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Diet, Pace of Biological Aging, and Risk of Dementia in the Framingham Heart Study

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Objective: People who eat healthier diets are less likely to develop dementia, but the biological mechanism of this protection is not well understood. We tested the hypothesis that healthy diet protects against dementia because it slows the pace of biological aging.

Methods: We analyzed Framingham Offspring Cohort data. We included participants ≥60 years-old, free of dementia and having dietary, epigenetic, and follow-up data. We assessed healthy diet as long-term adherence to the Mediterranean-Dash Intervention for Neurodegenerative Delay diet (MIND, over 4 visits spanning 1991–2008). We measured the pace of aging from blood DNA methylation data collected in 2005–2008 using the DunedinPACE epigenetic clock. Incident dementia and mortality were defined using study records compiled from 2005 to 2008 visit through 2018.

Results: Of n = 1,644 included participants (mean age 69.6, 54% female), n = 140 developed dementia and n = 471 died over 14 years of follow-up. Greater MIND score was associated with slower DunedinPACE and reduced risks for dementia and mortality. Slower DunedinPACE was associated with reduced risks for dementia and mortality. In mediation analysis, slower DunedinPACE accounted for 27% of the diet-dementia association and 57% of the diet-mortality association.

Interpretation: Findings suggest that slower pace of aging mediates part of the relationship of healthy diet with reduced dementia risk. Monitoring pace of aging may inform dementia prevention. However, a large fraction of the diet-dementia association remains unexplained and may reflect direct connections between diet and brain aging that do not overlap other organ systems. Investigation of brain-specific mechanisms in well-designed mediation studies is warranted.

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Introduction

People who eat healthier diets are less likely to develop dementia.[1,2] However, the biological mechanism of this protection is not well understood.[3] Understanding pathways mediating dietary impacts on dementia risk can inform development of therapeutic and prevention strategies.

By far the greatest risk factor for dementia is aging. Biological processes linked with both healthy diet and reduced dementia risk include reversal of several so-called hallmarks of aging, including metabolic regulation, reduced inflammation and oxidative stress, and vascular health, among others.[4–6] These observations, together with data from animal model and human observational studies relating healthy diet with health-span and lifespan,[7] suggest the hypothesis that 1 mechanism through which healthy diet reduces dementia risk is by slowing processes of biological aging.

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Biological aging is the progressive decline in system integrity that occurs with advancing age.[8] It arises from the accumulation of molecular changes that undermine the functioning and resilience capacity of tissues and organs, ultimately causing disease and death.[9,10] Recent advances in aging research have produced new measurements of this process.[11] The best-validated among these new measures of aging are algorithms that combine information from dozens to hundreds of chemical tags, called methylation marks, on the DNA sequence of white blood cells to estimate the pace and progress of multi-system biological aging.[12] These algorithms are known as epigenetic clocks. Several epigenetic clocks, in particular the PhenoAge, GrimAge, and DunedinPACE clocks, have accumulated substantial evidence as biomarkers predictive of health-span and lifespan.[13–15] These clocks also tend to indicate younger biological age and slower pace of aging in people who eat healthier diets.[16–19] In our study, we focused on DunedinPACE because evidence for associations with cognitive and brain aging outcomes, as well as with diet, were more consistent for DunedinPACE than for the PhenoAge and GrimAge clocks, including in the Framingham Offspring Cohort studied here.[15,19–23] We also conducted analysis of PhenoAge and GrimAge for comparison purposes. Epigenetic clock analysis tested the hypothesis that multi-system biological aging is a mechanism underlying diet-dementia associations.[4,24]

We analyzed data on diet, biological aging, and incidence of dementia collected over 3 decades of follow-up in the Framingham Heart Study Offspring Cohort. We assessed long-term healthy diet by the Mediterranean-Dash Intervention for Neurodegenerative Delay (MIND) diet, specifically developed for the prevention of dementia by emphasizing foods and nutrients with the most robust evidence for brain health.[25,26] We measured pace of biological aging using the DunedinPACE epigenetic clock. Dementia-free survival was determined from the Framingham Heart Study's records. We tested if participants with a healthier diet had slower pace of aging and improved dementia-free survival and if a slower pace of aging mediated the diet-dementia association. We also explored the specificity of this pathway for brain aging, as compared to overall aging, by contrasting the proportion mediated for dementia to the proportion mediated for all-cause mortality.

Patients and Methods

Study Population

The Framingham Heart Study is an ongoing population-based cohort following 3 generations of families recruited, starting 1948, within the town of Framingham, Massachusetts, USA.[27] We analyzed data from the second generation of participants, the Offspring Cohort. Initiated in 1971, the Offspring Cohort has been followed-up at 9 examinations, approximately every 4–7 years.[28] At each follow-up visit, data collection included physical examination, lifestyle-related questionnaires, blood sampling, and, starting in 1991, neurocognitive testing.[29] Additional annual neurologic and neuropsychological examinations were conducted for participants identified at risk for dementia.

Standard Protocol Approvals, Registrations, and Patient Consents

The study protocol was approved by the institutional Review Board for Human Research at Boston University Medical Center, and all participants provided written inform consent.

DNA Methylation

Whole-genome DNA methylation (DNAm) profiles were obtained from dbGaP (phs000724.v10.p14). For the Offspring Cohort, DNAm was measured from whole-blood buffy-coat samples collected at Visit 8 (2005–2008) using the Infinium HumanMethylation450 BeadChip (Illumina). Processing and normalization of DNAm data has been described previously.[30] Briefly, data were normalized using the "dasen" method in the 'wateRmelon' R package[31] and subjected to downstream quality control. Samples with missing rate > 1% at $p < 0.01$, poor SNP matching to the 65 SNP control probe locations, and outliers by multidimensional scaling techniques were excluded. Probes with missing rate of >20% at $p < 0.01$ were also excluded.

