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Chengchen Li Date

Association of Body Mass Index with DNA Methylation Age Acceleration
In African American Mothers: Cross-sectional Analysis from InterGEN Study

By

Chengchen Li
Master of Public Health

Epidemiology

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Yan V. Sun
Committee Chair

Association of Body Mass Index with DNA Methylation Age Acceleration In African American Mothers: Cross-sectional Analysis from InterGEN Study

By

Chengchen Li

B.A., University of Iowa, 2017

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An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2019

Abstract

Association of Body Mass Index with DNA Methylation Age Acceleration
In African American Mothers: Cross-sectional Analysis from InterGEN Study

By Chengchen Li

Background
African American women are affected by earlier onset of age-associated health deteriorations and obesity disproportionally, but little is known about the mechanism linking body mass index (BMI) and biological aging among this population. DNA methylation age acceleration (DNAm AA), measuring the difference between DNA methylation age and chronological age, is a novel biomarker of biological aging process, and predict aging-related disease outcomes.

Methods
The present study estimated cross-tissue DNA methylation age acceleration using saliva samples from 232 young African American women. Cross-sectional regression analyses were performed to assess the association of body mass index with age acceleration.

Results
The average chronological age and DNA methylation age were 31.67 years, and 28.79 years, respectively. After adjusting for smoking, hypertension diagnosis history, and socioeconomic factors (education, marital status, household income), 1 kg/m2 increase in BMI is associated with 0.14 years increment of DNAm AA (95% CI: (0.08, 0.21)).

Conclusion
In African American women, high BMI is associated with saliva-based DNA methylation age acceleration, after adjusting for smoking, hypertension, and socioeconomic status. This finding demonstrated the positive association between BMI and DNAm AA across tissue types and demographic groups, and supports the hypothesis that high BMI and obesity accelerate biological aging.

Length: 199 words

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Acknowledgements

I would like to express my deep gratitude to Dr. Yan Sun, my thesis advisor, for his patient guidance, enthusiastic encouragement and useful critiques of this thesis work. I would also like to thank Dr. Qin Hui and Mr. Yunfeng Huang, for their technique support in preliminary data processing. Finally, I wish to thank my mother for her support and encouragement throughout my study.

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CHAPTER I

Approximately 39.8% U.S. adults (age $\geq$ 20) were affected by obesity (BMI $\geq$ 30 kg/m^2) in 2015 – 2016 and adults obesity prevalence increased from 30.5% to 39.6% between 2003–2004 and 2015–2016. Overall, women (41.5%) had a higher prevalence of obesity than men (38%). Among women, the prevalence of obesity was the highest among non-Hispanic blacks (46.8%) compared with all other race and Hispanic origins (1). In 2014, annual medical cost of obesity was estimated to be $149.4 billion in the US. Obese individuals spent $1,910 more on healthcare comparing to those of normal weight on average per year (2). Obese individuals have been shown to be at greater risk for many age-related chronic diseases such as hypertension, coronary artery diseases, type2 diabetes, and certain types of cancer (3). In addition, obesity has shown to be associated with a 7-year decrease in life expectancy at the age of 40 years (4). Therefore, it has been hypothesized that obesity increases the risk of these chronic diseases through accelerated biological aging.

According to the most recent report, life expectancy for the black population (75.1 years) was 3.4 years less than the white population (78.9 years) in 2015. Among females, non-Hispanic blacks had the shortest life expectancy (78.1 years) comparing with other racial ethnic groups (Hispanic female: 84.3 years; Non-Hispanic white female: 81.0 years) (5). The racial discrepancy in life expectancy could be explained by earlier on-set of age-related chronic conditions and comorbidity among the disadvantaged groups (6, 7). Data from National Health and Nutrition Examination Survey (NHANES IV,

1999–2002) demonstrated that the average age-related allostatic load score for blacks in every age group, was comparable to that for whites who were chronologically 10 years older. Similar trends were observed for the predicted probability of having a high allostatic load score in blacks. These findings suggested that blacks are likely to experience health deterioration earlier in their lives, and hence, aging faster than whites (8).

Although chronological age is the strongest predictor for age-related health conditions, individuals of the same chronological age may experience different biological processes of aging. Biomarkers that capture inter-individual variations of biological aging may accurately assess an individual's aging-related disease risks. DNA methylation, one of the best-known epigenetic modifications, has been recently used to develop a novel biomarker of biological aging (17). Epigenetic changes are defined as DNA modifications that affect gene expression without changing the DNA sequences. Both genetic factors and environmental factors, such as exposure to pollutants, diet, and smoking, can affect epigenetic changes (9). During DNA methylation process, methyl groups are attached to segments of DNA in a particular gene and alter that gene's expression level (9). DNA methylation alterations can occur and accumulate over time. Recent research has identified specific genomic regions that are subject to substantial hypermethylation or hypomethylation associated with chronological age (10 - 14) and demonstrated its accuracy to predict one's age according to DNA methylation levels in specific tissues (e.g. saliva or blood) as well as cross multiple tissues (15 - 17).

