BIOLOGICAL AGING QUANTIFICATION AND ITS ASSOCIATION WITH SLEEP IN THE BOGALUSA HEART STUDY

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OF

DOCTOR OF PHILOSOPHY

BY

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Abstract

Background:

Human Biological Aging (BA) estimates are developed by human to better capture the gradual increase in the vulnerability of the aging body than chronological age. Human sleep dimensions have been suggested to be associated with human health indicators including cardiometabolic function, cognitive function and mortality. The objective of this study was to examine indicators of BA and their predictive validity using Klemera and Doubal’s Method (KDM), and Physiological Dysregulation Method (PDM) for quantifying BA, as well as to explore if phenotypical and genetic associations between sleep variables and BA estimates exist, using the Bogalusa Heart Study (BHS) – a community-based, cohort study.

Method:

In order to estimate BA, nineteen biomarkers were selected. Training datasets were from NHANES. The target dataset included 1,034 BHS subjects assessed between 2013-2016. Training was done separately for male and female, black and white participants. KDM and Mahalanobis Distance (D_M) based PDM methods were used. Cognitive and physical performance testing were used to examine predictive validity.

The association between three sleep dimension variables and BA estimates were explored using 953 black and white BHS 2013-2016 subjects. Sleep duration in hours, chronotype scores and social jetlag in hours were the independent variables. BA estimates were the dependent variables.
Genotyping information from the BHS 2013-2016 were included (n=646) for genetic association. Related SNPs on morning chronotype were used to compute a genetic risk score (GRS) for BHS participants. Association between chronotype GRS and chronotype phenotype were explored.

Multivariate linear regression was used for all association analyses.

Results:

BA estimates were calculated using both the KDM and PDM methods. Linear regression showed that PDM BA estimates were associated with lower cognitive function physical performance tests. The effect sizes of all associations between PDM BA estimates and performance tests were of greater magnitude than between KDM estimates and performance tests. Short sleep duration and evening chronotype was associated with larger PDM BA estimates. Morning chronotype GRS was not associated with morning chronotype phenotype among BHS participants.

Conclusion:

PDM BA estimates are robust measures of biological aging in black and white men and women enrolled in the BHS. Insufficient sleep duration and evening chronotype may advance biological aging, regardless of gender, race and CA. We did not find association between morning chronotype GRS and morning chronotype phenotype. PDM
BA estimates should be recommended for future aging studies using data from BHS participants.

Keywords:

Biological aging; Klemera and Doubal’s Method; Mahalanobis Distance; Sleep duration; Chronotype; Social jetlag; Genetic risk scores.
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List of abbreviations

AA: African ancestry

AK: Adenosine Kinase

BA: Biological Aging

BHS: Bogalusa Heart Study

BUN: blood urea nitrogen

CA: Calendar Age

CKD: chronic kidney diseases

CLOCK: Circadian Locomotor Output Cycles Kaput

CSM: Composite Scale of Morningness

DBP: diastolic blood pressure

DM: Mahalanobis Distance

DNAm PhenoAge: DNA Methylation Phenotypic Age

EA: European ancestry

GH: growth hormone

GRS: Genetic Risk Score

GWAS: Genome Wide Association Study

HD: Homeostatic Dysregulation
InCHIANTI: Invecchiare in Chianti

KDM: Klemera and Doubal’s Method

KNHANES: Korean National Health and Nutrition Examination Survey

LTL: leukocyte telomere length

MCQ: Munich Chronotype Questionnaire

MEQ: Morningness-Eveningness Questionnaire

MetS: Metabolic Syndrome

MR: Mendelian Randomization

NHANES: National Health and Nutrition Examination Survey

NIH: National Institute of Health

PCA: Principal Component Analysis

PDM: Physiological Dysregulation Method

PER: Period Circadian Regulator

PGS: polygenic score

PRS: polygenic risk score

REM: Rapid Eye Movement

RGS: Regulator of G protein Signaling

RMR: Resting Metabolic Rate
SBP: systolic blood pressure
SCN: suprachiasmatic nucleus
SD: standard deviation
SILJ: social jetlag
SNP: single nucleotide polymorphism
SRF: serum response factor
SPPB: the Short Physical Performance Battery
TSH: thyroid-stimulating hormone
UA: uric acid
VNTR: variable number tandem repeat
WAIS: Wechsler Adult Intelligence Scale
WMS: Wechsler Memory Scale
WRAT: Wide Range Achievement Test
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Chapter 1. Review of Literature

Biological Aging (BA) Introduction

Biological aging (BA) estimates are developed to better capture the gradual reduction in the viability of the organism and gradual increase in the vulnerability of the aging body than chronological age. Middle aged people with the same chronological age could have varied BA. Recent studies showed that BA is more accurate in relation to mortality, disease accumulation and physiological function than chronological age. BA is expected to be linearly associated with chronological age (CA), predict mortality morbidity and physical, cognitive function decline.

Gender and Race/Ethnicity Disparity on BA

BA differs between male and female individuals and across race/ethnicity groups. Cohen et al. discovered the “male–female dysregulation–frailty paradox” whereby men showed greater Physiological Dysregulation (PD, measured by Mahalanobis Distance - D_M) but show less susceptibility to frailty. People who identify as black were suggested to be 3 years older biologically than their white counterparts of the same CA.

Conversely, Blacks seem to have longer leukocyte telomere length (LTL) at birth but greater LTL shortening rate through their life course, compared to Whites. LTL is sensitive to social stress and shorter LTL is linked to premature mortality. Black Americans and Latino Americans expect less chronological age-related functional decline than Korean and Chinese Americans.
Introduction to Current BA Measures

BA is commonly estimated using two different methods. These methods use blood-based biomarkers and body examination measurements to quantify BA by algorithms. Other BA quantification measures include the telomere length, Frailty index, phenotypic age and DNA methylation age. These are summarized below.

Klemera and Doubal's Method (KDM)

The KDM uses mathematical algorithms to estimate BA for any battery of biomarkers. The KDM BA estimates are based upon minimizing the distance between m regression lines and m biomarker points, within an m dimensional space of all biomarkers. KDM has been regarded as one of the BA algorithms that has the highest sensitivity and most robust predictive power, despite including various biomarkers due to the availability in the datasets. The KDM first requires a reference/training database (often the NHANES) to calculate the algorithm parameters biomarkers based on their regression coefficients with CA. In previous studies, the chosen candidate biomarkers usually came from the domains of cardiovascular, lung, renal, hepatic, immune, cell blood count and metabolic function. Models were usually estimated separately for men and women. Second, the parameters are then used to calculate BA for individuals in the target dataset.
Physiological Dysregulation Method (PDM)

PDM is also referred to as homeostatic dysregulation. Mahalanobis Distance (\(D_M\)) is used as measure of PDM. \(D_M\) quantifies deviations of the biomarkers compared to their “normal” values \(^{14}\). Similar to the KDM, \(D_M\) also requires calculating the parameters based on reference population first and applying the parameters and the algorithm in target population. Arbeev et al. suggested selecting candidate biomarkers that are available in the study populations and those who are associated with mortality risk and risk of onset of “unhealthy life” \(^{15}\). Higher PD was associated with increased vulnerability to many diseases and precede their clinical manifestation \(^{15}\). PD could be a promising physical resilience and robustness measure in Aging studies \(^{15}\).

The Frailty Index-34 (\(FI_{34}\))

Kim et al. have constructed the \(FI_{34}\) with 34 health and function variables as a way to quantify BA \(^{16}\). Any individual’s FI score is calculated as the proportion of any deficient health variables amongst the 34 variables at a given age \(^{16}\). \(FI_{34}\) has been shown to be a good predictor of mortality and is positively associated with Resting Metabolic Rate (RMR) \(^{17}\). \(FI_{34}\) increases exponentially with chronological age and reflects individual variability or heterogeneity \(^{16-18}\). The \(FI_{34}\) is not dependent on blood test or complicated algorithm calculations, but the availability of the geriatric variables within the 34 variables is often limited among non-older populations.
**Phenotypic Age**

The idea of Phenotypic Age estimate was brought up by Levine et al. They first used NHANES as training data and produced parameters for each included biomarker (satisfying the threshold of significant association with aging-related mortality, plus CA) in the parametric proportional hazards model based on the Gompertz distribution. After that, the parameters and the algorithm were used in the target/validation dataset to estimate the Phenotypic Age for each individual. Phenotypic Age was significantly associated with mortality, morbidity and physical functions.

**DNA Methylation Phenotypic Age (DNAm PhenoAge)**

Epigenetic biomarkers such as DNA methylation (DNAm) has been potential BA biomarker recently. Unlike The blood-based DNAm algorithm by Hannum and the multi-tissue DNAm algorithm by Horvath, Levine et al. proposed the DNAm PhenoAge that produces BA estimates that correlate with Phenotypic Age well instead of CA. Similar to KDM and PD, reference/training dataset (the Invecchiare in Chianti (InCHIANTI) study) was needed to produce the linear regression parameters of candidate CpGs in the DNAm PhenoAge algorithm. Further analyses in the target/validation dataset suggested that DNAm PhenoAge strongly outperforms previous measures in regards to predictions for a variety of aging outcomes including mortality morbidity and many other diverse outcomes across multiple tissues and cells.
**Telomere Length**

Telomeres are long tracts of TTAGGG repeats at the end of chromosomes. They protect chromosome ends from being recognized as double-strand DNA damage \(^{21}\). Telomeres naturally undergo attrition with each division of human somatic cells. Telomere Length is heritable and its shortening may be a major determinant of human aging \(^{22}\). However, BA comparison studies have showed that Telomere length did not perform as well as KDM, PD or Phenotypic Age in terms of effect sizes \(^{23}\).

**Sleep Definitions**

For most humans in the world, sleep occupies about one third of their lifetime. Like diet and exercise, sleep is a behavior that largely influence people’s health, wellbeing, energy refill and performance \(^{24}\). Compared to 50 to 100 years ago, human beings at present sleep 1-2 fewer hours per night. Health consequences of sleep loss and other unhealthy sleep habits are estimated to effect 70 million people in the US \(^{24}\). In addition, direct and indirect costs of sleep-related problems are suggested to have significant cost in workplace and economy as well \(^{25}\). Study on five developed countries showed that employees with insufficient sleep have less productivity \(^{25}\). The U.S. loses approximately 1.23 million working days due to sleep deprivation \(^{25}\). Across the U.S., Canada, Japan, Germany, and United Kingdom, it is estimated that up to $680 billion is lost due to insufficient sleep annually \(^{25}\). The US Office of Disease Prevention and Health Promotion has been promoting sleep health as new focus objective of Healthy People 2020 \(^{26}\).
Sleep-related behaviors and features include sleep deprivation, chronotype, social jetlag, self-reported sleep problem and other sleep problems. Based on current research, non-optimal sleep behaviors likely lead to undesirable health outcomes through mechanisms such as unhealthy diet timing and preference, metabolic disturbance, hormone secretion and change of light exposure. Some of the detrimental health outcomes related to sleep disorders include obesity, metabolic syndrome, hypertension, diabetes, depression, and cardiovascular diseases (CVD).

Sleep Duration

Sleep duration refers to the total amount of obtained sleep. It can be either only during the nocturnal sleep, or across the 24-hour period. Sleep duration is usually determined by self-reporting, actigraphy, and polysomnography. The American Academy of Sleep Medicine and Sleep Research Society recommended no less than seven hours of sleep per night for adults. Long sleep duration is usually defined as more than eight or nine hours, moderate/normal sleep duration is usually six to eight hours, and having less than six or seven hours is short sleep duration or sleep deprivation. Worldwide, people sleep at least 30 minutes less than they did over 10 years ago. Thirty five percent of U.S. adults are reported to be habitual short sleepers.

Numerous studies have been performed to study the association between sleep duration and adverse health outcomes in the last two decades. Among older adults, it is indicated that long sleep duration, rather than short sleep duration was associated with worse cognitive function. Greater changes in sleep duration were also associated with
poor cognitive function\textsuperscript{40}. Short and long sleep duration were associated with metabolic syndrome (MetS) and MetS severity, especially in women\textsuperscript{41}. Data from NHANES found that mean-predicted 10-year cardiovascular risk was lowest among adults who reported sleeping 7 hours per night and increased as participants reported sleeping fewer and more hours\textsuperscript{42}. Both, short and long sleep duration were also associated with higher mortality rate, risk of hypertension, diabetes, depression, and fatty liver disease\textsuperscript{33, 34, 43-47}. Short sleep duration/Sleep deprivation was associated with higher risk of obesity and chronic kidney diseases (CKD)\textsuperscript{30, 48}.

\textit{Circadian Rhythm and Sleep Chronotype}

The circadian rhythm of humans encompasses an endogenous ‘biological clock’\textsuperscript{49} of about 24 hours that is mainly determined by the natural light-dark circle and mediated by the suprachiasmatic nucleus (SCN) in the hypothalamus\textsuperscript{50}. Following an approximately 24-hour period, both the human physiology-core body temperature and hormones especially melatonin, and human behavior, render oscillations that are subject to environmental influences such as light exposure\textsuperscript{51}. Distinguished from ‘Circadian rhythm’, sleep chronotype refers to the circadian preferences of individuals\textsuperscript{52}. The sleep-wake cycle is delayed in individuals with a late chronotype preference (or eveningness) compared to those with an early chronotype preference (or morningness). Sleep chronotype largely influences the time a person feels at their peak, and it is largely determined by individual differences in circadian rhythm\textsuperscript{53}. 
Without disruptions, individuals with a preference for late evenings, known as ‘Night owls’ are more comfortable to go to sleep late, wake up late and be energetic/productive at later time of a day’s cycle, while those with a preference for morning, or ‘Early birds’, act oppositely. The most common ways to measure chronotype are self-reported questionnaires such as the Morningness-Eveningness Questionnaire (MEQ), the Composite Scale of Morningness (CSM) and the Munich Chronotype Questionnaire (MCQ) 54.

Prior studies have found associations between eveningness/late chronotype and increased risk of a list of detrimental health outcomes including obesity, depression, cardiovascular stress, high blood pressure, anxiety and diabetes 55-57. Eveningness has also been reported to be associated with other negative health behaviors such as diet behaviors which would lead to more negative health outcomes 58.

Social Jetlag

Jetlag refers to the mismatch between the biological clocks in human bodies and external time cues 59,60. Social jetlag is another type of jetlag which emphasizes the discrepancy in sleep timing between our work days and free days, between biological timing and social timing 38. Social Jetlag is usually calculated as the difference between weekend sleep midpoint and weekday sleep midpoint (between bedtime and risetime). Human circadian rhythm is about 24 hours and sleep chronotype is the individual circadian preference manifested in behaviors 53,61. When late chronotype people are forced to have early arousals during work days, they accumulate sleep debt which will
usually be compensated on weekends by extending sleep duration and altering sleep onset and/or offset timing \textsuperscript{62}.

Up until now, large scale observational studies on social jetlag have been limited. A longitudinal study included 65,000 Europeans and showed that adults had extra-long sleep duration on free days and much short sleep duration on work days, and that the average sleep duration gaps between work days and free days significantly decreased among adults as their age increase \textsuperscript{24}. Roughly 80\% of people need an alarm to wake up on workdays, indicating high prevalence of the ‘social jetlag’ \textsuperscript{24}.

Similar to other sleep-related behaviors, social jetlag has been reported to be associated with a series of adverse health outcomes including depression, type 2 diabetes, metabolic syndrome, cardiovascular risk, work ability and ADHD \textsuperscript{63-67}.

Sleep and Health

The Conceptual model of sleep health

Daniel J. Buysse proposed the original sleep health definition ‘SATED’, which summarized five dimensions of sleep health based on existing sleep and health research: (1) Sleep duration (2) Sleep continuity or efficiency (3) Sleep timing (4) Alertness/sleepiness and (5) Satisfaction/quality \textsuperscript{68}. SATED was later expanded to ‘RU SATED’ by adding regularity as the sixth sleep health dimension \textsuperscript{69,70}.

