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Original Article

Body Size, Diet Quality, and Epigenetic Aging: Cross-Sectional and Longitudinal Analyses

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Abstract

Epigenetic age is an emerging marker of health that is highly predictive of disease and mortality risk. There is a lack of evidence on whether lifestyle changes are associated with changes in epigenetic aging. We used data from 1 041 participants in the Melbourne Collaborative Cohort Study with blood DNA methylation measures at baseline (1990–1994, mean age: 57.4 years) and follow-up (2003–2007, mean age: 68.8 years). The Alternative Healthy Eating Index-2010 (AHEI-2010), the Mediterranean Dietary Score, and the Dietary Inflammatory Index were used as measures of diet quality, and weight, waist circumference, and waist-to-hip ratio as measures of body size. Five age-adjusted epigenetic aging measures were considered: *GrimAge*, *PhenoAge*, *PCGrimAge*, *PCPhenoAge*, and *DunedinPACE*. Multivariable linear regression models including restricted cubic splines were used to assess the cross-sectional and longitudinal associations of body size and diet quality with epigenetic aging. Associations between weight and epigenetic aging cross-sectionally at both time points were positive and appeared greater for *DunedinPACE* (per *SD*: β ~0.24) than for *GrimAge* and *PhenoAge* (β ~0.10). The longitudinal associations with weight change were markedly nonlinear (U-shaped) with stable weight being associated with the lowest epigenetic aging at follow-up, except for *DunedinPACE*, for which only weight gain showed a positive association. We found negative, linear associations for AHEI-2010 both cross-sectionally and longitudinally. Other adiposity measures and dietary scores showed similar results. In middle-aged to older adults, declining diet quality and weight gain may increase epigenetic age, while the association for weight loss may require further investigation. Our study sheds light on the potential of weight management and dietary improvement in slowing aging processes.

Keywords: Adiposity, Biological age, Diet, Epigenetic aging, Lifestyle, Obesity

Biological aging is a process of cumulative deterioration of human organs, cells, and molecules (1). It takes into account the heterogeneity of the degenerative process between individuals and reflects physiological health status and risk of age-related health events (2). Recent technology developments allow biological age to be estimated with epigenomic, transcriptomic, proteomic, or metabolomic data (3). Based on DNA methylation (DNAm) data, epigenetic age measures have shown promise as predictors of age, age-related health conditions, and mortality (4). Hannum (5) and Horvath (6) were the "first generation" epigenetic aging measures that used methylation markers to predict chronological age. More recent measures including *GrimAge*, *PhenoAge*, and *DunedinPACE* (7–9), the "second and third generation epigenetic clocks," were designed to more directly estimate biological aging by predicting mortality or rate of aging.

Obesity and poor diet are well-established risk factors for many health outcomes including cardiovascular diseases, cancers, and all-cause mortality (10,11). The association between lifestyle factors, for example, body size and diet, and biological aging remains unclear (12,13). Several studies have assessed the cross-sectional associations of weight and diet with epigenetic aging. Kim et al. (14) found that higher diet quality was associated with lower *GrimAge*, *PhenoAge*, and *DunedinPoAm*. Intakes of specific food or nutrients such as fish, fruit, and vegetables were found to be associated with lower epigenetic aging (7,8,15). Analysis of data from 2 758 women in the Sister Study found positive associations of weight, waist circumference, and waist-to-hip ratio (WHR) with epigenetic aging (16).

In a 2-year randomized 4-armed trial of 219 women, 57 participants in the dietary intervention arm in which advice on healthy diet was given had reduced *GrimAge* compared with the control arm (17). Only a few studies have examined lifestyle changes using longitudinal methylation data. In the study by Quach et al. (15) based on 239 participants, an

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increase in BMI over 2.7 years was associated with increased Horvath age.

Most existing evidence on the associations of body size and diet quality with epigenetic aging was based on cross-sectional data. Many studies on diet focused on specific nutrients or foods, but fewer have considered overall diet quality. Most studies have assessed the first-generation epigenetic age measures, which are less effective in estimating biological age and predicting mortality (4,15,18–21).

This study aimed to add to the evidence on the potential causal effect of body size and diet quality on epigenetic aging through longitudinal data analysis, by assessing, in middle aged and older Australians, whether changes in body size and diet quality over a decade are associated with changes in epigenetic aging.

Method

Study Participants

We used data from participants in the Melbourne Collaborative Cohort Study (MCCS), a prospective cohort study with 41 513 Australians (59% females) recruited between 1990 and 1994 (22). All participants were of white European origin, with 99% aged between 40 and 69 years at recruitment. An in-person follow-up in 2003–2007 collected updated data on lifestyle and health, and physical measurements and blood samples were collected (22).

A subset of 1 100 participants who were selected as controls in 6 cancer case-control studies nested in MCCS was used in the current study. A flowchart of the participant selection process is shown in Supplementary Figure 1. Genome-wide DNA methylation was measured in these 1 100 participants who had blood samples (dried blood spot on Guthrie card) available at baseline and follow-up using the Illumina HumanMethylation450K BeadChip array (Supplementary Methods). The methods relating to DNA extraction and DNA methylation data processing have been described in previous MCCS studies (23,24).

