. First, mtDNA-CN was measured only once and we could not evaluate changes in mtDNA-CN after the baseline visit. In fact, the association between mtDNA-CN and incident HF decreased progressively over follow-up, although mtDNA-CN was inversely associated with HF for at least two decades after measurement. Second, systematic adjudication of HF events only occurred after 2005. However, the sensitivity and positive predictive value of ICD-9 codes for HF compared to adjudicated acute decompensated HF and chronic HF in ARIC were relatively high at 0.95 and 0.77, respectively, and the results from our sensitivity analyses were consistent for analyses based on discharge codes and for those based on adjudicated events.[25] Third, our

analysis of the specific association of mtDNA-CN levels and subtypes of HF was restricted to events occurring after 2005, and we had limited power to identify differences between HFpEF and HFrEF. Finally, we were not able to evaluate the association between mtDNA-CN level and the severity of HF symptoms as such information was not available for all participants.

The major strengths of this study include the prospective design with a long duration of follow-up to capture HF events, the large sample size, the high quality of field and laboratory methods of the ARIC study, and the ability to account for multiple potential confounders for the association between mtDNA-CN and incident HF. In addition, we used state-of-the art tools to measure mtDNA-CN.[23]

CONCLUSIONS

In this large community-based prospective cohort, mtDNA-CN was inversely associated with the risk of incident HF suggesting that reduced levels of mtDNA-CN, a biomarker of mitochondrial dysfunction, could reflect early susceptibility to HF. Further studies are needed to better understand the underlying mechanisms and to characterize the association of mtDNA-CN with different types of HF and their severity.

ACKNOWLEDGEMENTS

The authors thank the staff and participants of the ARIC study for their important contributions.

SOURCES OF FUNDING

US National Institutes of Health grants (R01HL131573 to E.G., R.J.L., C.A.C., D.E.A.; and R01HL111267 to R.J.L., D.E.A.). The Atherosclerosis Risk in Communities study has been funded in whole or in part with Federal funds from the National Heart, Lung, and Blood Institute, National Institutes of Health, Department of Health and Human Services (contract numbers HHSN268201700001I, HHSN268201700002I, HHSN268201700003I, HHSN268201700004I and HHSN268201700005I), R01HL087641, R01HL059367, R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research.

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FIGURE LEGENDS

Figure 1. Hazard ratios for incident heart failure by levels of mitochondrial DNA copy number.

The curves represent adjusted hazard ratios (solid line) and their 95% confidence intervals (dotted lines) based on restricted quadratic splines of mtDNA copy number with knots at 5th, 50th, and 95th percentiles of its distribution. The reference value (diamond dot) was set at the 10th percentile of the distribution. Results were obtained from a Cox model adjusted for age, sex, race/ethnicity, body mass index, smoking, alcohol intake, total and HDL cholesterol, cholesterol medication, hypertension, diabetes, and prevalent coronary heart disease. Histograms represent the frequency distribution of mtDNA copy number at baseline.

Figure 2. Hazard ratios for incident heart failure comparing the 90th to the 10th percentile of mitochondrial DNA copy number in selected subgroups.

The figure includes hazard ratios for comparing the 90th to the 10th percentile (reference) of mtDNA copy number. Pre-specified subgroups were age (<60 or ≥60 years), sex, race (White or Black), smoking status (never, former, or current), alcohol intake (never, former, current), BMI (underweight/normal, overweight, or obese), and prevalent CHD. Models were adjusted for age, sex, race/ethnicity, body mass index, smoking, alcohol intake, total and HDL cholesterol, cholesterol medication, hypertension, diabetes, and prevalent coronary heart disease.

Figure 3. Time-dependent hazard ratios for incident heart failure comparing the 90th to the 10th percentile of mitochondrial DNA copy number.

The curve represents time-dependent adjusted hazard ratios (solid line) and the gray band represents its corresponding 95% confidence interval comparing the 90th to the 10th percentile of

mtDNA copy number. The dashed line represents the time-fixed adjusted hazard ratio comparing the 90^{th} to the 10^{th} percentile of mtDNA copy number. Models were adjusted for age, sex, race/ethnicity, body mass index, smoking, alcohol intake, total and HDL cholesterol, cholesterol medication, hypertension, diabetes, and prevalent coronary heart disease.

Table 1. Baseline characteristics of study participants by quintile of mitochondrial DNA copy number.

	Overall (n = 10,802)	**Quintile 1 (n = 2,161)**	**Quintile 2 (n = 2,160)**	**Quintile 3 (n = 2,161)**	**Quintile 4 (n = 2,160)**	**Quintile 5 (n = 2,160)**	***P*-values[†]**
mtDNA-CN range	-5.16, 4.84	-5.16, -0.72	-0.72, -0.16	-0.16, 0.29	0.29, 0.78	0.78, 4.84	
Age (year)	57.3 (5.9)	57.3 (6.0)	57.3 (6.1)	57.3 (5.9)	57.2 (5.9)	57.3 (5.8)	0.78
BMI (kg/m^2)	27.8 (5.3)	27.8 (5.4)	27.6 (5.1)	28.1 (5.4)	27.7 (5.3)	27.9 (5.3)	0.78
Male (%)	45.4	45.2	44.9	46.3	44.5	46.2	0.71
Race (%)							0.61
White	79.2	80.3	79.1	78.9	78.3	79.3	
Black	20.8	19.7	20.9	21.2	21.7	20.7	
Smoking (%)							0.02
Never	38.6	36.6	40.2	39.0	38.8	38.5	
Former	38.5	37.7	36.8	38.8	39.7	40.0	
Current	22.7	25.6	23.1	22.2	21.5	21.5	
Current drinker (%)	58.0	55.3	59.0	57.5	61.1	57.9	0.003
TC (mg/dl)	209.7 (39.4)	209.5 (41.7)	208.8 (39.3)	209.3 (37.3)	209.5 (38.0)	211.6 (40.7)	0.07
HDL-C (mg/dl)	49.8 (17.1)	48.4 (16.8)	49.5 (17.1)	49.8 (16.7)	51.2 (17.7)	49.9 (17.2)	< 0.001
TG (mg/dl)[*]	115 (83–162)	117 (84–164.5)	116 (83–162)	115 (82–165)	109 (81–158)	118 (85–162)	0.003
SBP (mmHg)	122.0 (19.1)	122.7 (19.6)	122.0 (19.9)	122.0 (18.4)	121.6 (18.9)	121.6 (18.6)	0.05
Prevalent CHD (%)	4.8	6.5	5.0	4.8	3.9	4.0	0.001
Hypertension (%)	34.6	37.0	35.1	33.6	33.2	34.5	0.08
Diabetes (%)	14.0	17.3	14.2	13.5	12.1	12.9	< 0.001

Abbreviations: BMI, body mass index; CHD, coronary heart disease; HDL-C, high-density lipoprotein-cholesterol; mtDNA-CN, mitochondrial DNA copy number; SBP, systolic blood pressure; TC, total cholesterol; and TG, triglyceride.

[*] Median (interquartile range).

[†] *P* for trend for continuous variables; *P* values from χ^2 tests for categorical variables.

Table 2. Hazard ratios for incident heart failure by levels of mitochondrial DNA copy number.

mtDNA-CN	N events / N total	Incidence rate per 1,000	Model 1	Model 2	Model 3	Model 4
Overall	2,227 / 10,802	10.3				
Quintile 1	526 / 2,161	12.7	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Quintile 2	455 / 2,160	10.6	0.82 (0.73–0.93)	0.89 (0.78–1.01)	0.90 (0.80–1.03)	0.91 (0.80–1.04)
Quintile 3	427 / 2,161	9.9	0.77 (0.68–0.87)	0.79 (0.69–0.89)	0.81 (0.71–0.93)	0.82 (0.72–0.93)
Quintile 4	416 / 2,160	9.3	0.71 (0.63–0.81)	0.77 (0.68–0.88)	0.80 (0.70–0.91)	0.81 (0.71–0.92)
Quintile 5	403 / 2,160	9.2	0.70 (0.62–0.80)	0.74 (0.65–0.84)	0.73 (0.64–0.84)	0.74 (0.65–0.85)
***P* for trend**			< 0.001	< 0.001	< 0.001	< 0.001
90th vs. 10th percentile			0.71 (0.65–0.78)	0.73 (0.66–0.81)	0.75 (0.68–0.82)	0.76 (0.69–0.84)

Abbreviations: mtDNA-CN, mitochondrial DNA copy number.

