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Age and mitochondrial DNA copy number influence the association between outdoor temperature and cognitive function

Insights from the VA Normative Aging Study

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Background: General cognitive function deteriorates with aging, a change that has been linked to outdoor temperature. Older individuals have reduced ability to adapt to changes in outdoor temperature than younger people. However, to what extent short-term changes in outdoor temperature interact with mitochondria to affect cognition in older people has not yet been determined.
Methods: Our study included 591 participants of the Normative Aging Study who underwent multiple examinations between 2000 and 2013. Cognitive function was evaluated via the Mini-Mental State Examination. Outdoor temperature was estimated at residential addresses 1 day before the examination using on a validated spatiotemporal temperature model. Mitochondrial DNA copy number (mtDNAcn) was determined using buffy coat samples.
Results: We found an interaction between temperature, age, mtDNAcn, and cognition. In individuals 84 years of age or older, cooler temperature was associated with low cognition (odds ratio = 1.2; 95% confidence interval = 1.05, 1.35 for a 1°C decrease in temperature; $P = 0.007$). We found higher odds ratio per 1°C decrease in temperature among individuals with lower mtDNAcn ($\beta_3 = 0.12$; 95% confidence interval = 0.01, 0.22; $P_{interaction} = 0.02$).
Conclusions: Our findings, albeit potentially underpowered, suggest that older individuals may be more susceptible to the influence of short-term temperature exposure on cognition. Moreover, the level of mtDNAcn may also modify the association between temperature and cognitive function, indicating a possible role of these cellular elements in this relationship.

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Data and code are available upon request.

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Introduction

The rapidly growing population of older people is an increasingly relevant public health concern. Cognitive decline is a major challenge facing aging populations. As cognition deteriorates with age, independent of mental health status,[1] there may be a higher risk of falls,[2] loss of independence, increased risk of frailty,[3] and increased mortality.[4]

Older individuals also have an impaired ability to adapt to changes in outdoor temperature.[5] Previous studies have shown that even within a normal temperature range, cognitive function can be altered by temperature in elderly men.[6] However, results regarding the connection between outdoor temperature and cognitive function are discordant: some studies have reported a worsening effect of warmer temperature,[7] some have found a worsening effect of colder temperature[8] and some have found no measurable effect of temperature.[9]

What this study adds

There is some evidence that short-term temperature exposure may impact cognitive function. Our study builds upon previous studies to provide further insights for this association. Specifically, we found that among older males, cooler temperature was associated with low cognition. Furthermore, we found worse temperature effect estimates per 1°C decrease among individuals with lower mitochondrial DNA count. This observed interaction adds a new element to the relationship between cognition and temperature. Our findings may help with decision-making about policies in the public health field that concern the impact of temperature on human health.

Mitochondrial DNA copy number (mtDNAcn), a measure of mitochondrial genome abundance, is commonly used as a representation of the mitochondria's response to oxidative stress as well as general dysfunction.[10] Mitochondrial DNA (mtDNA) is sensitive to oxidative stress and mtDNA damage persists longer compared to genomic DNA due to the lack of a robust DNA repair system to restore oxidative stress-induced damage.[11] Typically, mtDNAcn will increase when the endogenous antioxidant response is no longer able to recover its redox balance,[10] possibly as a compensatory response for insufficient reactive oxygen species cleavage.[12]

Moreover, mitochondria are cellular organelles involved in energy production and thermal regulation of the human body. As such, they are critical for adaptive responses to external temperature changes. Adaptive thermogenesis, defined as the production of heat in response to changes in environmental temperature, is directly regulated by mitochondrial pathways.[13] Mitochondria have also been shown to impact brain function during aging through cognition and synaptic transmission,[14] the pathway through which mitochondrial bioenergetic processes act as primary acute regulators of cognitive functions.[15]

To evaluate the relationship between cognitive function and outdoor temperature, we utilized data from the Normative Aging Study (NAS), a US-based cohort of older males in eastern New England with longitudinal data on cognitive function. Due to the role of mitochondria in temperature adaptation, with our study we build upon a previous study of temperature and cognitive function in this cohort[6]; specifically, we evaluated whether cellular content of mitochondria, determined via the mtDNAcn, modified the association between temperature and cognitive function.

