Healthy lifestyle behaviors and biological aging in the US National Health and Nutrition Examination Surveys 1999-2018

Aline Thomas, PhD[1,2]; Daniel W. Belsky, PhD[3,4]; Yian Gu, PhD[1,2,4,5]

[1] Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University, New York, NY, USA.

[2] Department of Neurology, Columbia University, New York, NY, USA.

[3] Butler Columbia Aging Center, Columbia University Mailman School of Public Health, New York, NY, USA.

[4] Department of Epidemiology, Joseph P. Mailman School of Public Health, Columbia University, New York, NY, USA.

[5] Gertrude H. Sergievsky Center, Columbia University, New York, NY, USA.

Corresponding author: Yian Gu, Departments of Neurology and Epidemiology, Taub Institute, and Sergievsky Center, Columbia University Irving Medical Center, 622 W 168th St., New York, NY 10032

Phone: 212-305-6684 Fax: 212-342-1838

Email: yg2121@cumc.columbia.edu

ABSTRACT

People who have a balanced diet and engage in more physical activity live longer, healthier lives. The aim of this study was to test the hypothesis that these associations reflect a slowing of biological processes of aging. We analyzed data from 42,625 participants (aged 20-84 years, 51% females) from the National Health and Nutrition Examination Surveys (NHANES), 1999-2018. We calculated adherence to a Mediterranean diet (MeDi) and level of leisure time physical activity (LTPA) using standard methods. We measured biological aging by applying the PhenoAge algorithm, developed using clinical and mortality data from NHANES-III (1988-1994), to clinical chemistries measured from a blood draw at the time of the survey. We tested the associations of diet and physical activity measures with biological aging, explored synergies between these health behaviors, and tested heterogeneity in their associations across strata of age, sex, and body mass index (BMI). Participants who adhered to the MeDi and who did more LTPA had younger biological ages compared with those who had less-healthy lifestyles (high vs low MeDi tertiles, β = 0.14 SD [95% CI, -0.18; -0.11]; high vs sedentary LTPA, β = 0.12 SD [-0.15; -0.09], in models controlled for demographic and socioeconomic characteristics. Healthy diet and regular physical activity were independently associated with lower clinically defined biological aging, regardless of age, sex, and BMI category.

Keywords: diet; physical activity; NHANES; epidemiology

INTRODUCTION

Healthy lifestyle behaviors, including balanced diet and regular physical activity, are associated with reduced risk for multiple chronic diseases and longer lifespan (1–3). Diet and exercise are also associated with preservation of metabolic, immunologic, and physical functioning with aging (4,5). These observations suggest the hypothesis that healthy lifestyle behaviors may slow the pace of biological aging.

Biological aging is the progressive loss of system integrity that occurs with advancing age (6). It is thought to arise from the accumulation of molecular changes or "hallmarks" that undermine the functioning and resilience capacity of tissues and organs, ultimately leading to disease and death (7,8). Experiments with animals indicate that biological aging is modifiable; a range of behavioral and pharmacologic interventions that modify molecular hallmarks of aging prolong healthy lifespan in animals (9). In humans, variation in the pace and progress of biological aging is observable from at least young adulthood, and possibly much earlier in the life-course (10,11).

From a public health perspective, lifestyle interventions to slow biological aging have the potential to prevent or delay multiple diseases simultaneously, thus prolonging years of healthy life more efficiently that targeting individual diseases (12). Early efforts to test the hypothesis that healthy lifestyle behaviors could slow biological aging focused on leukocyte telomere length (13–15). However, findings are mixed and lack of clarity over whether telomere length functions as a biomarker of aging and concerns about measurement reliability complicate interpretation of the data (16,17). A new generation of measurements to quantify biological aging uses machine-learning algorithms to integrate information across panels of physiological and/or molecular measures to summarize the overall pace or state of aging of the organism (18–21).

Quantifications of biological aging have been proposed at different biological levels of analysis and in different types of data, including epigenetic, proteomic, metabolomic, and clinical-lab datasets (21). In general, these algorithm-based measurements have proven both more technically reliable and more precise in their predictions of morbidity and mortality as compared with previous generations of aging biomarkers, such as telomere length (22). There are promising findings from analysis of DNA methylation algorithms, several of which indicate slower aging and younger biological age associated with healthy lifestyle factors (23–26).

We evaluated the associations of healthy lifestyle behaviors with biological aging using data from large representative samples of the US adult population. We measured biological aging from clinical laboratory data; a more feasible approach to implement at scale within the setting of public health surveillance. Clinical laboratory-based measures of biological aging have the further advantage of providing information more proximate to disease processes and are as or more predictive of morbidity and mortality as compared with molecular approaches (18,27–29). We tested if participants who adhered more closely to a Mediterranean diet (MeDi) and who were more physically active in their leisure time exhibited signs of delayed biological aging relative to those with less healthy lifestyle. We further explored potential synergies between diet and physical activity and variation in the impact of healthy lifestyle behaviors across strata of age, sex, and body-mass index (BMI).

METHODS

Study design and participants

The NHANES study is an ongoing nationally representative cross-sectional survey designed to assess the health and nutritional status of the non-institutionalized US population (30). Since 1999, the survey is conducted biennially with the recruitment of a stratified, multistage, probability clustered sample of about 5000 participants in 15 counties across the country. The survey includes an in-home interview with collection of demographics, socio-economic, and lifestyle information; followed by a physical examination consisting in a dietary interview, medical and physical measurements, and laboratory tests conducted by trained medical personnel in a mobile examination center. Details of the study design, recruitment procedure and data collection are available from US Centers of Disease Control and Prevention (30). The protocol of NHANES was approved by the National Center for Health Statistics Research Ethics Review Board, and all participants provided written informed consent.

We combined ten biennial NHANES datasets from 1999 to 2018. We included non-pregnant participants aged ≥20 years-old, and participants ≥85 years-old were not considered because ages ≥85 years-old were recorded as 85 to maximize confidentiality in the survey. Of the 50,313 non-pregnant individuals aged 20 to 84 years-old who were seen at the medical examination, we excluded participants with missing data for diet or leisure time physical activity (LTPA) (n = 4,181) and blood chemistries (n = 3,507) (**Figure 1**). In total, 42,625 participants were included in the analysis.