The MCCS was approved by the Human Research Ethics Committee of the Cancer Council Victoria, Melbourne, VIC, Australia, and informed consent was provided by all participants according to the Declaration of Helsinki.

Exposures

Physical measurements of weight, waist circumference, and hip circumference were performed by trained personnel at both baseline and follow-up; height was measured at baseline only.

Diet quality was primarily analyzed using the Alternative Healthy Eating Index 2010 (AHEI-2010) score, which was calculated based on food frequency questionnaires (FFQ) specifically designed for the MCCS at baseline and follow-up (Supplementary Methods); it is a literature-based score that includes dietary components associated with lower risk of chronic diseases and can range between 0 and 110 (25). We also considered the Mediterranean Dietary Score (MDS, ranging between 0 and 9) and the Dietary Inflammatory Index (DII, ranging between –8.9 and 8.0) in secondary analyses. The MDS indicates the degree of adherence to the traditional Mediterranean diet (26). The DII is a literature-based dietary score that was developed based on foods and nutrients associated with 6 selected inflammatory markers; a higher DII score indicates a pro-inflammatory potential of the diet. DII scores were calculated by Connecting Health Innovations LLC using 29 out of 45 foods and nutrients available from the FFQs (27).

For the longitudinal analysis, absolute changes in exposures were calculated for both body size measures and dietary scores.

Outcomes

The outcome of interest, epigenetic aging, was estimated with 3 age-adjusted epigenetic aging measures: *GrimAge*, *PhenoAge* and their improved versions *PCGrimAge* and *PCPhenoAge*, and *DunedinPACE* (21,28). Although many other epigenetic aging measures have been developed, these 3 are widely used and were found to be strongly and consistently associated with mortality and disease risk. *GrimAge* was developed based on chronological age, sex, and 8 DNAm-based biomarkers including a biomarker of smoking pack-years (8), although *PhenoAge* was developed with 10 clinical markers to predict mortality (7). These epigenetic aging measures were based on individual CpGs where methylation measurement is differentially reliable (23). To improve their reliability, Higgins-Chen et al. (29) developed a method based on principal components which we used to calculate *PCGrimAge* and *PCPhenoAge*. *DunedinPACE* was generated with 173 CpGs based on the Pace of Aging, which was calculated based on the changes in 19 biomarkers across 4 time points during a 20-year follow-up among participants from the Dunedin study who were of the same chronological age of 26 at baseline, and free of age-related diseases (9). We calculated *GrimAge* and *PhenoAge* using the Horvath Lab's web tool (6), *PCGrimAge* and *PCPhenoAge* using the *dnaMethyAge* package (30), and *DunedinPACE* using the R code provided in the original publication (9).

Batch effects were considered and investigated using linear mixed effects models with random effects for study, and assay plate and slides (24,31,32), but not retained as they produced virtually identical results because a small proportion of epigenetic aging variation was explained by technical factors at either baseline or follow-up 2.

For each measure, the residuals of a linear regression model of epigenetic age on chronological age were extracted to obtain age-adjusted estimates of aging and were used in all analyses.

Covariates

The covariates we considered were collected via questionnaires during interviews at baseline and follow-up, including sex (male, female), country of birth (Australia/New Zealand, Northern Europe, Southern Europe), self-reported health status (excellent, very good, good, fair, and poor), smoking status (never smoked, former smoker, and current smoker), smoking pack-years (log transformed), socioeconomic status (socioeconomic index for areas (SEIFA) score, decile), alcohol consumption (g/day), height (m), and physical activity (log transformed; baseline: frequency score (Supplementary Methods); follow-up: metabolic equivalent of task (MET) score).

Data Preparation

Of 1 100 initially selected participants with methylation data measured at baseline and follow-up, 1 041 were included in the data analysis after the exclusion of samples that did not pass quality controls or were outliers (Supplementary Figure 1).

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There were 0%–12% missing values at baseline and follow-up (Supplementary Table 1) which were imputed using the *missForest* method. *MissForest* is a simple imputation approach where a random forest is fitted to observed values to predict missing values iteratively until there is an increase in the difference between 2 imputation results or preset criteria are met (33). It performs well with mixed data types and a relatively small proportion of missing data (33).

Statistical Analysis

Pearson correlation coefficients were calculated between height-adjusted adiposity measures (residuals of the linear regression of each measure on height) and dietary scores, and between epigenetic aging measures. For easier comparison of the results, exposures and outcomes were standardized to *z*-scores (mean of 0 and *SD* of 1).

Multivariable linear regression models were used to assess cross-sectional and longitudinal associations with epigenetic aging. For each model, potential nonlinearity was assessed using restricted cubic splines (using 3 knots at the 10th, 50th, and 90th percentiles), comparing models with splines or linear terms using a likelihood ratio test (LRT). When nonlinearity was detected ($p < .05$), linear terms were not presented, and models including splines were presented graphically (including scatter plots). To avoid other potential age-related effects, such as cohort effects (34), chronological age was adjusted in all models even though epigenetic aging measures were independent of age.