Materials and methods

Study participants

The Veterans Affairs' NAS, established in 1963 by the Department of Veterans Affairs, is an ongoing longitudinal study of 2,280 community-dwelling, healthy men living in the Greater Boston area.[16] At the time of enrollment, participants were free of known chronic medical conditions. Participants continue to undergo examinations every 3–5 years. All participants have provided written informed consent.

Cognitive testing did not start until 1993, by which time many NAS study participants had already died (N = 1,044). Of the 1,236 remaining participants, we excluded from analysis all subjects who had experienced stroke (N = 49), as stroke can severely impact a person's ability to successfully complete the Mini-Mental State Examination (MMSE)—the instrument used to assess cognitive function in this study—and has been linked to alterations in mtDNAcn. We further restricted our study population to participants with mtDNAcn information (N = 710). We did not have complete information on cognitive function for 56 and on temperature exposure for 63 participants, resulting in a final population of 591 subjects included in our analyses.

This study was reviewed and approved by the Institutional Review Boards of the participating institutions.

Cognitive assessment

The MMSE is a common instrument used to determine the presence of cognitive impairment and screen for dementia.[17] The MMSE assesses several cognitive domains, including orientation, immediate and short-term recall, attention and calculation, word-finding, figure construction, reading and writing skills, and the ability to follow a 3-step command. The MMSE is scored on a scale of 0–30, corresponding to the lowest and highest cognitive performance, respectively. In this study, the maximal MMSE score was 29 due to exclusion of the MMSE question regarding the participant's county of residence.[18]

Temperature assessment

To generate temperature data for the northeastern USA, we used a spatiotemporal prediction model that incorporates satellite remote sensing, land-use regression, meteorological variables, and spatial smoothing. Details can be found elsewhere.[19] We used predictions from this model to assign outdoor temperature estimates at each participant's residential address and estimated 24-hour mean temperature at participant residences.[6]

We used a 1-day lag of outdoor temperature to reflect recent, short-term exposure to outdoor temperature.

Determination of blood mtDNAcn

We measured blood mitochondrial abundance, a widely used biomarker, by comparing the relative amplification of nuclear and mitochondrial segments of DNA.[10,20] At every study visit, fasting whole blood samples were collected and stored at −80°C until mtDNAcn analysis. DNA was extracted using standard techniques and normalized to 2 ng/µl. DNA yield and purity were quantified on a Nanophotometer Pearl (Implen, Munich, Germany). We adapted a multiplex quantitative real-time polymerase chain reaction method with minor modifications.[21] To measure mtDNAcn, we used the mtDNA 12S ribosomal ribonucleic acid (RNA) TaqMan (Applied Biosystems, Waltham, Massachusetts) probe (6FAM-5′ TGCCAGCCACCGCG 3′-MGB). Simultaneously, mtDNA was amplified with the following primers mtF805 (5′CCACGGGAAACAGCAGTGATT3′) and mtR927 (5′CTATTGACTTGGGTTAATCGTGTGA3′), while a single copy nuclear Ribonuclease P gene was amplified for a nuclear DNA (nDNA) (TaqMan RNase P Control Reagents Kit; Applied Biosystems) comparison. Real-time polymerase chain reaction assays were performed following a previously published protocol,[21] using Bio-Rad CFX384 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, California). All samples were run in triplicate and the mean of the 3 measurements was used for statistical analysis. The coefficient of variation was calculated for both within-run and between-run assay variation and were 3.35% and 3.26%, respectively. A laboratory reference DNA sample—comprised of equal DNA from 300 test samples (20 µl taken from each sample, final concentration: 40 ng/µl)—was used to construct a standard curve (mtDNA and nDNA, $R^2 \geq 0.99$). The standard curve was used to quantify and standardize the mtDNAcn and nDNAcn measurements across all test samples.[22] The relative abundance of mtDNAcn, controlled for plate effects, was calculated by dividing the starting quantity of mtDNA by the starting quantity of nDNA. A ratio value of 1 indicates that the mtDNA/nDNA of the test sample is equal to the mtDNA/nDNA in the reference DNA pool used in the assay.

