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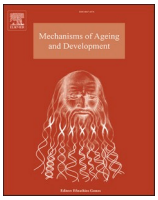

Cardiovascular health is associated with the epigenetic clock in the Berlin Aging Study II (BASE-II)

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ABSTRACT

The *epigenetic clock* parameter DNAm age acceleration is a promising biomarker of aging. We have recently described an epigenetic clock based on only seven cytosine-phosphate-guanine sites, which is highly associated with chronological age. The aim of this study was to examine this epigenetic clock with respect to its relationship with cardiovascular health (CVH) in older adults.

We used data from the Berlin Aging Study II (BASE-II; 1,671 participants; 68.8 ± 3.7 years old). CVH was operationalized using two different CVH scores, the Framingham Risk Score (FRS), and the Life's simple 7 (LS7). To adjust for potential confounding, e.g. by sex, we performed regression analyses.

The LS7 score was higher, i.e. more favorable, in woman than in men (8.8 ± 2 vs. 8.2 ± 2, $p < 0.001$). DNAm age acceleration was associated with the FRS ($\beta = 0.122$, $p = 0.028$) and with the LS7 ($\beta = -0.804$, $p = 0.032$). In more detail, physical activity ($\beta = -0.461$, $p = 0.05$), HDL-cholesterol ($\beta = 0.343$, $p = 0.03$) and total cholesterol ($\beta = -0.364$, $p = 0.002$) were associated with epigenetic age acceleration. We present evidence suggesting that better CVH is associated with decelerated biological aging measured by the epigenetic clock.

1. Introduction

Age is the paramount risk factor for cardiovascular disease. However, biological ageing occurs at different rates in different persons (Ferrucci et al., 2020). Therefore, chronological age is not a perfectly adequate marker of individual biological aging. Biomarkers of aging are required to determine an individual's biological age and to better understand aging processes. Furthermore, accurate biomarkers of aging are important to measure the success of new anti-aging and regenerative medical interventions (Horvath and Raj, 2018). A promising biomarker is epigenetic age, i.e. the DNA methylation age (DNAm age). *Epigenetic clocks* estimate age with high accuracy on the basis of DNA methylation fractions (Horvath and Raj, 2018). DNAm age is computed by the DNA methylation level of a specified number of cytosin-phosphate-guanine nucleotides, selected by a stepwise penalized regression analysis (elastic net or lasso) (Hui and Hastie, 2005). Many different epigenetic clocks, using different selections and numbers of cytosin-phosphate-guanine sites (CpGs), like the original Horvath clock with 353 CpGs and the Hannum clock with 71 CpGs, have been described (Horvath, 2013; Hannum et al., 2013).

Tightly linked to DNAm age, DNAm age acceleration represents the deviation of DNAm age from the chronological age. An individual's DNAm age may be higher or lower than its chronological age, resulting in a positive or negative value for the DNAm age acceleration (Horvath and Raj, 2018). A negative DNAm age acceleration indicates a slower epigenetic aging rate, whereas a positive DNAm age acceleration reflects

Abbreviations: CpGs, cytosine-phosphate-guanine sites; BASE- II, Berlin Aging study II; DNAm, DNA methylation; LS7, Life's simple 7 score; FRS, Framingham Risk Score; CVH, cardiovascular health; EEAA, extrinsic epigenetic age acceleration; IEAA, intrinsic epigenetic age acceleration; HbA1c, glycosylated hemoglobin, type A1C; Total-c, total cholesterol; HDL-c, high density lipoprotein cholesterol; BP, blood pressure; BMI, body mass index.

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faster epigenetic aging. There are different methods to determine DNAm age acceleration (Horvath and Raj, 2018). A common approach is to calculate the residuals of a linear regression analysis of the DNAm age on chronological age, adjusted for leukocytes cell composition (Vetter et al., 2019), thus yielding the proportion of the epigenetic age, which cannot be explained by chronological age and leukocytes cell distribution. Consequently, DNAm age acceleration can be interpreted as an estimate of the pace of biological aging (Horvath and Raj, 2018).