Model 1: Adjusted for age, sex, race, and enrollment center; Model 2: Model 1 + body mass index, smoking, and alcohol intake; Model 3: Model 2 + total cholesterol, HDL-cholesterol, cholesterol medication, hypertension, and diabetes; and Model 4: Model 3 + prevalent coronary heart disease.

Table 3. Hazard ratios for incident heart failure at 10, 20, and 30 years since mtDNA copy number measurement.

	Year 10	Year 20	Year 30
Quintile 1	1 (reference)	1 (reference)	1 (reference)
Quintile 2	0.90 (0.78–1.03)	0.94 (0.83–1.08)	0.97 (0.82–1.14)
Quintile 3	0.78 (0.66–0.93)	0.87 (0.75–1.02)	0.91 (0.72–1.16)
Quintile 4	0.76 (0.61–0.93)	0.89 (0.74–1.06)	0.95 (0.69–1.32)
Quintile 5	0.68 (0.53–0.88)	0.84 (0.69–1.02)	0.92 (0.61–1.39)
90th vs. 10th percentile	0.65 (0.54–0.79)	0.89 (0.76–1.04)	0.99 (0.72–1.37)

Time-dependent model adjusted for age, sex, race, enrollment center, body mass index, smoking, alcohol intake, total cholesterol, HDL-cholesterol, cholesterol medication, hypertension, diabetes, and prevalent coronary heart disease.

Table 4. Hazard ratios for incident heart failure with preserved ejection fraction (HFpEF) and heart failure with reduced ejection fraction (HFrEF) by quintiles of mitochondrial DNA copy number.

mtDNA-CN	N events / N total	Incidence rate per 1,000	Model 1	Model 2	Model 3	Model 4
HFpEF						
Overall	564 / 8,892	6.2				
Quintile 1	103 / 1,701	6.0	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Quintile 2	111 / 1,776	6.1	1.05 (0.80–1.37)	1.09 (0.83–1.43)	1.10 (0.94–1.45)	1.12 (0.85–1.47)
Quintile 3	120 / 1,762	6.6	1.16 (0.89–1.51)	1.16 (0.89–1.51)	1.17 (0.90–1.53)	1.17 (0.90–1.53)
Quintile 4	122 / 1,846	6.2	1.12 (0.86–1.45)	1.15 (0.88–1.49)	1.17 (0.89–1.52)	1.13 (0.86–1.48)
Quintile 5	108 / 1,807	5.8	1.01 (0.77–1.32)	1.02 (0.78–1.34)	1.02 (0.77–1.34)	0.97 (0.74–1.29)
***P* for trend**			0.80	0.76	0.77	0.89
HFrEF						
Overall	504 / 8,892	5.5				
Quintile 1	114 / 1,701	6.7	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Quintile 2	103 / 1,776	5.7	0.87 (0.66–1.13)	0.88 (0.68–1.16)	0.88 (0.67–1.15)	0.88 (0.67–1.16)
Quintile 3	94 / 1,762	5.1	0.80 (0.64–1.05)	0.81 (0.62–1.07)	0.81 (0.62–1.07)	0.79 (0.59–1.04)
Quintile 4	100 / 1,846	5.1	0.82 (0.63–1.07)	0.85 (0.64–1.11)	0.85 (0.65–1.12)	0.87 (0.66–1.15)
Quintile 5	93 / 1,807	5.0	0.76 (0.58–1.00)	0.77 (0.58–1.02)	0.77 (0.58–1.02)	0.80 (0.60–1.05)
***P* for trend**			0.06	0.07	0.08	0.14

Abbreviations: HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; and mtDNA-CN, mitochondrial DNA copy number.

Model 1: Adjusted for age, sex, and race/enrollment center; Model 2: Model 1 + body mass index, smoking, alcohol intake, and physical activity; Model 3: Model 2 + total cholesterol, HDL-cholesterol, cholesterol medication, hypertension, diabetes; Model 4: Model 3 + prevalent coronary heart disease.

Figure 1. Hazard ratios for incident heart failure by levels of mitochondrial DNA copy number.

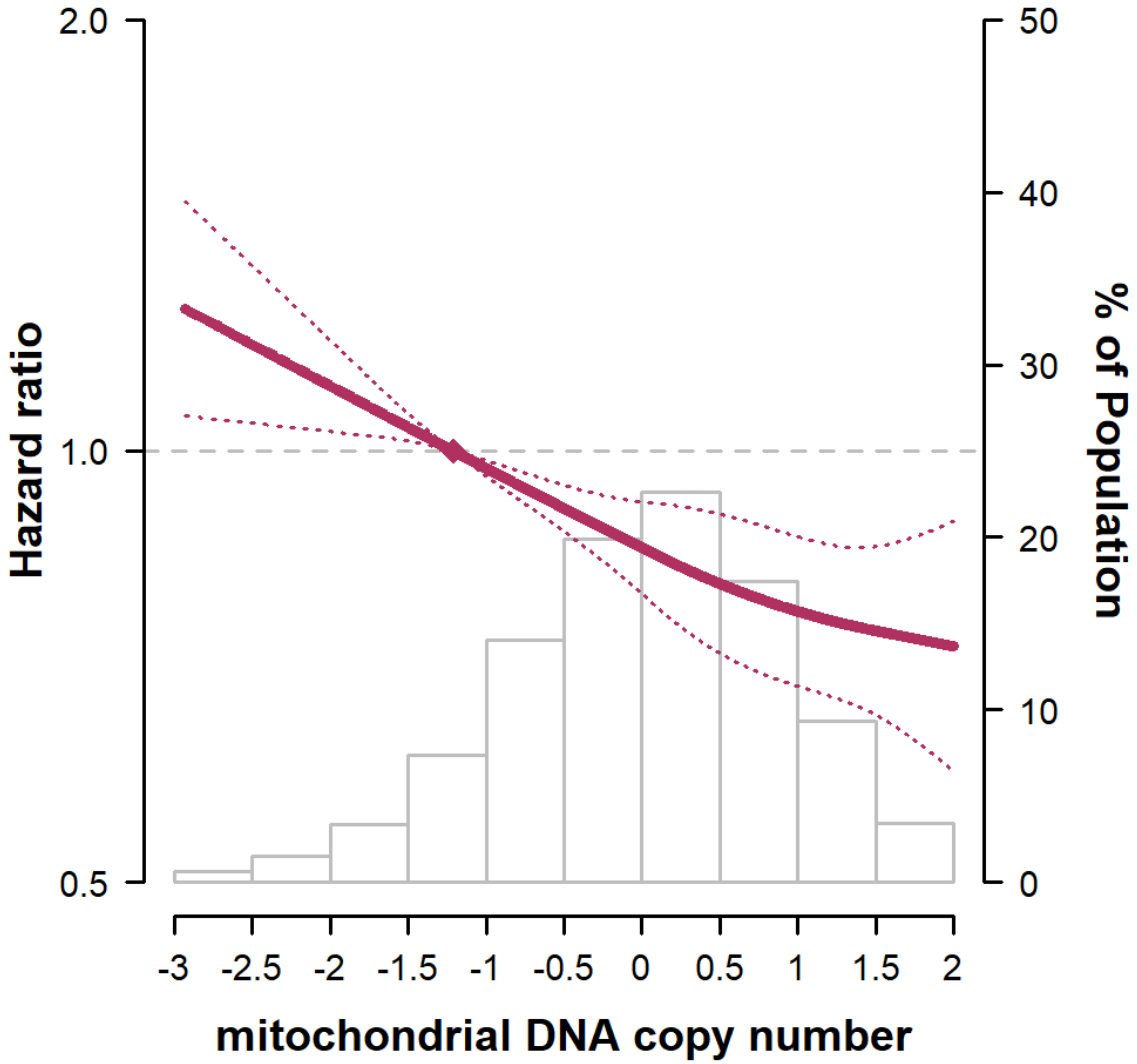


Figure 2. Hazard ratios for incident heart failure comparing the 90th to the 10th percentile of mitochondrial DNA copy number in selected subgroups.

