The American Journal of Clinical Nutrition 120 (2024) 1143–1155

The American Journal of CLINICAL NUTRITION

journal homepage: https://ajcn.nutrition.org/

Original Research Article

Evaluating the connection between diet quality, EpiNutrient intake and epigenetic age: an observational study

Laura Bordoni [1,*], João Agostinho de Sousa [2], Jingran Zhuo [2], Ferdinand von Meyenn [2,**]

[1] *Unit of Molecular Biology and Nutrigenomics, School of Pharmacy, University of Camerino, Camerino, Italy;* [2] *Laboratory of Nutrition and Metabolic Epigenetics, Department of Health Sciences and Technology, ETH Zurich, Switzerland*

A B S T R A C T

Background: DNA methylation (DNAm) has unique properties which makes it a potential biomarker for lifestyle-related exposures. Epigenetic clocks, particularly DNAm-based biological age predictors [epigenetic age (EA)], represent an exciting new area of clinical research and deviations of EA from chronological age [epigenetic age acceleration (EAA)] have been linked to overall health, age-related diseases, and environmental exposures.
Objectives: This observational study investigates the relationships between biological aging and various dietary factors within the LifeLines-DEEP Cohort. These factors include diet quality, processed food consumption, dietary glycemic load, and intake of vitamins involved in maintaining the epigenetic homeostasis (vitamins B-9, B-12, B-6, B-2, and C).
Methods: Dietary records collected using food-frequency questionnaires were used to estimate diet quality [LifeLines Diet Score (LLDS)], measure the intake of unprocessed/ultraprocessed food according to the NOVA food classification system, and the adequacy of the dietary intake of vitamins B-9, B-12, B-2, B-6, and C. EA using Horvath, Hannum, Levine, and Horvath2 epigenetic clock models and DNAm-predicted telomere length (DNAm-TL) were calculated from DNAm data in 760 subjects. Associations between dietary factors and EAA were tested, adjusting for sex, energy intake, and body composition.
Results: LLDS was associated with EAA (EAA_Horvath: β: −0.148; $P = 1 \times 10^{-4}$; EAA_Hannum: β: −0.148; $P = 9 \times 10^{-5}$; EAA_Levine: β: −0.174; $P = 1 \times 10^{-5}$; and EAA_Horvath2: β: −0.176; $P = 4 \times 10^{-6}$) and DNAm-TL (β: 0.116; $P = 0.003$). Particularly, EAA was associated with dietary glycemic load (EAA_Horvath: β: 0.476; $P = 9 \times 10^{-10}$; EAA_Hannum: β: 0.565; $P = 1 \times 10^{-13}$; EAA_Levine: β: 0.469; $P = 5 \times 10^{-9}$; EAA_-Horvath2: β: 0.569; $P = 1 \times 10^{-13}$; and DNAmTL adjusted for age: β: −0.340; $P = 2 \times 10^{-5}$) and different measures of food processing (NOVA classes 1 and 4). Positive EAA was also associated with inadequate intake of vitamin B-12 (EAA_Horvath: β: −0.167; $P = 0.002$; EAA_Hannum: β: −0.144; $P = 0.007$; and EAA_Horvath2: β: −0.126; $P = 0.019$) and C (EAA_Hannum: β: −0.136; $P = 0.010$ and EAA_Horvath2: β: −0.151; $P = 0.005$).
Conclusions: Our findings corroborate the hypothesis that nutrition plays a pivotal role in influencing epigenetic homeostasis, especially DNAm, thereby contributing to individual health trajectories and the pace of aging.

Keywords: epigenetic clocks, nutritional epigenomics, DNA methylation, diet, 1-carbon metabolism, nutrigenomics, aging

Introduction

DNA methylation (DNAm) [1,2] is a stable chemical modification with a central role in cell fate decisions, identity [3], and function in normal development [4], physiology, and disease [3,5]. DNAm serves as a responsive and dynamic mechanism allowing cells and organisms to adapt to environmental stimuli throughout their lifespan. Environmental stress accumulated over the lifetime may alter the epigenetic landscape and cause noisy transcription, which has been suggested to contribute to the aging phenotype [6]. Indeed, both environmental exposures [7] and aging [8] have been shown to modulate the cell methylome, and changes in the DNAm landscape correlate with health trajectories [9–11]. Owing to its capacity for inheritance during cell replication and susceptibility to modification by environmental influences, DNAm emerges as a promising tool to record the past and predict the future [12,13].

Abbreviations: 1CM, one-carbon metabolism; AI, adequate intake; BMR, basal metabolic rate; DASH, Dietary Approaches to Stop Hypertension; DNAm, DNA methylation; DNAmTL, telomere length estimated from DNAm; DNAmTL_adjAge, DNAmTL adjusted for age; DRV, dietary reference value; EA, epigenetic age; EAA, epigenetic age acceleration; FFQ, food-frequency questionnaire; LLDS, LifeLines Diet Score; PRI, population reference intake; TET, 10-11 translocating; TL, telomere length; WHR, waist-to-hip ratio.

* Corresponding author.
** Corresponding author.
E-mail addresses: laura.bordoni@unicam.it (L. Bordoni), ferdinand.vonmeyenn@hest.ethz.ch (F. von Meyenn).

https://doi.org/10.1016/j.ajcnut.2024.08.033
Received 22 February 2024; Received in revised form 25 August 2024; Accepted 30 August 2024; Available online 11 October 2024
0002-9165/© 2024 The Authors. Published by Elsevier Inc. on behalf of American Society for Nutrition. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

DNAm-based biological age [epigenetic age (EA)] has been suggested as a useful biomarker of age-related conditions [14], with a number of models developed to predict lifespan. Epigenetic clocks are age estimators that are based on combinations of methylation values that change with age at specific CpGs in the genome. In 2013, Hannum [9] and Horvath [10] proposed the first epigenetic clocks trained to predict chronological age from DNAm data. Since then, an updated iteration of the Horvath clock has been released [15]. The Levine clock estimates PhenoAge [16], or "phenotypic age," defined as a function of both chronological age and a number of clinical biomarkers related to mortality (for example, C reactive protein and blood composition). The deviation between predicted DNAm age and chronological age is termed epigenetic age acceleration (EAA), and found to be a biomarker for aging and related to overall survival and age-related diseases onset [17–19].

Numerous biological, social, and environmental factors have been investigated for their relationship to epigenetic age acceleration and deceleration [20,21]. Among environmental factors, nutrition has been demonstrated to impact life expectancy [22]. The field of nutriepigenomics has revealed that food and nutrients possess the capability of modulating DNAm patterns [23–25]. This occurs through the alteration of substrate and cofactor availability and the modulation of epigenetic enzyme activity [26–28]. Notably, the 1-carbon metabolism (1CM) plays a pivotal role in this context, with folate (vitamin B-9) serving as the main substrate of S-adenosyl-methionine, the universal methyl donor, and other B vitamins (for example, vitamins B-2, B-6, and B-12) acting as cofactors [29]. It has been proposed that nutrients involved in the 1CM may impact epigenetic regulations and EA [23,29–34]. Vitamin C can affect DNAm by modulating the activity of 10-11 translocating (TET) enzymes [35,36] and maintaining redox balance [37], which is tightly related to the epigenetic homeostasis [38].

Several studies indicate an association between epigenetic aging and specific nutrient intake, such as ω-3 polyunsaturated fatty acids [39], as well as specific foods like vegetables, fish, and poultry [39,40], but investigations related to the impact of overall diet quality on EA remain limited, with few studies addressing this aspect [41–43]. Furthermore, the effect of ultraprocessed food intake (which has been linked to various adverse health outcomes [44,45]) on EA has not been studied.

A recent systematic review [46] has summarized the evidence suggesting the ability of nutrition to affect telomere length (TL). It is worth noting that all the studies measured TL using PCR-based methods (which has various limitations [47–50]), and none of them explored the correlation between diet and TL estimated from DNAm (DNAmTL), which has demonstrated higher performance than TL in correlating with age, mortality, cardiovascular diseases, and even some dietary parameters [51].

The primary objective of this study is to offer additional insights into the association between biological aging estimators based on DNAm (specifically EAA and DNAmTL) and diet quality and consumption of processed foods. As secondary outcomes, this investigation explores the link between EAA and specific dietary features, such as the intake of nutrients involved in the 1CM and epigenetic homeostasis and dietary glycemic load.

Methods

Recruitment and samples collection

The study presented here analyzed data from the LifeLines Cohort, a large prospective cohort and Biobank designed to facilitate FAIR (findable, accessible, interoperable, and reusable) research on healthy aging [52]. LifeLines is a multidisciplinary prospective population-based cohort study examining in a unique 3-generation design the health and health-related behaviors of 167,729 persons living in the North of the Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, sociodemographic, behavioral, physical, and psychological factors that contribute to the health and disease of the general population, with a special focus on multimorbidity and complex genetics. The LifeLines protocol was approved by the UMCG Medical ethical committee under number 2007/152. All participants signed an informed consent form. The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Recruitment started with a basal assessment carried out from 2007 to 2013. Follow-up assessments were conducted until 2023. Additional information on the LifeLines Cohort can be found at dedicated website (https://www.lifelines-biobank.com/).

Data on sex, age, dietary habits (see details below), and smoking and physical activity were obtained from questionnaires. Anthropometric measurements were conducted by well-trained staff at the LifeLines research facilities. Height (cm) and body weight (kg) were measured without shoes and heavy clothing. Height was measured using the SECA 222 stadiometer (Seca GmbH); body weight was measured using the SECA 761 scale (Seca GmbH). Waist (cm) and hip circumferences (cm) were measured to calculate the waist-to-hip ratio (WHR) as an estimator of body composition and predictor of obesity complications [53,54]. Basal metabolic rate (BMR) [55] was calculated according to the Schofield equation [56]. Caloric surplus over the basal metabolic rate (kcal/BMR) has been determined by dividing the daily caloric intake by the BMR. A subset comprising 1500 participants from the LifeLines study actively participated in the LifeLines-DEEP Cohort [57]. For these participants, additional molecular data has been generated, facilitating a more comprehensive exploration of the association between genetic and phenotypic variation. For this study, the following inclusion/exclusion criteria were applied. Inclusion criteria for this study are: available data on chronological age and sex, and available DNAm data, fasting at baseline assessment. Exclusion criteria for this study are: diagnosis of cancer (from data collected at baseline and during 6y follow up); missing information about sex or age; no fasting at baseline assessment; missing DNAm data. A flowchart showing the selection of participants included in the study is available as Supplemental Figure 1.

Data collection on dietary habits, diet quality estimation, and processed food intake

Within the LifeLines Cohort Study, data on habitual dietary intake were collected using the Flower food-frequency questionnaire (FFQ) [58]. The questionnaire was developed by Wageningen University using the Dutch FFQTOOL™, in which food items were selected on the basis of the Dutch National Food Consumption Survey of 1997/1998 [59]. The reference period of the FFQs is 1 mo, assuming that food consumption patterns detected are stable over a longer period of time [60]. The validation of this tool in estimating the habitual dietary intake of participants, including data on the intake of energy, several macro- and micronutrients, and food items, has been previously published [58]. Questions pertaining to frequency were completed by selecting answers ranging from "never" to "6–7 d/wk." Portion sizes were estimated using natural portions (for example, slices of bread and pieces of fruit) and commonly used household measures (for example, cups and spoons). From data on food consumption obtained with the Flower FFQ, average daily intake of foods was calculated. Data on food consumption were also converted into daily energy and nutrient intake using data from the Dutch food composition database of 2011 [61].

