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Original article

Associations between dietary food categories, epigenetic age acceleration and low-grade inflammation in the Lifelines-DEEP cohort

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SUMMARY

Background and aim: Low-grade inflammation (LGI) and metabolic dysfunction are tightly linked processes central to the onset and progression of complex diseases and aging. LGI acts both as a driver and a consequence of metabolic disturbances and represents a hallmark of "inflammaging", the chronic, systemic inflammatory state arising from sustained immune activation that characterizes aging and underlies many age-related pathologies. Targeting LGI through lifestyle changes, particularly diet, offers a promising approach for healthy aging. Epigenetic clocks have recently emerged as biomarkers of biological aging, capturing the cumulative effects of environmental and lifestyle factors, including diet. This study aims to assess whether the inflammatory potential of the diet influences epigenetic age acceleration (EAA), as measured by several next-generation epigenetic clocks, and whether this relationship is mediated by LGI.

Methods: This study included 691 participants from the Lifelines-DEEP cohort. Habitual dietary intake was assessed using food frequency questionnaires to derive overall diet quality and the intake of pro- and anti-inflammatory dietary components. Circulating biomarkers of LGI were measured and combined into composite inflammatory scores alongside individual markers. EAA was estimated from blood DNA methylation data using next-generation principal component–based epigenetic clocks (PCPhenoAge and PCGrimAge) and the DunedinPACE clock, selected for their enhanced sensitivity to lifestyle-related exposures compared with traditional clocks. Multivariable regression models were applied to examine associations between dietary inflammatory profiles and EAA, and mediation analyses were conducted to evaluate the contribution of LGI to these relationships.

Results: Overall diet quality was significantly associated with EAA, particularly when assessed using the PCGrimAge clock, which showed the strongest and most consistent associations among the analyzed clocks. PCGrimage EAA was further related specific food categories: consumption of high-energy beverages (HHB) and NOVA4 foods was positively associated with EAA, whereas intake of vegetables and tea showed inverse associations. Mediation analyses indicated that the link between overall diet quality and PCGrimage EAA involved both direct and indirect components. Specifically, the positive associations of HHB and NOVA4 foods with EAA were largely explained by higher LGI levels, whereas the inverse associations observed for beneficial food categories (i.e., NOVA1, vegetables, tea) reflected both direct effects and indirect effects mediated by lower LGI. For the other clocks, associations with dietary features were no longer significant after accounting for LGI mediation.

Conclusions: These findings support PCGrimAge as a particularly informative epigenetic clock for capturing nutritional influences on the epigenome, emphasizing that diet, inflammation, and epigenetic aging are interrelated yet distinct processes that should be jointly considered when assessing long-term health risk.

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1. Introduction

Aging and age-related diseases burden modern society, making strategies to slow aging a global priority. The exposome (which encompasses lifelong environmental and lifestyle exposures, including dietary habits) plays a central role in shaping health trajectories. Accordingly, tools for monitoring the impact of lifestyle and environmental factors on biological aging are essential. Such tools hold promises for enabling risk stratification and guiding precision interventions that target modifiable factors to promote healthy aging and prevent age-associated diseases.

Investigating and mapping the intricate molecular pathways through which the exposome impacts aging cannot overlook epigenetics [1]. Epigenetic modifications change with aging across the genome, reflecting the impact of social, environmental, and behavioural factors on health [2,3]. These changes often also drive detrimental processes such as genomic instability, chronic inflammation, and oxidative stress, which are recognized hallmarks of aging and major drivers of age-related diseases [4]. To assess the impact of the exposome on the epigenome, researchers have developed a range of epigenetic clocks aimed at predicting age-related and phenotypic outcomes [5,6]. The most widely used epigenetic clocks focus on DNA methylation (DNAm) at specific CpG sites, where methylation levels are regressed against chronological or phenotypic age. Biological age acceleration is measured by estimating the discrepancy between an individual's epigenetic age and their actual chronological age [7]. This difference, known as epigenetic age acceleration (EAA), reflects shifts in the methylome beyond the age-related drift typically observed over lifespan, potentially estimating the impact of environmental and lifestyle exposures on health [3,8].

Epigenetic clocks have evolved through successive generations, each designed to improve the estimation of biological aging and its responsiveness to health-related exposures. Among the various epigenetic clocks developed [9], second and third-generation clocks (or next-generation clocks), have been identified as the most powerful in tracking the impact of lifestyle-associated exposures on the epigenome [10,11]. Clocks emerging as the most responsive to lifestyle interventions include the PhenoAge, Grimage, and DunedinPace clocks [12]. PhenoAge was developed by incorporating DNAm patterns from multiple CpG sites along with clinical biomarkers related to aging, such as cholesterol levels, blood pressure, and inflammation markers. GrimAge was defined as a composite biomarker (weighted linear combination) of seven DNAm surrogates of plasma proteins, a DNAm-based estimator of smoking pack-years, age, and sex [13]. GrimAge relied on the fact that some plasma protein levels can be estimated based on cytosine methylation levels. Thus, it combines DNAm-based plasma protein estimates, DNAm pack-year estimates, and observed age and sex as a function of mortality risk [13]. GrimAge has been shown to be a very powerful morbidity and mortality predictor, outperforming other widely used DNAm biomarkers of aging [13]. DunedinPACE is derived from analysis of longitudinal data collected from a cohort of individuals who are all the same chronological age [14]. It reflects differences in the rate of deterioration in system integrity occurring in the individuals over a fixed time interval, age 26 to age 45 years [14]. Additionally, to further minimize technical noise and enhance the application of epigenetic clocks in both basic and translational research, principal component-based clocks (PCClocks) have been developed. These PCClocks are trained using principal components instead of individual CpGs, significantly improving reliability and sensitivity while reducing background noise [15]. As a result, PCClocks are considered the most suitable tools for studies aimed at tracking the lifestyle footprint on the epigenome.

Considering the central role of inflammaging, the chronic, low-grade systemic inflammation (LGI) state driven by persistent immune activation that emerges with age [16], in shaping aging trajectories [17], systemic inflammation is likely an interdependent or co-occurring process with the factors on which these clocks were trained. As a result, inflammation may account for a significant proportion of the variance in these clocks [18]. This suggests that part of the effectiveness of these clocks in tracking the influence of lifestyle exposures on future pathophysiologies may lie in their ability to capture inflammaging. However, studies suggested that, while both systemic inflammation and DNAm age acceleration are risk factors for mortality, they may represent different biological processes that independently capture different dimensions of overall risk [18,19].

LGI is a well-established common state that may link various non-communicable diseases to lifestyle habits, particularly diet [20–23]. Numerous studies have emphasized the pro- or anti-inflammatory effects of different diets [24–29], indicating that dietary impact on sterile inflammation could be a critical factor in mediating the onset of complex diseases [30,31]. Of note, this aspect might also differ between metabolically healthy or unhealthy individuals, both within the context of normal weight and obese phenotypes. Indeed, individuals living with metabolically healthy obesity (MHO) or metabolically unhealthy obesity (MUO) have been suggested to be different in terms of their epigenetic patterns [32].

Despite evidence linking diet to inflammation and inflammation to epigenetic aging, the extent to which dietary habits relate to EAA, and the degree to which LGI may account for or modify such associations, remains unclear. Based on prior literature, we hypothesized that dietary patterns and specific food categories previously linked to pro- or anti-inflammatory properties would also show corresponding associations with EAA, and that LGI would explain part of these relationships by acting as a potential biological intermediary between dietary intake and epigenetic aging. Accordingly, we first focused on overall diet quality and subsequently extended the analyses to specific dietary components, including EDIP-related pro- and anti-inflammatory food groups and NOVA food categories, to identify the dietary drivers underlying the observed associations. The EDIP-related categories were selected because they represent food components empirically associated with inflammatory potential in multiple epidemiological investigations[33–35]. NOVA groups were included because highly-processed foods have emerged as an independent predictor of metabolic risk and LGI [36–40] and has recently been associated with adverse trajectories of healthy aging [40–42]. This approach allowed us to capture both broad dietary patterns and the contribution of distinct food groups, offering a comprehensive evaluation of how different facets of diet relate to LGI and EAA.

The main objective of this study was therefore to examine the associations between overall diet quality and specific pro- or anti-inflammatory food category intake with next-generation EAA measures in the Lifelines-DEEP cohort [43]. We further evaluated whether these associations remained significant after accounting for LGI by assessing its potential mediating role. Finally, we explored how LGI and EAA relate to broader metabolic status. By testing these hypotheses using multiple epigenetic clocks and established LGI markers, our study aims to clarify how diet, inflammation, and biological aging interrelate, providing new insights into modifiable pathways relevant for healthy aging.

2. Methods

2.1. Recruitment and samples collection

This study utilized data from the Lifelines Cohort, a large prospective cohort and biobank designed to support FAIR (findable, accessible, interoperable, and reusable) research on healthy aging [44]. Lifelines is a multidisciplinary, population-based cohort study that uniquely examines the health and health-related behaviors of 167729 individuals living in the northern Netherlands through a three-generation design. The study employs a wide range of investigative methods to assess biomedical, socio-demographic, behavioral, physical, and psychological factors contributing to the health and disease of the general population, with a particular focus on multi-morbidity and complex genetics. The Lifelines protocol was approved by the University Medical Centre Groningen (UMCG) Medical Ethical Committee (approval number 2007/152), and all participants provided informed consent. The research was conducted in accordance with the World Medical Association's Code of Ethics (Declaration of Helsinki). Recruitment began with a baseline assessment conducted between 2007 and 2013, followed by follow-up assessments until 2023. Additional details about the Lifelines cohort can be found on its dedicated website (https://www.lifelines-biobank.com/). A subset of 1500 participants from the LifeLines study actively participated in the LifeLines-DEEP cohort [43], contributing additional molecular data to better understand the relationship between genetic and phenotypic variations. These include analysis of DNAm from whole blood. For this study, the following inclusion and exclusion criteria were applied. Inclusion criteria: Participants with available data on chronological age, sex, and DNAm suitable for second- and third-generation epigenetic clock calculations, as well as confirmed fasting status at baseline. Exclusion criteria: Diagnosis of cancer (based on baseline and 6-year follow-up data), missing information on sex or age, no fasting during the baseline assessment, missing DNAm data.

2.2. Anthropometry and body composition

Data on sex, age, dietary and smoking habits were collected through questionnaires. Anthropometric measurements were taken by trained staff at the Lifelines research facilities. Height (cm) and body weight (kg) were measured without shoes or heavy clothing, using the SECA 222 stadiometer (Seca GmbH, Hamburg, Germany) for height and the SECA 761 scale (Seca GmbH, Hamburg, Germany) for body weight. Waist (cm) and hip circumferences (cm) were measured to calculate the waist-to-hip ratio (WHR), an indicator of body composition and a predictor of obesity-related complications [45]. Basal metabolic rate (BMR) [46] was calculated using the Schofield equation [47]. To adopt a conservative approach and in line with relevant literature indicating that central obesity is a more accurate marker of metabolic health than BMI [48,49], we used WC to classify metabolic status in subsequent analyses.

2.3. Blood composition data and clinical parameters

Assessments of blood composition of fresh blood samples were performed at the laboratory center of the UMCG, that is certified according to NEN-EN-ISO 9001:2015 and NEN-EN-ISO 15189:2012 standards. We retrieved information concerning Basophilic Granulocytes (10^9/L), Erythrocytes (10^{12}/L), Eosinophilic Granulocytes (10^9/L), Leukocytes (10^9/L), Lymphocytes (10^9/L), Mononuclear Cells (10^9/L), Neutrophilic Granulocytes (10^9/L), Thrombocytes (10^9/L) in EDTA Sysmex. Also, we retrieved data about high-sensitivity CRP (hs-CRP) in heparin (mg/L), HDL cholesterol in heparin (mmol/L), LDL cholesterol in heparin (mmol/L) and triglycerides in heparin (mmol/L). Detailed information can be found in the dedicated and publicly available LifelinesWiki page (https://wiki.lifelines.nl/doku.php?id=blood_analyses).

2.4. LGI quantification and metabolic health status definition

Definition of LGI is a controversial topic, we therefore employed a multi-biomarker approach to assess this complex state. Primary among these was high-sensitivity CRP (hs-CRP). Although hs-CRP is classically considered an acute-phase reactant, persistent mild elevation (3–10 mg/L) is thought to be consistent with LGI [50]. Despite its limited specificity, chronically elevated hs-CRP serves as both an LGI marker and an independent predictor of vascular- and all-cause mortality [51]. Also, LGI can be estimated by the neutrophils to lymphocyte ratio (NLR). NLR is firstly an important marker of immune homeostasis, reflecting the balance between innate immunity (via neutrophils) and adaptive immunity (via lymphocytes). Zahorec [52] has suggested a normal range for NLR of 1–2, with values falling <0.7 or >3.0 considered abnormal, and an NLR of 2–3 considered a borderline range consistent with subclinical inflammation or LGI. A normal NLR is considered to be 1 to 2, although a wide range of cut-offs has been used in the literature to define an elevated NLR) [52,53]. In addition, given previous literature suggesting the ability of Mediterranean diet to modulate risk for CVD through the effect on neutrophil-to-erythrocyte (NEER) and neutrophil-to-HDL (NHR) ratios, we also calculated these parameters as indices of risk [31]. Also, the systemic immune-inflammation index (SII), a novel biomarker for inflammation previously associated with malignancy and coronary artery disease prognosis [54,55], was calculated as follows: (neutrophil count) × (platelet count)/(lymphocyte count). Finally, we calculated the INFLA-score proposed by Shivappa and colleagues [56], which quantitatively assesses chronic LGI by integrating CRP, white blood cell count, platelet count and granulocyte-to-lymphocyte ratio [56]). The granulocyte count is inclusive of neutrophil, eosinophil and basophil counts. Each biomarker was categorised into scoring levels based on their decile range: the highest range (7th to 10th deciles) received a positive score of + 1 to + 4, the fifth and sixth deciles were assigned a value of 0 and the lowest range (1st to 4th deciles) was assigned a negative score of −4 to − 1. The INFLA-score is the sum of the scores obtained for these four indicators, ranging from −16 to +16, reflecting an individual's chronic LGI level, with a higher score suggesting heightened inflammatory status [57,58]. This score has previously been used to investigate the association between overall LGI and dietary habits [36,59], as well as to examine the relationship between LGI and disease risk [58,60–62]. Also, in order to evaluate LGI in the specific context of obesity and metabolic health, data about circulating levels of adipokines (leptin, adiponectin and resistin), previously determined as described by Kurilshikov et al. [63], were retrieved from the Lifelines Biobank. The use of individual circulating cytokines (e.g., IL-1β, IL-6, IL-8, IL-10, IL-12, and TNF-α) was considered but ultimately excluded because of the limited availability of data and the highly skewed and zero-inflated distributions of these measures, which would have compromised the statistical reliability and interpretability of the analyses.

As for the categorization of the metabolic status based on the Adult Treatment Panel III criteria [64], we divided the cohort into 4 groups according to the presence of central obesity (WC > 102 in man or WC > 88 cm in women) and to the metabolic health status (metabolically healthy vs unhealthy). Metabolically unhealthy individuals were defined by the presence of at least 2 of

the following risk factors [65]: diastolic blood pressure >85 mmHg; systolic blood pressure >130 mmHg; fasting glucose >100 mg/dl; TG > 150 mg/dl; HDL < 1 mmol/L for man or HDL <1.3 mmol/L for women; currently being administered with medication for diabetes or hypertension or high cholesterol. As a result, the cohort was divided in to 4 groups: 1) lean and metabolically healthy (healthy); 2) lean but metabolically unhealthy (MUL); 3) affected by central obesity but metabolically healthy (MHO); 4) affected by central obesity and metabolically unhealthy (MUO).

2.5. Data collection on dietary habits

In the Lifelines cohort study, data on participants' habitual dietary intake were gathered using the Flower Food Frequency Questionnaire (FFQ). This questionnaire was developed by Wageningen University utilizing the Dutch FFQTOOL™ [66], with food items selected based on the Dutch National Food Consumption Survey of 1997/1998 [67]. The FFQ reference period is one month, based on the assumption that food consumption patterns remain stable over a longer period [68]. The validation of this tool for estimating habitual dietary intake, including data on energy intake, macronutrients, micronutrients, and food items, has been previously published [66]. The food consumption data were then converted into daily energy and nutrient intake using the Dutch food composition database from 2011 [69].

