Associations of five obesity metrics with epigenetic age acceleration: Evidence from 2,474 Taiwan Biobank participants

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Short title: Obesity and epigenetic age acceleration

Word count: 3,267

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Study Importance

What is already known?

- With recent advances in epigenetics, several studies have shown that body mass index (BMI) is associated with epigenetic age acceleration (EAA).

What does this study add?

- This study evaluated the associations of 5 obesity metrics with male EAA and female EAA. The 5 obesity metrics included BMI, body fat percentage, waist circumference, hip circumference, and waist-hip ratio (WHR).
- The obesity metric associated with biological aging varies with sex. “Abdominal obesity” (indicated by WHR) and “general obesity” (indicated by BMI) are significantly associated with male EAA and female EAA, respectively.

How might these results change the direction of research?

- Prevention of abdominal obesity is associated with a lower risk of EAA in men, whereas prevention of general obesity is associated with a lower risk of EAA in women.

Abstract

Objective: Obesity is associated with epigenetic age acceleration (EAA), resulting in an increased risk of many age-related disorders. However, most studies have focused on the relationship of EAA with body mass index (BMI). Whether any other obesity metric is more relevant to EAA remains unknown.

Methods: Here, we calculated the methylation age of 2,474 Taiwan Biobank (TWB) subjects according to Levine's PhenoAge and Lu's GrimAge. Residuals from regressing methylation age on chronological age were used to quantify PhenoEAA and GrimEAA. Five obesity metrics were evaluated, namely, BMI, body fat percentage, waist circumference, hip circumference, and waist-hip ratio (WHR). Sex-stratified EAA was regressed on each of the 5 obesity metrics.

Results: For males, an increase of one standard deviation (S.D.) in WHR (0.06) was associated with a 0.602-year PhenoEAA (p = 6.3E-6) and a 0.481-year GrimEAA (p = 1.2E-8). For females, every S.D. increase in BMI (3.7 kg/m^2) was associated with a 0.600-year PhenoEAA (p = 3.3E-5) and a 0.305-year GrimEAA (p = 3.1E-5).

Conclusions: "Abdominal obesity" and "general obesity" are significantly associated with male and female EAA, respectively. Prevention of abdominal obesity and general obesity is associated with a lower risk of EAA in men and women, respectively.

Keywords: Biological aging; chronological age; DNA methylation; methylation clock.

Introduction

Currently, obesity is a public health issue worldwide. The World Health Organization (WHO) estimated that more than 1.9 billion adults were overweight (body mass index [BMI] ≥ 25 kg/m^2) in 2016 and that over 650 million of the 1.9 billion overweight adults were obese (BMI ≥ 30

kg/m^2) (https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight). Obesity is associated with an increased risk of many chronic diseases, such as cardiovascular disorders, type 2 diabetes, hypertension, and several types of cancers (1, 2).

Obesity is also related to an increased risk of many age-related disorders (1). With the advancement of epigenetics, obesity has been shown to accelerate epigenetic aging (3, 4). Epigenetics is a mechanism by which environmental factors may influence gene expression without changing the DNA sequences. DNA methylation (DNAm) is one type of epigenetic manifestation, and it is associated with biological aging and health (5). DNAm occurs predominantly at cytosine-phosphate-guanine (CpG) sites (6). Environmental factors such as smoking are associated with changes in methylation levels (7, 8, 9, 10). In contrast to DNA sequences, which remain stable throughout the lifetime, DNAm levels are dynamic and can reflect physiological alterations (11).

Because DNAm levels can reflect physiological and pathological conditions, they have been used to estimate biological age (5, 12, 13, 14). For example, Hannum *et al.* selected 71 methylation sites that were highly predictive of chronological age and found that the aging rate of men was 4% faster than that of women (12). Horvath further showed that an average acceleration of 36 years of age was observed in 20 cancer types (13). By regressing a transformed chronological age on 21,369 CpG probes (the intersection of the Illumina 450K and 27K platforms) with elastic net regularized regression (15), Horvath selected 353 CpG sites that can predict chronological age. The weighted average of the methylation levels at these 353 sites forms an epigenetic clock (i.e., the so-called Horvath clock) (13). Moreover, Horvath *et al.* found that ethnicity and sex are significantly associated with epigenetic aging rates (16). Women generally have slower epigenetic aging rates than men in blood, brain tissue, and saliva (16). McCrory *et al.* investigated allostatic load and epigenetic clocks and found that men and women

have different biological aging rates (17).