The cross-sectional analyses were carried out separately for baseline and follow-up data. Model 1 adjusted for age, sex, and country of birth, and additionally for height when assessing associations with body size measures. Model 2 additionally adjusted for socioeconomic status, smoking status, smoking pack-years, alcohol consumption, and physical activity, and additionally for BMI when assessing associations with diet quality, and for AHEI-2010 for the associations with body size measures.

To assess the ability of our longitudinal analyses to detect epigenetic aging changes over a decade, we first examined the association between quitting smoking and *GrimAge* at follow-up (which is highly influenced by smoking because it includes a methylation-based predictor of smoking pack-years (8)) in the 103 participants who reported being current smokers at baseline. The associations of *PhenoAge* and *DunedinPACE* were also calculated for comparison.

For longitudinal analyses of associations of weight change and diet quality change with follow-up 2 epigenetic aging, adjusting for respective baseline epigenetic aging measures, 3 models were considered: (i) adjusting for age, sex, country of birth, baseline epigenetic aging, as well as baseline BMI when assessing associations with body size changes, and baseline diet score for associations with diet quality change; (ii) additionally adjusting for socioeconomic status, physical activity, smoking status, smoking pack-years, alcohol consumption, as well as the height and baseline AHEI-2010 for associations with adiposity measures, and baseline BMI for associations with diet quality; (iii) for the association with weight change (diet quality change, respectively), additionally adjusting for AHEI-2010 change (weight change, respectively). It is noted that weight change is likely a mediator, rather than a confounder of the diet change/epigenetic aging association. Although a formal mediation analysis would be needed to determine the mediation effect of weight change, comparing Model 2 and Model 3 still provides an approximation of the proportion of the effect of diet change on epigenetic aging that may be attributed to weight change. As secondary analyses, the same longitudinal analyses were repeated using waist circumference and WHR to measure body size, and MDS and DII to measure diet quality. Baseline epigenetic age was adjusted in all models to account for baseline differences between participants. Baseline AHEI-2010 and BMI were adjusted in each model according to the directed acyclic graphs (Supplementary Figures 2 and 3), considered as being associated with changes in AHEI-2010 and weight during follow-up (Supplementary Figures 4 and 5).

We performed several sensitivity analyses to assess the robustness of our findings: (i) To explore potential noise in epigenetic aging markers, we repeated the cross-sectional and longitudinal analyses using *PCGrimAge* and *PCPhenoAge*; (ii) to control for weight changes caused by health deterioration (eg, unintentional weight loss), we added self-reported health status to Model 2; (iii) we explored and minimized the influence of potential outliers via 2 sensitivity analyses, first by excluding participants whose weight changed by more than 20 kg over follow-up, and second by restricting the analysis to participants with a BMI between 20 and 35 kg/m^2 at baseline. The thresholds were chosen based on a study showing that the BMI-mortality association for individuals with BMI below 20 kg/m^2 or over 35kg/m^2 either deviated from a linear trend or was largely affected by smoking and health conditions (35).

All statistical analyses were conducted with Stata version 17.0 and R version 4.3.0 and all *p*-values were two-sided.

Results

Sample Characteristics

Of 1 041 MCCS participants in this study, 68.4% were male. The average age was 57.4 years at baseline and 68.8 years at follow-up. The majority (76.4%) of participants were born in Australia or New Zealand, and 14.7% were born in Southern Europe. Ten percent were smokers at baseline and 5% at follow-up (Table 1).

The correlations between dietary scores and adiposity measures (height-adjusted), and between epigenetic aging markers, are shown in Supplementary Tables 2–4. Moderate to strong correlations were found between height-adjusted adiposity measures (eg, at baseline: 0.83 between weight and WC and 0.49 between weight and WHR). Weight change was strongly correlated with waist circumference change ($r = 0.65$) and moderately correlated with the change in WHR ($r = 0.34$). DII and MDS at baseline were strongly correlated with each other ($r = -0.63$), but only moderately with AHEI-2010 ($r = -0.29$ and 0.30, respectively). Epigenetic aging markers were moderately correlated, and the correlation was strongest between *GrimAge* and *DunedinPACE* (follow-up: $r = 0.59$).

Cross-Sectional Associations at Baseline and Follow-Up

There was no evidence of departure from linearity at either time point (LRT $p > .32$ for weight and $p > .39$ for AHEI-2010).

Weight was positively associated with epigenetic aging at both baseline and follow-up, with no major attenuation of

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Table 1. Characteristics of the Study Participants (*N* = 1 041, Melbourne Collaborative Cohort Study)

