

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | | |
|-------------------------------------|--|
| n/a | Confirmed |
| ☐ | ☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ A description of all covariates tested |
| ☐ | ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	Data collection was conducted using REDCap (Research Electronic Data Capture).
Data analysis	Data analysis was performed with R (version 4.4.2). Quality control and normalization analyses were performed using the R Bioconductor package SeSAMe (R version 4.3.0; Bioconductor version 3.17; SeSAMe version 1.18.4). Batch effects were additionally identified using singular value decomposition analysis and then corrected using Combat in sva R package. PC versions of the Horvath, and Hannum, GrimAge, PhenoAge epigenetic clocks were computed according to the method described by Higgins-Chen et al. using the R code hosted on GitHub (https://github.com/MorganLevineLab/PC-Clocks) using R (version 4.4.2). DunedinPACE was calculated according to the method described by Belsky et al. using the R code hosted on GitHub (https://github.com/danbelsky/DunedinPACE/) using R (version 4.4.2). Statistical analyses were performed using lmer, lmerTest, mmrm, and emmeans R packages.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Due to participant confidentiality and privacy concerns, COSMOS data are available upon request, with formal applications submitted to the COSMOS research committee, to ensure data security and ethical considerations. Please refer to <https://cosmostrial.org/for-investigators/> for various forms, information, and resources to propose a manuscript/abstract, or to request data using COSMOS resources. Proposals to access and analyze the data will be reviewed by the COSMOS research committee based on whether the hypothesis has been tested, data availability and cleaning requirements, and the programming time required to prepare the data. A response to a request for access is typically provided within two weeks. De-identified data may be subsequently made available to external researchers subject to a controlled-access system and a data-sharing agreement.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

We collected the biological sex of participants, which was self-reported. All study participants provided written informed consent. As predefined in the study protocol, we tested whether the treatment effects varied by sex (Extended Data Figure 1). Since the interaction for the main findings was not statistically significant, the findings of the present study apply to all sexes.

Reporting on race, ethnicity, or other socially relevant groupings

Race/ethnicity were self-reported by participants. As predefined in the study protocol, we collected and tested whether the treatment effects varied by race.

Population characteristics

Table 1 presents the baseline characteristics in the overall study population and stratified by randomized multivitamin assignment. The mean age was 70.2±5.6 years. 482 (50.3%) were female and 854 (89.1%) were white participants.

Recruitment

Recruitment included mailings to 71,521 active Women's Health Initiative (WHI) Extension Study participants and mailings by Brigham and Women's Hospital (BWH) to 237,736 women and men contacted for, but not randomly assigned to, the VITamin D and Omega-3 Trial (VITAL); mass mailings to 2,616,343 US men and women; and mailings to 3380 volunteers through other sources, including responses to media stories and advertisements. Of 191,796 participants completing a brief screening questionnaire, 120,629 were initially eligible and willing; then 63,025 completed another questionnaire with written informed consent before enrollment with oversight by the Human Subjects Committee at BWH. Participants were required to forego cocoa supplements (chocolate intake was not restricted) and multivitamins during the trial (vitamin D was limited to ≤1000 IU/d and calcium to ≤1200 mg/d from all supplements). Safety exclusions included renal failure or dialysis, cirrhosis, other serious conditions that precluded participation, and reported extreme sensitivity to caffeine, as the cocoa extract supplement had modest theobromine (~50 mg/d) and caffeine (~15 mg/d) content. A total of 35,669 eligible, willing, and consenting participants began at least a 2-mo placebo run-in to eliminate poor compliers (defined as taking <75% of the study pills) before randomization to increase study power. At the end of the run-in, participants returned a final compliance, eligibility, and risk factor questionnaire and semi-quantitative food-frequency questionnaire. Finally, a total of 21,442 US adults, including 12,666 women aged ≥65 y and 8776 men aged ≥60 y were included in COSMOS. Baseline biospecimens were obtained during the run-in period from 6867 (32.0%) of 21,442 randomized participants, of whom 1,829 participants provided bloods at baseline, Year 1, and Year 2 of follow-up. In this ancillary study, we randomly selected a total of 998 participants for DNA methylation assessment, with oversampling of non-White participants and balanced by biological sex. We originally based our analyses upon 600 participants through one funded project, then added participants through additionally obtained support. Participants included in the current study were representative of the overall COSMOS Blood subcohort and the overall COSMOS trial.