Buysse also proposed a conceptual model of sleep health (Figure below), which integrated the genetic, social, environmental, behavioral, and health care factors into the
association between sleep and health. Intermediate level processes including genetic and molecular, and system-level processes including inflammation and hormonal responses explain how the multiple sleep dimensions can affect health and function outcome.

**Circadian Rhythm, Sleep, and Hormone**

Several hormone secretions are affected by sleep and the light-dark cycle. Growth hormone secretion increases during sleep and peak soon after falling into sleep. Melatonin levels are higher at night than in the day. Thyroid-stimulating hormone (TSH) concentrations usually top in the middle of the night and dip during the afternoon. Cortisol level keeps rising during the night and peaks in the morning. Ghrelin secretion increases prior to and decreases after meal times. Leptin secretion has
been suggested to increase at night and peak in the morning, but results are not consistent 28, 81.

**Sleep and Immunity**

Sleep and immunity affect each other. Microbial challenges to the immune system trigger inflammatory response and leads to increase in sleep duration, sleep intensity and also sleep disruption 82. During an infection, sleep enhancement is associated with reduced infection risk, improved infection outcome and improved vaccine responses 82. It is likely that in these circumstances, sleep gives feedback to the immune system to improve host defense through hormonal constellation induction 82, 83. In addition, prolonged sleep deprivation and sleep disturbance were suggested to lead to chronic low-grade inflammation and diseases (such as neurodegeneration, atherosclerosis and diabetes) via inflammatory mediators like cytokines 82.

**Sleep and Aging**

Physiological and activity rhythms were found to decrease with aging. Activity rhythms generally phase advance towards early bedtime and early morning wakening, as well as a longer latency to fall asleep, reduced length of Rapid Eye Movement (REM) and reduced period of stage 3 & 4 of None REM 84-91. For example, daily rhythms in hormones like melatonin and cortisol are decreased among older adults. In addition, sleep and body temperature rhythms also tend to fade in older individuals 92.
As people age, they tend to shift into morningness preference\textsuperscript{87, 93, 94}. With advanced sleep phase, older adults tend to have reduced sleep efficiency, self-reported alertness and the core body temperature rhythm amplitude\textsuperscript{87, 95, 96}. In addition, metabolism rhythms that are regulated by circadian clocks within liver and pancreas tissues lessen with progressed age and likely contribute to diabetes, hypertension and dyslipidemia\textsuperscript{87, 97-101}. Inflammatory processes are likely rhythmic and are directly regulated by clock genes\textsuperscript{87, 102}. Therefore, age-related circadian clockwork changes may contribute to chronic inflammation and pathologies\textsuperscript{87}.

Sleep duration, sleep chronotype, and social jetlag have been suggested to be associated with cardiometabolic function, mental health, cognitive function, obesity and mortality\textsuperscript{38, 62, 103, 104, 105}. Since BA predicts mortality, morbidity and functional decline\textsuperscript{20}, it is likely that BA works as a proxy between sleep phenotypes and the adverse health outcomes. Older adults experience sleep changes including advanced sleep timing, shorter sleep duration, more fragile sleep and reduced slow wave activity\textsuperscript{106}. Chronotype is influenced by aging. More specifically, sleep phase advances with aging\textsuperscript{107}. On the other hand, eveningness was suggested to be associated with an increased risk of hypertension, depression, smoking and alcohol usage as well as mortality\textsuperscript{107}. Acute sleep deprivation has been shown to be associated with aging brain – effected attention, working memory, default mode network, disrupted incentive processing, and inaccurate expression of emotions\textsuperscript{108}. Experimental studies showed that even one night of partial sleep deprivation could activate the BA genetic process – DNA damage response and the senescence-associated secretory phenotype in older adults\textsuperscript{109}. In addition, sleep characteristics including sleep duration and sleep onset timing have been associated with
aging indexes, BA and telomere length. It is worthwhile to explore the associations between sleep duration, chronotype, social jetlag and aging in multi-racial, middle-aged populations, using BA estimates as the aging indicator.

In addition, melatonin was found to be protective against aging. Melatonin has been found to have antioxidant, immunomodulatory, anti-proliferate, oncostatic and endocrine-modulatory effects. It is suggested that melatonin is able to lessen the consequences of aging through (1) regulation of circadian rhythm (2) decreasing energy expenditure (3) mitochondrial biogenesis and (4) immune remodeling (reducing inflammation). More specifically, melatonin has been suggested to be protective against brain aging, cardiovascular aging, ovary aging, liver aging, skin aging, kidney aging, bone aging, colon aging, lung aging, neural system aging, adipose tissue aging and ovary aging.

Chronotype and Genetic Factors

Most sleep chronotype related genetic studies focused on circadian clock genes. The clock genes PER1 and PER2 have been reported to be associated with morningness or early chronotype. The 3111C allele of the Circadian Locomotor Output Cycles Kaput (CLOCK) gene and the length polymorphisms of PER3 gene was associated with eveningness or delayed sleep onset/offset. In addition, the variable number tandem repeat (VNTR) polymorphisms in PER3 gene have also been widely studied in association with sleep chronotype.
Large scale genetic analyses were lacking until 2016, when 3 large GWAS studies on sleep chronotype were published. Hu et al. discovered 15 loci that were significantly associated with chronotype using data of 89,283 European participants from the 23andMe cohort 50. Jones et al. found 16 variants that were associated with chronotype using 128,266 white British individuals from the UK Biobank study 118. Lane et al. found 12 loci significantly associated with chronotype, using data of 100,420 individuals from the UK Biobank cohort 119. PER2, RGS16, FBXL13 and AK5 genes were associated with chronotype in all three GWASs. In 2019, Jones and Lane jointly published the largest chronotype GWAS meta-analysis so far using 697,828 European ancestry participants from UK Biobank and 23andMe 120. They found morningness was associated with 351 loci including RGS16, PER1, PER2, PER3 and CRY1 120.

**Bogalusa Heart Study**

The Bogalusa Heart Study is a prospective study following the same black and white residents of the community of Bogalusa, Louisiana for the natural history of health conditions especially for atherosclerotic cardiovascular disease, from 1973 until now 121. The current cohort includes 1,298 participants who were screened at least twice during childhood and twice during adulthood, born between 1959 and 1979, and completed a comprehensive set of sleep questionnaires administered during the 2013-2016 in-person examination 122. The mean age of this population is 48.2 years old, ranges from 34 years old to 58 years old. 41.14% of this population are male, and 65.48% are white.
Bogalusa Heart Study Genotyping

Approximately eight hundred subjects amongst the current BHS cohort (n=1,298) were genotyped\textsuperscript{123}. Genotyping was performed using the Illumina Human610 BeadChip\textsuperscript{123}. Stringent quality control removed SNPs with low call rate or a poor cluster separation score. Genotypes were phased using SHAPEIT\textsuperscript{124} software and untyped markers were imputed using the 1000 Genomes phase 3, version 5 combined ancestry reference panel with Minimac software\textsuperscript{125}. Variants with imputation quality <0.3 were removed. Genomic ancestry principal components (PCs) were also calculated for white and black participants, separately.
Chapter 2. Research questions

Theoretical Framework

Based on the Conceptual Model of Sleep Health, the theoretical framework for this dissertation was established (Figure below). The hypothesis is that aging is represented by quantified biological aging, and sleep (at least in the dimensions of duration, timing and regularity) is associated with aging both phenotypically and genetically (through intermediate process/proxy, which is GRS).

Aims

Aim 1: To explore the association between BA estimates and mortality, and cardiovascular disease events.
Aim 1.1 To develop sex and race specific algorithms of BA.

Aim 1.2 To examine the association between KDM BA estimates, PDM BA estimates and mortality, and cardiovascular disease events.

Aim 2: To explore the association between sleep duration, sleep chronotype, social jetlag phenotypes and BA estimates.

Aim 3: To explore the possible causal association between evening chronotype and BA.

Rationale

The rationale of the proposed study includes: (1) BA estimates reflect the aging body better than chronological age. BA estimates offer an opportunity to explore aging research in BHS participants whose chronological age is younger than 65 years old. However, no “Gold standard” of BA quantification has been established and gender and race specific BA quantification studies have not been conducted previously. (2) KDM and PDM are some of the most robust algorithms to quantify BA. (3) In recent years, studies have been demonstrated the association between short/long sleep duration, eveningness, social jetlag and adverse health outcomes including CVD, mortality, obesity, diabetes and problematic cognitive function. Sleep related phenotypes are likely associated with BA through altered or distinctive circadian rhythm, however these potential associations have rarely been studied. (4) Mendelian Randomization (MR) may provide evidence for a causal relationship between sleep chronotype and BA estimates. (5) GRS has the advantage of integrating a group of
relevant genetic loci instead of individual locus. The proposed study will be the first to perform MR on sleep chronotype and BA estimates using GRS.
Chapter 3. ESTIMATING BIOLOGICAL AGING IN THE BOGALUSA HEART STUDY USING TWO METHODS

Abstract

**Background:** Biological Aging (BA) estimates are developed to better capture the gradual increase in the vulnerability of the aging body, and more accurately reflect the risk of mortality, and decrease in physiological function than Calendar Age (CA). Common methods used to estimate BA include Klemera and Doublal’s Method (KDM), Physiological Dysregulation Method (PDM) and telomere length. Research in the past 10 to 15 years has suggested KDM and Mahalanobis Distance (D_M) methods based on PDM as some of the more accurate ways to assess BA. The objective of this study was to examine common ways to estimate BA and their predictive validity in the Bogalusa Heart Study (BHS) a community-based, cohort study.

**Methods:** Nineteen biomarkers were selected and applied in the training datasets (non-Hispanic black and white subjects from NHANES 2011-2018 for the KDM algorithm and a subset of younger and healthy non-Hispanic black and white subjects from NHANES 2011-2018 for DM based PDM algorithm.). The target dataset included BHS subjects assessed between 2013-2016 (n=1,034). Training was done separately for male and female, black and white participants. During the validation, cognitive (standardized neuropsychologic battery including digit span forward and backward, digit symbol coding, logical memory immediate and delayed, word reading and vocabulary tests from the Wide Range Achievement Test 3, and Trail making tests A&B) and
physical performance (Short Physical Performance Battery [SPPB], grip strength test, knee extension test, pegboard test, chair stands and 6-minute walk) testing were used to examine predictive validity. Linear regression was performed, and effect sizes were compared.

**Results:** The mean CA was 48.0 (± 5.3) years old, 39.5% were male, and 67.3% were white. KDM BA estimates and PDM BA estimates were calculated. Only PDM BA estimates were correlated with CA (Pearson r=0.85, p<0.001). Linear regression showed that PDM BA estimates were associated with lower cognitive function (p<0.001) and 5 of the 7 physical performance tests (all p<0.001), after adjusting for CA, gender and race. KDM BA estimates were associated with only 3 of the 7 physical performance measures (p<0.05), and not associated with cognitive function, after adjusting for CA, gender and race. Higher CA was also associated with lower cognitive function (p<0.001) and 6 out of the 7 physical performance tests (p<0.001 or p<0.01) after adjusting for gender and race. However, the effect sizes of all associations between PDM BA estimates and performance tests were of greater magnitude than between CA and the performance tests.

**Discussion:** The results are consistent with current literature and show that biomarkers and algorithm-based estimates are valid measures for BA. This is the first time that KDM BA estimates and PDM BA estimates have been trained separately in race groups in addition to gender groups. In this study PDM BA estimates were of greater magnitude and more robustly associated with performance testing than KDM BA estimates or CA.
**Conclusion:** PDM BA estimates are robust measures of biological aging processes in black and white men and women enrolled in the BHS. PDM BA estimates should be recommended for future aging studies using data from BHS participants.

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**Keywords:** Biological aging; Klemera and Doubal’s Method; Mahalanobis Distance

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**Background**

Biological aging (BA) estimates are developed to better capture the gradual reduction in the viability of the organism and gradual increase in the vulnerability of the aging body than chronological age\(^1\). Middle aged people with the same chronological age could have varied BA\(^3\). Recent studies showed that BA is more accurate in relation to mortality, disease accumulation and physiological function than chronological age\(^4\). BA is expected to be linearly associated with chronological age (CA), predict mortality morbidity and physical, cognitive function decline\(^4,5\).

The Klemera and Doubal's Method (KDM) has been regarded as one of the BA algorithms that have the highest sensitivity and most robust predictive power\(^4,5,10-13\). The
KDM first requires a training database (often the NHANES) to calculate the algorithm parameters biomarkers based on their regression coefficients with CA. The chosen candidate biomarkers usually came from the domains of cardiovascular, lung, renal, hepatic, immune, cell blood count and metabolic function. Models were usually estimated separately for men and women. After that, the parameters are used to calculate BA for individuals in the target dataset.

Physiological dysregulation is also called homeostatic dysregulation. Physiological Dysregulation Method (PDM) often uses Mahalanobis Distance ($D_M$) as measurement. $D_M$ quantifies deviations of the biomarkers compared to their “normal” reference. Similar to the KDM, $D_M$ also requires calculating the parameters based on training population first and applying the parameters and the algorithm to target population. Higher PDM BA estimates was associated with increased vulnerability to many diseases and precede their clinical manifestation. PDM BA estimates could be a promising physical resilience and robustness measure in Aging studies.

BA differs between male and female individuals and across race/ethnicity groups. Cohen et al. discovered the “male–female dysregulation–frailty paradox” whereby men showed greater physiological dysregulation but show less susceptibility to frailty. Black people were suggested to be 3 years older biologically than their white counterparts of the same CA. Conversely, Blacks seem to have longer leukocyte telomere length (LTL) at birth but greater LTL shortening rate through their life course, compared to Whites. LTL is sensitive to social stress and shorter LTL is linked to premature mortality. Black Americans and Latino Americans expect less chronological age-related functional decline than Korean and Chinese Americans.
Methods

Study population

The training population was selected from participants from the National Health and Nutrition Examination Survey (NHANES) conducted between 2011-2018. For estimating KDM BA, the training population inclusion criteria include adults aged between 20-80 years old, and self-identified as non-Hispanic white or black. Subjects who were missing information in any selected biomarkers or CA, race, gender were excluded. To estimate PDM BA, training population additionally excluded subjects aged above 30 years old, and whose selected biomarkers were outside of the normal range defined by the Mayo Clinic.

The target population were participants from the Bogalusa Heart Study (BHS) who completed a comprehensive set of sleep questionnaires administered during the 2013-2016 in-person examination. The Bogalusa Heart Study is a prospective study following the same black and white residents of the community of Bogalusa, Louisiana for the natural history of health conditions related to atherosclerotic cardiovascular disease, from 1973 until now. Sociodemographic, lifestyle information, and anthropometric measurements were also obtained. Subjects missing information in CA, gender, race, or any of the selected biomarkers (selected based on training population) were excluded. The final target population included 1034 participants. Written informed consent was obtained from each participant and all data collection protocols were approved by the Institutional Review Board of Tulane University (IRB # 356359).
Biomarker selection

Biomarkers were selected based upon their availability in the training and target dataset, and their associations with aging \(^{132-151}\). In addition, only the biomarkers that were significantly associated with CA were included (Pearson correlation coefficient \(r>0.1\) or \(r<-0.1,\ p<0.05\) \(^4\) and without redundancy. In addition, Pearson correlation coefficients were determined for all pairs of candidate biomarkers and high redundancy was defined as having a Pearson correlation coefficient \(r>0.7\) \(^{152}\). Within each redundant pair, only the biomarker that had a stronger correlation with CA (larger absolute value of Pearson correlation coefficient \(r\)) remained. As a result, 19 biomarkers were included for both KDM and PDM BA estimation. By functional domains, they are: (1) Cardiac Function: systolic blood pressure, pulse at rest; (2) Liver Function: albumin, alkaline phosphatase, total protein; (3) Metabolic Function: glycohemoglobin, waist circumference; (4) Immune and Inflammation: lymphocyte percent, monocyte percent; (5) Kidney Function: creatinine, blood urea nitrogen (BUN), uric acid; (6) Cell Blood Count: red blood cell counts, mean cell volume, red cell distribution width, platelet count; (7) Electrolyte: potassium, chloride, bicarbonate (Table 2). Amongst all biomarkers, Alkaline Phosphotase, BUN, creatinine, glycohemoglobin, monocyte percentage and red cell distribution width were log transformed (all biomarker values were greater than zero) due to being severely skewed (|skewness|>2 or |kurtosis|>7) \(^{153}\).