DNA methylation age (DNAm age) can be estimated based on DNA methylation levels of 353 CpG dinucleotides across multiple-tissues in Horvath's clock (17). Biological aging can be measured by the discrepancy between DNAm age and chronological age (Δage). Since DNAm age is strongly linearly associated with chronological age, the residual resulting from regressing DNAm age against chronological age in a linear model can also be defined as DNA methylation age acceleration (DNAm AA). DNAm AA is independent of chronological age, and can be used as an instrument to accurately measure how fast an individual ages (18). A positive DNAm AA suggests that an individual is experiencing accelerated aging.

Previous studies reported that DNAm AA and/or Δage derived from a specific tissue were linked to age-related diseases. For example, a study consisting of 700 Caucasian subjects suggested that DNAm AA of dorsolateral prefrontal cortex is associated with multiple neuropathological measurements and global cognitive function decline (19). Additionally, a German case cohort of 1863 older people found that every 5-year increment of Horvath Δage is associated with 23% higher all-cause mortality, 22% higher cancer morality, and 19% higher cardiovascular mortality (20). Consistent results were observed in a sub-cohort of 2543 African Americans enrolled in the Atherosclerosis Risk in Communities (ARIC) Study. Researcher found that 5-year increment of Horvath DNAm AA in blood is associated with 0.01 mm increase in carotid intima-media thickness (IMT) and 17% higher hazard of fatal coronary heart disease (21).

Recent studies have characterized the association of BMI or obesity with DNA methylation at specific genomic regions in a particular tissue (**Error! Reference source not found.** - 40). For example, studies identified and validated the association of increased BMI with hypermethylation of gene HIF3A in blood cells and adipose tissues (41, 42). DNA methylation at 2825 genes (e.g. FTO, ITIH5, CCL18, MTCH2, IRS1 and SPP1) in adipose tissue were also identified to be correlated with BMI in Rönn et al's study (32). Although these findings suggested that BMI affects site-specific DNA methylation levels, it is a separate question whether similar pattern can be observed for the association of BMI and DNAm Age and/or DNAm AA.

Only few studies investigated the relationship between BMI and accelerated aging measured by DNA methylation levels among adults (43 - 47). In a subset of 4,173 postmenopausal women from Women's Health Initiative (WHI) including 2,045 Caucasians, 1,192 African Americans, and 717 Hispanics, researcher observed a statistically significant correlation between BMI and DNAm AA among African American ($r = 0.05 – 0.06$). Longitudinal data in WHI also suggested that 1 kg/m^2 increase in BMI over time is associated with 0.40 years increase in DNAm AA ($\beta_{\text{Extrinsic DNAm AA}} = 0.40$; $\beta_{\text{Intrinsic DNAm AA}} = 0.27$)(47). Similar results were found in liver samples collected from German patients, where DNAm AA increased by 0.33 years for 1 kg/m^2 increase in BMI (46). In a British birth cohort consisting of 790 women, 1 kg/m^2 increase in BMI at age 53 was found to be positively associated with 0.085 (95% CI: 0.014 to 0.156) years increase in Δage in buccal tissue, and 0.044 (95% CI: −0.065 to 0.154) years increase in Δage for blood tissue (43). A prospective cohort study nested within the Melbourne

Collaborative Cohort collected blood samples from 2,818 participants, and observed higher DNAm AAs in overweight (BMI ≥ 25) and obese individuals (BMI ≥ 30) compared with lean individuals (BMI < 25) ($\beta_{\text{Overweight vs Lean}}$ = 0.40 years; $\beta_{\text{Obesity I vs Lean}}$ = 0.15 years; $\beta_{\text{Obesity II \& III vs. Lean}}$ = 2.38 years) (45). Similar to previous described studies, data from Young Finns Study indicated that BMI is positively associated with ∆age measured in blood tissues in middle-age individuals (r = 0.281). However, this study failed to observe this association among young adult and the nonagenarian populations (44).

Despite the high prevalence of obesity in the U.S., only one of studies described above was conducted among the U.S. adults. Moreover, this study only evaluated the association of BMI and blood-based DNAm AA among postmenopausal women. It is unclear whether findings from this study are generalizable to young women, or other tissue types. Considering African American women are disproportionally affected by high BMI, it is important to evaluate the association between BMI and DNA methylation age acceleration among African American women.

Using data from the Intergenerational Impact of Genetic and Psychological Factors on Blood Pressure (InterGEN) Study (49, 50) which began in April 2015, this study estimated DNA methylation age acceleration by applying Horvath's clock on saliva samples from 250 African American women. Further, this study explored 1) whether BMI associates with DNA methylation age acceleration (DNAm AA) measured in saliva samples among 250 African American women; 2) the association of weight status and

DNAm AA. Overall, we hypothesize that participants with higher BMI are likely to experience larger DNA methylation age acceleration calculated by Horvath's clock.