To generate an index of overall diet quality, the LifeLines Diet Score (LLDS) was developed [61]. Briefly, FFQ items were categorized into 22 food groups, based on the food-based 2015 Dutch Dietary Guidelines [62]. This categorization is based on current internationally available scientific evidence on the relation of foods and dietary patterns with chronic diseases and gone through extensive revision in meta-analyses [61]. The food groups were categorized as positive, negative, neutral, or unknown. Nine "positive" groups (vegetables, fruit, whole grain products, legumes and nuts, fish, oils and soft margarines, unsweetened dairy, coffee, and tea), 1 "neutral" group (eggs), 3 "negative" groups (red and processed meat, butter and hard margarines, and sugar-sweetened beverages), and 9 "unknown" groups, for which health evidence is either absent or weak (potatoes, refined grain products, white unprocessed meat, cheese, savory and ready products, sugary products, soups, sweetened dairy, and artificially sweetened products) were defined. The 9 positive and 3 negative food groups were combined into the LLDS. The LLDS represents relative diet quality, and takes into account differences in energy intake between individuals. The intake of the food groups was expressed in grams per 1000 kilocalories (kcal) instead of grams per day. The sum of the component scores resulted in an LLDS score ranging from 0 to 48, where higher scores are associated with better diet quality. The reliability of this index have been previously validated [59,61].

In addition, the consumption of processed food was recorded according to the NOVA food intake classification [63,64]. NOVA is one of the most popular systems used to classify foods according to processing-related criteria [65]. Its purpose is to classify "all foods according to the nature, extent, and purposes of the industrial processes they undergo" [66]. According to the NOVA classification, ultra-processed food is mostly formulated from food substances and industrial ingredients that undergo a series of chemical and physical manufacturing processes. The NOVA classification categorizes all food items into 4 categories: *1*) unprocessed or minimally processed food (for example, fresh vegetables/fruits and unprocessed meat), *2*) processed culinary ingredient (for example, butter/oil for cooking, sugar, and salt), *3*) processed food (for example, canned vegetables/fish and fruits in syrup), and *4*) ultraprocessed food (for example, processed meat and soft drinks) [63]. The intake energy ratio, which indicates the energy ratio (g/1000 kcal) of a specific category in the total diet of a day, was calculated. Also, the proportion (weight ratio) of the intake of ultraprocessed food to the total weight of food and beverages consumed per day (g/d) was calculated. The weight ratio of ultra-processed food intake accounts also for the food that does not provide energy (for example, artificially sweetened beverages) as well as non-nutritional factors (for example, additives and by-products during processing).

The adequacy of vitamin intakes was defined according to the EFSA dietary reference values (DRVs): vitamin B-9 [population reference intake (PRI): <330 μg/d]; vitamin B-12 [adequate intake (AI): 4 μg/d]; intake of vitamin B-2 (PRI: 1.6 mg/d); vitamin B-6 (PRI: 1.6 mg/d), and vitamin C (PRI: 95 mg/d) [67].

Methylation data and epigenetic clock calculation

Genome-wide DNAm data were generated by Bonder et al. [68], as previously described. Briefly, 500 ng of genomic DNA extracted from whole blood was bisulfite treated using the EZ DNA Methylation kit (Zymo Research) and then hybridized to Illumina 450K arrays according to the manufacturer's protocols. The original IDAT files were generated by the Illumina iScan BeadChip scanner. QC for both probes and samples was performed as detailed in Bonder et al. [68]. *M* values for 820 samples from the LifeLines-DEEP Cohort have been calculated for samples that passed QC and have been deposited in the LifeLines data repository. From this sample set, 4 nonfasting samples and 10 samples missing for phenotypic variables (for example, sex or chronological age) were eliminated from further analysis. Because methylation patterns are strongly altered in cancer [69], we removed additional 46 samples that had cancer before or after blood sampling (data from prospective observations). Thus, 760 samples were used for the following analyses (Supplemental Figure 1). *M* values were used to calculate the epigenetic clocks using the Methylclock package [70] from Bioconductor [71]. Normalization was performed according to the BMIQ method, proposed by Teschendorff et al. [72] using Horvath's robust implementation that consists of an optimal R code implementation and optimization procedures. This normalization is recommended by Horvath because it improves the predictions for his clock. The imputation of missing data was performed by a fast imputation method that imputes missing values by the median of required CpGs (as recommended in Bohlin et al. [73] and recommended for large datasets by the Methylclock package developers [70]). The function DNAmAge computes age in years. The following epigenetic clocks were used for calculation:

- *Horvath's clock*: It uses 353 CpGs described in Horvath [10]. It was trained using 27K and 450K arrays in human samples from 51 healthy tissues and cell types.
- *Hannum's clock*: It uses 71 CpGs described in Hannum et al. [9]. It was trained using 450K arrays in blood samples.
- *Horvath's skin+blood clock (Horvath2)*: Epigenetic clock for skin and blood cells. It uses 391 CpGs described in Horvath et al. [15]. It was trained using 450K and EPIC arrays in skin and blood samples.
- *Levine's clock* (also known as *PhenoAge*): It uses 513 CpGs described in Levine et al. [16]. It was trained using 27K, 450K and EPIC arrays in blood samples.

EAA was computed both as the difference between DNAm and chronological ages (EAA) and as the epigenetic age calculated as residuals from regressing predicted epigenetic age on chronological age (EAA_UR). Results described in the main manuscript rely on EAA. Results based on EAA_UR are shown in supplementary materials. Individuals with negative EAA are considered to be biologically younger than their chronological age, and vice versa.

In addition, DNAm-TL was computed according to Lu et al. [51]. This epigenetic biomarker was developed by regressing measured TL on blood methylation data from 2256 individuals and then validated in another large dataset ($N = 9345$) [51]. Although both DNAm age and TL were associated with chronological age, they exhibit only weak correlations with each other after adjusting for age [74–76]. This suggests that the underlying mechanisms of DNAm aging and TL are distinct [51].

Statistical analysis

Descriptive statistics to explore the distribution of participants' dietary patterns, processed food intake (according to NOVA), 1CC vitamins intake as well as their age, sex, BMI, and WHR was performed. The normality of data distribution was evaluated using the Kolmogorov–Smirnov test. Parameters with skewed distributions were appropriately log-transformed before the analyses. Associations between continuous variables were tested by multivariate linear regression models by including dietary parameters as independent variables and different measures of EAA as an outcome. Variance inflation factor was calculated to check for multicollinearity. All linear

models meet the assumptions of linearity, independence, homoscedasticity, and normality for linear regression. Multivariate logistic regression was used to test the association between EAA and adequacy of vitamin intakes (as categorical variable, that is, 0: suboptimal intake; 1: AI). Models were adjusted for covariates as specified in each single test and displayed in the table describing the results. Tables show both nominal P values and adjusted P values according to the Benjamini–Hochberg procedure to correct for multiple comparisons, setting a false discovery rate of 0.05. For missing data on chronological or biological age, a listwise deletion procedure was implemented. A pairwise deletion procedure was employed for missing data regarding other variables. Data analysis and visualization was performed using R Studio (version 4.2.2) and Statistics for Data Analysis (SPSS V.28.0.1.1).

Results

Descriptive statistics

We calculated the EA for 760 individuals from the LifeLines-DEEP Cohort [see Measurements of biological aging (EA, EAA, and DNAmTL) section for details]. A total of 42.8% of the subjects were male and 57.2% were female. The cohort showed a broad age distribution (min = 18; max = 80; mean = 46 ± 13; median = 46; IQR = 20; modal = 49 y/o), rendering it a well-suited sample for EA assessments. The average BMI was 25.55 ± 4.18 (IQR: 4.81) and the mean WHR was 0.92 ± 0.07 (IQR: 0.10). According to BMI values, 1.2% of individuals are underweight, 49.2% are of normal weight, 35.9% are overweight, and 13.7% are obese. This confirms that this cohort is representative of the European population because WHO estimates that 59% of adults are living with overweight or obesity, with more than half of adults in 50 out of 53 Member States in the European Region living with overweight or obesity [77,78]. Regarding dietary patterns, the average total energy intake for the entire group was 2024.39 ± 678.5 kcal/d. The overall diet quality (LLDS) was negatively correlated with the total energy intake ($\rho = -0.323$; $P = 1 \times 10^{-10}$). Descriptive statistics of diet quality (LLDS), processed food intake (NOVA classification), and other features of the dietary habits of the participants are shown in Table 1. LLDS was significantly higher in females than that in males (M: 23.18 ± 5.69 compared with F: 26.32 ± 5.70; 2-sided t test $P = 2.7 \times 10^{-13}$) and was correlated with chronological age ($\rho = 0.177$; $P = 2 \times 10^{-6}$). Coherently, chronological age was correlated with the intake of NOVA food group 1 ($\rho = 0.346$; $P = 4.9 \times 10^{-22}$) and group 4 ($\rho = -0.274$; $P = 4.3 \times 10^{-14}$), but not with group 2 ($\rho = 0.055$; $P = 0.136$) and group 3 ($\rho = 0.122$; $P = 0.4 \times 10^{-4}$). The intake of NOVA food group 1 ($P = 2.6 \times 10^{-18}$) and 2 ($P = 8 \times 10^{-5}$) was higher in females than that in males, whereas no differences between sex were observed for NOVA food group 3 ($P = 0.061$) or 4 ($P = 0.647$). Age was also negatively associated with total diet glycemic load ($\rho = -0.172$; $P = 2 \times 10^{-6}$).

The intake of vitamins B-9, B-12, B-2, B-6, and C estimated from the FFQ is shown in Table 2. According to the DRVs for the analyzed vitamins [67], 86.7% of the subjects had a suboptimal intake of vitamin B-9 (PRI: <330 μg/d); 58.1% had a suboptimal intake of vitamin B-12 (AI: 4μg/d); 71.5% had a suboptimal intake of vitamin B-2 (PRI: 1.6 mg/d); 71.3% had a suboptimal intake of vitamin B-6 (PRI: 1.6 mg/d); and 46% had a suboptimal intake of vitamin C (PRI: 95 mg/d).

LLDS was positively correlated with the intake of vitamin B-9 ($\rho = 0.233$; $P = 5 \times 10^{-6}$) and vitamin C ($\rho = 0.292$; $P = 8 \times 10^{-9}$) but not associated with the intake of vitamin B-12 ($\rho = -0.092$;$P = 0.075$), vitamin B-2 ($\rho = -0.001$; $P = 0.988$) or vitamin B-6 ($\rho = -0.074$; $P = 0.151$).

Measurements of biological aging (EA, EAA, and DNAmTL)

Descriptive statistics for EA calculated from DNAm with Horvath's, Hannum's, Levine's, and Horvath2 clocks, as well as DNAmTL are shown in Table 3A. Strong correlations between chronological age and all assessed epigenetic biomarkers of aging were found (Figure 1). EAA, measured as the difference between EA and chronological age, as well as DNAmTL adjusted for age (DNAmTL_adjAge) are shown in Table 3B. Pairwise correlations between different measures of biological aging are shown in Supplemental Figure 2. Measures of EAA as residuals from regression (EEA_UR) and pairwise correlations between them are shown in Supplemental Table 1 and Supplemental Figure 3, respectively. Also, correlations between EAA and EAA_UR for the same clock are shown in Supplemental Figure 4.

Overall diet quality (LLDS), total caloric intake, and measures of biological age (EAA and DNAmTL_adjAge)

Significant correlations between EAA measurements and LLDS were detected for all analyzed epigenetic clocks (Figure 2). Because of the potential role of confounding factors such as sex, body composition, or energy intake (especially if in excess compared with BMR), we tested the association between EAA and LLDS in multivariate linear regression models. Total caloric intake was associated with EAA measurements (except for AA_Levine) adjusting the model for sex and WHR

TABLE 1
Descriptive statistics of total energy intake, diet quality (LLDS) and processed food intake (NOVA classification) in the overall population, including both g/1000 kcal and g/d classifications. Also, descriptive statistics for glycemic load are shown.

