On-line Supplementary Material

Supplementary Methods

Diet quality score

The Alternate Health Eating Index (AHEI) (1) and a modified Mediterranean-style Diet Score (MDS) (2) were used to confirm the association between diet quality and epigenetic age acceleration. The AHEI score essentially reflects concordance of an individual's diet with the current Dietary Guidelines for Americans (1, 3), while the MDS represents the major characteristics of the Mediterranean-type diet (2).

Eleven components considered in the AHEI score are vegetables, fruits, nuts and legumes, whole grains, red and processed meat, sugar-sweetened beverages and fruit juice, EPA and DHA, PUFA (without EPA and DHA), trans-fatty acids, sodium, and alcohol (4). Each component scores from 0 (unhealthy) to 10 (healthiest), with a higher score assigned to moderate alcohol drinking and higher intakes of fruits, vegetables, whole grains, nuts and legumes, EPA and DHA, and PUFA, as well as to lower intakes of sugar-sweetened beverages, red and processed meat, trans-fatty acids, and sodium (1). It comprises a total of 110 scores with a higher score reflecting a healthier diet.

The MDS incorporates 9 components, including vegetables, fruits, nuts, legumes, whole grains, fish, red and processed meat, ratio of MUFA to SFA, and alcohol (2). Consumption of each component was categorized into sex-specific quartiles (5). A score of 0 to 3 was assigned

according to the quartile rank to each component except for red and processed meat and alcohol. For red and processed meat, the order of the scores was reversed (i.e. the highest quartile was assigned a score of 0). Moderate alcohol consumption (i.e., 10–25 grams/day for men and 5–15 grams/day for women) was assigned a value of one. The MDS comprises a total of 25, with a higher score reflecting better adherence to the overall Mediterranean dietary pattern.

Reference

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Higher diet quality relates to decelerated epigenetic aging; Y Kim et al.

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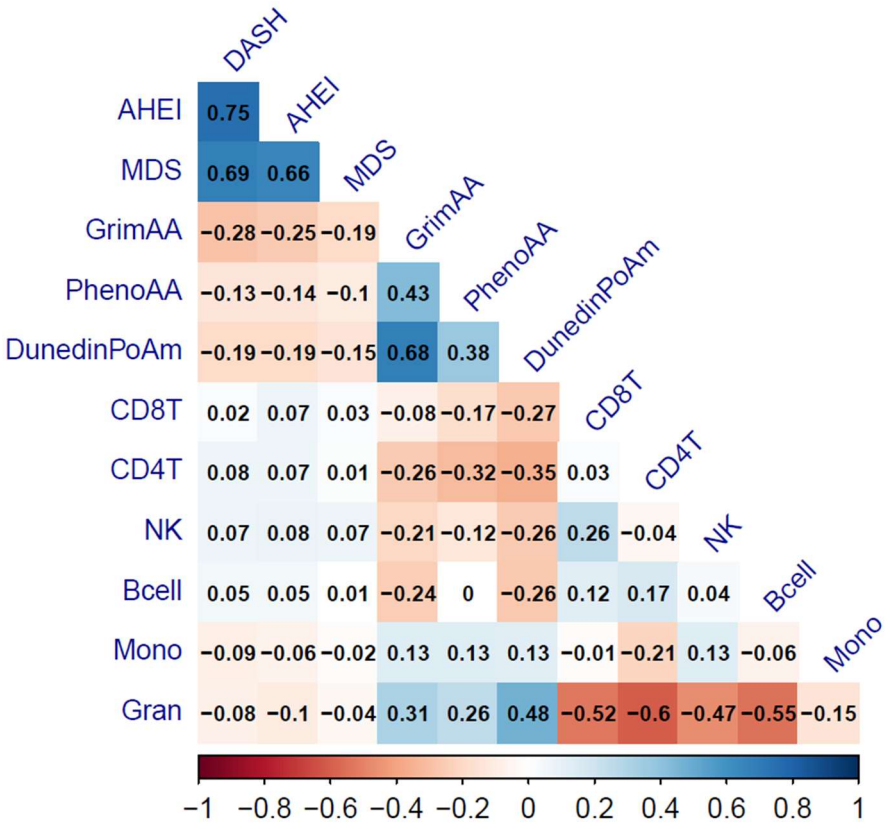