CHAPTER II

Association of Body Mass Index with DNA Methylation Age Acceleration In African American Mothers: Cross-sectional Analysis from InterGEN Study

By Chengchen Li

ABSTRACT

Background

African American women are affected by earlier onset of age-associated health deteriorations and obesity disproportionally, but little is known about the mechanism linking body mass index (BMI) and biological aging among this population. DNA methylation age acceleration (DNAm AA), measuring the difference between DNA methylation age and chronological age, is a novel biomarker of biological aging process, and predict aging-related disease outcomes.

Methods

The present study estimated cross-tissue DNA methylation age acceleration using saliva samples from 232 young African American women. Cross-sectional regression analyses were performed to assess the association of body mass index with age acceleration.

Results

The average chronological age and DNA methylation age were 31.67 years, and 28.79 years, respectively. After adjusting for smoking, hypertension diagnosis history, and socioeconomic factors (education, marital status, household income), 1 kg/m^2 increase in BMI is associated with 0.14 years increment of DNAm AA (95% CI: (0.08, 0.21)).

Conclusion

In African American women, high BMI is associated with saliva-based DNA methylation age acceleration, after adjusting for smoking, hypertension, and socioeconomic status. This finding demonstrated the positive association between BMI and DNAm AA across tissue types and demographic groups, and supports the hypothesis that high BMI and obesity accelerate biological aging.

MeSH: DNA methylation age acceleration, aging, obesity, BMI, African American

INTRODUCTION

Approximately 39.8% U.S. adults (age $\geq$ 20) were affected by obesity (BMI $\geq$ 30 kg/m^2) in 2015 – 2016 and adults obesity prevalence increased from 30.5% to 39.6% between 2003–2004 and 2015–2016. Overall, women (41.5%) had a higher prevalence of obesity than men (38%). Among women, the prevalence of obesity was the highest among non-Hispanic blacks (46.8%) compared with all other race and Hispanic origins (1). Obese individuals have been shown to be at greater risk for many age-related chronic diseases such as hypertension, coronary artery diseases, type2 diabetes, and certain types of cancer (3). Among females, non-Hispanic blacks had the shortest life expectancy (78.1 years) comparing with other racial ethnic groups (Hispanic female: 84.3 years; Non-Hispanic white female: 81.0 years) (5), which could be explained by earlier on-set of age-related chronic conditions and comorbidity among the disadvantaged groups (6, 7). Data from National Health and Nutrition Examination Survey (NHANES IV, 1999–2002) suggested that blacks are likely to experience health deterioration earlier in their lives, and hence, aging faster than whites (8).

Biomarkers that capture inter-individual variations of biological aging may accurately assess an individual's aging-related disease risks. DNA methylation, one of the best-known epigenetic modifications, has been recently used to develop a novel biomarker of biological aging (17). Epigenetic changes are defined as DNA modifications that affect gene expression without changing the DNA sequences. Both genetic factors and environmental stimuli, such as diet and smoking, can affect epigenetic changes.

During DNA methylation process, methyl groups are attached to segments of DNA in a particular gene and alter that gene's expression level (9). Recent research has identified specific genomic regions that are subject to substantial hypermethylation or hypomethylation associated with chronological age (10 - 14) and demonstrated its accuracy to predict one's age according to DNA methylation levels in specific tissues (e.g. saliva or blood) as well as cross multiple tissues (15 - 17). DNA methylation age (DNAm age) can be estimated based on DNA methylation levels of 353 CpG dinucleotides across multiple-tissues in Horvath's clock (17). Biological aging can be measured by the discrepancy between DNAm age and chronological age (Δage). Since DNAm age is strongly linearly associated with chronological age, the residual resulting from regressing DNAm age against chronological age in a linear model can also be defined as DNA methylation age acceleration (DNAm AA). DNAm AA is independent of chronological age, and can be used as an instrument to accurately measure how fast an individual ages (18).

Only few studies investigated the relationship between BMI and accelerated aging measured by DNA methylation levels among adults (43 - 47) and only one was conducted among the U.S. adults despite of the high prevalence of adults obesity in the U.S. Moreover, it is unclear whether findings from this study are generalizable to young women, or other tissue types. In the present study, we 1) estimated DNA methylation age acceleration by applying Horvath's clock on saliva samples from 250 African American women enrolled in the Intergenerational Impact of Genetic and Psychological Factors on Blood Pressure (InterGEN) Study which began in April 2015 (49, 50); 2)

explored the relationship of BMI and weight status, with DNA methylation age acceleration.

MATERIALS AND METHODS

STUDY SAMPLE

The InterGEN study is a longitudinal cohort study based in Southwest and Central Connecticut that examines the independent and interaction effects of genomic and environmental factors on blood pressure among African American mother-child dyads over time. After study procedures were reviewed and approved by New York University's institutional Review Broad (approval #1311012986), a total of 250 African American mothers and their biological children were recruited in the InterGEN study. Eligibility criteria include women a) speak English, b) are 21 years of age or older, c) self-identify as African American or black, d) have no active psychological or cognitive impairment, e) have a biological child aged 3-5 years. Detailed study procedures have been described elsewhere (16, 17). In brief, members of trained research team collect saliva samples, height and weight measurements, and blood pressure readings for both mothers and children. Demographic and socioeconomic information, psychological measures, and health history are obtained from mothers using audio self-assisted interviewing software. Researchers collect the clinical data and psychological measures during the baseline visit and three follow-ups approximately 6 months apart over the 2-year span. Data collected at baseline are included in the present study.