The DunedinPACE epigenetic clock was computed from the DNAm dataset using the code available on GitHub (https://github.com/danbelsky/DunedinPACE) following the procedure described in Belsky and al.[15] Briefly, DunedinPACE was developed in the Dunedin Longitudinal Birth Cohort Study from analysis of the Pace of Aging, an integrative summary of rate-of-change estimates for 19 blood biomarker and organ-function-tests at ages 26, 32, 38, and 45 of the 1,037 study participants.[23,32,33] DunedinPACE algorithm was then developed from elastic-net regression of Pace of Aging on blood DNAm data collected from participants at age 45. The resulting algorithm included 173 cytosine-phosphate-guanine (CpG) sites, and allows computation of Pace of Aging from a single DNAm assessment rather than relying on longitudinal measurement of multiple blood biomarkers. We computed DunedinPACE for Framingham participants using the publicly available R package and the DNA methylation dataset published by the Framingham Heart Study on NIH dbGaP

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(accession phs000724.v10.p14). DunedinPACE values are normed to the Dunedin Study birth cohort, in which a value of 1 corresponds to expected biological decline per calendar year over the age 26–45 follow-up interval. Values greater than 1 biological year per chronological year indicate a faster Pace of Aging. Values below 1 indicate a slower Pace of Aging. For analysis, the DunedinPACE values were standardized to mean = 0, standard deviation (SD) = 1, so that positive values indicate faster-than-average Pace of Aging within the Framingham Offspring cohort and negative values indicate slower-than-average Pace of Aging. Standardized DunedinPACE was analyzed as a continuous variable in regression analysis and categorized by tertiles for descriptive analysis.

Dietary Assessment

Dietary information was collected from Offspring Cohort participants at every study visit using the validated 126-item Harvard semi-quantitative Food Frequency Questionnaire (FFQ), recording habitual food consumptions over the past year. The frequency of consumption of each food items (ie, from never or <1 per month to >6 per day) was converted into servings-per-week using common portion size. Nutrient intakes were estimated by multiplying the frequency of consumption of each food item by the nutrient content of the specified portions.

The MIND diet was developed in 2015 in the US Rush Memory and Aging Project and is specifically tailored for dementia prevention.[25,26] MIND diet adherence is associated with slower cognitive decline, reduced risk of dementia, and preservation of brain structure in multiple cohorts.[2,34–36] The MIND diet score is computed from consumption of 15 food groups, including 10 brain-healthy food groups (green leafy vegetables, other vegetables, nuts, berries, beans, whole grains, fish, poultry, olive oil, and moderate wine) and 5 unhealthy food groups (red meats, butter/margarine, cheese, pastries/sweets, fried/fast food). Following the original definition,[26] we computed participants' MIND diet scores in 2 steps. First, we assigned a score of 0, 0.5, or 1 to each food group according to frequency of consumption (for healthy foods, higher scores correspond to more-frequent consumption; for unhealthy foods, higher scores correspond to less-frequent consumption; olive oil consumption was scored 1 if used as the primary oil and 0 otherwise). Second, the 15 component sub-scores were summed to compute the final MIND diet score (range = 0–15). Higher MIND diet scores reflect greater MIND diet adherence.

We computed participants' MIND diet scores from data collected at the 5th (1991–1995), 6th (1995–1998), 7th (1998–2001), and 8th (2005–2008) visits. For analysis, we averaged MIND diet scores across examination cycles to measure participants' long-term adherence to healthy eating patterns (72% of participants had completed FFQs at all 4 exams, 20% completed 3 FFQs, and the remainder completed 2 FFQs). The MIND diet score was analyzed as a continuous variable (denominated in SD units) in regression analysis and categorized by tertiles for descriptive analyses.

Dementia

Dementia diagnosis was made following a 3-step procedure. First, participants were flagged for further examination if (1) they performed lower than the education-based cutoff scores for the Mini-Mental State Examination assessed at each visit; (2) subjective cognitive impairment was reported by the participant or a family member; (3) referred by a treating physician or by an ancillary investigator of the cohort; or (4) after review of outside medical records. Second, flagged participants underwent additional annual neurologic and neuropsychological examinations. Third, all cases of possible cognitive decline and dementia were reviewed by a committee to determine the presence of dementia and its etiology based on criteria of the Diagnostic and Statistical Manual of Mental Disorders-IV and the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association. Date of onset was determined by the committee after reviewing systematic tracking of annual cognitive status before and after dementia diagnosis. For participants without diagnosis of dementia, the censoring date was set at date of last available neurocognitive assessment. Incident cases were adjudicated through 2018.

Mortality

All-cause mortality data were obtained from continuous surveillance of medical and hospital records, death certificates, communication with personal physicians, and next-of-kin interviews. Three physicians reviewed and adjudicated the information through 2018.