Klemera and Doubal’s Method (KDM)

Klemera and Doubal’s method uses mathematical algorithms to estimate BA for any battery of biomarkers \(^{10}\). If \(m\) is the number of included functionally uncorrelated biomarkers, \(X_j\) \((j = 1, \ldots, m)\) is the battery of biomarkers, \(B\) is the values of hypothetical
BA, $C$ is calendar age, $s_B^2$ is the variance of the difference between $B$ and $C$, $s_j^2$ is the variance of the biomarker’s fluctuation (away from the linear function predicted by $B$), $k$ and $q$ are the slope and intercept with respect to each linear function $F_x$ expressing the dependence of $X$ on $B$. The estimated KDM BA is equal to $BA_{KDM} = \frac{\sum_{j=1}^{m} (x_j - q_j) k_j + c}{\sum_{j=1}^{m} (s_j)^2 + \frac{1}{s_B^2}}$.

The BA estimates are based upon minimizing the distance between $m$ regression lines and $m$ biomarker points, within an $m$ dimensional space of all biomarkers. The function above was optimum and had greater precision of estimate than multiple linear regression, Hochschild’s method, principal component analysis and the sister KDM BA function without CA, according to simulations and existing population studies.

Advantages of using the function above also include: The possibility to evaluate BA estimates precision of any group of functionally uncorrelated biomarkers, the applicability even when some biomarkers are not linearly associated with BA, the flexibility of using any subset of original biomarkers, and the resistance to the “paradox of biomarkers.” The unit of KDM BA estimates is year.

Physiological Dysregulation Method (PDM)

Mahalanobis Distance ($D_M$) was used to estimate PDM BA. The method uses the joint distribution of multiple biomarkers to assign individuals a score indicating how normal or abnormal their overall profile is relative to a reference population, based on how close or faraway their biomarkers are from the reference average. $BA_{DM}(x) = \sqrt{(x - \mu)^T S^{-1} (x - \mu)} = \sqrt{\sum_{i=1}^{B} \frac{(x_i - \mu_i)^2}{\sigma^2(x_i)}}$, where $x$ is a multivariate observation of selected biomarkers, $\mu$ is the training population means for each selected biomarker. $B$ is
the number of selected biomarkers and \( \sigma^2(x_i) \) is the variance in the \( i \) th biomarker. The unit of PDM BA estimates is standard deviation (of the reference population).

**Physiological function**

Cognitive function was assessed by using a neurocognitive battery that is compatible with the recommended domains of the NIH toolbox and is similar to the battery used in the Framingham Heart Study. The neurocognitive battery includes: the digit span subtest and digit symbol coding subtest from the Wechsler Adult Intelligence Scale–Third Edition (WAIS-III), the logical memory I&II test and the logical memory recognition test from the Wechsler Memory Scale–Third Edition (WMS-III), the reading and vocabulary test from the Wide Range Achievement Test-3 (WRAT-3), and the Trail-making tests A & B. The sum score of the standardized scores of each subtest from the neurocognitive battery was used. If a subtest measures the completed quantity, the standardized score was added to the total cognitive function score. If a subtest measures the time taken to complete it, the standardized score was subtracted from the total cognitive function score. Higher sum scores represent better cognitive function.

Physical performance was assessed by: (1) The Short Physical Performance Battery (SPPB): SPPB is a standard physical function assessment tool, it has three parts -a balance test (scores ranging 0-4), a 4-meter walk test (scores ranging 0-4) and a chair stand test (scores ranging 0-4). The total scores from three parts were used as discreet variables. A higher score represents better physical performance. (2) Grip strength: The average of two reads in kilograms were recorded (3) Knee extension strength: is the average of six reads from both legs in kilograms (repeated three times each leg). (4) Pegboard challenge: The time taken to complete the pegboard challenge
using the dominant and non-dominant hands. (5) Chair stand: The time taken to complete ten chair stands recorded in seconds. (6) 6-minute walk: The distance walked in 6 minutes were recorded in meters. All functional test scores were standardized before linear regressions to make the linear regression coefficients comparable.

Statistical analysis

SAS 9.4 (SAS Institute, Cary, NC, USA) and R language version 3.6.3 were used in this study. Means and standard deviations were used to describe the demographic and biomarkers information of the training and target dataset, and the KDM and PDM BA estimates of the target dataset. KDM BA estimates and PDM BA estimates were trained separately in race and gender groups, using an R language package from Github by Kwon and Belsky. Pearson correlations were used during the biomarker selection, redundancy test, and while examining the correlation between the BA estimates and CA. For the validation process, all KDM and PDM BA estimates and CA were first standardized, then linear regressions were performed in unadjusted and adjusted models for the association between BA estimates and physiological functions. Models were adjusted for gender, race and calendar age if applicable. In addition, sensitivity analyses were done by training KDM and PDM BA estimates separately in gender groups only. Level of significance for all analyses is p<0.05.
Results

Training and Target population characteristics

A total of 10,156 participants from NHANES 2011-2018 were included as the training population for KDM BA estimating. Mean age was 50.6 years old. Among them, 49.7% were male and 63.3% were white. From the 10,156 NHANES participants, 1,802 were included as the training population for PDM BA estimating. Mean age was 24.9 years old. Among them, 49.2% were male and 59.2% were white. A total of 1,034 participants from the BHS were included as the target population for both KDM and PDM BA estimating. Among the target population, the mean age was 48.0 (± 5.3) years old, 38.5% were male and 67.3% were white.

KDM and PDM BA estimates

KDM, PDM BA estimates were calculated (Table 5). The overall mean KDM BA estimates were 47.88 years ± 6.11, and ranged from 7.60 to 70.84 years. White males have the highest KDM BA estimates (48.30 years ± 5.67), and black males have the lowest (47.30 years ± 9.32). The overall median PDM BA estimates was 2.28 units, ranging from 0.87 to 7.15. White males have the highest PDM BA estimates (median=2.82), and Black females have the lowest (median=1.96). White males have the highest PDM BA estimates (2.93 ± 0.88), and black females have the lowest (2.16 ± 0.91). Pearson correlations were calculated between calendar age and KDM BA estimates, PDM BA estimates respectively (Figure 1, scatter plots). Only KDM BA estimates were correlated with CA (R=0.85, p<0.001), but not PDM BA estimates (R=0.055, p=0.078).
Linear regression estimates of BA and functional tests results

All KDM and PDM BA estimates as well as CA were standardized first. All functional test results were standardized too. Linear regression was used to examine the relationship between standardized calendar age and the standardized cognitive and physical tests as reference. Unadjusted models were developed first, then race, gender and CA (if applicable) were included for adjustment. Linear regression was used to examine standardized KDM BA estimates. Standardized KDM BA was found to be associated with cognitive function (p<0.01), SPPB (p<0.001), grip strength (p<0.05), knee extension (p<0.05), pegboard challenge dominant hand (p<0.001), pegboard challenge nondominant hand (p<0.001), and chair stand test (p<0.001).

Standardized KDM BA estimates were associated with standardized cognitive function score and 6 of the 7 standardized physical function scores (p<0.05). Standardized PDM BA estimates were associated with all cognitive and physical performance tests (p<0.001 or p<0.01). Standardized CA was associated with standardized cognitive function score as well as 4 of the 7 standardized physical function scores (p<0.01).

After adjusting for CA, gender and race, standardized KDM BA estimates were only associated with SPPB (β=-0.136, p<0.01), chair stand test (β=0.121, p<0.05) and 6-minute walk (β=-0.121, p<0.05). Standardized PDM BA estimates were associated with standardized cognitive function (β=-0.103, p<0.001), SPPB (β=-0.132, p<0.001), pegboard challenge dominant hand (β=0.166, p<0.001), pegboard challenge nondominant hand (β=0.193, p<0.001), chair stand (β=0.149, p<0.001) and 6-minute walk remained (β=-0.254, p<0.001). After adjusting for gender and race, standardized CA was
associated with cognitive function ($\beta=-0.113$, $p<0.001$), SPPB ($\beta=-0.091$, $p<0.01$), grip strength ($\beta=-0.090$, $p<0.001$), knee extension ($\beta=-0.077$, $p<0.01$), pegboard challenge dominant hand ($\beta=0.158$, $p<0.001$), pegboard challenge nondominant hand ($\beta=0.152$, $p<0.001$), and chair stand ($\beta=0.125$, $p<0.001$). The $\beta$ effect sizes of the associations between standardized PDM BA estimates and performance tests were of greater magnitude than standardized KDM BA estimates and standardized CA.

Discussion

This is the first time that biological aging was quantified in the BHS using KDM and D$_M$ based PDM. Using NHANES 2011-2018 population as the training dataset and the BHS 2013-2016 population as the target dataset, BA of the target population was quantified using two methods – KDM, and the D$_M$ based PDM. A battery of 19 biomarkers was created for both quantification methods. Amongst the target population, only the KDM BA estimates were correlated with CA. During the validation process, after adjusting for confounders, PDM BA estimates and CA were associated with 6 out of the 8 cognitive and physical performance subtest standardized results. KDM BA estimates were only associated with 3 out of the 8 subtest standardized results. In addition, the linear association coefficients ($\beta$) of PDM BA estimates and subtest standardized results were higher than those of KDM BA estimates or even CA, and subtest standardized results. Both KDM BA estimates and PDM BA estimates were able to indicate cognitive and physiological aging, but the PDM BA estimates exceeded the other two methods in the strength of the estimates.
KDM and/or Mahalanobis distance based PDM BA quantification, and their predictive validity has been performed in various studies and populations. Physiological Dysregulation (PD) has sometimes been referred to as Homeostatic Dysregulation (HD). Both the KDM BA estimates and the Mahalanobis distance based PDM BA estimates have been suggested to be valid quantifications for individual biological aging, similar to the result of the current research. Levine et al. used data from over 9,000 participants from NHANES III whose CAs were between 30-75 years old and compared the predictive ability of KDM, Principal Component Analysis (PCA) and Multiple Linear Regression (MLR) \(^4\). She found that the BA estimates generated by KDM were most reliable in predicting mortality and had the best predictive sensitivity on CA \(^4\). Hastings et al. used data from 6,731 participants from NHANES over 20 years old, and compared the BA estimates calculated by KDM, Mahalanobis distance-based HD, Phenotypic Age and leukocyte telomere length \(^23\). They found that BA estimates derived from the first three algorithms were correlated with each other but not with the estimates from telomere length \(^23\). In addition, the effect sizes of the associations between BA estimates and physical, cognitive, perceptual functioning, limitations to daily activities, pain, and self-rated health were larger for KDM, PDM, and phenotypic Age than telomere length \(^23\).

Using the NHANES 2007-2010 population as training dataset and the Comprehensive Assessment of the Long-term Effects of Reducing Intake of Energy (CALERIE) Biobank as the target dataset, Belsky et al. found that both the KDM Biological Age and the Mahalanobis distance based homeostatic dysregulation (HD) were associated with physical limitation \(^164\). Similar studies had been performed in East Asian populations as well. Gaydosh et al. used data from several hundreds of older Taiwanese adults (mean
age about 67 years old) in the Social Environment and Biomarkers of Aging Study (SBAS) and evaluated the BA estimated calculated by KDM and Mahalanobis distance based Homeostatic Dysregulation (HD)\(^5\). They found that the BA estimates by KDM and HD predicted mortality, physical and cognitive function as well as the biomarker index based on the norms within their analysis sample\(^5\). Using the China Nutrition and Health Survey (CHNS) 2009 wave population as the training dataset and the China Health and Retirement Longitudinal Study (CHARLS) population as target dataset, Liu found that both KDM and Mahalanobis distance based physiological dysregulation BA estimates were predictive of mortality and disease counts after adjusting for confounders\(^{165}\).

Moreover, two Korean National Health and Nutrition Examination Surveys (KNHANES) based studies found that BA estimates calculated by KDM were most reliable and stable compared to MLR and PCA, both in men (n=940, age between 30-80) and women (n=912, age between 30-80)\(^{12,13}\). Arbeev et al. found that Mahalanobis distance based PDM estimates was associated with both increased mortality and increased calendar age\(^{167}\). Cohen et al. found that using 14 biomarkers, Mahalanobis distance based PDM estimates was associated with both calendar age and mortality, and that including larger number of biomarkers within the battery produce stronger signal\(^{168}\). Leung et al. also found that Mahalanobis distance based PDM estimates was associated with calendar age and mortality\(^{169}\). Even non-human study supports the association between PDM BA estimates and aging. Dansereau et al. found that Mahalanobis distance based PDM estimates using biomarkers were strongly associated with mortality risk in multiple nonhuman primates\(^{166}\).
Distinguished from previous research, the current study has two unique findings. First, the effect sizes of all associations between PDM BA estimates and standardized performance test results were of much greater magnitude than KDM BA estimates. Most other published research had different results. In the study of Hastings et al. and Gaydosh et al., the effect sizes of the Pearson correlations between KDM age and physiological function performance were similar to those between Mahalanobis distance-based HD age and functional performance. In the study of Hastings et al. and Gaydosh et al., the effect sizes of the Pearson correlations between KDM age and physiological function performance were similar to those between Mahalanobis distance-based HD age and functional performance. In the study of Belsky et al., the association between KDM Biological Age and physical limitations had the effect size \( r=0.34 \) almost twice as that between Mahalanobis distance-based HD and physical limitations \( r=0.16 \). In the study of Liu, the Hazard Ratio of physiological dysregulation on mortality \( 1.50 \) was moderately larger than that of KDM biological age on mortality \( 1.14 \).

Second, the effect sizes of all associations between PDM BA estimates and standardized performance test results exceeded that of CA as well. So far, no existing studies have directly compared the BA predictive validity of CA to that of KDM or PDM BA estimates. It is necessary to use CA to challenge the computed BA estimates for the predictive validity of biological aging. In theory, BA estimates that do not outperform CA in predicting biological aging should have limited applied value, where CA is already available and ready to use.

The reason behind the outstanding effect sizes of the PDM BA estimates in the current study is unknown. Unlike KDM BA estimates, the PDM BA estimates were not correlated with CA \( r=0.051, p=0.1 \). It is not surprising because (1) CA is part of the KDM formula, and (2) one of the basic hypotheses of KDM is that the scale of KDM BA estimates is set for a given population so that all subjects with CA equal to a certain
number has an average value of BA estimates equal to that number. The Mahalanobis
distance computation, however, is independent of CA. It is possible that within our target
population of the BHS 2013-2016, the CA, by nature, is less indicative of physical and
cognitive function than the log transformed, Mahalanobis distance based PDM BA
estimates. KDM BA estimates however, are highly correlated with CA and do not exceed
the PDM BA estimates or even CA, in predicting physical and cognitive function in the
BHS 2013-2016.

In fact, from biomarker battery selection to data training, both KDM and
Mahalanobis distance based PDM are highly dependent on the chosen training population
and target population. Disparities in the results of BA estimate predictive validities across
different studies should be expected. Limitations while comparing results of different BA
quantifications using the same methods should be considered.

Following publications of Levine’s, Belsky et al.’s, Liu’s and Jee’s 4,11,12,164,165,
one of the selecting rules of KDM biomarker battery candidates we applied is that the
candidate biomarkers must be linearly associated with calendar age. This is not the only
biomarkers selection method. Hasting’s et al. and Gaydosh et al. did not assess the
associations between individual biomarkers and calendar age, instead they selected
biomarkers based on their inclusion in published biological age papers 5,23. Due to the
lack of sufficient target population data in common BA biomarker battery candidates
such as C-reactive protein, mean corpuscular volume, Cytomegalovirus optical density
and forced expiratory volume, we ran the linear regression on all available biomarkers
that fit our inclusion criteria, including those never mentioned in previous studies.
For PDM, other variable screening criteria are available. Cohen et al. prefer the biomarkers whose deviance from baseline mean were linearly associated with calendar age over those who themselves were linearly associated with calendar age as the biomarker battery candidates. Following a number of other studies, we used the same battery of KDM biomarkers in PDM BA estimates computation, to make the KDM and PDM results comparable.