Supplementary Figure 1. Pearson correlation matrix of diet quality scores and blood cell counts. The Houseman algorithm was used for estimating blood cell counts. AHEI, Alternative healthy eating index; DASH, Dietary Approaches to Stop Hypertension score; DunedinPoAm, Dunedin Pace of Aging Methylation; GrimAA, DNA methylation GrimAge acceleration; Gran, Granulocytes; MDS, Mediterranean-style dietary score; Mono, monocytes; NK, natural killer; PhenoAA, DNA methylation PhenoAge acceleration.

Supplementary Table 1. Predictors for developing epigenetic aging measures

Measures	Biomarkers
DunedinPoam	Glycated hemoglobin, maximum oxygen uptake, waist-hip ratio, forced expiratory volume in one second, the ratio of the forced expiratory volume in the first one second to the forced vital capacity, mean arterial pressure, body mass index, leukocyte telomere length, creatinine clearance, urea nitrogen, lipoprotein (a), triglycerides, gum health, total cholesterol, white blood cell count, high-sensitivity C-reactive protein, high-density lipoprotein-cholesterol, and apolipoprotein B/A1 ratio
GrimAA	DNA methylation-based markers of plasma proteins (adrenomedullin, beta-2-microglobulim, cystatin C, growth differentiation factor 15, leptin, plasminogen activation inhibitor 1, and tissue inhibitor metalloproteinases 1), DNA methylation-based estimator of smoking pack-years, chronological age, and sex
PhenoAA	Albumin, creatinine, serum glucose, C-reactive protein, percentage of lymphocyte, mean red cell volume, red cell distribution width, alkaline phosphatase, white blood cell count, and chronological age

DenedinPoAm, Dunedin Pace of Aging Methylation; GrimAA, DNAm GrimAge Acceleration; PhenoAA, accelerated DNAm PhenoAge Acceleration.

Supplementary Table 2. Association between diet quality score and epigenetic age acceleration

	β	SE	*p* -value
DASH			
DunedinPoAm	-0.05	0.02	0.007
GrimAA	-0.09	0.02	4.9E-07
PhenoAA	-0.07	0.02	0.001
AHEI			
DunedinPoAm	-0.07	0.02	4.6E-04
GrimAA	-0.10	0.02	9.9E-09
PhenoAA	-0.10	0.02	3.4E-05
MDS			
DunedinPoAm	-0.08	0.02	3.7E-04
GrimAA	-0.08	0.02	2.9E-05
PhenoAA	-0.07	0.02	0.002

AHEI, Alternate Health Eating Index; DASH, Dietary Approaches to Stop Hypertension; DunedinPoAm, Dunedin Pace of Aging Methylation; GrimAA, DNA methylation GrimAge Acceleration; LCI, lower value of the 95 % confidence interval; UCI, upper value of the 95 % confidence interval; PhenoAA, DNA methylation PhenoAge Acceleration; MDS, Mediterranean-style Diet Score. Linear mixed effects models were fitted to estimate regression coefficients (βs) and 95% CIs with a diet quality score as a fixed effect and family structure as a random effect. The DASH score and epigenetic age acceleration measures were standardized to a mean of 0 and SD of 1. Model was adjusted for categorical smoking status, age, sex, body mass index, physical activity score, alcohol consumption, and energy intake.