Covariates

Demographic, socioeconomic, and lifestyle variables were collected at Visit 8 (DNAm baseline assessment), and included age, sex, educational level (less than high school; high school graduate; some college; college graduate), marital status (never married; married or cohabitating; separated, divorced, or widowed). Apolipoprotein E (*APOE*) ϵ4 allele carrier status was considered dichotomously (carrying at least 1 ϵ4 allele versus no ϵ4 allele). Body mass index (BMI) was calculated as weight divided by height squared (in kg/m^2; continuous). A physical activity index was computed as a composite score of time spent in 5

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types of activities, assessed by a physical activity questionnaire, weighted by their intensity level (ie, numbers of hours per day spent sleeping [weight factor = 1], in sedentary [weight factor = 1.1], light [weight factor = 1.5], moderate [weight factor = 2.4], or heavy [weight factor = 5.0] activities).[37] Smoking status was defined as current if the individual had reported smoking during the year preceding baseline examination, as former if the individual had reported smoking at any previous examination visit, and never otherwise. Histories of cardiovascular disease and diabetes were self-reported at each visit. White blood cell composition was estimated from the DNAm data using the algorithms developed by Houseman et al. to estimate relative abundances of CD4 T cells, CD8 T cells, natural killer cells, B lymphocytes, monocytes, and granulocytes.[38]

Statistical Analysis

Our analysis examined the potential mediating role of a slower pace of biological aging linking healthy diet with reduced risk of dementia. For comparative purposes, we conducted parallel analysis with all-cause mortality as the outcome.

Analytic Sample. For the present study, baseline was set at Visit 8 (year 2005–2008), when DNAm to compute the DunedinPACE measure of pace of aging (mediator) was collected. Dietary data (exposure) were collected during the 16-year period prior to baseline (Visits 5 [1991–1995], 6 [1995–1998], 7 [1998–2001], and 8 [2005–2008]). Follow-up for dementia and mortality (outcomes) were conducted from Visit 8 through 2018, the most recent date for which data were available. See Fig 1A for study design and data collection timeline. Our analysis sample included participants for whom DNAm and dementia follow-up data were available, who were aged 60 or older and free of dementia at baseline Visit 8, and who completed at least 2 FFQs at the Visits 5, 6, 7 and/or 8.

The Offspring Cohort Visit 8 included 2,798 participants, of whom 2,479 (89%) had DNAm data passing quality control (Fig 1B). Among them, 1,871 (75%) participants were over 60 years-old. We excluded participants not followed for dementia (n = 2), with prevalent dementia at baseline (n = 160), or with less than 2 valid FFQs (either because dietary intake data not available, or an abnormal estimated total energy intake [<600 or >3,999 kcal for women; or < 600 or >4,199 kcal for men] and/or > 13 missing items; n = 65). The final sample for analysis included 1,644 participants.

Analysis of Mediation by DunedinPACE. Causal mediation analysis was performed using Aalen additive hazard model for survival analysis in a counterfactual framework, as proposed by Lange et al.[39] Compare to the more widely used Cox proportional hazards model, the Aalen additive hazard model is a flexible semi-parametric approach which does not require the proportional hazards assumption and which has a straightforward interpretation of effect sizes as absolute number of events.[39] In a counterfactual framework, the method allows estimation of the number of additional dementia cases per unit of time (time-to-event outcome) that would be observed by increasing the MIND diet score (exposure), and to decompose this number into a part attributable to the direct pathway and a part mediated by DunedinPACE (mediator). First, the coefficient β_M for the exposure-mediator association of MIND diet score with DunedinPACE was estimated by adjusted linear regression. Second, the effect of MIND diet score on dementia was estimated using an adjusted Aalen additive hazard model, with time-dependent coefficients tested for exposure and covariates (only age showed time-dependent effect). In the mediation analysis, the Natural Direct Effect (NDE) measures the change in the number of dementia cases (coefficient β_{NDE}) for each 1-SD increase in MIND diet score, controlling for the DunedinPACE pathway and all other covariates. The Natural Indirect Effect (NIE) represents the change in dementia cases (β_{NIE}) attributable to the change in DunedinPACE associated with a 1-SD increase in MIND diet score. The Total causal Effect (β_{TE}) of diet on dementia is the sum of the NDE and the NIE. Proportion mediated is calculated as β_{NIE}/β_{TE}. The 95% confidence interval (CI) for NDE was computed via the Aalen additive model. CIs for NIE, TE and proportion mediated were constructed by combining the covariance matrices for the parameter estimates of the linear regression and the Aalen model across 100,000 Monte Carlo simulations. The same mediation analysis was run with all-cause mortality as the outcome. All models included the following covariates: age, sex, total energy intake, and the DNA methylation processing facility.

Sensitivity Analyses. We ran a series of supplementary analyses. First, we repeated analysis using 2 alternative healthy-diet patterns, the Mediterranean diet and the U.S. 2010 Dietary Guidelines for Americans (Dietary Guidelines Adherence Index) (see Supporting information for details). Second, we tested robustness of findings to additional covariate adjustment for socioeconomic factors (education level and marital status), *APOE*ε4 status, other lifestyle factors (physical activity index, BMI, and smoking status), history of medical conditions (cardiovascular disease and diabetes), and DNAm-estimated blood cell composition. Third, we evaluated the modification effect of