	Baseline		Follow-up	
Age (y), mean (*SD*)	57.4	(7.9)	68.8	(8.1)
Sex, *N* (%)				
Male	712	(68.4)		
Female	329	(31.6)		
Country of birth, *N* (%)				
Australia/New Zealand	795	(76.4)		
Northern Europe	93	(8.9)		
Southern Europe	153	(14.7)		
SEIFA score, median (IQR)	7	(3, 9)	7	(4, 9)
BMI (kg/m^2), mean (*SD*)	26.7	(3.8)	27.1	(4.2)
Weight (kg), mean (*SD*)	76.2	(12.9)	77.4	(13.9)
Height (cm), mean (*SD*)	168.9	(9.0)		
Weight change (kg), mean (*SD*)			1.1	(5.9)
Physical activity				
METS (ln(METS + 1)), median (IQR)			3	(2.2, 3.6)
Physical activity frequency score* (ln(frequency + 1)), median (IQR)	1.6	(0.9, 1.9)		
Alcohol consumption (g/d), median (IQR)	7.4	(0.0, 19.1)	9.4	(0.0, 22.7)
Smoking				
Current, *N* (%)	103	(9.9)	55	(5.3)
Former, *N* (%)	407	(39.1)	456	(43.8)
Never, *N* (%)	531	(51.0)	530	(50.9)
Quantity (log(pack/day)), median (IQR)	0	(0, 5.9)		
Diet scores				
AHEI-2010, mean (*SD*)	64	(11.1)	52.6	(10.2)
MDS, mean (*SD*)	4.4	(1.7)	4.7	(1.6)
DII, median (IQR)	−1	(−2, 0)	0	(−1, 1)
Diet change (AHEI), mean (*SD*)			−11.4	(10.7)
Waist circumference (cm), mean (*SD*)	88.2	(11.9)	94.4	(12.1)
WHR, mean (*SD*)	0.88	(0.1)	0.91	(0.1)
Self-reported health status, *N* (%)				
Excellent			169	(16.2)
Very good			411	(39.5)
Good			330	(31.7)
Fair			111	(10.7)
Poor			20	(1.9)
Age-adjusted epigenetic age, mean (*SD*)				
GrimAge (y)	−0.04	(4.26)	−0.05	(4.10)
PhenoAge (y)	−0.07	(7.03)	−0.09	(6.65)
DunedinPACE	−0.001	(−0.115)	−0.003	(−0.114)

Notes: AHEI-2010 = Alternative Healthy Eating Index 2010; BMI = body mass index; DII = dietary inflammatory index; IQR = interquartile range; MDS = mediterranean dietary score; MET = metabolic equivalent of task; *SD* = standard deviation; SEIFA = socioeconomic index for areas; WHR = waist-to-hip ratio.
*Physical activity frequency score was developed according to the frequency of different types of physical activity, including walking, less vigorous activity, and vigorous activity, ranges between 0 and 16, where: 0 = none, 1.5 = 1 or 2 times per week, 4 = more than 3 times per week, twice the weight was assigned to vigorous activity.

the coefficients after confounder adjustment. In Model 2, the strongest associations were found between weight and *DunedinPACE*, with coefficients per *SD* of 0.23 (95% confidence interval (CI): 0.16, 0.30; $p = 10^{-10}$) at baseline and 0.24 (95% CI: 0.18, 0.31; $p = 10^{-12}$) at follow-up. Weaker associations were found with *GrimAge* and *PhenoAge* at both time points ($p \leq .04$, β per *SD* between 0.06 and 0.12). Overall similar associations were observed for waist circumference and WHR, Table 2.

The regression coefficients between AHEI-2010 and epigenetic aging in Model 1 ranged between −0.04 and −0.17; compared with weight, these were more substantially attenuated after adjustment for other lifestyle factors. There was evidence of an association with *PhenoAge* at follow-up (Model 2, per *SD*: β = −0.09, 95% CI: 0.15, −0.03) but not at baseline (Model 2: β= −0.02; $p = .63$). Similar associations were observed for the MDS (negative) and the DII (positive), Table 2.

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Longitudinal Associations

Of 103 participants who were smokers at baseline, 54 (52%) had quit at follow-up and their *GrimAge* was estimated to be 0.4-*SD* (per *SD*: 95% CI: −0.7, −0.1), ie, 1.6 *GrimAge* "years" lower at follow-up than those who had continued smoking. Similar associations, albeit less robust, were found with *PhenoAge* (per *SD*: β= −0.3; 95% CI: −0.6, 0.1) and *DunedinPACE* (per *SD*: β= −0.3; 95% CI: −0.7, 0.0), Table 3.

Longitudinal associations between weight and epigenetic aging were nonlinear (LRT $p < .022$). Only minimal changes were observed between the 3 spline models adjusting for different sets of confounders, therefore, only the restricted cubic splines models adjusting for all confounders except diet change (Model 2) were presented graphically, using non-standardized weight values (Figure 1). Overall, the U-shaped curves were quite similar across the 3 epigenetic aging measures with stable weight being associated with lowest epigenetic age. The U shape appeared more pronounced for *PhenoAge*, and only weight gain, but not weight loss, was associated with higher *DunedinPACE*.

The associations of diet quality changes with epigenetic aging at follow-up were consistent with linear associations (LRT $p > .09$), with improvement in diet quality being associated with decreased epigenetic aging. In Model 2, 1 *SD*

Table 2. Cross-Sectional Associations of Body Size Measures and Dietary Scores With Epigenetic Aging (*N* = 1 041)