Supplementary Table 3. Association between diet quality score and epigenetic age acceleration: Sensitivity analyses considering blood cell counts

	β	SE	*p* -value
DASH			
DunedinPoAm	-0.042	0.018	0.020
GrimAA	-0.083	0.017	9.2E-07
PhenoAA	-0.059	0.022	0.009
AHEI			
DunedinPoAm	-0.052	0.018	0.004
GrimAA	-0.098	0.017	4.8E-09
PhenoAA	-0.082	0.022	1.8E-04
MDS			
DunedinPoAm	-0.068	0.019	3.3E-04
GrimAA	-0.080	0.018	6.4E-06
PhenoAA	-0.054	0.023	0.020

AHEI, Alternate Health Eating Index; DASH, Dietary Approaches to Stop Hypertension; DunedinPoAm, Dunedin Pace of Aging Methylation; GrimAA, DNA methylation GrimAge Acceleration; LCI, lower value of the 95 % confidence interval; UCI, upper value of the 95 % confidence interval; PhenoAA, DNA methylation PhenoAge Acceleration; MDS, Mediterranean-style Diet Score. Linear mixed effects models were fitted to estimate regression coefficients (βs) and 95% CIs with a diet quality score as a fixed effect and family structure as a random effect. The DASH score and epigenetic age acceleration measures were standardized to a mean of 0 and SD of 1. Model was adjusted for age, sex, smoking status, body mass index, physical activity score, alcohol consumption, energy intake, and blood cell counts (CD8+ T cells, CD4+ T, natural killer, B cells, and granulocytes).

Supplementary Table 4. Interaction between DASH and potential effect modifiers on epigenetic age acceleration

Effect modifier \ Epigenetic age acceleration	DunedinPoAm			GrimAA			Age-/Smoking pack-year-adjusted GrimAA			PhenoAA		
	β	SE	*p* -value	β	SE	*p* -value	β	SE	*p* -value	β	SE	*p* -value
Age	-0.004	0.002	0.070	-0.003	0.002	0.099	-0.001	0.003	0.636	-0.004	0.003	0.118
Sex	0.063	0.040	0.119	0.054	0.034	0.114	0.068	0.044	0.124	0.009	0.046	0.847
BMI	0.008	0.004	0.042	0.005	0.003	0.119	0.013	0.004	0.002	0.006	0.004	0.162
Smoking status												
Never-/Ever-smokers	-0.178	0.041	1.3E-05	-0.231	0.036	1.1E-10	-0.258	0.035	2.4E-13	-0.068	0.045	0.131
Never/Former/Current-smokers	-0.130	0.031	2.8E-05	-0.165	0.026	2.2E-10	-0.180	0.024	6.4E-14	-0.042	0.035	0.221
Physical activity	0.005	0.003	0.112	0.004	0.003	0.119	-0.001	0.004	0.798	0.008	0.004	0.032

DASH, Dietary Approaches to Stop Hypertension; DunedinPoAm, Dunedin Pace of Aging Methylation; GrimAA, DNA methylation GrimAge Acceleration; PhenoAA, DNA methylation PhenoAge Acceleration. Linear mixed effects models were fitted to estimate regression coefficients (βs) and standard errors (SE) with the DASH score, each effect modifier variable (age, sex, BMI, smoking status, and physical activity), and interactions between the DASH score and effect modifier variable as fixed effects and family structure as a random effect. The DASH score and epigenetic age acceleration measures were standardized to a mean of 0 and SD of 1. Model was adjusted for age, sex, BMI, smoking status, physical activity score, alcohol consumption, and energy intake.

Supplementary Table 5. Association between DASH and epigenetic age acceleration according to smoking status

Epigenetic age acceleration / Smoking status	DunedinPoAm				GrimAA				PhenoAA			
	β	LCI	UCI	*p* -value	β	LCI	UCI	*p* -value	β	LCI	UCI	*p* -value
Never smoker (*n* = 1,149)	-0.016	-0.065	0.032	0.511	-0.030	-0.066	0.007	0.115	-0.062	-0.121	-0.003	0.040
Ever smoker (*n* = 846)	-0.178	-0.249	-0.106	1.4E-06	-0.249	-0.317	-0.180	4.2E-12	-0.104	-0.174	-0.034	0.004
Former smoker (*n* = 687)	-0.082	-0.153	-0.011	0.023	-0.133	-0.201	-0.065	1.6E-04	-0.076	-0.158	0.006	0.070
Current smoker (*n* = 159)	-0.152	-0.357	0.054	0.142	-0.256	-0.407	-0.105	0.002	-0.108	-0.257	0.041	0.150