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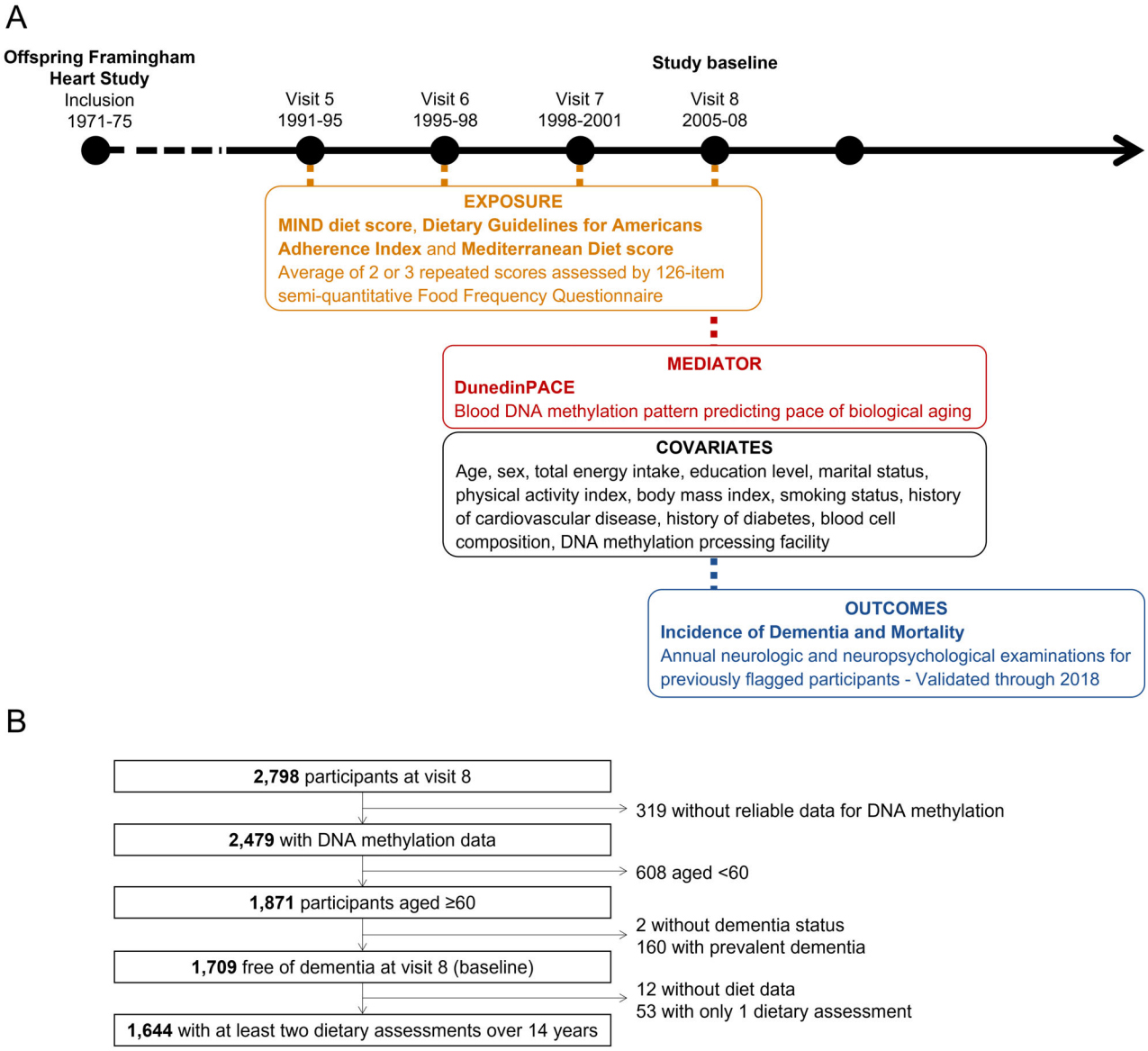

FIGURE 1: Study design (panel A) and flowchart of the study participants (panel B). Panel A: For the present study, baseline was set at Visit 8, when DNA methylation data to compute the DunedinPACE measure of pace of aging (mediator) was collected. Dietary data (exposure) were collected during the 16-year period prior to baseline (Visits 5 [1991–1995], 6 [1995–1998], 7 [1998–2001], and 8 [2005–2008]). Follow-up for dementia and mortality (outcomes) were conducted from Visit 8 through 2018, the most recent date for which data were available. Covariates were collected at study baseline (Visit 8). [Color figure can be viewed at www.annalsofneurology.org]

diet by sex, *APOE*ε4 status, and smoking status (which was previously shown to be associated with DNA methylation profiles and to interact with diet in its association with biological aging).[17] Fourth, we evaluated the 17-year stability of dietary behavior across the 4 study visits at which dietary assessment was conducted, using intraclass correlation coefficient (ICC; with the following specifications: 2-way mixed-effects model, absolute agreement, and average measures).[40] We also considered proximate and distant dietary habits by studying visit-specific MIND diet scores at each time point (ie, at Visits 5, 6, 7, and 8 separately). Fifth, we evaluated the etiologic specificity of findings by restricting analysis to consider only dementia cases attributed to Alzheimer's disease (AD). Sixth, we evaluated sensitivity of results to cases already experiencing cognitive impairment at the time of DNAm collection by restricting analysis to participants classified as cognitively healthy at baseline (Mini-Mental State Examination score ≥ 26 at Visit 8). Finally, we explored 2 alternative DNA methylation measures of biological age: PhenoAge and GrimAge (see Supporting information for computation details).

Statistical analyses were performed using R version 4.3.1 (R Foundation for Statistical Computing, Vienna,

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Austria). Analyses were performed on participants with all available covariates (none of which was missing for the primary model). The significance level was set at 0.05 for all tests.

Results

Among the 1,644 participants included, 54% were women and the mean age at baseline Visit 8 was 69.6 (±6.9) years (Table). The mean MIND diet score was 7.3 (±1.6) points. Participants with higher adherence to the MIND diet were more often women, had higher educational level, were less likely to be smokers, practiced more physical activity, and had lower BMI.