In this study, we adopted a food-category-based approach to examine how dietary characteristics are associated with biological outcomes. Accordingly, we first quantified the intake of food categories that have been empirically identified as key contributors to the proinflammatory potential of the diet within the dietary inflammatory patter (EDIP) score [33,34]. This score based on food groups (expressed as portion/day) was developed in a US-based prospective cohort and its validity evaluated by using data from 2 independent US-based prospective cohorts of health professionals [33]. In the original paper from Tabung et al., the component food groups comprising the EDIP are as follows: intakes of processed meat, red meat, organ meat, fish (other than dark-meat fish), other vegetables (i.e., vegetables other than green-leafy vegetables and dark-yellow vegetables), refined grains, high-energy beverages (cola and other carbonated beverages with sugar, fruit drinks) (HEB), low-energy beverages (low-energy cola and other low-energy carbonated beverages) (LEB), and tomatoes were positively related to concentrations of the inflammatory markers. Intakes of beer, wine, tea, coffee, dark-yellow vegetables (comprising carrots, yellow squash, and sweet potatoes), green-leafy vegetables, snacks, fruit juice, and pizza were inversely related to concentrations of the inflammatory markers. From Lifelines FFQ, we calculated the intake of the same variables considered in the EDIP score expressed as portion/day as defined in the original article [34]. In particular, each Lifelines FFQ variable was mapped to a corresponding EDIP food group using detailed food descriptions from the Dutch Healthy Diet index (DHD) variables and FFQ metadata. Because EDIP variables are defined in portions/day rather than grams/day, we converted Lifelines food intake into EDIP-equivalent portions using either: (i) Direct correspondence, when serving sizes were the same (e.g., 1 glass of wine, 1 cup of coffee, 1 glass of fruit juice), or (ii) Gram-based conversion, using mean portion sizes from USDA [70] or DHD [71–73] references. Specific details for each food group, including mappings, serving sizes, and conversion formulas, are provided in Supplementary Table 1. In the present study, we analysed these components individually rather than computing the cumulative EDIP score. This choice reflects both the methodological focus of our work (i.e., assessing the associations between EAA and the intake of specific food categories) and the fact that calculation of the composite EDIP score requires complete data for all included food items. Because the presence of missing values prevents computation of the cumulative score, and imputation of dietary variables was deemed inappropriate in this context, the full EDIP index could be derived for only a limited number of participants within the selected cohort, preventing its use in subsequent analyses.

Consumption of processed food was also estimated according to the NOVA food intake classification [74,75]. NOVA is one of the most popular systems used to classify foods according to processing-related criteria, dividing food items into four categories: (1) unprocessed or minimally processed foods (e.g., fresh vegetables/fruits, unprocessed meat), (2) processed culinary ingredients (e.g., butter/oil for cooking, sugar, salt), (3) processed foods (e.g., canned vegetables/fish, fruits in syrup), and (4) ultra-processed foods (UPF) (e.g., processed meat, soft drinks). NOVA food categories are expressed in grams of the intake of that specific food categories over 1000 kcal of the total food intake per day (energy ratio). In addition, an index of overall diet quality, the Lifelines Diet Score (LLDS) was calculated as described elsewere [69,76]. LLDS is a fully food-based and evidence-based tool to assess relative diet quality. Its highest scores represent diets expected to be most beneficial in light of the prevention of nutrition-related chronic diseases. The reliability of this index has been previously validated [67,69] and numerous studies used this index to test association between diet quality and several health outcomes [77–82]. In addition, we retrieved from the Lifelines dataset also the DHD2015-index to evaluate overall diet quality using an additional metric and to assess adequacy of the intake of vegetables, processed meat and red meat. The DHD2015-index is a measure of adherence to the 2015 Dutch dietary guidelines [83,84] and has been previously validated and associated with health outcomes [85–88]. The index consists of sixteen components, each scored with a minimum of 0 points to a maximum of 10 points (with a higher score indicating a better adherence to the guidelines) [71,73]. As for vegetables, DHD is based on the recommendation to consume at least 200 g of vegetables daily. The cut-off was set at 200 g as quantified in the guideline. Concerning red meat, this group included beef, pork, duck, pheasant, offal and game products. The Netherlands Nutrition Centre advises to consume less than 300 g of red meat weekly (about 45 g/d). The cut-off value was thus set at an intake of 45 g/d. The Health Council of the Netherlands indicated that with a consumption of 100 g/d or more negative health effects were observed. Consequently, the threshold value was set at an intake of 100 g/d. As for processed meat (both red and white), the Health Council of the Netherlands indicated that negative health effects of processed meat are observed at intakes of 50 g/d or more, and therefore this was set as threshold value [73]. Further methodological details are provided in references [73,83,84].

Additionally, in a subset of the cohort of 383 individuals, dietary intakes of vitamin C, folate (vitamin B9), vitamin B12, riboflavin (vitamin B2), and vitamin B6 were examined, given their established roles in one-carbon metabolism and their previously reported associations with epigenetic regulation and obesity-related traits [89–94]. Moreover, lipid quality was estimated by calculating the atherogenicity index (AI) = SFA/(MUFA + PUFA) [95,96], and the dietary intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was evaluated. These additional analyses were motivated by evidence indicating that both one-carbon metabolism and fatty acid composition influence inflammatory pathways, DNA methylation processes, and metabolic health, all of which are implicated in healthy aging [41,97–104].

2.6. Methylation data analysis: second- and third-generation epigenetic clocks calculation

Genome-wide DNAm data from Lifelines-DEEP cohort participants were generated as described previously [105]. The original IDAT files were generated using the Illumina iScan BeadChip scanner. Quality control for both probes and samples was performed as outlined in Bonder et al. [105]. B-values deposited in the Lifelines data repository were used to calculate epigenetic age according to several second- or third-generation clocks. We selected the principal component–based epigenetic clocks (PCClocks), developed by Higgins-Chen and colleagues from the Levine's Laboratory, due to the higher sensitivity, which makes them more suitable for tracing the impact of lifestyle-related factors [15]. In detail, we focused our analyses on PCPhenoAge, PCGrimAge, and DunedinPACE clocks, which have shown stronger and more consistent associations with lifestyle-related factors and aging-related outcomes in previous studies [14,15,106,107]. PCPhenoAge and PCGrimAge have been developed to improve the reliability of DNA methylation–based aging measures by reducing technical noise while preserving biologically meaningful signals. Unlike conventional CpG-based clocks, which rely on a limited subset of individual CpGs selected through supervised machine learning and may be affected by substantial technical variability, PCClocks apply principal component analysis to genome-wide CpG methylation data to capture shared patterns of covariation across many CpGs and dilute noise from individual sites. Elastic net regression is subsequently used to retain components that contribute to prediction while minimizing the influence of noise-related components [15]. By minimizing technical variation, PC-based clocks yield more stable estimates of epigenetic age and EAA and show stronger and more consistent associations with aging-related phenotypes, behavioral factors, and environmental exposures [14,15], supporting their use in epidemiological and clinical research settings. This improved reliability is particularly important in observational studies, where technical noise may obscure true but modest associations between environmental or lifestyle-related exposures, such as diet, and biological aging. The original training outcomes of the clocks (e.g., mortality risk, clinical outcomes, or pace of aging) were not modeled as dependent variables in the present analyses, as these aspects have been previously validated in the original development studies. In this study, epigenetic clocks were applied as established continuous biological aging measures to examine their associations with dietary exposures, in accordance with the study objectives.

Calculation was performed using the *methyAge* function from the publicly available and peer-reviewed 'dnaMethyAge' R package [108], following the developers' recommended implementation and default parameters. No retraining, recalibration, or validation of the underlying epigenetic clock algorithms was performed in the present study. As described in the original publication by Wang et al. [108], the *methyAge* function requires DNA methylation β-values as input and returns continuous epigenetic age estimates for each sample. EAA was obtained directly from the package output, which internally calculates EAA as the residuals from the regression of DNA methylation–based age on chronological age. Additional details on the underlying algorithms and exact computational steps are provided in the original reference [108]. A flowchart illustrating the participant selection process is provided in Supplementary Fig. 1.

2.7. Statistical analysis

Data analysis and visualization was performed using R Studio (version 4.2.2) and Statistics for Data Analysis (SPSS V.28.0.1.1). Adjusted p values have been calculated according to the Benjamini–Hochberg procedure to correct for multiple comparisons, setting a false discovery rate of 0.05, where indicated. This method is widely regarded as appropriate for controlling the expected proportion of false positives in settings involving multiple hypothesis testing [109–111]. A pairwise deletion procedure was employed for missing data in correlation analyses, while listwise deletion was applied in regression models. Dietary variables with extensive missingness (e.g., refined grains and tomatoes) were excluded from the analyses, as their inclusion would have caused substantial reductions in sample size due to listwise deletion, markedly compromising statistical power and the stability of estimates. Multiple imputation was not considered appropriate, given the large inter-individual variability and non-random missingness typical of FFQ-derived dietary data. The difference in mean EAA across quartiles of intake for each EDIP variable or metabolic health status was tested using the Kruskal–Wallis test, followed by the Dunn post-hoc test for multiple pairwise comparisons between groups, with p-values adjusted according to the Holm method. Associations between continuous variables were tested by multivariate robust linear regression models by including dietary parameters as independent variables and different measures of inflammation or EAA as an outcome. Robust linear regression models were fitted using the *lmrob* function from the *robustbase* package (version 0.99.6), which estimates regression coefficients through an MM-estimator initialized by a highly robust S-estimator. This approach provides robustness to outliers and deviations from normality, thereby yielding more stable and reliable estimates in datasets characterized by skewed distributions and heterogeneous variance. Models were adjusted for covariates as specified in each single test and displayed in the tables describing results. Since WHR is considered a more accurate indicator of metabolic health than BMI and better reflects levels of abdominal fat (including dangerous visceral fat) [45,48], we used WHR as covariate to adjust for body composition. Principal Component Analysis (PCA) was employed to reduce the dimensionality of the blood composition data (erythrocytes, platelets basophilic, eosinophilic and neutrophilic granulocytes, mononuclear cells, lymphocytes). The first three principal components (PC1, PC2, and PC3), which cumulatively explained 62% of the variance and had eigenvalues greater than 1, were selected based on a scree plot and used as confounding factors in the linear models where indicated. To identify the LGI marker showing the most robust and consistent pattern of associations with dietary exposures, we performed a comparative summary analysis across all diet–inflammation regression models adjusted for age, sex, smoking status, and WHR. From these models, four summary metrics were derived: (i) the number of significant associations after false discovery rate correction (FDR-adjusted $p < 0.05$); (ii) the aggregate signal strength, calculated as the sum of $-\log_{10}$(FDR-adjusted p-values) across associations surviving FDR correction; (iii) the mean absolute standardized regression coefficient, reflecting the average magnitude of diet–marker associations on a standardized scale, and (iv) a global p-value obtained from Fisher's combined probability test applied to the set of raw p-values for each marker. Together, these complementary metrics were used to quantitatively compare the strength, robustness, and consistency of diet–inflammation association patterns across LGI biomarkers and to guide the selection of the primary LGI marker for subsequent analyses. *Lavaan* package (version 0.6.20) was used to perform mediation analysis. Mediation analysis was conducted to examine whether the association between dietary habits (independent variable, *X*) and PCGrimAge-derived epigenetic age acceleration (EAA; dependent variable, *Y*) was mediated by systemic low-grade inflammation, quantified by the INFLA-score (mediator, *M*) [112].

Structural equation modelling (SEM) was employed to estimate the direct effect of each dietary component on EAA, the indirect effect via INFLA-score, and the total effect (sum of direct and indirect effects). All models were adjusted for relevant covariates, including age, sex, smoking status, WHR and caloric surplus. Parameter estimation was performed using maximum likelihood (ML) with bootstrapped standard errors and bias-corrected 95% confidence intervals based on 5000 resamples to ensure robustness against non-normality. Model adequacy was evaluated using conventional fit indices, Comparative Fit Index (CFI), Tucker–Lewis Index (TLI), Root Mean Square Error of Approximation (RMSEA), and Standardized Root Mean Square Residual (SRMR), with acceptable fit defined as CFI and TLI $\geq$0.90 and RMSEA and SRMR $\leq$0.08. When models were just-identified (degrees of freedom = 0), fit indices could not be interpreted. In such cases, inference was based on parameter estimates, their bootstrap confidence intervals, and information criteria (Akaike Information Criterion [AIC] and Bayesian Information Criterion [BIC]) for comparative model evaluation.

3. Results

3.1. Descriptive statistics

In this study, we analyzed a subset (N = 691) of the Lifelines-DEEP cohort. Among participants, 42.3% were male and 57.5% were female. Mean age of participants was 44.8 years, with a range of 18–80 years. 17.5% of the participants were smokers. According to the Adult Treatment Panel III criteria [64], individuals defined as metabolically unhealthy (MU) were 248 over 443 individuals metabolically healthy (MH). As for body composition, 12.7% (N = 88) had a BMI>30, while 31.5% (N = 218) were affected by central obesity considering waist circumference (WC) as criterion (i.e., WC > 102 cm for men or WC > 88 cm for women). By crossing these two variables, 133 individuals having a BMI<30 were classified as affected by central obesity according to the WC. Considering WC as criterion to define central obesity, 103 subjects were classified as affected by central obesity but metabolically healthy (MHO), while 133 were lean but metabolically unhealthy (MUL). 340 subjects were lean and healthy (MHL), while 115 were both affected by central obesity and metabolically unhealthy (MUO).

Parameters defining LGI and circulating adipokines in this cohort are shown in Table 1. Correlations between different measures of LGI are shown in Supplementary Materials Fig. 2. While all parameters were significantly correlated, none demonstrated complete concordance across all others. Among these, INFLA-score emerged as the biomarker that most comprehensively summarizes LGI in our dataset. From a statistical perspective, INFLA-score showed the strongest and most consistent correlations across the panel of inflammatory markers, with correlation coefficients ranging from r = 0.596 to r = 0.805 for single immune-cell ratios (NLR, NHR, SII, NEER) and r = 0.613 for hs-CRP, all with extremely small p-values ($p < 10^{-60}$), indicating robust and stable associations. Importantly, INFLA-score also correlated in the expected direction with adipokines, positively with leptin (r = 0.327) and resistin (r = 0.289), and negatively with adiponectin (r = −0.119), demonstrating that it captures not only immune-cell derived inflammation but also metabolic–inflammatory pathways. Additional descriptive statistics related to metabolic characteristics and blood composition of the cohort are provided in Supplementary Materials Table 2.

Regarding EAA, we computed epigenetic age estimates from raw DNA methylation data using the PCPhenoAge, PCGrimAge, and DunedinPACE clocks for the 691 participants included in the analysis, and subsequently derived EAA measures from these estimates. All clocks and corresponding EAA measures were successfully obtained. Descriptive correlations with chronological age, as well as pairwise correlations among the different biological age measures, were then assessed and are presented in Supplementary Fig. 3. Chronological age showed strong positive correlations with the epigenetic age estimates mAge_PCPhenoAge (r = 0.897, p < 0.001) and mAge_PCGrimAge (r = 0.955, p < 0.001), indicating a close agreement between these biomarkers and chronological aging. A more moderate but still significant association was observed between chronological age and mAge_DunedinPACE (r = 0.286, p < 0.001), consistent with its interpretation as a measure of the pace of aging rather than accumulated age. Additionally, mAge_PCPhenoAge and mAge_PCGrimAge were highly correlated with each other (r = 0.923, p < 0.001), while both showed moderate correlations with mAge_DunedinPACE (r = 0.431, p < 0.001), suggesting that these metrics capture overlapping but distinct aspects of biological aging.

Table 1
Descriptive statistics of biomarkers of LGI and adipokines in the cohort analyzed. Min = minimum; Max = maximum; SD = standard deviation.