It is worth mentioning that the nature of KDM allows for computing individual BA estimates using any subset of the original biomarker battery. For Mahalanobis distance based PDM, larger biomarker battery was suggested to provide higher predictive power. Future sensitivity analyses using different subsets of the original biomarker battery are needed to compare the predictive power of the KDM and Mahalanobis distance based PDM BA estimates.

To our knowledge this is the first study to construct a BA index and conduct validation studies separately across race/ethnicity groups. Due to the gender and race/ethnicity disparities in BA, it is reasonable to develop an index of BA and calculate estimates separately for each gender and racial group. The Bogalusa Heart Study (BHS), a community cohort with nearly 2:1 white to black population ratio, provides an excellent database for gender and race specific BA research.

There are advantages of this study. First, the assessment of the training population and the target population occurred around the same time period. This potentially reduces confounders related to general lifestyle changes that occurred during the time period. Second, BA quantification was performed separately in different gender and race groups to achieve higher accuracy. Third, the BA predictive validity of two BA quantifications
were compared with those of CA. Results strongly support the superiority of using PDM estimates over CA to indicate biological aging, which is a straightforward but overlooked area of analysis. There are several weaknesses of this study as well. First, physical and cognitive function performance tests are less direct indicators of aging than mortality. Mortality data was not available during the period of analysis. Second, the size of the target population is relatively small especially comparing to the training population, so the study power could be limited. Third, the blood test devices during the 2011-2018 NHANES assessment were not consistent. The NHANES used Beckman Coulter MAXM was used for hematology analysis during 2011-2012 but the device was changed to Beckman Coulter DXH 800 in 2013. The standard biochemistry profile was assessed using Roche Cobas 6000 (c501 module) during the 2017-2018 cycle instead of the Beckman Coulter DxC800 analyzer in all previous cycles. The difference in equipment may affect the blood test results and cause undetected bias. In addition, some commonly used BA biomarkers such as C-reactive protein and forced expiratory volume (FEV) are not available in the total population of BHS 2013-2016.

Conclusion

PDM BA estimates are robust measures of biological aging processes in Black and White men and women enrolled in the BHS. They outperform both KDM BA estimates in predicting human physical and cognitive function aging. PDM BA estimates should be recommended for future aging studies using data from BHS participants. Further studies in the predictive validity of PDM BA estimates on mortality and disease specific mortality will be great additions once the mortality data becomes available.
Tables and figures

Table 1. Baseline biomarker information of eligible participants from the training dataset – NHANES 2011-2018.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Male, white</th>
<th>Male, black</th>
<th>Female, white</th>
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<tbody>
<tr>
<td>N</td>
<td>10,156</td>
<td>3,236</td>
<td>1,814</td>
<td>3,193</td>
<td>1,913</td>
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<tr>
<td>Age (yrs)</td>
<td>50.55 ± 18.05</td>
<td>51.80 ± 18.60</td>
<td>49.50 ± 17.30</td>
<td>51.40 ± 18.50</td>
<td>48.00 ± 16.70</td>
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<td>Albumin (g/dL)</td>
<td>4.21 ± 0.35</td>
<td>4.34 ± 0.34</td>
<td>4.23 ± 0.34</td>
<td>4.18 ± 0.34</td>
<td>4.02 ± 0.33</td>
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<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>69.44 ± 26.62</td>
<td>68.00 ± 26.40</td>
<td>69.70 ± 23.40</td>
<td>69.10 ± 29.00</td>
<td>72.10 ± 25.50</td>
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<td>Blood urea nitrogen (mg/dL)</td>
<td>14.08 ± 6.25</td>
<td>15.50 ± 6.11</td>
<td>13.80 ± 6.75</td>
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<td>Bicarbonate (mmol/L)</td>
<td>26.16 ± 2.38</td>
<td>25.30 ± 2.31</td>
<td>25.70 ± 2.40</td>
<td>24.90 ± 2.37</td>
<td>24.90 ± 2.40</td>
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<td>Creatinine (mg/dL)</td>
<td>0.94 ± 0.43</td>
<td>1.02 ± 0.35</td>
<td>1.16 ± 0.68</td>
<td>0.80 ± 0.22</td>
<td>0.86 ± 0.41</td>
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<td>Total protein (g/dL)</td>
<td>7.09 ± 0.47</td>
<td>7.05 ± 0.44</td>
<td>7.29 ± 0.48</td>
<td>6.94 ± 0.43</td>
<td>7.23 ± 0.47</td>
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<td>Uric acid (mg/dL)</td>
<td>5.49 ± 1.45</td>
<td>6.02 ± 1.29</td>
<td>6.10 ± 1.40</td>
<td>4.89 ± 1.29</td>
<td>5.03 ± 1.44</td>
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<tr>
<td>Potassium (mmol/L)</td>
<td>4.01 ± 0.36</td>
<td>4.10 ± 0.36</td>
<td>4.01 ± 0.37</td>
<td>3.99 ± 0.35</td>
<td>3.89 ± 0.35</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>103.10 ± 3.16</td>
<td>103.00 ± 3.10</td>
<td>103.00 ± 3.09</td>
<td>103.00 ± 3.23</td>
<td>104.00 ± 3.13</td>
</tr>
<tr>
<td>Glycohemoglobin (%)</td>
<td>5.75 ± 1.05</td>
<td>5.69 ± 0.96</td>
<td>5.94 ± 1.26</td>
<td>5.58 ± 0.81</td>
<td>5.91 ± 1.25</td>
</tr>
<tr>
<td>Lymphocyte percent (%)</td>
<td>30.81 ± 9.17</td>
<td>28.10 ± 8.21</td>
<td>33.80 ± 9.76</td>
<td>29.30 ± 7.89</td>
<td>35.10 ± 9.71</td>
</tr>
<tr>
<td>Monocyte percent (%)</td>
<td>8.28 ± 2.45</td>
<td>8.68 ± 2.57</td>
<td>8.92 ± 2.58</td>
<td>7.71 ± 2.08</td>
<td>7.93 ± 2.44</td>
</tr>
<tr>
<td>Red blood cell count (million cells/uL)</td>
<td>4.65 ± 0.50</td>
<td>4.86 ± 0.46</td>
<td>4.83 ± 0.55</td>
<td>4.47 ± 0.38</td>
<td>4.41 ± 0.45</td>
</tr>
<tr>
<td>Mean cell volume (fL)</td>
<td>89.27 ± 6.05</td>
<td>90.70 ± 4.72</td>
<td>88.50 ± 6.52</td>
<td>90.10 ± 5.25</td>
<td>86.10 ± 7.39</td>
</tr>
<tr>
<td>Red cell distribution width (%)</td>
<td>13.68 ± 1.39</td>
<td>13.40 ± 1.03</td>
<td>13.80 ± 1.37</td>
<td>13.50 ± 1.28</td>
<td>14.30 ± 1.80</td>
</tr>
<tr>
<td>Platelet count (1000 cells/uL)</td>
<td>236.10 ± 62.61</td>
<td>222.00 ± 57.20</td>
<td>219.00 ± 57.50</td>
<td>247.00 ± 60.00</td>
<td>258.00 ± 69.90</td>
</tr>
<tr>
<td>60 sec. pulse (30 sec. pulse * 2)</td>
<td>72.21 ± 11.94</td>
<td>71.30 ± 12.20</td>
<td>69.70 ± 11.70</td>
<td>73.60 ± 11.60</td>
<td>73.80 ± 11.70</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>101.20 ± 17.12</td>
<td>103.00 ± 16.00</td>
<td>99.90 ± 17.50</td>
<td>97.70 ± 16.90</td>
<td>103.00 ± 18.10</td>
</tr>
<tr>
<td>Mean SBP across 3 measures</td>
<td>124.81 ± 18.04</td>
<td>124.00 ± 15.70</td>
<td>130.00 ± 18.10</td>
<td>122.00 ± 18.50</td>
<td>126.00 ± 19.80</td>
</tr>
</tbody>
</table>
Table 1.2. Baseline biomarker information, as well as physical performance and cognitive function information of eligible participants from the target dataset – BHS 2013-2016.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Male, white</th>
<th>Male, black</th>
<th>Female, white</th>
<th>Female, black</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1,034</td>
<td>284</td>
<td>114</td>
<td>412</td>
<td>224</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>48.04 ± 5.31</td>
<td>48.70 ± 5.02</td>
<td>47.60 ± 5.96</td>
<td>48.00 ± 5.14</td>
<td>47.50 ± 5.56</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.27 ± 0.30</td>
<td>4.38 ± 0.26</td>
<td>4.34 ± 0.31</td>
<td>4.27 ± 0.29</td>
<td>4.09 ± 0.26</td>
</tr>
<tr>
<td>Alkaline phosphotase (IU/L)</td>
<td>74.75 ± 24.58</td>
<td>73.40 ± 23.90</td>
<td>73.40 ± 24.70</td>
<td>75.00 ± 25.10</td>
<td>76.60 ± 24.50</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>13.63 ± 4.31</td>
<td>15.10 ± 4.13</td>
<td>13.60 ± 4.94</td>
<td>13.20 ± 3.79</td>
<td>12.40 ± 4.57</td>
</tr>
<tr>
<td>Bicarbonate (mmol/L)</td>
<td>22.63 ± 2.14</td>
<td>22.70 ± 1.95</td>
<td>22.40 ± 2.08</td>
<td>22.50 ± 2.14</td>
<td>23.00 ± 2.34</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.88 ± 0.38</td>
<td>1.00 ± 0.37</td>
<td>1.17 ± 0.83</td>
<td>0.76 ± 0.13</td>
<td>0.81 ± 0.17</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>6.96 ± 0.43</td>
<td>6.91 ± 0.39</td>
<td>7.17 ± 0.48</td>
<td>6.87 ± 0.40</td>
<td>7.09 ± 0.46</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>5.47 ± 1.47</td>
<td>6.19 ± 1.27</td>
<td>6.53 ± 1.35</td>
<td>4.88 ± 1.21</td>
<td>5.07 ± 1.52</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.24 ± 0.43</td>
<td>4.41 ± 0.43</td>
<td>4.20 ± 0.34</td>
<td>4.25 ± 0.40</td>
<td>4.02 ± 0.42</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>102.50 ± 2.66</td>
<td>102.00 ± 2.57</td>
<td>102.00 ± 2.90</td>
<td>103.00 ± 2.57</td>
<td>103.00 ± 2.81</td>
</tr>
<tr>
<td>Glycohemoglobin (%)</td>
<td>5.84 ± 1.10</td>
<td>5.73 ± 0.91</td>
<td>6.03 ± 1.32</td>
<td>5.75 ± 1.10</td>
<td>6.02 ± 1.17</td>
</tr>
<tr>
<td>Lymphocyte percent (%)</td>
<td>33.41 ± 9.06</td>
<td>31.10 ± 7.93</td>
<td>36.70 ± 11.00</td>
<td>32.40 ± 8.20</td>
<td>36.50 ± 9.60</td>
</tr>
<tr>
<td>Monocyte percent (%)</td>
<td>8.04 ± 2.28</td>
<td>8.81 ± 2.25</td>
<td>8.98 ± 2.84</td>
<td>7.53 ± 1.97</td>
<td>7.54 ± 2.13</td>
</tr>
<tr>
<td>Red blood cell count (million cells/uL)</td>
<td>4.64 ± 0.47</td>
<td>4.97 ± 0.41</td>
<td>4.83 ± 0.58</td>
<td>4.50 ± 0.33</td>
<td>4.40 ± 0.43</td>
</tr>
<tr>
<td>Mean cell volume (fL)</td>
<td>88.18 ± 5.78</td>
<td>89.20 ± 4.02</td>
<td>87.30 ± 7.40</td>
<td>89.20 ± 5.13</td>
<td>85.40 ± 6.80</td>
</tr>
<tr>
<td>Red cell distribution width (%)</td>
<td>14.03 ± 1.22</td>
<td>13.70 ± 0.67</td>
<td>14.30 ± 1.10</td>
<td>13.80 ± 1.01</td>
<td>14.70 ± 1.75</td>
</tr>
<tr>
<td>Platelet count (1000 cells/uL)</td>
<td>268.60 ± 68.64</td>
<td>237.00 ± 56.00</td>
<td>255.00 ± 67.80</td>
<td>279.00 ± 63.10</td>
<td>297.00 ± 75.80</td>
</tr>
<tr>
<td>60 sec. pulse (30 sec. pulse*2)</td>
<td>72.79 ± 11.24</td>
<td>70.30 ± 10.60</td>
<td>74.30 ± 11.90</td>
<td>73.90 ± 10.70</td>
<td>73.10 ± 12.10</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>95.76 ± 19.21</td>
<td>101.00 ± 15.80</td>
<td>98.00 ± 20.60</td>
<td>89.90 ± 18.50</td>
<td>99.40 ± 20.80</td>
</tr>
<tr>
<td>Mean SBP across 3 measures</td>
<td>121.77 ± 14.86</td>
<td>125.00 ± 12.60</td>
<td>130.00 ± 13.50</td>
<td>116.00 ± 13.40</td>
<td>124.00 ± 17.30</td>
</tr>
<tr>
<td>Cognitive function</td>
<td>0.46 ± 5.13</td>
<td>0.80 ± 4.75</td>
<td>-3.14 ± 4.08</td>
<td>2.24 ± 4.73</td>
<td>-1.39 ± 5.25</td>
</tr>
<tr>
<td>SPPB</td>
<td>11.09 ± 1.23</td>
<td>11.30 ± 1.07</td>
<td>10.80 ± 1.50</td>
<td>11.20 ± 1.11</td>
<td>10.70 ± 1.35</td>
</tr>
<tr>
<td>Grip strength (kg)</td>
<td>34.91 ± 11.67</td>
<td>46.80 ± 9.16</td>
<td>44.40 ± 9.65</td>
<td>27.50 ± 5.72</td>
<td>28.70 ± 7.09</td>
</tr>
<tr>
<td>Test</td>
<td>Mean ± SD 1</td>
<td>Mean ± SD 2</td>
<td>Mean ± SD 3</td>
<td>Mean ± SD 4</td>
<td>Mean ± SD 5</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Knee Extension (kg)</td>
<td>53.23 ± 20.61</td>
<td>70.10 ± 19.80</td>
<td>62.30 ± 22.70</td>
<td>45.40 ± 13.50</td>
<td>41.70 ± 14.80</td>
</tr>
<tr>
<td>Pegboard Dominant Time (s)</td>
<td>73.33 ± 21.47</td>
<td>71.30 ± 14.50</td>
<td>81.70 ± 27.70</td>
<td>67.50 ± 16.60</td>
<td>82.50 ± 27.90</td>
</tr>
<tr>
<td>Pegboard Nondominant Time (s)</td>
<td>79.05 ± 25.13</td>
<td>75.60 ± 16.00</td>
<td>88.10 ± 36.90</td>
<td>73.20 ± 19.90</td>
<td>89.60 ± 30.80</td>
</tr>
<tr>
<td>Time Completing 10 Chair Stands (s)</td>
<td>23.08 ± 6.77</td>
<td>21.60 ± 6.60</td>
<td>23.80 ± 7.32</td>
<td>22.60 ± 6.44</td>
<td>25.40 ± 6.66</td>
</tr>
<tr>
<td>6-Minute Walk Distance (m)</td>
<td>429.20 ± 86.56</td>
<td>461.00 ± 83.90</td>
<td>425.00 ± 70.20</td>
<td>435.00 ± 83.80</td>
<td>380.00 ± 81.10</td>
</tr>
</tbody>
</table>
Table 1.3. Pearson correlation between calendar age and selected biomarkers that are kept at this step in KDM & PDM algorithms among NHANES 2011-2018 participants (n=10,156).