Findings regarding the association between cardiovascular disease (CVD) and DNAm age acceleration are inconsistent. While Roetker et al. (2018), Banszerus et al. (2020), Lu et al. (2019) and Perna et al. (2016) found CVD to be associated with an accelerated DNAm age, Horvath et al. (2016), and Dugue et al. (2018) reported no association between DNAm age acceleration and incident CVD. Lind et al. found CVD to be associated with DNAm age acceleration according to Horvath's epigenetic clock, but not according to Hannum's clock (Lind et al., 2018). Furthermore, reports on the association of DNAm age acceleration with lifestyle factors and cardiovascular risk factors are inconsistent. Associations between DNAm age acceleration and cardiovascular risk factors, like hypertension (Lind et al., 2018; Quach et al., 2017; McCartney et al., 2018), diabetes (Roetker et al., 2018; Horvath et al., 2016; Quach et al., 2017), low high density lipoprotein (HDL) cholesterol Roetker et al. (2018), obesity (Horvath et al., 2014; Li et al., 2019), increased BMI (Quach et al., 2017; Fiorito et al., 2019; Ryan et al., 2019; Nevalainen et al., 2017), and smoking (Roetker et al., 2018; Fiorito et al., 2019) have been described. Moreover, lower education (Fiorito et al., 2019; Ryan et al., 2019), frailty (Breitling et al., 2016; Gale et al., 2018; McCrory et al., 2021), unhealthy diet (Quach et al., 2017), insufficient levels of Vitamin D (Vetter et al., 2020) and low physical activity (Roetker et al., 2018; Quach et al., 2017; Fiorito et al., 2019; McCrory et al., 2021) have been associated with accelerated DNAm age. Though, there were other studies, which found no association between DNAm age acceleration and cardiovascular risk factors, e.g. hypertension (for the Horvath clock estimate) (McCartney et al., 2018), unhealthy diet (Dugue et al., 2018; Simons et al., 2016) physical inactivity (Dugue et al., 2018; Simons et al., 2016). Also, a meta-analysis by Ryan et al. (2019) found no association between DNAm age acceleration and smoking.

Here we examined the association between DNAm age acceleration measured by the 7-CpG epigenetic clock and cardiovascular health, using the Life's simple 7 (LS7) and the Framingham risk score (FRS) to operationalize CVH. The FRS is based on age, sex, systolic blood pressure, HDL cholesterol, total cholesterol, and diabetes mellitus to estimate the ten-year cardiovascular risk (D'Agostino et al., 2008), and has been previously computed for the BASE-II cohort by Berger et al. (Berger, 2019). The LS7 was developed by the American Heart Association (AHA) in 2010 (Lloyd-Jones et al., 2010). This score takes the following seven items into account: smoking, body mass index (BMI), physical activity, diet, blood pressure, glucose and total cholesterol. Different studies have shown that a LS7 score in the optimal range (ranging from 10 to 14), is associated with a lower risk of cardiovascular disease (Peng and Wang, 2017; Ogunmoroti et al., 2017). While the LS7 is a lifestyle score and includes only modifiable factors, the FRS is a clinical risk prognosis instrument, which also includes the non-modifiable factors age and sex.

We hypothesized that more favorable results in the LS7 and FRS scores are associated with a lower biological age, estimated by DNAm age acceleration. Moreover, we hypothesized that the LS7 maybe associated more strongly with DNAm age acceleration than the FRS, because it includes physical activity and diet, which are believed to strongly impact epigenetic aging (Roetker et al., 2018; Quach et al., 2017; Fiorito et al., 2019; Levine et al., 2018).

2. Materials and methods

2.1. The Berlin Aging Study II

The aim of the multidisciplinary Berlin Aging Study II (BASE-II) is to explore factors impacting healthy aging positively and negatively. Between 2009 and 2014 the medical examination of 2,171 residents of the greater Berlin metropolitan area (~75 % aged 60–84 years, and ~25 % aged 20–37 years) was conducted. For the current analysis, we only used data from the older group of participants aged 60 and over (N = 1,671).

Participants were included after providing written informed consent. BASE-II was approved by the Ethics Committee of the Charité-Universitätsmedizin Berlin, approval number EA2/029/09. During the two-day medical investigation, the objective and subjective health of the participants was assessed. For further details on the BASE-II study, refer to Bertram et al., Demuth et al. and Gerstorf et al. (Demuth et al., 2019; Bertram et al., 2014; Gerstorf et al., 2016).

2.2. Life's simple 7 (LS7)

We calculated the Life's simple 7 according to the American Heart Association (AHA) (Lloyd-Jones et al., 2010) with minor adaptions (Konig et al., 2018). Accordingly, we adjusted the measurement of diet (see below) and physical activity. We used glycosylated hemoglobin, type A1C (=HbA1c) instead of fasting plasma glucose. The score used in this study includes the following items: BMI, systolic and diastolic blood pressure, HbA1c, total cholesterol, smoking, physical activity and diet. The items were structured in the following way: poor results (0 points), intermediate results (1 point) and ideal results (2 points), resulting in a total score ranging between 0–14 points. The total score was categorized into three groups reflecting the degree of cardiovascular health (CVH): inadequate (0−4 points), average (5−9 points), and optimal (10−14 points) (Table 2).