	N	Min	Max	Mean	SD
hs-CRP (mg/L)	609	0.20	9.90	2.19	1.75
INFLA-score	609	−15.00	16.00	−0.23	6.11
NEER	691	0.23	1.93	0.69	0.23
SII	691	73.78	1560.32	401.42	195.99
NHR	691	0.46	8.15	2.29	1.16
NLR	691	0.50	7.98	1.67	0.69
Adiponectin (μg/mL)	644	0.2	14.4	4.05	2.06
Resistin (ng/mL)	644	5	53	15.64	6.06
Leptin (ng/mL)	644	0.8	100.00	16.35	17.01

3.2. Association between diet and LGI markers and circulating adipokines

Association with LGI metrics was tested for the intake of food groups previously linked to the pro- or anti-inflammatory potential of diet according to the EDIP score categories (EDIP_variables). Descriptive statistics for the EDIP_variables are shown in Supplementary Materials Table 3. Due to missing data and a limited number of cases successfully calculated for refined grains (N = 285) and tomatoes (N = 175), these specific EDIP_variables were excluded from subsequent analyses. The EDIP_variables were categorized into quartiles to account for non-parametric distributions, and mean differences between quartiles for each variable were analyzed for various indices of LGI (Supplementary Materials Table 4). Among EDIP food categories, the most pronounced and consistent differences in LGI biomarkers across intake quartiles were observed for tea, beer, and HEB, each showing statistically significant differences in more than three inflammatory markers (Supplementary Table 4). In particular, HEB displayed the clearest pattern of quartile differences, with highly significant effects for NHR (adj-p = 5.1×10^{-7}), NEER (adj-p = 0.004), and INFLA-score (adj-p = 0.049), and an additional difference observed for adiponectin (adj-p = 0.049). Beer intake also showed strong evidence of quartile differences, particularly for leptin (adj-p = 8.1×10^{-24}), adiponectin (adj-p = 2.7×10^{-5}), and NLR (adj-p = 0.037). Tea consumption demonstrated a comparable multi-marker profile, with significant differences across quartiles for leptin (adj-p = 1.8×10^{-4}), adiponectin (adj-p = 0.013), resistin (adj-p = 0.049), and NHR (adj-p = 0.008). Full detailed results of all EDIP–LGI quartile comparisons are reported in Supplementary Table 4. To validate and further investigate the association between food categories and LGI controlling for confounding factors, we used robust linear regression models (adjusted for age, sex,

Table 2
Associations between LGI markers and different measure of EAA tested by robust linear regression adjusted for sex, age, WHR, and smoking status. Effect sizes are reported as raw and standardized beta coefficients (Raw β; Std β). P-values were corrected for multiple testing using the Benjamini–Hochberg procedure (Adj-p).

EAA	LGI descriptor	Raw β	Std β	SE	Statistic	p	Adj-p
PCPhenoage	lnAdiponectin (μg/mL)	−0.464	−0.049	0.411	−1.128	0.260	0.270
PCPhenoage	INFLA-score	0.261	0.335	0.031	8.476	1.9E-16	**1.0E-15**
PCPhenoage	lnLeptin (ng/mL)	0.988	0.209	0.255	3.874	1.2E-04	**1.9E-04**
PCPhenoage	lnResistin (ng/mL)	1.899	0.141	0.568	3.342	0.001	**0.001**
PCPhenoage	lnhs-CRP (mg/L)	0.816	0.148	0.228	3.571	3.8E-04	**0.001**
PCPhenoage	NEER	8.105	0.392	0.767	10.571	2.9E-24	**4.0E-23**
PCPhenoage	SII	0.012	0.469	0.001	11.687	7.6E-29	**2.1E-27**
PCPhenoage	NHR	1.419	0.334	0.167	8.513	1.1E-16	**7.5E-16**
PCPhenoage	NLR	3.916	0.542	0.439	8.924	4.3E-18	**3.9E-17**
PCGrimage	lnAdiponectin (μg/mL)	−0.123	−0.021	0.208	−0.592	0.554	0.554
PCGrimage	INFLA-score	0.098	0.202	0.017	5.677	2.2E-08	**3.9E-08**
PCGrimage	lnLeptin (ng/mL)	0.191	0.066	0.142	1.347	0.178	0.193
PCGrimage	lnResistin (ng/mL)	0.805	0.097	0.271	2.973	0.003	**0.004**
PCGrimage	lnhs-CRP (mg/L)	0.303	0.090	0.114	2.658	0.008	**0.009**
PCGrimage	NEER	3.611	0.280	0.456	7.922	9.7E-15	**3.3E-14**
PCGrimage	SII	0.004	0.278	0.001	8.289	6.2E-16	**2.8E-15**
PCGrimage	NHR	0.671	0.252	0.094	7.130	2.6E-12	**7.1E-12**
PCGrimage	NLR	1.527	0.338	0.214	7.137	2.5E-12	**7.1E-12**
DunedinPACE	lnAdiponectin (μg/mL)	−0.025	−0.124	0.009	−2.673	0.008	**0.009**
DunedinPACE	INFLA-score	0.004	0.231	0.001	5.761	1.3E-08	**2.6E-08**
DunedinPACE	lnLeptin (ng/mL)	0.036	0.353	0.005	6.829	2.0E-11	**4.3E-11**
DunedinPACE	lnResistin (ng/mL)	0.028	0.096	0.012	2.309	0.021	**0.024**
DunedinPACE	lnhs-CRP (mg/L)	0.032	0.272	0.005	7.048	4.9E-12	**1.2E-11**
DunedinPACE	NEER	0.121	0.270	0.018	6.818	2.1E-11	**4.3E-11**
DunedinPACE	SII	9.8E-05	0.178	2.5E-05	3.934	9.2E-05	**1.6E-04**
DunedinPACE	NHR	0.028	0.302	0.003	7.993	5.8E-15	**2.2E-14**
DunedinPACE	NLR	0.034	0.214	0.010	3.488	0.001	**0.001**

Table 3
Associations between different dietary features and EAA resulting from robust linear regression models adjusted for sex, age, WHR, caloric surplus and smoking. Effect sizes are reported as raw and standardized beta coefficients (Raw β; Std β). P-values were corrected for multiple testing using the Benjamini–Hochberg procedure (Adj-p).

EAA	Diet feature	Raw β	Std β	SE	statistics	p	Adj-p
PCPhenoage	LLDS	−0.058	−0.069	0.034	−1.728	0.084	0.147
PCPhenoage	DHD index score	−0.035	−0.104	0.013	−2.606	0.009	**0.034**
PCPhenoage	sqrtNOVA1	−0.083	−0.086	0.047	−1.769	0.077	0.142
PCPhenoage	sqrtNOVA4	0.172	0.099	0.068	2.531	0.012	**0.038**
PCPhenoage	Processed meat (DHD score)	0.007	0.004	0.069	0.098	0.922	0.922
PCPhenoage	sqrtHEB (EDIP_portion/day)	0.659	0.078	0.333	1.981	0.048	0.106
PCPhenoage	sqrtLEB (EDIP_portion/day)	0.414	0.028	0.511	0.810	0.418	0.482
PCPhenoage	sqrtTea (EDIP_servings/day)	−0.179	−0.025	0.302	−0.593	0.554	0.571
PCPhenoage	Vegetable intake (DHD score)	−0.088	−0.047	0.070	−1.255	0.210	0.289
PCPhenoage	sqrtCoffee (EDIP_portion/day)	0.250	0.038	0.313	0.800	0.424	0.482
PCPhenoage	Red meat intake (DHD score)	0.283	0.067	0.184	1.540	0.124	0.195
PCGrimage	LLDS	−0.053	−0.101	0.017	−3.082	0.002	**0.015**
PCGrimage	DHD index score	−0.023	−0.113	0.007	−3.554	0.000	**0.007**
PCGrimage	sqrtNOVA1	−0.049	−0.080	0.024	−2.070	0.039	0.092
PCGrimage	sqrtNOVA4	0.100	0.092	0.034	2.914	0.004	**0.017**
PCGrimage	Processed meat (DHD score)	−0.044	−0.044	0.034	−1.304	0.193	0.277
PCGrimage	sqrtHEB (EDIP_portion/day)	0.530	0.100	0.175	3.033	0.003	**0.015**
PCGrimage	sqrtLEB (EDIP_portion/day)	0.172	0.019	0.268	0.640	0.522	0.556
PCGrimage	sqrtTea (EDIP_servings/day)	−0.337	−0.076	0.148	−2.283	0.023	**0.058**
PCGrimage	Vegetable intake (DHD score)	−0.113	−0.097	0.037	−3.051	0.002	**0.015**
PCGrimage	sqrtCoffee (EDIP_portion/day)	0.265	0.065	0.138	1.924	0.055	0.113
PCGrimage	Red meat intake (DHD score)	0.123	0.046	0.074	1.663	0.097	0.160
DunedinPACE	LLDS	−0.002	−0.092	0.001	−2.278	0.023	**0.058**
DunedinPACE	DHD index score	−0.001	−0.111	0.000	−2.999	0.003	**0.015**
DunedinPACE	sqrtNOVA1	−0.001	−0.044	0.001	−0.863	0.389	0.475
DunedinPACE	sqrtNOVA4	0.004	0.100	0.001	2.677	0.008	**0.031**
DunedinPACE	Processed meat (DHD score)	−0.001	−0.026	0.001	−0.663	0.508	0.556
DunedinPACE	sqrtHEB (EDIP_portion/day)	0.030	0.161	0.007	4.049	0.000	**0.002**
DunedinPACE	sqrtLEB (EDIP_portion/day)	0.016	0.051	0.011	1.509	0.132	0.198
DunedinPACE	sqrtTea (EDIP_servings/day)	−0.011	−0.074	0.006	−1.851	0.065	0.125
DunedinPACE	Vegetable intake (DHD score)	−0.004	−0.088	0.001	−2.443	0.015	**0.045**
DunedinPACE	sqrtCoffee (EDIP_portion/day)	0.007	0.047	0.006	1.165	0.245	0.323
DunedinPACE	Red meat intake (DHD score)	−0.003	−0.031	0.003	−0.909	0.364	0.462

smoking and WHR) to examine the association between LGI and dietary parameters commonly linked to the pro- or anti-inflammatory properties of the diet described as continuous variables. The variables examined included indicators of overall diet quality (LLDS and DHD index), intake of specific food categories (vegetables, unprocessed and ultraprocessed foods as

Table 4
Mediation analysis testing the impact of overall diet quality on EAA measured by different clocks, accounting for the mediation of INFLA-score and adjusting the models for sex, age, WHR, smoking status and energy surplus. SE: Standard Error; Z: z-score; p: p value; CI: confidence interval.

Model	Effect	Estimate	SE	z	p	CI low	CI up
LLSD - PCPhenoage	Indirect	−0.073	0.015	−4.862	**1.12e-06**	−0.103	−0.045
LLSD - PCPhenoage	Direct	−0.004	0.033	−0.117	0.906	−0.070	0.062
LLSD - PCPhenoage	Total	−0.076	0.035	−2.208	**0.027**	−0.145	−0.010
LLDS - PCGrimage	Indirect	−0.035	0.008	−4.533	**5.8e-06**	−0.051	−0.021
LLDS - PCGrimage	Direct	−0.046	0.019	−2.292	**0.021**	−0.086	−0.007
LLDS - PCGrimage	Total	−0.081	0.020	−3.936	**8.3e-05**	−0.122	−0.040
LLDS - DunedinPACE	Indirect	−0.0009	0.00023	−3.880	**1.0e-04**	−0.001	−5.0e-04
LLDS - DunedinPACE	Direct	−0.0001	0.00076	−0.157	0.874	−0.001	0.001
LLDS - DunedinPACE	Total	−0.0010	0.00073	−1.418	0.156	−0.002	−4.0e-04
DHD - PCPhenoage	Indirect	−0.024	0.005	−4.506	**6.6e-06**	−0.035	−0.014
DHD - PCPhenoage	Direct	−0.015	0.013	−1.109	0.267	−0.040	0.011
DHD - PCPhenoage	Total	−0.039	0.014	−2.807	**0.005**	−0.065	−0.011
DHD - PCGrimage	Indirect	−0.008	0.002	−3.638	**2.7e-04**	−0.013	−0.004
DHD - PCGrimage	Direct	−0.015	0.007	−2.159	**0.031**	−0.029	−0.001
DHD - PCGrimage	Total	−0.024	0.007	−3.308	**0.001**	−0.038	−0.010
DHD - DunedinPACE	Indirect	−3.1e-04	8.5e-05	−3.670	**2.4e-04**	−4.9e-04	−1.6e-04
DHD - DunedinPACE	Direct	−3.0e-04	2.8e-04	−1.094	0.274	−0.001	2.5e-04
DHD - DunedinPACE	Total	−0.001	2.7e-04	−2.244	**0.025**	−0.001	−7.1e-05

defined by NOVA classification, HEB, LEB, red meat, processed meat, wine, coffee, and tea), as well as selected nutrient intakes (EPA + DHA, lipid quality, carbohydrate quality, alcohol, and vitamins B9 and C). Full results of the adjusted regression models are reported in Fig. 1. The heatmap revealed a heterogeneous but biologically coherent pattern. The heatmap revealed a heterogeneous but biologically coherent pattern of associations between dietary factors and LGI. Intake of NOVA4 group was positively associated with multiple LGI markers, including INFLA-score (Std $\beta = 0.200$; adj-p $= 1.2 \times 10^{-4}$), NEER (Std $\beta = 0.190$; adj-p < 0.002), NHR (Std $\beta = 0.140$; adj-p < 0.002), and circulating leptin (Std $\beta = 0.160$; adj-p $= 5 \times 10^{-5}$). In particular, HEB intake was positively associated with INFLA-score (Std $\beta = 0.150$; adj-p $= 0.0036$), leptin (Std $\beta = 0.110$; adj-p < 0.003), and NHR (Std $\beta = 0.100$; adj-p < 0.01). LEB intake also showed positive associations with INFLA-score (Std $\beta = 0.140$; adj-p $= 0.0035$), NEER (Std $\beta = 0.140$; adj-p < 0.01), and NHR (Std $\beta = 0.110$; adj-p < 0.01). In contrast, higher adherence to dietary guidelines, as captured by the LLDS and the DHD index, was associated with lower INFLA-score (Std $\beta = -0.210$; adj-p $< 4 \times 10^{-5}$), NEER (Std $\beta = -0.160$; adj-p $< 5 \times 10^{-5}$), hs-CRP (Std $\beta = -0.130$ to -0.140; adj-p < 0.005), and leptin (Std $\beta \approx -0.120$; adj-p < 0.01). Finally, selected micronutrients involved in one-carbon metabolism were also inversely associated with inflammatory markers. Vitamin B9 and B2 were inversely associated with INFLA-score (Std $\beta = -0.280$ and -0.270, respectively; both adj-p $< 1 \times 10^{-4}$), NEER (Std $\beta \approx -0.180$ to -0.200; adj-p < 0.001), and hs-CRP (Std $\beta \approx -0.150$; adj-p < 0.01). Pyridoxine (vitamin B6) also showed a modest inverse association with INFLA-score (Std $\beta = -0.160$; adj-p $= 0.044$). Among all LGI biomarkers, INFLA-score exhibited the most consistent and multi-domain associations with dietary exposures, showing significant relationships with several independent dietary factors. Summary metrics for each LGI biomarker were derived from robust linear regression models relating dietary exposures to inflammatory outcomes, with the aim of identifying which LGI biomarker most consistently captured associations with dietary features (Supplementary Materials Table 5). Based on these comparative metrics, the INFLA-score showed a strong and consistent overall performance, with 11 significant associations (FDR <0.05), a high cumulative $-\log_{10}$(adjusted p-value) ($\Sigma = 31.276$), the largest mean absolute standardized effect size (mean $|\text{Std } \beta| = 0.124$), and a highly significant Fisher global p-value ($p = 8.8 \times 10^{-29}$). While some individual biomarkers exhibited a similar or higher number of significant associations, they were characterized by smaller average standardized effect sizes and/or lower cumulative statistical evidence. Based on this multi-metric comparison, the INFLA-score was selected as the primary indicator of diet-related LGI for subsequent analyses.