<table>
<thead>
<tr>
<th>Biomarker name</th>
<th>Function</th>
<th>Pearson r</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/dL)</td>
<td>Liver</td>
<td>-0.190</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Alkaline phosphotase (IU/L)</td>
<td>Liver</td>
<td>0.140</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>Kidney</td>
<td>0.411</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bicarbonate (mmol/L)</td>
<td>Electrolyte</td>
<td>0.154</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>Kidney</td>
<td>0.184</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>Liver</td>
<td>-0.145</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>Kidney</td>
<td>0.149</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>Electrolyte</td>
<td>0.173</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>Electrolyte</td>
<td>-0.123</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glycohemoglobin (%)</td>
<td>Metabolic</td>
<td>0.275</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lymphocyte percent (%)</td>
<td>Immune and inflammation</td>
<td>-0.164</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Monocyte percent (%)</td>
<td>Immune and inflammation</td>
<td>0.150</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Red blood cell count (million cells/uL)</td>
<td>Cell blood count</td>
<td>-0.176</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean cell volume (fL)</td>
<td>Cell blood count</td>
<td>0.187</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Red cell distribution width (%)</td>
<td>Cell blood count</td>
<td>0.157</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Platelet count (1000 cells/uL)</td>
<td>Cell blood count</td>
<td>-0.145</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>60 sec. pulse (30 sec. pulse*2)</td>
<td>Cardiac</td>
<td>-0.148</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>Metabolic</td>
<td>0.181</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean SBP across 3 measures</td>
<td>Cardiac</td>
<td>0.420</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 1.4. A list of all included biomarkers for BA estimating by functional categories.

<table>
<thead>
<tr>
<th>Function</th>
<th>Biomarkers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac</td>
<td>Systolic Blood Pressure, Pulse (at rest)</td>
</tr>
<tr>
<td>Liver</td>
<td>Albumin, Alkaline phosphatase, Total protein</td>
</tr>
<tr>
<td>Metabolic</td>
<td>Glycohemoglobin, Waist Circumference</td>
</tr>
<tr>
<td>Immune and Inflammation</td>
<td>Lymphocyte percent, Monocyte percent</td>
</tr>
<tr>
<td>Kidney</td>
<td>Creatinine, Blood Urea Nitrogen (BUN), Uric Acid</td>
</tr>
<tr>
<td>Cell Blood Count</td>
<td>Red Blood Cell counts, Mean Cell Volume, Red Cell Distribution Width, Platelet count</td>
</tr>
<tr>
<td>Electrolyte</td>
<td>Potassium, Chloride, Bicarbonate</td>
</tr>
</tbody>
</table>
Table 1.5. The mean ± SD, median, minimum, maximum of the KDM BA estimates, PDM BA estimates, PDM BA estimates and Calendar Age (CA) among BHS 2013-2016 population (n=1,034).

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Total</th>
<th>Male, white</th>
<th>Male, black</th>
<th>Female, white</th>
<th>Female, black</th>
</tr>
</thead>
<tbody>
<tr>
<td>KDM BA</td>
<td>Mean ± SD</td>
<td>47.88 ± 6.11</td>
<td>48.30 ± 5.67</td>
<td>47.30 ± 9.32</td>
<td>48.00 ± 5.37</td>
<td>47.40 ± 5.86</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>48.27</td>
<td>48.40</td>
<td>47.70</td>
<td>48.30</td>
<td>48.30</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>70.84</td>
<td>70.50</td>
<td>70.80</td>
<td>61.10</td>
<td>58.70</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>7.60</td>
<td>34.10</td>
<td>7.60</td>
<td>36.00</td>
<td>33.80</td>
</tr>
<tr>
<td>PDM BA</td>
<td>Mean ± SD</td>
<td>2.50 ± 1.00</td>
<td>2.93 ± 0.88</td>
<td>2.88 ± 0.89</td>
<td>2.28 ± 1.00</td>
<td>2.16 ± 0.91</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>2.28</td>
<td>2.82</td>
<td>2.66</td>
<td>2.04</td>
<td>1.96</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>7.15</td>
<td>6.92</td>
<td>6.98</td>
<td>7.15</td>
<td>5.61</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>0.87</td>
<td>1.44</td>
<td>1.13</td>
<td>1.08</td>
<td>0.87</td>
</tr>
<tr>
<td>CA</td>
<td>Mean ± SD</td>
<td>48.04 ± 5.31</td>
<td>48.70 ± 5.02</td>
<td>47.60 ± 5.96</td>
<td>48.00 ± 5.14</td>
<td>47.50 ± 5.56</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>49.00</td>
<td>49.00</td>
<td>48.50</td>
<td>48.00</td>
<td>48.00</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>58.00</td>
<td>57.00</td>
<td>58.00</td>
<td>58.00</td>
<td>57.00</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
<td>34.00</td>
<td>36.00</td>
<td>34.00</td>
<td>34.00</td>
<td>35.00</td>
</tr>
</tbody>
</table>

Note: The units of KDM BA estimates and CA are years, and the unit of PDM BA estimates is standard deviation.
Table 1.6. Linear regression (effect sizes and standard errors) of standardized CA and BA estimates, with standardized cognitive and physical function scores, BEFORE and AFTER adjusting for covariates.

<table>
<thead>
<tr>
<th></th>
<th>KDM BA estimates</th>
<th>Calendar Age</th>
<th>PDM BA estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
<td>β</td>
</tr>
<tr>
<td>cognitive function</td>
<td>-0.084**</td>
<td>0.031</td>
<td>-0.096**</td>
</tr>
<tr>
<td>SPPB</td>
<td>-0.105***</td>
<td>0.028</td>
<td>-0.077**</td>
</tr>
<tr>
<td>grip strength</td>
<td>-0.076*</td>
<td>0.031</td>
<td>-0.051</td>
</tr>
<tr>
<td>knee extension</td>
<td>-0.065*</td>
<td>0.031</td>
<td>-0.040</td>
</tr>
<tr>
<td>pegboard dominant time</td>
<td>0.127***</td>
<td>0.028</td>
<td>0.141***</td>
</tr>
<tr>
<td>pegboard nondominant time</td>
<td>0.126***</td>
<td>0.030</td>
<td>0.133***</td>
</tr>
<tr>
<td>time completing 10 chair stands</td>
<td>0.127***</td>
<td>0.030</td>
<td>0.107***</td>
</tr>
<tr>
<td>6-minute walk distance</td>
<td>-0.044</td>
<td>0.029</td>
<td>-0.008</td>
</tr>
</tbody>
</table>

Note: *** p<0.001; ** p<0.01; * p<0.05.
T: Functional scores were standardized.
Figure 1.1. Scatter plots showing the Pearson correlation between (1) KDM BA estimates and CA (left) and (2) PDM BA estimates and CA (right), among eligible BHS 2013-2016 participants. The regression lines, correlation coefficients (R) and p values are included.
Chapter 4. The association between sleep duration, sleep chronotype, social jetlag (SJL) phenotypes and Biological Aging (BA) estimates

Abstract

Background: Human sleep is multidimensional. Sleep duration, sleep chronotype and social jetlag (SJL) have been suggested to be associated with human health indicators including cardiometabolic function, mental health, cognitive function, obesity and mortality. It is not known if an association between sleep and estimates of biological aging exist.

Method: Black and White Bogalusa Heart Study (BHS) subjects assessed between 2013-2016 were included (n=953). NHANES 2011-2018 population was used to create the biomarker battery and to train the algorithms. Klemera and Doubal’s Method (KDM), Mahalanobis Distance (DM) based Physiological Dysregulation Method (PDM) were used to construct BA estimates. BA estimates were the dependent variables. Sleep duration in hours, chronotype (cumulative scores of the reduced Morningness and Evennessness Questionnaire) and SJL in hours (calculated by weekday and weekend bedtime and wake time) were the independent variables. Linear regression was performed.

Results: Using PDM, shorter sleep duration and evening chronotype was associated with larger BA estimates (p<0.01 and p<0.001, respectively).
Conclusion: Insufficient sleep duration and evening chronotype may advance biological aging, regardless of gender, race and CA. Future molecular and genetic studies are recommended for validation.

Keywords: Sleep duration, chronotype, social jetlag, Biological Aging, Klemera and Doubal’s Method, Mahalanobis Distance.

BACKGROUND

Sleep duration, sleep chronotype and social jetlag (SJL) are some of the most common variables that are used to measure the length, time preference and time frame oscillation of human sleep behavior. Sleep chronotype refers to the individual preferences of the sleep-wake cycle, being early or late \(^52\). SJL is the discrepancy of sleep timing between our work days and free days \(^38\).

With increased age, people tend to shift into having morning chronotype and reduced sleep efficiency \(^87,93-96\). The metabolism rhythms, core body temperature rhythm, and gene-regulated rhythmic inflammatory processes are also likely to lose regulation due to advancing age and subsequent circadian clockwork changes \(^87,95-102\). In the past few decades, sleep duration, sleep chronotype, and social jetlag have been suggested to be associated with cardiometabolic function, mental health, cognitive function, obesity and mortality \(^38,62,103-105\). Attempts have been made to assess the association between sleep and biological aging (BA) directly. Sleep duration and sleep onset timing has been suggested to be associated with BA, telomere length and other aging indexes \(^110-112\).
Biological aging (BA) estimates are developed to better capture the gradual increase in the vulnerability of the aging body than chronological age\textsuperscript{1,2}. Klemera and Doubal’s Method (KDM) and Mahalanobis Distance (\(D_M\)) based physiological dysregulation method (PDM) are two of the most reliable methods to quantify BA and predict mortality as well as physiological functions\textsuperscript{4,5,12,13,23}. In the previous study, KDM BA estimates and PDM BA estimates were generated in the Bogalusa Heart Study (BHS). The objective of this study is to explore the association between sleep duration, sleep chronotype and SJL and KDM, PDM BA estimates in the bi-racial, middle-aged population of BHS.

**METHOD**

*Study subjects*

The study population were participants from the Bogalusa Heart Study (BHS) who completed a comprehensive set of sleep questionnaires administered during the 2013-2016 in-person examination\textsuperscript{121,122}. Sociodemographic, lifestyle information, and anthropometric measurements were also obtained. Subjects missing information in CA, gender, race, sleep duration, chronotype, social jetlag or any of the selected biomarkers (selected based on training population, same as in the previous chapter) were excluded. The final target population included 953 participants. Written informed consent was obtained from each participant and all data collection protocols were approved by the Institutional Review Board of Tulane University.
Sleep variables

Sleep duration was calculated as a daily average from the total hours of self-reported sleep during the weekdays plus the total hours of sleep on the weekends divided by seven. In addition, sleep duration was categorized into “Short” (<6h), “Medium” (6-8h) and “Long” (>8h) three groups for further analysis. “Medium” was set to be the reference group.

Sleep chronotype: A 5-item self-reported questionnaire – the reduced Morningness-Eveningness Questionnaire (rMEQ), was used in this study. The cumulative scores of this questionnaire were used directly as a discreet variable, ranging from 4 to 26. Subjects reporting higher scores indicate a morning chronotype. In addition, based on rMEQ score, chronotype was also categorized into “Definitely morning” (22-25), “More morning than evening” (18-21), “Neither” (12-17), “More evening than morning” (8-11) and “Definitely evening” (4-7) five groups.

Social jetlag: The absolute value of SJL in hours is the difference between the midsleep time on free days (MSF) and the midsleep time on workdays (MSW). SJL |MSF – MSW| = |(sleep offset on free days – sleep onset on free days) – (sleep offset on workdays – sleep onset on workdays)|. In this study, the absolute value of SJL in hours will be used because any discrepancy between midsleep on workdays and free days regardless of the directions should be considered having SJL in general (versus not having SJL). In addition, social jetlag was categorized into “Least” (0-1 hour), “More” (1-2 hours) and “Most” (>2 hours) three groups.
**BA estimates**

Nineteen biomarkers were select to quantify BA. These biomarkers are: systolic blood pressure, pulse at rest, albumin, alkaline phosphatase, total protein, glycohemoglobin, waist circumference, lymphocyte percent, monocyte percent, creatinine, blood urea nitrogen (BUN), uric acid, red blood cell counts, mean cell volume, red cell distribution width, platelet count, potassium, chloride, bicarbonate. These variables were available in both the training population and the target population, they were suggested to be associated with aging, they were associated with calendar age in BHS with Pearson correlation coefficient >0.1, and they don’t have collinearity with each other.

Klemera and Doubal’s Method (KDM) BA estimates: If \( m \) is the number of included functionally uncorrelated biomarkers, \( X_j \ (j =1, \ldots, m) \) is the battery of biomarkers, \( B \) is the values of hypothetical BA, \( C \) is calendar age, \( s_B^2 \) is the variance of the difference between \( B \) and \( C \), \( s_j^2 \) is the variance of the biomarker’s fluctuation (away from the linear function predicted by \( B \)), \( k \) and \( q \) are the slope and intercept with respect to each linear function \( F_x \) expressing the dependence of \( X \) on \( B \). The estimated KDM BA is equal to

\[
BA_{KDM} = \frac{\sum_{j=1}^{m} (x_j - q) \frac{k_j}{s_j} + \frac{C}{s_B^2}}{\sum_{j=1}^{m} (\frac{k_j}{s_j})^2 + \frac{1}{s_B^2}}.
\]

The unit of KDM BA estimates is years.

Physiological Dysregulation Method (PDM) BA estimates: Mahalanobis Distance (D_M) was used to estimate PDM BA. \( BA_{D_M}(x) = \sqrt{(x - \mu)^T S^{-1} (x - \mu)} = \sqrt{\sum_{i=1}^{B} \frac{(x_i - \mu_i)^2}{\sigma^2(x_i)}} \), where \( x \) is a multivariate observation of selected biomarkers, \( \mu \) is the training population mean for each selected biomarker. \( B \) is the number of selected
biomarkers and \( \sigma^2(x_i) \) is the variance in the \( i^{th} \) biomarker. The unit of PDM BA estimates is standard deviations.

Covariates

Gender (male, female), calendar age (years) and race (black, white) were used as covariates for adjustment in the association analyses.

In addition, social economic status including educational status and annual income was adjusted as well. Educational status was self-reported. It was categorized into 4 groups: “Less than high school” (11\textsuperscript{th} grade or under), “High school” (12\textsuperscript{th} grade), “Attended college didn’t graduate” (13-15\textsuperscript{th} grade), and “College graduate or above” (16\textsuperscript{th} to 20\textsuperscript{th} grade). Annual income was self-reported as well. It was categorized into 3 groups: “Under 25,000 USD”, “25,000 – 50,000 USD”, and “Over 50,000 USD”.

Statistical analysis

Means (± SD) were used to describe characteristics for demographic information, sleep variables and BA estimates in the total population and among each gender and race group (white female, white male, black female, black male) separately. Linear regression was used to estimated BA using KDM and PDM separately with sleep duration, sleep chronotype scores, SJL as independent variables. In secondary analyses, calendar age, gender and race were added to the linear regression models to adjust for covariates. The regression coefficients and standard errors (SE) were reported. SAS 9.4 (SAS Institute, Cary, NC, USA) and R language version 3.6.3 were used for all analyses. Level of significance for all analyses is \( p < 0.05 \).
RESULTS

Characteristics of the study population

Among the 953 participants, average CA was 48.23 ± 5.29 years. 41.61% were male, 66.82% were white (Table 2.1). Average sleep duration was 7.00 ± 1.45 hours per night, white females had the longest sleep duration (7.08 ± 1.32 hours) and white males had the shortest sleep duration (6.91 ± 1.41 hours). Average sleep chronotype score was 16.45 ± 3.82, black males had the highest chronotype scores (17.00 ± 3.81) and white females had the lowest scores (16.10 ± 3.85). The average social jetlag was 1.27 ± 1.40 hours, black females had the largest SJL (1.36 ± 1.49 hours) and black males had the lowest (1.18 ± 1.23 hours). Average KDM BA estimate was 48.16 ± 6.34 years old, white males had the highest KDM BA estimates (48.80 ± 5.99) while black females had the lowest (47.40 ± 5.96). Average PDM BA estimate was 2.55 ± 1.00 (unit is standard deviation), white males had the highest PDM BA estimates (2.99 ± 0.94) while white and black females had the lowest (mean = 5.68).