	N	Min.	Max.	Mean	SD	IQR
Total energy intake	742	18.6	7768.02	2024.39	678.5	786.8
LifeLines diet score (LLDS)	728	8.0	40.0	25.0	5.9	8.0
NOVA classification 1: unprocessed or minimally processed food (g/1000 kcal)	731	118.8	1775.1	665.4	260.5	330.3
NOVA classification 2: processed culinary ingredient (g/1000 kcal)	737	0.0	29.5	4.6	5.6	4.8
NOVA classification 3: processed food (g/1000 kcal)	738	0.0	53.0	14.9	9.5	11.0
NOVA classification 4: ultraprocessed food (g/1000 kcal)	737	0.0	837.4	380.1	119.8	135.3
NOVA classification 1: unprocessed or minimally processed food (g/d)	742	238.9	2771.7	1266.7	422.6	527.8
NOVA classification 2: processed culinary ingredient (g/d)	742	0.0	120.4	10.3	14.5	9.9
NOVA classification 3: processed food (g/d)	742	0.0	187.3	30.4	22.9	24.1
NOVA classification 4: ultraprocessed food (g/d)	742	0.0	3257.1	784.7	355.2	421.5
Glycemic load (all food item per day)	742	0.4	411.3	128.1	46.4	54.7

Abbreviations: Max., maximum; Min., minimum; SD, standard deviation.

TABLE 2
Descriptive statistics of vitamins intake available for the analyzed population.

Vitamin intake	*N*	Min.	Max.	Mean	SD
Vitamin B-2 (mg/d)	383	0.041	3.723	1.397	0.483
Vitamin B-6 (mg/d)	383	0.096	3.284	1.431	0.445
Vitamin B-12 (μg/d)	383	0.431	14.158	4.125	2.084
Vitamin B-9 (μg/d)	383	10.2	648.8	250.7	81.7
Vitamin C (mg/d)	383	0.385	428.46	103.34	48.61

Abbreviations: Max., maximum; Min., minimum; SD, standard deviation.

TABLE 3
Descriptive statistics for (A) chronological age, epigenetic age, and DNAmTL and (B) age acceleration measured as the difference between different epigenetic clocks and chronological age (EAA) and DNAmTL adjusted for age.

A	*N*	Min.	Max.	Mean	SD
Age of participants (y)	760	18	80	46.1	13.5
Horvath age (y)	760	19.7	69.5	44.9	9.5
Hannum age (y)	760	32.5	97.4	57.7	10.4
Levine age (y)	760	20.5	91.3	53.9	11.5
Horvath2 age (y)	760	15.2	66.7	39.7	10.0
DNAmTL	760	7.2	8.5	7.9	0.2
B	***N***	**Min**	**Max**	**Mean**	**SD**
AA_Horvath (y)	760	−21.3	11.9	−1.2	5.7
AA_Hannum (y)	760	−8.0	27.4	11.6	5.4
AA_Levine (y)	760	−11.0	24.7	7.9	5.8
AA_Horvath2 (y)	760	−23.0	4.9	−6.4	4.5
DNAmTL_adjAge	760	−24.6	26.6	−2.6	7.3

Abbreviations: AA, age acceleration; DNAmTL_adjAge, DNA methylation-predicted telomere length adjusted for chronological age; Max, maximum; Min., minimum; SD, standard deviation.

(Table 4). On the contrary, surplus of caloric intake compared with BMR (kcal/BMR) was not directly linked to EAA (Table 4). To exclude that the effect of diet on EAA was driven by imbalanced caloric intake rather than diet quality, we incorporated kcal/BMR as a covariate when assessing the relationship between EAA and LLDS. Of note, the association between LLDS and all the EAA was significant even adjusting the model for sex, WHR, and energy surplus (kcal/BMR) (Table 4). This evidence demonstrates that EAA is more closely associated with diet quality rather than overnutrition in terms of calories. A significant correlation with LLDS was also observed for DNAmTL_adjAge ($r = 0.143$; $P = 0.003$). Of note, the association remained significant even after adjusting the model for sex, WHR, and kcal/BMR (Table 4). Partial correlation plots for multivariate linear regression testing the association between LLDS and EAA or DNAmTL_adjAge are shown in Supplemental Figures 5–9. Results for EAA_UR are shown in Supplemental Figure 10 and Supplemental Table 2.

Intake of processed food (NOVA food groups) and measures of biological age (EAA and DNAmTL_adjAge)

Intake (NOVA_energyratio, that is, grams per 1000 kcal of the total food intake per day) of minimally processed food (NOVA class 1) was independently and negatively associated with EAA, adjusting the model for sex, WHR, and overall diet quality (LLDS) (Table 5). No significant association with EAA and NOVA class 2 and 3 food was detected after the correction for multiple comparisons (Table 5A), except for AA_Hannum and NOVA class 3. A significant positive associations was observed between EAA and NOVA class 4 foods (that is, highly processed food) (Table 5A). The association between EAA and NOVA group 1 or 4 remains confirmed even when considering the intake of NOVA groups measured in grams per kilogram of food per day (NOVA_weightratio), which accounts for noncaloric food and beverages (Table 5B).

DNAmTL_adjAge was associated with the intake of NOVA food groups 1 and 4, both in terms of grams per 1000 kcal of the total food intake (NOVA_energyratio) and grams per kilogram of food per day (NOVA_weightratio) (Table 5). Results for EAA_UR are shown in Supplemental Table 3.

Association between intake of vitamins linked to the epigenetic homeostasis and biological age (EAA and DNAmTL_adjAge)

Associations between EAA and intake of various vitamins involved in epigenetic regulations (vitamins B-9, B-12, B-2, B-6, and C) (descriptive statistics in Table 2) were tested. The cohort was divided into 2 groups on the basis of adequate or inadequate intake of vitamins according to the EFSA DRVs. Notably, suboptimal intake of vitamin B-12 (observed in 58.1% of the population) was significantly associated with increased EAA across the majority of DNAm clocks (Table 6). We also found a significant association between suboptimal vitamin C intake (observed in 54% of the cohort) and increased Hannum and Horvath2 EAA (Table 6). In contrast, no associations between EAA and inadequate intake of vitamin B-9 (observed in 13.3% of the cohort), vitamin B-2 (observed in 28.5% of the cohort), or vitamin B-6 (observed in 28.7% of the cohort) were measured (Table 6). Also, no associations between the adequacy of vitamin intakes and TL estimated by DNAm were detected (Table 6). Results for EAA_UR are shown in Supplemental Table 4.

Measurements of biological age (EAA and DNAmTL_adjAge) and diet glycemic load

The associations between diet glycemic load (from total intake of food in a day) and EAA measured by different clocks, adjusted for sex, WHR, overall diet quality (LLDS), and kcal/BMR are shown in Table 7. Significant positive associations were measured for all DNAm clocks. Also, dietary glycemic load was negatively associated with DNAmTL_adjAge (Table 7). Results for EAA_UR are shown in Supplemental Table 5.

Discussion

Several nutriepigenomic studies have revealed that nutrition influences and shapes the DNAm landscape [79–85], providing compelling reasoning to expect possible effects of environmental factors (diet in particular) on fundamental biological processes, including aging [23]. Our study reinforces the potential of healthy diets to influence the (healthy) aging process. Using the LifeLines-DEEP Cohort, we observed an association between EAA and diet quality estimated by the LLDS. Of note, we showed that diet quality, rather than caloric intake (considering the BMR) is associated with EAA in our cohort. Our findings are in agreement with previous observational studies using different estimators of diet quality. Kim et al. [42] showed a correlation between EAA and quartiles of the Dietary Approaches to Stop Hypertension (DASH) score, which is a diet score targeted to control hypertension. Kresovic et al. [41] found an association between EAA and the DASH index, the Healthy Eating Index, and the Alternative Mediterranean (aMed) index, in a cohort of non-Hispanic white females. Confirmation of associations between different dietary

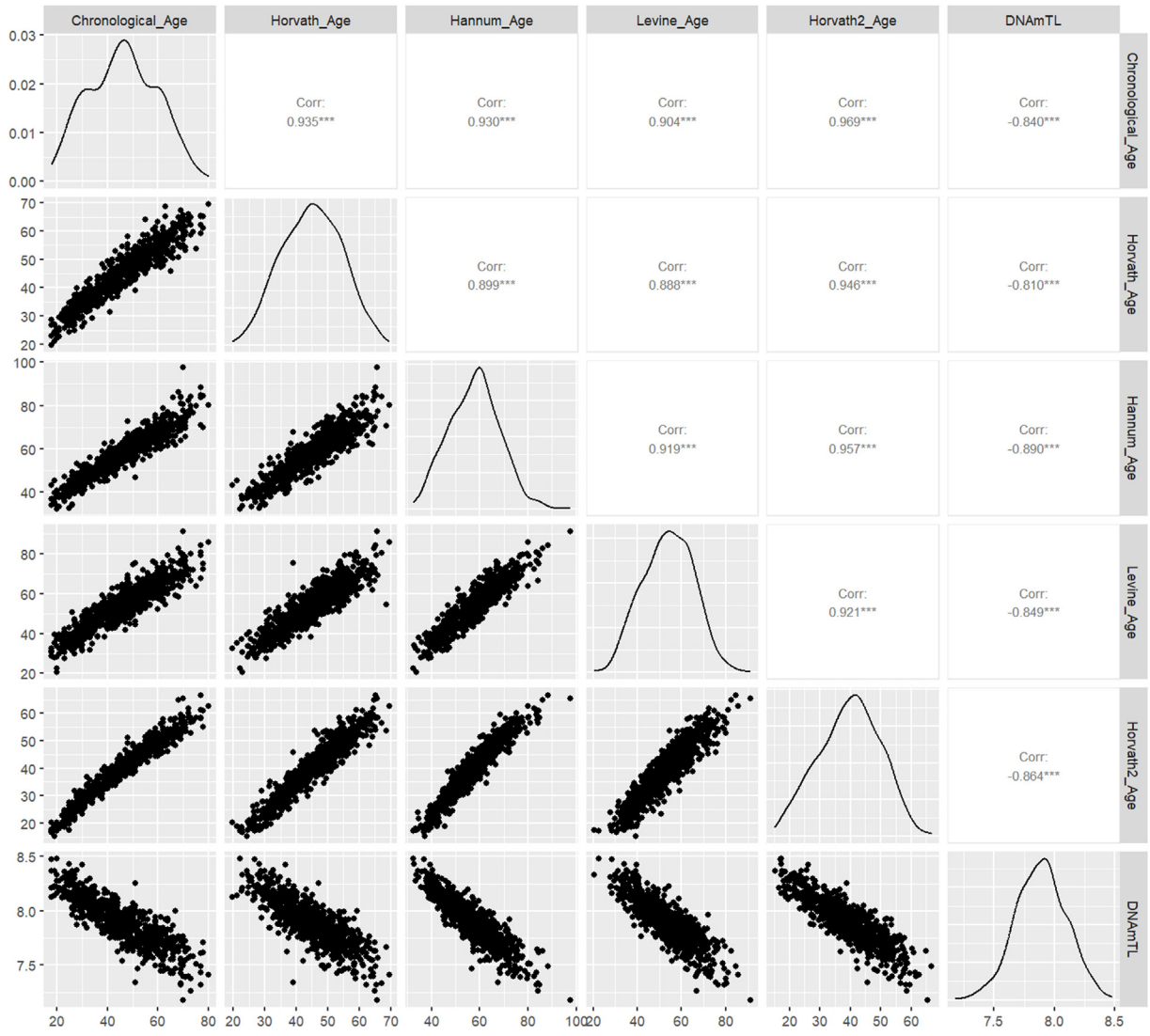

FIGURE 1. Pairwise plot showing Pearson's correlations between chronological age and EA [based on different epigenetic clocks] or DNAmTL. DNAmTL: DNA methylation-predicted telomere length; EA, epigenetic age. $N = 760$.

indexes and EAA in a cohort of post-menopausal females was recently provided by Reynolds et al. [43].