Phenotypes

BMI category was assigned to each individual according to the World Health Organization standard (48): underweight (<18.5 kg/m^2), normal-weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), obese (≥30.0 kg/m^2). Smoking was categorized as current smokers or non-current smokers. Marital status was classified into married, single, or other (divorced, separated, widowed, living with but not married to a significant other). Individuals were classified as a college graduate and no college degree according to their self-report. Annual household income was grouped into < $15,000, or ≥$15,0000.

DNA methylation profiling and data processing

Genome-wide DNA methylation was profiled in saliva samples from 250 African American women using the Illumina Infinium Methylation EPIC (850K) BeadChip which quantifies DNA methylation at over 850,000 CpG dinucleotides with near complete coverage of all known genes. Fluorescent signals from the M (methylated) and U (unmethylated) probes were measured and used to determine DNA methylation level (β=max (M,0) /|U|+|M| $+ 100$) for each CpG site. Using detection p-value threshold of 0.001 for each CpG site, we excluded DNA methylation sites of which missing genotype call rate is above 10%. Additional quality control procedures of DNA methylation data were described previously including probe-level quality control and normalization (49). We calculated DNAm Age for each individual using the Horvath epigenetic clock based on the methylation levels of 353 CpGs (18). Due to the upper limit for uploading large files, DNAm levels (β values) of 28,587 CpGs from each sample were selected according

to a reference file containing CpG probes present on the Illumina 450K array. Among all preselected CpG sites, 353 sites were used to calculate the DNAm age, to estimate tissue type, and to predict sex using an online age calculator (https://dnamage.genetics.ucla.edu/). DNAm AA, the measure of age acceleration is defined as the residual term of a univariate model regressing estimated DNAm Age on chronological age.

Statistical analysis

We excluded individuals flagged with inconsistent prediction sex, and/or unrealistic estimated DNAm Age (|Chronological Age – DNAm Age| > 15, likely sample swaped between mother and child dyad). In addition, individuals with missing information on covariates are excluded. The final sample for the DNAm age analysis included 232 African American mothers. We conducted multiple linear regression analysis to evaluate the association between DNAm AA and BMI considering a range of phenotypic variables , which were selected based on prior studies of DNAm AA (19 - 21, 43 - 47) and availability in the InterGEN study. We compared DNAm AA across BMI categories, adjusting for smoking, household income, education, marital status, and previous hypertension diagnosis. We also investigated the linear relationship between BMI and DNAm AA using spline regression analysis. All analyses were conducted in R studio with R version 3.4.3.

RESULTS

The present study comprised 232 individuals from the cohort for whom DNAm AA, BMI, demographic information, health history is completed. As shown in Figure 1, chronological age was strongly correlated with DNAm Age ($r = 0.77$; $p < 0.001$). Table 1 summarizes sample characteristics and results of regression analysis of factors related to DNAm AA. The DNA methylation age (28.8±6.7 years) was younger than the chronological age (31.7±5.7 years) among studied African American mothers. The median BMI was 28.58 kg/m^2 (IQR: 23.61 - 34.14) with 58 overweight individuals (25.0%) and 102 obese individuals (44.0%). 53 individuals (22.8%) reported themselves to currently smoke cigarettes, and 47 individuals (20.3%) reported to be previously diagnosed with hypertension.

In univariate regression analysis (Table 1), we observed a statistically significant positive association between BMI and DNAm AA (0.15 years increase in DNAm AA per 1 kg/m^2 increase in BMI ($p < 0.001$)). Obese individuals (BMI ≥ 30 kg/m²) were 1.71 years "older" (95%CI: 0.34, 3.08) than individuals with normal weight. On average, overweight ($25 \leq$ BMI < 30 kg/m²) and underweight (BMI < 18.5 kg/m²) individuals were 0.83 years "older" (95%CI: -0.71, 2.38) and 0.91 years "younger" (95%CI: -3.47, 1.66), respectively, than individuals with normal weight. However, we only observed statistically significant associations among obese individuals. Compared with individuals with no hypertension history, individuals with hypertension diagnosis were 1.49 years "older" measured by DNAm AA (95%CI: 0.11, 2.86). Mothers who identified themselves as current smokers were 0.73 years "younger" than non-smoker mothers, however, the association was not

significant (95% CI: -2.05, 0.59). No significant associations were observed between socioeconomic factors (education level, annual household income, and marital status) and DNAm AA.

To better understand the association of BMI and DNAm AA, we fit BMI as the independent variable and DNAm AA as the dependent variable in a spline regression model. We observed that DNAm AA increases more rapidly per 1 kg/m² increment of BMI among low BMI group (BMI < 20 kg/m²) and extreme high BMI group (BMI > 45 kg/m²). This suggested that the association of BMI and DNAm AA might be stronger in individuals of low or extreme high BMI (Figure 2).