Recently, Levine *et al.* searched for epigenetic biomarkers of a "phenotypic age" (instead of chronological age), which can better reflect one's physiological condition (5). With clinical data from the third National Health and Nutrition Examination Survey (NHANES III), Levine *et al.* used a Cox regularized regression model to regress the hazard of aging-related mortality on 43 markers, including 42 clinical markers and chronological age. Through this step, they developed a "phenotypic age" (so-called "PhenoAge") as a weighted average of 9 clinical markers and chronological age. Moreover, based on the Invecchiare in Chianti (InCHIANTI) study data (18), Levine *et al.* used elastic net regression (15) with 10-fold cross-validation and selected 513 CpGs (from 20,169 CpGs) as predictors of PhenoAge. A linear combination of these 513 CpGs forms one's methylation age (i.e., the so-called Levine clock) (5).

Epigenetic clocks are informative for predicting human lifespan and healthspan (5, 14). However, such studies have been overwhelmingly performed in Western populations (5, 12, 13, 14). Here, we evaluated whether 513 CpGs from Levine *et al.* can also predict aging in a Taiwanese population. If this is the case, we will then calculate the epigenetic age acceleration (EAA) of 2,474 Taiwan Biobank (TWB) participants according to Levine *et al.*'s 513 CpGs.

In addition, we evaluated EAA according to the GrimAge clock (14, 19). The GrimAge clock (14) is based on 1,030 CpGs that are associated with DNAm-based biomarkers for 7 plasma proteins and smoking pack-years. GrimAge is useful for predicting mortality and age-related phenotypes (14, 19). Here, we focused on PhenoAge (5) and GrimAge (14) because both can reflect one's physiological conditions better than chronological age can.

Obesity is associated with acceleration of epigenetic aging (3, 4, 20, 21, 22, 23, 24, 25). However, most studies have evaluated the relationship of EAA with body mass index (BMI) (3, 4, 20, 21, 23, 24, 25), except two studies discussing other obesity metrics such as waist

circumference (WC) and waist-hip ratio (WHR) (22, 24). Which obesity metric is more relevant to EAA remains unknown. Moreover, the obesity metrics associated with EAA may be different for men and women. To address this issue, we evaluated 5 obesity metrics, including BMI, body fat percentage (BFP), WC, hip circumference (HC), and WHR. Although BMI is the most commonly used obesity indicator, it incorporates no information on lean body mass or abdominal obesity (26). BFP is the percentage of total fat mass out of a person's total body mass. HC is one of the body size metrics that can predict type 2 diabetes (27). WHR is a dimensionless ratio of WC to HC. While BMI and BFP measure general obesity, WC and WHR are indicators of abdominal obesity. Here, we investigated which obesity metrics were associated with male EAA and female EAA.

Methods

Taiwan Biobank

The TWB was approved by both the Institutional Review Board on Biomedical Science Research/IRB-BM at Academia Sinica and the Ethics and Governance Council of Taiwan Biobank. Each individual provided written informed consent before participating in the TWB, following institutional requirements and the principles of the Declaration of Helsinki. Our application to access the TWB data was approved on February 18, 2020, with an application number of "TWBR10810-07". The current study also received approval from the Research Ethics Committee of National Taiwan University Hospital (NTUH-REC no. 201805050RINB).