Associations between Sleep variables and BA estimates

Unadjusted linear regression models using continuous sleep variables as predictor and BA estimates as outcome were created first. Longer sleep duration was associated with lower PDM BA estimates (beta=-0.065, p<0.01), higher sleep chronotype scores was associated with higher KDM BA estimates (beta=0.144, p<0.01) and longer SJL was associated with lower KDM BA estimates (beta=-1.855, p<0.05). After adjusting for covariates CA, gender, race, education and annual income, longer sleep duration was still
associated with lower PDM BA estimates (beta=-0.049, p<0.05). In addition, higher sleep chronotype scores, which represents earlier sleep chronotype, was associated with lower PDM BA estimates (beta=-0.016, p<0.05). Details are in Table 2.2.

Similar linear regression models were created for white and black BHS participants next. After adjusting for calendar age, gender, education and annual income, longer sleep duration was associated with lower PDM BA estimates (beta=-0.086, SE=0.035 for black participants), Details are in Table 2.3.

Using categorized sleep variables (short sleep duration, medium sleep duration (reference group) and long sleep duration; Definitely morning, more morning than evening, neither, more evening than morning and definitely evening (reference group); Least SJL (reference group), More SJL and Most SJL), adjusted (covariates were CA, gender, race, education and annual income) linear regression models showed that compared to people with medium sleep duration, people with short sleep duration on average are 0.193 units higher (SE=0.088) in PDM BA estimates. Compared to people who are “Definitely evening” type, “Definitely morning” people are 0.895 units lower (SE=0.298) in PDM BA estimates, “More morning than evening” people are 0.772 units lower (SE=0.285) in PDM BA estimates, “Neither” people are 0.798 units lower (SE=0.283) in PDM BA estimates, and “More evening than morning” people are 0.620 units lower (SE=0.296) in PDM BA estimates. Details are in Table 2.4. Similar associations between short sleep duration and higher PDM BA estimates were observed in only black participants. Associations between categorized chronotype and PDM BA estimates were only observed among black participants as well. Details are in Table 2.5 and 2.6.
Discussion

In this study, the associations between sleep and BA were explored. After adjusting for calendar age, gender, race, education and annual income, a reduction of 2.94 minutes in sleep duration was associated with a 1 unit increase in BA (using log transformed PDM BA estimates). This indicates that shorter sleep duration was associated with older BA. Additionally, a reduction of 0.016 in rMEQ (sleep chronotype) score was associated with a 1 unit increase in BA (using log transformed PDM BA estimates), which means later sleep chronotype was associated with older BA.

Based on previous research, short (usually less than 5 or 6 hours per night) and/or long (usually more than 8 or 9 hours per night) sleep duration tends to have a U-shape relation with increased risk of cardiovascular events, hypertension, obesity, diabetes, metabolic syndrome, cognitive performance, and depression\textsuperscript{30,31,34,171-179}. Late chronotype was suggested to be associated with obesity, low high-density lipoprotein (HDL), type 2 diabetes, hypertension, poor cardiovascular health, depression, anxiety, substance use disorders, attention issues, and aggression\textsuperscript{57,104,122,180-184}. Studies on social jetlag suggested that more severe social jetlag was associated with obesity, low HDL, triglycerides, fasting plasma insulin, fasting glucose, metabolic syndrome (MetS), diabetes, cognitive performance and attention deficit hyperactivity disorder (ADHD) symptoms and impulsivity\textsuperscript{38,64,67,104,185,186}.

Having increased risk of chronic and complicated health issues is one of the manifestations of human aging. Wang et al. found that both sleeping less than 6 hours
and more than 8 hours were associated with increased risk of the composite outcome of deaths and major cardiovascular events. Yaffe et al. found that sleeping less than 6 hours per night was associated with worse markers of brain white matter integrity in midlife, which indicates increased risk of dementia and stroke. Mortality, disease specific mortality and functional declines are important aging indicators as well.

Akerstedt et al. found that among people under 65 years old, both short (≤5h) and long (≥8h) sleep duration was associated with increased mortality. They also found that among people under 65 years old, short weekend sleep (<5h) was associated with a 52% increase in mortality. Long weekend sleep may compensate for short weekday sleep.

Ma et al. found that sleeping less than 4 hours per night or more than 10 hours per night was associated with increased cognitive decline. Dominguez et al. found that very short sleep duration (<6h) was associated with higher atherosclerotic burden.

Measurable biological aging is another avenue to estimate human aging before outcome events of aging ever occur. So far, only telomere length has been used to quantify biological aging in sleep research. Shorter telomere length indicates more advanced biological age. Wynchank et al. found that delayed circadian rhythm was strongly associated with shorter telomere length, while extremely early chronotype was associated with significantly less telomere shortening. For 9-year-old children, James et al. found that each hour less of nightly sleep duration is associated with having telomeres that are 0.015 log-kilobases per chromosome shorter. However, Nguyen et al. only found a weak association between longer telomere length with later sleep timing but not with sleep duration. Our study further supports the association between late...
chronotype and more advanced biological aging, and suggests the association between short sleep duration and more advanced biological aging.

Based on the Conceptual Model of Sleep Health proposed by Buysse, sleep dimensions including satisfaction, alertness, timing, efficiency, duration and regularity, eventually have impact on human health, disease and functional status through intermediate epigenetic, molecular and cellular processes first, then through the systems-level processes including inflammation, sympathetic nervous system activation, hormonal responses and neural circuitry responses. 

So far, intermediate level evidence suggest that sleep health is associated with epigenetic, molecular and cellular modifications that could be related with aging. Studies among adolescents have found that short sleep duration is associated with leukocyte DNA methylation patterns of metabolism genes. Longer sleep duration was associated with better endothelial health and lower levels of endothelial-derived microparticles. In addition, even temporary sleep deprivation was associated with long term changes of genomic regulation, through cellular components such as Serum response factor (SRF). Prolonged wakefulness was suggested to have direct impact on clock gene expression at molecular levels.

In addition, system-level evidence suggest that sleep health is associated with aging related inflammation, sympathetic nervous system activation, hormonal responses and neural circuitry responses. Sleep deprivation was associated with increased oxidative stress in brain regions. Sleep deprivation was associated with increased endothelial oxidative stress and suppressed antioxidant responses due to reduced expression of SRF during daytime and subsequently decreased amount of DCUN1D3 protein in animal
models. Prolonged sleep deprivation and sleep disturbance were suggested to lead to chronic low-grade inflammation via inflammatory mediators like cytokines. In addition, secretion of growth hormone (GH), melatonin, thyroid-stimulating hormone (TSH) and cortisol increase during sleep. Consequently, sleep deprivation would reduce the level of these hormones. Controversy has been observed between GH and aging, that while older individuals exhibit decreased GH secretion, animal models found increased life span with congenital GH deficiency. Melatonin was found to be protective against aging. Melatonin as a molecule has been found to be antioxidant, immunomodulatory, anti-proliferate, oncostatic and endocrine-modulatory. It is suggested that melatonin is able to lessen the consequences of aging through (1) regulation of circadian rhythm (2) decreasing energy expenditure (3) mitochondrial biogenesis and (4) immune remodeling (reducing inflammation). More specifically, melatonin was suggested to be protective against brain aging, cardiovascular aging, ovary aging, liver aging and so on. Aging was suggested to be associated with TSH regardless of any thyroid disease. Cortisol is a stress hormone. Low level of cortisol is suggested to trigger persistent inflammatory response in human body, the latter being one of the most important mechanisms in aging. Reduced level of melatonin, TSH and cortisol induced by sleep deprivation are thus likely associated with progressed aging.

Physiological and activity rhythms were found to decrease with aging. Activity rhythms generally phase advance towards early bedtime and early morning wakening. Thus, older adults experience sleep changes including advanced sleep timing, and chronotype shifts towards morningness with aging. On the contrary, we found association between late chronotype and biological aging. As we mentioned earlier, late
chronotype and delayed circadian rhythm has been suggested to be associated with shorter telomere length, which is indicative of more advanced aging\textsuperscript{110,112,194}. Furthermore, survival analysis found that eveningness increased the likelihood of mortality among older adults\textsuperscript{202}. Eveningness is suggested to be associated with lower cortisol secretion. Evidence linking late chronotype and aging at molecular, cellular, and physiological level is not sufficient. Nevertheless, it is possible that chronotype and aging are associated at genetic level since the heritability of chronotype is estimated to be 13.7\% (95\% CI: 13.3–14.0\%)\textsuperscript{120}. Aim 3 of this thesis will be exploring if there are genetic association between chronotype and biological aging.

We observed race difference in the association between sleep duration, chronotype and PDM BA estimates. After categorizing the total population in to white and black, the association between sleep duration, chronotype and BA estimates only persists among black participants. It is not clear why the race difference in association occurred, but it is possible that there are factors other than sleep that are more influential to biological aging among white participants. Sleep related behavioral interventions and modifications may be significant for promoting health and resisting biological aging, especially among black participants.

This is the first study on the association between sleep and aging in BHS population. Strength of this study also include proper association analyses categorized by race and consideration of social economic status confounders adjustment. There are several limitations in this study as well. First, all sleep measures were self-reported, which are prone to self report bias. Second, the cross-sectional nature of the study means that the result is not sufficient for causal relation evidence. Similar studies with
prospective or retrospective longitudinal data would in theory provide more evidence in
the potential causal relations between sleep duration, chronotype and aging.

Conclusion

In sum, the current study found that among the black participants of BHS 2013-2016, both short sleep duration and late chronotype contributed to more advanced biological age. Future longitudinal studies are needed to explore the potential causal relationships between sleep duration, chronotype and biological age, as well as to explore protective sleep behaviors to compensate for individuals with late chronotype, and those who are not able to adjust their sleep duration due to external responsibilities and other conditions.
Table 2.1. Means ± standard deviations, and n (%) of demographic and sleep information, and BA estimates in the BHS (n=953).

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>White, male</th>
<th>Black, male</th>
<th>White, female</th>
<th>Black, female</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>953</td>
<td>291</td>
<td>108</td>
<td>371</td>
<td>183</td>
</tr>
<tr>
<td>Calendar Age (years)</td>
<td>48.23 ± 5.29</td>
<td>48.90 ± 5.06</td>
<td>47.80 ± 6.13</td>
<td>48.30 ± 5.00</td>
<td>47.40 ± 5.59</td>
</tr>
<tr>
<td>Sleep duration (hours)</td>
<td>7.00 ± 1.45</td>
<td>6.91 ± 1.41</td>
<td>6.93 ± 1.83</td>
<td>7.08 ± 1.32</td>
<td>7.01 ± 1.52</td>
</tr>
<tr>
<td>Sleep chronotype</td>
<td>16.45 ± 3.82</td>
<td>16.90 ± 3.73</td>
<td>17.00 ± 3.81</td>
<td>16.10 ± 3.85</td>
<td>16.20 ± 3.82</td>
</tr>
<tr>
<td>Social Jetlag (hours)</td>
<td>1.27 ±1.40</td>
<td>1.27 ± 1.50</td>
<td>1.18 ± 1.23</td>
<td>1.25 ± 1.33</td>
<td>1.36 ± 1.49</td>
</tr>
<tr>
<td>KDM BA estimates</td>
<td>48.16 ± 6.34</td>
<td>48.80 ± 5.99</td>
<td>47.40 ± 9.86</td>
<td>48.30 ± 5.36</td>
<td>47.40 ± 5.96</td>
</tr>
<tr>
<td>PDM BA estimates</td>
<td>2.55 ± 1.00</td>
<td>2.99 ± 0.94</td>
<td>2.84 ± 0.90</td>
<td>2.26 ± 0.92</td>
<td>2.26 ± 1.03</td>
</tr>
<tr>
<td>Education – less than high school</td>
<td>97 (10.18)</td>
<td>25 (8.60)</td>
<td>23 (21.30)</td>
<td>29 (7.82)</td>
<td>20 (10.93)</td>
</tr>
<tr>
<td>High school</td>
<td>345 (36.20)</td>
<td>112 (38.49)</td>
<td>50 (46.30)</td>
<td>105 (28.30)</td>
<td>78 (42.62)</td>
</tr>
<tr>
<td>Attended college didn’t graduate</td>
<td>263 (27.60)</td>
<td>68 (23.37)</td>
<td>25 (23.15)</td>
<td>114 (30.73)</td>
<td>56 (30.60)</td>
</tr>
<tr>
<td>College graduate or above</td>
<td>248 (26.02)</td>
<td>86 (29.55)</td>
<td>10 (9.26)</td>
<td>123 (33.15)</td>
<td>29 (15.85)</td>
</tr>
<tr>
<td>Annual income – Under 25,000 USD</td>
<td>414 (43.44)</td>
<td>72 (24.74)</td>
<td>75 (69.44)</td>
<td>142 (38.27)</td>
<td>125 (68.31)</td>
</tr>
<tr>
<td>25,000 – 50,000 USD</td>
<td>260 (27.28)</td>
<td>69 (23.71)</td>
<td>18 (16.67)</td>
<td>129 (34.77)</td>
<td>44 (24.04)</td>
</tr>
<tr>
<td>Over 50,000 USD</td>
<td>279 (29.28)</td>
<td>150 (51.55)</td>
<td>15 (13.89)</td>
<td>100 (26.95)</td>
<td>14 (7.65)</td>
</tr>
</tbody>
</table>

Note: BA is biological aging, BHS is Bogalusa Heart Study, KDM is Klemera and Doubal’s Method, PDM is Physiological Dysregulation Method.
Figure 2.1. Distribution of sleep duration in hours (left), chronotype in rMEQ score (middle), and social jetlag in hours (right)
Table 2.2. Linear regression (effect sizes, standard errors) between sleep duration, sleep chronotype, social jetlag and Biological Aging (BA) estimates calculated using the KDM and PDM methods (n=953).

<table>
<thead>
<tr>
<th></th>
<th>KDM estimates</th>
<th></th>
<th>PDM BA estimates</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
<td>β</td>
<td>SE</td>
</tr>
<tr>
<td>Sleep duration</td>
<td>-0.064</td>
<td>0.142</td>
<td>-0.065**</td>
<td>0.022</td>
</tr>
<tr>
<td>Sleep chronotype</td>
<td>0.144**</td>
<td>0.054</td>
<td>0.009</td>
<td>0.009</td>
</tr>
<tr>
<td>Social Jetlag</td>
<td>-1.855*</td>
<td>0.933</td>
<td>0.032</td>
<td>0.148</td>
</tr>
</tbody>
</table>

Adjusted for calendar age, gender, race, education, annual income

<table>
<thead>
<tr>
<th></th>
<th>KDM estimates</th>
<th></th>
<th>PDM BA estimates</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
<td>β</td>
<td>SE</td>
</tr>
<tr>
<td>Sleep duration</td>
<td>-0.062</td>
<td>0.076</td>
<td>-0.049*</td>
<td>0.021</td>
</tr>
<tr>
<td>Sleep chronotype</td>
<td>0.015</td>
<td>0.029</td>
<td>-0.016*</td>
<td>0.008</td>
</tr>
<tr>
<td>Social Jetlag</td>
<td>-0.083</td>
<td>0.504</td>
<td>0.200</td>
<td>0.138</td>
</tr>
</tbody>
</table>

Note: *** p<0.001; ** p<0.01; * p<0.05.

*T*: Variable was transformed using log10(x+1).
Table 2.3. Linear regression (effect sizes, standard errors) between sleep duration, sleep chronotype, social jetlag and Biological Aging (BA) estimates calculated by the KDM and PDM method, in white and black participants of the Bogalusa Heart Study, separately (n=953).