Lifestyle exposures

Mediterranean Diet score

Dietary information was obtained from validated 24-hour dietary recalls delivered by trained dietary interviewers, using the computer-assisted US Department of Agriculture's (USDA) Multi-Pass Method. A first 24-hour recall was administrated during the in-person medical examination, and a second was conducted by telephone within 3 to 10 days of the in-person interview (except for the first two NHANES waves 1999-2002 for which only the first dietary recall was collected). Reported foods and beverages were grouped into 37 food components, in the USDA's Food Patterns Equivalents Database. For computation of energy-adjusted dietary intakes, we used the average of the two 24-hour recalls whenever possible (for 75% of the study sample), and only the first recall for participants who did not complete the second one.

The MeDi score, reflecting adherence to the traditional Mediterranean diet, was calculated from nine food components, with sex-specific medians of energy-adjusted intakes used as cut-off values (31). For beneficial components (vegetables, fruits, legumes, cereals, fish, and ratio of mono-unsaturated to saturated fats), one point was given for an intake equal or greater than the median. For components presumed to be detrimental (dairy products, and meat), one point was awarded for an intake less than the median. For alcohol, one point was given for a mild-to-moderate consumption (i.e.,]0-1] drink per day for females, and]0-2] drink per day for males). The total MeDi score ranges from 0 to 9, with higher score indicating greater adherence. The MeDi score was studied as both continuous and categorized (empirical tertiles defining three score categories: 0-3 [low adherence], 4-5 [moderate adherence], and 6-9 [high adherence]) variables.

Leisure Time Physical Activity level

Self-reported physical activity was assessed by two different questionnaires depending on the NHANES wave (1999-2006, and 2007-2018), both administrated during the medical examination. From 1999 to 2006, the physical activity questionnaire detailed the engagement in 62 specific LTPA over the past 30 days, with recording of the frequencies, durations (in minutes), and intensities (moderate or vigorous) for each activity. The frequency was multiplied by the duration, and the resulting value (total minutes/month) divided by 4.33 (weeks/month) to obtain the number of minutes per week for each LTPA. The total duration of LTPA per week was then computed separately for activities of moderate and vigorous intensities. Starting 2007, the WHO Global Physical Activity Questionnaire reported the number of days and minutes of participating for at least 10 minutes continuously in moderate and vigorous LTPA (sports, fitness, and recreational activities) in a typical week. The total number of minutes per week for each intensity of LTPA was calculated as the frequency multiplied by the duration.

For both questionnaires, the total moderate-to-vigorous LTPA was then coded in metabolic equivalent of task (MET) minutes per week by multiplying the duration of the activities and the intensity-specific MET scores (4.0 MET for moderate and 8.0 MET for vigorous intensity LTPA, as suggested by the NHANES guidelines). Finally, LTPA was classified into four levels according to the 2018 national physical activity guidelines: sedentary (no regular physical activity, i.e. 0 MET minutes/week), low (insufficient regular activity, <500 MET min/week, i.e. ~2 hours/week of moderate LTPA), moderate (500-1000 MET min/week), and high (>1000 MET min/week, i.e. ~4 hours/week of moderate LTPA) (32).

Biological aging

Biological age was measured from clinical laboratory blood chemistries using the PhenoAge algorithm (33,34). We selected PhenoAge because this is the best-validated measure of biological age that is feasible to implement within NHANES. PhenoAge is highly predictive of morbidity and mortality, outperforming alternative blood-chemistry-based biological age algorithms and algorithms derived from DNA methylation (27,28). Moreover, PhenoAge is modified by caloric restriction, an intervention established to slow biological aging (33). Briefly, the PhenoAge was developed from analysis of NHANES III data (collected 1988-1994) using elastic-net regression to develop a mortality predictor from a comprehensive database of clinical laboratory data and age. The resulting PhenoAge algorithm consisted of age and eight biomarkers: albumin, alkaline phosphatase, creatinine, glycated hemoglobin (HbA1C), white blood cell count, lymphocyte percentage, mean cell volume, and red cell distribution width. Values of the PhenoAge can be interpreted as the age at which a participant's mortality risk would match the average in the NHANES III training sample. The PhenoAge algorithm was implemented using the 'BioAge' R package (33).

For analysis, we computed PhenoAge advancement as the difference between predicted biological age and chronological age. PhenoAge advancement was standardized to have a mean of 0 and a standard deviation of 1. A positive PhenoAge advancement value indicates an advanced state of biological aging and increased risk of diseases and mortality; a negative PhenoAge advancement indicates a delayed biological aging.

Covariates

Demographic characteristics obtained from the in-home interview included age, self-reported sex, race/ethnicity (non-Hispanic Whites, non-Hispanic Blacks, Hispanics [including Mexican-American and other Hispanics], and others [including Asians, others, and mixed race/ethnicities]). Socioeconomic information included educational attainment (under high school, high school or some college, and bachelor's degree or above), marital status (married/cohabitating, divorced/widowed/separated, and never married), and ratio of family income to poverty (below the federal poverty level [≤1], middle income [1-4], high-income [≥4], according to the Patient Protection and Affordable Care Act and previous studies) (35). Lifestyle factors included smoking status (never [did not smoke 100 cigarettes in life], former [smoked at least 100 lifetime cigarettes but do not smoke now], and current), BMI category (normal weight [<25 kg/m^2], overweight [25-30 kg/m^2], and obesity [≥30 kg/m^2]), and total energy intake from the 24-h recall (in kcal).

Statistical analysis

NHANES complex survey design was taken into account in weighted analyses using dietary survey weights (WTDR2D) which address unequal selection probabilities, pattern of non-response to the survey and to the dietary component, and incorporate the day of the week of recall, to obtain nationally representative estimates. As recommended, weights were combined across survey cycles using the 4-year dietary survey weights for 1999-2002 period and the 2-years dietary weights for the following waves.

The characteristics of participants were presented as means with standard-deviation (SD) and percentages in the total population and across categories of MeDi adherence. The standardized

PhenoAge advancement was described as mean and 95% confidence intervals (CI) across categories of MeDi, LTPA levels and their interactions.