Since October 2012, the TWB has recruited 122,071 community-based volunteers with ages from 30 to 70 years. After signing informed consent, these participants provided blood and urine samples and underwent physical examinations. They were further interviewed by TWB

researchers to record their lifestyle factors, such as physical exercise, alcohol consumption status and cigarette smoking (28). The body height and weight of each individual were measured by TWB researchers. BMI was then calculated as weight (kg)/[height (m)]^2. BFP was measured by bioelectrical impedance analysis using a TANITA Body composition analyzer BC-420MA (Tokyo, Japan). As recommended by the WHO, WC is the circumference of the midpoint between the iliac crest and lowest rib, measured by a nonelastic tape (29). HC was measured by a nonelastic tape at the largest circumference around buttocks in a standing position. WHR was obtained as the ratio of WC to HC.

During 2016-2021, TWB researchers randomly selected 2,474 TWB participants and submitted their blood samples for DNAm quantification. Blood DNAm levels were analyzed with the Illumina Infinium MethylationEPIC BeadChip (Illumina, Inc., San Diego, CA), which included ~860,000 CpG sites.

The methylation β-value has an intuitive biological interpretation (30). It is the ratio of methylated probe intensity to the overall intensity of both methylated and unmethylated probes (31). β-values range from 0 to 1. A higher value indicates hypermethylation (relatively more methylation), whereas a lower value denotes hypomethylation (relatively less methylation). The β-values at some CpG sites have been found to be associated with biological aging, and therefore, they were recently used to gauge biological age (5, 12, 13, 14).

Calculation of methylation age

Here, we calculated the methylation age based on Levine's (5) and Lu's (14) clocks. While some epigenetic clocks identified CpG sites according to chronological age (12, 13), Levine *et al.* (5) detected 513 CpG sites that were associated with PhenoAge. PhenoAge has been linked to aging-related mortality (mortality related to heart diseases, chronic lower respiratory disease,

malignant neoplasms, Alzheimer's disease, diabetes mellitus, cerebrovascular disease, nephritis, nephrotic syndrome, and nephrosis); therefore, it can better reflect one's health condition.

To compute GrimAge, we uploaded TWB DNAm data to the online DNAm Age Calculator that was developed by Horvath's laboratory, https://dnamage.genetics.ucla.edu/new. A total of 30,084 CpGs were listed in the annotation file "datMiniAnnotation3.csv" under "Advanced Analysis". The Illumina Infinium MethylationEPIC BeadChip covered 27,526 CpGs, 91.5% of the 30,084 sites. All 513 sites composing PhenoAge were also included in the set of 27,526 CpGs.

Regarding quality control of DNAm data, a small detection *p* value indicated a reliable signal, whereas a large *p* value (say, > 0.01) generally indicated a poor quality signal. As suggested by the workflow of methylation data analysis (32), we calculated the average detection *p* value to summarize the quality of signals across 27,526 CpGs for each sample. The highest average detection *p* value across 27,526 CpGs for all 2,474 samples was 0.0013, which was below the recommended 0.01 in the workflow (32). Therefore, we kept all 2,474 samples in this step.

On the other hand, we also checked the quality of signals across all 2,474 samples for each CpG site. β-values with detection *p* values higher than 0.05 were regarded as missing values. In total, 27,480 sites (among 27,526 CpGs) had missing rates of less than 1%. A total of 2 sites with a missing rate > 25% (i.e., more than 25% of β-values with detection *p* values > 0.05) were removed from the analysis, resulting in 27,524 reliable CpGs in the final dataset. As suggested by a recent study, the replacement of missing β-values at a CpG with the average of all known β-values at that site (the so-called "mean" approach) is a better choice than sophisticated methods (31). Therefore, we used this "mean" approach to impute all missing β-values.

Regarding normalization of DNAm data, raw intensity data were obtained from IDAT files and further processed using the R package minfi v1.36 (33). To account for technical variation in the background fluorescence signal, intensity data were normalized by normal-exponential out-of-band (noob) (34) using the *preprocessNoob* function in minfi. The normalized intensities were then used to calculate the β-value according to $\frac{max(M,0)}{max(M,0)+max(U,0)+100}$, where M is the methylated intensity and U is the unmethylated intensity. Therefore, β-values ranged from 0 to 1.