In multivariable analysis (Table 2), we further examined whether the association of BMI and DNAm AA is independent of socioeconomic status, smoking status and hypertension history. After adjusting for socioeconomic status, 1 kg/m^2 increase in BMI is associated with 0.16 years increment of DNAm AA (95% CI: (0.09, 0.22)). After further adjusting for smoking status and hypertension history, BMI remained positively associated with DNAm AA. (0.14 years increase in DNAm AA per 1 kg/m^2 increase in BMI, (95% CI: (0.08, 0.21)).

Similar analyses were conducted to investigate the relationship between weight status and DNAm AA (Table 3). As shown in Model 3 (Table 3), after adjusting for socioeconomic status, smoking status and hypertension history, obese and overweight individuals were 1.58 years (95%CI: 0.17, 2.99) and 0.66 years (95%CI: -0.92, 2.25) “older” than individuals with normal weight. In contrast, underweight individuals were

0.77 years “younger” (95%CI: -3.38, 1.83) than normal weight individuals. However, the difference was statistically significant among obese individuals only.

DISCUSSION

DNAm AA and BMI

To my knowledge, this study is the first to report a significant positive association between BMI and DNAm age acceleration using saliva samples from a cohort of young African American women. We also observed this association is independent of smoking status, socioeconomic factors, and hypertension status. Our main finding that high BMI is associated with accelerated epigenetic aging aligns with other studies (21, 43 - 47). In a subset of 4,173 postmenopausal women from Women’s Health Initiative (WHI) including 2,045 Caucasians, 1,192 African Americans, and 717 Hispanics, researcher observed a statistically significant correlation between BMI and DNAm AA among African American ($r = 0.05 – 0.06$). Longitudinal data available for a small subset of individuals in WHI also suggested that increase in BMI over time is associated with increase in DNA methylation age acceleration ($\beta_{\text{Extrinsic DNAm AA}} = 0.40$; $\beta_{\text{Intrinsic DNAm AA}} = 0.27$) (47). In another sub-cohort of 2543 African Americans with mean age of 57 years enrolled in the ARIC Study, BMI was also statistically significantly correlated with DNAm AA ($r=0.02$) (21). Positive association between BMI and DNAm AA was also found in liver samples collected from German patients, where DNAm AA increased by 0.33 years for 1 kg/m^2 increase in BMI (46).

Although the direction of association or correlation between BMI and DNAm AA in our study is consistent with previous studies, we observed smaller estimated effect of BMI on DNAm AA in the present study (0.14 years increment in DNAm AA per 1 kg/m^2 increase in BMI (95% CI: (0.08, 0.21)). Additionally, our findings in the correlation between BMI and DNA methylation age acceleration in saliva (r = 0.28) (Figure 2) is weaker than that has been previously reported for human liver (r=0.42) but stronger than that for blood ($0.02 \leq r \leq 0.08$) (21, 46, 47). Such tissue-dependent correlations suggest that the DNAm AA estimate may not be completely consistent across tissue types. Furthermore, unlike most of the previous studies in which most enrolled participants were old individuals (21, 46, 47), our study population consists of young African American women only. This association and/or correlation strength might vary depending on age group, sex and ethnicity.

DNAmm and Weight Status

Moreover, examination of BMI categories reveals the trend in DNAm AA across weight groups. Comparing with normal weight individuals, obese and overweight individuals had higher average DNAm AA and underweight individuals have average lower DNAm AA. The difference was statistically significant among obese individuals, but not in overweight and underweight group which could be due to smaller sample size of these two groups in the present study. This trend is consistent with findings from a prospective cohort study nested within the Melbourne Collaborative Cohort. This cohort study collected blood samples from 2,818 participants, and observed higher DNAm AAs in overweight (BMI ≥ 25) and obese individuals (BMI ≥ 30) compared with lean

individuals (BMI < 25) ($\beta_{\text{Overweight vs Lean}}$ = 0.40 ; $\beta_{\text{Obesity I vs Lean}}$ = 0.15 years; $\beta_{\text{Obesity II \& III vs. Lean}}$ = 2.38 years) (45).

Hence, DNA methylation could be a potential molecular connection between obesity and accelerating aging. Although mechanisms of this connection are unclear, one potential explanation is that oxidative stress introduced by obesity could alter the regulation of DNA methylation, especially at CpGs related to cell growth and death, organism development and aging.

DNAm AA and other covariates

Extending finding that hypertension is significantly associated with accelerated epigenetic aging, is consistent with other studies (19,51). Results for self-reported smoking status and DNA methylation age acceleration are consistent with 3 recent reports that have failed to find robust associations (52 - 54). Of 3 socioeconomic factors assessed, we observed no significant association between annual household income, marital status, and DNAm AA. This contrasts with results from a study of a U.S. sample of middle age Black women where they found significant associations of low income, marital status, with epigenetic aging estimated by Hannum's clock (55). This might be explained by the possible greater economic hardship experienced by participants in the previous study. Additionally, consistent with other studies (55 - 57), we failed to observe a significant association between educational attainment and DNA methylation age acceleration.