BMI, Body mass index; DASH, Dietary Approaches to Stop Hypertension diet score; DenedinPoAm, Dunedin Pace of Aging Methylation; GrimAA, DNAm GrimAge Acceleration; LCI, lower value of the 95 % confidence interval; UCI, upper value of the 95 % confidence interval; PhenoAA, accelerated DNAm PhenoAge Acceleration. Linear mixed effects models were fitted to estimate regression coefficients (βs) and 95% CIs with the DASH score as a fixed effect and family structure as a random effect. The DASH score and epigenetic age acceleration measures were standardized to a mean of 0 and SD of 1. Model was adjusted for age, sex, BMI, physical activity score, alcohol consumption, and energy intake. Each stratification factor was removed from the covariates.

Supplementary Table 6. Longitudinal associations of diet quality score and epigenetic age acceleration on the risk of all-cause mortality

	HR	LCI	UCI	*p* -value
Epigenetic age acceleration				
DunedinPoAm	1.394	1.206	1.610	6.8E-06
GrimAA	1.617	1.405	1.860	1.9E-11
PhenoAA	1.226	1.093	1.375	0.001
Diet quality score				
DASH	0.880	0.778	0.996	0.043
AHEI	0.859	0.757	0.974	0.018
MDS	0.856	0.753	0.974	0.018

AHEI, Alternate Healthy Eating Index score; DASH, Dietary Approaches to Stop Hypertension diet score; DunedinPoAm, Dunedin Pace of Aging Methylation; GrimAA, DNA methylation GrimAge Acceleration; HR, Hazard ratio; LCI, lower value of the 95 % confidence interval; MDS, Mediterranean-style diet score; PhenoAA, DNA methylation PhenoAge Acceleration; SD, standard deviation; UCI, upper value of the 95 % confidence interval. The sample size is 1,899 participants in the analysis for all-cause mortality. Mxed effect Cox proportional hazards model was applied to estimate HRs with 95% CIs for each independent variable per 1 SD increase. Continuous standardized values of each diet quality score or epigenetic age acceleration were entered as independent variables in separate models. The model was adjusted for age, sex, smoking status, BMI, physical activity score, alcohol consumption, energy intake, systolic blood pressure, use of antihypertension medications, high density lipoprotein cholesterol, total cholesterol, type 2 diabetes, and history of cardiovascular disease and cancer. For measures of epigenetic age acceleration, models were further adjusted for differential blood counts (CD8+ T cells, CD4+ T, natural killer, B cells, and granulocytes).

Supplementary Table 7. Mediation effect of epigenetic age acceleration on the association of diet quality scores with all-cause mortality

DNAmAge	AHEI					MDS				
	HR	LCI	UCI	p-value	Proportion mediated (%)	HR	LCI	UCI	*p* -value	Proportion mediated (%)
All										
DunedinPoAm	0.975	0.956	0.990	0.012	20.8%	0.972	0.952	0.988	0.009	27.5%
AgeAccelGrim	0.957	0.937	0.975	2.2E-04	37.5%	0.967	0.948	0.984	0.002	29.5%
AgeAccelPheno	0.975	0.958	0.988	0.005	15.7%	0.980	0.964	0.993	0.019	13.6%
Never smoker										
DunedinPoAm	0.986	0.964	1.002	0.156	23.9%	0.980	0.957	0.998	0.095	0.0%
AgeAccelGrim	0.981	0.961	0.996	0.061	25.5%	0.984	0.965	1.000	0.106	54.1%
AgeAccelPheno	0.979	0.957	0.995	0.056	53.8%	0.980	0.957	0.996	0.070	19.7%
Ever smoker										
DunedinPoAm	0.928	0.884	0.965	0.005	23.6%	0.930	0.886	0.968	0.007	23.8%
AgeAccelGrim	0.891	0.844	0.933	1.6E-04	43.2%	0.915	0.870	0.954	0.001	28.2%
AgeAccelPheno	0.966	0.937	0.989	0.024	11.0%	0.978	0.951	1.001	0.102	7.0%