Diet, Pace of Aging, and Risk of Dementia

Over a median follow-up of 6.9 years (with maximum 13.6 years, and a total of 11,740 person-years), 140 participants (8.5%) were diagnosed with dementia. Participants with greater DunedinPACE values at baseline were at increased risk of developing dementia over follow-up (each 1-SD increase in DunedinPACE was associated with 45.4 [95% CI, 21.9; 69.0] additional dementia cases per 10,000 person-years at risk; Fig 2C and Fig 3A). Participants with a healthier diet over the preceding decades had a slower pace of aging at baseline and a lower risk of dementia (each 1-SD increase in MIND diet score was associated with a 0.20 SD [95% CI, −0.25; −0.15] slower DunedinPACE and 33.6 [−55.6; −11.7] fewer incident dementia cases per 10,000 person-years of follow-up; Figs 2A and 3A). Mediation analysis suggested that 27% [0.11; 0.79] of the total effect of MIND diet score on the risk of dementia was attributed to DunedinPACE ($\beta_{NDE} = -24.4$ [−46.5; −4.2] and $\beta_{NIE} = -9.1$ [−14.7; −4.2]; Fig 3A).

Diet, Pace of Aging, and Risk of All-Cause Mortality

A total of 471 participants (28.6%) died during the follow-up (median, 12.1 years; maximum, 13.8 years). Participants with faster DunedinPACE at baseline were at increased risk of all-cause mortality over follow-up (Fig 2D and Fig 3B). Healthier diet was associated with lower risk of mortality (each 1-SD increase in MIND diet score was associated with 47.3 [−74.8; −20.0] fewer deaths per 10,000 person-years of follow-up; Fig 2B and Fig 3B). Mediation analysis suggested that 57% [0.34; 1.32] of the total effect of MIND diet score on mortality risk was attributed to DunedinPACE ($\beta_{NDE} = 20.1$ [−47.2; 6.9] and $\beta_{NIE} = -27.2$ [−36.7; −18.8]; Fig 3B).

Sensitivity Analyses

We conducted sensitivity analyses. First, as a test of the robustness of findings to overall healthy diet, we repeated our analysis using 2 alternative healthy eating indices, the Mediterranean diet score and the Dietary Guideline Adherence Index (Supporting information). All 3 diet scores correlated with each other (r = 0.62 to 0.72, $p < 0.001$), and yield similar results (Table S1).

Next, we tested further adjustment on participants' socioeconomic characteristics, *APOE*ε4 status, lifestyle factors, diabetes and cardiovascular diseases, and DNAm estimates of blood cell composition. Results were similar to those from our primary model specifications (Tables S2 and S3). We found no evidence of effect modification by sex, *APOE*ε4 status, or smoking status (all *p*-values for product terms testing interactions >0.16).

Participants' dietary scores were stable across the 4 visits at which measures were collected, spanning 17 years of follow-up (MIND diet score, mean = 7.0 to 7.6, ICC = 0.87 [95% CI, 0.86; 0.88]; Mediterranean diet score, mean = 4.20 to 4.23, ICC = 0.80 [0.78; 0.82]; Dietary Guidelines Adherence Index, mean = 61.0 to 61.9, ICC = 0.84 [0.83; 0.85]). Furthermore, in addition to long-term dietary habits, we considered MIND diet scores from each single visit to explore dietary exposure at different periods in life, from mid-life (Visit 5, mean age 56.3 ± 6.9) to older age (Visit 8, mean age 69.8 ± 6.8). Results from this visit-specific analyses were similar to those for our primary analysis of the cross-visit average, with the exception that Visit-7 MIND diet score was not associated with mortality risk (Fig S1).

In etiology-specific analysis, 106 dementia cases were classified as AD. DunedinPACE and MIND diet associations with AD were similar to, but slightly weaker than, associations with all-cause dementia (effect sizes were smaller by roughly 10 fewer cases per 10,000 person-years of follow-up; Fig S2). Mediation results for AD were similar to results for all-cause dementia, with a proportion mediated of 22% [0.08; 0.62] ($\beta_{NDE} = -24.4$ [−44.0; −4.6] and $\beta_{IDE} = -7.0$ [−11.6; −2.8]). Analysis excluding 126 participants with evidence of cognitive impairment at Visit 8 yielded results similar to our primary analysis (Fig S3).

Finally, we repeated our analysis using 2 alternative epigenetic clocks. There are many proposed methods to quantify biological aging. We focused on DunedinPACE because of prior evidence establishing associations with diet, brain aging, and risk of dementia.[15,19–23] For comparison purposes, we also report results for 2 other DNAm measures of aging with robust evidence of association with morbidity and mortality, but mixed evidence of association with brain aging and dementia, the PhenoAge and GrimAge epigenetic clocks. Briefly, patterns of associations were similar for these 2 clocks, although results for dementia were less consistent and effects were of smaller magnitude relative to DunedinPACE (Table S4).