3.3. Epigenetic age acceleration, LGI and blood composition

Associations between EAA and LGI markers were assessed using robust linear regression models adjusted for sex, age, waist-to-hip ratio, and smoking. Results are reported in Table 2 and revealed consistent associations across all three EAA measures. PCPhenoAge displayed the strongest overall associations, with the largest effect sizes observed for NLR (Std $\beta = 0.542$; adj-p $= 3.9 \times 10^{-17}$), SII (Std $\beta = 0.469$; adj-p $= 2.1 \times 10^{-27}$), and NEER (Std $\beta = 0.392$; adj-p $= 4.0 \times 10^{-23}$), alongside robust associations with INFLA-score (Std $\beta = 0.335$; adj-p $= 1.0 \times 10^{-15}$) and NHR (Std $\beta = 0.334$; adj-p $= 7.5 \times 10^{-16}$). PCGrimAge showed a similar profile, with its strongest associations observed for NLR (Std $\beta = 0.338$; adj-p $= 7.1 \times 10^{-12}$), NEER (Std $\beta = 0.280$; adj-p $= 3.3 \times 10^{-14}$), and SII (Std $\beta = 0.278$; adj-p $= 2.8 \times 10^{-15}$), together with significant associations for NHR (Std $\beta = 0.252$; adj-p $= 7.1 \times 10^{-12}$) and INFLA-score (Std $\beta = 0.202$; adj-p $= 3.9 \times 10^{-8}$). DunedinPACE exhibited a partially distinct pattern, with the strongest associations involving leptin (Std $\beta = 0.353$; adj-p $= 4.3 \times 10^{-11}$), NHR (Std $\beta = 0.302$; adj-p $= 2.2 \times 10^{-14}$), hs-CRP (Std $\beta = 0.272$; adj-p $= 1.2 \times 10^{-11}$), and NEER (Std $\beta = 0.270$; adj-p $= 4.3 \times 10^{-11}$), as well as a robust association with INFLA-score (Std $\beta = 0.231$; adj-p $= 2.6 \times 10^{-8}$).

After additional adjustment for blood cell composition (Supplementary Materials Table 6), the associations between EAA and LGI markers that are independent of blood composition, namely hs-CRP, leptin, adiponectin, and resistin, were largely confirmed. Importantly, INFLA-score remained significantly associated with all three epigenetic clocks (PCPhenoAge: Std $\beta = 0.519$, adj-p $= 1.7 \times 10^{-17}$; PCGrimAge: Std $\beta = 0.256$, adj-p $= 1.1 \times 10^{-6}$; DunedinPACE: Std $\beta = 0.280$, adj-p $= 4.6 \times 10^{-6}$), indicating that the relationship between systemic LGI and biological aging is robust to adjustment for circulating immune cell distributions.

3.4. Epigenetic age acceleration and LGI in different metabolic status

EAA values were compared across groups defined by metabolic status (Healthy, MUL, MHO, MUO) using Dunn's post-hoc tests

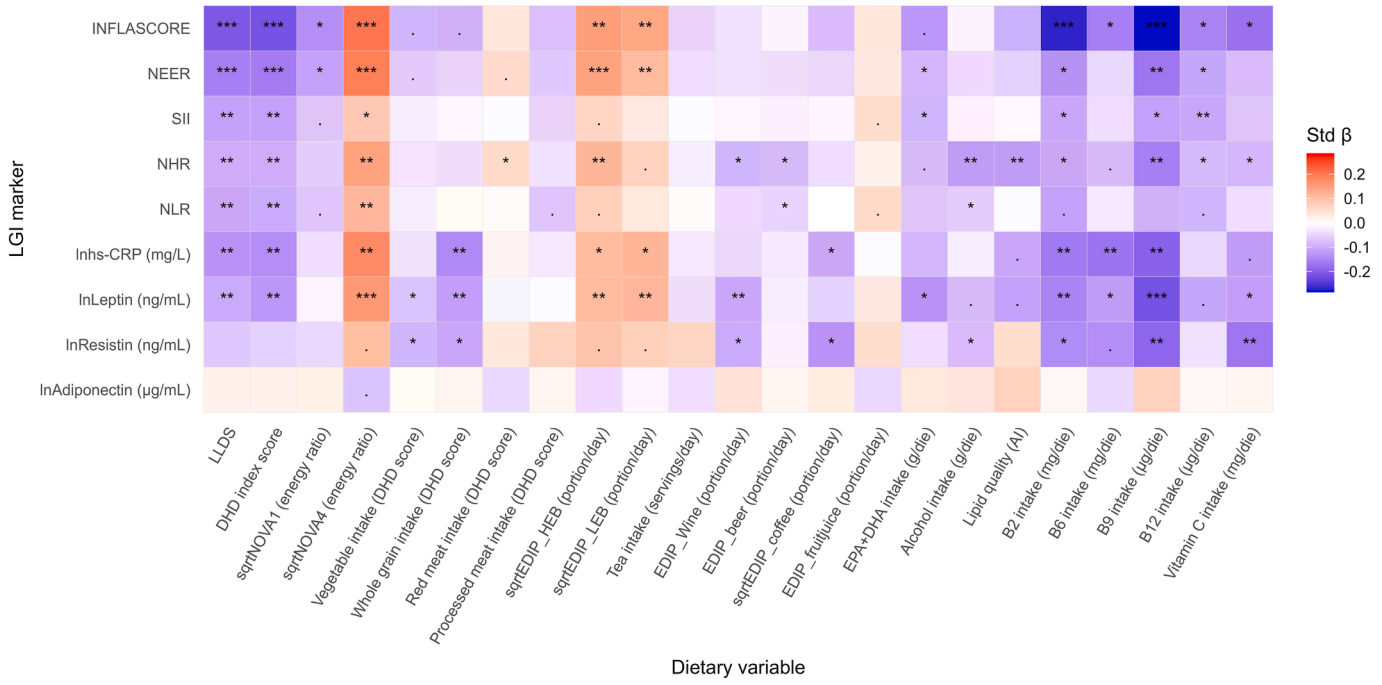

Fig. 1. Heatmap summarizing the associations between LGI markers and dietary parameters, based on robust linear regression models adjusted for age, sex, smoking status, and WHR. The dietary variables include components commonly linked to the pro- or anti-inflammatory properties of the diet, as well as EDIP-derived food categories identified as significant in prior analyses. Colours represent standardized beta coefficients (Std β), and BH-adjusted p-values are indicated by significance symbols within each cell.

following Kruskal–Wallis analysis. While PCPhenoAge and PCGrimAge showed only modest discrimination across metabolic groups (Figure 2A and B), clear differences emerged when EAA was assessed using the DunedinPACE clock (Fig. 2C). In particular, DunedinPACE values were significantly higher in all metabolically altered groups compared with Healthy individuals, with increases of approximately 3.3% in MUL ($p = 0.007$), 5.1% in MHO ($p = 3.3 \times 10^{-4}$), and 7.1% in MUO ($p = 3.6 \times 10^{-8}$) relative to healthy controls (Fig. 2C, overall $p = 3.1 \times 10^{-9}$). No EAA measure differed significantly between MHO and MUO groups across any of the clocks, indicating that these epigenetic clocks did not detect significant differences in the epigenetic aging process between these two metabolic phenotypes in this cohort. To assess whether LGI varied according to metabolic status, we compared INFLA-score across the same groups (Fig. 2D, overall $p = 2.3 \times 10^{-7}$). Compared with healthy individuals, INFLA-score values were significantly higher in both MHO and MUO participants (mean differences = +3.35 and + 3.20 units, respectively; $p = 2.4 \times 10^{-5}$ and $p = 3.9 \times 10^{-5}$), whereas no significant difference was detected between Healthy and MUL subjects ($p > 0.05$). INFLA-score was also higher in MHO than in MUL ($p = 0.045$), with a trend toward higher values in MUO compared with MUL ($p = 0.066$), while no difference emerged between MHO and MUO ($p > 0.05$). Notably, DunedinPACE was the only biomarker capable of discriminating all metabolically altered groups (MUL, MHO, MUO) from healthy participants, indicating a sensitivity to metabolic dysregulation that was not observed for the LGI score or for the other epigenetic clocks.

3.5. Diet pro- or anti-inflammatory features and epigenetic age acceleration

Dividing the population into quartiles for each EDIP variable (portion/day) revealed significant differences in EAA distribution across groups, especially for PCGrimage (Supplementary Materials Table 7). This analysis confirms that PCGrimage is the most sensitive among the selected clocks to detect associations between EAA and EDIP food categories (i.e., processed meat, organ meat, dark yellow and green leafy vegetables, coffee, tea, HEB and beer). Notably, HEB intake was the only EDIP category associated with EAA across all three clocks (PCGrimAge, PCPhenoAge, and DunedinPACE). Supplementary Fig. 4 illustrates the differences in PCGrimage across quartiles for the variables that showed significant associations.

For each dietary feature previously linked to inflammatory responses, we fitted a dedicated robust linear regression model adjusted for age, sex, smoking status, WHR, and caloric surplus (Table 3). Results indicate that the association between EAA and several dietary features within this cohort (measured with first-generation clocks in a previous study [76]) is confirmed even when using second-generation clocks and PC versions, particularly for overall diet quality measures and for ultra-processed food intake (NOVA4), including HEB. Across epigenetic clocks, higher intake of NOVA4 foods was consistently associated with higher EAA, with significant positive associations observed for PCPhenoAge (raw $\beta = 0.172$; Std $\beta = 0.099$; adj-p = 0.038), PCGrimAge (raw $\beta = 0.100$; Std $\beta = 0.092$; adj-p = 0.017), and DunedinPACE (raw $\beta = 0.004$; Std $\beta = 0.100$; adj-p = 0.031). This pattern was particularly evident for HEB, which showed robust positive associations with PCGrimAge (raw $\beta = 0.530$; Std $\beta = 0.100$; adj-p = 0.015) and DunedinPACE (raw $\beta = 0.030$; Std $\beta = 0.161$; adj-p = 0.002), whereas the corresponding association with PCPhenoAge did not remain significant after multiple-testing correction. Among the clocks, PCGrimAge exhibited the most comprehensive dietary association profile, showing significant inverse associations with overall diet quality (LLDS: raw $\beta = -0.053$; Std $\beta = -0.101$; adj-p = 0.015; DHD index: raw $\beta = -0.023$; Std $\beta = -0.113$; adj-p = 0.007) and vegetable intake (raw $\beta = -0.113$; Std $\beta = -0.097$; adj-p = 0.015), alongside positive associations with NOVA4 and HEB intake. Two additional inverse associations

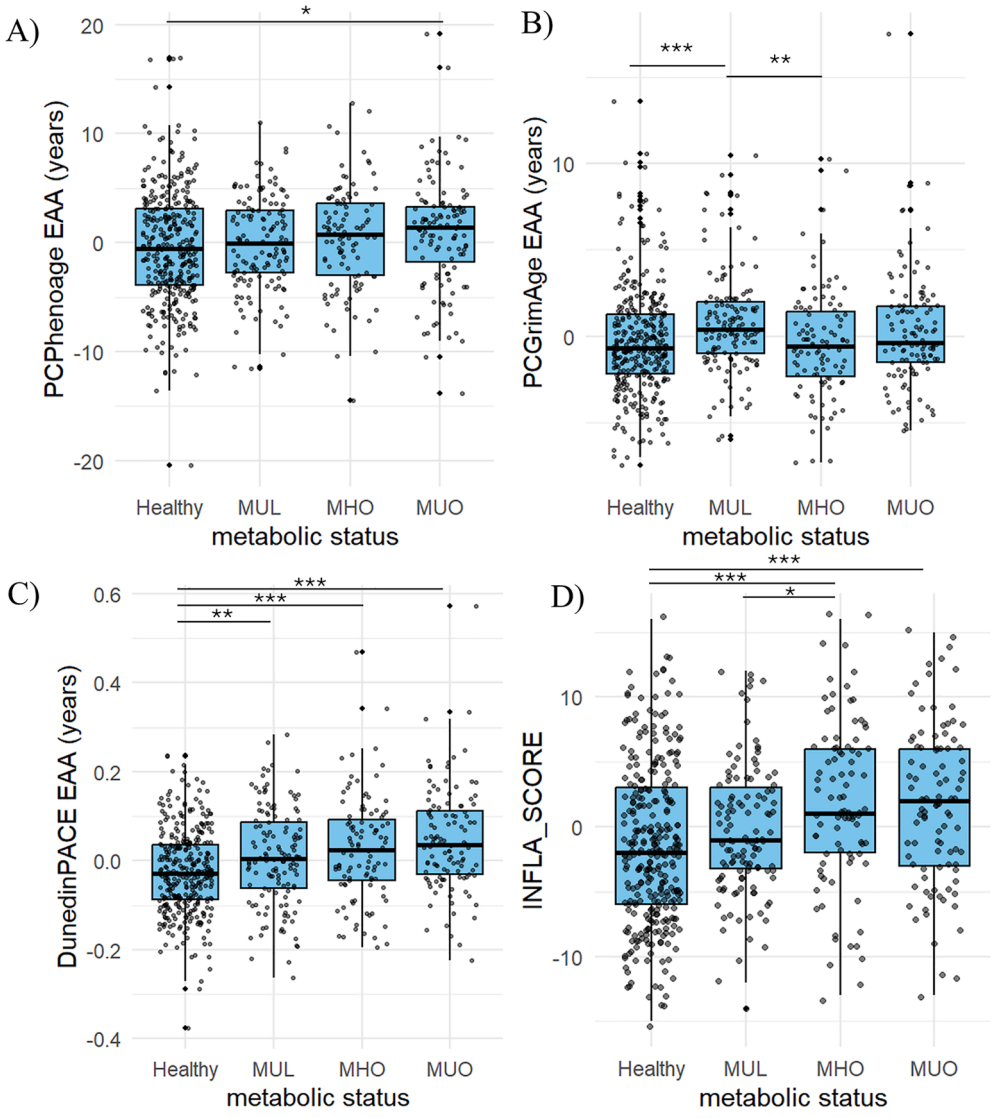

Fig. 2. Differences in EAA measured by PCPhenoage (A), PCGrimage (B), DunedinPACE (C) or INFLA-score (D) in different metabolic health status. Healthy (MHL), N = 340; Metabolically Unhealthy Lean (MUL), N = 133; Metabolically Healthy Obese (MHO), N = 103; Metabolically Unhealthy Obese (MUO), N = 115. *p < 0.05; **p < 0.01; ***p < 0.001.

were observed for NOVA1 and tea consumption that were statistically significant at the nominal level (sqrtNOVA1: p = 0.039; tea: p = 0.023), although they showed only a trend after FDR correction (adj-p = 0.092 and adj-p = 0.058, respectively). DunedinPACE also showed significant associations with overall diet quality as assessed by the DHD index (Std β = −0.111; adj-p = 0.015), vegetable intake (Std β = −0.088; adj-p = 0.045), NOVA4 intake (Std β = 0.100; adj-p = 0.031), and HEB intake (Std β = 0.161; adj-p = 0.002), whereas the association with LLDS was only nominal (adj-p = 0.058). In contrast, PCPhenoAge displayed fewer and weaker associations, with diet quality assessed by the DHD index (Std β = −0.104; adj-p = 0.034) and NOVA4 intake (Std β = 0.099; adj-p = 0.038) emerging as the most consistent correlates. Full details are provided in Table 3.

Given the strong associations observed for PCGrimAge and its known sensitivity to smoking-related methylation signatures, we conducted a sensitivity analysis restricted to non-smokers to further assess potential residual confounding by smoking that cannot be captured by considering the smoking status as covariate. This approach was adopted because quantitative smoking data (number of cigarettes per day) were available for only 108 participants, which was insufficient for stable dose–response modelling. Results showed that associations observed in the full cohort were largely confirmed in the non-smoker subgroup (Supplementary Materials Table 8), indicating that residual confounding by smoking is unlikely to be a major driver of the observed relationships. Although residual confounding by smoking in the full cohort cannot be completely excluded, this risk is expected to be minimal because smoking status captures the major behavioural distinction relevant for confounding control, and the main findings were confirmed in the sensitivity analysis restricted to non-smokers.