Of the essential nutrients consumed, vitamins (especially those involved in the 1CM) show important roles in regulating epigenetic homeostasis. Changes in DNAm have been associated with higher intake or increased levels in the blood of folate, vitamins B-2, B-6, or B-12 [32,86–89]. However, conflicting evidence can be found in literature [31,90]. Sae-Lee et al. [30] studied the effect of 2 y of daily supplementation with 400 μg folic acid + 500 μg vitamin B-12 in 44 individuals. In this small sample set, EA estimated by the Horvath clock did not significantly change following the intervention. However, differential methylation at the 353 epigenetic clock loci was observed. In our study, the inadequate intake of vitamin B-12 is associated with accelerated EA. As a cofactor of the methionine synthase, vitamin B-12 plays a central role in the 1CM [91], and vitamin B-12 deficiency is not uncommon in human diets [92,93]. The risk is particularly high in vegans [94], but also in any other diet with low intakes of animal-sourced foods as well as in many less-industrialized countries [95]. Malabsorption can also lead to vitamin B-12 deficiency. In addition, we measured a significant association between EAA and inadequate dietary intake of vitamin C. This finding reinforces the well-established correlation between redox balance, epigenomics, and healthy aging. In addition to its role as an antioxidant, vitamin C also plays an important role for Fe^{2++} and α-ketoglutarate-dependent dioxygenases [96]. This class of enzyme includes the Jumonji-C domain-containing histone demethylases and the TET family of DNA hydroxylases. Thus, vitamin C can contribute to the regulation of both histones and DNA (de)methylation, having a direct impact on the epigenetic homeostasis both in health and disease [97,98]. Our findings support the hypothesis that AI of vitamin B-12 and C contributes to the maintenance of epigenetic homeostasis and healthy aging.

Additionally, an independent association between EAA and the consumption of foods with varying degrees of processing was identified in our cohort. Specifically, a higher intake of ultraprocessed foods (NOVA group 4) was linked to EAA, whereas a higher intake of raw foods (NOVA group 1) was associated with a decelerated EA. This latter finding is particularly intriguing, considering that NOVA food group 1 is rich in vitamins and bioactive molecules that may promote health, potentially through the regulation of epigenetic homeostasis. Despite the established association between the consumption of

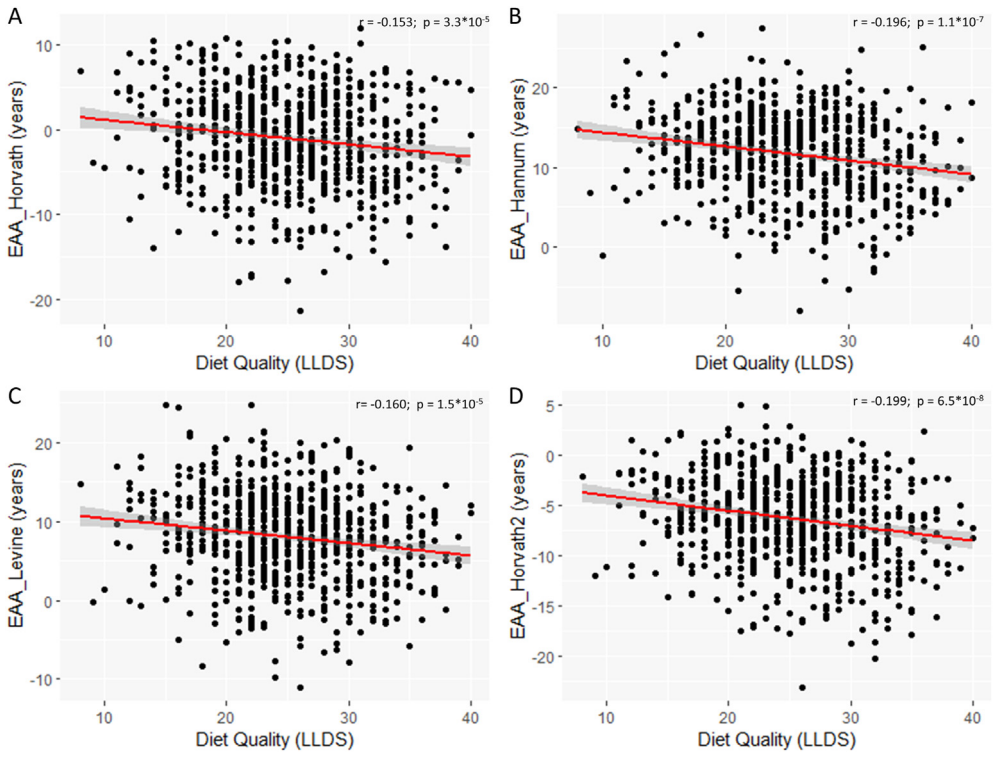

FIGURE 2. Pearson's correlations between EAA measured by different clocks (A and B) and diet quality measured according to the LLDS. $N = 728$. EAA, epigenetic age acceleration; LLDS, LifeLines Diet Score.

TABLE 4
Multivariate linear regression describing associations between EAA measured by different clocks, total kcal intake, and LLDS.

Outcome	Predictor	Covariates	*N*	Adj *R* squared	*P* value of the model	VIF	*B*	CI (95%)	β	*P*	FDR-adj *P*
AA_Horvath	kcal	Sex, WHR	742	0.077	2×10^{-13}	1.177	0.001	0.001, 0.002	0.139	3×10^{-4}	7×10^{-4}
AA_Hannum	kcal	Sex, WHR	742	0.096	6×10^{-17}	1.177	0.001	0.000, 0.001	0.113	0.003	0.007
AA_Levine	kcal	Sex, WHR	742	0.015	0.003	1.177	0.001	0.000, 0.001	0.069	0.083	0.129
AA_Horvath2	kcal	Sex, WHR	742	0.088	3×10^{-15}	1.177	0.001	0.001, 0.001	0.153	6×10^{-5}	1×10^{-4}
DNAmTL_adjAge	kcal	Sex, WHR	742	0.041	2×10^{-7}	1.177	−0.001	−0.001, 0.000	−0.057	0.147	0.220
AA_Horvath	kcal/BMR	Sex, WHR	742	0.062	8×10^{-11}	1.010	0.501	−0.472, 1.474	0.036	0.312	0.410
AA_Hannum	kcal/BMR	Sex, WHR	742	0.090	6×10^{-15}	1.010	0.275	−0.634, 1.183	0.021	0.553	0.648
AA_Levine	kcal/BMR	Sex, WHR	742	0.015	0.011	1.010	−0.256	−1.287, 0.776	−0.018	0.627	0.691
AA_Horvath2	kcal/BMR	Sex, WHR	742	0.074	3×10^{-12}	1.010	0.493	−0.274, 1.259	0.045	0.207	0.292
DNAmTL_adjAge	kcal/BMR	Sex, WHR	742	0.039	5×10^{-7}	1.010	0.176	−1.106, 1.1459	0.010	0.787	0.831
AA_Horvath	LLDS	Sex, WHR, kcal/BMR	728	0.081	3×10^{-13}	1.161	−0.140	−0.212, −0.069	−0.148	1×10^{-4}	2×10^{-4}
AA_Hannum	LLDS	Sex, WHR, kcal/BMR	728	0.106	1×10^{-17}	1.161	−0.133	−0.199, −0.066	−0.148	9×10^{-5}	3×10^{-4}
AA_Levine	LLDS	Sex, WHR, kcal/BMR	728	0.036	4×10^{-6}	1.161	−0.170	−0.245, −0.095	−0.174	1×10^{-5}	3×10^{-5}
AA_Horvath2	LLDS	Sex, WHR, kcal/BMR	728	0.097	4×10^{-16}	1.161	−0.132	−0.187, −0.076	−0.176	4×10^{-6}	2×10^{-4}
DNAmTL_adjAge	LLDS	Sex, WHR, kcal/BMR	728	0.049	4×10^{-8}	1.161	0.143	0.238, 0.049	0.116	0.003	0.006

Abbreviations: AA, age acceleration; Adj, adjusted; BMR, basal metabolic rate; CI, confidence intervals (lower bound, upper bound); DNAmTL_adjAge, DNA methylation-predicted telomere length adjusted for chronological age; FDR-adj, false discovery rate adjusted; LLDS, LifeLines diet score; VIF, variance inflation factor; WHR, waist-to-hip ratio.

ultraprocessed food and the risk of numerous complex pathologies [99], the current challenge lies in defining the concept of ultraprocessed food when assessing its impact on health [100]. The deleterious effects of ultraprocessed food consumption on health can likely be ascribed to the increased presence of harmful components prevalent in foods categorized as ultraprocessed, such as excessive calories, sugars, and saturated fats, among others.

Additionally, diet quality was associated with TL predicted by DNAm (DNAmTL_adjAge), adjusting the model for sex, body composition, and energy surplus. DNAmTL was also linked to the intake of NOVA food groups 1 and 4. These findings point out a significant association between diet and TL, in line with evidence from other studies [46], even where TL is estimated from DNAm data, and not by PCR-based methods.

Lastly, we observed a significant association between total glycemic load and both EAA and DNAmTL. Although the total intake of carbohydrates may not necessarily indicate an unhealthy diet, it is widely recognized that a high dietary glycemic load can contribute to the onset of insulin resistance, resulting in unfavorable long-term health consequences [101]. Our results provide further evidence for this hypothesis, indicating that a high dietary glycemic load may contribute to the acceleration of aging biomarkers.

TABLE 5

Multivariate linear regression models describing associations between measures of biological age acceleration (that is, EAA and DNAmTL_adjAge) and intake of NOVA food groups (1–4) expressed as (A) g/1000 kcal of the total food intake per day (NOVA_energyratio) or (B) g/kg of food per day (NOVA_weightratio).