The association between MeDi score or LTPA level and PhenoAge advancement (standardized) was evaluated by linear regressions. Adjustment for covariates was performed in several models: Model 1 was adjusted for demographics (i.e., age, sex, race/ethnicity, total energy intake, and NHANES wave); Model 2 was additionally adjusted for socio-demographic status (i.e., education, income-to-poverty ration, and marital status); Model 3 was adjusted for covariates of Model 1 and smoking status and BMI category; Model 4 was adjusted for Model 1 and mutually adjusted for MeDi score and LTPA level; and Model 5 was fully adjusted for all above covariates and mutually adjusted for dietary score and LTPA level. The MeDi score was analyzed as a continuous variable (for each 1-point increase) and as a categorical variable (moderate and high vs low adherence), and LTPA was modeled as a categorical variable (low, moderate, and high levels vs sedentary). The linear trends across categories of MeDi or LTPA were tested by assigning the median value to each category and treating it as a continuous variable in the models.

The interaction of MeDi score and regular LTPA on PhenoAge, and the potential modification effects by age (age groups 20-40, 40-60, and 60-84 years old), sex, BMI category were separately examined by testing the interaction between the variable and the primary exposures (continuous MeDi or regular LTPA [binary variable, ≥low level vs sedentary]), and stratified analyses were performed. Interaction tests and stratifications were analyzed in Model 1.

In a sensitivity analysis, we accounted for possible reverse causality by excluding participants with history of chronic or major diseases that could influence their diet and practice of physical

activity (i.e., diabetes, hypertension, hypercholesterolemia, stroke, cardiovascular disease, chronic bronchitis, liver condition, pulmonary emphysema, thyroid disease, and arthritis), resulting in a subsample of 10,682 disease-free participants. Second, we investigated an alternative dietary pattern score, the Healthy Eating Index 2015 (HEI-2015), assessing diet quality by evaluating the adherence to the 2015-2020 Dietary Guidelines for Americans (see **eMethods** in the Supplement for computation details) (36). Finally, we performed separate analyses in two NHANES cycles defined by the LTPA questionnaire used: 1999-2006 (n = 14,568) and 2007-2018 (n = 28,057).

Statistical analyses were performed using R version 4.2.0 (R Foundation).

RESULTS

Study population characteristics

The 42,625 participants of the analytic sample were representative of 197,323,426 U.S. adults, with a mean age of 47.0 (±16.7) years and 51.0% of females (**Table 1**). Overall, the mean MeDi score was 3.97 (±1.6) points and 17.5% of the participants had a high adherence to MeDi, 43.2% a moderate adherence, and 39.3% a low adherence. Regarding physical activity, 29.4% of the participants had a high level of LTPA, 12.4% a moderate level, 17.4% a low level and, 40.8% did not practice regular LTPA. The mean PhenoAge advancement was -3.62 (±4.6) years, indicating that, on average, participants PhenoAge scores were 3.62 years younger than their chronological age.

Participants with higher adherence to MeDi were older, had higher education level, were more often married or cohabitating, had better socioeconomic conditions, were less likely to smoke or to have obese BMI, and had higher levels of LTPA (**Table 1**). Similarly, participants with higher LTPA level were more educated with higher income-to-poverty ratio, were more likely to be non-smokers, to have normal BMI, and to have higher adherence to MeDi; they also tended to be younger and were more often males (**eTable 1** in Supplement). Participants with greater adherence to MeDi and higher level of LTPA had younger biological age, indicated by lower mean PhenoAge advancement (**Figure 2, panels A and B**).

Association of MeDi and LTPA with PhenoAge advancement

Healthier behaviors were significantly associated with lower biological aging. Each 1-point higher MeDi score was associated with a 0.07 SD (95%CI, -0.08; -0.06) younger PhenoAge. Compared to sedentary participants, those with a high level of LTPA had a 0.31 SD (-0.34; -0.28) younger PhenoAge, after adjustment for demographics (**Table 2**).

The associations were only slightly attenuated when adjusting for socioeconomic status in Model 2. For both MeDi score and LTPA level, the greatest change in effect-size was observed when smoking status and BMI category were added in Model 3, but the associations remained significant, suggesting a partial mediation by these factors. The associations of MeDi score and LTPA level with PhenoAge advancement were only minimally attenuated in the mutually adjusted model (Model 4), indicating independent associations of diet and physical activity with biological aging. In the fully adjusted Model 5, compared to individuals in the lowest tertile of adherence to MeDi, those in the highest tertile had a PhenoAge advancement decreased by 0.14 SD (-0.18; -0.11), which was about the same magnitude of the effect-size of LTPA with

decreases of 0.11 SD (-0.14; -0.08), 0.14 SD (-0.19; -0.10), and 0.12 SD (-0.15; -0.09) respectively for low, moderate and high LTPA level compared to sedentarity.

Synergistic effect between healthy diet and physical activity on PhenoAge advancement

The association of MeDi adherence with PhenoAge advancement was observed across categories of LTPA, and a higher LTPA level was associated with lower PhenoAge advancement across the MeDi categories, with no evidence of interaction (interaction p-values > .05) (**Figure 2, panel C**, and **eTables 2** and **3**).

Effect modification by sex, sex, and BMI category

The associations of MeDi and LTPA level with biological aging were significant for all age groups, for males and females, and for all BMI categories (**Figure 3** and **eTable 4**). However, some heterogeneity was observed.

Effect-sizes for healthy-diet associations with biological aging were slightly stronger for females as compared with males (β = -0.08 SD [-0.09; -0.07] and -0.06 SD [-0.07; -0.05] for each 1-point increase in MeDi, respectively; interaction p-value = .001) and for participants with normal weight as compared to those with overweight or obese BMI (β = -0.07 SD [-0.09; -0.06], -0.05 SD [-0.06; -0.04], and -0.05 SD [-0.07; -0.04], respectively; interaction p-value = .02).

For physical activity, effect-sizes were larger for older as compared with younger participants: compared to sedentarity, practice of some LTPA was associated with 0.17 SD (-0.21; -0.13) younger PhenoAge for those aged 20-40, 0.32 SD (-0.36; -0.27) younger PhenoAge for those aged 40-60, and 0.38 SD (-0.43; -0.33) younger PhenoAge for those older than 60 (interaction p-value < .001).

Sensitivity analysis

The associations of diet and physical activity with biological aging were only slightly attenuated and remained significant among the 10,682 disease-free participants (**eTable 5**). The HEI-2015, which was correlated with the MeDi score (Pearson correlation coefficient = 0.38, $p < .001$), yielded similar results with a decrease of 0.06 SD (-0.09; -0.04) and 0.17 SD (-0.20; -0.14) in PhenoAge advancement for the second and third tertiles compared to the lowest respectively, in the fully adjusted model (**eTables 6** and **7**). Stratification on NHANES cycles, in accordance to which LTPA questionnaire was used, did not change the results (**eTable 8**).