<table>
<thead>
<tr>
<th></th>
<th>KDM estimates</th>
<th>PDM BA estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>white</td>
<td>black</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
</tr>
<tr>
<td>Sleep duration</td>
<td>-0.156</td>
<td>0.161</td>
</tr>
<tr>
<td>Sleep chronotype</td>
<td>0.170**</td>
<td>0.057</td>
</tr>
<tr>
<td>Social Jetlag†</td>
<td>-1.790</td>
<td>1.022</td>
</tr>
</tbody>
</table>

Adjusted for calendar age, gender, education, annual income

<table>
<thead>
<tr>
<th></th>
<th>KDM estimates</th>
<th>PDM BA estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>white</td>
<td>black</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
</tr>
<tr>
<td>Sleep duration</td>
<td>-0.139</td>
<td>0.071</td>
</tr>
<tr>
<td>Sleep chronotype</td>
<td>0.008</td>
<td>0.026</td>
</tr>
<tr>
<td>Social Jetlag†</td>
<td>-0.237</td>
<td>0.456</td>
</tr>
</tbody>
</table>

Note: *** p<0.001; ** p<0.01; * p<0.05.
†: Variable was transformed using log10(x+1).
Table 2.4. Linear regression between sleep duration, sleep chronotype, social jetlag and Biological Aging (BA) estimates calculated by the KDM and PDM method (n=953). All sleep variables were categorized

<table>
<thead>
<tr>
<th></th>
<th>KDM BA estimates</th>
<th>PDM BA estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
</tr>
<tr>
<td><strong>Sleep duration</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short</td>
<td>-0.125</td>
<td>0.590</td>
</tr>
<tr>
<td>Medium (ref)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Long</td>
<td>-0.488</td>
<td>0.461</td>
</tr>
<tr>
<td><strong>Sleep chronotype</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definitely morning</td>
<td>1.537</td>
<td>2.027</td>
</tr>
<tr>
<td>More morning than evening</td>
<td>0.924</td>
<td>1.937</td>
</tr>
<tr>
<td>Neither</td>
<td>0.242</td>
<td>1.928</td>
</tr>
<tr>
<td>More evening than morning</td>
<td>-0.958</td>
<td>2.015</td>
</tr>
<tr>
<td>Definitely evening (ref)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Social jetlag</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Least (ref)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>More</td>
<td>-1.301**</td>
<td>0.472</td>
</tr>
<tr>
<td>Most</td>
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<td><strong>Adjusted for calendar age, gender, race, education, annual income</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sleep duration</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short</td>
<td>0.303</td>
<td>0.320</td>
</tr>
<tr>
<td>Medium (ref)</td>
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</tr>
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<td>Long</td>
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<td>0.248</td>
</tr>
<tr>
<td><strong>Sleep chronotype</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definitely morning</td>
<td>0.955</td>
<td>1.091</td>
</tr>
<tr>
<td>More morning than evening</td>
<td>0.518</td>
<td>1.042</td>
</tr>
<tr>
<td>Neither</td>
<td>0.503</td>
<td>1.036</td>
</tr>
<tr>
<td>More evening than morning</td>
<td>0.795</td>
<td>1.082</td>
</tr>
<tr>
<td>Definitely evening (ref)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Social jetlag</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Least (ref)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>More</td>
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</tr>
<tr>
<td>Most</td>
<td>0.024</td>
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</tr>
</tbody>
</table>

Note: *** p<0.001; ** p<0.01; * p<0.05.
Table 2.5. Linear regression between sleep duration, sleep chronotype, social jetlag and Biological Aging (BA) estimates calculated by the KDM and PDM method, among white participants (n=662). All sleep variables were categorized.

<table>
<thead>
<tr>
<th></th>
<th>KDM BA estimates</th>
<th></th>
<th>PDM BA estimates</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
<td>β</td>
<td>SE</td>
</tr>
<tr>
<td><strong>Sleep duration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short</td>
<td>0.530</td>
<td>0.654</td>
<td>0.198</td>
<td>0.115</td>
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<tr>
<td>Medium (ref)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Long</td>
<td>-0.019</td>
<td>0.493</td>
<td>-0.039</td>
<td>0.087</td>
</tr>
<tr>
<td><strong>Sleep chronotype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definitely morning</td>
<td>-0.275</td>
<td>2.110</td>
<td>-0.704</td>
<td>0.375</td>
</tr>
<tr>
<td>More morning than evening</td>
<td>0.273</td>
<td>2.015</td>
<td>-0.756*</td>
<td>0.358</td>
</tr>
<tr>
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<td>-1.055</td>
<td>2.006</td>
<td>-0.769*</td>
<td>0.356</td>
</tr>
<tr>
<td>More evening than morning</td>
<td>-2.566</td>
<td>2.106</td>
<td>-0.776*</td>
<td>0.374</td>
</tr>
<tr>
<td>Definitely evening (ref)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Social jetlag</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Least (ref)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>More</td>
<td>-1.592**</td>
<td>0.492</td>
<td>-0.290***</td>
<td>0.086</td>
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<tr>
<td>Most</td>
<td>-1.545**</td>
<td>0.583</td>
<td>0.135</td>
<td>0.102</td>
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<td><strong>Adjusted for calendar age, gender, education, annual income</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Sleep duration</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Short</td>
<td>0.823**</td>
<td>0.291</td>
<td>0.080</td>
<td>0.107</td>
</tr>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Long</td>
<td>0.145</td>
<td>0.217</td>
<td>-0.044</td>
<td>0.079</td>
</tr>
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<td><strong>Sleep chronotype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>-0.305</td>
<td>0.945</td>
<td>-0.649</td>
<td>0.343</td>
</tr>
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<td>-0.417</td>
<td>0.898</td>
<td>-0.597</td>
<td>0.326</td>
</tr>
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<td>0.943</td>
<td>-0.481</td>
<td>0.343</td>
</tr>
<tr>
<td>Definitely evening (ref)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Social jetlag</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Least (ref)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>More</td>
<td>-0.201</td>
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<td>0.082</td>
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<td>-0.156</td>
<td>0.262</td>
<td>0.175</td>
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Note: *** p<0.001; ** p<0.01; * p<0.05.
Table 2.6. Linear regression between sleep duration, sleep chronotype, social jetlag and Biological Aging (BA) estimates calculated by the KDM and PDM method, among black participants (n=291). All sleep variables were categorized.

<table>
<thead>
<tr>
<th></th>
<th>KDM BA estimates</th>
<th>PDM BA estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
</tr>
<tr>
<td><strong>Sleep duration</strong></td>
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<td></td>
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<tr>
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<td>Long</td>
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<td>1.006</td>
</tr>
<tr>
<td><strong>Sleep chronotype</strong></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>6.297</td>
<td>4.666</td>
</tr>
<tr>
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<td>4.465</td>
</tr>
<tr>
<td>Neither</td>
<td>3.763</td>
<td>4.449</td>
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<td>More evening than morning</td>
<td>3.236</td>
<td>4.607</td>
</tr>
<tr>
<td>Definitely evening (ref)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Social jetlag</strong></td>
<td></td>
<td></td>
</tr>
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<td>Least (ref)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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</tr>
<tr>
<td>Most</td>
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<td>1.076</td>
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<td><strong>Adjusted for calendar age, gender, education, annual income</strong></td>
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<td></td>
</tr>
<tr>
<td>Sleep duration</td>
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<td></td>
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<tr>
<td>Short</td>
<td>-0.699</td>
<td>0.776</td>
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<tr>
<td>Medium (ref)</td>
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<td>-</td>
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<td>Long</td>
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<td>0.655</td>
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<td>Sleep chronotype</td>
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<tr>
<td>Definitely morning</td>
<td>4.353</td>
<td>2.979</td>
</tr>
<tr>
<td>More morning than evening</td>
<td>2.480</td>
<td>2.836</td>
</tr>
<tr>
<td>Neither</td>
<td>2.915</td>
<td>2.824</td>
</tr>
<tr>
<td>More evening than morning</td>
<td>4.216</td>
<td>2.924</td>
</tr>
<tr>
<td>Definitely evening (ref)</td>
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<td>-</td>
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<td></td>
</tr>
<tr>
<td>Least (ref)</td>
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<td>-</td>
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<tr>
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</tr>
<tr>
<td>Most</td>
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</tr>
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</table>

Note: *** p<0.001; ** p<0.01; * p<0.05.
Chapter 5. Genetic Association between Chronotype and Physiological Dysregulation Method (PDM) Biological Aging (BA) Estimates in the Bogalusa Heart Study (BHS) Population

Abstract

Background: Sleep chronotype was suggested to be associated with biological aging estimates in our previous study. With the help of genetic risk scores (GRS) computed by chronotype variants, mendelian randomization will be an effective method to explore whether there is a genetic association between chronotype and biological aging.

Method: Participants from the Bogalusa Heart Study (BHS) measured in 2013-2016, with genotyping information available were used (n=646). For GRS determination, morning chronotype related SNPs (n=351) proposed by a GWAS meta-analysis using 697,828 European ancestry participants were used to compute the morning chronotype GRS for BHS participants. Multivariate linear models were created to explore the association between morning chronotype GRS and morning chronotype phenotype. All analyses were performed in African ancestry participants and European ancestry participants separately.

Result: Six hundred and forty-six BHS participants were included. Morning chronotype GRS was not associated with morning chronotype phenotype among BHS participants.
Conclusion: We did not find any association between morning chronotype GRS and morning chronotype phenotype, however there is no evidence to validate true negative results due to the small percentage that morning chronotype accounts for the morning chronotype phenotype variance, and the small sample size.

Keywords: Chronotype; Biological aging. Genetic risk scores.
**Background**

Sleep chronotype refers to the individual preferences of the sleep-wake cycle, being early or late. Short telomere length is associated with aging. Late chronotype, or eveningness, has been suggested to be associated with shorter telomere length. Previously, we found that the association between late chronotype and more advanced estimates of BA using the Mahalanobis distance based physiological dysregulation method (PDM), among the black and white participants of the Bogalusa Heart Study (BHS) 2013-2016. However, causal correlation requires additional analysis and verification. Genetic association analysis on chronotype and biological aging will likely provide insight into causal relations between chronotype and biological aging, especially if using mendelian randomization (MR). Mendelian Randomization (MR) is one of the most popular approaches to validate causal associations because it requires evaluating the association between the proxy and both the exposure and the outcome, as well as excluding any possible link between the proxy and confounders.

Most sleep chronotype related genetic studies focused on circadian clock genes. Large scale genetic analyses were lacking until 2016, when 3 large GWAS studies on sleep chronotype were published. Hu et al. discovered 15 loci that were significantly associated with chronotype using data of 89,283 European participants from the 23andMe cohort. Jones et al. found 16 variants were associated with chronotype using 128,266 white British individuals from the UK Biobank study. Lane et al. found 12 loci significantly associated with chronotype, using data of 100,420 individuals from the UK Biobank cohort. PER2, RGS16, FBXL13 and AK5 genes were associated with chronotype in all three GWASs. In 2019, Jones and Lane jointly published the largest
chronotype GWAS meta-analysis so far using 697,828 European ancestry participants from UK Biobank and 23andMe \(^{120}\). 351 loci including RGS16, PER1, PER2, PER3 and CRY1 were found to be associated with morningness \(^{120}\).

Genetic Risk Score (GRS) integrates a group of relevant genetic loci for phenotypes of complex traits and provide the overall genetic risk estimates for individuals \(^{204}\). Utilizing GRS instead of single loci will likely improve the power for causal linkage. Summary statistics of the large GWAS meta-analysis by Jones et al., including positions, effect alleles, and effect sizes could be used to construct the GRS for sleep chronotype.

The objective of this study is to test if in BHS: (1) Individuals with higher evening chronotype GRS are more likely to have evening chronotype. (2) Evening chronotype GRS is not associated with confounders like age, gender and race. (3) Higher evening chronotype GRS is associated with increased BA estimates.

**Method**

**Study subjects**

The study population was participants from the Bogalusa Heart Study (BHS) who completed a comprehensive set of sleep questionnaires administered during the 2013-2016 in-person examination \(^{121,122}\). Only subjects with complete PDM BA estimates, reduced Morningness-Eveningness Questionnaire (rMEQ) total scores, and calculated morning chronotype GRS scores were included. Subjects missing information in calendar age, gender, race were excluded. The final target population included 646 participants.
Written informed consent was obtained from each participant and all data collection protocols were approved by the Institutional Review Board of Tulane University.

**Sleep chronotype**

A 5-item self-reported questionnaire – the rMEQ, was used in this study. The cumulative scores of this questionnaire were used directly as a discreet variable, ranging from 4 to 26. Subjects reporting higher scores tend to be more of morning person. Furthermore, one of the five self-reported questions (See supplementary information) – “One hears about “morning” and “evening” types of people, which one of the types do you consider yourself to be?” was given special attention and analyzed separately due to the fact that the lead SNPs discovered by GWAS meta-analysis were selected by a similar self-reported question among the UK Biobank and 23andme participants. To be consistent with the corresponding question in the chronotype GWAS, we recoded “Definitely a morning type” and “more a morning than an evening type” into “morning”, “more an evening type than a morning type” and “definitely an evening type” into “evening”, and “neither a morning nor evening type” into missing.

**BA estimates**

Nineteen biomarkers were select to quantify BA. These biomarkers are: systolic blood pressure, pulse at rest, albumin, alkaline phosphatase, total protein, glycohemoglobin, waist circumference, lymphocyte percent, monocyte percent, creatinine, blood urea nitrogen (BUN), uric acid, red blood cell counts, mean cell volume,
red cell distribution width, platelet count, potassium, chloride, bicarbonate. These variables were available in both the training population and the target population, they were suggested to be associated with aging, they were associated with calendar age in BHS with Pearson correlation coefficient >0.1, and they don’t have collinearity with each other.

BA estimates using the Physiological Dysregulation Method (PDM) uses \(^{14}\) Mahalanobis Distance (\(D_M\)) to estimate BA. \(BA_{D_M}(x) = \sqrt{(x - \mu)^T S^{-1} (x - \mu)} = \sqrt{\sum_{i=1}^{B} \frac{(x_i - \mu_i)^2}{\sigma^2(x_i)}}, \) where \(x\) is a multivariate observation of selected biomarkers, \(\mu\) is the training population means for each selected biomarker, \(B\) is the number of selected biomarkers and \(\sigma^2(x_i)\) is the variance in the \(i^{th}\) biomarker. The unit of PDM BA estimates is standard deviation.

**Morningness loci identified by GWAS meta-analysis**

Jones et al. published a large chronotype GWAS meta-analysis using over 697,828 participants of UK Biobank and 23andMe and discovered and validated 351 morning chronotype associated loci (p<5\(^{-8}\)) \(^{120}\). Details about the GWAS meta-analysis and summary statistics of the significant loci are available online \(^{120}\).

**BHS Genotyping and imputation**

Genotyping was performed using the Illumina Human610 BeadChip \(^{123}\). Stringent quality control removed SNPs with low call rate or a poor cluster separation score.
Genotypes were phased using SHAPEIT software \(^{124}\) and untyped markers were imputed using the 1000 Genomes phase 3, version 5 combined ancestry reference panel with Minimac software \(^{125}\). Variants with imputation quality<0.3 were removed. To identify and adjust for ancestry differences among participants, genomic ancestry principal components (PCs) were also calculated for white and black participants, separately.

**GRS calculation**

Based on the GWAS meta-analysis by Jones et al., we developed a weighted GRS for morning chronotype. Among the 351 morningness SNPs that were discovered by the chronotype GWAS, we used 330 that are available in BHS. To create the GRS, we summed all included minor alleles weighted by their corresponding regression coefficients for risk of being a morning type for each participant.