A	Outcome	Predictor	Covariates	*N*	Adj *R* squared	*P* value of the model	VIF	*B*	CI (95%)	β	*P*	FDR-adj *P*
	AA_Horvath	NOVA_energyratio_1	Sex, WHR, LLDS	727	0.175	6×10^{-30}	1.857	−0.474	−0.575, −0.374	−0.426	2×10^{-19}	1×10^{-17}
	AA_Hannum	NOVA_energyratio_1	Sex, WHR, LLDS	727	0.178	2×10^{-30}	1.857	−0.391	−0.486, −0.296	−0.37	3×10^{-15}	7×10^{-14}
	AA_Levine	NOVA_energyratio_1	Sex, WHR, LLDS	727	0.075	2×10^{-12}	1.857	−0.327	−0.437, −0.217	−0.284	8×10^{-9}	5×10^{-8}
	AA_SkinHorvath	NOVA_energyratio_1	Sex, WHR, LLDS	727	0.18	8×10^{-31}	1.857	−0.351	−0.430, −0.272	−0.399	2×10^{-17}	7×10^{-16}
	DNAmTL_adjAge	NOVA_energyratio_1	Sex, WHR, LLDS	727	0.091	6×10^{-15}	1.857	0.422	0.284, 0.559	0.290	3×10^{-9}	2×10^{-8}
	AA_Horvath	NOVA_energyratio_2	Sex, WHR, LLDS	722	0.088	2×10^{-14}	1.049	−0.358	−0.711, −0.004	−0.072	0.047	0.080
	AA_Hannum	NOVA_energyratio_2	Sex, WHR, LLDS	722	0.108	9×10^{-18}	1.049	−0.05	−0.381, 0.281	−0.011	0.766	0.820
	AA_Levine	NOVA_energyratio_2	Sex, WHR, LLDS	722	0.034	9×10^{-6}	1.049	−0.076	−0.451, 0.300	−0.015	0.693	0.753
	AA_Horvath2	NOVA_energyratio_2	Sex, WHR, LLDS	722	0.104	4×10^{-17}	1.049	−0.277	−0.554, 0.000	−0.071	0.05	0.083
	DNAmTL_adjAge	NOVA_energyratio_2	Sex, WHR, LLDS	722	0.048	6×10^{-8}	1.049	0.040	−0.431, 0.511	0.006	0.866	0.877
	AA_Horvath	NOVA_energyratio_3	Sex, WHR, LLDS	727	0.08	4×10^{-13}	1.035	−0.092	−0.419, 0.234	−0.02	0.579	0.668
	AA_Hannum	NOVA_energyratio_3	Sex, WHR, LLDS	727	0.155	4×10^{-19}	1.035	−0.451	−0.754, −0.148	−0.104	0.004	0.008
	AA_Levine	NOVA_energyratio_3	Sex, WHR, LLDS	727	0.033	1×10^{-5}	1.035	−0.041	−0.386, 0.304	−0.009	0.816	0.85
	AA_Horvath2	NOVA_energyratio_3	Sex, WHR, LLDS	727	0.101	9×10^{-17}	1.035	−0.227	−0.532, −0.023	−0.077	0.033	0.061
	DNAmTL_adjAge	NOVA_energyratio_3	Sex, WHR, LLDS	727	0.048	5×10^{-8}	1.035	0.275	−0.157, 0.707	0.046	0.212	0.294
	AA_Horvath	NOVA_energyratio_4	Sex, WHR, LLDS	723	0.105	3×10^{-17}	1.174	0.334	0.185, 0.484	0.167	1×10^{-5}	3×10^{-5}
	AA_Hannum	NOVA_energyratio_4	Sex, WHR, LLDS	723	0.131	7×10^{-22}	1.174	0.319	0.180, 0.458	0.169	8×10^{-6}	3×10^{-5}
	AA_Levine	NOVA_energyratio_4	Sex, WHR, LLDS	723	0.054	8×10^{-9}	1.174	0.311	0.152, 0.469	0.151	1×10^{-4}	2×10^{-4}
	AA_Horvath2	NOVA_energyratio_4	Sex, WHR, LLDS	723	0.128	3×10^{-21}	1.174	0.298	0.182, 0.415	0.189	7×10^{-7}	3×10^{-6}
	DNAmTL_adjAge	NOVA_energyratio_4	Sex, WHR, LLDS	723	0.066	8×10^{-11}	1.174	−0.396	−0.594, −0.197	−0.153	9×10^{-5}	2×10^{-4}
B	Outcome	Predictor	Covariates	*N*	Adj *R* squared	*P* value of the model	VIF	*B*	CI (95%)	β	*P*	FDR-adj *P*
	DNAmTL_adjAge	NOVA_energyratio_4	Sex, WHR, LLDS	723	0.066	8×10^{-11}	1.174	−0.396	−0.594, −0.197	−0.153	9×10^{-5}	2×10^{-4}
	AA_Horvath	NOVA_weightratio_1	Sex, WHR, LLDS	728	0.127	2×10^{-21}	1.301	−0.232	−0.305, −0.159	−0.247	7×10^{-10}	8×10^{-9}
	AA_Hannum	NOVA_weightratio_1	Sex, WHR, LLDS	728	0.134	1×10^{-6}	1.301	−0.17	−0.239, −0.102	−0.192	1×10^{-6}	4×10^{-6}
	AA_Levine	NOVA_weightratio_1	Sex, WHR, LLDS	728	0.061	4×10^{-10}	1.301	−0.183	−0.260, −0.105	−0.189	5×10^{-6}	2×10^{-5}
	AA_Horvath2	NOVA_weightratio_1	Sex, WHR, LLDS	728	0.134	1×10^{-21}	1.301	−0.152	−0.209, −0.094	−0.205	3×10^{-7}	2×10^{-6}
	DNAmTL_adjAge	NOVA_weightratio_1	Sex, WHR, LLDS	728	0.079	5×10^{-13}	1.301	0.248	0.151, 0.346	0.204	7×10^{-7}	3×10^{-6}
	AA_Horvath	NOVA_weightratio_4	Sex, WHR, LLDS	726	0.117	2×10^{-19}	1.441	0.228	0.145, 0.310	0.227	8×10^{-8}	5×10^{-7}
	AA_Hannum	NOVA_weightratio_4	Sex, WHR, LLDS	726	0.135	1×10^{-22}	1.441	0.194	0.117, 0.271	0.205	9×10^{-7}	4×10^{-6}
	AA_Levine	NOVA_weightratio_4	Sex, WHR, LLDS	726	0.048	5×10^{-8}	1.441	0.152	0.064, 0.240	0.148	7×10^{-4}	0.001
	AA_Horvath2	NOVA_weightratio_4	Sex, WHR, LLDS	726	0.141	1×10^{-23}	1.441	0.198	0.134, 0.262	0.251	2×10^{-9}	1×10^{-8}
	DNAmTL_adjAge	NOVA_weightratio_4	Sex, WHR, LLDS	726	0.062	4×10^{-10}	1.441	−0.193	−0.303, −0.083	−0.149	7×10^{-4}	0.002

Abbreviations: AA, age acceleration; Adj, adjusted; BMR, basal metabolic rate; CI, confidence intervals (lower bound, upper bound); DNAmTL_adjAge, DNA methylation-predicted telomere length adjusted for chronological age; FDR-adj, false discovery rate adjusted; LLDS: LifeLines diet score; VIF, variance inflation factor; WHR, waist-to-hip ratio.

TABLE 6
Multivariate linear regression models describing associations between EAA and adequacy of vitamin intakes according to the EFSA dietary reference values (DRVs) for the analyzed vitamins: B-9 [population reference intake (PRI): <330 μg/d]; B-12 [adequate intake (AI): 4 μg/d]; intake of vitamin B-2 (PRI: 1.6 mg/d); vitamin B-6 (PRI: 1.6 mg/d); and vitamin C (PRI: 95 mg/d); $N = 375$.

Outcome	Predictor	Covariates	Adj R squared	P value of the model	VIF	B	CI (95%)	β	P	FDR-adj P
AA_Horvath	Vitamin B-9	Sex, LLDS, kcal	0.039	8×10^{-4}	1.338	0.639	−1.165, 2.442	0.041	0.487	0.589
AA_Hannum	Vitamin B-9	Sex, LLDS, kcal	0.083	3×10^{-7}	1.338	−1.181	−2.912, 0.550	−0.077	0.181	0.266
AA_Levine	Vitamin B-9	Sex, LLDS, kcal	0.012	0.079	1.338	−0.041	−2.042, 1.960	−0.002	0.969	0.969
AA_Horvath2	Vitamin B-9	Sex, LLDS, kcal	0.069	3×10^{-5}	1.338	0.382	−1.048, 1.812	0.030	0.600	0.671
DNAmTL_adjAge	Vitamin B-9	Sex, LLDS, kcal	0.048	2×10^{-4}	1.338	2.270	−0.131, 4.679	0.188	0.065	0.105
AA_Horvath	Vitamin B-12	Sex, LLDS, kcal	0.063	1×10^{-5}	1.137	-1.809	-2.945, -0.672	-0.167	0.002	0.004
AA_Hannum	Vitamin B-12	Sex, LLDS, kcal	0.096	2×10^{-8}	1.137	−1.530	−2.631, −0.430	−0.144	0.007	0.014
AA_Levine	Vitamin B-12	Sex, LLDS, kcal	0.023	0.012	1.137	−1.373	−2.645, −0.102	−0.116	0.034	0.062
AA_Horvath2	Vitamin B-12	Sex, LLDS, kcal	0.072	2×10^{-6}	1.137	−1.088	−1.994, −0.182	−0.126	0.019	0.036
DNAmTL_adjAge	Vitamin B-12	Sex, LLDS, kcal	0.042	5×10^{-4}	1.137	0.971	−0.575, 2.517	0.067	0.218	0.297
AA_Horvath	Vitamin B-2	Sex, LLDS, kcal	0.049	2×10^{-4}	1.322	−1.378	−2.725, −0.031	−0.117	0.045	0.078
AA_Hannum	Vitamin B-2	Sex, LLDS, kcal	0.085	2×10^{-7}	1.322	−1.068	−2.365, 0.230	−0.092	0.106	0.162
AA_Levine	Vitamin B-2	Sex, LLDS, kcal	0.014	0.058	1.322	−0.664	−2.164, 0.836	−0.051	0.384	0.488
AA_Horvath2	Vitamin B-2	Sex, LLDS, kcal	0.066	6×10^{-6}	1.322	−1.000	−2.069, 0.069	−0.106	0.067	0.106
DNAmTL_adjAge	Vitamin B-2	Sex, LLDS, kcal	0.040	7×10^{-4}	1.322	0.717	−1.097, 2.532	0.045	0.437	0.546
AA_Horvath	Vitamin B-6	Sex, LLDS, kcal	0.041	8×10^{-4}	1.400	0.668	−0.720, 2.056	0.057	0.345	0.446
AA_Hannum	Vitamin B-6	Sex, LLDS, kcal	0.078	7×10^{-7}	1.400	0.115	−1.221, 1.451	0.010	0.866	0.877
AA_Levine	Vitamin B-6	Sex, LLDS, kcal	0.013	0.064	1.400	0.561	−0.979, 2.101	0.044	0.474	0.582
AA_Horvath2	Vitamin B-6	Sex, LLDS, kcal	0.059	3×10^{-5}	1.400	0.298	−0.803, 1.400	0.032	0.595	0.671
DNAmTL_adjAge	Vitamin B-6	Sex, LLDS, kcal	0.040	8×10^{-4}	1.400	0.575	−1.288, 2.432	0.036	0.544	0.647
AA_Horvath	Vitamin C	Sex, LLDS, kcal	0.049	1×10^{-4}	1.153	−1.191	−2.339, −0.043	−0.110	0.042	0.075
AA_Hannum	Vitamin C	Sex, LLDS, kcal	0.095	3×10^{-8}	1.153	−1.441	−2.541, −0.341	−0.136	0.010	0.019
AA_Levine	Vitamin C	Sex, LLDS, kcal	0.016	0.040	1.153	−0.829	−2.107, 0.448	−0.070	0.202	0.291
AA_Horvath2	Vitamin C	Sex, LLDS, kcal	0.078	7×10^{-7}	1.153	−1.304	−2.210, −0.398	−0.151	0.005	0.010
DNAmTL_adjAge	Vitamin C	Sex, LLDS, kcal	0052	5×10^{-4}	1.153	0.912	−0.633, 2.457	0.063	0.246	0.329

Abbreviations: AA, age acceleration; Adj, adjusted; CI, confidence intervals (lower bound, upper bound); FDR-adj, false discovery rate adjusted; LLDS: LifeLines diet score; VIF, variance inflation factor.

TABLE 7
Multivariate linear regression models describing associations between measures of biological aging and total glycemic load (total intake of food in a day); $N = 728$.

Outcome	Predictor	Covariates	Adj R squared	P value of the model	VIF	B	CI (95%)	β	P	FDR-adj P
AA_Horvath	Total glycemic load	Sex, WHR, LLDS, kcal/BMR	0.107	4×10^{-18}	4.899	0.057	0.039, 0.075	0.476	9×10^{-10}	1×10^{-9}
AA_Hannum	Total glycemic load	Sex, WHR, LLDS, kcal/BMR	0.150	7×10^{-26}	4.899	0.064	0.047, 0.081	0.565	1×10^{-13}	1×10^{-12}
AA_Levine	Total glycemic load	Sex, WHR, LLDS, kcal/BMR	0.055	3×10^{-9}	4.899	0.058	0.039, 0.077	0.469	5×10^{-9}	4×10^{-8}
AA_Horvath2	Total glycemic load	Sex, WHR, LLDS, kcal/BMR	0.140	4×10^{-23}	4.899	0.054	0.040, 0.068	0.569	1×10^{-13}	1×10^{-12}
DNAmTL_adjAge	Total glycemic load	Sex, WHR, LLDS, kcal/BMR	0.061	3×10^{-10}	4.899	−0.053	−0.077, −0.029	−0.340	2×10^{-5}	6×10^{-5}

Abbreviations: AA, age acceleration; Adj, adjusted; BMR, basal metabolic rate; CI, confidence intervals (lower bound, upper bound); DNAmTL_adjAge, DNA methylation-predicted telomere length adjusted for chronological age; FDR-adj, false discovery rate adjusted; LLDS, LifeLines Diet Score; VIF, variance inflation factor; WHR, waist-to-hip ratio.