DISCUSSION

We analyzed data from a population-representative sample of US adults drawn across the first two decades of the 21st Century, NHANES 1999-2018. We measured biological age using the PhenoAge blood-chemistry algorithm (18,33). We tested if people with healthier diet and who engaged in more physical activity tended to be biologically younger as compared to peers with less healthy behavior. Across NHANES waves and for all individuals (younger and older, males and females, lean and participants with obesity), a healthier diet and a higher level of physical activity were consistently associated with younger biological age. Associations were robust to potential confounders, including demographic and socioeconomic factors, smoking, and BMI.

Associations of healthy diet and physical activity with younger biological age were independent and additive; healthier diet was associated with younger biological age at all levels of physical

activity, and higher activity levels were associated with younger biological age at all levels of healthy diet adherence. Nevertheless, we note that among individuals with the less healthy diet, those who practiced physical activity, even at low level, exhibited delayed biological aging; while among sedentary people, even the healthiest diet did not completely overcome the detrimental effects of sedentarity (**Figure 2, panel C** and **eTable 2**).

Effect-sizes for healthy-lifestyle associations with biological age seemed to be small; participants with the healthiest diet and practicing at least some physical activity were about 1-year biologically younger than those with the least healthy dietary habits and sedentary behaviors. However, this small effect has public health significance. In a prior study of a diverse sample of US adults aged 50 and older, using the same measure as in this study, we found that a 1-year younger biological age corresponded to a 7% decrease in mortality, a 3% decrease in incident disability, and a 2% decrease in incident chronic disease over two years of follow-up (28). Improving healthy lifestyle behaviors in the population therefore has potential to generate non-trivial improvements in healthy lifespan.

Our findings are significant for public health and public policy. In the context of the aging global population, prevention strategies to delay age-related diseases and prolong healthy lifespan are needed (37). Changes in diet and exercise behavior represent low-cost and scalable strategies to promote population health (38). Our results suggest they can contribute to promoting healthy lifespan via slowed biological aging. Coming from a large, diverse, population-based sample of US adults accumulated over two decades, our findings build on smaller scale studies focusing on genomic measures of biological aging (23–26,39–41) to establish the link between healthy

behaviors and processes of biological aging spanning young adulthood to midlife to old age. Programs and policies to promote healthy lifestyle represent a critical component of strategies to maintain health and productivity of aging populations.

Our study has some limitations. First, there is no gold standard for measuring biological aging. Nevertheless, the PhenoAge measure we analyzed is well validated as a predictor of age-related risk for disease, disability, and mortality in diverse populations (18,19,27–29). PhenoAge is quantified from easily accessible clinical indicators that directly reflect the integrity organ systems involved in age-related disease; and it shows evidence of modifiability by caloric restriction, known to modify biological aging in animal models (33). Second, the cross-sectional design prevents us to confirm the temporal relationship between lifestyle factors and biological aging or the causality of their association; although, the associations were observed among participants without history of chronic or major diseases. Confirmation of findings in randomized trials of healthy-lifestyle interventions in the general population is needed. Moreover, because data were collected at a single time-point in both young and older adults, the healthy lifestyles we observed likely reflect a mixture of individuals with long-term and more recently adopted healthy behaviors. Longitudinal studies that recording life-course patterns of health lifestyle and how those patterns relate to biological aging are needed. Third, LTPA (activities over the previous month) and diet (24-h recalls) information was self-reported and therefore subject to recall and social-desirability biases that may lead to misclassification of behavioral exposures. However, social-desirability bias in particular should be strongest among higher-SES individuals and those sampled in more recent years, when public health messaging about healthy lifestyle has grown more prominent. Our findings were robust to adjustment for

socioeconomic measures and were consistent across the two-decade period of our study. Residual misclassification bias would drive effects toward the null, making our estimates conservative.

In conclusion, in the US adult population (≥20 years-old), a healthy diet and regular physical activity were independently associated with younger biological age across the first two decades of the 21st Century. These associations were consistent regardless of age, sex, and BMI category, encouraging the promotion of diet and physical activity for overall healthy aging to the general population.

ACKNOWLEDGEMENTS

Authors contributions: AT, DWB and YG designed and conceptualized the study and wrote the manuscript. AT analyzed the data. DWB and YG supervised the research project. All authors had access to all of the data, contributed to the interpretation of findings, and revised and approved the final manuscript.

Funding: This work was supported by the National Institute on Aging (grant numbers R01AG061378, R01AG059013, and R01AG061008). The funders had no role in the study design, data collection, data analysis, data interpretation, or writing of the manuscript.

Conflict of Interest and Funding Disclosure: All authors declare no conflicts of interest.

REFERENCES

1. Dempsey PC, Rowlands AV, Strain T, Zaccardi F, Dawkins N, Razieh C, et al. Physical activity volume, intensity, and incident cardiovascular disease. European Heart Journal. 2022 Oct 27;ehac613. https://doi.org/10.1093/eurheartj/ehac613

2. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet. 2020 Aug 8;396(10248):413–46. https://doi.org/10.1016/S0140-6736(20)30367-6

3. Papadimitriou N, Markozannes G, Kanellopoulou A, Critselis E, Alhardan S, Karafousia V, et al. An umbrella review of the evidence associating diet and cancer risk at 11 anatomical sites. Nat Commun. 2021 Jul 28;12(1):4579. https://doi.org/10.1038/s41467-021-24861-8

4. Longo VD, Anderson RM. Nutrition, longevity and disease: From molecular mechanisms to interventions. Cell. 2022 Apr 28;185(9):1455–70. https://doi.org/10.1016/j.cell.2022.04.002

5. Daskalopoulou C, Stubbs B, Kralj C, Koukounari A, Prince M, Prina AM. Physical activity and healthy ageing: A systematic review and meta-analysis of longitudinal cohort studies. Ageing Research Reviews. 2017 Sep 1;38:6–17. https://doi.org/10.1016/j.arr.2017.06.003

6. Kirkwood TBL. Understanding the odd science of aging. Cell. 2005 Feb 25;120(4):437–47. https://doi.org/10.1016/j.cell.2005.01.027

7. Kennedy BK, Berger SL, Brunet A, Campisi J, Cuervo AM, Epel ES, et al. Geroscience: linking aging to chronic disease. Cell. 2014 Nov 6;159(4):709–13. https://doi.org/10.1016/j.cell.2014.10.039

8. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013 Jun 6;153(6):1194–217. https://doi.org/10.1016/j.cell.2013.05.039

9. Campisi J, Kapahi P, Lithgow GJ, Melov S, Newman JC, Verdin E. From discoveries in ageing research to therapeutics for healthy ageing. Nature. 2019 Jul;571(7764):183–92. https://doi.org/10.1038/s41586-019-1365-2

10. Belsky DW, Caspi A, Houts R, Cohen HJ, Corcoran DL, Danese A, et al. Quantification of biological aging in young adults. Proc Natl Acad Sci U S A. 2015 Jul 28;112(30):E4104-4110. https://doi.org/10.1073/pnas.1506264112

11. Gladyshev VN. The Ground Zero of Organismal Life and Aging. Trends Mol Med. 2021 Jan;27(1):11–9. https://doi.org/10.1016/j.molmed.2020.08.012

12. Burch JB, Augustine AD, Frieden LA, Hadley E, Howcroft TK, Johnson R, et al. Advances in Geroscience: Impact on Healthspan and Chronic Disease. The Journals of Gerontology: Series A. 2014 Jun 1;69(Suppl_1):S1–3. https://doi.org/10.1093/gerona/glu041

13. Siopis G, Porter J. Contribution of Biological Age-Predictive Biomarkers to Nutrition Research: A Systematic Review of the Current Evidence and Implications for Future Research and Clinical Practice. Adv Nutr. 2022 May 24;nmac060. https://doi.org/10.1093/advances/nmac060

14. Canudas S, Becerra-Tomás N, Hernández-Alonso P, Galié S, Leung C, Crous-Bou M, et al. Mediterranean Diet and Telomere Length: A Systematic Review and Meta-Analysis. Advances in Nutrition. 2020 Nov 15;11(6):1544–54. https://doi.org/10.1093/advances/nmaa079

15. Chilton W, O'Brien B, Charchar F. Telomeres, Aging and Exercise: Guilty by Association? Int J Mol Sci. 2017 Nov 29;18(12):E2573. https://doi.org/10.3390/ijms18122573

16. Nettle D, Gadalla SM, Lai TP, Susser E, Bateson M, Aviv A. Measurement of Telomere Length for Longitudinal Analysis: Implications of Assay Precision. American Journal of Epidemiology. 2021 Jul 1;190(7):1406–13. https://doi.org/10.1093/aje/kwab025

17. Lindrose AR, McLester-Davis LWY, Tristano RI, Kataria L, Gadalla SM, Eisenberg DTA, et al. Method comparison studies of telomere length measurement using qPCR approaches: A critical appraisal of the literature. PLoS One. 2021;16(1):e0245582. https://doi.org/10.1371/journal.pone.0245582

18. Belsky DW, Moffitt TE, Cohen AA, Corcoran DL, Levine ME, Prinz JA, et al. Eleven Telomere, Epigenetic Clock, and Biomarker-Composite Quantifications of Biological Aging: Do They Measure the Same Thing? Am J Epidemiol. 2018 Jun 1;187(6):1220–30. https://doi.org/10.1093/aje/kwx346

19. Hastings WJ, Shalev I, Belsky DW. Comparability of biological aging measures in the National Health and Nutrition Examination Study, 1999-2002. Psychoneuroendocrinology. 2019 Aug;106:171–8. https://doi.org/10.1016/j.psyneuen.2019.03.012

20. Verhulst S, Susser E, Factor-Litvak PR, Simons MJ, Benetos A, Steenstrup T, et al. Commentary: The reliability of telomere length measurements. International Journal of Epidemiology. 2015 Oct 1;44(5):1683–6. https://doi.org/10.1093/ije/dyv166

21. Rutledge J, Oh H, Wyss-Coray T. Measuring biological age using omics data. Nat Rev Genet. 2022 Jun 17;1–13. https://doi.org/10.1038/s41576-022-00511-7

22. Li X, Ploner A, Wang Y, Magnusson PK, Reynolds C, Finkel D, et al. Longitudinal trajectories, correlations and mortality associations of nine biological ages across 20-years follow-up. Harper DM, Franco E, Moskalev A, editors. eLife. 2020 Feb 11;9:e51507. https://doi.org/10.7554/eLife.51507

23. He L. Epigenetic Clock: A Novel Tool for Nutrition Studies of Healthy Ageing. J Nutr Health Aging. 2022 Apr 1;26(4):316–7. https://doi.org/10.1007/s12603-022-1773-0

24. Kim Y, Huan T, Joehanes R, McKeown NM, Horvath S, Levy D, et al. Higher diet quality relates to decelerated epigenetic aging. Am J Clin Nutr. 2022 Jan 11;115(1):163–70. https://doi.org/10.1093/ajcn/nqab201

25. Kresovich JK, Garval EL, Martinez Lopez AM, Xu Z, Niehoff NM, White AJ, et al. Associations of Body Composition and Physical Activity Level With Multiple Measures of Epigenetic Age Acceleration. Am J Epidemiol. 2021 Jun 1;190(6):984–93. https://doi.org/10.1093/aje/kwaa251

26. Kresovich JK, Park YMM, Keller JA, Sandler DP, Taylor JA. Healthy eating patterns and epigenetic measures of biological age. Am J Clin Nutr. 2022 Jan 11;115(1):171–9. https://doi.org/10.1093/ajcn/nqab307

27. Liu Z, Kuo PL, Horvath S, Crimmins E, Ferrucci L, Levine M. A new aging measure captures morbidity and mortality risk across diverse subpopulations from NHANES IV: A cohort study. PLoS Med. 2018 Dec;15(12):e1002718. https://doi.org/10.1371/journal.pmed.1002718

28. Graf GH, Crowe CL, Kothari M, Kwon D, Manly JJ, Turney IC, et al. Testing Black-White Disparities in Biological Aging Among Older Adults in the United States: Analysis of DNA-Methylation and Blood-Chemistry Methods. Am J Epidemiol. 2022 Mar 24;191(4):613–25. https://doi.org/10.1093/aje/kwab281

29. Parker DC, Bartlett BN, Cohen HJ, Fillenbaum G, Huebner JL, Kraus VB, et al. Association of Blood Chemistry Quantifications of Biological Aging With Disability and Mortality in Older Adults. The Journals of Gerontology: Series A. 2020 Sep 1;75(9):1671–9. https://doi.org/10.1093/gerona/glz219

30. Centers of Disease Control and Prevention. National Health and Nutrition Examination Survey - NHANES [Internet]. [cited 2022 Aug 4]. Available from: https://www.cdc.gov/nchs/nhanes/index.htm

31. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. N engl J med. 2003;2003(348):2599–608.