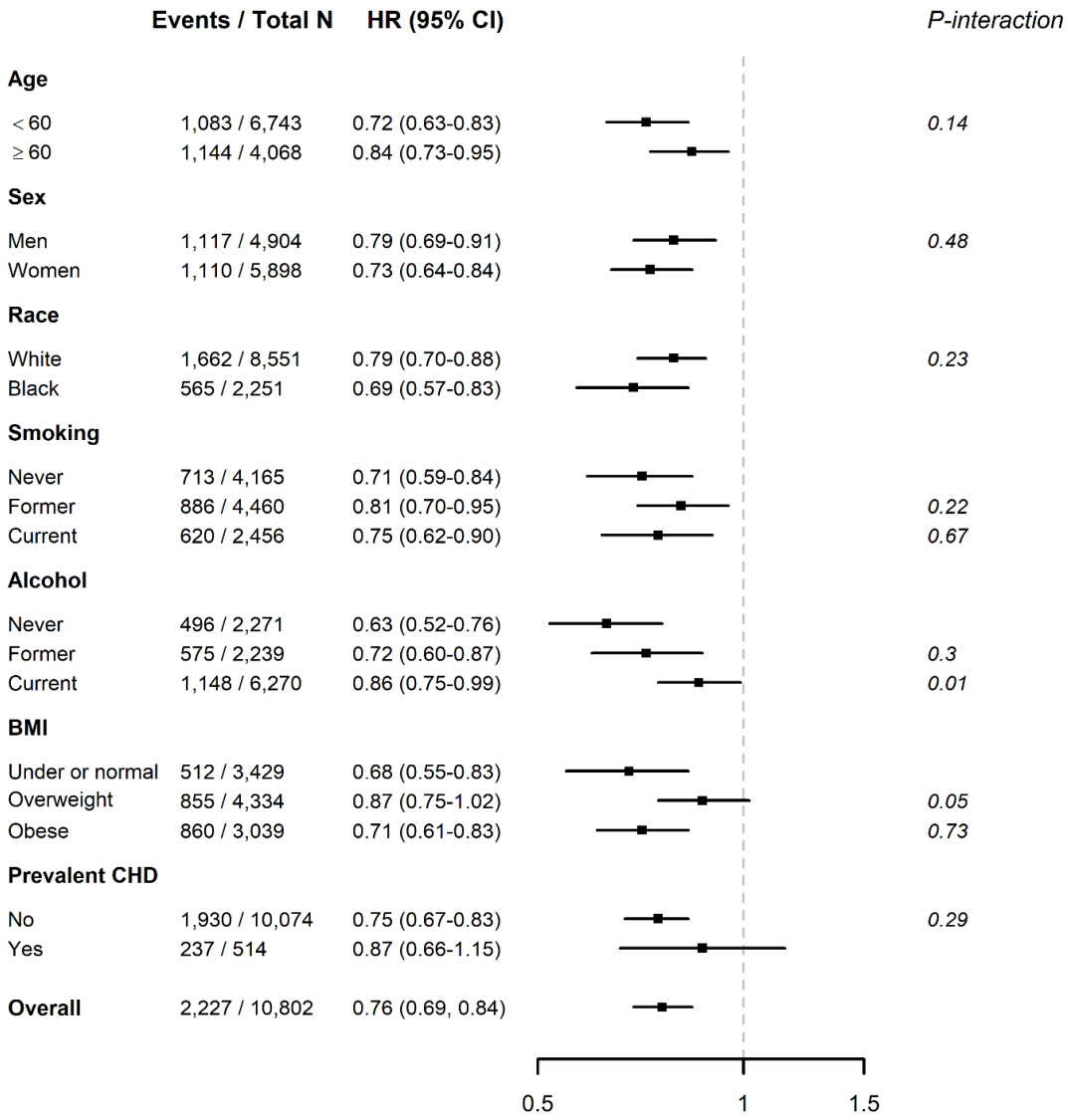
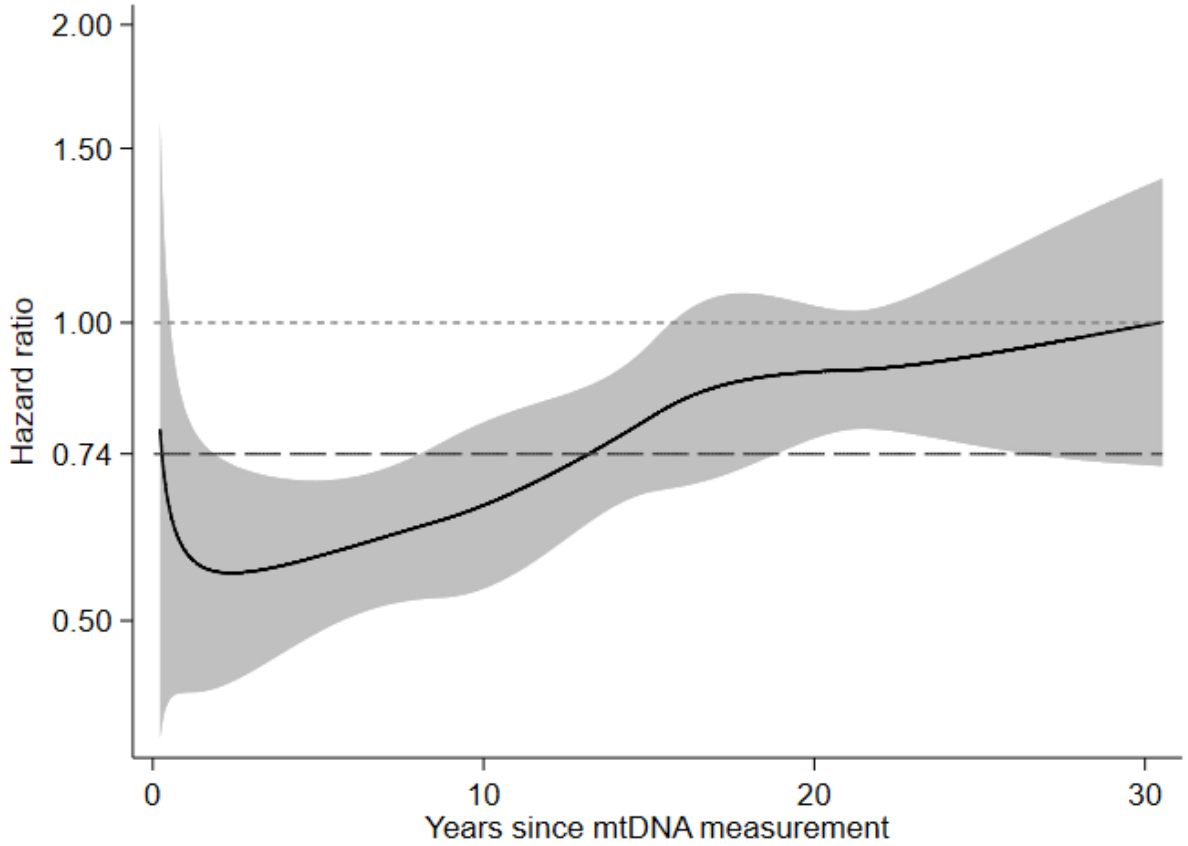


Figure 3. Time-dependent hazard ratios for incident heart failure comparing the 90th to the 10th percentile of mitochondrial DNA copy number.



Supplementary Table 1. Hazard ratios for incident heart failure (identified from discharge codes for the entire follow-up period) by levels of mitochondrial DNA copy number.

mtDNA-CN	N events / N total	Incidence rate per 1,000	Model 1	Model 2	Model 3	Model 4
Overall	2,410 / 10,802	11.2				
Quintile 1	560 / 2,161	13.6	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Quintile 2	491 / 2,160	11.5	0.83 (0.74–0.94)	0.90 (0.79–1.01)	0.91 (0.80–1.03)	0.92 (0.81–1.04)
Quintile 3	463 / 2,161	10.8	0.80 (0.69–0.88)	0.79 (0.70–0.90)	0.82 (0.72–0.93)	0.83 (0.73–0.94)
Quintile 4	463 / 2,160	10.4	0.74 (0.66–0.84)	0.81 (0.71–0.91)	0.83 (0.74–0.94)	0.84 (0.74–0.96)
Quintile 5	433 / 2,160	9.9	0.71 (0.62–0.80)	0.74 (0.65–0.84)	0.73 (0.65–0.83)	0.75 (0.66–0.86)
***P* for trend**			< 0.001	< 0.001	< 0.001	< 0.001
90th vs. 10th percentile			0.72 (0.66–0.79)	0.74 (0.68–0.81)	0.76 (0.69–0.83)	0.78 (0.71– 0.85)

Abbreviations: mtDNA-CN, mitochondrial DNA copy number.