Finally, in light of the significant associations observed between multiple dietary features and PCGrimAge, we extended the analysis for this clock to additional dietary components with known pro- or anti-inflammatory properties, as well as to key dietary intermediates of one-carbon metabolism, whose intake may be relevant to epigenetic aging. As shown in Supplementary Materials Table 9, vitamin B9 and vitamin C intake were inversely associated with PCGrimAge EAA. Specifically, higher vitamin B9 intake was associated with lower PCGrimAge acceleration (raw β = −0.006; standardized β = −0.156; p = 0.006; adj-p = 0.028), and a similar

Table 5
Mediation analysis testing the impact of different dietary features on EAA measured by PCGrimage accounting for the mediation of INFLA-score adjusting the models for sex, age, WHR, smoking status and energy surplus. SE: Standard Error; Z: z-score; p: p value; CI: confidence interval.

Dietary feature	Effect	Estimate	SE	z	p	CI_lower	CI_upper
NOVA1 (energy ratio)	Indirect	0.041	0.011	3.621	**2.9E-04**	0.019	0.063
NOVA1 (energy ratio)	Direct	0.041	0.038	1.060	0.289	−0.035	0.116
NOVA1 (energy ratio)	Total	0.082	0.039	2.118	**0.034**	0.006	0.158
NOVA4 (energy ratio)	Indirect	−0.024	0.007	−3.348	**0.001**	−0.039	−0.010
NOVA4 (energy ratio)	Direct	−0.030	0.026	−1.172	0.241	−0.081	0.020
NOVA4 (energy ratio)	Total	−0.055	0.026	−2.094	**0.036**	−0.106	−0.004
Vegetables intake (DHD score)	Indirect	−0.019	0.009	−2.011	**0.044**	−0.037	0.000
Vegetables intake (DHD score)	Direct	−0.122	0.038	−3.182	**0.001**	−0.197	−0.047
Vegetables intake (DHD score)	Total	−0.141	0.039	−3.594	**3.3E-04**	−0.218	−0.064
HEB (EDIP_portion/day)	Indirect	0.174	0.052	3.307	**0.001**	0.071	0.276
HEB (EDIP_portion/day)	Direct	0.219	0.191	1.148	0.251	−0.155	0.592
HEB (EDIP_portion/day)	Total	0.392	0.192	2.042	**0.041**	0.016	0.769
Tea (EDIP_portion/day)	Indirect	−0.061	0.038	−1.615	0.096	−0.135	0.013
Tea (EDIP_portion/day)	Direct	−0.409	0.157	−2.599	**0.009**	−0.717	−0.101
Tea (EDIP_portion/day)	Total	−0.470	0.161	−2.917	**0.004**	−0.785	−0.154
Vitamin C (mg/die)	Indirect	−0.001	0.001	−1.409	0.159	−0.003	0.000
Vitamin C (mg/die)	Direct	−0.010	0.003	−3.204	**0.001**	−0.016	−0.004
Vitamin C (mg/die)	Total	−0.011	0.003	−3.477	**0.001**	−0.017	−0.005
B9 intake (μg/die)	Indirect	−0.045	0.021	−2.152	**0.031**	−0.086	−0.004
B9 intake (μg/die)	Direct	−0.162	0.076	−2.132	**0.033**	−0.311	−0.013
B9 intake (μg/die)	Total	−0.207	0.077	−2.681	**0.007**	−0.358	−0.056

inverse association was observed for vitamin C (raw $\beta = -0.009$; standardized $\beta = -0.135$; $p = 0.005$; adj-$p = 0.027$). In contrast, no significant associations were detected for vitamins (B12, B2, B6), EPA + DHA intake, or alcohol consumption after correction for multiple testing (Supplementary Materials Table 9).

3.6. *Effect of low-grade inflammation in mediating the impact of diet on EAA: a mediation analysis*

Given the observed associations between dietary features and LGI markers, we next evaluated whether the relationship between diet quality and EAA could be explained, at least in part, by LGI. Because INFLA-score emerged as the most responsive and comprehensive LGI indicator in this cohort, integrating both circulating inflammatory biomarkers and blood cell composition and showing the most consistent associations with dietary features (Supplementary Table 5), it was selected as the LGI estimator to be used as the mediator in this analysis. We first tested whether INFLA-score mediated the association between EAA measured with different clocks and the two overall diet quality measures (LLDS and DHD) (Table 4). Mediation models showed significant direct effects only for PCGrimAge, for both LLDS (direct estimate $= -0.046$, $p = 0.021$) and DHD (direct estimate $= -0.015$, $p = 0.031$), resulting in significant total effects. In contrast, for PCPhenoAge and DunedinPACE, direct effects were not statistically significant, suggesting that the associations between diet quality and EAA for these clocks were largely explained by the indirect pathway through LGI. Complete mediation estimates, including effect sizes, confidence intervals, and statistical significance for all models, are reported in Table 4.

Since the PCGrimage was the most responsive to diet in previous analyses, we further tested in mediation analysis the association of EAA measured by PCGrimage with specific dietary features (i.e., NOVA1, NOVA4, HEB, vegetables intake, tea, vitamins B9 and C), considering INFLA-score as mediator. Results indicated a significant mediating effect of INFLA-score on the relationship between multiple dietary features and PCGrimAge EAA, as reflected by significant indirect effects across all tested dietary variables (Table 5). In particular, dietary components typically considered pro-inflammatory, such as NOVA4 energy ratios and HEB, showed significant indirect effects through INFLA-score (e.g., NOVA4 indirect effect $= -0.024$, $p = 0.001$; HEB indirect effect $= 0.174$, $p = 0.001$), while their direct effects on PCGrimAge were not statistically significant ($p > 0.05$). This pattern suggests that the associations of these dietary features with EAA were largely explained by their relationship with LGI. In contrast, several beneficial dietary components displayed significant direct associations on PCGrimAge that were independent of INFLA-score. In particular, vegetable intake showed both a significant indirect effect ($p = 0.044$) and a direct effect (estimate $= -0.122$, $p = 0.001$), resulting in a robust total association ($p = 3.3 \times 10^{-4}$). Similarly, tea intake, vitamin C, and B9 exhibited significant direct effects on PCGrimAge (tea: $p = 0.009$; vitamin C: $p = 0.001$; vitamin B9: $p = 0.033$). Overall, these findings indicate that both pro- and anti-inflammatory dietary features relate to EAA through their association with LGI, but that beneficial dietary components rich in vitamins and bioactive compounds also show direct associations with PCGrimAge beyond LGI mediation. Complete mediation estimates, including indirect, direct, and total effects with confidence intervals, are reported in Table 5.

4. Discussion

LGI and metabolic health critically influence complex disease development. LGI both results from and exacerbates metabolic dysregulation, and it represents a core feature of inflammaging, a consequence of intricate immune interactions involving cascades and feedback loops [113]. Given the chronic and multifactorial nature of these processes, addressing LGI is considered a promising strategy for disease prevention and promotion of healthy aging. Lifestyle modifications have been proposed as practical approaches to control LGI and improve metabolic health, thereby supporting healthy aging. Among these, diet represents a key modifiable factor, acting as a source of food-derived signals relevant to biological aging within a systems and precision nutrition framework [114]. Evidence from a large prospective cohort of US women and men followed for 30 years has recently strengthened the evidence linking diet to healthy aging [41]. Tessier and colleagues have showed that higher intakes of fruits, whole grains, vegetables, added unsaturated fats, nuts, legumes, and low-fat

dairy were associated with greater odds of healthy aging, whereas higher intakes of trans fats, sodium, total meats, and red and processed meats were associated with lower odds [41].

The ability to track the impact of diet on the aging process provides a promising approach to evaluate the effectiveness of nutritional interventions and monitor progress. In this context, emerging evidence suggests that epigenetic clocks may serve as promising tools to monitor the biological impact of dietary changes on aging and health. Data corroborating the hypothesis that dietary intervention can shape EAA has been growing [115–120]. For a comprehensive review on the impact of nutrition strategies on DNAm, including global DNAm and epigenetic clocks, see García-García et al. [121]. Our previous findings from the Lifelines-DEEP cohort identified a significant link between certain dietary factors and first-generation aging clocks [76]. In the present study, we extended the investigation to second- and third-generation clocks in their PC version to further validate the relationship between diet and these aging markers.

Our findings specifically confirmed the association between overall diet quality and EAA as measured by PCGrimage, that emerged as the more sensitive to dietary features among the analyzed clocks, based on the magnitude and consistency of statistically significant associations observed across multiple dietary features. Moreover, our results highlight several associations between EAA and food categories with proposed pro- or anti-inflammatory properties. These include HHB and foods classified under the NOVA4 category (which showed consistent positive associations across all the clocks) but also intake of vegetables, NOVA1 category, tea, folate and vitamin C which were inversely associated with PCGrimAge EAA in adjusted models. In this cohort, processed meat, red meat, and coffee were associated with the selected estimators of EAA when comparing quartiles of their intake; however, these associations lost significance in robust linear regression after adjusting for covariates, indicating that these relationships were not robust to multivariable adjustment. Effect sizes were small. Nevertheless, modest slowing of the pace of aging can have profound effects on population health [122,123], as discussed also by Waziri et al. [124]. We did not observe significant associations between EAA and fat quality, nor did we detect robust associations with omega-3 intake in multivariable models, and thus we were unable to further support findings from recent studies reporting an association between EAA and omega-3 supplementation [125,126]. However, our results are consistent with existing evidence demonstrating the beneficial effects of vegetable consumption and associated bioactive molecules and vitamins, such as folate and vitamin C, on EAA. Specifically, our findings align with studies reporting the positive impact of diets rich in raw and nutrient-dense foods on epigenetic aging, as shown in the randomized controlled trial DIRECT PLUS [127], that partly attributed the effect also to their high polyphenol content. The importance of adequate vitamin intake in mitigating EAA is reinforced by an additional trial, which measured a normalization of EAA in individuals receiving tHcy-lowering B-vitamin treatment (N = 107) compared to the placebo group (N = 110) [104]. Accordingly, Bozack and colleagues recently showed that serum folate concentration was associated to lower EAA in the NHANES cohort [103]. Our findings also support previous evidence showing that diets high in fast food, processed red meat, and HHB and low in fruits and vegetables are associated with accelerated biological aging even in young adulthood [118]. Recent evidence from an observational study has confirmed a direct association between the intake of various bioactive molecules and micronutrients and EAA in a cohort of 3969 postmenopausal women, utilizing a data-driven exploratory analysis approach [128]. An additional recent trial suggests a possible benefit of combined nutritional supplementation (including vitamin B3, C, D, omega 3 fish oils, and other bioactive molecules) especially in individuals with an accelerated epigenetic age and inflammaging [116]. All these findings are consistent with current knowledge indicating that food-derived macro- and micronutrients, along with dietary phytochemicals, play a crucial role in reducing oxidative stress and inflammation [129].

Notably, in this cohort, overall diet quality (measured by LLDS and DHD score), as well as specific food categories and nutritional components, were also associated with LGI levels, as demonstrated by statistically significant associations with several LGI biomarkers in multivariable models, particularly the composite INFLA-score. This was particularly evident for HHB, NOVA4 foods, folate and vitamin C intake, though not limited to these factors. Given that both epigenetic modifications and inflammation are key molecular mechanisms influencing the rate of aging [130], and that both can be affected by dietary habits [129,131–138], it is particularly interesting to consider how diet may modulate EAA by influencing LGI, which is also entangled with changes in blood composition. This is a clinically relevant question, as controlling LGI might represent a key strategy for protecting against complex diseases and promoting healthy aging. This hypothesis is supported by evidence from Sanchez-Cabo et al. reporting a mediatory role for proinflammatory pathways in association between Grimage EAA and subclinical atherosclerosis [139]. Key biomarkers for increased systemic inflammation, including an increase in total number of platelets and white blood cells, plasma C-reactive protein level, and neutrophil to lymphocyte ratio, were found to be strongly correlated with EAA. In parallel, previous evidence highlights the pivotal role of diet in regulating subclinical inflammation [140,141], a precursor to chronic diseases [23,56,142], which is also intrinsically connected to inflammaging [143]. Therefore, we investigated whether the impact of diet on PCGrimage EAA was primarily mediated by its effect on LGI levels using mediation analysis. Our results revealed that overall diet quality is associated with PCGrimage EAA through both direct and indirect pathways. Specifically, when examining individual dietary features, we found that the association between EAA and unhealthy dietary habits, such as the intake of HHB and NOVA4 foods, was largely accounted for by variation in the INFLA-score (used as an indicator of LGI), with negligible residual direct associations with EAA in this experimental setting. In contrast, beneficial dietary features, including the intake of vegetables, tea, and folate, were associated with lower EAA both in relation to lower INFLA-score values and through additional direct pathways independent from this marker. Our findings align with previous studies demonstrating association between epigenetic aging and inflammation, suggesting that inflammaging and EAA may represent two different biological processes, connected but not completely overlapped, that independently capture complementary aspects of overall risk [18].

Also, it should be noticed that a link between diet and blood cell composition exists, with findings indicating a significant mediating role in the beneficial effects of dietary patterns on non-communicable disease prevention. For example, recent evidence from the CARDIOPREV intervention study has highlighted the protective role of healthy dietary patterns, particularly the Mediterranean diet, in mitigating complex diseases like cardiovascular disease (CVD), potentially through modulation of neutrophil count [144]. In this study, adherence to a Mediterranean diet is associated with lower neutrophil levels and regression of atherosclerosis over time (decreased carotid intima-media thickness), underscoring a novel dietary strategy for atherosclerosis management via neutrophil-targeted interventions and suggesting a mechanistic pathway for CVD risk reduction. While dietary patterns are known to influence LGI and shape blood cell composition, our

findings indicate that these factors alone do not fully account for the observed associations with EAA.

Coming back to the relationship between diet and inflammation, our findings are in accordance with prior observations on a large cohort of 4510 adults from the Moli-Sani Study, where biological aging was estimated from 36 blood parameters. In this cohort, a high adherence to well-known anti-inflammatory dietary patterns (e.g., Mediterranean Diet and DASH) and dietary polyphenols consumption were associated with delayed biological aging, while diet pro-inflammatory potential was linked to an acceleration of this parameter [21,145,146]. Previous studies have also shown an association between EAA and elevated levels of inflammatory markers, including cytokines and molecules such as IL-6, CRP, TNF-α, IL-18, IL-18BP, and leptin. [106,147–149]. A recent study of 22 plasma-based inflammatory markers and four clocks (PhenoAge, GrimAge, DunedinPoAm, and Zhang) found associations between higher CRP and IL-6 and DNAm EAA, but other indicators of inflammation were inconsistent [19]. They also found that epigenetic aging and inflammaging were strongly and independently associated with mortality. Other authors, using data from the Health and Retirement Study (N = 3113), generated a continuous latent variable for systemic inflammation (from seven measured indicators of inflammation) which was positively associated with DNAm age acceleration for 10 of the 13 epigenetic clocks measured adjusting for covariates (adjusting for sociodemographics and chronic disease risk factors) [18]. However, they did not consider dietary habits in the analysis. Notably, the authors observed stronger associations for inflammation with clocks that were trained on mortality, mitotic division, or biomarkers of aging, including GrimAgeAA, than for clocks trained on chronological age or phenotypic aging. They suggest that systemic inflammation is likely an interdependent or co-occurring process with the factors used to train these clocks, causing the inflammation latent variable to explain a larger proportion of variance in these models. Our findings align with this observation and further suggest that LGI cannot fully explain the association between diet and EAA.

Indeed, our study also underscore the importance of leveraging multiple biomarkers to characterize the effect of diet on inflammaging in aging research. In our analysis, INFLA-score values were significantly higher in individuals with obesity (both MHO and MUO) compared with healthy participants, indicating a greater inflammatory burden in these groups. However, no significant differences were observed between healthy subjects and metabolically unhealthy lean (MUL) individuals, suggesting that INFLA-score may be more sensitive to adiposity-related inflammatory changes than to metabolic impairments occurring in the absence of excess fat mass. In contrast, DunedinPACE captured distinctions that were not detectable through inflammatory profiling alone. DunedinPACE values were higher not only in obese groups but also in MUL compared with healthy subjects, revealing sensitivity to metabolic dysregulation independent of adiposity and suggesting a faster pace of aging in these groups. This pattern aligns with previous evidence showing that DunedinPACE exhibits stronger associations with metabolic dysfunction, insulin resistance, and adiposity-related biological aging than other epigenetic clocks [150]. Overall, these findings suggest that epigenetic aging metrics (particularly DunedinPACE) may provide additional resolution beyond traditional inflammatory markers when distinguishing metabolic phenotypes, underscoring the potential value of combining inflammation-based and methylation-based biomarkers to more comprehensively assess metabolic health in aging research.