In conclusion, it is becoming evident that loss of epigenetic homeostasis and altered epigenetic signatures can contribute to aging. Novel approaches to reverse these changes can improve health in experimental animal models [102]. The use of epigenetic clocks to identify strategies to slow down the aging process is met with enthusiasm [11,103]. However, the specific biological mechanisms linked to the alterations of DNAm remain limited, despite some initial attempts [104]. Currently the sensitivity of the epigenetic clocks is unclear. We observed differences related to the EAA between different epigenetic clocks. It remains to be verified how sensitive these epigenetic clocks are to small interventions. Given the high stability and faithful maintenance of DNAm patterns, we expect that long-term exposure is more likely to be measured than those of recent exposures, but this requires further investigations. Recent interventional studies have indicated that diet can have causal effects on epigenetic age acceleration/deceleration [105,106]. Caloric restriction, in particular, has been shown to affect EAA [107]. However, some findings associating dietary changes with EA reversal are based on few individuals and short-term interventions [108,109].

Limitations: Because of the observational nature of this study, causality on the effects of diet on EAA cannot be demonstrated. Dietary

intake measured by FFQ has drawbacks in terms of reliability [110]. The associations between EAA and vitamin intakes has been validated in a reduced number of samples. Future studies using accurate biomarkers of food intake will help to verify the associations with EAA. Because environmental factors related to EAA are numerous, we decided to adjust the analysis only for covariates that are tightly related to dietary habits (that is, body composition and caloric intake over BMR). Further studies addressing the role of the cumulative effect of the exposome on EAA should also consider other environmental factors that might contribute to the EAA (that is, smoking, physical activity, and drugs). Finally, the association between diet features and EAA are limited in their effect size. Because it is not clear if these may have clinical implications, results should be interpreted with caution and additional confirmatory studies are essential before considering their translation into clinical practice.

Despite these limitations, our study emphasizes the value of epigenetic clocks in human nutrition research as a cutting-edge tool capable of revealing novel associations and assisting in the assessment of the effectiveness of nutritional interventions. It may develop into an early biological sensor capable of capturing diverse pathways [111–113] and allowing us to monitor the influence of lifestyle (including dietary habits) on human health. This holds the potential to anticipate unfavorable outcomes of lifestyle, including accelerated aging and chronic diseases. Our findings corroborate the hypothesis that overall diet quality (rather than specific food items) has a central role in shaping human health and that its impact can be traced by using molecular biomarkers. Further interventional studies aimed at validating this hypothesis and elucidating which dietary interventions can actively decelerate the epigenetic clock are warranted. These studies will help to translate findings into effective dietary guidelines that may effectively promote health and prevent nutrition-related complex diseases.

Authors contributions

The authors' contributions were as follows – LB: conception and study design, data curation, and formal analysis; funding acquisition; writing—original draft; JAdS, JZ: bioinformatics analysis and data curation; FvM: supervision, writing—review and editing, funding acquisition; and all authors: read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

Funding

The LifeLines initiative has been made possible by subsidy from the Dutch Ministry of Health, Welfare and Sport, the Dutch Ministry of Economic Affairs, the University Medical Center Groningen (UMCG), Groningen University and the Provinces in the North of the Netherlands (Drenthe, Friesland, Groningen). This work was supported by European Union – NextGenerationEU and Ministry of University and Research – Promotion and Development Fund – Ministerial Decree No. 737/2021, The Mi.Me.Si. Project, ERC UNICAM Grant, Swiss National Science Foundation (Project 320030E_215870), and European Research Council (BRITE).

Data availability

Data may be obtained from a third party and are not publicly available. Researchers can apply to use the LifeLines data used in this study. More information about how to request LifeLines data and the conditions of use can be found on their website (https://www.lifelines.nl/researcher/how-to-apply).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2024.08.033.

References

[1] A. Bird, Perceptions of epigenetics, Nat. Engl. 447 (7143) (2007) 396–398.
[2] A. Bird, DNA methylation patterns and epigenetic memory, Genes Dev. 16 (1) (2002) 6–21.
[3] M. Kim, J. Costello, DNA methylation: an epigenetic mark of cellular memory, Exp. Mol. Med. 49 (4) (2017), https://doi.org/10.1038/emm.2017.10 e322–e322.
[4] M. Iurlaro, F. von Meyenn, W. Reik, DNA methylation homeostasis in human and mouse development, Curr. Opin. Genet. Dev. 43 (2017) 101–109, https://doi.org/10.1016/j.gde.2017.02.003.
[5] K.D. Robertson, A.P. Wolffe, DNA methylation in health and disease, Nat. Rev. Genet. 1 (1) (2000) 11–19, https://doi.org/10.1038/35049533.
[6] W. Zhang, J. Qu, G.-H. Liu, J.C.I. Belmonte, The ageing epigenome and its rejuvenation, Nat. Rev. Mol. Cell Biol. 21 (3) (2020) 137–150.
[7] H. Wu, C.M. Eckhardt, A.A. Baccarelli, Molecular mechanisms of environmental exposures and human disease, Nat. Rev. Genet. 24 (5) (2023) 332–344.
[8] S. Pal, J.K. Tyler, Epigenetics and aging, Sci. Adv. 2 (7) (2016) e1600584, https://doi.org/10.1126/sciadv.1600584.
[9] G. Hannum, J. Guinney, L. Zhao, L. Zhang, G. Hughes, S. Sadda, et al., Genome-wide methylation profiles reveal quantitative views of human aging rates, Mol. Cell. 49 (2) (2013) 359–367.
[10] S. Horvath, DNA methylation age of human tissues and cell types, Genome Biol 14 (10) (2013) R156, https://doi.org/10.1186/gb-2013-14-10-r115.
[11] K. Wang, H. Liu, Q. Hu, L. Wang, J. Liu, Z. Zheng, et al., Epigenetic regulation of aging: implications for interventions of aging and diseases, Signal Transduct. Target. Ther. 7 (1) (2022) 374, https://doi.org/10.1038/s41392-022-01211-8.
[12] G. Kelsey, O. Stegle, W. Reik, Single-cell epigenomics: recording the past and predicting the future, Science 358 (6359) (2017) 69–75, https://doi.org/10.1126/science.aan6826.
[13] C. Ladd-Acosta, M.D. Fallin, DNA methylation signatures as biomarkers of prior environmental exposures, Curr. Epidemiol. Rep. 6 (1) (2019) 1–13, https://doi.org/10.1007/s40471-019-0178-z.
[14] S. Horvath, K. Raj, DNA methylation-based biomarkers and the epigenetic clock theory of ageing, Nat. Rev. Genet. 19 (6) (2018) 371–384, https://doi.org/10.1038/s41576-018-0004-3.
[15] S. Horvath, J. Oshima, G.M. Martin, A.T. Lu, A. Quach, H. Cohen, et al., Epigenetic clock for skin and blood cells applied to Hutchinson Gilford Progeria Syndrome and ex vivo studies, Aging (Albany NY) 10 (7) (2018) 1758–1775, https://doi.org/10.18632/aging.101508.
[16] M.E. Levine, A.T. Lu, A. Quach, B.H. Chen, T.L. Assimes, S. Bandinelli, et al., An epigenetic biomarker of aging for lifespan and healthspan, Aging (Albany NY) 10 (4) (2018) 573–591, https://doi.org/10.18632/aging.101414.
[17] C. Topart, E. Werner, P.B. Arimondo, Wandering along the epigenetic timeline, Clin. Epigenetics. 12 (1) (2020) 97, https://doi.org/10.1186/s13148-020-00893-7.
[18] Y. Salameh, Y. Bejaoui, N. El Hajj, DNA methylation biomarkers in aging and age-related diseases, Front. Genet. 11 (2020) 171, https://doi.org/10.3389/fgene.2020.00171.
[19] K. Declerck, W. Vanden Berghe, Back to the future: epigenetic clock plasticity towards healthy aging, Mech. Ageing Dev. 174 (2018) 18–29, https://doi.org/10.1016/j.mad.2018.01.002.
[20] L. Oblak, J. van der Zaag, A.T. Higgins-Chen, M.E. Levine, M.P. Boks, A systematic review of biological, social and environmental factors associated with epigenetic clock acceleration, Ageing Res. Rev. 69 (2021) 101348, https://doi.org/10.1016/j.arr.2021.101348.
[21] F. Galkin, O. Kovalchuk, D. Koldasbayeva, A. Zhavoronkov, E. Bischof, Stress, diet, exercise: common environmental factors and their impact on epigenetic age, Ageing Res. Rev. 88 (2023) 101956, https://doi.org/10.1016/j.arr.2023.101956.
[22] L.T. Fadnes, C. Celis-Morales, J.-M. Økland, S. Parra-Soto, K.M. Livingstone, F.K. Ho, et al., Life expectancy can increase by up to 10 years following sustained shifts towards healthier diets in the United Kingdom, Nat. Food. 4 (11) (2023) 961–965, https://doi.org/10.1038/s43016-023-00868-w.