Body weight was measured in kg with a precision limited to one decimal digit, and height was determined to the nearest 0.1 cm by using an electronic weighing and measuring station (seca 764, seca, Hamburg, Germany). The BMI was calculated as weight in kilograms divided by height in meters squared. The blood pressure was measured in a seating position on both arms with an electronic sphygmomanometer (bosomedicus memory, Jung Willingen, Germany). The mean of the two measurements was used in the analysis. The smoking status (never or quit >12 months ago, quit <12 months ago or current smoker) and the medication were assessed by a study physician. The physical activity item was quantified according to the Baecke physical activity questionnaire (Baecke et al., 1982). The physical activity item was categorized into never/seldom (0 points), sometimes (1 point) and often/always (2 points). Finally, the healthy diet item was divided into 6 components:

1 fruits $\geq$ 1 serving per day,
2 vegetables $\geq$ 1 serving per day,
3 fish $\geq$ 2 servings per week,
4 sugar sweetened beverages $\leq$ 1 per week,
5 processed meat $\leq$ 1.5 servings per week,
6 unprocessed red meat $\leq$ 1.5 servings per week.

The applicability of five to six components was defined as *ideal* healthy diet (2 points), three to four components were considered as *intermediate* (1 point), and zero to two components were rated as *poor* (0 points). The diet item was retrieved from data collected by the EPIC-food frequency questionnaire (Nothlings et al., 2007). The original diet score by the AHA for the LS7 included following items: fiber-rich whole

grains, sodium intake, saturated fat intake and the intake of nuts, legumes, and seeds, but does not include the intake of unprocessed red meats.

2.3. Framingham Risk Score (FRS)

The current Framingham Risk Score was published 2008 by D'Agostino et al. (2008). This established cardiovascular risk score is based on longitudinal data from the population of Framingham (Massachusetts) (Anderson et al., 1991a, b). The score estimates the risk of developing cardiovascular disease (coronary heart disease, cerebrovascular events, peripheral artery disease and heart failure) within the upcoming decade. It is applicable for both sexes and for persons between 30 and 75 years. The score considers seven items: age, smoking, total cholesterol, high density lipoprotein (HDL) cholesterol, diabetes mellitus, and treated or non-treated systolic hypertension. Moreover, the score controls for biological sex by awarding different values for each component ranging from −3 to 12 points for women and −2 to 15 points for men. The final sum of added points from the different categories defines the cardiovascular disease risk within the next ten years in percent. For women a sum of ≤ −2 points corresponds to the lowest cardiovascular risk of <1 % and a sum of ≥21 points implies a cardiovascular risk of >30 %. For men a sum of ≤ −3 points corresponds to the lowest cardiovascular risk of <1% and a sum of ≥18 points corresponds to the highest cardiovascular risk of >30 %. The FRSs for the BASE-II dataset investigated here, were previously reported (Berger, 2019).

2.4. DNA methylation age and DNAm age acceleration

The epigenetic clock parameters, DNAm age and DNAm age acceleration, were determined in our laboratory (Vetter et al., 2019). Briefly, we employed a slightly adapted version of the DNA methylation protocol from Vidal-Bralo et al. (2016, 2017) to determine the methylation fraction at eight CpG sites. After a stepwise multiple linear regression analysis, one CpG site (cg10917602) was excluded from the DNAm age calculation, because it did not improve the model of chronological age prediction, resulting in a seven-CpG epigenetic clock (Vetter et al., 2019).

We defined DNAm age acceleration as the proportion of the DNAm age, which is not explained by the chronological age or the leukocytes cell distribution. Therefore, the DNAm age acceleration was calculated as the residual from a linear regression analysis of DNAm age on chronological age and leukocyte cell distribution (monocytes, lymphocytes, neutrophils and eosinophils) (Vetter et al., 2019). The DNAm age acceleration calculated here employs a slightly modified version of the intrinsic epigenetic age acceleration (IEAA) proposed by Quach et al. (Vetter et al., 2019; Quach et al., 2017). In brief, individuals with a higher epigenetic age than chronological age have a positive value for DNAm age acceleration, whereas persons whose epigenetic age is lower than their chronological age, have a negative value for DNAm age acceleration (Horvath and Raj, 2018).