Then, we evaluated whether the 513 CpGs could reflect aging in Taiwanese individuals. Levine *et al.* (5) reported Pearson's correlations between chronological age and each of the 513 CpGs. Here, we regressed the normalized β-value of each CpG on chronological age while adjusting for sex (male vs. female), BMI (continuous), smoking status (yes vs. no), drinking status (yes vs. no), regular exercise (yes vs. no), educational attainment (1, 2, …, or 7), and three random batch effects (which 96-well plate during the methylation quantification; which row and which column on the plate). The definitions of the covariates, such as smoking, drinking, regular exercise, and educational attainment, can be found in Table 1.

Among the 513 CpGs, 226 (44.1%) were significantly associated with chronological age in the TWB ($p < 0.05/513 = 9.7 \times 10^{-5}$). Among the 226 CpGs, 223 (98.7%) exhibited the same direction of the correlations (Column G of Table S1) shown by Levine *et al.* (5). This suggests that many of the 513 aging-related CpGs identified by Levine *et al.* (5) can be well replicated in the TWB. Among the 513 CpGs, 102 (19.9%) showed different directions from the correlations (Column G of Table S1) of Levine *et al.* (5). However, 89 of these 102 sites (87.3%) were not significantly associated with chronological age in the TWB ($p > 0.05$).

PhenoAge and GrimAge were calculated by the online DNAm Age Calculator (https://dnamage.genetics.ucla.edu/new). In addition, we also computed PhenoAge based on the

513 CpGs. That is,

$$PhenoAge = \hat{\alpha}_0 + \sum_{j=1}^{513} \hat{\alpha}_j X_j, \quad (1)$$

where $\hat{\alpha}_j$s (j = 0, 1, …, 513) were extracted from Levine *et al.*'s (5) study (Column F of Table S1. $\hat{\alpha}_0 = 60.664$, $\hat{\alpha}_1 = 63.124$, etc.), and X_j is the normalized β-value (based on noob (34)) at the j^{th} CpG site. Our calculation based on formula (1) was consistent with that computed by the online DNAm Age Calculator.

Epigenetic age acceleration

Instead of using the difference between methylation age and chronological age, we calculated residuals from regressing methylation age on chronological age. In this way, the residuals are robust to various normalization methods and measurement platforms (35). These residuals were used to quantify EAA, with positive values indicating that an individual is epigenetically older than his/her chronological age (36). As men and women are different in body composition and risks of metabolic syndromes (37), we performed regression analyses for males and females to explore sex-specific obesity metrics that are associated with EAA.

We regressed EAA on each obesity metric, while adjusting for smoking status (yes vs. no), drinking status (yes vs. no), regular exercise (yes vs. no), educational attainment (1, 2, …, or 7), and the current region of residence (the northern, central, southern, or eastern regions). Taiwan can be divided into four regions according to the National Development Council of Taiwan. The northern region includes Taipei city and the surrounding cities and counties. Taipei has been Taiwan's center of government and business since 1945. The northern region has received more resources in regard to medical and economic development. To adjust for regional differences, we considered region of residence to be a covariate in the regression models. Lifestyle factors such

as smoking, drinking, and performing regular exercise might influence EAA; therefore, we also considered them to be covariates. In addition, a higher level of education was associated with better performance on cognitive tests and a slower decline in mental conditions (38). To adjust for its potential effects, we also considered education to be a covariate in the regression models. On the other hand, because dietary information was not available in the TWB, it was not adjusted for in our regression models.

To compare the effect sizes of the five obesity metrics, we performed a *z*-score transformation on each obesity measure before running the regression. Because a total of 20 tests (2 DNAm ages, 2 sexes, 5 obesity metrics) were of interest, a *P*-value < 0.0025 (= 0.05/20) was considered indicative of significance according to the Bonferroni correction.

[Table 1 is approximately here]

Results

Table 1 presents the basic characteristics of the 2,474 TWB subjects. Except for BFP, males, on average, had larger values of the obesity metrics than did females. Extreme outliers were defined if an EAA > $Q_3 + 3 \times (Q_3 - Q_1)$ or EAA < $Q_1 - 3 \times (Q_3 - Q_1)$, where Q_1 and Q_3 were the 25^{th} and 75^{th} percentiles, respectively. A total of 2 and 6 extreme outliers were excluded from follow-up analysis, with regard to PhenoEAA and GrimEAA, respectively.