32. U.S. Department of Health and Human Services. Physical Activity Guidelines for Americans, 2nd edition. Washington, DC: U.S. Department of Health and Human Services; 2018.

33. Kwon D, Belsky DW. A toolkit for quantification of biological age from blood chemistry and organ function test data: BioAge. Geroscience. 2021 Dec;43(6):2795–808. https://doi.org/10.1007/s11357-021-00480-5

34. Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, et al. An epigenetic biomarker of aging for lifespan and healthspan. Aging. 2018 Apr 18;10(4):573–91. https://doi.org/10.18632/aging.101414

35. Zhang YB, Chen C, Pan XF, Guo J, Li Y, Franco OH, et al. Associations of healthy lifestyle and socioeconomic status with mortality and incident cardiovascular disease: two prospective cohort studies. BMJ. 2021 Apr 14;373:n604. https://doi.org/10.1136/bmj.n604

36. Krebs-Smith SM, Pannucci TE, Subar AF, Kirkpatrick SI, Lerman JL, Tooze JA, et al. Update of the Healthy Eating Index: HEI-2015. J Acad Nutr Diet. 2018 Sep;118(9):1591–602. https://doi.org/10.1016/j.jand.2018.05.021

37. Scott AJ. The longevity society. The Lancet Healthy Longevity. 2021 Dec 1;2(12):e820–7. https://doi.org/10.1016/S2666-7568(21)00247-6

38. Mehta N, Myrskylä M. The Population Health Benefits Of A Healthy Lifestyle: Life Expectancy Increased And Onset Of Disability Delayed. Health Affairs. 2017 Aug;36(8):1495–502. https://doi.org/10.1377/hlthaff.2016.1569

39. Senior AM, Legault V, Lavoie FB, Presse N, Gaudreau P, Turcot V, et al. Multidimensional associations between nutrient intake and healthy ageing in humans. BMC Biol. 2022 Sep 1;20(1):196. https://doi.org/10.1186/s12915-022-01395-z

40. Esposito S, Gialluisi A, Costanzo S, Di Castelnuovo A, Ruggiero E, De Curtis A, et al. Mediterranean diet and other dietary patterns in association with biological aging in the Moli-sani Study cohort. Clin Nutr. 2022 May;41(5):1025–33. https://doi.org/10.1016/j.clnu.2022.02.023

41. Gialluisi A, Di Castelnuovo A, Costanzo S, Bonaccio M, Persichillo M, Magnacca S, et al. Exploring domains, clinical implications and environmental associations of a deep learning marker of biological ageing. Eur J Epidemiol. 2022 Jan 1;37(1):35–48. https://doi.org/10.1007/s10654-021-00797-7

Table 1. Characteristics of the study participants, NHANES 1999-2018 (n = 42,625)

	Total population	Adherence to Mediterranean diet		
		Low [0 - 3] (n = 15 075)	Moderate [4 - 5] (n = 18 863)	High [6 - 9] (n = 8 687)
Representative population size	197 323 426	77 482 601	85 285 797	34 555 028
Age (years), mean (SD)	47.0 (16.7)	44.4 (16.3)	47.9 (16.8)	50.7 (16.8)
Females, n (%)	21357 (51.0)	7745 (51.7)	9496 (51.0)	4116 (49.3)
Race/ethnicity, n (%)				
Non-Hispanic White	19408 (69.4)	8073 (74.1)	8169 (68.0)	3166 (62.2)
Non-Hispanic Black	8470 (10.4)	3023 (10.0)	3826 (10.8)	1621 (10.1)
Hispanic	11064 (13.6)	3122 (11.1)	5269 (14.7)	2673 (16.5)
Others	3683 (6.6)	857 (4.7)	1599 (6.6)	1227 (11.3)
Education, n (%)				
Less than high school	10991 (16.3)	3556 (16.0)	5010 (16.3)	2425 (17.1)
High school or some college	22175 (55.6)	8768 (60.6)	9593 (54.5)	3814 (46.9)
Bachelor degree or higher	9417 (28.1)	2740 (23.4)	4241 (29.2)	2436 (36.0)
Marital status, n (%)				
Married or cohabitating	25783 (63.3)	8630 (60.9)	11480 (63.7)	5673 (67.4)
Divorced, widowed, or separated	9086 (18.4)	3231 (18.7)	4076 (18.4)	1779 (17.8)
Never married	7386 (18.4)	3082 (20.4)	3145 (17.9)	1159 (14.8)
Income-to-poverty ratio, n (%)				

Below poverty level	7725 (13.9)	2957 (14.9)	3392 (13.5)	1376 (12.4)
Middle-income	20878 (48.9)	7602 (50.6)	9186 (48.4)	4090 (46.2)
High-income	10514 (37.2)	3440 (34.5)	4655 (38.0)	2419 (41.3)
Smoking status, n (%)				
Never	22943 (53.4)	7408 (49.7)	10485 (55.1)	5050 (57.7)
Former	10621 (25.0)	3399 (22.6)	4761 (25.8)	2461 (28.3)
Current	9032 (21.6)	4260 (27.8)	3606 (19.0)	1166 (14.0)
BMI category, n (%)				
Normal weight	12306 (31.0)	4360 (30.1)	5283 (30.5)	2663 (34.4)
Overweight	14353 (33.5)	4759 (32.3)	6438 (33.8)	3156 (35.7)
Obesity	15497 (35.4)	5823 (37.5)	6917 (35.7)	2757 (30.0)
Dietary calories (kcal), mean (SD)	2133.5 (894.0)	2335.3 (897.4)	2053.3 (881.6)	1878.7 (817.6)
MeDi score, mean (SD)	3.97 (1.6)	2.35 (0.8)	4.45 (0.5)	6.42 (0.6)
LTPA level, n (%)				
Sedentary	20411 (40.8)	7506 (43.5)	9022 (40.0)	3883 (36.5)
Low	6777 (17.4)	2385 (17.4)	3013 (17.8)	1379 (16.4)
Moderate	4794 (12.4)	1632 (12.1)	2100 (12.4)	1062 (13.2)
High	10643 (29.4)	3552 (27.0)	4728 (29.9)	2363 (33.9)
NHANES wave, n (%)				
1999-2000	3410 (8.2)	1256 (9.1)	1503 (7.8)	651 (7.1)
2001-2002	3858 (9.3)	1410 (9.6)	1706 (9.3)	742 (9.0)