2.5. Statistical analysis

All statistical calculations were conducted using IBM SPSS Statistics for Windows, Version 25.0. (IBM Corp., Armonk, USA). All figures were created with GraphPad Prism Version 8.2.1 (Graphpad Software Inc., San Diego, USA). All participants with missing values for one of the LS7 metrics, one of the FRS components, DNAm age, or for DNAm age acceleration were excluded (Fig. S1). For 11 % of the total sample the LS7 could not be calculated due to missing data. We have, however, no reason to assume that participants excluded from the analyses for this reason differed significantly from the final sample studied here. To assess differences in the distribution of normally distributed variables, the *t*-test for independent samples was conducted, and the X^2 -test was used were appropriate. For all calculations including the LS7 score we used the three LS7 categories: optimal (10−14 points), average (5−9 points), and inadequate (0−4 points). For better visualization, figures including the LS7 (Figs. S3/S4) were generated using the LS7 points (0–14). For both the analyses and the histogram (Fig. S2) Framingham Risk Score (FRS) sumpoints were used. To study the relationship between the two scores on the one hand and DNAm age acceleration on the other hand, we first conducted Spearman Rho correlation analyses. For the correlations between the metric cardiovascular risk factors and the epigenetic clock parameters, we calculated the Pearson correlation coefficient. Subsequently, we performed linear regression analyses with DNAm age acceleration as the dependent variable and the LS7 score as the independent variable with adjustment for sex. We did not adjust for chronological age, because the correlations between chronological age and the LS7 (r = 0.007, N = 1396) and DNAm age acceleration (r=−0.045, N = 1395) were marginal, and conceptually chronological age cannot be a confounder in the relationship between CVH and DNAm age acceleration. We did not adjust for sex and chronological age in the linear regression analyses with the FRS as explanatory variable, because both parameters are already included within the FRS. To assess interaction by sex, we also conducted linear regression analyses stratified by sex. Furthermore, to examine which item of the cardiovascular risk/health scores is driving the association between CVH and epigenetic age acceleration, we calculated a regression model (Table 4, Model 1), in which we regressed DNAm age acceleration on all LS7 items and, in this case, we used the pairwise deletion method for missing values. Similarly, we regressed DNAm age acceleration on the FRS components (Table 5). We used the pairwise deletion method, so we would not have to exclude cases from the multivariable regression analysis, because of one missing variable in the analysis.

3. Results

3.1. Characteristics of the studied BASE-II dataset

Data from 1,671 participants (51.6 % women) with a mean age of 68.8 ± 3.7 were available. Women were on average 0.5 years younger than men (p = 0.004), while their estimated epigenetic age was on average 2.7 years lower than the average men's epigentic age (p< 0.001) (Table 1). There was also evidence of a difference between the DNAm age acceleration in women (-1.0 ± 6.6 years) and in men (1.0 ± 7.0 years) (p< 0.001) (Table 1).

One hundred and forty-eight participants (9.3 %) had evidence of CVD, including coronary artery disease, history of myocardial infarction, peripheral arterial disease, stroke or other diseases of brain supplying vessels. The prevalence of CVD was significantly lower in women than in men (7.5 % vs. 11.2 %, p = 0.009). The mean systolic blood pressure was comparable in men (144.5 ± 17.9 mmHg) and women (142.8 ± 19.6 mmHg, p = 0.061) (Table 1), while the prevalence of diabetes mellitus type II was significantly lower in women than in men (Table 1). The body mass index was significantly higher in men than in women (Table 1), while the HDL-cholesterol levels and total cholesterol levels were significantly higher in women than in men (Table 1). Overall, there were 170 (10.2 %) active smokers among the studied BASE-II participants.

3.2. Framingham Risk Score (FRS) and Life's simple 7 (LS7)

The FRS was normally distributed among men and women (Fig. S2). The mean FRS was significantly lower in women (14.8 ± 3.5, p < 0.001) compared to men (16.8 ± 3.1) (Table 1, Fig. 1). One hundred and ninety-eight (26.7 %) women had a low predicted ten-year cardiovascular (CV) risk between 0% and 9%, whereas only 15 (2.2 %) men had a predicted CV risk within this range (Fig. 1). Nearly half of the women (n = 367, 49.5 %), but only 136 men (20.1 %) had a CV risk between 10 % and 19 %. One hundred and seventy-six women (23.8 %) and the majority of men (n = 527, 77.7 %) had a high CV risk (20 % and higher).

Table 1
Characteristics of the BASE-II participants.

Variables	Men	n	Women	n	Total	n	*p*-value
Chronological age (years)	69.0 ± 3.8	809	68.5 ± 3.6	862	68.8 ± 3.7	1671	0.004*
DNAm age (years)	67.6 ± 7.7	745	64.9 ± 7.3	726	66.3 ± 7.6	1471	<0.001*
DNAm age acceleration (years)	1.0 ± 7.0	707	−1.0 ± 6.6	688	0.02 ± 6.9	1395	<0.001*
LS7 sum points	8.2 ± 2.0	702	8.8 ± 2.0	694	8.5 ± 2.0	1396	<0.001*
Framingham Score points	16.8 ± 3.1	678	14.8 ± 3.5	741	15.7 ± 3.4	1419	<0.001*
BP systolic (mmHg)	144.5 ± 17.9	804	142.8 ± 19.6	857	143.7 ± 18.8	1661	0.061*
BP diastolic (mmHg)	84.2 ± 10.4	804	82.2 ± 11.2	857	83.1 ± 10.9	1661	<0.001*
Body mass index (kg/m^2)	27.2 ± 3.6	789	26.4 ± 4.7	789	26.83 ± 4.2	1578	<0.001*
HDL-cholesterol (mg/dl)	55.3 ± 14.4	778	69.4 ± 16.2	816	62. 52 ± 16.9	1594	<0.001*
Total cholesterol (mg/dl)	203.2 ± 37.8	796	226.0 ± 38.6	835	214.9 ± 39.9	1631	<0.001*
HbA1c (%)	5.7 ± 0.6	783	5.6 ± 0.5	820	5.6 ± 0.6	1603	0.005*
Diabetes mellitus type 2	123 (15.8 %)	778	70 (8.6 %)	816	193 (12.1 %)	1594	<0.001**
Smoker***	96 (11.9 %)	804	74 (8.6 %)	856	170 (10.2 %)	1660	0.027**
BP treatment	368 (47.3 %)	778	343 (42.0 %)	816	711 (44.6 %)	1594	0.034**