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TABLE. Baseline (Visit 8, year 2005–2008) Characteristics of the Study Participants, The Framingham Offspring Cohort (n = 1,644)

		Category of MIND Diet Score		
Parameter	**Total Population**	**Low (n = 562)**	**Moderare (n = 586)**	**High (n = 496)**
Age (years), mean (SD)	69.6 (6.9)	69.9 (6.9)	69.6 (7.0)	69.4 (6.8)
Female, n (%)	891 (54.2)	241 (42.9)	309 (52.7)	341 (68.8)
Education level, n (%)				
Less than high school	53 (3.6)	30 (5.9)	15 (2.9)	8 (1.8)
High school	356 (24.0)	159 (31.3)	120 (22.9)	77 (17.1)
Some college	409 (27.6)	134 (26.4)	152 (29.0)	123 (27.3)
College graduate	666 (44.9)	185 (36.4)	238 (45.3)	243 (53.9)
Marital status, n (%)				
Never married	75 (4.6)	35 (6.3)	22 (3.8)	18 (3.7)
Married/cohabitating	1,137 (69.6)	381 (68.4)	425 (72.8)	331 (67.3)
Separated/divorced/widowed	421 (25.8)	141 (25.3)	137 (23.5)	143 (29.1)
APOEε4, n (%)	250 (16.7)	89 (17.4)	90 (17.1)	71 (15.5)
Smoking status, n (%)				
Never	877 (53.3)	262 (46.6)	316 (53.9)	299 (60.3)
Former	662 (40.3)	247 (44.0)	230 (39.2)	185 (37.3)
Current	105 (6.4)	53 (9.4)	40 (6.8)	12 (2.4)
Physical activity index, mean (SD)	35.2 (5.1)	34.5 (5.1)	35.2 (5.1)	36.0 (5.4)
Body mass index (kg/m^2), mean (SD)	28.1 (5.1)	28.5 (5.3)	28.6 (5.1)	27.2 (4.9)
History of cardiovascular disease	305 (18.6)	121 (21.5)	120 (20.5)	64 (12.9)
Diabetes	275 (16.8)	114 (20.4)	103 (17.7)	58 (11.7)
Total energy intake (kcal), mean (SD)	1829.8 (507.6)	1893.2 (541.4)	1813.2 (508.5)	1777.6 (457.8)
MIND diet score, mean (SD)	7.3 (1.6)	5.6 (0.8)	7.3 (0.4)	9.1 (0.8)
Standardized DunedinPACE, mean (SD)	0.1 (1.0)	0.3 (1.0)	0.1 (1.0)	−0.2 (0.9)

Note: Categories of MIND diet score are defined by tertiles of score (ie, low adherence for the 1st tertile [≤6.5], moderate adherence for the 2nd tertile [6.5–8], and high adherence for the 3rd tertile [>8]). Participants' characteristics were assessed at baseline (ie, Visit 8), when blood DNA methylation data were collected. Means and percentages are of non-missing values. Missing values: 9.7% for education level; 9.1% for *APOEε4*; 1.0% for physical activity index; 0.7% for marital status; 0.6% for diabetes; and 0.3% for body mass index.

Abbreviations: *APOEε4* = *ε4* allele of the apolipoprotein E gene; MIND = Mediterranean-DASH Intervention for neurodegenerative delay; SD = standard deviation.

Discussion

We analyzed data from the large population-based Framingham Heart Study Offspring Cohort to investigate the potential mediating role of biological aging in the relationship of diet with dementia risk. We found that participants with greater adherence to the MIND diet had a slower pace of biological aging, as measured by the DunedinPACE epigenetic clock, and lower risks of dementia and of all-cause mortality. In turn, faster DunedinPACE was associated with greater risks of dementia and mortality. Mediation analysis indicated that 27% of the healthy-diet association with dementia risk was mediated by slower biological aging. The parallel mediation proportion for all-cause mortality was 57%. Associations were robust to

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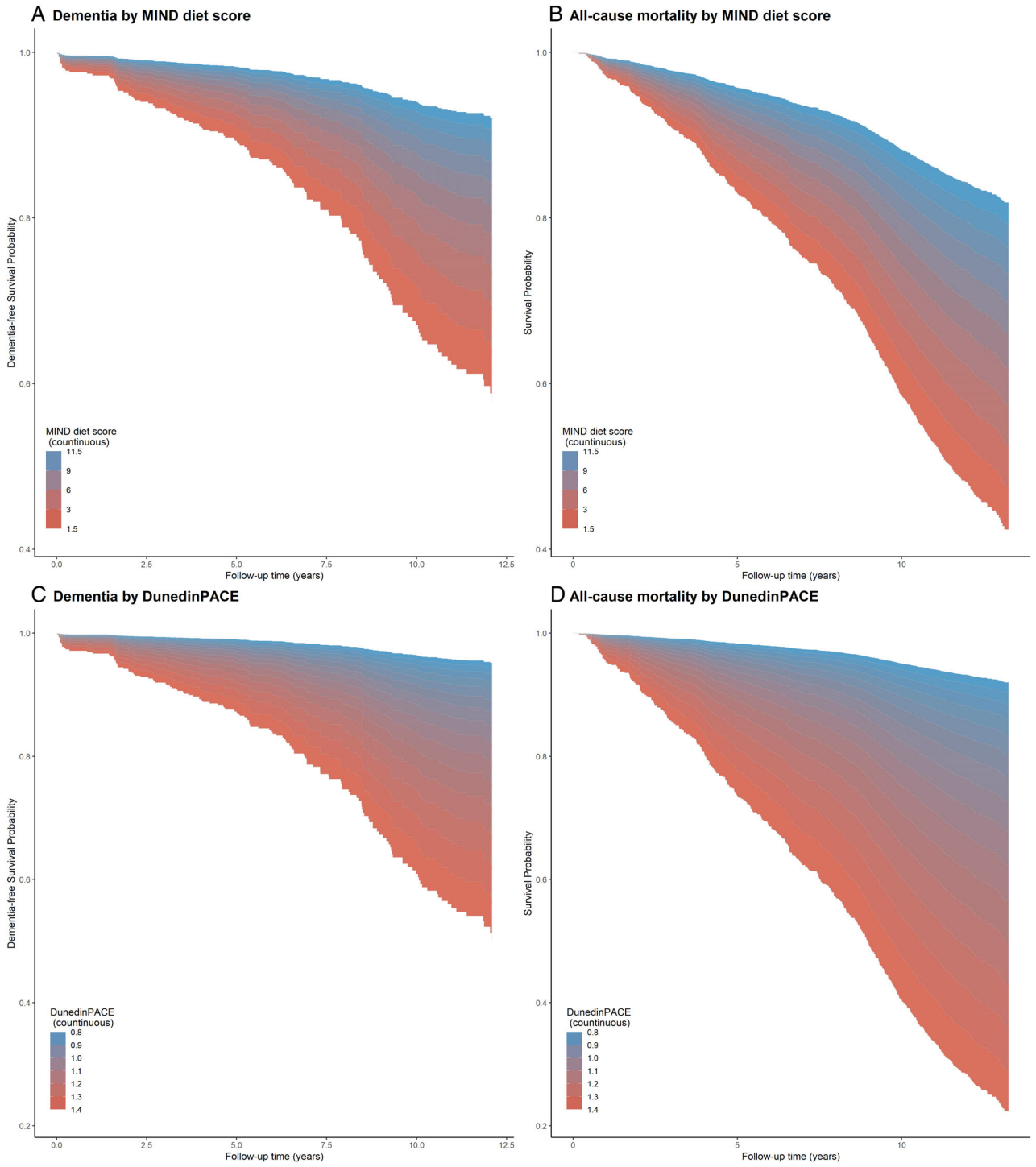