A key limitation of this study is the observational design, which prevents determining the directionality of the inflammation-EAA relationship. Additionally, EAA was linked to UPF intake using the NOVA classification, which has known limitations [151]. The deleterious effects of UPFs consumption on health can likely be ascribed to the increased presence of harmful components prevalent in foods categorized as ultra-processed, such as excessive calories, sugars, and saturated fats, among others. This attribution extends beyond the outcomes of the technological processes employed by the food industry alone. As a result, we refrain from directly linking industrial food processing *per se* to the acceleration of epigenetic or biological age. Nonetheless, our findings show that the consumption of foods classified under NOVA group 4 (especially HHB) exhibit an acceleration of this parameter. Considering that consumption of UPFs is reaching high prevalence all over the world, especially in USA [152], and given the association between UPFs intake and the risk of numerous complex pathologies [40,153–158], these findings underscore the urgent need for additional research to enable the translation of this evidence into actionable dietary recommendations for healthy aging.

In conclusion, this study extends previous evidence on diet and biological aging by showing that dietary patterns and specific food categories are differentially associated with EAA depending on the epigenetic clock considered. Among next-generation clocks, PCGrimAge and DunedinPACE emerged as the most responsive to dietary variation, with consistent associations observed for overall diet quality and NOVA4 food intake, particularly HHB. PCPhenoAge showed weaker and less consistent relationships in this cohort. Importantly, our findings indicate that LGI, captured by a composite score integrating circulating biomarkers and blood cell composition, accounts for a substantial part of the association between diet quality and EAA. However, mediation analyses also revealed residual direct associations for selected beneficial food categories, suggesting that inflammatory pathways alone do not fully explain the link between diet and EAA. Given the intricate interplay between chronic inflammation and other hallmarks of aging, which collectively drive cellular decline [159], targeting chronic inflammation, including through dietary interventions, holds high translational potential for mitigating age-related diseases. While the observational design precludes causal inference, this work provides a framework for future longitudinal and interventional studies including multiple biomarkers of inflammaging aimed at disentangling the biological pathways through which diet relates to aging and age-related disease risk.

Author contributions

LB: Conceptualization, study design, methodology, dietary and methylation data analysis, statistics, Manuscript writing: writing original draft, found acquisition; FM: Dietary data analysis, Manuscript writing: editing&revision; CR: Clinical data analysis, Manuscript writing: editing&revision; JAdS: Support in statistical analysis, Manuscript writing: editing&revision; FvM: Manuscript writing: editing&revision, Supervision. No one eligible for authorship has been excluded from the list of authors.

Data availability statement

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).

Declaration of generative AI and AI-assisted technologies in the writing process

None of the text was automatically generated by AI. During the preparation of this work the authors used ChatGPT in order to improve readability of some sentences. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

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References

[1] Benayoun BA, Pollina EA, Brunet A. Epigenetic regulation of ageing: linking environmental inputs to genomic stability. Nat Rev Mol Cell Biol 2015;16(10):593–610. https://doi.org/10.1038/nrm4048. 2015;16.
[2] Wang K, Liu H, Hu Q, Wang L, Liu J, Zheng Z, et al. Epigenetic regulation of aging: implications for interventions of aging and diseases. Signal Transduct Targeted Ther 2022;7:374. https://doi.org/10.1038/s41392-022-01211-8.
[3] Galkin F, Kovalchuk O, Koldasbayeva D, Zhavoronkov A, Bischof E. Stress, diet, exercise: common environmental factors and their impact on epigenetic age. Ageing Res Rev 2023;88:101956. https://doi.org/10.1016/j.arr.2023.101956.
[4] Kabacik S, Lowe D, Fransen L, Leonard M, Ang S-L, Whiteman C, et al. The relationship between epigenetic age and the hallmarks of aging in human cells. Nat Aging 2022;2:484–93. https://doi.org/10.1038/s43587-022-00220-0.
[5] Walaszczyk E, Luijten M, Spijkerman AMW, Bonder MJ, Lutgers HL, Snieder H, et al. DNA methylation markers associated with type 2 diabetes, fasting glucose and HbA1c levels: a systematic review and replication in a case–control sample of the Lifelines study. Diabetologia 2018;61. https://doi.org/10.1007/s00125-017-4497-7.
[6] Warner B, Ratner E, Datta A, Lendasse A. A systematic review of phenotypic and epigenetic clocks used for aging and mortality quantification in humans. Aging (Albany NY) 2024;16:12414. https://doi.org/10.18632/AGING.206098.
[7] Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nat Rev Genet 2018;19:371–84. https://doi.org/10.1038/S41576-018-0004-3.
[8] Oblak L, van der Zaag J, Higgins-Chen AT, Levine ME, Boks MP. A systematic review of biological, social and environmental factors associated with epigenetic clock acceleration. Ageing Res Rev 2021;69. https://doi.org/10.1016/J.ARR.2021.101348.
[9] Liang R, Tang Q, Chen J, Zhu L. Epigenetic clocks: beyond biological age, using the past to predict the present and future. Aging Dis 2024. https://doi.org/10.14336/AD.2024.1495. 0-.
[10] Harris KM, Levitt B, Gaydosh L, Martin C, Meyer JM, Mishra AA, et al. Sociodemographic and lifestyle factors and epigenetic aging in US young adults: NIMHD social epigenomics program. JAMA Netw Open 2024;7:e2427889. https://doi.org/10.1001/JAMANETWORKOPEN.2024.27889.
[11] Johnson AA, Shokhirev MN. First-generation versus next-generation epigenetic aging clocks: differences in performance and utility. Biogerontology 2025;26. https://doi.org/10.1007/S10522-025-10265-4.
[12] Bergsma T, Rogaeva E. DNA methylation clocks and their predictive capacity for aging phenotypes and healthspan. Neurosci Insights 2020;15. https://doi.org/10.1177/2633105520942221/ASSET/IMAGES/LARGE/10.1177_2633105520942221-FIG2.JPEG.
[13] Lu AT, Quach A, Wilson JG, Reiner AP, Aviv A, Raj K, et al. DNA methylation GrimAge strongly predicts lifespan and healthspan. Aging 2019;11:303–27. https://doi.org/10.18632/aging.101684.
[14] Belsky DW, Caspi A, Corcoran DL, Sugden K, Poulton R, Arseneault L, et al. DunedinPACE, a DNA methylation biomarker of the pace of aging. eLife 2022;11:e73420. https://doi.org/10.7554/eLife.73420.
[15] Higgins-Chen AT, Thrush KL, Wang Y, Minteer CJ, Kuo PL, Wang M, et al. A computational solution for bolstering reliability of epigenetic clocks: implications for clinical trials and longitudinal tracking. Nat Aging 2022;2:644. https://doi.org/10.1038/S43587-022-00248-2.
[16] Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A. Inflammaging: a new immune–metabolic viewpoint for age-related diseases. Nat Rev Endocrinol 2018;14(10):576–90. https://doi.org/10.1038/s41574-018-0059-4. 2018;14.
[17] Baechle JJ, Chen N, Makhijani P, Winer S, Furman D, Winer DA. Chronic inflammation and the hallmarks of aging. Mol Metab 2023;74. https://doi.org/10.1016/j.molmet.2023.101755.
[18] Meier HCS, Mitchell C, Karadimas T, Faul JD. Systemic inflammation and biological aging in the Health and Retirement Study. GeroScience 2023;45:3257–65. https://doi.org/10.1007/s11357-023-00880-9.
[19] Cribb L, Hodge AM, Yu C, Li SX, English DR, Makalic E, et al. Inflammation and epigenetic aging are largely independent markers of biological aging and mortality. J Gerontol: Series A 2022;77:2378–86. https://doi.org/10.1093/GERONA/GLAC147.
[20] Stromsnes K, Correas AG, Lehmann J, Gambini J, Olaso-gonzalez G. Anti-inflammatory properties of diet: role in healthy aging. Biomedicines 2021;9. https://doi.org/10.3390/biomedicines9080922.
[21] Martínez CF, Esposito S, Di Castelnuovo A, Costanzo S, Ruggiero E, De Curtis A, et al. Association between the inflammatory potential of the diet and biological aging: a cross-sectional analysis of 4510 adults from the moli-sani Study cohort. Nutrients 2023;15:1503. https://doi.org/10.3390/NU15061503/S1.
[22] Shivappa N, Godos J, Hebert JR, Wirth MD, Piuri G, Speciani AF, et al. Dietary inflammatory index and cardiovascular risk and Mortality-A meta-analysis. Nutrients 2018;10. https://doi.org/10.3390/nu10020200.
[23] Christ A, Lauterbach M, Latz E. Western diet and the immune System: an inflammatory connection. Immunity 2019;51:794–811. https://doi.org/10.1016/J.IMMUNI.2019.09.020.
[24] Barbaresko J, Koch M, Schulze MB, Nöthlings U. Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review. Nutr Rev 2013;71. https://doi.org/10.1111/nure.12035.
[25] Lankinen M, Uusitupa M, Schwab U. Nordic diet and Inflammation—A review of observational and intervention studies. Nutrients 2019;11. https://doi.org/10.3390/nu11061369.
[26] Soltani S, Chitsazi MJ, Salehi-Abargouei A. The effect of dietary approaches to stop hypertension (DASH) on serum inflammatory markers: a systematic review and meta-analysis of randomized trials. Clinical Nutrition 2018;37. https://doi.org/10.1016/j.clnu.2017.02.018.
[27] Millar SR, Navarro P, Harrington JM, Shivappa N, Hébert JR, Perry IJ, et al. Dietary score associations with markers of chronic low-grade inflammation: a cross-sectional comparative analysis of a middle- to older-aged population. Eur J Nutr 2022;61. https://doi.org/10.1007/s00394-022-02892-1.
[28] Minihane AM, Vinoy S, Russell WR, Baka A, Roche HM, Tuohy KM, et al. Low-grade inflammation, diet composition and health: current research evidence and its translation. Br J Nutr 2015;114:999–1012. https://doi.org/10.1017/S0007114515002093.
[29] Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. Br J Nutr 2011;106(Suppl):S5–78. https://doi.org/10.1017/S0007114511005460.
[30] Delgado-Lista J, Alcala-Diaz JF, Torres-Peña JD, Quintana-Navarro GM, Fuentes F, Garcia-Rios A, et al. Long-term secondary prevention of cardiovascular disease with a Mediterranean diet and a low-fat diet (CORDIOPREV): a randomised controlled trial. Lancet 2022;399. https://doi.org/10.1016/S0140-6736(22)00122-2.
[31] Boughanem H, Torres-Peña JD, Arenas-de Larriva AP, Romero-Cabrera JL, Gómez-Luna P, Martín-Piedra L, et al. Mediterranean diet, neutrophil count, and carotid intima-media thickness in secondary prevention: the CORDIOPREV study. Eur Heart J 2024:ehae836. https://doi.org/10.1093/eurheartj/ehae836.