2003-2004	3647 (9.2)	1151 (8.4)	1617 (9.2)	879 (11.1)
2005-2006	3653 (9.3)	1300 (9.1)	1680 (9.8)	673 (8.7)
2007-2008	4931 (10.0)	1689 (9.7)	2182 (10.0)	1060 (10.7)
2009-2010	5262 (10.2)	1892 (10.2)	2289 (9.9)	1081 (11.2)
2011-2012	4313 (10.5)	1488 (9.8)	1911 (10.9)	914 (11.3)
2013-2014	4675 (10.9)	1747 (11.4)	1989 (10.6)	939 (10.8)
2015-2016	4553 (11.0)	1577 (11.0)	2090 (11.4)	886 (10.0)
2017-2018	4323 (11.2)	1565 (11.7)	1896 (11.2)	862 (10.2)
PhenoAge advancement (years), mean (SD)	-3.62 (4.6)	-3.27 (4.5)	-3.70 (4.6)	-4.22 (4.6)
Standardized PhenoAge advancement, mean (SD)	-0.09 (0.94)	-0.02 (0.92)	-0.11 (0.95)	-0.21 (0.94)

Notes: BMI = body mass index; LTPA = leisure-time physical activity; MeDi = Mediterranean diet; MET = metabolic equivalent of task; SD = standard deviation

Percentages, means, and standard deviations are of non-missing values and presented as weighted estimates to account for sampling design. Values were missing for: 8.2% of the sample for income-to-poverty ration, 1.1% for BMI, 0.9% for marital status, and 0.1% for education and smoking status.

BMI categories are defined as: normal weight (<25 kg/m^2), overweight (25-30 kg/m^2), and obesity (≥30 kg/m^2). LTPA levels are defined as: sedentary (0 MET minutes per week), low (1-500 MET min/week), moderate (500-1000 MET min/week), and high (>1000 MET min/week).

Table 2. Associations of MeDi and LTPA level with PhenoAge advancement, estimated by adjusted linear regressions, NHANES 1999-2018 (n = 42,625)

	Model 1 Demographics	Model 2 Socioeconomics	Model 3 Smoking and BMI	Model 4 LTPA adjusted	Model 5 Fully adjusted
MeDi score (for +1 point)	-0.07 [-0.08; -0.06]	-0.05 [-0.06; -0.05]	-0.04 [-0.05; -0.04]	-0.06 [-0.07; -0.05]	-0.03 [-0.04; -0.03]
Adherence to MeDi					
Low	Ref	Ref	Ref	Ref	Ref
Moderate	-0.13 [-0.16; -0.10]	-0.11 [-0.14; -0.07]	-0.08 [-0.11; -0.05]	-0.12 [-0.15; -0.09]	-0.07 [-0.10; -0.04]
High	-0.27 [-0.30; -0.24]	-0.22 [-0.26; -0.18]	-0.17 [-0.21; -0.14]	-0.23 [-0.27; -0.20]	-0.14 [-0.18; -0.11]
LTPA level					
Sedentary	Ref	Ref	Ref	Ref	Ref
Low	-0.21 [-0.24; -0.18]	-0.15 [-0.18; -0.11]	-0.15 [-0.18; -0.12]	-0.20 [-0.23; -0.17]	-0.11 [-0.14; -0.08]
Moderate	-0.29 [-0.33; -0.25]	-0.21 [-0.25; -0.17]	-0.20 [-0.24; -0.16]	-0.28 [-0.32; -0.23]	-0.14 [-0.19; -0.10]
High	-0.31 [-0.34; -0.28]	-0.23 [-0.26; -0.19]	-0.17 [-0.20; -0.14]	-0.28 [-0.32; -0.25]	-0.12 [-0.15; -0.09]

Notes: LTPA = leisure-time physical activity; MeDi = Mediterranean diet.

Results given as β coefficient and 95% confidence intervals.

PhenoAge advancement was standardized so that regression coefficients are relative to 1 SD (= 4.6 years) of PhenoAge advancement. Adherence to MeDi is defined as tertiles of MeDi score: low (0 to 3), moderate (4 to 5), and high (6 to 9). LTPA levels are defined as: sedentary (0 MET min/week), low (1 to 500 MET min/week), moderate (500 to 1000 MET min/week), and high (>1000 MET min/week).

Models were run on participants without missing data for covariates and separately for MeDi score or category and LTPA level (except for mutual adjustments in models 4 and 5). Model 1 was adjusted for age, sex, race/ethnicity, total energy intake, and NHANES wave (n = 42,625). Model 2 was adjusted for covariates of model 1, and education, income-to-poverty ratio, and marital status (n = 38,771). Model 3 was adjusted for covariates of model 1, and smoking status and body mass index category (n = 42,130). Model 4 was adjusted covariates of model 1 and mutually adjusted for MeDi score and LTPA level (n = 42,625). Model 5 was mutually adjusted for MeDi score and LTPA level and included all the above covariates (n = 38,328).

All associations were significant with a p-value and/or P for trend <0.001 (linear trends tested by assigning the median value to each category and treating it as a continuous variable in the models: medians MeDi scores were 3 points for low, 4 points for moderate, and 6 points for high adherence; medians MET minutes per week were 0 for sedentary, 249 for low, 721 for moderate, and 2400 for high level of LTPA).

Figure 1. Flow chart of participants selection

Figure 2. Mean standardized PhenoAge advancement by MeDi and LTPA categories, NHANES 1999-2018 (n = 42,625)

Notes: LTPA = leisure-time physical activity; MeDi = Mediterranean diet

Means and 95% confidence intervals of the standardized PhenoAge advancement weighted estimates accounting for sampling design, by categories of adherence to MeDi (Panel A; low [0-3], moderate [4-5], and high [6-9]), LTPA levels (Panel B; sedentary [0 MET min/week], low [1-500 MET min/week], moderate [500-1000 MET min/week], and high [>1000 MET min/week]), and the interaction of MeDi and LTAP categories (Panel C).