Covariate information

We used information on the following variables, assessed at each visit, to control for potential confounding in analyses: age (continuous), education (≤12 years, 13–15 years, ≥16 years), smoking status (never, former, current), alcohol consumption (≤2 drinks/day, >2 drinks/day), hypertension (yes/no), diabetes (yes/no), computer experience (yes/no), English as first language (yes/no) and body mass index (continuous). We also used information on percentage of the participant's census tract that is non-white (continuous), percentage of the adult residents in the participant's census tract with at least a college degree and above (continuous), and season (spring, summer, fall, winter) as previously described.[23,24]

Statistical analysis

Temperature and cognitive function

Due to an observed ceiling effect of MMSE scores, with many men achieving the maximal score and few men who exhibited

scores that would typically trigger further evaluation (<24), we dichotomized the MMSE as >25 and ≤25, as described in previous work within the same cohort.[6,25] Regression analyses were performed using generalized linear mixed models with a logit link between the dichotomized MMSE score as the dependent variable and 1-day lag temperature as the independent variable, adjusting for potential confounders. We used random intercepts to account for repeated measures and within-subject clustering. To assess any potential deviation from linearity, we also fit a generalized additive mixed model with a penalized spline for temperature and used generalized cross-validation to select the optimal number of degrees of freedom for this association. Generalized cross-validation estimated that the best-fitting number of degrees of freedom was 1, suggesting that a linear association was the best fit. We, thus, present linear results.

To evaluate the effects of temperature on the oldest portion of our cohort, we stratified the models by age, dichotomized at 84 years old, that is, at the 90th percentile of the age distribution in this study population.

We present all estimated effects as odds ratios (ORs) and 95% confidence intervals (CIs) per 1°C decrease in temperature, unless otherwise noted. We conducted all statistical analyses using the R Statistical Software, version 3.4.4 (Foundation for Statistical Computing, Vienna, Austria).

Effect modification of the association between temperature and cognitive function by mtDNAcn

It is possible that outdoor temperature could influence mtDNAcn, in which case mtDNAcn would be a mediator in the temperature—cognition association. Before assessing mtDNAcn as a potential modifier, thus, we evaluated whether temperature was associated with mtDNAcn in our population, using a linear mixed model, adjusting for potential confounders. We observed no association between temperature and mtDNAcn in our study population ($P = 0.9$). This suggests that mtDNAcn did not operate as an intermediate in the association between temperature and cognitive function and a model with an interaction term between temperature and mtDNAcn was appropriate.

We, therefore, run a model that included an interaction term between temperature (1-day lag) and mtDNAcn to determine whether mtDNAcn modifies the association between temperature and cognition. Specifically, we fit the following model:

$$\log\left(\frac{p_{it}}{1-p_{it}}\right) = \left(\beta_0 + b_{0,i}\right) + \beta_1 T_{it} + \beta_2 mtDNAcn_{it} + \beta_3 T_{it} mtDNAcn_{it} + \boldsymbol{\beta Z},$$

where p_{it} denotes the probability of low cognitive MMSE score for subject i at visit t, $b_{0,i} \sim N\left(0, \sigma_b^2\right)$ random intercepts to account for within-subject clustering, T_{it} the outdoor temperature, $mtDNAcn_{it}$ the mtDNA count, and $\boldsymbol{Z}$ a list of potential confounders included in the model. The term $\beta_3 T_{it} mtDNAcn_{it}$ denotes the interaction term we included in the model to evaluate whether mtDNAcn modifies the association between outdoor temperature and cognitive score.

Sensitivity analyses

As a sensitivity analysis, we evaluated the possible role of humidity on the relationship between temperature and cognitive function. We ran the same models described above using apparent temperature as the exposure of interest, which is used to describe how people perceive the combination of temperature and humidity.[26]

In addition, we assessed the influence of potentially informative loss to follow-up using inverse probability weighting (IPW), as previously described.[23] If loss to follow-up does not occur at random, conditional on the terms included in our regression models, it may lead to selection bias. To evaluate this potential bias, we repeated the analyses of the interaction model after weighting follow-up observations by the inverse probability of attaining a follow-up response.[25,27,28] A subject with full information across all visits in the study period was defined as complete, otherwise, the subject was defined as lost to follow-up. We predicted the probability of the response indicator (yes/no) at each visit using covariate information from the previous visit, using a logistic regression model. Subsequently, the interaction model was weighted by the inverse of the estimated probabilities of obtaining a follow-up response, that is, higher weights were assigned to observations that were more likely to be missing.