Model 1: Adjusted for age, sex, race, and enrollment center; Model 2: Model 1 + body mass index, smoking, and alcohol intake; Model 3: Model 2 + total cholesterol, HDL-cholesterol, cholesterol medication, hypertension, diabetes; Model 4: Model 3 + prevalent coronary heart disease.

Supplementary Table 2. Hazard ratios for incident heart failure (restricted to cases adjudicated by the ARIC Heart Failure Committee since 2005) by levels of mitochondrial DNA copy number.

mtDNA-CN	N events / N total	Incidence rate per 1,000	Model 1	Model 2	Model 3	Model 4
Overall	1,266 / 8,493	14.4				
Quintile 1	262 / 1,597	16.1	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Quintile 2	255 / 1,690	14.8	0.91 (0.77–1.08)	0.97 (0.82–1.16)	0.99 (0.83–1.18)	1.00 (0.84–1.20)
Quintile 3	251 / 1,698	14.3	0.89 (0.75–1.06)	0.91 (0.77–1.08)	0.92 (0.77–1.10)	0.92 (0.77–1.10)
Quintile 4	265 / 1,780	14.1	0.87 (0.73–1.03)	0.94 (0.79–1.12)	0.95 (0.80–1.13)	0.95 (0.80–1.13)
Quintile 5	233 / 1,728	13.0	0.80 (0.67–0.96)	0.84 (0.70–1.00)	0.83 (0.70–1.00)	0.82 (0.69–0.99)
***P* for trend**			0.02	0.05	0.05	0.03
90^{th} vs. 10^{th} percentile			0.85 (0.74– 0.97)	0.87 (0.77–1.00)	0.88 (0.77–1.01)	0.88 (0.77–1.00)

Abbreviations: mtDNA-CN, mitochondrial DNA copy number.

Model 1: Adjusted for age, sex, race, and enrollment center; Model 2: Model 1 + body mass index, smoking, and alcohol intake; Model 3: Model 2 + total cholesterol, HDL-cholesterol, cholesterol medication, hypertension, diabetes; Model 4: Model 3 + prevalent coronary heart disease.

Supplementary Table 3. Hazard ratios for incident heart failure (restricted to cases identified from discharge codes since 2005) by levels of mitochondrial DNA copy number.

mtDNA-CN	N events / N total	Incidence rate per 1,000	Model 1	Model 2	Model 3	Model 4
Overall	1,449 / 8,494	16.6				
Quintile 1	296 / 1,597	18.4	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Quintile 2	291 / 1,691	17.0	0.92 (0.78–1.08)	0.98 (0.83–1.15)	0.98 (0.83–1.15)	1.00 (0.85–1.18)
Quintile 3	287 / 1,697	16.4	0.89 (0.76–1.05)	0.91 (0.77–1.07)	0.92 (0.78–1.08)	0.92 (0.78–1.09)
Quintile 4	312 / 1,781	16.7	0.91 (0.77–1.06)	0.98 (0.83–1.15)	0.99 (0.84–1.16)	0.99 (0.84–1.17)
Quintile 5	263 / 1,728	14.8	0.79 (0.67–0.94)	0.82 (0.70–0.97)	0.82 (0.69–0.97)	0.83 (0.70–0.98)
***P* for trend**			0.01	0.04	0.05	0.04
90^{th} vs. 10^{th} percentile			0.85 (0.75–0.97)	0.88 (0.77–0.99)	0.88 (0.78–1.00)	0.89 (0.78–1.01)

Abbreviations: mtDNA-CN, mitochondrial DNA copy number.

Model 1: Adjusted for age, sex, race, and enrollment center; Model 2: Model 1 + body mass index, smoking, and alcohol intake; Model 3: Model 2 + total cholesterol, HDL-cholesterol, cholesterol medication, hypertension, diabetes; Model 4: Model 3 + prevalent coronary heart disease.

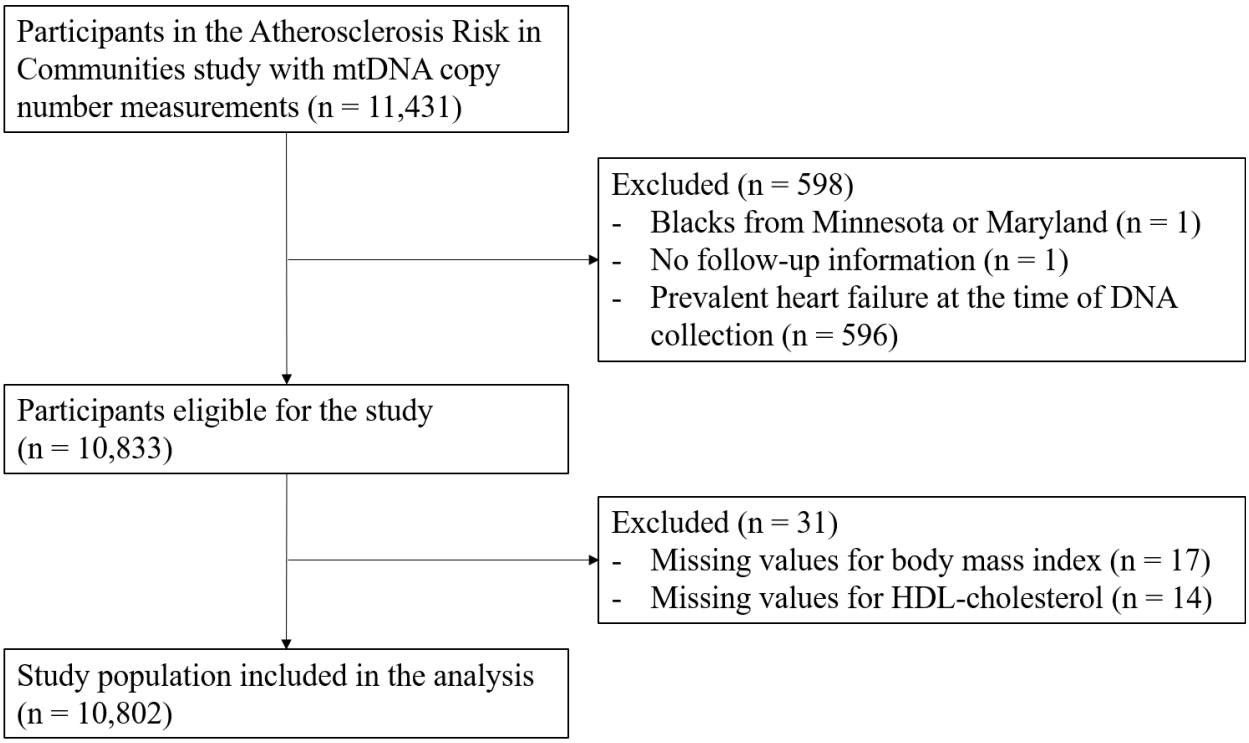
Supplementary Table 4. Hazard ratios for incident heart failure by levels of mitochondrial DNA copy number with competing risks for other causes of death.

mtDNA-CN	N events / N total	Incidence rate per 1,000	Model 1	Model 2	Model 3	Model 4
Overall	2,227 / 10,802	10.3				
Quintile 1	526 / 2,161	12.7	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Quintile 2	455 / 2,160	10.6	0.84 (0.74–0.96)	0.88 (0.77–1.01)	0.90 (0.79–1.03)	0.91 (0.80–1.04)
Quintile 3	427 / 2,161	9.9	0.79 (0.69–0.90)	0.80 (0.70–0.91)	0.82 (0.72–0.94)	0.83 (0.72–0.95)
Quintile 4	416 / 2,160	9.3	0.76 (0.67–0.87)	0.81 (0.71–0.93)	0.85 (0.75–0.97)	0.86 (0.75–0.99)
Quintile 5	403 / 2,160	9.2	0.73 (0.64–0.83)	0.75 (0.66–0.86)	0.76 (0.67–0.87)	0.78 (0.68–0.90)
***P* for trend**			< 0.001	< 0.001	< 0.001	< 0.001

Abbreviations: mtDNA-CN, mitochondrial DNA copy number.

Model 1: Adjusted for age, sex, and race/enrollment center; Model 2: Model 1 + body mass index, smoking, alcohol intake, and physical activity; Model 3: Model 2 + total cholesterol, HDL-cholesterol, cholesterol medication, hypertension, diabetes; Model 4: Model 3 + prevalent coronary heart disease.

Supplementary Figure 1. Flowchart of study participants.