Figure 1 presents the pairwise scatter plots of chronological age, PhenoAge, and GrimAge. The departure of each point from the regression line (the black line in Figure 1 (A) & (B)) represents the residual or EAA. Figure 2 (A) and (B) show the histograms of PhenoEAA and GrimEAA, respectively.

[Figures 1-2 are approximately here]

[Table 2 is approximately here]

Because the obesity metrics that are associated with EAA may be different for men and women, we performed regression analysis stratified by sex. Table 2 shows the coefficients when regressing EAA on each obesity metric while adjusting for smoking status (yes vs. no), drinking status (yes vs. no), regular exercise (yes vs. no), educational attainment (1, 2, …, or 7), and the current region of residence (the northern, central, southern, or eastern regions). The obesity metrics most strongly associated with EAA were WHR and BMI for males and females, respectively. This result was consistently supported by PhenoAge and GrimAge.

For males, an increase of one S.D. in WHR (0.06, as shown in Table 1) was associated with a 0.602-year PhenoEAA (p = 6.3E-6, 95% confidence interval [C.I.] = [0.341, 0.862]) and a 0.481-year GrimEAA (p = 1.2E-8, 95% confidence interval [C.I.] = [0.317, 0.645]). For females, every S.D. increase in BMI (3.7 kg/m^2, as shown in Table 1) was associated with a 0.600-year PhenoEAA (p = 3.3E-5, 95% C.I. = [0.317, 0.883]) and a 0.305-year GrimEAA (p = 3.1E-5, 95% confidence interval [C.I.] = [0.162, 0.448]).

Discussion

Studies have shown that obesity can shorten life expectancy by up to 20 years (39). In 2015, obesity (BMI $\geq$ 30 kg/m^2) and overweight (BMI $\geq$ 25 kg/m^2) accounted for 4.0 million deaths globally (95% C.I. = [2.7, 5.3] million deaths) (40). Obesity increases the risk of many age-related diseases (1) and several types of cancers, such as breast, gallbladder, colon, pancreas, bladder, renal, cervical, uterine, and prostate cancers (41). A plausible reason is that obesity accelerates epigenetic aging (3, 4). Recently, Kresovich *et al.* (22) evaluated the associations of 3 adiposity metrics (BMI, WC, and WHR) with EAA based on 2,758 women recruited from the United States and Puerto Rico between 2003–2009 (the Sister Study) (42). Both BMI and WHR were found to be associated with female EAA, of which the strongest association was found to be

between BMI and female EAA (this conclusion is in line with Table 2 in the current study, right column).

In 2017, Grant *et al.* analyzed DNAm at 3 time points, covering on average of 16 years for each of 43 women enrolled in the Women's Health Initiative. The authors found that female EAA was associated with BMI ($p = 0.0012$) and WC ($p = 0.033$ but no longer significant after multiple testing correction) (24). Both of the abovementioned studies focused on women, and the authors consistently found that BMI was more closely associated with female EAA than other obesity metrics (22, 24). This is in line with our finding for women in this study. However, which obesity metric is more relevant to EAA, as far as Asian individuals and males are concerned, remains unknown.

In this study, we evaluated the associations of 5 adiposity metrics (BMI, BFP, WC, HC, and WHR) with male and female EAA. We found that "abdominal obesity" (WHR as an indicator) and "general obesity" (BMI as an indicator) were significantly associated with male EAA and female EAA, respectively. Our finding for women is consistent with that from previous studies (22, 24). Additionally, we identified a relevant obesity metric (i.e., WHR) for male EAA. Future studies about the development of a specialized metabolic/obesity-focused aging clock will be beneficial to a specific investigation on metabolic and obesity-related disorders (43).