Finally, the measurements of blood mtDNAcn are correlated with the relative amount of different blood cell types. Therefore, in sensitivity analyses, we further adjusted the interaction model that included mtDNAcn for blood cells that showed associations with mtDNAcn in our data (i.e., neutrophils lymphocytes, monocytes, and platelets).

Results

Of the 591 NAS participants included in the analysis (Table 1), 428 (31.2%) had 2 visits, 260 had 3 visits (18.9%), 94 had 4 visits (6.8%), and 1 had 5 visits (0.07%). Of these participants, 15.9% had diabetes, 74.8% had hypertension, and 78.7% were overweight (body mass index >25 Kg/m^2). Also, 4.6% of the participants were current smokers and 18.9% were heavy alcohol drinkers (≥2 drinks/day). The mean age was 75.5 years (SD = 6.6). The prevalence of low cognitive function among all observations in our population was 28.3%. Outdoor temperature ranged between −5.8°C and 25.7°C, with a mean of 12°C (SD = 5.3°C). In our study population, mtDNAcn ranged from 0.09 to 7.8 with a mean of 1.08.

Temperature and risk of low cognitive score

We observed no association between outdoor temperature and lower cognitive scores on the MMSE (OR = 0.99; 95% CI = 0.94, 1.03; $P = 0.61$) among the full set of study participants. However, among older participants (those at or above the 90th percentile of the age distribution, ≥84 years), cooler temperature was associated with a higher OR for low cognitive score

Table 1.

Baseline characteristics of NAS participants.

	First exam characteristics[a]	
Characteristic	**N**	**%**
Alcohol usage (drinks/day)		
≤2	479	81.1
>2	112	18.9
Diabetes		
No	497	84.1
Yes	94	15.9
Hypertension		
No	149	25.2
Yes	442	74.8
BMI (Kg/m^2)		
≥25	465	78.68
<25	126	21.32
Smoking		
Never	178	30.1
Current	27	4.57
Former	386	65.3
Education (years)		
12–15	326	55.1
≥16	228	38.6
No data	7	1.2

[a]Characteristics at the first study visit included in the analysis.

BMI indicates body mass index.

(OR = 1.2; 95% CI = 1.05, 1.35; P = 0.007) (Figure 1). Among participants younger than 84, we observed no relationship between cooler temperature and impaired cognition (OR = 0.99; 95% CI = 0.94, 1.04; P = 0.85).

Blood mtDNA copy number modifies the interaction between outdoor temperature and cognitive function

The age- and visit-adjusted intraclass correlation coefficient for mtDNAcn was 0.14, indicating that most of the variability in mtDNAcn in our study population (86%) is within-subjects, independent of visit number and age.

We observed that mtDNAcn modified the association between outdoor temperature and cognitive function ($P_{interaction}$ = 0.02). In Figure 2, we present the OR per 1°C decrease in temperature across the mtDNAcn distribution, including the CIs. Among participants with higher mtDNAcn, we observed lower ORs per 1°C decrease in temperature compared to the ORs among participants with lower mtDNAcn.

Sensitivity analyses

When using apparent temperature in sensitivity analyses, our results between apparent temperature and cognition did not change in either the main model among the full set of study participants (OR = 0.99; 95% CI = 0.96, 1.02; P = 0.42) or in models stratified by age (OR = 1.1; 95% CI = 1.04, 1.21; P = 0.002, for participants ≥84 years old; OR = 1.00; 95% CI = 0.97, 1.03; P = 0.92, for participants <84 years old).

We used IPW to assess the robustness of our mtDNAcn—temperature interaction results to possible informative missingness due to loss to follow-up. The IPW-adjusted model produced very similar interaction term effect estimates as the non-IPW model ($\beta_{3,IPW}$ = 0.12; 95% CI = 0.015, 0.22 vs. $\beta_{3,main}$ = 0.12; 95% CI = 0.014, 0.22).