Exposure	Stage	Model	GrimAge			PhenoAge			DunedinPACE		
			β	95% CI	*p*-Value	β	95% CI	*p*-Value	β	95% CI	*p*-Value
Weight	Baseline	Model 1*	0.13	0.06–0.20	2×10^{-4}	0.12	0.05–0.20	.001	0.26	0.18–0.33	4×10^{-12}
		Model 2**	0.08	0.02–0.13	.01	0.12	0.04–0.19	.002	0.23	0.16–0.29	3×10^{-10}
	Wave 2	Model 1	0.11	0.04–0.18	.001	0.10	0.03–0.17	.004	0.27	0.20–0.34	1×10^{-14}
		Model 2	0.06	0.002–0.11	.04	0.08	0.01–0.15	.03	0.24	0.18–0.31	1×10^{-12}
Waist circumference	Baseline	Model 1	0.14	0.08–0.21	3×10^{-5}	0.12	0.04–0.19	.002	0.24	0.17–0.31	2×10^{-11}
		Model 2	0.08	0.02–0.14	.006	0.11	0.03–0.18	.004	0.21	0.14–0.28	2×10^{-9}
	Wave 2	Model 1	0.14	0.08–0.20	1×10^{-5}	0.10	0.03–0.16	.004	0.27	0.20–0.33	6×10^{-16}
		Model 2	0.06	0.01–0.11	.03	0.06	−0.01 to 0.13	.07	0.22	0.16–0.29	5×10^{-12}
WHR	Baseline	Model 1	0.16	0.08–0.24	1×10^{-4}	0.04	−0.05 to 0.13	.34	0.20	0.12–0.29	5×10^{-6}
		Model 2	0.05	−0.02 to 0.12	.14	0.02	−0.07 to 0.11	.64	0.16	0.07–0.24	3×10^{-4}
	Wave 2	Model 1	0.21	0.14–0.29	3×10^{-8}	0.12	0.04–0.20	.003	0.30	0.22–0.38	5×10^{-14}
		Model 2	0.06	−0.004 to 0.12	.07	0.06	−0.02 to 0.15	.12	0.22	0.14–0.29	3×10^{-8}
Diet quality (AHEI-2010)	Baseline	Model 1	−0.13	−0.18 to −0.07	2×10^{-5}	−0.04	−0.10 to 0.03	.25	−0.11	−0.17 to −0.05	6×10^{-4}
		Model 2	−0.06	−0.11 to −0.01	.01	−0.02	−0.08 to 0.05	.63	−0.08	−0.14 to −0.02	.01
	Wave 2	Model 1	−0.17	−0.22 to −0.11	7×10^{-9}	−0.13	−0.19 to −0.07	2×10^{-5}	−0.17	−0.23 to −0.12	6×10^{-9}
		Model 2	−0.07	−0.12 to −0.02	.004	−0.09	−0.15 to −0.03	.002	−0.09	−0.15 to −0.04	.001
MDS	Baseline	Model 1	−0.08	−0.14 to −0.02	.005	−0.03	−0.09 to 0.03	.27	−0.04	−0.10 to 0.02	.23
		Model 2	−0.06	−0.10 to −0.01	.02	−0.04	−0.10 to 0.02	.24	−0.02	−0.07 to 0.04	.57
	Wave 2	Model 1	−0.10	−0.16 to −0.05	3×10^{-4}	−0.10	−0.15 to −0.04	.001	−0.12	−0.18 to −0.06	4×10^{-5}
		Model 2	−0.05	−0.10 to −0.01	.02	−0.08	−0.14 to −0.02	.01	−0.07	−0.13 to −0.01	.01
DII	Baseline	Model 1	0.13	0.07–0.18	2×10^{-5}	0.02	−0.05 to 0.08	.63	0.06	−0.00 to 0.12	.07
		Model 2	0.05	0.01–0.10	.03	0.00	−0.06 to 0.06	1.00	0.01	−0.05 to 0.07	.71
	Wave 2	Model 1	0.15	0.10–0.21	1×10^{-7}	0.10	0.04–0.16	.001	0.14	0.08–0.20	2×10^{-6}
		Model 2	0.07	0.02–0.11	.01	0.07	0.01–0.13	.02	0.08	0.02–0.13	.007

*Model 1: Adjusted for age, sex, and country of birth, height was additionally adjusted for associations of body size measures.
**Model 2: Adjusted for age, sex, country of birth, SES, physical activity, smoking status, smoking pack-years, and alcohol consumption; cross-sectional analysis on body size measures also adjusted for diet quality (AHEI-2010); cross-sectional analysis on dietary scores also adjusted for BMI.
Epigenetic aging measures, body size measures, and dietary scores were standardized to a mean of 0 and *SD* of 1.
Epigenetic aging measures were age-adjusted.

Table 3. Longitudinal Association Between Quitting Smoking and Epigenetic Aging at Follow-up (*N* = 103).

Exposure	Models	GrimAge			PhenoAge			DunedinPACE		
		β	95% CI	*p*-Value	β	95% CI	*p*-Value	β	95% CI	*p*-Value
Quitting smoking (*N* = 54)*	Standardized	−0.38	−0.67 to −0.09	.01	−0.26	−0.64 to 0.12	.18	−0.34	−0.68 to 0.00	.05
	(Nonstandardized)	−1.58	−2.78 to −0.37	.01	−1.78	−4.39 to 0.83	.18	−0.04	−0.08 to 0.00	.05

*Of 103 smokers at baseline, 54 quitted smoking during follow-up. Reference category: smokers at baseline and follow-up.
The model adjusted for age, sex, country of birth, and baseline epigenetic aging measure.
Epigenetic aging measures were age-adjusted and standardized to a mean of 0 and *SD* of 1.