Taiwan adopts a more stringent standard than the WHO criterion to define overweight (24 $kg/m^2 \leq$ BMI $< 27\ kg/m^2$) and obesity (BMI $\geq 27\ kg/m^2$), according to the Ministry of Health and Welfare of Taiwan. In the Taiwanese population, a BMI over 24 kg/m^2 is a risk factor for type 2 diabetes and cardiovascular diseases (44, 45). The prevalence of overweight or obesity has been increasing over the past decade, and it reached 48.0% among Taiwanese adults (aged over 18 years) in 2019 (49.8% among our 2,474 TWB subjects, aged 30 to 70 years). Although obesity is commonly defined by BMI because of convenience, other obesity metrics may be more closely

associated with EAA. Here, we show that biological aging is more relevant to abdominal obesity than other metrics in Taiwanese men. Prevention of abdominal obesity and general obesity is associated with a lower risk of EAA in men and women, respectively.

There are three major strengths of this study, including the relatively large sample size, the availability of a range of obesity metrics, and the fact that this work was performed with participants from Asia (unlike most published studies, which were conducted on individuals of European ancestry). However, there are ethnic differences in the distribution of obesity metrics. According to the WHO, more people are obese than underweight globally, except in Asia and sub-Saharan Africa. For example, the mean BMI of 499,480 U.K. Biobank participants is 27.3 (S.D.= 4.8) kg/m^2 (https://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=21001), whereas it is 25.2 (S.D.= 3.4) kg/m^2 in male participants and 23.5 (S.D.= 3.7) kg/m^2 in female participants in the current study (Table 1). The results of this work may not be generalizable to residents of other countries. In addition, the associations shown here are cross-sectional, and causality cannot be inferred from our results.

Acknowledgments. The authors would like to thank the anonymous reviewers for their insightful and constructive comments, and the Taiwan Biobank for approving our application to access the data.

Funding agencies: This study was supported by the Ministry of Science and Technology of Taiwan (grant number MOST 107-2314-B-002-195-MY3 to W.-Y.L.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Disclosure: The authors declared no conflict of interest.

Supporting information: Additional Supporting Information may be found in the online version of this article.

Table S1 - The Levine *et al.*'s 513 CpGs

Column F, "Weight": $\hat{\alpha}_j$s (j = 0, 1, …, 513) in model 1, which were provided by Levine et al. (5).

Column G, "Univariate.Age.Correlation.Levine": the Pearson's correlations between methylation levels and chronological age, which were provided by Levine et al. (5).

Columns H & I, "Regression.Coef.TWB" and "P.value.TWB": the regression coefficient and *P*-value of regressing the normalized β-value of each CpG on chronological age, while adjusting for sex (male vs. female), BMI (continuous), smoking status (yes vs. no), drinking status (yes vs. no), regular exercise (yes vs. no), educational attainment (1, 2, …, or 7), and three random batch effects (which 96-well plate during the methylation quantification; which row and column on the plate).

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Tables

	Males	Females	*p*-value [1]
Total	1,243 (50.2%)	1,231 (49.8%)	
Age (years)	50.3±11.3	49.3±10.8	0.0246
Drinking [2]	147 (11.8%)	24 (1.9%)	7.7E-22
Smoking [3]	235 (18.9%)	48 (3.9%)	2.0E-31
Regular exercise [4]	595 (47.9%)	497 (40.4%)	2.0E-4
Educational attainment [5]	5.8±0.9	5.4±0.9	1.6E-22
Living in the northern region	563 (45.3%)	545 (44.3%)	0.6383
Living in the central region	233 (18.7%)	222 (18.0%)	0.6859
Living in the southern region	419 (33.7%)	436 (35.4%)	0.3944
Living in the eastern region	28 (2.3%)	28 (2.3%)	>0.999
BMI (kg/m^2)	25.2±3.4	23.5±3.7	2.3E-32
Body fat percentage (%)	22.9±5.4	31.8±6.5	4.5E-228
Waist circumference (cm)	87.9±9.3	80.5±9.8	6.8E-76
Hip circumference (cm)	98.2±6.7	95.6±6.9	2.1E-20
Waist-hip ratio	0.89±0.06	0.84±0.07	1.3E-90

Table 1. **Basic characteristics of the 2,474 subjects**

Data are presented in *n* (%) or mean±standard deviation.