Chapter 5

Conclusion

SUMMARY OF FINDINGS AND IMPLICATIONS

In *Chapter* 2, we evaluated the association between long-term exposure to PM ≤10 μm diameter (PM_{10}) and nitrogen dioxide (NO_2) with mtDNA-CN in 195,196 adults in the UK Biobank study. An increase in annual average PM_{10} and NO_2 exposure was inversely associated with mtDNA-CN. In the analysis using air pollutant concentrations and mtDNA-CN measured in the concurrent year (Lag 0), an increase in PM_{10} and NO_2 was also inversely associated with mtDNA-CN in both 2007 and 2010. The associations persisted for lags of up to 3 years but did not show a clear trend with increasing lag. $PM_{2.5-10}$ was also inversely associated with mtDNA-CN.

In this large-scale study, long-term exposure to PM_{10} and NO_2 were inversely associated with mtDNA-CN. These findings suggest that oxidative stress-induced mitochondrial dysfunction, reflected by reduced mtDNA-CN, may be an additional mechanism mediating the health effects of air pollution.

In *Chapter* 3, we evaluated the relationship between short-term exposure to ambient temperature with mtDNA-CN in association with metabolites in 374,397 adult men and women from the UK Biobank study. mtDNA-CN, on average, was lower in the warmer months and higher in the colder months. An increase in ambient temperature was inversely associated with mtDNA-CN. The inverse association between ambient temperature and mtDNA-CN persisted over 7 days with the strongest association at lag 0. This inverse association of ambient temperature with mtDNA-CN was most explained by metabolites produced during glucose and lipid metabolism, including pyruvate, lactate, and ketone bodies.

In this large-scale population-based study, short-term exposure to ambient temperature up to 7 days was inversely associated with mtDNA-CN. In addition, metabolites involved in glucose

and free fatty acid metabolism explained much of the temperature– mtDNA-CN association. This study supports the active involvement of mitochondria in thermoregulation and may explain the potential mechanism of various adverse health effects associated with ambient temperature.

In Chapter 4, we examined the association between mtDNA-CN and the risk of incident HF among 10,802 participants free of HF at baseline from the Atherosclerosis Risk in Communities (ARIC) study, a large bi-racial population-based cohort. Higher mtDNA-CN was inversely associated with incident HF with an approximately linear dose-response relationship. In stratified analyses, the associations between mtDNA-CN and HF were similar across subgroups defined by age, sex, race, smoking status, and history of CHD. The association between mtDNA-CN and HF was progressively attenuated from the time of mtDNA-CN measurement.

In this large community-based prospective cohort, mtDNA-CN was inversely associated with the risk of incident HF suggesting that reduced levels of mtDNA-CN, a biomarker of mitochondrial dysfunction, could reflect early susceptibility to HF. Further studies are needed to better understand the underlying mechanisms and to characterize the association of mtDNA-CN with different types of HF and their severity.

FUTURE DIRECTIONS

Based on our findings and previous literature, mitochondrial function appears to be associated with various environmental exposures, and lower levels of mtDNA-CN, a biomarker of mitochondrial dysfunction, is a useful predictor of HF in the near future. However, there remain several questions which require further research.

A need for longitudinal studies

In Chapters 2 and 3, we observed cross-sectional, inverse associations of environmental exposures on mtDNA-CN using the UK Biobank data. In the UK Biobank, mtDNA-CN metrics were measured on a single occasion at baseline, and thus, in these analyses, we were only able to evaluate the average difference of mtDNA-CN on a population level rather than the change in mtDNA-CN on an individual level in response to external environment. To date, the evidence on how mtDNA-CN changes after exposure to air pollution or ambient temperature from a large population-based study is lacking.

A longitudinal study with multiple measurements of mtDNA-CN is needed to evaluate the change in mtDNA-CN over time by exposure concentration. Moreover, for short-term exposures, a longitudinal study with repeated measurement of mtDNA-CN at a daily or sub-daily intervals would allow us to identify the most critical time window of exposure on the change of mtDNA-CN.

Measurement of exposures on multiple occasions will also allow a more detailed description of the exposure-outcome associations at different lags (lag effects). Additionally, a longitudinal study with information on environmental exposures, mtDNA-CN, and incident health outcomes will allow us to perform mediation analysis to better elucidate the role of mitochondrial function, or mtDNA-CN, on the association between environmental exposure and various health outcomes.

Precise measurement of environmental exposures

Most studies, including the analyses we present in Chapters 2 and 3, use air pollution concentrations and ambient temperature derived from prediction models. The predicted values do

not reflect an individual's actual levels of exposure and the quality and accuracy are dependent on the input data as well as model performance. In addition, the temporal and spatial resolution of exposure levels are determined by available data.

Improvement in the measurement of environmental exposures, including personal monitors, and higher temporal and spatial resolution of exposure levels are needed to more accurately assess the relationship between the mechanisms between environmental factors and health outcomes.

Broader generalizability to other geographical regions and populations

The results from Chapter 2 of this dissertation highlight the potential mechanism of adverse health effects from air pollution exposure. The air pollution levels in most areas of the UK are maintained at relatively low concentrations and the associations may not be generalizable to other countries. The relationship between ambient temperature and mtDNA-CN in Chapter 3 was also examined in one country within a single climate region. People residing in different climate regions adapt differently to their weather conditions so the findings may not be generalizable to other climate regions. Therefore, further investigation is needed in different countries, geographic areas, and climate regions to better understand the observed associations.

The UK Biobank recruited participants between the ages of 40 – 69 at baseline. Older individuals and children are at a higher risk of air pollution and temperature-associated illnesses and additional investigation in different age groups is warranted.

Moreover, the majority of participants in both the UK Biobank and ARIC study report themselves as White and participants of other races are underrepresented. Therefore, further

studies including diverse populations are needed to better understand variability by race and ethnicity group.

SUMMARY

This dissertation contributes to current knowledge of mtDNA-CN as a biomarker of both long-term and short-term environmental exposures. It is also a useful biomarker of incident HF, particularly as an early indicator for HF in the near future. Future research, in the form of experimental studies and longitudinal data with multiple measurements mtDNA-CN, is warranted to solidify the causal associations between environmental stressors, mitochondrial function, and health outcomes.

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Published as: Nitrergic pathway is the main contributing mechanism in the human gastric fundus relaxation: An in vitro study. [PubMed] [PDF] PMID: 27589594. PMCID: PMC5010257.

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TEACHING EXPERIENCE

- 2018–2021 Teaching Assistant for Principles of Epidemiology (340.601), Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health.
- 2017–2021 Teaching Assistant for Exposure Science & Environmental Epi Journal Club (185.801), Department of Environmental Health & Engineering, Johns Hopkins Bloomberg School of Public Health.
- 2017–2022 Teaching Assistant for Environmental and Occupational Epidemiology (340.680), Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health.
- 2017–2019 Teaching Assistant for Advanced Methods for Design and Analysis of Cohort Studies (340.728), Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health.
- 2017–2020 Advanced Clinical Research Design (ACRD), Center for Clinical Epidemiology, Samsung Medical Center, Seoul, Korea.
- 2017–2020 How to Write Good Paper (HTWGP), Center for Clinical Epidemiology, Samsung Medical Center, Seoul, Korea.
- 2016–2017 Teaching Assistant for Epidemiologic Methods 2 (340.752), Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health.

PROFESSIONAL ACTIVITIES

Society membership

- 2014 – Present The Korean Association of Internal Medicine.

- 2010 – Present The Korean Society of Neurogastroenterology and Motility.
- 2010 – Present Korean Medical Association.

AWARDS

- 2020 Winifred Hayes scholarship
- 2017 Robert Dyar Memorial scholarship.
- 2017 Maryland Genetics, Epidemiology, and Medicine (MD-GEM) Wolfe Street Competition, Johns Hopkins Bloomberg School of Public Health and School of Medicine.
- 2014 Outstanding Research Award for Resident, Samsung Medical Center, Seoul, Korea.
- 2014 In-training examination for residents, Department of Medicine, Samsung Medical Center, Seoul, Korea.
- 2013 Outstanding resident of the year, Department of Medicine, Samsung Medical Center, Seoul, Korea.

PUBLICATIONS

Journal Articles (Peer-reviewed)

***Co-first author.**

1. Han SJ, Kim H, **Hong YS**, Kim SW, Ku SY, Suh CS. Prediction model of persistent ovulatory dysfunction in Korean women with polycystic ovary syndrome. J Obstet Gynaecol Res. 2022 May 23. [PubMed] PMID: 35603765. doi: 10.1111/jog.15288. Epub ahead of print.