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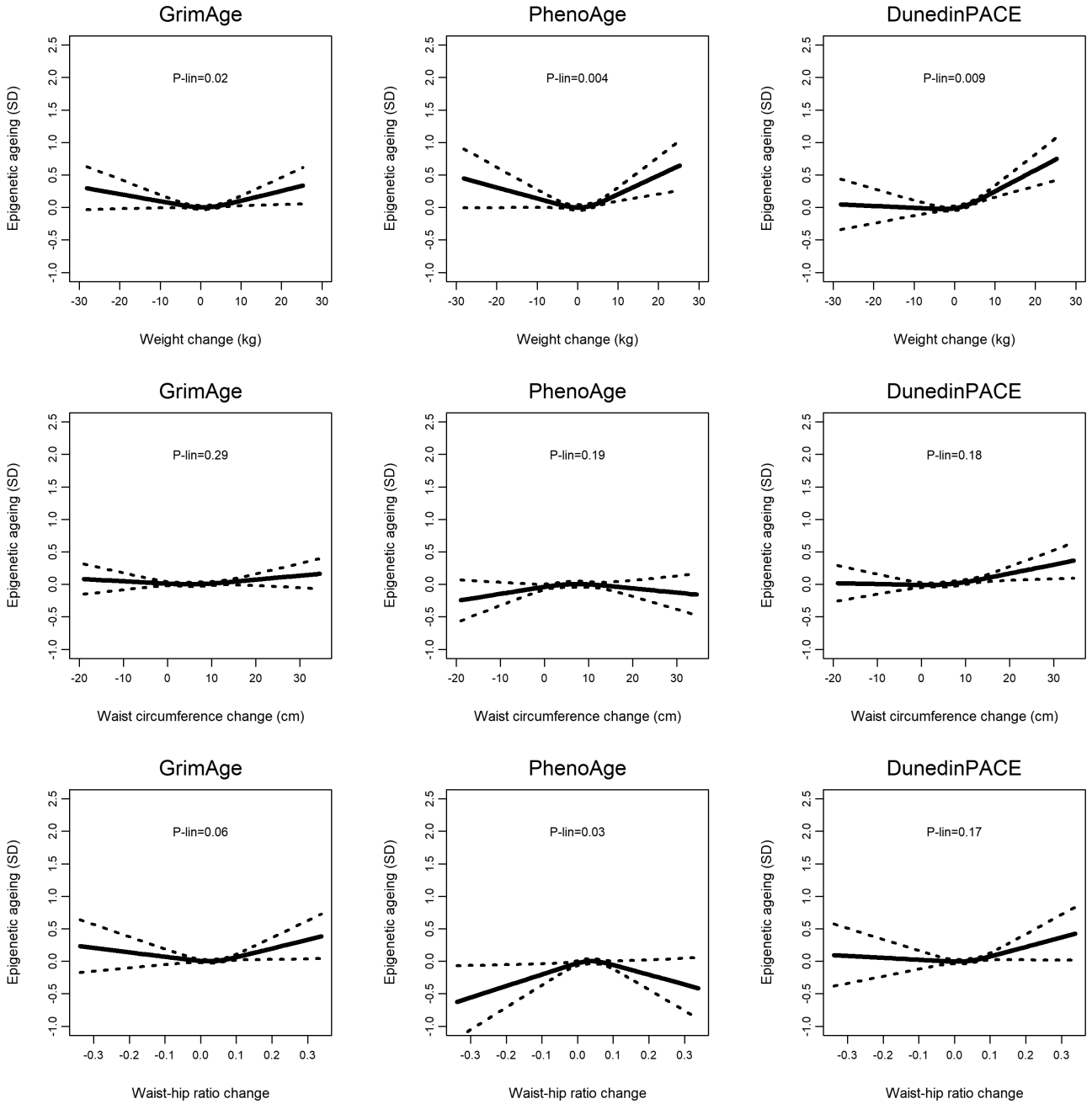


Figure 1. Restricted cubic splines for the longitudinal association (Model 2) between changes in body size and 3 epigenetic aging measures at follow-up (N = 1 041). Model adjusted for sex, age, country of birth, SES, baseline epigenetic age, baseline BMI, physical activity, smoking status, smoking pack-years, alcohol consumption, height, and AHEI-2010. Epigenetic aging measures were age-adjusted and standardized to a mean of 0 and *SD* of 1.

increase in the AHEI-2010 was associated with 0.10-*SD* (p = .002), 0.05-*SD* (p = .03), and 0.07-*SD* (p = .02) decrease in *PhenoAge*, *GrimAge*, and *DunedinPACE*, respectively. Additional adjustment for weight change left these estimates virtually unchanged (per *SD*: *PhenoAge*: β= −0.10; *GrimAge*: β= −0.05; *DunedinPACE*: β= −0.06).

Secondary Analyses

Weaker associations but similar U-shape curves were observed for the associations of changes in waist circumference and WHR with *GrimAge* and *DunedinPACE*, whereas for *PhenoAge*, the association appeared bell-shaped with stable waist circumference and stable WHR being associated with highest *PhenoAge* (Figure 1).