AHEI, Alternate Health Eating Index; DASH, Dietary Approaches to Stop Hypertension; DunedinPoAm, Dunedin Pace of Aging Methylation; GrimAA, DNA methylation GrimAge Acceleration; HR, Hazard ratio; LCI, lower value of the 95 % confidence interval; SD, standard deviation; UCI, upper value of the 95 % confidence interval; PhenoAA, DNA methylation PhenoAge Acceleration; MDS, Mediterranean-style Diet Score. Hazard ratios per 1 increase of SD of DASH score in standardized z-score and *P* values were derived from mixed effect Cox proportional hazard models. Linear mixed effect model was estimated for epigenetic age acceleration (mediator) conditional on the DASH score (exposure) and covariates. Mixed effect Cox proportional hazard model was estimated for all-cause mortality (outcome) conditional on the DASH score, epigenetic age acceleration, and covariates to estimate the indirect (mediation) effect. The proportion of mediation was calculated as the ratio of indirect effect to the sum of both direct and indirect effect using R package mediation. Models were adjusted for age, sex, body mass index, smoking status, physical activity score, alcohol intake, energy intake, body mass index, systolic blood pressure, hypertension medications, high-density lipoprotein cholesterol, total cholesterol, type 2 diabetes, and history of cardiovascular disease and cancer. For stratified analysis on smoking status, the same covariates, except for smoking status, were used as in the models for all participants.

Supplementary Table 8. Significance level for mediation effect of epigenetic age acceleration on the association of diet with all-cause mortality after additional adjustment for metformin use

DNAmAge	DASH	AHEI	MDS
DunedinPoAm	0.021	0.007	0.006
AgeAccelGrim	0.001	9.6E-05	0.001
AgeAccelPheno	0.022	0.005	0.018

AHEI, Alternate Health Eating Index; DASH, Dietary Approaches to Stop Hypertension; DunedinPoAm, Dunedin Pace of Aging Methylation; GrimAA, DNA methylation GrimAge Acceleration; PhenoAA, DNA methylation PhenoAge Acceleration; MDS, Mediterranean-style Diet Score. Linear mixed effect and mixed effect Cox proportional hazard models were estimated to estimate the indirect (mediation) effect. Models were further adjusted for metformin use (n = 111) in addition to age, sex, body mass index, smoking status, physical activity score, alcohol intake, energy intake, body mass index, systolic blood pressure, hypertension medications, high-density lipoprotein cholesterol, total cholesterol, type 2 diabetes, and history of cardiovascular disease and cancer.

Supplementary Table 9. Association between individual DASH components and epigenetic age acceleration

Diet	DunedinPoAm				GrimAA				PhenoAA			
	β	SE	*p* -value[1]	*p* -value[2]	β	SE	*p* -value[1]	*p* -value[2]	β	SE	*p* -value[1]	*p* -value[2]
Vegetables	-0.026	0.015	0.078	0.154	-0.029	0.013	0.020	0.027	-0.031	0.017	0.062	0.043
Fruits	-0.021	0.015	0.166	0.225	-0.039	0.013	0.002	0.003	-0.041	0.017	0.018	0.014
Nuts and Legumes	-0.047	0.014	0.001	0.001	-0.028	0.012	0.021	0.022	-0.042	0.016	0.011	0.010
Whole grains	-0.027	0.014	0.059	0.094	-0.025	0.012	0.044	0.056	-0.015	0.016	0.361	0.345
Low fat dairy	-0.003	0.015	0.838	0.855	-0.024	0.012	0.055	0.056	0.023	0.017	0.167	0.165
Red and processed meats	0.015	0.015	0.315	0.668	0.034	0.013	0.008	0.032	0.025	0.017	0.142	0.181
Sugar-sweetened beverages	0.007	0.015	0.633	0.838	0.017	0.013	0.184	0.430	0.050	0.017	0.004	0.009
Sodium	0.017	0.015	0.240	0.269	0.044	0.013	0.001	0.001	0.027	0.017	0.109	0.113