Figure 3. Mean standardized PhenoAge advancement by MeDi and LTPA categories, stratified by age group, sex, and BMI category, NHANES 1999-2018 (n = 42,625)

Notes: BMI = body mass index; LTPA = leisure-time physical activity; MeDi = Mediterranean diet

Means and 95% confidence intervals of the weighted estimates accounting for sampling design, by categories of adherence to MeDi and by LTPA levels, stratified by age group (Panel A), sex (Panel B), and by BMI categories (Panel C).

Figure 1

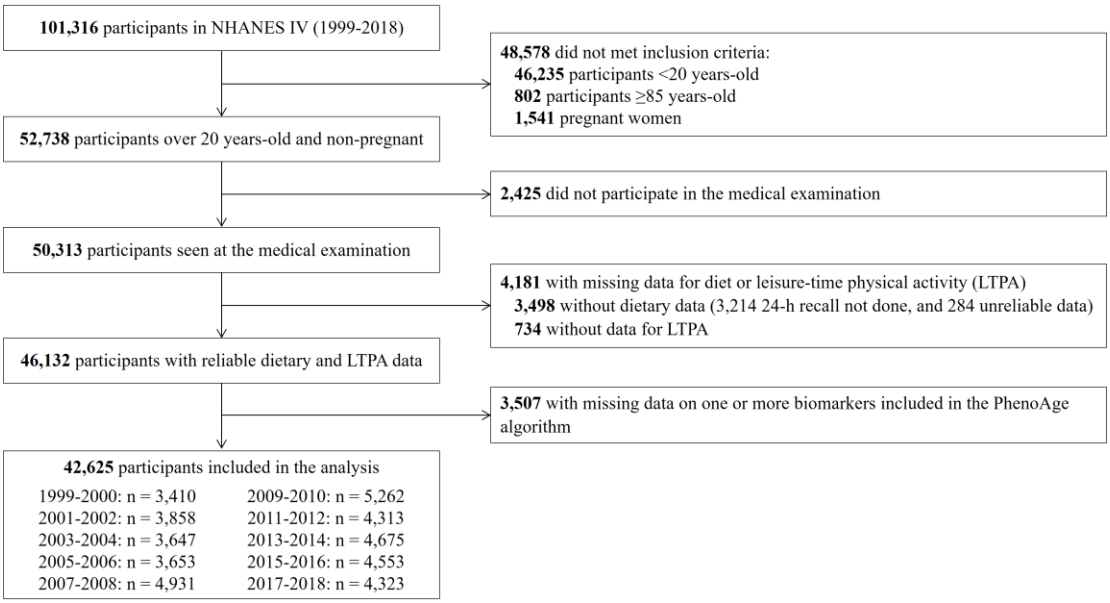

Figure 2

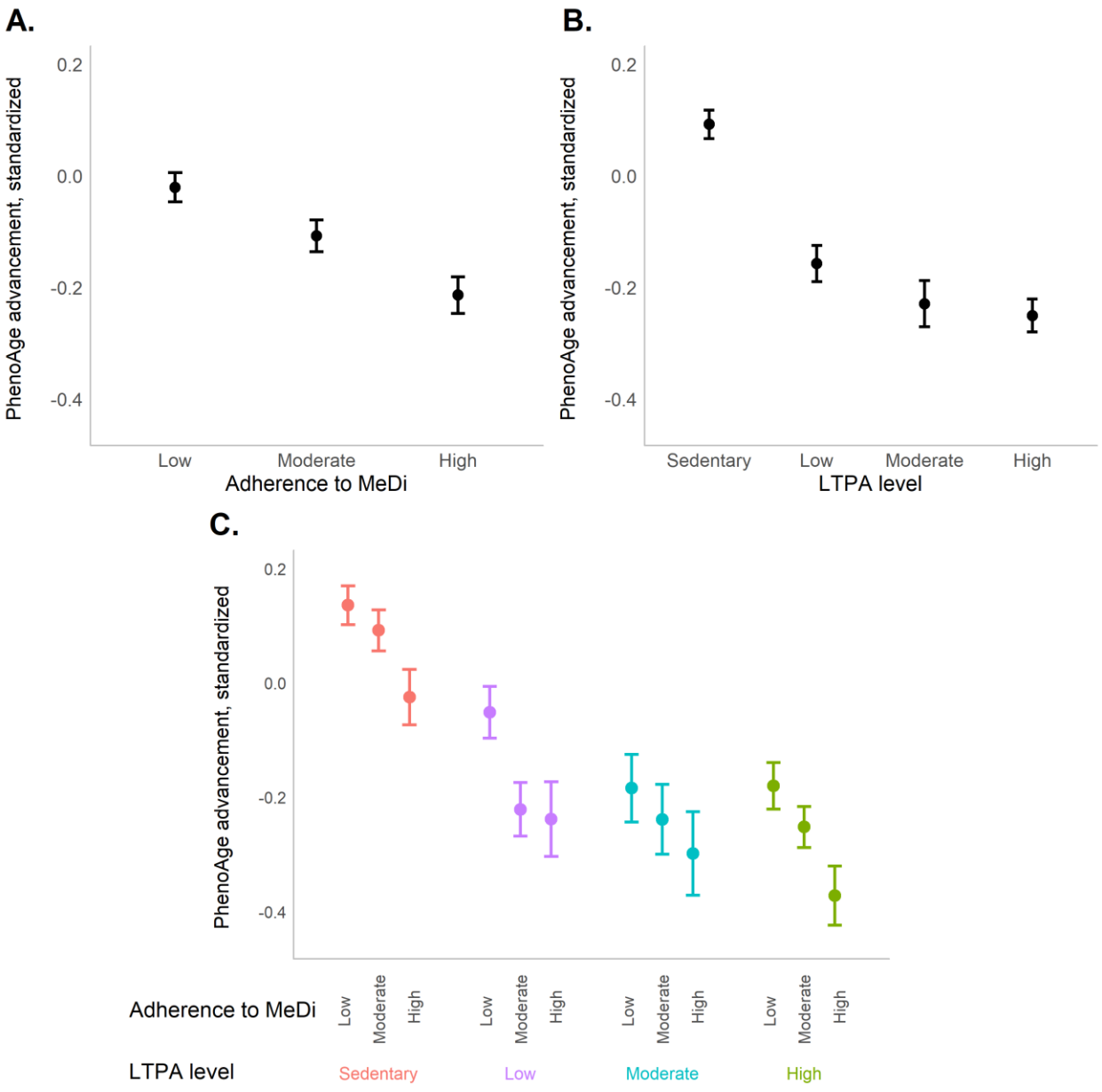

Figure 3