2. Sinn DH, Kang D, Kang M, Guallar E, **Hong YS**, Lee KH, Park J, Cho J, Gwak GY. Nonalcoholic Fatty Liver Disease and Accelerated Loss of Skeletal Muscle Mass: A Longitudinal Cohort Study. Hepatology. 2022 May 19. [PubMed] PMID: 35588190. doi: 10.1002/hep.32578. Epub ahead of print.

3. Lee J, **Hong YS***, Cho J, Lee J, Lee G, Kang D, Yun J, Jeon YJ, Shin S, Cho JH, Choi YS, Kim J, Zo JI, Shim YM, Guallar E, Kim HK. Reclassifying the International Association for the Study of Lung Cancer Residual Tumor Classification According to the Extent of Nodal Dissection for NSCLC: One Size Does Not Fit All. J Thorac Oncol. 2022 Apr 21:S1556-0864(22)00195-2. [PubMed] PMID: 35462086. doi: 10.1016/j.jtho.2022.03.015. Epub ahead of print.

4. Kang D, Kang M, **Hong YS**, Park J, Lee J, Seo HJ, Kim DW, Ahn JS, Park YH, Lee SK, Shin DW, Guallar E, Cho J. Temporal patterns of chronic disease

incidence after breast cancer: a nationwide population-based cohort study. Sci Rep. 2022 Mar 31;12(1):5489. [PubMed] PMID: 35361864. doi: 10.1038/s41598-022-09542-w.

5. Lee J, Lee J, **Hong YS**, Lee G, Kang D, Yun J, Jeon YJ, Shin S, Cho JH, Choi YS, Kim J, Zo JI, Shim YM, Guallar E, Cho J, Kim HK. Validation of the IASLC Residual Tumor Classification in Patients with Stage III-N2 Non-small Cell Lung Cancer Undergoing Neoadjuvant Chemoradiotherapy Followed by Surgery. Ann Surg. 2022 Feb 15. Epub ahead of print. [PubMed] PMID: 35166266. doi: 10.1097/SLA.0000000000005414.

6. Sinn DH, Kang D, Guallar E, **Hong YS**, Cho J, Gwak GY. Modest alcohol intake and mortality in individuals with elevated alanine aminotransferase levels: a nationwide cohort study. BMC Med. 2022 Jan 24;20(1):18. [PubMed] PMID: 35067226; PMCID: PMC8785562. doi: 10.1186/s12916-021-02215-x.

7. **Hong YS***, Jung KU, Rampal S, Zhao D, Guallar E, Ryu S, Chang Y, Kim HO, Kim H, Chun HK, Sohn CI, Shin H, Cho J. Risk factors for hemorrhoidal disease among healthy young and middle-aged Korean adults. Sci Rep. 2022 Jan 7;12(1):129. [PubMed] PMID: 34996957; PMCID: PMC8741788. doi: 10.1038/s41598-021-03838-z.
8. Kang D, Yoon SE, Shin D, Lee J, **Hong YS**, Lee SK, Lee JE, Park YH, Ahn JS, Guallar E, Kim WS, Lee J, Kim SJ, Cho J. Risk of non-Hodgkin lymphoma in breast cancer survivors: a nationwide cohort study. Blood Cancer J. 2021 Dec 14;11(12):200. [PubMed] PMID: 34907177; PMCID: PMC8671407. doi: 10.1038/s41408-021-00595-0.

9. **Hong YS**, Kim H, Zhao D, Cho A. Chronic Kidney Disease on Health-Related Quality of Life in Patients with Diabetes Mellitus: A National Representative Study. J Clin Med. 2021 Oct 10;10(20):4639. [PubMed] PMID: 34682762; PMCID: PMC8540120. doi: 10.3390/jcm10204639.

10. Sohn W, Chang Y, Cho YK, **Hong YS**, Shin H, Ryu S. Liver fibrosis scores and risk of liver-related mortality in young adults with chronic hepatitis B: A cohort study. J Viral Hepat. 2021 Sep 28. Epub ahead of print. [PubMed] PMID: 34582599. doi: 10.1111/jvh.13618.

11. **Hong YS***, Kim H. Hand grip strength and health-related quality of life in postmenopausal women: a national population-based study. Menopause. 2021 Sep 20. Epub ahead of print. [PubMed] PMID: 34547005. doi: 10.1097/GME.0000000000001863.

12. Sung KC, **Hong YS***, Lee JY, Lee SJ, Chang Y, Ryu S, Zhao D, Cho J, Guallar E, Lima JAC. Physical activity and the progression of coronary artery

calcification. Heart. 2021 Sep 20:heartjnl-2021-319346. Epub ahead of print. [PubMed] PMID: 34544807. doi: 10.1136/heartjnl-2021-319346.

13. Sinn DH, Kang D, **Hong YS**, Koh KC, Guallar E, Cho J, Gwak GY. Prior antiviral treatment and mortality among patients with hepatitis C virus-related hepatocellular carcinoma: A national cohort study. PLoS One. 2021 Aug 3;16(8):e0255624. [PubMed] [PDF] PMID: 34343200; PMCID: PMC8330890. doi: 10.1371/journal.pone.0255624.

14. Kim Y, Chang Y, Kwon MJ, **Hong YS**, Kim MK, Sohn W, Cho YK, Shin H, Wild SH, Byrne CD, Ryu S. Fasting Ketonuria and the Risk of Incident Nonalcoholic Fatty Liver Disease With and Without Liver Fibrosis in Nondiabetic Adults. Am J Gastroenterol. 2021 Jun 11. Epub ahead of print. [PubMed] PMID: 34114568. doi: 10.14309/ajg.0000000000001344.

15. **Hong YS**, Park HY, Chang Y, Jang EH, Zhao D, Kim S, Guallar E, Kim H, Cho J, Ryu S. Stages of menopause and abnormal lung function: a cross-sectional study of middle-aged women. Menopause. 2021 Apr 5;28(7):811-818. [PubMed] PMID: 33828036. doi: 10.1097/GME.0000000000001779.

16. Kim H, Han SJ, **Hong YS**, Kim SW, Ku SY, Suh CS, Kim SH. Optimal Oocyte Number in Controlled Ovarian Stimulation with Gonadotropin-Releasing Hormone Agonist/Antagonist and Day 3 Fresh Embryo Transfer. Reprod Sci. 2021 Mar 24. Epub ahead of print. [PubMed] PMID: 33763817. doi: 10.1007/s43032-021-00550-1.

17. Park HY, Chang Y, Kang D, **Hong YS**, Zhao D, Ahn J, Shin SH, Singh D, Guallar E, Cho J, Ryu S. Blood eosinophil counts and the development of obstructive lung disease: the Kangbuk Samsung Health Study. Eur Respir J. 2021 Mar 18:2003823. Epub ahead of print. [PubMed] PMID: 33737406. doi: 10.1183/13993003.03823-2020.

18. Moon SM, Lim JH, **Hong YS**, Shin KC, Lee CY, Kim DJ, Lee SH, Jung KS, Lee CH, Yoo KH, Lee H, Park HY. Clinical impact of forced vital capacity on exercise performance in patients with chronic obstructive pulmonary disease. J Thorac Dis. 2021 Feb;13(2):837-846. [PubMed] [PDF] PMID: 33717557; PMCID: PMC7947547. doi: 10.21037/jtd-20-1098a.

19. Ko RE, Moon SM, Kang D, Cho J, Chung CR, Lee Y, **Hong YS**, Lee SH, Lee JH, Suh GY. Translation and validation of the Korean version of the clinical frailty scale in older patients. BMC Geriatr. 2021 Jan 13;21(1):47. [PubMed] [PDF] PMID: 33441092; PMCID: PMC7805036. doi: 10.1186/s12877-021-02008-0.

20. Zhang Z, Kang J, **Hong YS**, Chang Y, Ryu S, Park J, Cho J, Guallar E, Shin HC, Zhao D. Long-Term Particulate Matter Exposure and Incidence of Arrhythmias: A Cohort Study. J Am Heart Assoc. 2020 Nov 17;9(22):e016885. Epub 2020 Nov 4. [PubMed] [PDF] PMID: 33146044. doi: 10.1161/JAHA.120.016885.

21. Sinn DH, Kang D, Guallar E, Chang Y, Ryu S, Zhao D, **Hong YS**, Cho J, Gwak GY. Alcohol Intake and Mortality in Patients With Chronic Viral Hepatitis: A Nationwide Cohort Study. Am J Gastroenterol. 2021 Feb 1;116(2):329-335. [PubMed] PMID: 33038136. doi: 10.14309/ajg.0000000000000966.