Finally, we further adjusted the mtDNAcn—temperature interaction models for blood cell subpopulations. We included cell types associated with mtDNAcn in bivariate analysis: neutrophils, lymphocytes, monocytes, and platelets. The cell type-adjusted estimates did not show meaningful differences in the estimates of the interaction terms ($\beta_{3,cell\_adj}$ = 0.11; 95% CI = 0.01, 0.21; P = 0.03).

Discussion

Among individuals 84 years of age or older, decreasing temperature was associated with higher risk of low cognitive score. This suggests that there may be an effect of older age on the association between cooler temperature and cognitive function. The level of mtDNAcn also modified the association between temperature and cognitive function, consistent with our hypothesis that mitochondria are involved in adaptation to temperature levels.

Our work closely relates to that published by Dai et al.[6] who, using the same cohort, reported an nonlinear association between short-term temperature exposure and cognition. However, there are a few important differences between the 2 studies. Our study is based on multiple study visits expanding the within-subject knowledge. Also, we excluded people who had experienced a stroke, while Dai et al. did not. We decided to exclude this subpopulation, as stroke can severely impact a person's ability to successfully complete an MMSE examination and has been linked to alterations in mtDNAcn,[29] an important component in our analysis that was not part of the Dai et al. analysis. Moreover, we excluded the subpopulation with stroke a population that was on average more likely to be diabetic and have hypertension, 2 conditions that could aggravate temperature impacts on cognitive health. Exclusion of this subpopulation could explain the null findings in our dataset when we considered all ages.

Multiple biological mechanisms may explain our findings. Colder temperature causes vasoconstriction and this decreased blood flow may be related to worse cognitive function. In older individuals, this may be compounded by an already weakened vascular system. Higher outdoor temperature could sustain a generalized inflammatory state through increased kinetic energy at the molecular level. This, in turn, could result in higher

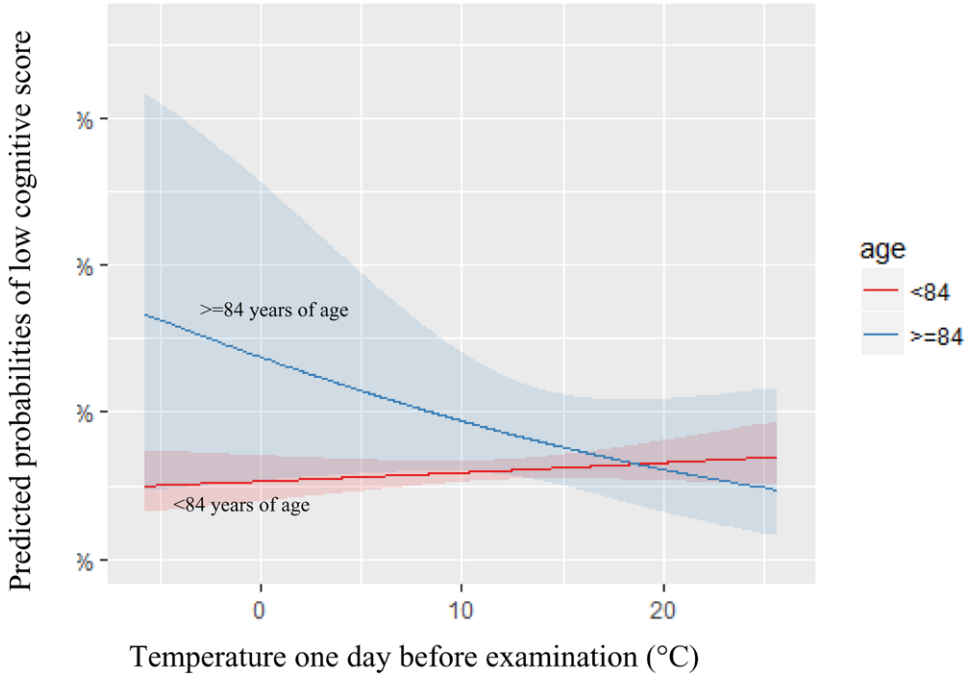

Figure 1. Probability of low MMSE cognitive score (≤25) associated with temperature (with a 1-day lag) by age at the time of examination (55–84 years or 84–99 years).