[23] S.D. Amenyah, M. Ward, J.J. Strain, H. McNulty, C.F. Hughes, C. Dollin, et al., Nutritional epigenomics and age-related disease, Curr. Dev. Nutr. 4 (7) (2020) nzaa097, https://doi.org/10.1093/cdn/nzaa097.
[24] J.A. McKay, J.C. Mathers, Diet induced epigenetic changes and their implications for health, Acta Physiol. (Oxf.) 202 (2) (2011) 103–118, https://doi.org/10.1111/j.1748-1716.2011.02278.x.
[25] I. Alam, F. Ali, F. Zeb, A. Almajwal, S. Fatima, X. Wu, Relationship of nutrigenomics and aging: involvement of DNA methylation, J. Nutr. Intermed. Metab. 16 (2019) 100098.
[26] F.Z. Kadayifci, S. Zheng, Y.-X. Pan, Molecular mechanisms underlying the link between diet and DNA methylation, Int. J. Mol. Sci. 19 (12) (2018) 4055, https://doi.org/10.3390/ijms19124055.
[27] N. El Omari, S. Bakrim, M. Bakha, J.M. Lorenzo, M. Rebezov, M.A. Shariati, et al., Natural bioactive compounds targeting epigenetic pathways in cancer: a review on alkaloids, terpenoids, quinones, and isothiocyanates, Nutrients 13 (11) (2021) 3714, https://doi.org/10.3390/nu13113714.
[28] Q.A. Abdul, B.P. Yu, H.Y. Chung, H.A. Jung, J.S. Choi, Epigenetic modifications of gene expression by lifestyle and environment, Arch. Pharm. Res. 40 (11) (2017) 1219–1237, https://doi.org/10.1007/s12272-017-0973-3.
[29] S.-W. Choi, S. Friso, Modulation of DNA methylation by one-carbon metabolism: a milestone for healthy aging, Nutr. Res. Pract. 17 (4) (2023) 597–615, https://doi.org/10.4162/nrp.2023.17.4.597.
[30] C. Sae-Lee, S. Corsi, T.M. Barrow, G.G.C. Kuhnle, V. Bollati, J.C. Mathers, et al., Dietary intervention modifies DNA methylation age assessed by the epigenetic clock, Mol. Nutr. Food Res. 62 (23) (2018) e1800092, https://doi.org/10.1002/mnfr.201800092.
[31] J.A. Chamberlain, P.-A. Dugué, J.K. Bassett, A.M. Hodge, M.T. Brinkman, J.E. Joo, et al., Dietary intake of one-carbon metabolism nutrients and DNA methylation in peripheral blood, Am. J. Clin. Nutr. 108 (3) (2018) 611–621, https://doi.org/10.1093/ajcn/nqy119.
[32] P.R. Mandaviya, R. Joehanes, J. Brody, J.E. Castillo-Fernandez, K.F. Dekkers, A.N. Do, et al., Association of dietary folate and vitamin B-12 intake with genome-wide DNA methylation in blood: a large-scale epigenome-wide association analysis in 5841 individuals, Am. J. Clin. Nutr. 110 (2) (2019) 437–450, https://doi.org/10.1093/ajcn/nqz031.
[33] L.K. Park, S. Friso, S.-W. Choi, Nutritional influences on epigenetics and age-related disease, Proc. Nutr. Soc. 71 (1) (2012) 75–83, https://doi.org/10.1017/S0029665111003302.
[34] H.W. Korsmo, X. Jiang, One carbon metabolism and early development: a diet-dependent destiny, Trends Endocrinol. Metab. 32 (8) (2021) 579–593, https://doi.org/10.1016/j.tem.2021.05.011.
[35] R. Yin, S.-Q. Mao, B. Zhao, Z. Chong, Y. Yang, C. Zhao, et al., Ascorbic acid enhances Tet-mediated 5-methylcytosine oxidation and promotes DNA demethylation in mammals, J. Am. Chem. Soc. 135 (28) (2013) 10396–10403, https://doi.org/10.1021/ja4028346.
[36] T.A. Hore, F. von Meyenn, M. Ravichandran, M. Bachman, G. Ficz, D. Oxley, et al., Retinol and ascorbate drive erasure of epigenetic memory and enhance reprogramming to naïve pluripotency by complementary mechanisms, Proc. Natl Acad. Sci. U S A. 113 (43) (2016) 12202–12207, https://doi.org/10.1073/pnas.1608679113.
[37] M.G. Traber, J.F. Stevens, Vitamins C and E: beneficial effects from a mechanistic perspective, Free Radic. Biol. Med. 51 (5) (2011) 1000–1013, https://doi.org/10.1016/j.freeradbiomed.2011.05.017.
[38] A. Guillaumet-Adkins, Y. Yañez, M.D. Peris-Diaz, I. Calabria, C. Palanca-Ballester, J. Sandoval, Epigenetics and oxidative stress in aging, Oxid. Med. Cell. Longev. 2017 (2017) 9175806, https://doi.org/10.1155/2017/9175806.
[39] A.T. Lu, A. Quach, J.G. Wilson, A.P. Reiner, A. Aviv, K. Raj, et al., DNA methylation GrimAge strongly predicts lifespan and healthspan, Aging (Albany NY) 11 (2) (2019) 303–327, https://doi.org/10.18632/aging.101684.
[40] A. Quach, M.E. Levine, T. Tanaka, A.T. Lu, B.H. Chen, L. Ferrucci, et al., Epigenetic clock analysis of diet, exercise, education, and lifestyle factors, Aging (Albany NY) 9 (2) (2017) 419–446, https://doi.org/10.18632/aging.101168.
[41] J.K. Kresovich, Y.-M.M. Park, J.A. Keller, D.P. Sandler, J.A. Taylor, Healthy eating patterns and epigenetic measures of biological age, Am. J. Clin. Nutr. 115 (1) (2022) 171–179, https://doi.org/10.1093/ajcn/nqab307.
[42] Y. Kim, T. Huan, R. Joehanes, N.M. McKeown, S. Horvath, D. Levy, et al., Higher diet quality relates to decelerated epigenetic aging, Am. J. Clin. Nutr. 115 (1) (2022) 163–170, https://doi.org/10.1093/ajcn/nqab201.
[43] L.M. Reynolds, D.K. Houston, M.B. Skiba, E.A. Whitsel, J.D. Stewart, Y. Li, et al., Diet quality and epigenetic aging in the women's health initiative, J. Acad. Nutr. Diet. (2024), https://doi.org/10.1016/j.jand.2024.01.002. S2212-2672(24)00002-9.
[44] A. Rico-Campà, M.A. Martínez-González, I. Alvarez-Alvarez, R.D. Mendonça, C. de la Fuente-Arrillaga, C. Gómez-Donoso, et al., Association between consumption of ultra-processed foods and all cause mortality: SUN prospective cohort study, BMJ 365 (2019) l1949, https://doi.org/10.1136/bmj.l1949.
[45] B. Srour, L.K. Fezeu, E. Kesse-Guyot, B. Allès, C. Méjean, R.M. Andrianasolo, et al., Ultra-processed food intake and risk of cardiovascular disease: prospective cohort study (NutriNet-Santé), BMJ 365 (2019) l1451, https://doi.org/10.1136/bmj.l1451.
[46] S. Galiè, S. Canudas, J. Muralidharan, J. García-Gavilán, M. Bulló, J. Salas-Salvadó, Impact of nutrition on telomere health: systematic review of observational cohort studies and randomized clinical trials, Adv. Nutr. 11 (3) (2020) 576–601, https://doi.org/10.1093/advances/nmz107.
[47] T. Steenstrup, J.V.B. Hjelmborg, J.D. Kark, K. Christensen, A. Aviv, The telomere lengthening conundrum – artifact or biology? Nucleic Acids Res 41 (13) (2013) e131 https://doi.org/10.1093/nar/gkt370.
[48] J. Denham, F.Z. Marques, F.J. Charchar, Leukocyte telomere length variation due to DNA extraction method, BMC Res. Notes. 7 (2014) 877, https://doi.org/10.1186/1756-0500-7-877.
[49] S. Verhulst, E. Susser, P.R. Factor-Litvak, M.J.P. Simons, A. Benetos, T. Steenstrup, et al., Commentary: the reliability of telomere length measurements, Int. J. Epidemiol. 44 (5) (2015) 1683–1686, https://doi.org/10.1093/ije/dyv166.
[50] A. Tolios, D. Teupser, L.M. Holdt, Preanalytical conditions and DNA isolation methods affect telomere length quantification in whole blood, PLOS ONE 10 (12) (2015) e0143889, https://doi.org/10.1371/journal.pone.0143889.
[51] A.T. Lu, A. Seeboth, P.-C. Tsai, D. Sun, A. Quach, A.P. Reiner, et al., DNA methylation-based estimator of telomere length, Aging (Albany NY) 11 (16) (2019) 5895–5923, https://doi.org/10.18632/aging.102173.
[52] A. Sijtsma, J. Rienks, P. van der Harst, G. Navis, J.G.M. Rosmalen, A. Dotinga, Cohort Profile Update: LifeLines, a three-generation cohort study and biobank, Int. J. Epidemiol. 51 (5) (2022) e295–e302, https://doi.org/10.1093/ije/dyab257.
[53] G. Vazquez, S. Duval, D.R.J. Jacobs, K. Silventoinen, Comparison of body mass index, waist circumference, and waist/hip ratio in predicting incident diabetes: a meta-analysis, Epidemiol. Rev. 29 (2007) 115–128, https://doi.org/10.1093/epirev/mxm008.
[54] L. de Koning, A.T. Merchant, J. Pogue, S.S. Anand, Waist circumference and waist-to-hip ratio as predictors of cardiovascular events: meta-regression analysis of prospective studies, Eur. Heart. J. 28 (7) (2007) 850–856, https://doi.org/10.1093/eurheartj/ehm026.
[55] A.E. Black, Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations, Int. J. Obes. Relat. Metab. Disord. 24 (9) (2000) 1119–1130, https://doi.org/10.1038/sj.ijo.0801376.
[56] W.N. Schofield, Predicting basal metabolic rate, new standards and review of previous work, Hum. Nutr. Clin. Nutr. 39 (suppl 1) (1985) 5–41.
[57] E.F. Tigchelaar, A. Zhernakova, J.A.M. Dekens, G. Hermes, A. Baranska, Z. Mujagic, et al., Cohort profile: LifeLines DEEP, a prospective, general population cohort study in the northern Netherlands: study design and baseline characteristics, BMJ Open 5 (8) (2015) e006772, https://doi.org/10.1136/bmjopen-2014-006772.
[58] E.M. Brouwer-Brolsma, C. Perenboom, D. Sluik, A. van de Wiel, A. Geelen, E.J. Feskens, et al., Development and external validation of the "Flower-FFQ": a FFQ designed for the LifeLines Cohort Study, Public Health Nutr 25 (2) (2022) 225–236, https://doi.org/10.1017/S1368980021002111.
[59] E. Siebelink, A. Geelen, J.H.M. de Vries, Self-reported energy intake by FFQ compared with actual energy intake to maintain body weight in 516 adults, Br. J. Nutr. 106 (2) (2011) 274–281, https://doi.org/10.1017/S0007114511000067.
[60] R.A. Goldbohm, P. van't Veer, P.A. van den Brandt, M.A. van't Hof, H.A. Brants, F. Sturmans, et al., Reproducibility of a food frequency questionnaire and stability of dietary habits determined from five annually repeated measurements, Eur. J. Clin. Nutr. 49 (6) (1995) 420–429.
[61] P.C. Vinke, E. Corpeleijn, L.H. Dekker, D.R.J. Jacobs, G. Navis, D. Kromhout, Development of the food-based LifeLines diet score (LLDS) and its application in 129,369 LifeLines participants, Eur. J. Clin. Nutr. 72 (8) (2018) 1111–1119, https://doi.org/10.1038/s41430-018-0205-z.
[62] D. Kromhout, C.J.K. Spaaij, J. de Goede, R.M. Weggemans, The 2015 Dutch food-based dietary guidelines, Eur. J. Clin. Nutr. 70 (8) (2016) 869–878, https://doi.org/10.1038/ejcn.2016.52.
[63] C.A. Monteiro, G. Cannon, P.C. Jaime, D.S. Canella, M.L. Louzada, G. Calixto, et al., The star shines bright. Food classification, Public health NOVA, World Nutr. 7 (1–3) (2016) 28–38.
[64] M.-J. Duan, P.C. Vinke, G. Navis, E. Corpeleijn, L.H. Dekker, Ultra-processed food and incident type 2 diabetes: studying the underlying consumption patterns to unravel the health effects of this heterogeneous food category in the prospective LifeLines Cohort, BMC Med 20 (1) (2022) 7, https://doi.org/10.1186/s12916-021-02200-4.