BP = blood pressure, HDL = high density lipoprotein, LS7 = Life's simple 7, DNAm age = DNA methylation age, HbA1c = glycosylated hemoglobin type A1C.
* *t*-test for independent samples.
** X^2 -test.
*** Current smoking or stopped smoking <12 months ago.

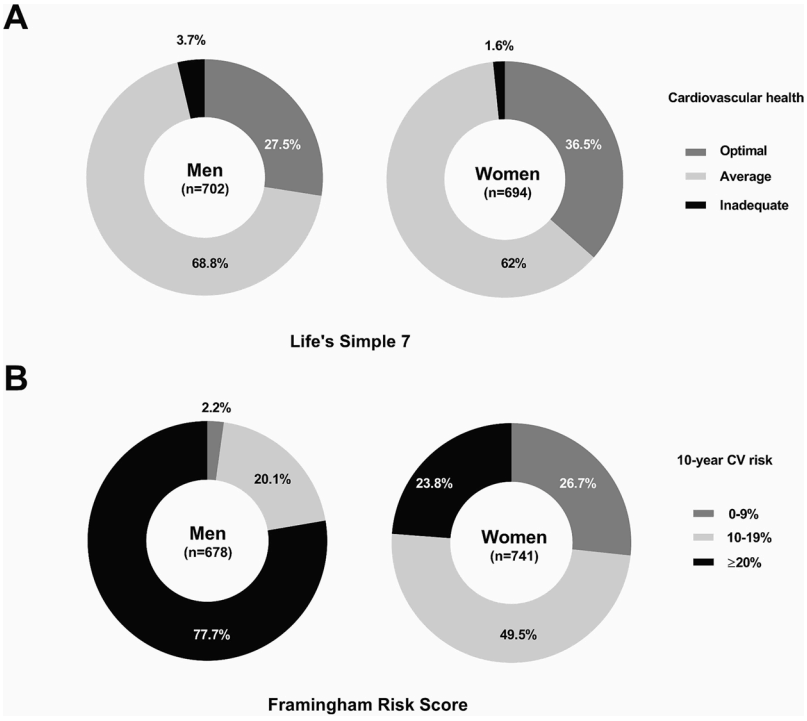

Fig. 1. Cardiovascular health and risk in men and women of the BASE-II cohort.
A) Percentage of men and women meeting the criteria for inadequate (black), average (light grey) or optimal (medium grey) cardiovascular health (CVH), according to the concept of the Life's Simple 7.
B) Percentage of men and women having a ten year cardiovascular risk of 0–9 % (medium grey), 10–19 % (light grey) and higher than 20 % (black).

The LS7 was normally distributed among the participants (Fig. S3). On average women had a higher, i.e. more favorable, LS7 score (8.8 ± 2.0) than men (8.2 ± 2.0, $p < 0.001$, Table 1). Two hundred and fifty-three women (36.5 %) and 193 men (27.5 %) were in the optimal range (Table 2, Fig. 1). The majority of both men (n = 483, 68.8 %) and women (n = 430, 62 %) were categorized in the average range. The CVH of 11 women (1.6 %) and 26 men (3.7 %) was classified as inadequate. An overview of the classification of the participants with respect to the LS7 categories is shown in Table 2.

3.3. Correlations between the cardiovascular scores and DNAm age acceleration

We found the LS7 to be weakly and inversely correlated with DNAm age acceleration (r= −0.078, N = 1243, Fig. S4, Table S1). The FRS was likewise weakly correlated with DNAm age acceleration (r = 0.060, N = 1232, Fig. S5, Table S1). Also in the sex-stratified analyses, there was very weak evidence of a correlation between the FRS and DNAm age acceleration among men (r= -0.049, N = 612, Table S2) and weak evidence for women (r = 0.084, N = 620, Table S3). We also found weak correlations between the LS7 and the epigenetic age acceleration for men (r= −0.076, N = 633, Table S2) and very weak correlations for women (r= −0.049, N = 610, Table S3). The LS7 score and the FRS were moderately correlated (r= −0.473, N = 1240). Chronological age was only marginally correlated with the LS7 (r = 0.007, N = 1396) and with DNAm age acceleration (r= −0.045, N = 1395). For further information concerning correlations between cardiovascular risk factors, LS7 items and the epigenetic clock parameters see Table S1.