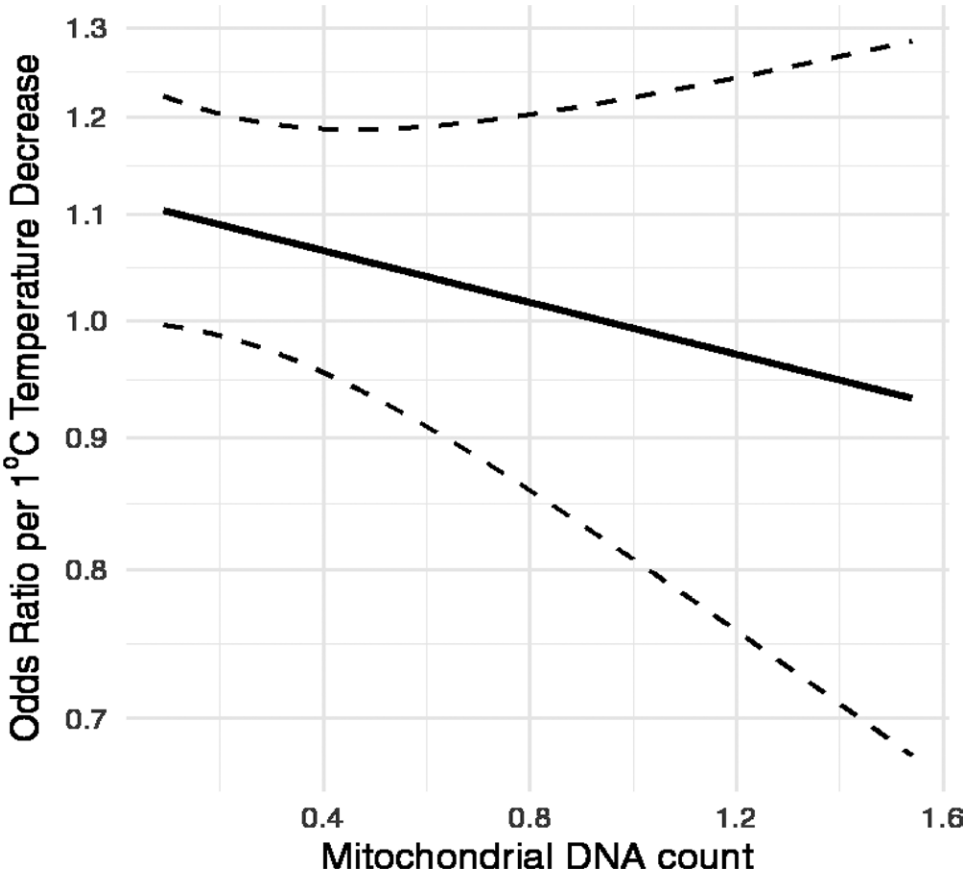

Figure 2. Adjusted ORs (solid line) and 95% CIs (dashed lines) per 1°C decrease in temperature across the mtDNAcn distribution. To facilitate visualization, we present results up to the 95th percentile of the mtDNAcn distribution.

production of reactive oxygen species, which may cause vasoconstriction[30] and worsen cognitive performance. In our sensitivity analyses, we used apparent temperature to take into consideration the role of humidity on the relationship between temperature and cognitive function, but no differences were seen compared to the outdoor temperature models.

MtDNAcn is particularly sensitive to oxidative stress.[31] Fluctuations in mtDNAcn, which are sensitive to exogenous stressors and involved in mitochondrial biogenesis and degradation processes, are thought to mediate an adaptive response to compensate for mitochondrial genome damage.[32] These capabilities could be compromised by aging, given that mitochondrial dysfunction is 1 of the 9 hallmarks of the aging process. Mitochondrial dysfunctions manifest during normal aging, but alternatively, the intensification of dysfunction can accelerate aging or the amelioration of dysfunction can extend lifespan in model organisms.[33] Consideration of these elements could help to better understand the association between temperature and mtDNAcn observed in our study population.