[32] Linares-Pineda TM, Boughanem H, Gutiérrez-Repiso C, Macías-González M, Andrés-León E, Rojo-Martínez G, et al. Epigenetic changes in the metabolically healthy obese: a case-control versus a prospective study. Eur J Clin Invest 2022;52. https://doi.org/10.1111/eci.13783.
[33] Tabung FK, Smith-Warner SA, Chavarro JE, Wu K, Fuchs CS, Hu FB, et al. Development and validation of an empirical dietary inflammatory index. J Nutr 2016;146:1560. https://doi.org/10.3945/JN.115.228718.
[34] Tabung FK, Smith-Warner SA, Chavarro JE, Fung TT, Hu FB, Willett WC, et al. An empirical dietary inflammatory pattern Score enhances prediction of circulating inflammatory biomarkers in adults. J Nutr 2017;147:1567–77. https://doi.org/10.3945/JN.117.248377.
[35] Li M, Zhang Z, Zhu Y, Zeng XF, Li H, Wang Y, et al. Association of dietary inflammatory potential with risk of overall and cause-specific mortality. Br J Nutr 2022;127:1878–87. https://doi.org/10.1017/S0007114521002907.
[36] Mignogna C, Costanzo S, Di Castelnuovo A, Ruggiero E, Shivappa N, Hebert JR, et al. The inflammatory potential of the diet as a link between food processing and low-grade inflammation: an analysis on 21,315 participants to the Moli-sani study. Clinical Nutrition 2022;41:2226–34. https://doi.org/10.1016/J.CLNU.2022.08.020.
[37] Melekoglu E, Mete B, Sadıkoglu HM, Demirhindi H, Barutcu A, Macka T, et al. Low-grade inflammation and ultra-processed foods consumption in childhood. Clin Nutr ESPEN 2024;63:1100–1. https://doi.org/10.1016/j.clnesp.2024.07.385.
[38] Tristan Asensi M, Napoletano A, Sofi F, Dinu M. Low-Grade inflammation and ultra-processed foods consumption: a review. Nutrients 2023;15. https://doi.org/10.3390/NU15061546.
[39] Esposito S, Gialluisi A, Di Castelnuovo A, Costanzo S, Pepe A, Ruggiero E, et al. Ultra-processed food consumption is associated with the acceleration of biological aging in the Moli-sani Study. Am J Clin Nutr 2024;120:1432–40. https://doi.org/10.1016/j.ajcnut.2024.10.006.
[40] Monteiro CA, Louzada ML, Steele-Martinez E, Cannon G, Andrade GC, Baker P, et al. Ultra-processed foods and human health: the main thesis and the evidence. Lancet 2025;406:2667–84. https://doi.org/10.1016/s0140-6736(25)01565-x.
[41] Tessier AJ, Wang F, Korat AA, Eliassen AH, Chavarro J, Grodstein F, et al. Optimal dietary patterns for healthy aging. Nat Med 2025 2025:1–9. https://doi.org/10.1038/s41591-025-03570-5.
[42] Bonaccio M, Di Castelnuovo A, Costanzo S, Ruggiero E, Esposito S, Cerletti C, et al. Combination of a traditional Mediterranean Diet with ultra-processed food consumption in relation to all-cause and cause-specific mortality: prospective findings from the Moli-sani Study. Clinical Nutrition 2025;50:83–91. https://doi.org/10.1016/J.CLNU.2025.04.024.
[43] Tigchelaar EF, Zhernakova A, Dekens JAM, Hermes G, Baranska A, Mujagic Z, et al. Cohort profile: lifelines DEEP, a prospective, general population cohort study in the northern Netherlands: study design and baseline characteristics. BMJ Open 2015;5:e006772. https://doi.org/10.1136/bmjopen-2014-006772.
[44] Sijtsma A, Rienks J, van der Harst P, Navis G, Rosmalen JGM, Dotinga A. Cohort Profile Update: Lifelines, a three-generation cohort study and biobank. Int J Epidemiol 2022;51:e295–302. https://doi.org/10.1093/ije/dyab257.
[45] Khan I, Chong M, Le A, Mohammadi-Shemirani P, Morton R, Brinza C, et al. Surrogate adiposity markers and mortality. JAMA Netw Open 2023;6:e2334836. https://doi.org/10.1001/jamanetworkopen.2023.34836.
[46] Black AE. 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 2000;24:1119–30. https://doi.org/10.1038/sj.ijo.0801376.
[47] Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. Hum Nutr Clin Nutr 1985;39(Suppl 1):5–41.
[48] Harris E. Study: Waist-to-Hip ratio might predict mortality better than BMI. JAMA 2023;330:1515–6. https://doi.org/10.1001/JAMA.2023.19205.
[49] de Koning L, Merchant AT, Pogue J, Anand SS. Waist circumference and waist-to-hip ratio as predictors of cardiovascular events: meta-regression analysis of prospective studies. Eur Heart J 2007;28:850–6. https://doi.org/10.1093/eurheartj/ehm026.
[50] Imhof A, Fröhlich M, Loewel H, Helbecque N, Woodward M, Amouyel P, et al. Distributions of C-reactive protein measured by high-sensitivity assays in apparently healthy men and women from different populations in Europe. Clin Chem 2003;49:669–72. https://doi.org/10.1373/49.4.669.
[51] Sharif S, Van der Graaf Y, Cramer MJ, Kapelle LJ, de Borst GJ, Visseren FLJ, et al. Low-grade inflammation as a risk factor for cardiovascular events and all-cause mortality in patients with type 2 diabetes. Cardiovasc Diabetol 2021;20. https://doi.org/10.1186/S12933-021-01409-0.
[52] Zahorec R. Neutrophil-to-lymphocyte ratio, past, present and future perspectives. Bratisl Lek Listy 2021;122:474–88. https://doi.org/10.4149/BLL_2021_078.
[53] Lucius K. Novel and emerging markers of chronic or low-grade inflammation, 29; 2023. p. 130–42. https://doi.org/10.1089/ICT.2023.29075.KLU. Https://HomeLiebertpubCom/Ict.
[54] Yang YL, Wu CH, Hsu PF, Chen SC, Huang SS, Chan WL, et al. Systemic immune-inflammation index (SII) predicted clinical outcome in patients with coronary artery disease. Eur J Clin Invest 2020;50. https://doi.org/10.1111/ECI.13230.
[55] Hu B, Yang XR, Xu Y, Sun YF, Sun C, Guo W, et al. Systemic immune-inflammation index predicts prognosis of patients after curative resection for hepatocellular carcinoma. Clin Cancer Res 2014;20:6212–22. https://doi.org/10.1158/1078-0432.CCR-14-0442.
[56] Shivappa N, Bonaccio M, Hebert JR, Di Castelnuovo A, Costanzo S, Ruggiero E, et al. Association of proinflammatory diet with low-grade inflammation: results from the Moli-sani study. Nutrition 2018;54:182–8. https://doi.org/10.1016/J.NUT.2018.04.004.
[57] Pounis G, Bonaccio M, Di Castelnuovo A, Costanzo S, De Curtis A, Persichillo M, et al. Polyphenol intake is associated with low-grade inflammation, using a novel data analysis from the Moli-sani study. Thromb Haemost 2016;115:344–52. https://doi.org/10.1160/TH15-06-0487.
[58] Shi H, Schweren LJS, ter Horst R, Bloemendaal M, van Rooij D, Vasquez AA, et al. Low-grade inflammation as mediator between diet and behavioral disinhibition: a UK Biobank study. Brain Behav Immun 2022;106:100–10. https://doi.org/10.1016/J.BBI.2022.07.165.
[59] Bonaccio M, Costanzo S, Di Castelnuovo A, Gialluisi A, De Curtis A, Persichillo M, et al. An upward trajectory in adherence to Mediterranean diet is associated with favourable changes in inflammation biomarkers. Nutr Metabol Cardiovasc Dis 2021;31:3249. https://doi.org/10.1016/j.numecd.2021.08.002.
[60] Liu S, Gu Y. INFLA score: a novel inflammatory marker for assessing cardiometabolic disease risk in obese individuals. Diabetol Metab Syndr 2024;16:151. https://doi.org/10.1186/S13098-024-01396-8.
[61] Cheng W, Du Z, Lu B. Chronic low-grade inflammation associated with higher risk and earlier onset of cardiometabolic multimorbidity in middle-aged and older adults: a population-based cohort study. Sci Rep 2024;14(1):22635. https://doi.org/10.1038/s41598-024-72988-7. 2024;14.
[62] Jiang S, Wang J, Guan X, An H, Tao N. Low-grade inflammation score (INFLA-score) associated with metabolic syndrome and its components in shift workers. Diabetol Metab Syndr 2025;17(1):381. https://doi.org/10.1186/S13098-025-01850-1. 2025;17.
[63] Kurilshikov A, Van Den Munckhof ICL, Chen L, Bonder MJ, Schraa K, Rutten JHW, et al. Gut microbial associations to plasma metabolites linked to cardiovascular phenotypes and risk: a cross-sectional Study. Circ Res 2019;124:1808–20. https://doi.org/10.1161/CIRCRESAHA.118.314642/SUPPL_FILE/CIRCRES_CIRCRES-2018-314642D_SUPP2.XLSX.
[64] Cleeman JI. Executive Summary of the third report of the National Cholesterol Education Program (NCEP) expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment Panel III). JAMA 2001;285:2486–97. https://doi.org/10.1001/JAMA.285.19.2486.
[65] Blüher M. Metabolically healthy obesity. Endocr Rev 2020;41:405–20. https://doi.org/10.1210/ENDREV/BNAA004.
[66] Brouwer-Brolsma EM, Perenboom C, Sluik D, Van De Wiel A, Geelen A, Feskens EJM, et al. Development and external validation of the 'Flower-FFQ': a FFQ designed for the Lifelines Cohort Study. Public Health Nutr 2022;25:225–36. https://doi.org/10.1017/S1368980021002111.
[67] Siebelink E, Geelen A, De Vries JHM. Self-reported energy intake by FFQ compared with actual energy intake to maintain body weight in 516 adults. Br J Nutr 2011;106:274–81. https://doi.org/10.1017/S0007114511000067.
[68] Goldbohm RA, van 't Veer P, van den Brandt PA, van 't Hof MA, Brants HA, Sturmans F, et al. Reproducibility of a food frequency questionnaire and stability of dietary habits determined from five annually repeated measurements. Eur J Clin Nutr 1995;49:420–9.
[69] Vinke PC, Corpeleijn E, Dekker LH, Jacobs DRJ, Navis G, Kromhout D. Development of the food-based Lifelines Diet Score (LLDS) and its application in 129,369 Lifelines participants. Eur J Clin Nutr 2018;72:1111–9. https://doi.org/10.1038/s41430-018-0205-z.
[70] Item - USDA National Nutrient Database for Standard Reference, Legacy release - ag data commons - figshare n.d. https://agdatacommons.nal.usda.gov/articles/dataset/USDA_National_Nutrient_Database_for_Standard_Reference_Legacy_Release/24661818 (accessed December 19, 2025).
[71] Kromhout D, Spaaij CJK, De Goede J, Weggemans RM, Brug J, Geleijnse JM, et al. The 2015 Dutch food-based dietary guidelines. Eur J Clin Nutr 2016;70:869–78. https://doi.org/10.1038/EJCN.2016.52.
[72] Van Lee L, Geelen A, Van Huysduynen E, De Vries JHM, Vant Veer P, Feskens EJM. The Dutch Healthy Diet index (DHD-index): an instrument to measure adherence to the Dutch Guidelines for a Healthy Diet. Nutr J 2012;11. https://doi.org/10.1186/1475-2891-11-49.
[73] Looman M, Feskens EJM, De Rijk M, Meijboom S, Biesbroek S, Temme EHM, et al. Development and evaluation of the Dutch Healthy Diet index 2015. Public Health Nutr 2017;20:2289–99. https://doi.org/10.1017/S136898001700091X.
[74] Duan MJ, Vinke PC, Navis G, Corpeleijn E, Dekker LH. 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 2022;20. https://doi.org/10.1186/S12916-021-02200-4.
[75] Monteiro CA, Cannon G, Levy R, Moubarac J-C, Jaime P, Paula Martins A, et al. NOVA. The star shines bright. World Nutrition 2016;7:28–38.
[76] Bordoni L, Agostinho de Sousa J, Zhuo J, von Meyenn F. Evaluating the connection between diet quality, EpiNutrient intake and epigenetic age: an observational study. Am J Clin Nutr 2024;120:1143–55. https://doi.org/10.1016/J.AJCNUT.2024.08.033.

[77] Khadem A, Shiraseb F, Mirzababaei A, Ghaffarian-Ensaf R, Mirzaei K. Association of Lifelines Diet Score (LLDS) and metabolically unhealthy overweight/obesity phenotypes in women: a cross-sectional study. BMC Womens Health 2022;22. https://doi.org/10.1186/S12905-022-01957-X.
[78] Akhavanfar R, Hojati A, Kahrizi MS, Farhangi MA, Ardekani AM. Adherence to lifelines diet score and risk factors of metabolic syndrome among overweight and obese adults: a cross-sectional study. Front Nutr 2022;9:961468. https://doi.org/10.3389/FNUT.2022.961468. 961468.
[79] Direksunthorn T, Abdelgawwad El-Sehrawy AAM, Hjazi A, Obaidur Rab S, Suliman Maashi M. The association between lifelines diet score and metabolic associated fatty liver disease: a case–control study. Front Nutr 2025;12:1569814. https://doi.org/10.3389/FNUT.2025.1569814/BIBTEX.
[80] Asiaei S, Sharifani MS, Ghobadian B, Baghdadi G, Biglari F, Rahimlou M. Association between lifelines diet score with odds of nonalcoholic fatty liver disease and some novel anthropometric indices among adults: a case–control study. Front Nutr 2024;11:1523651. https://doi.org/10.3389/FNUT.2024.1523651/BIBTEX.
[81] Abudari MO, Alreshidi NF, Alharbi SS, Alziyadi SH, Alanazi MA, Kambal N, et al. The association between Lifelines Diet Score and GDM: a case-control study. Front Nutr 2025;12:1625903. https://doi.org/10.3389/FNUT.2025.1625903/BIBTEX.
[82] Cai Q, Dekker LH, Vinke PC, Corpeleijn E, Bakker SJL, de Borst MH, et al. Diet quality and incident chronic kidney disease in the general population: the Lifelines Cohort Study. Clinical Nutrition 2021;40:5099–105. https://doi.org/10.1016/j.clnu.2021.07.033.
[83] Baart AM, Brouwer-Brolsma EM, de Jong HBT, de Vries JHM, Feskens EJM. Assessment of the Dutch Healthy Diet index 2015 in the Lifelines cohort study at baseline. Eur J Clin Nutr 2023;78(3):217–27. https://doi.org/10.1038/s41430-023-01372-x. 2023;78.
[84] Baart AM, Brouwer-brolsma EM, Perenboom CWM, de Vries JHM, Feskens EJM. Dietary intake in the lifelines cohort study: baseline results from the flower food frequency questionnaire among 59,982 participants. Nutrients 2022;14:48. https://doi.org/10.3390/NU14010048/S1.
[85] Van Lee L, Feskens EJM, Meijboom S, Hooft Van Huysduynen EJC, Van'T Veer P, De Vries JHM, et al. Evaluation of a screener to assess diet quality in the Netherlands. Br J Nutr 2016;115:517–26. https://doi.org/10.1017/S0007114515004705.
[86] Van Lee L, Feskens EJM, Hooft Van Huysduynen EJC, De Vries JHM, Van't Veer P, Geelen A. The Dutch Healthy Diet index as assessed by 24 h recalls and FFQ: associations with biomarkers from a cross-sectional study. J Nutr Sci 2014;2:1–8. https://doi.org/10.1017/JNS.2013.28.
[87] Kirk D, Baart AM, McLean J, Feskens EJM. Association between diet quality and all-cause mortality in a large Dutch cohort. PLoS One 2024;19:e0302905. https://doi.org/10.1371/JOURNAL.PONE.0302905.
[88] Van Lee L, Geelen A, Van Huysduynen E, De Vries JHM, Vant Veer P, Feskens EJM. The Dutch Healthy Diet index (DHD-index): an instrument to measure adherence to the Dutch Guidelines for a Healthy Diet. Nutr J 2012;11(1):49. https://doi.org/10.1186/1475-2891-11-49. 2012;11.
[89] Mlodzik-Czyzewska MA, Malinowska AM, Chmurzynska A. Low folate intake and serum levels are associated with higher body mass index and abdominal fat accumulation: a case control study. Nutr J 2020;19:53. https://doi.org/10.1186/s12937-020-00572-6.
[90] Jafari A, Gholizadeh E, Sadrmanesh O, Tajpour S, Yarizadeh H, Zamani B, et al. The effect of folic acid supplementation on body weight and body mass index: a systematic review and meta-analysis of randomized controlled trials. Clin Nutr ESPEN 2023;53:206–13. https://doi.org/10.1016/J.CLNESP.2022.11.020.
[91] Bird JK, Feskens EJ, Melse-Boonstra A. A systematized review of the relationship between obesity and vitamin C requirements. Curr Dev Nutr 2024;8:102152. https://doi.org/10.1016/J.CDNUT.2024.102152.
[92] Hore TA, Von Meyenn F, Ravichandran M, Bachman M, Ficz G, Oxley D, 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 2016;113:12202–7. https://doi.org/10.1073/PNAS.1608679113.
[93] Michels KB, Binder AM. Impact of folic acid supplementation on the epigenetic profile in healthy unfortified individuals – a randomized intervention trial. Epigenetics 2024;19. https://doi.org/10.1080/15592294.2023.2293410.
[94] Kok DEG, Dhonukshe-Rutten RAM, Lute C, Heil SG, Uitterlinden AG, Van Der Velde N, et al. The effects of long-term daily folic acid and vitamin B12 supplementation on genomewide DNA methylation in elderly subjects. Clin Epigenet 2015;7:1–14. https://doi.org/10.1186/S13148-015-0154-5/COMMENTS.
[95] Menotti A, Puddu PE, Geleijnse JM, Kafatos A, Tolonen H. Dietary atherogenicity and thrombogenicity indexes predicting cardiovascular mortality: 50-year follow-up of the Seven Countries Study. Nutr Metabol Cardiovasc Dis 2024;34:2107–14. https://doi.org/10.1016/J.NUMECD.2024.05.010.
[96] Ulbricht TLV, Southgate DAT. Coronary heart disease: seven dietary factors. Lancet 1991;338:985–92. https://doi.org/10.1016/0140-6736(91)91846-M.
[97] Dewhurst-Trigg R, Hulston CJ, Markey O. The effect of quantity and quality of dietary fat intake on subcutaneous white adipose tissue inflammatory responses. Proc Nutr Soc 2020;79:542–56. https://doi.org/10.1017/S0029665120000038.
[98] Lyon P, Strippoli V, Fang B, Cimmino L. B vitamins and one-carbon metabolism: implications in human health and disease. Nutrients 2020;12. https://doi.org/10.3390/nu12092867.
[99] Chamberlain JA, Dugué P-A, Bassett JK, Hodge AM, Brinkman MT, Joo JE, et al. Dietary intake of one-carbon metabolism nutrients and DNA methylation in peripheral blood. Am J Clin Nutr 2018;108:611–21. https://doi.org/10.1093/ajcn/nqy119.
[100] Choi S-W, Friso S. Modulation of DNA methylation by one-carbon metabolism: a milestone for healthy aging. Nutr Res Prac 2023;17:597–615. https://doi.org/10.4162/nrp.2023.17.4.597.
[101] Anderson OS, Sant KE, Dolinoy DC. Nutrition and epigenetics: an interplay of dietary methyl donors, one-carbon metabolism and DNA methylation. J Nutr Biochem 2012;23:853–9. https://doi.org/10.1016/j.jnutbio.2012.03.003.
[102] Młodzik-Czyżewska MA, Szwengiel A, Malinowska AM, Chmurzynska A. Comparison of associations between one-carbon metabolism, lipid metabolism, and fatty liver markers in normal-weight and overweight people aged 20-40 years. Ann Nutr Metab 2021;77:221–30. https://doi.org/10.1159/000517911.
[103] Bozack AK, Khodasevich D, Nwanaji-Enwerem JC, Gladish N, Shen H, Daredia S, et al. One-carbon metabolism-related compounds are associated with epigenetic aging biomarkers: results from the cross-sectional National Health and Nutrition Examination Survey 1999–2002. Am J Clin Nutr 2025. https://doi.org/10.1016/j.ajcnut.2025.05.029.
[104] Holmes HE, Valentin RE, Jernerén F, de Jager Loots CA, Refsum H, Smith AD, et al. Elevated homocysteine is associated with increased rates of epigenetic aging in a population with mild cognitive impairment. Aging Cell 2024;23. https://doi.org/10.1111/ACEL.14255.
[105] Bonder MJ, Luijk R, Zhernakova DV, Moed M, Deelen P, Vermaat M, et al. Disease variants alter transcription factor levels and methylation of their binding sites. Nat Genet 2017;49:131–8. https://doi.org/10.1038/ng.3721.
[106] Levine ME, Lu AT, Quach A, Chen BH, Assimes TL, Bandinelli S, et al. An epigenetic biomarker of aging for lifespan and healthspan. Aging 2018;10:573–91. https://doi.org/10.18632/aging.101414.
[107] Mavrommatis C, Belsky DW, Ying K, Moqri M, Campbell A, Richmond A, et al. An unbiased comparison of 14 epigenetic clocks in relation to 174 incident disease outcomes. Nat Commun 2025;16(1):11164. https://doi.org/10.1038/s41467-025-66106-y. 2025;16.
[108] Wang Y, Grant OA, Zhai X, Mcdonald-Maier KD, Schalkwyk LC. Insights into ageing rates comparison across tissues from recalibrating cerebellum DNA methylation clock. GeroScience 2024;46:39–56. https://doi.org/10.1007/S11357-023-00871-W/FIGURES/6.
[109] Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Roy Stat Soc B 1995;57:289–300. https://doi.org/10.1111/J.2517-6161.1995.TB02031.X.
[110] Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency, 29; 2001. p. 1165–88. https://doi.org/10.1214/AOS/1013699998. Https://DoiOrg/101214/Aos/1013699998.
[111] Benjamini Y. Discovering the false discovery rate. J R Stat Soc, Ser B Stat Methodol 2010;72:405–16. https://doi.org/10.1111/J.1467-9868.2010.00746.X.
[112] Rosseel Y. Lavaan: an R package for structural equation modeling. J Stat Software 2012;48:1–36. https://doi.org/10.18637/jss.v048.i02.
[113] Ferrucci L, Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. Nat Rev Cardiol 2018;15:505–22. https://doi.org/10.1038/S41569-018-0064-2.
[114] Carlberg C, Blüthner A, Schoeman-Giziakis I, Oosting A, Cocolin L. Modulating biological aging with food-derived signals: a systems and precision nutrition perspective. Npj Aging 2025;11(1):76. https://doi.org/10.1038/s41514-025-00266-5. 2025;11.
[115] Dwaraka VB, Aronica L, Carreras-Gallo N, Robinson JL, Hennings T, Carter MM, et al. Unveiling the epigenetic impact of vegan vs. omnivorous diets on aging: insights from the Twins Nutrition Study (TwiNS). BMC Med 2024;22:1–19. https://doi.org/10.1186/S12916-024-03513-W/FIGURES/8.
[116] McGee KC, Sullivan J, Hazeldine J, Schmunk LJ, Martin-Herranz DE, Jackson T, et al. A combination nutritional supplement reduces DNA methylation age only in older adults with a raised epigenetic age. GeroScience 2024;46:4333–47. https://doi.org/10.1007/S11357-024-01138-8/TABLES/3.
[117] Chiu DT, Hamlat EJ, Zhang J, Epel ES, Laraia BA. Essential nutrients, added sugar intake, and epigenetic Age in midlife Black and white Women: NIMHD Social Epigenomics Program. JAMA Netw Open 2024;7:e2422749. https://doi.org/10.1001/JAMANETWORKOPEN.2024.22749.
[118] Ravi S, Kankaanpää A, Bogl LH, Heikkinen A, Pietiläinen KH, Kaprio J, et al. Suboptimal dietary patterns are associated with accelerated biological aging in young adulthood: a study with twins. Clinical Nutrition 2025;45:10–21. https://doi.org/10.1016/J.CLNU.2024.12.018.
[119] Izquierdo AG, Lorenzo PM, Costa-Fraga N, Primo-Martin D, Rodriguez-Carnero G, Nicoletti CF, et al. Epigenetic aging acceleration in obesity is slowed Down by nutritional ketosis following very low-calorie Ketogenic diet (VLCKD): a new perspective to reverse biological Age. Nutrients 2025;17:1060. https://doi.org/10.3390/NU17061060. 2025;17:1060.
[120] Yi SY, Steffen LM, Jacobs DR, Joyce B, Guan W, Duprez D, et al. Dietary carbohydrate quality is associated with epigenetic Age acceleration: a