22. Lim JH, Kang D, **Hong YS**, Kim H, Ryu S, Chang Y, Park HY, Cho J. Association between reproductive lifespan and lung function among postmenopausal women. J Thorac Dis. 2020 Aug;12(8):4243-4252. [PubMed] [PDF] PMID: 32944336; PMCID: PMC7475608. doi: 10.21037/jtd-19-3726.

23. Zhao D, Bartz TM, Sotoodehnia N, Post WS, Heckbert SR, Alonso A, Longchamps RJ, Castellani CA, **Hong YS**, Rotter JI, Lin HJ, O'Rourke B, Pankratz N, Lane JA, Yang SY, Guallar E, Arking DE. Mitochondrial DNA copy number and incident atrial fibrillation. BMC Med. 2020 Sep 16;18(1):246. [PubMed] [PDF] PMID: 32933497; PMCID: PMC7493408. doi: 10.1186/s12916-020-01715-6.

24. **Hong YS**, Longchamps RJ, Zhao D, Castellani CA, Loehr LR, Chang PP, Matsushita K, Grove ML, Boerwinkle E, Arking DE, Guallar E. Mitochondrial DNA Copy Number and Incident Heart Failure: The Atherosclerosis Risk in Communities (ARIC) Study. Circulation. 2020 Jun 2;141(22):1823-1825. Epub 2020 Jun 1. [PubMed] PMID: 32479199; PMCID: PMC7295435. doi: 10.1161/CIRCULATIONAHA.120.046001.

25. Sinn DH, Kang D, Cho SJ, Chang Y, Ryu S, Song YB, Paik SW, **Hong YS**, Zhao D, Guallar E, Cho J, Gwak GY. Weight change and development of subclinical carotid atherosclerosis among metabolically healthy adults: A cohort study. J Clin Endocrinol Metab 2019. [PubMed] [PDF] PMID: 31545359. doi: 10.1210/clinem/dgz040.

26. Zhang Z, Zhao D, **Hong YS**, Chang Y, Ryu S, Kang D, Monteiro J, Shin HC, Guallar E, Cho J. Long-term particulate matter exposure and onset of depression in middle-aged men and women. Environ Health Perspect 2019 Jul; 127(7):77001. [PubMed] [PDF] PMID: 31268362. doi: 10.1289/EHP4094.

27. Dein E, Kuo PL, **Hong YS**, Hummers LK, Mecoli CA, McMahan ZH. Evaluation of risk factors for pseudo-obstruction in systemic sclerosis. Semin Arthritis Rheum 2019 May 23. [PubMed] [PDF] pii: S0049-0172(19)30038-1. doi:

10.1016/j.semarthrit.2019.05.005.

28. Kim S, Chang Y, Kang J, Cho A, Cho J, **Hong YS**, Zhao D, Ahn J, Shin H, Guallar E, Ryu S, Sung KC. Relationship of the Blood Pressure Categories, as Defined by the ACC/AHA 2017 Blood Pressure Guidelines, and the Risk of Development of Cardiovascular Disease in Low-Risk Young Adults: Insights From a Retrospective Cohort of Young Adults. J Am Heart Assoc 2019 Jun 4;8(11):e011946. [PubMed] [PDF] PMID: 31140347. PMCID: PMC6585354. doi: 10.1161/JAHA.119.011946.

29. Chang Y, Cho J, Cho YK, Cho A, **Hong YS**, Zhao D, Ahn J, Sohn CI, Shin H, Guallar E, Ryu S. Alcoholic and Nonalcoholic Fatty Liver Disease and Incident Hospitalization for Liver and Cardiovascular Diseases. Clin Gastroenterol Hepatol 2019 May 11. [PubMed] [PDF] PMID: 31085337. pii: S1542-3565(19)30514-2. doi: 10.1016/j.cgh.2019.05.004.

30. **Hong YS**,* Ryu S, Chang Y, Caínzos-Achirica M, Kwon MJ, Zhao D, Shafi T, Lazo M, Pastor-Barriuso R, Shin H, Cho J, Guallar E. Hepatitis B virus infection and development of chronic kidney disease: A cohort study. BMC Nephrol 2018 Dec 11; 19(1):353. [PubMed] [PDF] PMID: 30537940.

31. Kim S, Chang Y, Cho J, **Hong YS**, Zhao D, Kang J, Jung HS, Yun KE, Guallar E, Ryu S, Shin H. Life's simple 7 cardiovascular health metrics and progression of coronary artery calcium in a low-risk population. Arterioscler Thromb Vasc Biol 2019 Jan 31; ATVBAHA118311821. [PubMed] [PDF] doi: 10.1161/ATVBAHA.118.311821. [Epub ahead of print]

32. Hwangbo Y, Kang D, Kang M, Kim S, Lee EK, Kim YA, Chang YJ, Choi KS, Jung SY, Woo SM, Ahn JS, Sim SH, **Hong YS**, Pastor-Barriuso R, Guallar E, Lee ES, Kong SY, Cho J**.** Incidence of Diabetes After Cancer Development: A Korean National Cohort Study. JAMA Oncol 2018 Aug 1; 4(8):1099-105. [PubMed] [PDF] PMID: 29879271. PMCID: PMC6143049 [Available on 2019-06-07]. doi: 10.1001/jamaoncol.2018.1684.

33. **Hong YS**,* Chang Y, Ryu S, Cainzos-Achirica M, Kwon M, Zhang Y, Choi Y, Ahn J, Rampal S, Zhao D, Pastor-Barriuso R, Lazo M, Shin H, Cho J, Guallar E. Hepatitis B and C virus infection and diabetes mellitus: A cohort study. Scientific Reports 2017 Jul 4; 7(1):4606. [PubMed] [PDF] PMID: 28676706. PMCID: PMC5496892.

34. Min YW, **Hong YS**,* Ko EJ, Lee JY, Ahn KD, Bae JM, Rhee PL. Nitrergic pathway is the main contributing mechanism in the human gastric fundus relaxation: An in vitro study. *PLoS One* 2016 Sep 2; 11(9):e0162146.

[PubMed] [PDF] PMID: 27589594. PMCID: PMC5010257.

35. **Hong YS**, Min YW, Rhee PL. Two distinct types of hypercontractile esophagus: classic and spastic jackhammer esophagus. *Gut Liver* 2016 Sep 15;10(5):859-63.
[PubMed] [PDF] PMID: 27458179. PMCID: PMC5003212.

36. **Hong YS,** Sinn DH, Gwak GY, Cho J, Kang D, Paik YH, Choi MS, Lee JH, Koh KC, Paik SW. Characteristics and outcomes of chronic liver disease patients with acute deteriorated liver function by severity of underlying liver disease. *World J Gastroenterol* 2016 Apr 14; 22(14): 3785-92.
[PubMed] [PDF] PMID: 27076763. PMCID: PMC4814741.

37. **Hong YS**, Woo YS, Park G, Lee K, Kang SH, Lee HW, Kim ER, Hong SN, Chang DK, Kim YH, Rhee PL, Kim JJ. Endoscopic Findings of Enteropathy-Associated T-Cell Lymphoma Type II: A Case Series. *Gut Liver* 2016 Jan 23; 10(1): 147-51.
[PubMed] [PDF] PMCID: PMC4694747.

38. Lee JE, Lee K, **Hong YS**, Kim ER, Lee H, Min BH. Impact of carcinomatosis on clinical outcomes after expandable metallic stent placement for malignant gastric outlet obstruction. *PLoS One* 2015 Oct 14;10(10):e0140648.
[PubMed] [PDF] PMCID: PMC4605738.

39. Kang SH, Min BH, Lee K, Lee HW, Park GE, **Hong YS**. Delayed perforation occurring after endoscopic submucosal dissection for early gastric cancer. *Clinical Endoscopy* 2015 May;48(3):251-5.
[PubMed] [PDF] PMCID: PMC4461671.

40. Min YW, **Hong YS**,* Ko EJ, Lee JY, Min BH, Sohn TS, Kim JJ, Rhee PL. Impairment of the proximal to distal tonic gradient in the human diabetic stomach. *Neurogastroenterol Motil* 2014 Feb;26(2):229-36.
[PubMed] [PDF] PMID: 24165095.