Our study has many strengths. It is the first study to use mitochondrial genome content to study the relationship between temperature and cognitive function. Additionally, we assessed temperature exposure during the day before visit, an important measure of recent temperature exposure. Other strengths include the well-characterized cohort with information on multiple potential confounders and the longitudinal design.

This study also has some limitations. It is limited by the use of only 1 cognitive scoring system; therefore, results cannot be generalized to performance in specific cognitive domains. The MMSE may fail to detect changes in people with advanced dementia or poor education (known as floor effects). Also, our findings are based on a cohort of older white men and our findings, thus, may not generalize to other populations. In addition, the range of mtDNAcn in our study population could be smaller than in a younger population. We also lack information on indoor temperature, which is important as older people may spend more time indoors and may confound the assumptions made regarding outdoor temperatures. There were no examinations done at extreme temperatures, so we were unable to assess the temperature-cognition relationship under such conditions. It is probable that under extreme temperatures, study participants were likely to miss a visit. Finally, our analyses only included information on 591 participants and may have been underpowered.

Conclusions

In summary, we found that temperature can influence cognitive function in a specific age group. Among all our subjects, an association between warmer temperature and low cognitive scores was found for those with higher mtDNAcn; conversely, among individuals with lower mtDNAcn, cooler outdoor temperature was associated with low cognitive scores. Our findings suggest a kind of adaptation and compensatory effect to outdoor temperature in older individuals and those with relatively low mtDNAcn. Older people may lose an adaptation mechanism to an outdoor stressor like temperature, which could result in less efficient homeostatic processes, potentially resulting in decrease of cognitive function. Our results warrant further studies to better understand the effect of temperature on cognitive function and its possible interaction with mitochondrial function. Subsequently, this could help in understanding more clearly the biological mechanisms of the known decrease in homeostatic responses within an aging population and the contributions of outside stressors. Clarifying these questions could increase

our knowledge for disease prevention and help develop better healthy aging strategies, both of which are essential components of current public health policies that have to take into consideration role of climate change on human health.

Conflicts of interest statement

The authors declare that they have no conflicts of interest with regard to the content of this report.