cross-sectional Study of the CARDIA cohort. J Nutr 2025. https://doi.org/10.1016/J.TJNUT.2025.01.022.
[121] García-García I, Grisotto G, Heini A, Gibertoni S, Nusslé S, Gonseth Nusslé S, et al. Examining nutrition strategies to influence DNA methylation and epigenetic clocks: a systematic review of clinical trials. Frontiers in Aging 2024;5. https://doi.org/10.3389/FRAGI.2024.1417625.
[122] Goldman DP, Cutler D, Rowe JW, Michaud PC, Sullivan J, Peneva D, et al. Substantial health and economic returns from delayed aging may warrant a new focus for medical research. Health Aff 2013;32:1698–705. https://doi.org/10.1377/HLTHAFF.2013.0052.
[123] Scott AJ, Ellison M, Sinclair DA. The economic value of targeting aging. Nat Aging 2021;1:616–23. https://doi.org/10.1038/S43587-021-00080-0.
[124] Waziry R, Ryan CP, Corcoran DL, Huffman KM, Kobor MS, Kothari M, et al. Effect of long-term caloric restriction on DNA methylation measures of biological aging in healthy adults from the CALERIE trial. Nat Aging 2023;3:248–57. https://doi.org/10.1038/s43587-022-00357-y.
[125] Bischoff-Ferrari HA, Gängler S, Wieczorek M, Belsky DW, Ryan J, Kressig RW, et al. Individual and additive effects of vitamin D, omega-3 and exercise on DNA methylation clocks of biological aging in older adults from the DO-HEALTH trial. Nat Aging 2025;5(3):376–85. https://doi.org/10.1038/s43587-024-00793-y. 2025;5.
[126] Bozack AK, Khodasevich D, Nwanaji-Enwerem JC, Gladish N, Shen H, Daredia S, et al. Dietary fatty acids and epigenetic aging in US adults: results from the National Health and Nutrition Examination Survey. Npj Aging 2025;12(1):3. https://doi.org/10.1038/s41514-025-00298-x. 2025;12.
[127] Yaskolka Meir A, Keller M, Hoffmann A, Rinott E, Tsaban G, Kaplan A, et al. The effect of polyphenols on DNA methylation-assessed biological age attenuation: the DIRECT PLUS randomized controlled trial. BMC Med 2023;21:1–15. https://doi.org/10.1186/S12916-023-03067-3/FIGURES/5.
[128] Grootswagers P, Bach D, Biemans Y, Behrouzi P, Horvath S, Kramer CS, et al. Discovering the direct relations between nutrients and epigenetic ageing. J Nutr Health Aging 2024;28:100324. https://doi.org/10.1016/J.JNHA.2024.100324.
[129] Szarc Vel Szic K, Declerck K, Vidaković M, Vanden Berghe W. From inflammaging to healthy aging by dietary lifestyle choices: is epigenetics the key to personalized nutrition? Clin Epigenet 2015;7:1–18. https://doi.org/10.1186/S13148-015-0068-2/FIGURES/4.
[130] Han JDJ. The ticking of aging clocks. Trends Endocrinol Metabol 2024;35:11–22. https://doi.org/10.1016/J.TEM.2023.09.007/ASSET/C73BAA15-D875-4BC3-90FF-6E8A042433FA/MAIN.ASSETS/GR2.SML.
[131] García-Calzón S, Zalba G, Ruiz-Canela M, Shivappa N, Hébert JR, Martínez JA, et al. Dietary inflammatory index and telomere length in subjects with a high cardiovascular disease risk from the PREDIMED-NAVARRA study: cross-sectional and longitudinal analyses over 5 y. Am J Clin Nutr 2015;102:897. https://doi.org/10.3945/AJCN.115.116863.
[132] Ramos-Lopez O, Milagro FI, Riezu-Boj JI, Martinez JA. Epigenetic signatures underlying inflammation: an interplay of nutrition, physical activity, metabolic diseases, and environmental factors for personalized nutrition. Inflamm Res 2021;70:29–49. https://doi.org/10.1007/S00011-020-01425-Y/TABLES/4.
[133] Zhang Y, Kutateladze TG. Diet and the epigenome. Nat Commun 2018;9(1):1–3. https://doi.org/10.1038/s41467-018-05778-1. 2018;9.
[134] Evans LW, Stratton MS, Ferguson BS. Dietary natural products as epigenetic modifiers in aging-associated inflammation and disease. Nat Prod Rep 2020;37:653. https://doi.org/10.1039/C9NP00057G.
[135] Lorenzo PM, Izquierdo AG, Rodriguez-Carnero G, Fernández-Pombo A, Iglesias A, Carreira MC, et al. Epigenetic effects of healthy foods and lifestyle habits from the Southern european Atlantic diet pattern: a narrative review. Adv Nutr 2022;13:1725–47. https://doi.org/10.1093/ADVANCES/NMAC038.
[136] Crous-Bou M, Molinuevo JL, Sala-Vila A. Plant-Rich dietary patterns, plant foods and nutrients, and telomere length. Adv Nutr 2019;10:S296–303. https://doi.org/10.1093/ADVANCES/NMZ026.
[137] Lee Y, Kang D, Lee SA. Effect of dietary patterns on serum C-reactive protein level. Nutr Metabol Cardiovasc Dis 2014;24:1004–11. https://doi.org/10.1016/J.NUMECD.2014.05.001.
[138] Ren Z, Zhao A, Wang Y, Meng L, Szeto IMY, Li T, et al. Association between dietary inflammatory index, C-Reactive protein and metabolic syndrome: a cross-sectional Study. Nutrients 2018;10:831. https://doi.org/10.3390/NU10070831.
[139] Sánchez-Cabo F, Fuster V, Silla-Castro JC, González G, Lorenzo-Vivas E, Alvarez R, et al. Subclinical atherosclerosis and accelerated epigenetic age mediated by inflammation: a multi-omics study. Eur Heart J 2023;44:2698–709. https://doi.org/10.1093/EURHEARTJ/EHAD361.
[140] Hébert JR, Shivappa N, Wirth MD, Hussey JR, Hurley TG. Perspective: the dietary inflammatory index (DII)—Lessons learned, improvements made, and future directions. Adv Nutr 2019;10:185–95. https://doi.org/10.1093/ADVANCES/NMY071.
[141] Mello RN de, Gois BP de, Acp Kravchychyn, Dâmaso AR, Horst MA, Lima GC, et al. Dietary inflammatory index and its relation to the pathophysiological aspects of obesity: a narrative review. Arch Endocrinol Metab 2023;67. https://doi.org/10.20945/2359-3997000000631.
[142] Zhou N, Xie ZP, Liu Q, Xu Y, Dai SC, Lu J, et al. The dietary inflammatory index and its association with the prevalence of hypertension: a cross-sectional study. Front Immunol 2023;13:1097228. https://doi.org/10.3389/FIMMU.2022.1097228/BIBTEX.
[143] Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol Sci Med Sci 2014;69(Suppl 1):S4–9. https://doi.org/10.1093/GERONA/GLU057.
[144] Guasch-Ferré M. Connecting the dots: the Mediterranean diet, neutrophil count, and atherosclerosis. Eur Heart J 2025;46:730–2. https://doi.org/10.1093/EURHEARTJ/EHAE799.
[145] Esposito S, Gialluisi A, Costanzo S, Di Castelnuovo A, Ruggiero E, De Curtis A, et al. Dietary polyphenol intake is associated with biological aging, a novel predictor of cardiovascular disease: Cross-Sectional findings from the molisani Study. Nutrients 2021;13:1701. https://doi.org/10.3390/NU13051701. 2021;13:1701.
[146] Esposito S, Gialluisi A, Costanzo S, Di Castelnuovo A, Ruggiero E, De Curtis A, et al. Mediterranean diet and other dietary patterns in association with biological aging in the Moli-sani Study cohort. Clinical Nutrition 2022;41:1025–33. https://doi.org/10.1016/J.CLNU.2022.02.023.
[147] Huang RC, Lillycrop KA, Beilin LJ, Godfrey KM, Anderson D, Mori TA, et al. Epigenetic Age acceleration in adolescence associates with BMI, inflammation, and risk Score for middle Age cardiovascular disease. J Clin Endocrinol Metab 2019;104:3012. https://doi.org/10.1210/JC.2018-02076.
[148] Irvin MR, Aslibekyan S, Do A, Zhi D, Hidalgo B, Claas SA, et al. Metabolic and inflammatory biomarkers are associated with epigenetic aging acceleration estimates in the GOLDN study. Clin Epigenet 2018;10:1–9. https://doi.org/10.1186/S13148-018-0481-4/TABLES/4.
[149] Stevenson AJ, McCartney DL, Harris SE, Taylor AM, Redmond P, Starr JM, et al. Trajectories of inflammatory biomarkers over the eighth decade and their associations with immune cell profiles and epigenetic ageing. Clin Epigenet 2018;10. https://doi.org/10.1186/S13148-018-0585-X.
[150] Li DL, Hodge AM, Cribb L, Southey MC, Giles GG, Milne RL, et al. Body size, diet quality, and epigenetic aging: Cross-Sectional and longitudinal analyses. J Gerontol A Biol Sci Med Sci 2024;79. https://doi.org/10.1093/GERONA/GLAE026.
[151] Sadler CR, Grassby T, Hart K, Raats M, Sokolović M, Timotijevic L. Processed food classification: conceptualisation and challenges. Trends Food Sci Technol 2021;112:149–62. https://doi.org/10.1016/J.TIFS.2021.02.059.
[152] Wolfson JA, Tucker AC, Leung CW, Rebholz CM, Garcia-Larsen V, Martinez-Steele E. Trends in adults' intake of Un-processed/Minimally processed, and ultra-processed foods at home and away from home in the United States from 2003–2018. J Nutr 2025;155:280–92. https://doi.org/10.1016/J.TJNUT.2024.10.048.
[153] Vitale M, Costabile G, Testa R, D'Abbronzo G, Nettore IC, Macchia PE, et al. Ultra-Processed foods and human health: a systematic review and meta-analysis of prospective cohort studies. Adv Nutr 2024;15:100121. https://doi.org/10.1016/j.advnut.2023.09.009.
[154] Pacheco LS, Tobias DK, Li Y, Bhupathiraju SN, Willett WC, Ludwig DS, et al. Sugar-sweetened or artificially-sweetened beverage consumption, physical activity, and risk of cardiovascular disease in adults: a prospective cohort study. Am J Clin Nutr 2024;119:669–81. https://doi.org/10.1016/J.AJCNUT.2024.01.001.
[155] Cordova R, Viallon V, Fontvieille E, Peruchet-Noray L, Jansana A, Wagner KH, et al. Consumption of ultra-processed foods and risk of multimorbidity of cancer and cardiometabolic diseases: a multinational cohort study. The Lancet Regional Health - Europe 2023;35. https://doi.org/10.1016/j.lanepe.2023.100771.
[156] Lane MM, Gamage E, Du S, Ashtree DN, McGuinness AJ, Gauci S, et al. Ultra-processed food exposure and adverse health outcomes: umbrella review of epidemiological meta-analyses. BMJ 2024;384. https://doi.org/10.1136/BMJ-2023-077310.
[157] Kim Y, Cho Y, Kim JE, Lee DH, Oh H. Ultra-Processed food intake and risk of type 2 diabetes mellitus: a dose-response meta-analysis of prospective studies. Diabetes Metab J 2025. https://doi.org/10.4093/DMJ.2024.0706.
[158] Juul F, Martinez-Steele E, Parekh N, Monteiro CA. The role of ultra-processed food in obesity. Nat Rev Endocrinol 2025 2025:1–14. https://doi.org/10.1038/s41574-025-01143-7.
[159] Baechle JJ, Chen N, Makhijani P, Winer S, Furman D, Winer DA. Chronic inflammation and the hallmarks of aging. Mol Metab 2023;74:101755. https://doi.org/10.1016/J.MOLMET.2023.101755.