We observed associations for changes in MDS and DII with *PhenoAge* and *DunedinPACE* at follow-up of similar strengths as for AHEI-2010. The strongest associations were observed with *PhenoAge* (MDS: β= −0.09; p = .03; DII: β = 0.09; p = .01), Table 4.

Sensitivity Analyses

For *PCGrimAge* and *PCPhenoAge*, the effect estimates for the cross-sectional associations of weight and AHEI-2010 were very similar to the main analysis using *GrimAge* and

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Table 4. Longitudinal Association Between Diet Quality Change and Epigenetic Aging (N = 1 041).

Exposure	Model	GrimAA			PhenoAA			AA.DunedinPACE		
		β	95% CI	*p*-Value	β	95% CI	*p*-Value	β	95% CI	*p*-Value
Diet quality change (AHEI-2010)	Model 1*	−0.07	−0.12 to −0.02	.005	−0.11	−0.18 to −0.05	5×10^{-4}	−0.09	−0.14 to −0.03	.002
	Model 2**	−0.05	−0.10 to −0.01	.03	−0.10	−0.16 to −0.04	.002	−0.07	−0.12 to −0.01	.02
	Model 3***	−0.05	−0.10 to −0.005	.03	−0.10	−0.16 to −0.03	.03	−0.06	−0.11 to −0.004	.04
Change in MDS	Model 1*	−0.05	−0.10 to 0.01	.10	−0.10	−0.17 to −0.03	.004	−0.09	−0.15 to −0.02	.01
	Model 2**	−0.04	−0.09 to 0.01	.14	−0.09	−0.16 to −0.02	.01	−0.07	−0.13 to −0.01	.03
Change in DII	Model 1	0.07	0.02–0.12	.01	0.11	0.04–0.18	.001	0.09	0.03–0.15	.003
	Model 2	0.05	0.01–0.10	.03	0.09	0.03–0.16	.01	0.07	0.01–0.13	.03

*Model 1: adjusted for age, sex, country of birth, baseline diet, and baseline epigenetic age.
**Model 2: adjusted for age, sex, country of birth, SES, baseline epigenetic age, physical activity, smoking status, smoking pack-years, alcohol consumption, baseline diet, and BMI.
***Model 3: adjusted for age, sex, country of birth, SES, baseline epigenetic age, physical activity, smoking status, smoking pack-years, alcohol consumption, BMI, baseline diet, and weight change.
Epigenetic aging measures were age-adjusted; epigenetic aging measures and dietary scores were standardized to a mean of 0 and *SD* of 1.

PhenoAge, respectively (Supplementary Table 5), as were the estimates for the longitudinal associations with weight change and AHEI-2010 change (Supplementary Table 5 and Supplementary Figure 6).

After adjustment for self-reported health, exclusion of weight change over 20 kg, or exclusion of extreme baseline BMI, there was only minimal attenuation at the left tail of the spline curves for all 3 epigenetic aging measures (Supplementary Figures 7–9).

Discussion

This study assessed the cross-sectional and longitudinal associations of body size and diet quality with epigenetic aging in a large sample of middle-aged and older Australian adults followed over approximately a decade. Cross-sectionally, both weight and AHEI-2010 were linearly associated with epigenetic aging, and the associations were consistent between baseline and follow-up analyses. A particularly strong association was found between weight and *DunedinPACE*. Similar associations were observed for other measures of body size (waist circumference and WHR) and diet quality (MDS and DII).

In longitudinal analyses, both weight gain and weight loss were associated with a higher epigenetic age at follow-up. Participants with stable weight over a decade had the lowest epigenetic age, except for *DunedinPACE* for which weight loss and stable weight both showed lowest biological aging values. Taken together, the results for *DunedinPACE,* for example, stronger cross-sectional associations with body size and longitudinal association more consistent with a linear association, suggest that this measure captures more adiposity-related aspects of biological aging than do *GrimAge* and *PhenoAge*. Changes in waist circumference and WHR were less robustly associated with epigenetic aging than weight changes. Self-reported health status (as well as socioeconomic and lifestyle factors) did not appear to be a major confounder of the weight change associations, in particular it did not explain the increased epigenetic age associated with weight loss. Participants with extreme weight change or extreme baseline BMI did not appear to exert significant impact on the associations either. Improvement in AHEI-2010 was associated with decreased in the 3 epigenetic aging measures and was unlikely mediated by resulting changes in weight. The MDS and DII scores showed very similar associations as AHEI-2010 with *PhenoAge* and *DunedinPACE* both cross-sectionally and longitudinally.

Our cross-sectional findings are consistent with previous studies. One study based on the National Health and Nutrition Examination Survey 1999–2010 used weight data measured at recruitment and recalled at age 25 years and 10 years before recruitment and found a similar U-shaped association of *PhenoAge* (version not based on DNAm) with categorized weight changes among middle-aged and older participants, although when using absolute weight changes, they found no association with weight loss (36). However, with *PhenoAge* measured only at recruitment, they were unable to establish effect estimates for the longitudinal association between body size and biological aging. Two studies using different subsets of MCCS participants found that higher BMI was associated with older epigenetic age (19,37). Similar positive associations with epigenetic aging were also observed using data from 2 758 female participants in the Sister Study (United States) (16). In the same cohort, negative associations were found between diet quality and age-adjusted epigenetic aging (38). Negative associations between diet quality and epigenetic aging of similar size as the present study (per *SD*, for AHEI: β ranging between −0.07 for *DunedinPoAm* to −0.10 for *GrimAge* and *PhenoAge*; for MDS: β ranging between −0.07 for *PhenoAge* to −0.08 for *GrimAge* and *DunedinPoAm*) were reported by Kim et al. using data collected from 1995 participants in the Framingham Heart Study Offspring Cohort (14).