**Statistical analysis**

Means and standard deviations (SD) were used to describe characteristics for demographic information, sleep variable, BA estimates and principal components, in the BHS 2013-2016 African ancestry population and European ancestry population, separately. MR testing consists of (1) The association between chronotype GRS and chronotype phenotype (the exposure). (2) The association between chronotype GRS and BA estimates (the outcome). (3) The association between chronotype GRS and potential confounders\(^{203}\). Multivariate linear regression models and logistic regression models were created, using morning chronotype GRS as the independent variable, calendar age,
gender, and the top ten genetic principal components as covariates, and chronotype scores as the dependent variable. Models were built separately in African ancestry participants and European ancestry participants. For the multivariate linear regression models, the regression coefficients, standard errors (SE), P values, as well as the linear model coefficients of determination were reported. For the logistic regression models, Odds ratios, 95% confidence intervals and p values were reported. P-values <0.05 were considered statistically significant. SAS 9.4 (SAS Institute, Cary, NC, USA) and R language version 3.6.3 were used for all analyses.

Results

Characteristics of the participants

Among the 646 participants, 197 (30.5%) were of African ancestry (AA), 449 (69.5%) were of European ancestry (EA). Among the AA subjects, the mean calendar age was 49.03 years old, 55 (27.92%) of them were male, the mean rMEQ score was 16.53, the mean morning chronotype GRS was -1.26, and the mean PDM BA estimate was 2.44 standard deviations. Among the EA subjects, the mean calendar age was 49.55 years old, 188 (41.87%) of them were male, the mean rMEQ score was 16.37, the mean morning chronotype GRS was -0.75, and the mean PDM BA estimates was 2.58. See details in Table 1.

Morningness GRS and morning chronotype phenotype

No association was found between morning chronotype GRS and morning chronotype phenotype in AA or EA subjects (Figure 1). In AA participants, the effect
size of chronotype morningness GRS is -0.601 and the multivariate linear model explains 0.47% of the morning chronotype phenotype variation (Table 2). In EA participants, the effect size of morning chronotype GRS is 0.464, and the multivariate linear model explains 3.79% of the morning chronotype phenotype variation (Table 2). No association was found between morning chronotype GRS and binary chronotype phenotype as well (Table 3).

**Discussion**

We did not show any association between the morning chronotype GRS and morning chronotype phenotype (using both continuous and binary measures), in EA or AA participants. Hence, Mendelian randomization will not be performed between morning chronotype GRS and PDM BA estimates outcome variable using current BHS genotype data.

To eliminate the possibility of human calculation error, we made sure that the genotyping of both the GWAS meta-analysis population genotyping and BHS population used the 1000 Genomes reference panel with genome assembly GRCh37. In addition, efforts were made to ensure that for the effect alleles in GWAS that are different from the corresponding alternative alleles in the BHS, their beta estimate signs were flipped correctly. Furthermore, we performed sensitivity analysis and created multivariate linear models excluding all SNPs with “AT” or “CG” alleles, to rule out the possibility of strand issues. The sensitivity analysis results did not show association between morning
chronotype GRS and morning chronotype phenotype in any ancestry group either, and the regression effect sizes of the predictors only attenuated by a little (data not shown).

So far, only a limited number of publications have calculated chronotype GRS. Maukonen et al. included 8,433 Finnish adult men and women from population-based cross-sectional studies, and created eveningness GRS using 313 SNPs out of the 351 significant chronotype SNPs proposed by the GWAS meta-analysis of Jones et al. 206. They found associations between eveningness GRS and all three chronotype phenotype measures including continuous sMEQ score, binary sMEQ score and single-item chronotype result. Merikanto et al. included 17,243 Finnish participants from the same cross-sectional studies, and computed polygenic scores (PGS) for morningness, using the significant chronotype SNPs proposed by the GWAS meta-analysis of Jones et al. 207. They found that morningness PGS were weakly associated with short MEQ score, and that morningness PGS only explains between one to two percent of the variation in diurnal preference. 207. Morales-Muñoz et al. included over 3,000 Finnish infants and calculated their diurnal preference polygenic risk score (PRS) using the significant chronotype SNPs proposed by the GWAS meta-analysis of Jones et al. 208. However, they did not assess the association between diurnal preference PRS and self-reported diurnal preference. Vera et al. included 1,693 overweight and obese adult men and women, and calculated chronotype GRS using 15 SNPs out of the 18 significant chronotype SNPs proposed by the GWAS of Lane et al. 209. They found association between chronotype GRS and MEQ score (beta=-0.121, p=0.037) 209.

There are several possible reasons for the negative results.
First, we compared the linear regression beta coefficients of predictors – morning chronotype GRS, in the current study versus previous publications. Maukonen et al. used eveningness GRS as the predictor and continuous shortened MEQ score as the outcome. The predictor beta regression coefficient was reported to be -0.49, which is similar to morning chronotype GRS beta regression coefficient among EA subjects in our study. Both studies were cross-sectional and population-based. Both study populations had a mean age around 50 years old. However, the final study sample of Maukonen et al. included 8,433 EA subjects, while our study included 449 EA subjects. It is possible that the sample size was not sufficient to demonstrate differences between morning chronotype GRS and chronotype phenotype in the present study.

Perhaps more importantly, the chronotype GRS only explains 0.09% of the variance of the total score of MEQ among EA participants, and 0.15% among AA participants, in the current BHS population. In other words, despite that the estimated heritability of chronotype was as much as 13.7% (95% CI:13.3-14.0%) among the 697,828 EA individuals of the UK Biobank and 23andMe, the GRS composed by 330 variants discovered by the GWAS only explains an extremely small proportion of the heritability of the morning chronotype phenotype represented by the rMEQ, in the 646 EA and AA subjects in BHS. Had the association results been positive or negative, the chronotype GRS was not strong enough to serve as a proxy of chronotype phenotype in our study.

In addition to rMEQ scores, we also used chronotype measured by a self-reported binary variable that was same as the variable used in chronotype GWAS meta-analysis. No association was found between morning chronotype GRS and binary morning
chronotype. It is unlikely that the chronotype measurement method discrepancy between the GWAS and the current study is responsible for our negative results.

In addition, since the morning chronotype SNPs were identified by a GWAS of all EA participants, any identified variants potentially specific to AA individuals would likely be missing and EA specific variants may not be associated with the phenotype among AA individuals. Hence, the effect sizes, SEs and R²s we calculated for AA participants are more likely to be non-representative of the true heritability of chronotype in BHS.

This is the first time exploring the association between chronotype GRS and chronotype phenotype among BHS participants especially AA participants. Strengths of this study also include proper quality control and performing association analysis in AA and EA separately. Small sample size is one of the most significant limitations of this study. In addition, the only available chronotype GWAS meta-analysis was performed in exclusively EA subjects, and the identified SNPs may not be representative for AA individuals.

If more chronotype GWAS meta-analysis (especially ones that included multiracial participants) or more genotyping data among more BHS participants become available, it will be meaningful to perform replication association studies on chronotype GRS and phenotype, as well as mendelian randomization on chronotype and BA.
Conclusion

Morning chronotype GRS was not associated with morning chronotype phenotype among BHS participants. We were not able to perform MR between morning chronotype GRS and PDM BA estimates.
Table 3.1. Characteristics of the included BHS 2013-2016 participants (n=646)

<table>
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<tr>
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<td>SD/(%)</td>
<td>Mean/n</td>
<td>SD/(%)</td>
</tr>
<tr>
<td>Calendar age (years)</td>
<td>49.03</td>
<td>4.68</td>
<td>49.55</td>
<td>4.36</td>
</tr>
<tr>
<td>Male</td>
<td>55</td>
<td>27.92</td>
<td>188</td>
<td>41.87</td>
</tr>
<tr>
<td>Chronotype score</td>
<td>16.53</td>
<td>3.50</td>
<td>16.37</td>
<td>3.79</td>
</tr>
<tr>
<td>Morning chronotype</td>
<td>87</td>
<td>44.16</td>
<td>185</td>
<td>41.20</td>
</tr>
<tr>
<td>Neither chronotype</td>
<td>94</td>
<td>47.72</td>
<td>217</td>
<td>48.33</td>
</tr>
<tr>
<td>Evening chronotype</td>
<td>16</td>
<td>8.12</td>
<td>47</td>
<td>10.47</td>
</tr>
<tr>
<td>PDM BA estimates*</td>
<td>2.44</td>
<td>1.11</td>
<td>2.58</td>
<td>1.00</td>
</tr>
<tr>
<td>GRS scores</td>
<td>-1.26</td>
<td>0.28</td>
<td>-0.75</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Note: *: standard deviation is the unit.
SD: standard deviation.
Table 3.2. Linear regression estimates for morning chronotype GRS and morning chronotype phenotype using rMEQ score.

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th></th>
<th></th>
<th></th>
<th>EA</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
<td>P</td>
<td>R²</td>
<td>β</td>
<td>SE</td>
<td>P</td>
<td>R²</td>
</tr>
<tr>
<td>Chronotype scores</td>
<td>-0.601</td>
<td>0.937</td>
<td>0.522</td>
<td>0.09%</td>
<td>0.464</td>
<td>0.576</td>
<td>0.421</td>
<td>0.15%</td>
</tr>
</tbody>
</table>

Note: All linear regressions were adjusted for calendar age, gender and the first 10 principal components.

The unadjusted R²s for the multivariate linear regression models were 6.58% for EA participants and 7.07% for AA participants.
Table 3.3. Logistic regression models between morning chronotype GRS and morning chronotype phenotype using binary variable.

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th></th>
<th></th>
<th></th>
<th>EA</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>P</td>
<td>OR</td>
<td>95% CI</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Chronotype binary</td>
<td>1.17</td>
<td>0.63 - 2.17</td>
<td>0.8</td>
<td></td>
<td>0.24 - 3.50</td>
<td>0.81</td>
<td></td>
</tr>
</tbody>
</table>

Note: All logistic regressions were adjusted for calendar age, gender and the first 10 principal components.
Figure 3.1. Pearson correlation between morning chronotype GRS and morning chronotype phenotype, among AA subjects (left) and EA subjects (right)
Chapter 6. Holistic view

Looking back at the work that has been done for this dissertation, several major contributions were made. First, this research quantified and testified BA for BHS participants using KDM and D_M algorithms for the first time. Second, this study filled the research gap and explored the phenotypical associations between sleep and BA for the first time. In addition, this research tested the instrumental strength of morning chronotype GRS amongst BHS participants for the first time.

Implications for health and aging

The results of this dissertation research have made it possible to estimate BHS subjects’ true bodily degradation stage only by a blood draw and a few measures. It has also made it possible to identify those who are aging much more than peers with same CA, and provide personalized health suggestions to them.

This dissertation results could also be useful for others to provide sleep advice for a more generalized population with anti-aging goals. For example, avoiding sleep deprivation would be a good strategy to help fight against excessive biological degradation and aging. The results of this research would encourage others to explore protective sleep behaviors to compensate for individuals with late chronotype, and those who are not able to adjust their sleep duration due to external responsibilities and other conditions.

Implications for future research directions

Future research is needed to test the predictive validity of KDM and PDM BA estimates in BHS using mortality data. In addition, exploring the association between sleep quality, sleep apnea and BA would be meaningful as well. As part of sensitivity analysis, future studies could use the summary statistics of the other three chronotype GWAS published in 2016, and assess the instrumental strength of the new chronotype GRS. After more chronotype GWAS becomes available, or when genetic information of more BHS participants is obtained in the future, it would be necessary to calculate new chronotype GRS for BHS participants, to perform new instrumental strength testing, and to do MR on chronotype GRS and BA. Furthermore, future chronotype GWASs using objectively measured chronotype phenotype, and future fine-mapping studies on chronotype genes are needed. GWASs on other sleep dimensions will be extremely useful to help humans understand the inheritability patterns and the potential fundamental link between human sleep and human health aspects.
Supplementary material
Reduced MEQ questionnaire that was used in BHS.

1. Considering only your own “feeling best” rhythm, at what time would you get up if you were entirely free to plan your day?
   
<table>
<thead>
<tr>
<th>Time</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>5:00-6:30 am</td>
<td>[5]</td>
</tr>
<tr>
<td>6:30-7:45 am</td>
<td>[4]</td>
</tr>
<tr>
<td>7:45-9:45 am</td>
<td>[3]</td>
</tr>
<tr>
<td>9:45-11:00 am</td>
<td>[2]</td>
</tr>
<tr>
<td>After 11:00 am</td>
<td>[1]</td>
</tr>
</tbody>
</table>

2. During the first half hour after having woken in the morning, how tired do you feel?
   
<table>
<thead>
<tr>
<th>Tired Level</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Tired</td>
<td>[1]</td>
</tr>
<tr>
<td>Fairly Tired</td>
<td>[2]</td>
</tr>
<tr>
<td>Fairly Refreshed</td>
<td>[3]</td>
</tr>
<tr>
<td>Very Refreshed</td>
<td>[4]</td>
</tr>
</tbody>
</table>

3. At what time in the evening do you feel tired and in need of sleep?
   
<table>
<thead>
<tr>
<th>Time</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00-9:00 pm</td>
<td>[5]</td>
</tr>
<tr>
<td>9:00-10:15 pm</td>
<td>[4]</td>
</tr>
<tr>
<td>10:15-12:45 am</td>
<td>[3]</td>
</tr>
<tr>
<td>12:45-2:00 am</td>
<td>[2]</td>
</tr>
<tr>
<td>After 2:00 am</td>
<td>[1]</td>
</tr>
</tbody>
</table>

4. At what time of the day do you think that you reach your “feeling best” peak?
   
<table>
<thead>
<tr>
<th>Time</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>5:00-8:00 am</td>
<td>[5]</td>
</tr>
</tbody>
</table>
8:00-10:00 am [4]
10:00-04:45 pm [3]
4:45-9:45 pm [2]
After 9:45 pm [1]

5. One hears about “morning” and “evening” types of people. Which ONE of these types do you consider yourself to be?

- Definitely a “morning” type [6]
- More a “morning” than an “evening” type [4]
- More an “evening” type than a “morning” type [2]
- Definitely an “evening” type [0]
- NEITHER a “morning” nor “evening” type [3]
Bibliography


doi:10.1161/01.HYP.0000217362.34748.e0.


76. Allan JS, Czeisler CA. Persistence of the circadian thyrotropin rhythm under constant conditions and after light-induced shifts of circadian phase. J Clin Endocrinol Metab. 1994. doi:10.1210/jc.79.2.508


90. Hayashi Y, Endo S. All-night sleep polygraphic recordings of healthy aged persons: REM and slow-wave sleep. Sleep. 1982. doi:10.1093/sleep/5.3.277


104. Wong PM, Hasler BP, Kamarck TW, Muldoon MF, Manuck SB. Social Jetlag, chronotype, and cardiometabolic risk. J Clin Endocrinol Metab. 2015. doi:10.1210/jc.2015-2923


127. Chen JC, Espeland MA, Brunner RL, et al. Sleep duration, cognitive decline, and
doi:10.1016/j.jalz.2015.03.004

Obesity. 2008. doi:10.1038/oby.2007.118

129. Wong PM, Hasler BP, Kamarck TW, Muldoon MF, Manuck SB. Social Jetlag,
chronotype, and cardiometabolic risk. J Clin Endocrinol Metab. 2015.
doi:10.1210/jc.2015-2923

130. Centers for Disease Control and Prevention (CDC). National Center for Health
Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease

131. Test Catalog - Mayo Clinic Laboratories. https://www.mayocliniclabs.com/test-


133. Kotchen JM, McKean HE, Kotchen TA. Blood pressure trends with aging.
Hypertension. 1982;4(5_pt_2). doi:10.1161/01.hyp.4.5_pt_2.iii128

134. Waldstein SR, Rice SC, Thayer JF, Najjar SS, Scuteri A, Zonderman AB. Pulse
Pressure and Pulse Wave Velocity Are Related to Cognitive Decline in the Baltimore
doi:10.1161/hypertensionaha.107.093674

doi:10.3177/jnsv.53.37


doi:10.1089/rej.2020.2335

doi:10.1080/07315724.2019.1580169


doi:10.1002/oby.20651


185. Mota MC, Silva CM, Balieiro LC, Fahmy WM, Crispim CA. Social jetlag and metabolic control in non-communicable chronic diseases: a study addressing different obesity statuses. Scientific Reports. 2017;7(1). doi:10.1038/s41598-017-06723-w