DNA methylation age acceleration

This cohort of young African American mothers had an average ΔAge of -2.41 years and a median DNAm AA of -0.14 years. They appear to be biologically "younger" despite of high burden of many health risk factors. This could be explained by a lower level of global methylation among non-Hispanic blacks comparing to other race ethnicities (57). However, potential molecular mechanisms such as genetic polymorphisms associated with activities of enzymes involved in DNA methylation process could be responsible for race ethnic differences in global DNA methylation and DNA methylation age acceleration.

Telomere length and BMI

Telomere length is another established aging biomarker used in population studies. Telomere length progressively shortens with advancing age and thus estimates biological aging (22). Previous studies found BMI is negatively correlated with leukocyte telomere length (23 - 27). It demonstrated that BMI is associated with an age acceleration measured by telomere length. However, biological age quantified by telomere lengths is weakly correlated with DNAm age ($|r|=0.02 – 0.03$) (28), and these two estimates are independent predictors of chronological age, morality and frailty (29, 30). Therefore, the impact of BMI on age acceleration measured by DNA methylation might be independent of aging predicted by telomere length.

Strengths and limitations

The present study has both strengths and limitations. One strength is that InterGEN cohort consists of young African American women only. This minimizes potential confounding effects by genetic stratification and sex. Moreover, the

availability of both comprehensive genotype and phenotype information in InterGEN cohort has permitted the evaluation of both genetic and environmental risk factors of accelerated epigenetic aging which has not been traditionally studied in an all African American sample. The available DNA source, a saliva sample, poses a potential limitation as its limited generalizability to other tissues. Different tissues might age at different rates. Another limitation is the development of Horvath's clock relies on DNA methylation levels measured by two different platforms: the Illumina Infinium27K and the Infinium450K platform. Other limitations include the cross-sectional design of the present study as it does not allow to assess the impact of BMI on changes in DNA methylation age acceleration.

CONCLUSION

Overall, we observed that high BMI is associated with saliva-based DNA methylation age acceleration of young African American women, after adjusting for smoking, hypertension, and socioeconomic status. This finding demonstrated the positive association between BMI and DNAm AA across tissue types and demographic groups, and supports the hypothesis that high BMI and obesity accelerate biological aging. Future studies with longitudinal study designs are needed to assess the impact of BMI change over time on DNA methylation age acceleration, and potential interventions to prevent biological aging process.

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Tables and Figures

Table 1. Population characteristic of study sample (n=232) from Intergeneration Blood Pressure Study and results of univariate regression analysis between multiple factors and DNA methylation age acceleration

Characteristics		Mean (SD)/Median (IQR) / N (%)	β (95% CI)
Chronological age, years		31.67 (5.70)	NA
DNA methylation age, years		28.79 (6.75)	NA
ΔAge, years		-2.41 (4.33)	NA
DNAm AA, years		-0.14 (-3.04 – 2.65)	NA
Body Mass Index, kg/m^2		28.58 (23.61 - 34.14)	0.15* (0.08, 0.21)
Maternal cigarettes use			
	No	179 (77.20)	0 (Ref)
	Yes	53 (22.80)	-0.73 (-2.05, 0.59)
Education			
	< College	94 (40.50)	0 (Ref)
	College graduate or higher	138 (59.50)	0.77 (-0.36, 1.90)
Annual household income, $			
	< 15,000	122 (52.60)	0 (Ref)
	≥ 15,000	110 (47.40)	0.19 (-0.93, 1.30)

Marital Status			
	Married	55 (23.70)	0 (Ref)
	Single	152 (65.50)	-0.28 (-1.62, 1.06)
	Others	25 (10.80)	-0.41 (-2.46, 1.64)
Ever diagnosed with hypertension			
	No	185 (79.70)	0 (Ref)
	Yes	47(20.30)	1.49* (0.11, 2.86)
BMI category			
	Underweight (< 18.5 kg/m²)	13 (5.60)	-0.91 (-3.47, 1.66)
	Normal weight (18.5 - 24.9 kg/m²)	59 (25.40)	0 (Ref)
	Overweight (25 - 29.9 kg/m²)	58 (25.00)	0.83 (-0.71, 2.38)
	Obese (≥ 30 kg/m²)	102 (44.00)	1.71* (0.34, 3.08)

Abbreviations: CI, confidence interval; BMI, Body mass index; ΔAge, the discrepancy between DNA methylation age and chronological age; DNAm AA, DNA methylation age acceleration, the residual resulting from regression DNA methylation age on chronological age in a linear model.