Table 2
The Life's simple 7 in BASE-II (N = 1396).

Life's simple 7 (LS7)	Score	Relative frequencies
Blood pressure, mmHg		
<120/80, unmedicated	2	4.2 %
120–139/80–89 or treated to goal	1	37.4 %
>140/90	0	58.4 %
Total serum cholesterol, mg/dL		
<200 mg/dL, unmedicated	2	35.7 %
200–239 mg/dL or treated to <200 mg/dL	1	38.0 %
>240 mg/dL	0	26.3 %
Glycosylated Hemoglobin A1C, %		
<5.7 %, unmedicated	2	60.6 %
5.7–6.4 or treated to <5.7 %	1	32.1 %
>6.4 %	0	7.3 %
Smoking		
Never or quit >12 months ago	2	89.8 %
quit <12 months	1	0.9 %
Current smoker	0	9.3 %
Body mass index, kg/m2		
<25	2	36.1 %
25–29.9	1	45.4 %
> =30	0	18.6 %
Physical activity		
often/always	2	58.3 %
sometimes	1	18.5 %
seldom/never	0	23.3 %
Healthy diet		
Healthy diet score 5–6/6	2	23.2 %
Healthy diet score 3–4/6	1	57.4 %
Healthy diet score 0–2/6	0	19.4 %
Cardiovascular health		
Optimal	10–14	31.9 %
Average	5–9	65.4 %
Inadequate	0–4	2.7 %
Mean and standard deviation		8.47 ± 2
Median		9.00
Minimum		2.00
Maximum		14.00

Table 3
Linear Regression analysis of DNAm age acceleration on Life's simple 7 and Framingham Risk Score.

	β	SE	*p*-Value	R^{2}*
Linear regression analyses of DNAm age acceleration on LS7				
Model 1	−0.984	0.375	0.009	0.005
Model 2	−0.804	0.374	0.032	0.020
Linear regression analyses of DNAm age acceleration on FRS				
Total sample	0.122	0.055	0.028	0.003
Women	0.138	0.074	0.064	0.004
Men	−0.079	0.091	0.385	0.000

* R^2 is indicated as adjusted R^2, Adjustment: Model 1: none, Model 2: sex, sample size for linear regression analyses of DNAm age acceleration on FRS: full sample n = 1232, women: n = 620, men: n = 612.

3.4. Linear regression analysis of DNAm age acceleration on the Life's simple 7 score and the Life's simple 7 score items

To further explore the association between the LS7 score and DNAm age acceleration and to adjust for potential confounding by sex we calculated linear regression models (Table 3). According to the unadjusted model there was evidence that the LS7 score was inversely associated with DNAm age acceleration (Model 1: β = −0.984, p = 0.009), an association which persisted after adjustment for sex (Table 3, Model 1–2). Per one category increase in the LS7, DNAm age acceleration decreased by 0.8 years, e.g. from participants with only average CVH to participants with optimal CVH (Table 3, Model 2). There was no evidence of effect modification by sex in the association between the LS7 and DNAm age acceleration.

In order to examine which items of the LS7 score were driving the association between the LS7 and DNAm age acceleration, we computed a multivariable linear regression analysis of the DNAm age acceleration on all single LS7 components (diet, activity, BMI, HbA1c, cholesterol, smoking status, blood pressure), without (Table 4, Model 1) and with (Table 4, Model 2) adjustment for sex. This revealed that the physical activity component of the LS7 was most strongly associated with DNAm age acceleration (Table 4, Model 2: β = −0.461, p = 0.05).

3.5. Linear regression analysis of epigenetic age acceleration on the Framingham Risk score and the Framingham Risk score items

According to linear regression analysis, the Framingham risk score (FRS) was significantly associated with DNAm age acceleration (β = 0.122, p = 0.028). Per ten points lower FRS participants showed on average a one year decelerated epigenetic age acceleration (Table 3). We also tested interaction between FRS and sex by including an interaction term. The likelihood-ratio test provided no evidence of important interaction between the effects of FRS and sex on DNAm age acceleration (p = 0.685).

Again, we were interested to see which of the score's items were driving the detected association with the FRS. DNAm age acceleration was positively associated with the HDL- cholesterol component (β = 0.343, p = 0.030, Table 5) and negatively associated with the total cholesterol item (β = −0.364, p = 0.002, Table 5). That means that there was evidence that the HDL-cholesterol component was in parts driving the positive association between DNAm age acceleration and the FRS.