[1] *P*-value of testing the mean difference between males and females, based on the two-sample *t*-test (for continuous variables) or proportion test (for drinking, smoking, regular exercise, and living region).

[2] Drinking was defined as a person having a weekly intake of more than 150 mL of alcohol for at least 6 months and having not stopped drinking at the time he/she participated TWB.

[3] Smoking was defined as a person who had smoked cigarettes for at least 6 months and had not quit smoking at the time he/she participated TWB.

[4] Regular exercise was defined as performing 30 minutes of “exercise” three times a week. “Exercise” includes leisure-time activities such as jogging, swimming, cycling, dancing, weight training, mountain climbing, etc.

[5] Educational attainment ranges from 1 to 7: 1 “illiterate”, 2 “no formal education but literate”, 3 “primary school graduate”, 4 “junior high school graduate”, 5 “senior high school graduate”, 6 “college graduate”, and 7 “Master’s or higher degree”.

	Males			Females		
PhenoEAA						
	$\hat{\beta}$	95% C.I.	*P*-value	$\hat{\beta}$	95% C.I.	*P*-value
BMI [1, 2]	0.356	[0.096, 0.616]	0.007	**0.600**	**[0.317, 0.883]**	**3.3E-5**
Body fat percentage [1, 2]	0.313	[0.042, 0.583]	0.024	0.510	[0.225, 0.795]	4.7E-4
Waist circumference [1, 2]	0.427	[0.167, 0.687]	0.001	0.453	[0.170, 0.736]	0.002
Hip circumference [1, 2]	0.111	[-0.150, 0.372]	0.404	0.344	[0.064, 0.624]	0.016
Waist-hip ratio [1, 2]	**0.602**	**[0.341, 0.862]**	**6.3E-6**	0.349	[0.063, 0.634]	0.017
GrimEAA						
BMI [1, 2]	0.199	[0.034, 0.365]	0.018	**0.305**	**[0.162, 0.448]**	**3.1E-5**
Body fat percentage [1, 2]	0.213	[0.042, 0.384]	0.015	0.251	[0.107, 0.396]	6.7E-4
Waist circumference [1, 2]	0.277	[0.112, 0.442]	0.001	0.280	[0.137, 0.423]	1.3E-4
Hip circumference [1, 2]	-0.006	[-0.172, 0.160]	0.940	0.159	[0.017, 0.301]	0.028
Waist-hip ratio [1, 2]	**0.481**	**[0.317, 0.645]**	**1.2E-8**	0.271	[0.126, 0.415]	2.4E-4

Table 2. The coefficients when regressing male (or female) EAA on each obesity metric (the largest effect sizes and the smallest *P*-values were highlighted)

1 We performed a *z*-score transformation on each obesity measure before running the regression.

2 Covariates adjusted in all regression models included smoking status (yes vs. no), drinking status (yes vs. no), regular exercise (yes vs. no), educational attainment (1, 2, …, or 7), and the current region of residence (the northern, central, southern, or eastern regions).