** p-value < 0.05.*

Table 2. Results of cross-sectional multivariable analysis of association of BMI (kg/m^2) and DNAm AA (year) in study sample (n = 232) from InterGEN study

	Model 1	**Model 2**	**Model 3**
$\hat{\beta}_1$	0.16 ***	0.15 ***	0.14 ***
(95%CI)	(0.09, 0.22)	(0.09, 0.22)	(0.08, 0.21)
Covariates adjusted for in the model	Annual household income Education Marital Status	Annual household income Education Marital Status Smoking	Annual household income Education Marital Status Smoking Hypertension Diagnosis

Abbreviations: CI, confidence interval; BMI, Body mass index; DNAm AA, DNA methylation age acceleration.
**** p-value < 0.001.*

Table 3. Results of cross-sectional multivariable analysis of association of weight status and DNAm AA (year) in study sample (n = 232) from InterGEN study

		Model 1	**Model 2**	**Model 3**
$\hat{\beta}_1$ **(95%CI)**	Normal weight[a]		0 (Ref)	
	Obese[b]	1.83 * (0.44, 3.23)	1.80 * (0.40, 3.20)	1.58 * (0.17, 2.99)
	Overweight[c]	0.88 (-0.70, 2.46)	0.87 (-0.72, 2.45)	0.66 (-0.92, 2.25)
	Underweight[d]	-0.80 (-3.41, 1.82)	-0.80 (-3.42, 1.82)	-0.77 (-3.38, 1.83)
Covariates adjusted for in the model		Annual household income Education Marital Status	Annual household income Education Marital Status Smoking	Annual household income Education Marital Status Smoking Hypertension Diagnosis

Abbreviations: CI, confidence interval; BMI, Body mass index; DNAm AA, DNA methylation age acceleration.

** p-value < 0.05.*

[a] *Normal weight was defined a BMI of ≥ 18.5 kg/m² and < 24.9 kg/m².*

[b] *Obesity was defined a BMI of ≥ 30 kg/m².*

[c] *Overweight was defined a BMI of ≥25 kg/m² and < 30 kg/m².*

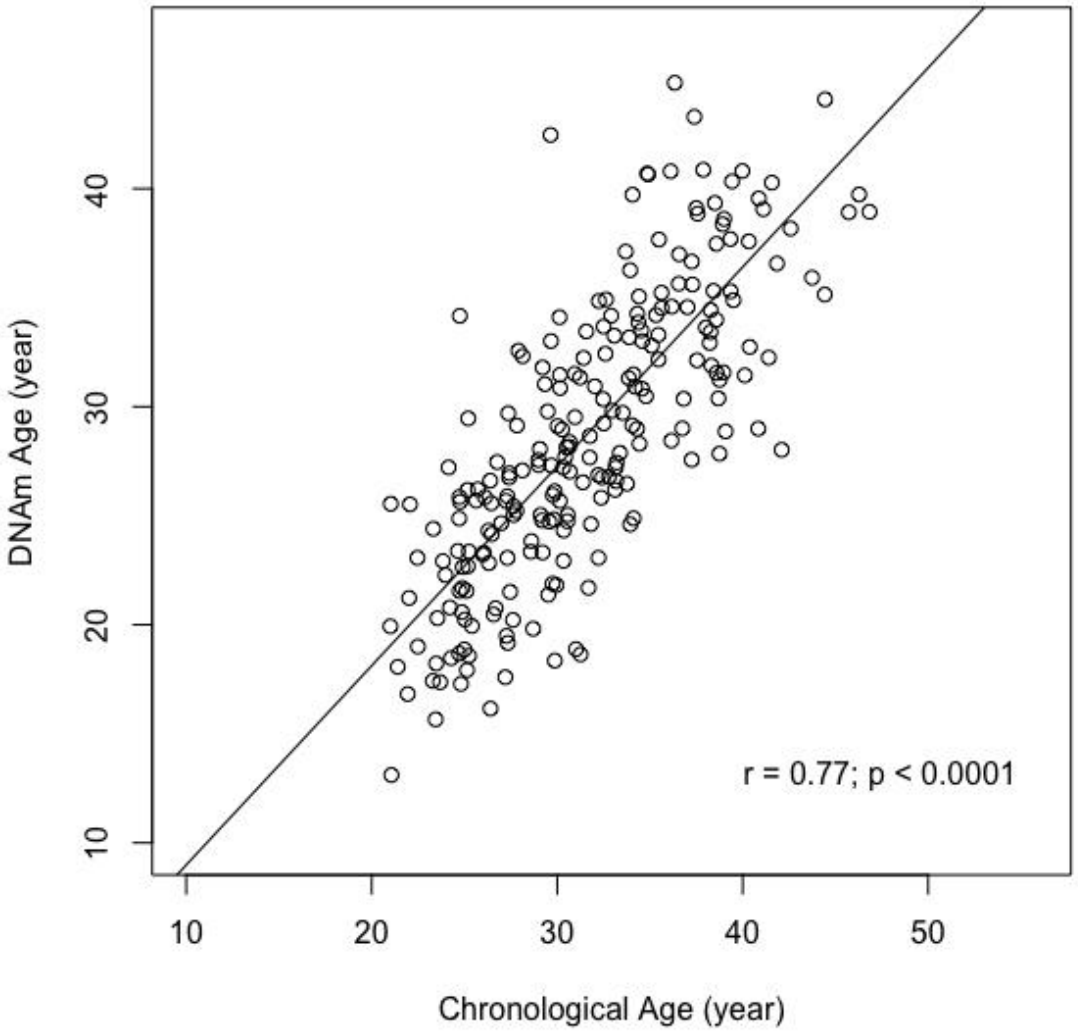

Figure 1. Significant correlation between the chronological age and DNA methylation age in 232 African American women from InterGEN study.