Ethics oversight

COSMOS trial activities were overseen by the Human Subjects Committee at BWH/Mass General Brigham (2014P002768).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	In this ancillary study, we randomly selected a total of 998 participants for DNA methylation assessment, with oversampling of non-White participants and balanced by biological sex. We originally based our analyses upon 600 participants through one funded project plus participants through additionally obtained support. Participants included in the current study were representative of the overall COSMOS Blood subcohort and the overall COSMOS trial.
Data exclusions	Data were excluded from the analysis due to low quality of DNA extraction, quality control (mismatched sex estimation, poor quality samples, and disagreement in genetic fingerprint), and absence of baseline and follow-up measurements. More details can be found in Figure 1.
Replication	For quality control purpose, we tested 42 blinded split samples from 14 participants as technical replicates. The probe-level pairwise comparison showed excellent reliability for DNA methylation measurements (Mean value: 0.992 ± 0.008 for Pearson correlation; 0.996 ± 0.016 for R square; 0.002 ± 0.002 for mean absolute error; 0.027 ± 0.013 for mean squared error).
Randomization	From April 2016 to March 2018, 21,442 participants meeting all eligibility criteria were randomized according to a schedule prepared and implemented by WHI staff to 1 of 4 arms in equal proportions: 1) active cocoa extract and active multivitamin, 2) active cocoa extract and multivitamin placebo, 3) active multivitamin and cocoa extract placebo, or 4) both placebos, using a computer-generated permuted block approach blinded to investigators and stratified by sex (women, men), age (separate 5-y age blocks for women and men), and recruitment source (WHI, BWH) in blocks of 12. Participants within a household were randomly assigned to the same intervention, when possible, to reduce cross-contamination risk.
Blinding	Participants, investigators, staff dispensing study pills and collecting outcomes, and data analysts were masked to group assignment.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	NCT02422745; NCT05510375
Study protocol	The study protocol has been previously published in Rist PM, Sesso HD, Johnson LG, Aragaki AK, Wang L, Rautiainen S, Hazra A, Tobias DK, LeBoff MS, Schroeter H, Friedenber G, Copeland T, Clar A, Tinker LF, Hunt RP, Bassuk SS, Sarkissian A, Smith DC, Pereira E, Carrick WR, Wion ES, Schoenberg J, Anderson GL, Manson JE; COSMOS Research Group. Design and baseline characteristics of participants in the COcoa Supplement and Multivitamin Outcomes Study (COSMOS). Contemp Clin Trials. 2022 May;116:106728. doi: 10.1016/j.cct.2022.106728. Epub 2022 Mar 12. PMID: 35288332; PMCID: PMC9133193. The full study protocol can be found in the website: https://cdn.clinicaltrials.gov/large-docs/45/NCT02422745/Prot_000.pdf .
Data collection	The COSMOS randomized a total of 21,442 older adults across US from April 2016 to March 2018, and the randomized treatments continued through 31 December 2020, ending as scheduled, with a median (IQR) treatment period of 3.6 (3.2, 4.2) y.
Outcomes	We focused on five common DNAm measures of biological aging, including the first-generation clocks predicting chronological age (Horvath and Hannum), second-generation clocks predicting mortality outcomes (PhenoAge and GrimAge), and third-generation clocks of epigenetic metrics developed to predict system integrity and pace of aging (DunedinPACE). To ensure that values of the first two generations of clocks were highly reproducible, we used the principal component (PC) versions using the method developed by Higgins-Chen et al. Then we residualized these clocks for chronological age to estimate epigenetic age acceleration at each time point, with positive values meaning accelerated aging and vice versa. As DunedinPACE was initially created using CpGs with good

replication performance to achieve high technical reliability, it was directly calculated using the method developed by Belsky et al. DunedinPACE corresponds to the number of biologic years per chronological year, with values above 1 indicating faster pace of aging.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A