4. Discussion

The main finding of this study was that individuals with more favorable results in two different scores reflecting cardiovascular health, the Framingham Risk Score and the Life's simple 7, showed lower DNAm age acceleration compared to participants with less favorable score results.

4.1. CVH scores and DNAm age acceleration

Our finding that CVH is associated with DNAm age acceleration is in

Table 4
Multivariable Linear Regression analysis of DNAm age acceleration on LS7 components.

DNAm age acceleration						
	Model 1**			Model 2***		
Covariates	β	SE	*p*- Value	β	SE	*p*- Value
Diet*	0.231	0.300	0.440	0.590	0.305	0.053
Activity*	−0.566	0.236	0.016	−0.461	0.235	0.050
BMI*	−0.454	0.279	0.104	−0.411	0.276	0.138
HbA1c*	−0.177	0.316	0.575	−0.123	0.313	0.695
Total-C*	0.151	0.249	0.545	−0.182	0.255	0.477
Smoking*	−0.024	0.334	0.943	0.061	0.331	0.853
BP*	−0.503	0.340	0.139	−0.312	0.339	0.357
sex	–	–	–	*−2.090*	*0.414*	*<0.001*

BMI = body mass index, Total-c: total cholesterol, BP = blood pressure, HbA1c = glycosylated hemoglobin, type A1C.
* LS7 items.
** Adjusted R^2 for Model 1: 0.006.
*** Adjusted R^2 for Model 2: 0.023.

Table 5
Multivariable Linear Regression analysis of DNAm age acceleration on the FRS items.

DNAm Age Acceleration*			
Covariates (FRS items)	β	SE	*p*- Value
Age	0.090	0.120	0.455
HDL-C	0.343	0.158	0.030
Total-C	−0.364	0.116	0.002
Diabetes mellitus	0.117	0.202	0.561
Systolic BP	0.082	0.090	0.362
Smoking	−0.149	0.186	0.422

Total-C = total cholesterol; HDL-C = High density lipoprotein cholesterol; BP = blood pressure.
* Adjusted R^2 is 0.011.

line with previous studies, which have investigated the association between cardiovascular risk factors and epigenetic clocks (Roetker et al., 2018; Horvath et al., 2016; Quach et al., 2017; Fiorito et al., 2019; Ryan et al., 2019; Nevalainen et al., 2017; Levine et al., 2018). Several studies found an association between the incidence and prevalence of CVD (Roetker et al., 2018; Lu et al., 2019), CVD mortality (Perna et al., 2016) and DNAm age acceleration. Lind et al., found CVD to be associated with Horvath's, but not with Hannum's DNAm age acceleration (Lind et al., 2018). Furthermore, some studies found no evidence for an association between incidence of CVD and DNAm age acceleration (Horvath et al., 2016; Dugue et al., 2018). Likewise, a number of studies did not find associations between DNAm age acceleration and cardiovascular risk or protective factors such as hypertension (McCartney et al., 2018), healthy diet (Dugue et al., 2018; Simons et al., 2016), total cholesterol (Roetker et al., 2018) and smoking (e.g. meta-analysis by Ryan et al. Ryan et al. (2019)).

Therefore, we and others suppose that some epigenetic clocks, like the one with seven CpG sites employed here, may reflect CVH and single indicators of CVH, such as physical activity, HDL cholesterol and total cholesterol, better than others (Liu et al., 2020).

4.2. Differences between the sexes

As expected, according to both the LS7 and the FRS women had on average better CVH than men. Likewise, women's estimated epigenetic age was on average 2.7 years lower than men's. Furthermore, women had a two-year lower DNAm age acceleration than men. These results are consistent with findings made by Horvath et al. (2016) and replicate findings from the LipidCardio study, where a nearly identical epigenetic clock as in the current study was used (Banszerus et al., 2019). We did not find evidence of important effect modification in the association between the FRS and epigenetic age acceleration.

4.3. Physical activity and epigenetic age acceleration

We could show that the association of LS7 and DNAm age acceleration was mainly driven by the physical activity component (Table 4, Models 1 and 2). This result is comparable to the association between another epigenetic clock (DNAm PhenoAge age acceleration) and exercise reported by Levine et al. (N = 9926) and McCrory et al. (McCrory et al., 2021; Levine et al., 2018). Moreover, GrimAge age acceleration and PhenoAge age acceleration have been both shown to be associated with physical activity (according to the international physical activity questionnaire-short form) and walking speed (McCrory et al., 2021). Likewise, Quach et al. (N = 4173), and Roetker et al. (N = 2543) found associations between extrinsic epigenetic age acceleration (EEAA, a version of the Hannum clock estimate) and physical activity, while they could not show an association between physical activity and intrinsic epigenetic age acceleration (IEAA, a Horvath clock estimate) (Roetker et al., 2018; Quach et al., 2017). Thus, again, the Hannum clock may reflect the relationship between physical activity and DNAm age acceleration better than the Horvath clock.