Very few studies have assessed longitudinal associations. One longitudinal analysis was performed by Quach et al. based on 239 participants over an average 2.7-year follow-up observed positive association between BMI and epigenetic aging (15). However, with relatively small sample size and short follow-up time, they were unable to capture the nonlinearity (39,40).

The strengths of our study include the use of longitudinal data from a large sample of general population participants with detailed information on a broad range of covariates. We investigated several adiposity measures and dietary scores and 3 epigenetic clocks that are the most widely used and highly predictive of mortality, providing a comprehensive

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picture of the different dimensions of our exposures and outcomes. Smoking status together with smoking pack-years were both adjusted for in our models, which provides a more thorough adjustment on smoking, particularly for the associations with *GrimAge*, which includes a methylation-based estimator of smoking pack-years (8), compared to most studies where only smoking status was adjusted for (14,16,36,38). The validity of the longitudinal analyses was confirmed by first showing that participants who had quit smoking from baseline to follow-up had reduced epigenetic age at follow-up. Moreover, we used restricted cubic spline curves to describe the nonlinear associations between changes in body size and epigenetic aging which provided us with more intuitive visualization of the associations (Supplementary Figure 10). However, the interpretation of the curves can be somewhat subjective. In addition, the evidence for associations observed in our study was generally strong. The effect estimates, although not very large, were consistent across different measures of body size, diet quality, and epigenetic aging in the present study and to what has been observed in previous studies (41–43). In absolute terms, our estimates per *SD* corresponded to for example, a *GrimAge* difference of approximately 1.0 and 1.4 years for participants in the highest quartile of weight and AHEI-2010, respectively, compared with the lowest quartiles (not shown). Therefore, despite some uncertainty in the associations with weight loss, our study provides robust evidence on the cross-sectional and longitudinal associations of body size, diet quality, and epigenetic aging.

The present study also has limitations. First, measurement error is inherent to the use of biomarkers and self-reported dietary data from FFQs. Even though dietary scores were calculated in the same way at baseline and follow-up, the FFQs at the 2 time points included slightly different dietary items (22,44). The significant reduction in *GrimAge* (which was developed in part based on biomarkers of smoking) for participants who had quit smoking during follow-up is nevertheless a strong indication that our longitudinal analyses were valid. Similarly, the FFQs used in the MCCS were evaluated for their validity and reliability (45–47) and performed well in previous studies (48,49). It remains that associations with diet quality might have been underestimated (biased towards the null), more so than for body size indicators, which were objectively measured. Regarding measurement error in the epigenetic aging measures, we considered the PC-based versions of *PhenoAge* and *GrimAge*, which may have improved test–retest reliability (29), but the estimates were not meaningfully different, which is consistent with previous observations (50) and further supports that our findings were not due to technical bias. Second, the MCCS only included participants of White European origin and people who take part in cohort studies are usually healthier than the general population, which may restrict the generalizability of our findings. Participants in our sample further attended follow-up 2, so they might have been healthier than the rest of the cohort. However, their median BMI at baseline was similar to the entire cohort (26.3 vs 26.4 kg/m^2), indicating low potential for attrition bias. The impact of selection bias due to nonparticipation or loss to follow-up is generally considered to be small (51) so it is reasonable to assume that our findings would extend to other populations, but studies in other population subgroups, particularly those most vulnerable to biological aging, would generate additional insights. An important limitation of our study and others is the lack of information about whether weight loss was intentional or unintentional which are known to be very different processes (52). For example, intentional weight loss is usually caused by modifications of lifestyle factors such as diet and physical activity which might improve health, and unintentional weight loss may be due to malnutrition or illnesses which in turn threaten one's healthy life-span and are associated with higher risk of all-cause mortality (53). Although we can confidently infer a detrimental effect of weight gain on biological aging, our results for weight loss should be interpreted with caution. With methylation data collected only at 2 time points, we were not able to assess temporal trends accurately; longitudinal analyses with measurements at 3 or more time points would allow better modeling of changes in lifestyle factors and aging markers.

To conclude, cross-sectionally, body size was positively associated with epigenetic aging, particularly for *DunedinPACE*, and diet quality was negatively associated with epigenetic aging. Longitudinally, weight gain and decreased diet quality were found to be associated with higher epigenetic aging, as well as weight loss for *GrimAge* and *PhenoAge*. These findings highlight the importance of maintaining a healthy weight and diet throughout adulthood to slow biological aging and extend a healthy life-span. Future studies including additional time points are needed to confirm and extend these findings. Further exploration is also needed of the reasons for weight change and their implication in terms of epigenetic aging, as well as the extent to which benefits of epigenetic aging translate into disease and mortality prevention.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

None.

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