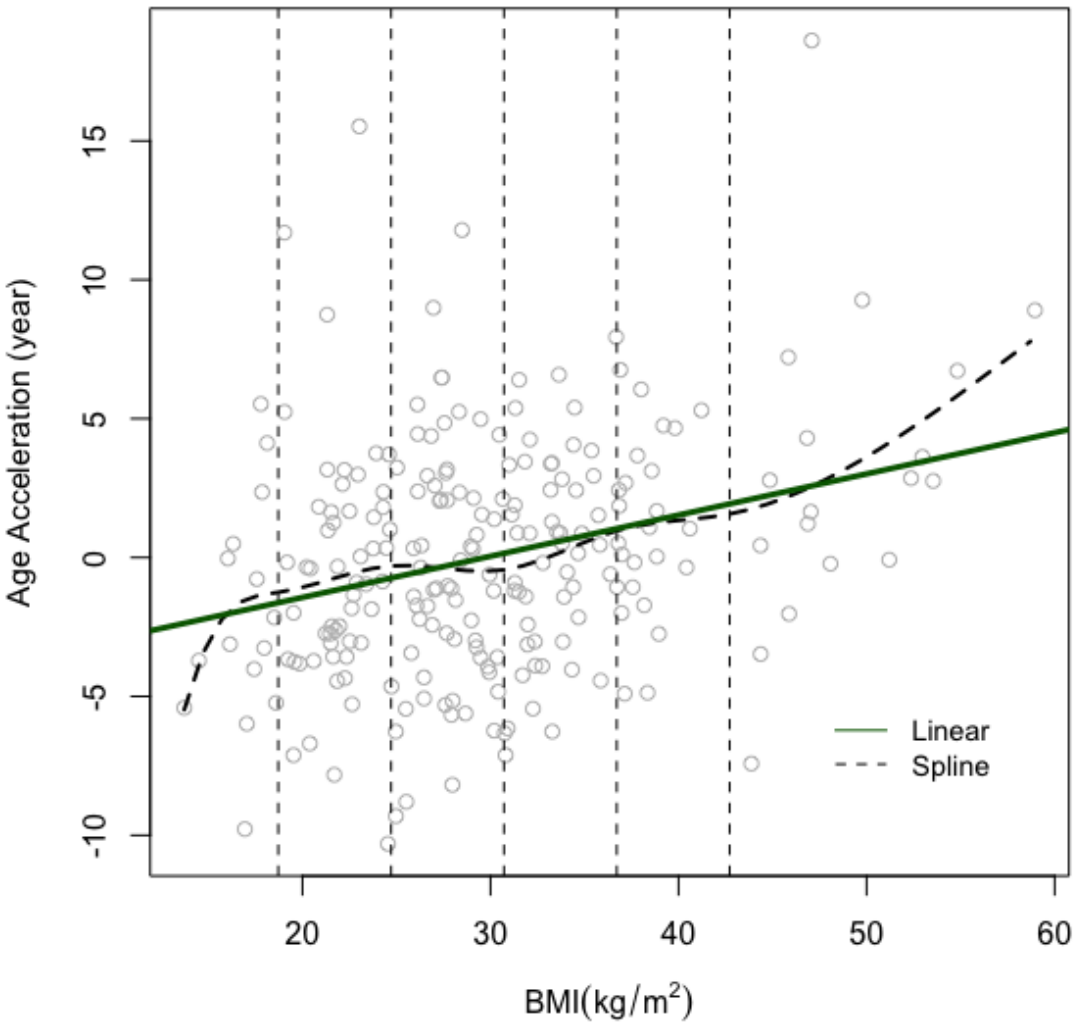

Figure 2. Association between BMI and DNA methylation age acceleration with **a)** a univariate linear model (0.15 years increase in DNAm AA per 1 kg/m^2 increase in BMI ($p < 0.001$)) **b)** a cubic smoothing spline in 232 African American women from InterGEN study.

CHAPTER III

Findings from this present study supports the hypothesis that obesity accelerates human aging and addressed the importance of maintain a healthy weight according to a sample of young adults. I would investigate the same association among children enrolled in InterGEN cohort, as well as whether BMI affects DNA methylation age acceleration intergenerationally. Moreover, future studies with longitudinal study designs can be implemented to assess whether change in BMI over time affects an individual's epigenetic aging rate. In addition, I would explore the relationship of psychological factors with DNA methylation age acceleration as stress is a known risk factor for many chronic diseases. Accelerated aging could be a biological mechanism linking stress and chronic disease risks. Lastly, I would examine whether DNA methylation age acceleration could be a predictor for chronic conditions highly prevalence among African American females, such as hypertension.