References

1. Hoogendam YY, Hofman A, van der Geest JN, van der Lugt A, Ikram MA. Patterns of cognitive function in aging: the Rotterdam Study. Eur J Epidemiol. 2014;29:133–140.
2. Muir SW, Gopaul K, Montero Odasso MM. The role of cognitive impairment in fall risk among older adults: a systematic review and meta-analysis. Age Ageing. 2012;41:299–308.
3. Almeida OP, Flicker L. The mind of a failing heart: a systematic review of the association between congestive heart failure and cognitive functioning. Intern Med J. 2001;31:290–295.
4. Bassuk SS, Wypij D, Berkman LF. Cognitive impairment and mortality in the community-dwelling elderly. Am J Epidemiol. 2000;151:676–688.
5. Hajat S, Kovats RS, Lachowycz K. Heat-related and cold-related deaths in England and Wales: who is at risk? Occup Environ Med. 2007;64:93–100.
6. Dai L, Kloog I, Coull BA, et al. Cognitive function and short-term exposure to residential air temperature: A repeated measures study based on spatiotemporal estimates of temperature. Environ Res. 2016;150:446–451.
7. Leavitt VM, Sumowski JF, Chiaravalloti N, Deluca J. Warmer outdoor temperature is associated with worse cognitive status in multiple sclerosis. Neurology. 2012;78:964–968.
8. Muller MD, Gunstad J, Alosco ML, et al. Acute cold exposure and cognitive function: evidence for sustained impairment. Ergonomics. 2012;55:792–798.
9. O'Brien C, Tharion WJ, Sils IV, Castellani JW. Cognitive, psychomotor, and physical performance in cold air after cooling by exercise in cold water. Aviat Space Environ Med. 2007;78:568–573.
10. Malik AN, Czajka A. Is mitochondrial DNA content a potential biomarker of mitochondrial dysfunction? Mitochondrion. 2013;13:481–492.
11. Yakes FM, Van Houten B. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. Proc Natl Acad Sci U S A. 1997;94:514–519.
12. Wei YH, Lee HC. Oxidative stress, mitochondrial DNA mutation, and impairment of antioxidant enzymes in aging 1. Exp Biol Med. 2002;227:671–682.
13. Rosenbaum M, Leibel RL. Adaptive thermogenesis in humans. Int J Obes (Lond). 2010;34 (Suppl 1):S47–S55.
14. Yuk F, Hara Y, Morrison JH, et al. Presynaptic mitochondrial morphology in monkey prefrontal cortex correlates with working memory and is improved with estrogen treatment. Proc Natl Acad Sci. 2013;111:486–491.
15. Hebert-Chatelain E, Desprez T, Serrat R, et al. A cannabinoid link between mitochondria and memory. Nature. 2016;539:555–559.
16. Damon A, Seltzer CC, Stoudt HW, Bell B. Age and physique in health white veterans at Boston. J Gerontol. 1972;27:202–208.
17. Tombaugh TN, McIntyre NJ. The Mini-Mental State Examination: a comprehensive review. J Am Geriatr Soc. 1992;40:922–935.
18. Weisskopf MG, Proctor SP, Wright RO, et al. Cumulative lead exposure and cognitive performance among elderly men. Epidemiology. 2007;18:59–66.
19. Kloog I, Chudnovsky A, Koutrakis P, Schwartz J. Temporal and spatial assessments of minimum air temperature using satellite surface temperature measurements in Massachusetts, USA. Sci Total Environ. 2012;432:85–92.
20. Malik AN, Shahni R, Iqbal MM. Increased peripheral blood mitochondrial DNA in type 2 diabetic patients with nephropathy. Diabetes Res Clin Pract. 2009;86:e22–e24.
21. Andreu AL, Martinez R, Marti R, García-Arumí E. Quantification of mitochondrial DNA copy number: pre-analytical factors. Mitochondrion. 2009;9:242–246.
22. Baccarelli A, Tarantini L, Wright RO, et al. Repetitive element DNA methylation and circulating endothelial and inflammation markers in the VA normative aging study. Epigenetics. 2010;5:222–228.
23. Wilker EH, Baccarelli A, Suh H, Vokonas P, Wright RO, Schwartz J. Black carbon exposures, blood pressure, and interactions with single nucleotide polymorphisms in MicroRNA processing genes. Environ Health Perspect. 2010;118:943–948.
24. Power MC, Weisskopf MG, Alexeeff SE, et al. Modification by hemochromatosis gene polymorphisms of the association between traffic-related air pollution and cognition in older men: a cohort study. Environ Health. 2013;12:16.
25. Power MC, Weisskopf MG, Alexeeff SE, Coull BA, Spiro A III, Schwartz J. Traffic-related air pollution and cognitive function in a cohort of older men. Environ Health Perspect. 2011;119:682–687.
26. Steadman RG, Steadman RG. A universal scale of apparent temperature. J Clim Appl Meteorol. 1984.
27. Robins JM, Hernán MA, Brumback B. Marginal structural models and causal inference in epidemiology. Epidemiology. 2000;11:550–560.
28. van der Wal WM, Geskus RB. IPW: an R package for inverse probability weighting. J Stat Softw. 2011;43:1–23.
29. Song L, Liu T, Song Y, et al. mtDNA copy number contributes to all-cause mortality of lacunar infarct in a Chinese prospective stroke population. J Cardiovasc Transl Res. 2019. doi: 10.1007/s12265-019-09943-9. Online ahead of print.
30. Heijenbrok FJ, Mathy MJ, Pfaffendorf M, van Zwieten PA. The influence of chronic inhibition of nitric oxide synthesis on contractile and relaxant properties of rat carotid and mesenteric arteries. Naunyn Schmiedebergs Arch Pharmacol. 2000;362:504–511.
31. Shaughnessy DT, McAllister K, Worth L, et al. Mitochondria, energetics, epigenetics, and cellular responses to stress. Environ Health Perspect. 2014;122:1271–1278.
32. Zhong J, Cayir A, Trevisi L, et al. Traffic-related air pollution, blood pressure, and adaptive response of mitochondrial abundance. Circulation. 2016;133:378–387.
33. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153:1194–1217.