41. Lee K, Kang SH, Lee HW, Park GE, **Hong YS**, Lee JE, Kim YG. A case of light chain deposition disease in a patient with diabetes and nodular glomerulosclerosis. *Korean Journal of Medicine* 2015;88(3):318-23.
[PDF] KMID: 0882420150880030318. Article in Korean.

42. Park GE, **Hong YS**, Lee K, Kang SH, Lee HY, Cho YY, Kim JH. A functioning adrenal adenoma and a pheochromocytoma in the same adrenal gland. *Korean J Intern Med* 2015 Jan;20(1):114-7.
[PubMed] [PDF] PMCID: PMC4293550.

43. Lee HW, Kang SH, Park GE, Lee KH, **Hong YS**, Lee K, Lee JH, Lee DH. Survey on the endoscopic diagnosis of chronic gastritis. *Korean J Helicobacter Up Gastrointest Res* 2014;14(3):194-98.
[Full Text]

44. Lee HW, Kang SH, Park GE, Lee KH, **Hong YS**, Kwon GY, Jung CW, Lee JE. Membranous nephropathy concurrent with interstitial nephritis after allogenic hematopoietic stem cell transplantation in a patient with acute lymphoblastic leukemia. *Korean J Blood Transfus* 2014;25(2):152-59.
[Full Text] Article in Korean.

45. Cho YY, Joung JY, Jeong H, Je D, **Hong YS**, Suh S, Kim SW. Postinfectious Guillain-Barre syndrome in a patient with methimazole-induced agranulocytosis. *Korean J Intern Med* 2013;28:724-27.
[PubMed] [PDF] PMID: 24307850. PMCID: PMC3847000.

46. Moon SM, **Hong YS**, Lee DS, Chung CR. Purpura fulminans on the nose with septic abortion. Intensive Care Med. 2015 Jun;41(6):1122.
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Under review

1. Lee J, **Hong YS***, Cho J, Lee J, Lee G, Kang D, Yun J, Jeon YJ, Shin S, Cho JH, Choi YS, Kim J, Zo JI, Shim YM, Guallar E, Kim HK, Reclassifying the IASLC R Classification According to the Extent of Nodal Dissection for Non-Small Cell Lung Cancer: One Size Does Not Fit All

2. Kang D, **Hong YS***, Lee J, Guallar E, Shin D, Kim HC, Lee H, Youn J, Jeon J, Jang HR, Ahn JS, Cho J. Incidence of end-stage renal disease after cancer development: A nationwide cohort study.

In preparation

***Co-first author.**

1. **Hong YS**, Zhao D, Castellani CA, Arking DE, Guallar E. Air pollution and mitochondrial DNA Copy Number in the UK Biobank.

2. **Hong YS**, Zhao D, Castellani CA, Arking DE, Guallar E. Ambient temperature, seasonal patterns and mitochondrial DNA Copy Number.

3. **Hong YS**, Kang S, Park HY, Kim WJ, Kang SW, Guallar E. The association between air pollution and COPD mortality.

4. **Hong YS**, Park HY, Chang Y, Kang D, **Hong YS**, Zhao D, Ahn J, Shin SH, Singh D, Guallar E, Cho J, Ryu S. Eosinophil count and lung function decline.

5. **Hong YS**, Park J, Kim H. Association of endometriosis with genital human papilloma virus infection is US women: a national population-based study.

6. Sinn DH, Kang D, Kang M, Guallar E, **Hong YS**, Cho J, Gwak GY. Nonalcoholic fatty liver disease is associated with accelerated loss of skeletal muscle mass: A longitudinal cohort study

7. Zhao D, **Hong YS**, Castellani CA, Arking DE, Guallar E. Mitochondrial DNA copy number and peripheral artery disease.

8. Zhao D, **Hong YS**, Castellani CA, Arking DE, Guallar E. Mitochondrial DNA copy number and cancer incidence.

9. **Hong YS**, Park J, Zhao D, Kang D, Cho J, Guallar E. Blood lead, cadmium and blood pressure trajectory.

10. **Hong YS**, Park J, Zhao D, Kang D, Cho J, Guallar E. Blood lead, cadmium and trajectory of kidney function.

11. Cho AJ, **Hong YS,** Shafi T, Guallar E. Trend of sudden cardiac death in hemodialysis patients, USRDS.

12. S Shin, **Hong YS,** Lee JH, Cho J. Surgical approach and differences in post-operative lung function in lung cancer patients.

ACADEMIC PRESENTATIONS

Scientific Meetings

Plenary sessions

1. **Hong YS**, Min YW, Ko EJ, Lee JY, Ahn KD, Bae JM, Koh SD, Rhee PL. Nitrergic pathway is the main contributing mechanism in the human gastric fundus relaxation. Asian Postgraduate course on Neurogastroenterology and Motility (APNM), Seoul, Korea, April, 2014. **(Best Research Award)**

2. **Hong YS**, Ko EJ, Lee JY, Kim ER, Min BH, Sinn DH, Son TS, Koh SD, Rhee PL. Mechanism of electrical field stimulation-induced relaxation in both circular and

longitudinal muscle strips of human gastric fundus. Korean Society of Neurogastroenterology and Motility (KSNM), Annual Conference, Seoul, Korea, April, 2011.

3. **Hong YS**, Ko EJ, Lee JY, Min BH, Sinn DH, Noh JH, Koh SD, Ree PL. Effect of acetylcholine in the human gastric circular muscle with diabetes mellitus. Korean Society of Neurogastroenterology and Motility (KSNM), Annual Conference, Seoul, Korea, November 2010.

Presentations

1. **Hong YS**, Chang Y, Ryu S, Caínzos-Achirica M, Kwon MJ, Zhang Y, Zhao D, Pastor-Barriuso R, Lazo M, Shin H, Cho J, Guallar E. Hepatitis B virus infection and development of chronic kidney disease: A cohort study. Society for Epidemiologic Research, Baltimore, USA, June, 2018.

2. **Hong YS**, Sinn DH, Gwak GY, Cho J, Kang D, Paik YH, Choi MS, Lee JH, Koh KC, Paik SW, Woo BC. Characteristics, outcomes and prognostic factors of acute-on-chronic liver failure (ACLF) according to a new classification. Seoul International Digestive Disease Symposium (SIDDS) & World Congress of Internal Medicine (WCIM), Seoul, Korea, October, 2014.

3. **Hong YS**, Shin I, Min YW, Rhee PL. The roles of multiple rapid swallow in the assessment of esophageal motor function. Seoul International Digestive Disease Symposium (SIDDS) & World Congress of Internal Medicine (WCIM), Seoul, Korea, October, 2014.

4. Min YW, Shin I, **Hong YS**, Rhee PL. The utility of multiple rapid swallow in patients showing normal swallow on esophageal high resolution manometry. Seoul International Digestive Disease Symposium (SIDDS) & World Congress of Internal Medicine (WCIM), Seoul, Korea, October, 2014.

5. **Hong YS**, Choi K, Min YW, Min BH, Lee JH, Kim JJ, Rhee PL. The analysis of causes and outcome of inpatient consulted for nausea and/or vomiting: a tertiary center experience. Federation of Neurogastroenterology and Motility Meeting (FNM), Guangzhou, China, September, 2014.

6. **Hong YS**, Choi K, Min YW, Min BH, Lee JH, Kim JJ, Rhee PL. Two distinct types of hypercontractile esophagus: classic and spastic Jackhammer esophagus. Federation of Neurogastroenterology and Motility Meeting (FNM), Guangzhou, China, September, 2014.

7. **Hong YS**, Kim ER, Min BH, Kim KM, Park CK, Min YW, Lee JH, Rhee PL, Rhee JC, Kim JJ. Long-term outcomes of endoscopic resection for early gastric cancer:

appropriate surveillance strategy based on the incidence and patterns of local, metachronous and extragastric recurrence. Digestive Disease Week (DDW), Chicago, USA, May, 2014.

8. **Hong YS**, Ko EJ, Lee JY, Min YW, Min BH, Koh SD, Rhee PL. Role of nitrergic responses in gastric accommodation in the human gastric fundus. American Neurogastroenterology and Motility Society (ANMS), Annual Conference, Huntington Beach, USA, September, 2013.

9. **Hong YS**, Min YW, Min BH, Lee JH, Kim JJ, Rhee PL. The effect of cimetropium bromide on esophageal motor function in patients with Jackhammer esophagus. Korean Society of Gastroenterology, Annual Conference, Seoul, Korea, November, 2013.

Detailed information is available upon request.

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