Plasminogen activator inhibitor-1 is associated with leukocyte telomere length in American Indians: findings from the Strong Heart Family Study

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Abstract

Background—Plasminogen activator inhibitor-1 (PAI-1) promotes cellular aging both in *vitro* and in *vivo*. Telomere length is a marker of biological aging.

Objectives—To examine the cross-sectional and longitudinal associations between plasma PAI-1 and leukocyte telomere length in a large-scale epidemiological study of American Indians.

Methods—We measured leukocyte telomere length (LTL) and plasma PAI-1 in 2,560 American Indians who were free of overt CVD and participated in the Strong Heart Family Study (SHFS) clinical exam in 2001–2003. LTL and PAI-1 were repeatedly measured in 475 participants who attended SHFS clinical visits in both 2001–2003 and 1998–1999. Generalized estimating equation model was used to examine the cross-sectional and longitudinal associations between PAI-1 and LTL, adjusting for known risk factors.

Results—A higher level of plasma PAI-1 was negatively associated with shorter age-adjusted LTL ($\beta=-0.023$, 95%CI: $-0.034 \sim -0.013$). This association was attenuated ($\beta=-0.015$, 95%CI: $-0.029 \sim -0.002$) after adjustments for demographics, study site, lifestyle (smoking, drinking,

physical activity), and metabolic factors (obesity, blood pressure, fasting glucose, insulin, lipids, kidney function). Further adjustment for hsCRP did not change this association (β=−0.015, 95%CI: −0.029 ~ −0.001). Longitudinal analysis revealed that change in plasma PAI-1 was also inversely associated with change in LTL after adjusting for demographics, follow-up years, lifestyle factors, changes in metabolic factors, baseline levels of PAI-1 and LTL (β=−0.0005, 95%CI: −0.0009 ~ −0.0001).

Conclusions—A higher level of plasma PAI-1 was associated with shorter LTL in American Indians. This finding may suggest a potential role of PAI-1 in biological aging among American Indians.

Keywords

Aging; Fibrinolysis; Indians; South American; Plasminogen Activator Inhibitor 1; Thrombosis; Telomere

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INTRODUCTION

Telomeres are repetitive DNA sequences and their associated proteins at the end of chromosomes.[1] Telomere length shortens during each round of cell division, and when it reaches a critical value, cells stop division and enter senescence.[2] Thus, telomere length has been considered a cellular marker of biological aging. Shorter telomere length has been associated with various age-related diseases including cardiovascular disease (CVD),[3, 4] obesity,[5] diabetes,[4] and cancer.[6] Among American Indians participating in the Strong Heart Study (SHS), our group has recently reported associations of leukocyte telomere length with incident diabetes [7], onset and progression of carotid atherosclerosis [8], obesity [9], arterial aging [10], and life simple 7 factors [11].

Plasminogen activator inhibitor-1 (PAI-1), the primary physiological inhibitor of plasminogen activator,[12] plays a critical role in regulating fibrinolysis [13], fibrosis [14], and thrombosis [15]. Experimental studies have demonstrated a critical role of PAI-1 in cellular senescence both *in vitro* and *in vivo*.[16, 17] Human studies showed that plasma PAI-1 increased with age [13] and an elevated level of plasma PAI-1 was associated with multiple age-related conditions, including CVD,[18] obesity,[19, 20] insulin resistance,[20, 21] and vascular remodeling,[22] all of which have been related to shorter telomere length. [23] While existing evidence clearly suggests a potential role of PAI-1 in cellular aging and age-related conditions, little is known about the relationship between plasma PAI-1 and telomere length, a marker of biological aging, in large-scale epidemiological studies. To date, we are aware of two cross-sectional studies examining the association between plasma PAI-1 and leukocyte telomere length (LTL) in Africans and Europeans,[24, 25] but the sample size of these studies was rather small and the associations were substantially attenuated after adjustments for metabolic factors in both studies. A large-scale epidemiological study with extensive control of potential confounding variables is needed to confirm or refute the relationship between plasma PAI-1 and LTL. Moreover, previous research indicated that elevated plasma PAI-1 was almost always accompanied by inflammation,[26] a condition associated with shorter telomere length as well.[27–29] Thus, it is possible that the previously reported association between plasma PAI-1 and LTL could

be modulated or confounded by inflammation. However, this has not been examined in previous studies. Further, no study has investigated the relationship between plasma PAI-1 and LTL in American Indians, a minority group suffering high rates of age-related diseases, e.g., diabetes, obesity, and CVD.[30, 31]

The goals of this study are twofold: (1) to investigate the cross-sectional and longitudinal associations between plasma PAI-1 and LTL in American Indians in the Strong Heart Family Study (SHFS); and (2) to examine whether the relationship between PAI-1 and LTL is modulated by inflammation assessed by high sensitive C-reactive protein (hsCRP).