FIGURE 2: Dementia-free survival and all-cause mortality survival according to MIND diet score and DunedinPACE, estimated by Kaplan–Meier estimator, The Framingham Offspring cohort (n = 1,644). Abbreviation: MIND = Mediterranean-DASH Intervention for Neurodegenerative Delay. Kaplan–Meier survival curves for dementia (panels A and C) and for all-cause mortality (panels B and D) displayed by continuous MIND diet score (panels A and B) and DunedinPACE (panels C and D), with time from DNAm data collection (baseline) as a time scale. Participants with the healthier diet and slowest pace of aging (indicated in blue) show lower rates of incident dementia and mortality compared to participants with less healthy diet and faster pace of aging (indicated in red). [Color figure can be viewed at www.annalsofneurology.org]

potential confounders, including demographic, socioeconomic, and lifestyle factors, including smoking status, as well as to exclusion of participants who were cognitively impaired at baseline. Similar results were also observed for alternative dietary scores and for 2 other epigenetic clocks, supporting the interpretation that healthy dietary habits are beneficial

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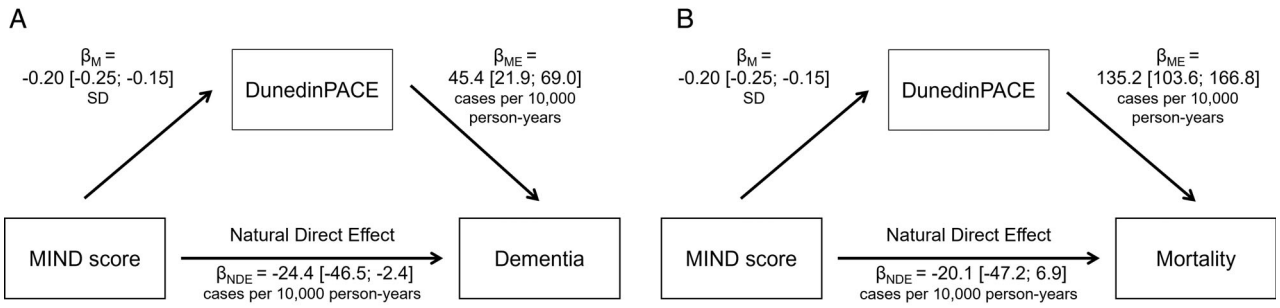

Natural Indirect Effect,	β_{NIE} = -9.1 [-14.7; -4.2] cases per 10,000 person-years	β_{NIE} = -27.2 [-36.7; -18.8] cases per 10,000 person-years
Total Effect,	β_{TE} = -33.6 [-55.6; -11.7] cases per 10,000 person-years	β_{TE} = -47.3 [-74.8; -20.0] cases per 10,000 person-years
Proportion mediated,	0.27 [0.11; 0.79]	0.57 [0.34; 1.32]

FIGURE 3: Mediation analysis of diet associations with dementia (panel A) and mortality (panel B) by the DunedinPACE epigenetic clock measure of the pace of aging, The Framingham Offspring cohort (n = 1,644). Abbreviations: CI = confidence interval; M = exposure-mediator association; ME = mediator effect on outcome; MIND = Mediterranean-DASH Intervention for Neurodegenerative Delay; NDE = natural direct effect; NIE = natural indirect effect; SD = standard deviation; TE = total effect. The figure shows effect estimates from analysis of mediation of MIND diet associations with risks of dementia and mortality, by DunedinPACE. All models were adjusted for age, sex, total energy intake, and an indicator of DNA methylation processing facility. Effect estimates are reported for 1-SD increments of MIND diet score and DunedinPACE, as estimated by linear regression (for analysis of DunedinPACE) and by Aalen additive hazard models (for analysis of dementia and mortality). β_M, is the "exposure-mediator" association of MIND diet score with DunedinPACE. β_{ME} is the "mediator-outcome" association of DunedinPACE with dementia/mortality. β_{NDE} is the "natural direct effect" association of MIND diet score with dementia/mortality, that is independent of DunedinPACE. β_{NIE} is the "natural indirect effect" representing the portion of the MIND diet score association with dementia/mortality that is mediated by DunedinPACE, estimated as the product of $\beta_{NDE} \times \beta_{ME}$. β_{TE}, is the "total effect" association of MIND diet score with dementia/mortality, estimated as the sum of β_{NDE} and β_{NIE}. The proportion mediated is estimated as β_{NIE}/β_{TE}. The 95% CIs for NIE, TE, and proportion mediated are estimated from 100,000 Monte-Carlo simulations.

for systematic biological aging and protective against dementia.