Figure Legends

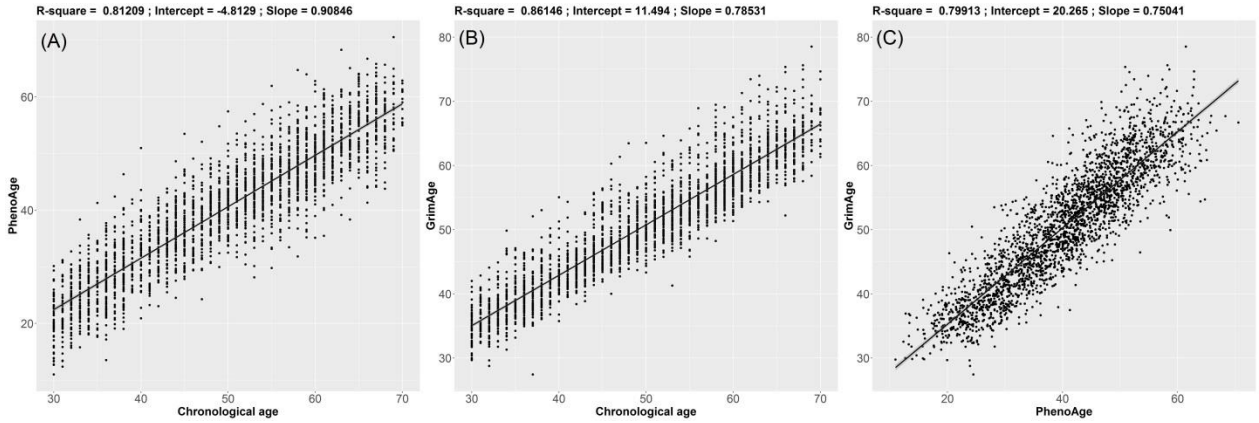

Figure 1 - Pairwise scatter plots of chronological age, PhenoAge, and GrimAge

The black lines depict the regression lines: (A) $\text{PhenoAge} = -4.8129 + 0.90846 \times$ chronological age, (B) $\text{GrimAge} = 11.494 + 0.78531 \times$ chronological age, and (C) $\text{GrimAge} = 20.265 + 0.75041 \times \text{PhenoAge}$. Subjects above the regression lines of Figures (A) & (B) are epigenetically older, whereas subjects below the lines are epigenetically younger.

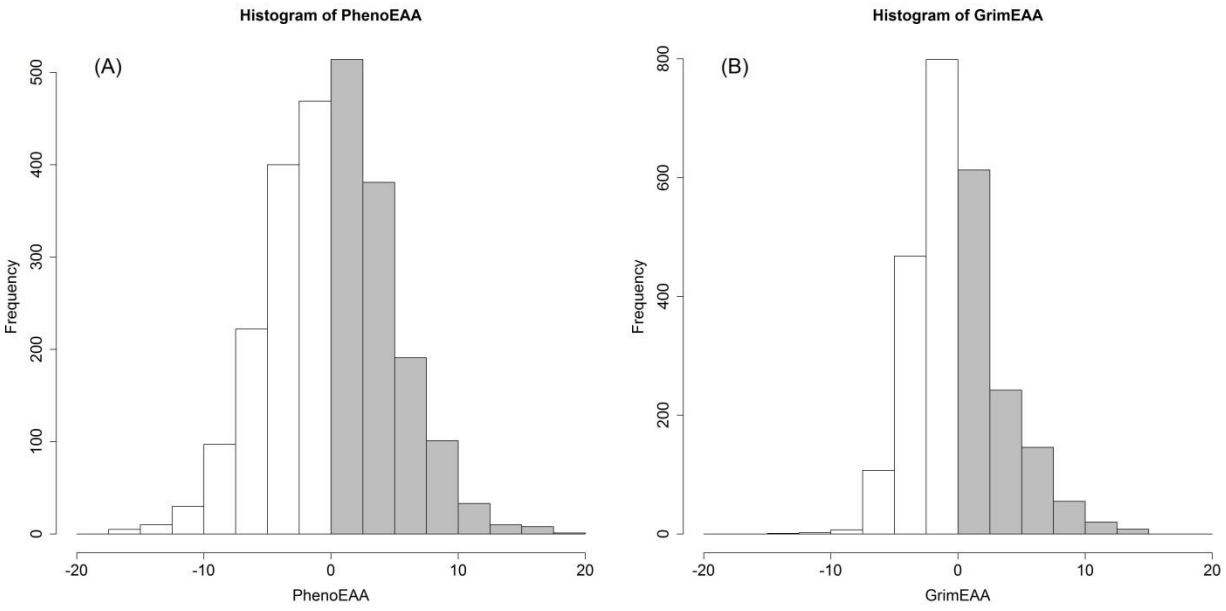

Figure 2 - Histogram of epigenetic age acceleration (EAA)

EAA > 0 (the grey part) means epigenetically older, whereas EAA < 0 (the white part) indicates

epigenetically younger.