It should be mentioned that some studies did not find evidence of an association between DNAm age acceleration and physical activity (Banszerus et al., 2020; Dugue et al., 2018; Simons et al., 2016). A possible reason could be that they have used different assessment methods for physical activity e.g. the RAPA (Rapid assessment for physical activity) questionnaire (Banszerus et al., 2020), a metabolic equivalent for physical activity (Dugue et al., 2018) and a self-created physical activity score (Simons et al., 2016). Besides, the study by Simons et al. had a rather small sample size (N = 100) and therefore had low statistical power to detect an association (Simons et al., 2016).

4.4. HDL-cholesterol, total cholesterol and DNAm age acceleration

We found evidence that the HDL-cholesterol item of the FRS was associated with DNAm age acceleration (Table 5). Epigenetic aging was decelerated by 0.34 years in participants with higher HDL-cholesterol levels, e.g. between 50−59 mg/dl compared to participants with HDL-cholesterol levels between 40−49 mg/dl. This was in line with the observed association between HDL-cholesterol and epigenetic age acceleration (Hannum and Horvath clock estimate) reported by Levine et al. (2018) and Roetker et al. (2018). Also some other studies have reported significant correlations between HDL-cholesterol and epigenetic age acceleration (Quach et al., 2017; McCartney et al., 2018).

Further, the total cholesterol item of the FRS was negatively associated with DNAm age acceleration, i.e. that participants with total serum cholesterol levels of e.g. 240 mg/dl appeared to be aging epigenetically at a lower rate than participants with lower total cholesterol levels of 200 mg/dl. This finding is difficult to interpret without considering other lipid parameters, namely LDL-cholesterol and HDL-cholesterol, which both and in conjuction with other lipoproteins contribute to the measured total cholesterol. Indeed, the observed association between the total cholesterol item and DNAm age acceleration could be explained by the fact, that total cholesterol includes HDL-cholesterol and in the current study high HDL-cholesterol levels were associated with lower DNAm age acceleration. Furthermore, previous studies have suggested that higher LDL-cholesterol levels may be beneficial with respect to mortality and morbidity in older age. E.g. a systematic review including 19 cohort studies with 68,094 elderly people, found LDL-cholesterol levels to be inversely associated with mortality (Ravnskov et al., 2016). In addition, Maihofer et al. found higher LDL-cholesterol levels to be positively associated with intact mobility to the age of 90 years in 3567 women of the Women's Health Initiative Study (Maihofer et al., 2020). In contrast, some studies reported total cholesterol to be positively associated with IEAA (Quach et al., 2017; McCartney et al., 2018), while they did not find evidence of an association between total cholesterol and EEAA (Roetker et al., 2018; Quach et al., 2017; McCartney et al., 2018) or IEAA (Roetker et al., 2018).

5. Conclusions

In conclusion, our results provide evidence of a weak association of the epigenetic clock (DNAm age acceleration) with cardiovascular health in the BASE-II cohort. We were able to confirm our initial hypothesis that more favorable results in the Life's simple 7 (LS7) and the Framingham Risk Score (FRS) would be associated with a lower DNAm age acceleration. The DNAm age acceleration of participants with more favorable scores in the well-established instruments FRS and LS7 resulted to be lower compared to participants with less favorable score results. Moreover, we found our hypothesis that the LS7 maybe associated more strongly with DNAm age acceleration than the FRS because it includes physical activity and diet at least partially confirmed. Physical activity resulted to be the driving force in the association between

DNAm age acceleration and the Life's simple 7. As expected, women displayed more favorable results in the CVH scores and had significantly lower epigenetic age acceleration than men. The above findings add to the growing body of evidence supporting the epigenetic clock's potential as a biomarker of aging in the context of CVH and lifestyle factors.

Author contributions

E.L. computed the LS7 score, performed the statistical analysis, and wrote the original draft of the manuscript; V.M.V. generated the epigenetic clock data used in the current project; N. B. computed the Framingham Risk Score; V.L.B. validation; M.K. supervision and project administration. I.D. funding acquisition, conceptualization, methodology, project administration and supervision of the study; all authors contributed to revision and editing.

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Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Charité-Universitätsmedizin Berlin, approval number EA2/029/09.

Informed consent statement

Participants were included in the study after providing written informed consent.

Data availability statement

Due to concerns for participant privacy, data are available only upon reasonable request. External scientists may apply to the Steering Committee of BASE-II for data access. Please refer to the BASE-II website (https://www.base2.mpg.de/7549/data-documentation) for additional information.

Declaration of Competing Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mad.2021.111616.

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