[65] V. Braesco, I. Souchon, P. Sauvant, T. Haurogné, M. Maillot, C. Féart, et al., Ultra-processed foods: how functional is the NOVA system? Eur. J. Clin. Nutr. 76 (9) (2022) 1245–1253, https://doi.org/10.1038/s41430-022-01099-1.
[66] C.A. Monteiro, G. Cannon, R.B. Levy, J.-C. Moubarac, M.L.C. Louzada, F. Rauber, et al., Ultra-processed foods: what they are and how to identify them, Public Health Nutr 22 (5) (2019) 936–941, https://doi.org/10.1017/S1368980018003762.
[67] EFSA, Dietary reference values for nutrients: summary report, EFSA J 14 (2017) e15121E, https://doi.org/10.2903/sp.efsa.2017.e15121.
[68] M.J. Bonder, R. Luijk, D.V. Zhernakova, M. Moed, P. Deelen, M. Vermaat, et al., Disease variants alter transcription factor levels and methylation of their binding sites, Nat. Genet. 49 (1) (2017) 131–138, https://doi.org/10.1038/ng.3721.
[69] A. Nishiyama, M. Nakanishi, Navigating the DNA methylation landscape of cancer, Trends Genet 37 (11) (2021) 1012–1027, https://doi.org/10.1016/j.tig.2021.05.002.
[70] D. Pelegí-Sisó, P. de Prado, J. Ronkainen, M. Bustamante, J.R. González, methylclock: a Bioconductor package to estimate DNA methylation age, Bioinformatics 37 (12) (2021) 1759–1760, https://doi.org/10.1093/bioinformatics/btaa825.
[71] R.C. Gentleman, V.J. Carey, D.M. Bates, B. Bolstad, M. Dettling, S. Dudoit, et al., Bioconductor: open software development for computational biology and bioinformatics, Genome Biol 5 (10) (2004) R80, https://doi.org/10.1186/gb-2004-5-10-r80.
[72] A.E. Teschendorff, F. Marabita, M. Lechner, T. Bartlett, J. Tegner, D. Gomez-Cabrero, et al., A beta-mixture quantile normalization method for correcting probe design bias in Illumina Infinium 450K DNA methylation data, Bioinformatics 29 (2) (2013) 189–196, https://doi.org/10.1093/bioinformatics/bts680.
[73] J. Bohlin, S.E. Håberg, P. Magnus, S.E. Reese, H.K. Gjessing, M.C. Magnus, et al., Prediction of gestational age based on genome-wide differentially methylated regions, Genome Biol 17 (1) (2016) 207, https://doi.org/10.1186/s13059-016-1063-4.
[74] R.E. Marioni, S.E. Harris, S. Shah, A.F. McRae, T. von Zglinicki, C. Martin-Ruiz, et al., The epigenetic clock and telomere length are independently associated with chronological age and mortality, Int. J. Epidemiol. 45 (2) (2018) 424–432, https://doi.org/10.1093/ije/dyw041.
[75] A.T. Lu, L. Xue, E.L. Salfati, B.H. Chen, L. Ferrucci, D. Levy, et al., GWAS of epigenetic aging rates in blood reveals a critical role for TERT, Nat. Commun. 9 (1) (2018) 387, https://doi.org/10.1038/s41467-017-02697-5.
[76] L.P. Breitling, K.-U. Saum, L. Perna, B. Schöttker, B. Holleczek, H. Brenner, Frailty is associated with the epigenetic clock but not with telomere length in a German cohort, Clin. Epigenetics. 8 (2016) 21, https://doi.org/10.1186/s13148-016-0186-5.
[77] WHO European Regional Obesity Report 2022, WHO Regional Office for Europe, Copenhagen, 2022.
[78] NCD Risk Factor Collaboration (NCD-RisC), Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults, Lancet 390 (10113) (2017) 2627–2642, https://doi.org/10.1016/S0140-6736(17)32129-3.
[79] P. Dominguez-Salas, S.E. Moore, M.S. Baker, A.W. Bergen, S.E. Cox, R.A. Dyer, et al., Maternal nutrition at conception modulates DNA methylation of human metastable epialleles, Nat. Commun. 5 (2014) 3746, https://doi.org/10.1038/ncomms4746.
[80] C.F. Nicoletti, C. Cortes-Oliveira, N.Y. Noronha, M.A.S. Pinhel, W.S. Dantas, A. Jácome, et al., DNA methylation pattern changes following a short-term hypocaloric diet in women with obesity, Eur. J. Clin. Nutr. 74 (9) (2020) 1345–1353, https://doi.org/10.1038/s41430-020-0660-1.
[81] I. Karabegović, E. Portilla-Fernandez, Y. Li, J. Ma, S.C.E. Maas, D. Sun, et al., Epigenome-wide association meta-analysis of DNA methylation with coffee and tea consumption, Nat. Commun. 12 (1) (2021) 2830, https://doi.org/10.1038/s41467-021-22752-6.
[82] A. Yaskolka Meir, H. Yun, M.J. Stampfer, L. Liang, F.B. Hu, Nutrition, DNA methylation and obesity across life stages and generations, Epigenomics 15 (19) (2023) 991–1015, https://doi.org/10.2217/epi-2023-0172.
[83] E.W. Tobi, J.J. Goeman, R. Monajemi, H. Gu, H. Putter, Y. Zhang, et al., DNA methylation signatures link prenatal famine exposure to growth and metabolism, Nat. Commun. 5 (2014) 5592, https://doi.org/10.1038/ncomms6592.
[84] Y. Zhang, T.G. Kutateladze, Diet and the epigenome, Nat. Commun. 9 (1) (2018) 3375, https://doi.org/10.1038/s41467-018-05778-1.
[85] S. Maegawa, Y. Lu, T. Tahara, J.T. Lee, J. Madzo, S. Liang, et al., Caloric restriction delays age-related methylation drift, Nat. Commun. 8 (1) (2017) 539, https://doi.org/10.1038/s41467-017-00607-3.
[86] Y. Ba, H. Yu, F. Liu, X. Geng, C. Zhu, Q. Zhu, et al., Relationship of folate, vitamin B12 and methylation of insulin-like growth factor-II in maternal and cord blood, Eur. J. Clin. Nutr. 65 (4) (2011) 480–485, https://doi.org/10.1038/ejcn.2010.294.
[87] G. Fiorito, S. Guarrera, C. Valle, F. Ricceri, A. Russo, S. Grioni, et al., B-vitamins intake, DNA-methylation of one carbon metabolism and homocysteine pathway genes and myocardial infarction risk: the EPICOR study, Nutr. Metab. Cardiovasc. Dis. 24 (5) (2014) 483–488, https://doi.org/10.1016/j.numecd.2013.10.026.
[88] R.E. Irwin, S.-J. Thursby, M. Ondičová, K. Pentieva, H. McNulty, R.C. Richmond, et al., A randomized controlled trial of folic acid intervention in pregnancy highlights a putative methylation-regulated control element at ZFP57, Clin. Epigenetics. 11 (1) (2019) 31, https://doi.org/10.1186/s13148-019-0618-0.
[89] A. Caffrey, R.E. Irwin, H. McNulty, J.J. Strain, D.J. Lees-Murdock, B.A. McNulty, et al., Gene-specific DNA methylation in newborns in response to folic acid supplementation during the second and third trimesters of pregnancy: epigenetic analysis from a randomized controlled trial, Am. J. Clin. Nutr. 107 (4) (2018) 566–575, https://doi.org/10.1093/ajcn/nqx069.
[90] F. Perrier, V. Viallon, S. Ambatipudi, A. Ghantous, C. Cuenin, H. Hernandez-Vargas, et al., Association of leukocyte DNA methylation changes with dietary folate and alcohol intake in the EPIC study, Clin. Epigenetics. 11 (1) (2019) 57, https://doi.org/10.1186/s13148-019-0637-x.
[91] P. Lyon, V. Strippoli, B. Fang, L. Cimmino, B vitamins and one-carbon metabolism: implications in human health and disease, Nutrients 12 (9) (2020) 2867, https://doi.org/10.3390/nu12092867.
[92] L.H. Allen, How common is vitamin B-12 deficiency? Am. J. Clin. Nutr. 89 (2) (2009) 693S–696S, https://doi.org/10.3945/ajcn.2008.26947A.
[93] R. Green, L.H. Allen, A.-L. Bjørke-Monsen, A. Brito, J.-L. Guéant, J.W. Miller, et al., Vitamin B(12) deficiency, Nat. Rev. Dis. Prim. 3 (2017) 17040, https://doi.org/10.1038/nrdp.2017.40.
[94] R. Pawlak, S.E. Lester, T. Babatunde, The prevalence of cobalamin deficiency among vegetarians assessed by serum vitamin B12: a review of literature, Eur. J. Clin. Nutr. 68 (5) (2014) 541–548, https://doi.org/10.1038/ejcn.2014.46.
[95] S. Gebremedhin, Trends in vitamin B12 supply and prevalence of inadequate intake in Africa: regional and country-specific estimates, Food Nutr. Bull 42 (4) (2021) 467–479, https://doi.org/10.1177/03795721211043353.
[96] J.I. Young, S. Züchner, G. Wang, Regulation of the epigenome by vitamin C, Annu. Rev. Nutr. 35 (2015) 545–564, https://doi.org/10.1146/annurev-nutr-071714-034228.
[97] T. Lee Chong, E.L. Ahearn, L. Cimmino, Reprogramming the epigenome with vitamin C, Front. Cell Dev. Biol. 7 (2019) 128, https://doi.org/10.3389/fcell.2019.00128.
[98] V. Camarena, G. Wang, The epigenetic role of vitamin C in health and disease, Cell Mol. Life Sci. 73 (8) (2016) 1645–1658, https://doi.org/10.1007/s00018-016-2145-x.
[99] M. Vitale, G. Costabile, R. Testa, G. D'Abbronzo, I.C. Nettore, P.E. Macchia, et al., Ultra-processed foods and human health: a systematic review and meta-analysis of prospective cohort studies, Adv. Nutr. 15 (1) (2024) 100121, https://doi.org/10.1016/j.advnut.2023.09.009.
[100] L. Sanchez-Siles, S. Roman, V. Fogliano, M. Siegrist, Naturalness and healthiness in "ultra-processed foods": a multidisciplinary perspective and case study, Trends Food Sci. Technol. 129 (2022) 667–673, https://doi.org/10.1016/j.tifs.2022.11.009.
[101] A.W. Barclay, P. Petocz, J. McMillan-Price, V.M. Flood, T. Prvan, P. Mitchell, et al., Glycemic index, glycemic load, and chronic disease risk—a meta-analysis of observational studies, Am. J. Clin. Nutr. 87 (3) (2008) 627–637, https://doi.org/10.1093/ajcn/87.3.627.
[102] J.-H. Yang, M. Hayano, P.T. Griffin, J.A. Amorim, M.S. Bonkowski, J.K. Apostolides, et al., Loss of epigenetic information as a cause of mammalian aging, Cell 186 (2) (2023) 305–326.e27, https://doi.org/10.1016/j.cell.2022.12.027.
[103] B. Pereira, F.P. Correia, I.A. Alves, M. Costa, M. Gameiro, A.P. Martins, et al., Epigenetic reprogramming as a key to reverse ageing and increase longevity, Ageing Res. Rev. 95 (2024) 102204, https://doi.org/10.1016/j.arr.2024.102204.
[104] S. Kabacik, D. Lowe, L. Fransen, M. Leonard, S.-L. Ang, C. Whiteman, et al., The relationship between epigenetic age and the hallmarks of aging in human cells, Nat. Aging. 2 (6) (2022) 484–493, https://doi.org/10.1038/s43587-022-00220-0.
[105] N. Gensous, P. Garagnani, A. Santoro, C. Giuliani, R. Ostan, C. Fabbri, et al., One-year Mediterranean diet promotes epigenetic rejuvenation with country- and sex-specific effects: a pilot study from the NU-AGE project, GeroScience 42 (2) (2020) 687–701, https://doi.org/10.1007/s11357-019-00149-0.

[106] G. Fiorito, S. Caini, D. Palli, B. Bendinelli, C. Saieva, I. Ermini, et al., DNA methylation-based biomarkers of aging were slowed down in a two-year diet and physical activity intervention trial: the DAMA study, Aging Cell 20 (10) (2021) e13439, https://doi.org/10.1111/acel.13439.
[107] R. Waziry, C.P. Ryan, D.L. Corcoran, K.M. Huffman, M.S. Kobor, M. Kothari, et al., Effect of long-term caloric restriction on DNA methylation measures of biological aging in healthy adults from the CALERIE trial, Nat. Aging. 3 (3) (2023) 248–257, https://doi.org/10.1038/s43587-022-00357-y.
[108] K.N. Fitzgerald, T. Campbell, S. Makarem, R. Hodges, Potential reversal of biological age in women following an 8-week methylation-supportive diet and lifestyle program: a case series, Aging (Albany NY) 15 (6) (2023) 1833–1839, https://doi.org/10.18632/aging.204602.
[109] K.N. Fitzgerald, R. Hodges, D. Hanes, E. Stack, D. Cheishvili, M. Szyf, et al., Potential reversal of epigenetic age using a diet and lifestyle intervention: a pilot randomized clinical trial, Aging (Albany NY) 13 (7) (2021) 9419–9432. https://pubmed.ncbi.nlm.nih.gov/33844651.
[110] A. Naska, A. Lagiou, P. Lagiou, Dietary assessment methods in epidemiological research: current state of the art and future prospects, F1000Res 6 (2017) 926, https://doi.org/10.12688/f1000research.10703.1.
[111] Z. Liu, D. Leung, K. Thrush, W. Zhao, S. Ratliff, T. Tanaka, et al., Underlying features of epigenetic aging clocks in vivo and in vitro, Aging Cell 19 (10) (2020) e13229, https://doi.org/10.1111/acel.13229.
[112] D.J. Simpson, T. Chandra, Epigenetic age prediction, Aging Cell 20 (9) (2021) e13452, https://doi.org/10.1111/acel.13452.
[113] C.G. Bell, R. Lowe, P.D. Adams, A.A. Baccarelli, S. Beck, J.T. Bell, et al., DNA methylation aging clocks: challenges and recommendations, Genome Biol 20 (1) (2019) 249, https://doi.org/10.1186/s13059-019-1824-y.