DNA methylation clocks that aim to measure processes of biological aging have emerged as novel measures of risk for aging-related diseases, including dementia.[20–22] Initial data suggest that they may provide useful biomarkers at the interface between the environment and aging biology.[41] However, little is known about how these measures relate to cognitive aging and dementia or the extent to which they are shaped by environmental exposures, such as diet. Our findings, showing associations of the DunedinPACE epigenetic clock with dietary exposure and dementia, suggest that this DNAm measure can provide a tool to better understand how environmental factors, such as diet, may contribute to brain aging.

Healthy diet is associated with preservation of cognitive function and brain integrity with aging.[1,2] Our findings complement those of other studies in elucidating the role of systemic biological aging in mediating this path. However, DunedinPACE mediated less than half as much of the diet-dementia association as compared to the diet-mortality association. The pathways from diet to dementia are diverse and likely include both direct effects of micro- and macro-nutrients on brain health and indirect effects mediated through metabolic, immune, and vascular health.[42] The systemic aging processes captured by DunedinPACE may relate primarily to the indirect pathways. The large unexplained fraction of the diet-dementia association may therefore reflect, in part, direct effects of nutrients on brain aging. For example, the brain has the higher metabolic demand relative to other organs consuming ~20% of glucose-derived energy provided by diet, while accounting for only ~2% of the body weight.[43] Nutrients play critical roles in neurotransmission, synaptic functioning, brain vasculature, and adult neurogenesis; eg, omega-3 fatty acids are major components if neuronal membranes.[42,44,45] Some nutrients, such as polyphenols or vitamin A, also have anti-amyloid and anti-tau properties.[34,42,46,47] While there are associations of nutrients with brain structure and white matter integrity phenotypes,[36,46,48] these features of brain aging may also have connections with indirect pathways. In midlife adults, slower pace of aging is associated with greater brain volumes and brain vascular health related to dementia risk.[23,49] Further research is needed to distinguish direct and indirect effects of diet on brain aging.

We acknowledge limitations. Self-reported dietary data are subject to recall bias and measurements errors, which might cause misclassification. We measured diet from 2 to 4 validated FFQs over 17 years of follow-up. The use of repeated measures may limit measurement error. Moreover, the beneficial effects of diet on biological

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aging and dementia risk were consistent for long-term healthy diet, as well as for healthy diet defined within the specific life-course periods of midlife and later life. These observations may reflect overall stable dietary habits across adulthood in healthy individuals,[50] as described over our study period using repeated scores for 3 distinct dietary patters, and suggest that even a single dietary assessment can be useful to evaluate the association of diet with age-related outcomes later in life. There is no gold standard measure of biological aging. DunedinPACE was developed from analysis that integrates rates of age-related decline across multiple organ systems, including metabolic, immune, cardiovascular, pulmonary, renal, hepatic, and periodontal systems.[15] Nutrition is involved in the regulation of many of these processes. Previous research has shown the involvement of brain metabolism,[4,51] inflammation and oxidative processes,[24] and periodontitis[52] in the relationship of diet with cognitive aging. Our findings of the mediation role of DunedinPACE on the effect of healthy diet on the risk of dementia hence are consistent with these previous findings, but in a more comprehensive way by capturing multiple systems through DunedinPACE. However, our data cannot distinguish a process in which diet improves organ health, which in turn results in a slower pace of aging, from one in which diet promotes healthy aging at the cellular level, and thereby preserves the health and functioning of organs. The Framingham Offspring cohort enrolled mainly White participants. This lack of diversity limits the generalizability of our results. Replication of findings in more diverse and non-US cohorts is a priority. As in any observational study, residual confounding may persist despite adjustment for multiple potential confounders in the analysis.

Our study shows that the association of healthier diet with lower risk of dementia is partly mediated by a slower pace of biological aging. If our observations are confirmed in more diverse populations, monitoring biological aging may inform dementia prevention. However, the pace of aging pathway does not fully explain the association of diet and dementia, suggesting the presence of other, more direct, pathways. Observational studies well-designed to conduct mediation analysis are needed to investigate direct associations of nutrients with brain aging that may operate independently of systemic biological aging.

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Potential Conflicts of Interest

A.C., T.E.M., K.S., and D.W.B. are listed as inventors of DunedinPACE, a Duke University and University of Otago invention licensed to TruDiagnostic Inc. A.T., C.P.R., Z.L., J.Z., and Y.G. declare no conflicts of interest.

Authors Contribution

A.T., D.W.B., and Y.G. contributed to the conception and design of the study; A.T., C.P.R., A.C., Z.L., T.E.M., K.S., J.Z., and D.W.B. contributed to the acquisition and analysis of data; A.T., D.W.B., and Y.G. contributed to drafting the text or preparing the figures.

Data Availability

Data for the Framingham Offspring Study were obtained from dbGaP (phs000007.v33.p14).

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