DASH, Dietary Approaches to Stop Hypertension diet score; GrimAA, DNAm GrimAge Acceleration; PhenoAA, accelerated DNAm PhenoAge Acceleration; DenedinPoAm, Dunedin Pace of Aging Methylation; SE, standard errors. Linear mixed effects models were fitted to estimate regression coefficients (βs) and SEs with a continuous DASH component score as a fixed effect and family structure as a random effect.

[1] Model was adjusted for age, sex, BMI, smoking status, physical activity score, alcohol consumption, and energy intake.

[2] Model was further adjusted for modified DASH score by excluding each of the individual components from the total DASH score

Supplementary Table 10. Association between individual DASH components and epigenetic age acceleration according to smoking status

Diet	DunedinPoAm			GrimAA			PhenoAA		
	β	SE	*p* -value	β	SE	*p* -value	β	SE	*p* -value
Never smoker (*n* =1,149)									
Vegetables	-0.016	0.017	0.360	-0.004	0.013	0.756	-0.022	0.021	0.301
Fruits	-0.013	0.018	0.454	-0.011	0.013	0.414	-0.020	0.022	0.356
Nuts and Legumes	-0.027	0.017	0.107	-0.026	0.013	0.042	-0.054	0.020	0.008
Whole grains	0.009	0.017	0.593	-0.005	0.013	0.696	-0.013	0.021	0.522
Low fat dairy	0.015	0.017	0.368	0.006	0.013	0.617	0.025	0.021	0.235
Red and processed meats	0.018	0.017	0.300	0.025	0.013	0.053	0.048	0.021	0.023
Sugar-sweetened beverages	-0.001	0.018	0.950	0.007	0.014	0.598	0.033	0.022	0.131
Sodium	0.001	0.017	0.959	0.012	0.013	0.380	0.018	0.021	0.403
Ever smoker (*n* =846)									
Vegetables	-0.057	0.025	0.026	-0.069	0.025	0.005	-0.031	0.025	0.218
Fruits	-0.060	0.026	0.023	-0.099	0.025	1.1E-04	-0.065	0.026	0.012
Nuts and Legumes	-0.096	0.025	1.8E-04	-0.076	0.025	0.002	-0.047	0.025	0.059
Whole grains	-0.099	0.025	9.3E-05	-0.097	0.024	8.5E-05	-0.028	0.025	0.257
Low fat dairy	-0.052	0.026	0.045	-0.086	0.025	0.001	0.006	0.025	0.827
Red and processed meats	0.027	0.026	0.310	0.059	0.025	0.022	0.016	0.026	0.547
Sugar-sweetened beverages	0.046	0.027	0.082	0.078	0.026	0.002	0.069	0.026	0.008
Sodium	0.046	0.025	0.072	0.087	0.024	4.4E-04	0.017	0.025	0.496

DASH, Dietary Approaches to Stop Hypertension diet score; DenedinPoAm, Dunedin Pace of Aging Methylation; GrimAA, DNAm GrimAge Acceleration; PhenoAA, accelerated DNAm PhenoAge Acceleration; SE, standard errors. For stratified analysis, in each smoking status group, linear mixed effects models were fitted to estimate regression coefficients (βs) and SEs with a continuous DASH component score as a fixed effect and family structure as a random effect. Three measures of epigenetic age acceleration were standardized to a mean of 0 and SD of 1. Model was adjusted for age, sex, BMI, physical activity score, alcohol consumption, and energy intake.