MATERIALS AND METHODS

Study population

The Strong Heart Study (SHS) is a multicenter, population-based longitudinal study of CVD, diabetes and their risk factors in American Indians in tribes and communities residing in Arizona, North/South Dakota, and Oklahoma. The study design, survey methods, and laboratory techniques of the SHS have been described previously.[32] The SHFS, a component of the SHS, was initiated in 1997 by enrolling 967 participants from 30 families of the original SHS cohort members. The study was expanded in 2001–2003 by enrolling a total of 3,665 participants from 94 families including re-examination of most of the 967 members enrolled in 1998–1999. All participants provided written informed consent. The SHFS protocol has been described elsewhere [33] and was approved by the Institutional Reviews Boards from the Indian Health Service and the participating institutions. In the current study, 874 participants from one community were removed due to withdraw of consent. Our cross-sectional analysis finally included 2,560 participants who attended the clinical visit in 2001–2003, with exclusion of participants with overt CVD (N=135) or missing data on either LTL or plasma PAI-1 (N=96). The longitudinal analysis included 475 participants with repeated measurements of both LTL and plasma PAI-1 in samples collected at two visits (1998–1999, 2001–2003). Selection of study participants is shown in Figure 1.

Measurement of LTL

Detailed methods for the measurement of LTL in the SHFS have been described previously. [7] Briefly, genomic DNA from peripheral blood was isolated according to standard protocols. LTL was quantified by quantitative PCR at the University of California, San Francisco using a high-throughput telomere length assay system. The telomere length assay determines the ratio of telomeric product/single copy gene (T/S) obtained using quantitative PCR according to protocols reported previously.[34] T/S ratio was calculated by taking the ratio between the mean of two T values and two S values attained for each of the three replicates. The three T/S ratios were averaged, and standard deviation and coefficient of variation (%CV, standard deviation/mean) were calculated. The T/S ratios were normalized to the mean of all samples and reported. For quality control, we included seven control DNA samples from various cancer cell lines in each assay plate. These control samples allowed us to create 8 standard curves, which were then integrated into a composite standard curve, used for T and S concentration calculations. For the purpose of quality control, about 20% of

the samples selected randomly were measured twice. Intra- and inter-assay %CV was 4.6% and 6.9%, respectively.

Measurement of plasma PAI-1 and hsCRP

Plasma PAI-1 was measured using an enzyme-linked immunosorbent assay (ELISA) according to standard protocols described previously.[35] All antibodies and reagents were obtained from the Center for Thrombosis and Vascular Research, University of Leuven, Belgium. The assay %CV of this method was <5%. Serum hsCRP was measured by an ELISA assay developed in-house using purified CRP and anti-CRP antibodies from Calbiochem at the University of Vermont laboratory.[36] The assay %CV was 8%.

Assessment of other factors

Demographic information (age, sex, education level) was collected by standard questionnaires. Cigarette smoking was classified as current smoking, past smoking and never smoking. Current smoking was defined as having smoked at least 100 cigarettes in the entire life, having smoked cigarettes regularly, and smoking currently. Past smoking was defined as having smoked at least 100 cigarettes in the entire life, having smoked cigarettes regularly in the past, but not smoking currently. Never smoking was defined as never smoked or having smoked fewer than 100 cigarettes in the entire life. Alcohol drinking was classified as current drinkers, former drinkers and never drinkers. Current drinkers were those who had consumed any alcohol during the past year, former drinkers had stopped consuming alcohol for 12 months, and never drinkers were those who reported never drinking alcohol in their life time. Each participant was asked to wear a pedometer for seven consecutive days and to record the number of the steps taken daily in an activity diary. Physical activity was assessed by the mean number of steps per day calculated by averaging the total number of steps recorded in 7 consecutive days. Body weight (kg) and height (cm) were measured when participants wore light clothes and no shoes by trained staff. Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters (kg/m^2). Waist circumference was measured at the level of the umbilicus while the participant was in a supine position. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured three times by trained staff using a standard mercury sphygmomanometer after the participants had been resting for at least 5 minutes and the mean of the last two measurements was used in statistical analysis. Fasting glucose, insulin, serum creatinine, and blood lipids including total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C), were measured by standard laboratory methods.[32] Estimated glomerular filtration rate (eGFR) was estimated based on serum creatinine by using the Modification of Diet in Renal Disease Study equation.[37]

Statistical analysis

Cross-sectional analysis—Given the strong correlation between age and LTL, we first regressed LTL on age and the residual from regression (i.e. age-adjusted LTL) was used in subsequent statistical analyses. PAI-1 and hsCRP were log-transformed (i.e. log-PAI-1, log-hsCRP) to improve normality.

To examine the cross-sectional association between plasma PAI-1 and LTL, we constructed a generalized estimating equation (GEE) model in which age-adjusted LTL was the dependent variable and plasma PAI-1 (continuous log-PAI-1 or categorized into quartiles) was the independent variable, adjusting for demographics (sex, education level), study site, lifestyle (smoking, drinking, physical activity), and metabolic factors (waist circumference, SBP, fasting glucose, insulin, LDL-C, HDL-C, eGFR). GEE regression model was used here to account for the family relatedness among study participants by including "family ID" as a cluster variable in the model. To examine whether and to what extent inflammation may potentially modulate the association between plasma PAI-1 and LTL, we additionally adjusted for log-hsCRP in the regression model.

Longitudinal analysis—Changes in LTL, plasma PAI-1, and metabolic factors were calculated by subtracting the follow-up values from their corresponding baseline measurements. To examine the longitudinal association between plasma PAI-1 and LTL, we constructed multivariate GEE model by regressing change in LTL (dependent variable) on change in plasma PAI-1 (independent variable), adjusting for demographics (age, sex, education level), study site, lifestyle (smoking, drinking) at baseline, and changes in metabolic factors (waist circumference, SBP, fasting glucose, insulin, LDL-C, HDL-C, eGFR) during the same time period, as well as follow-up years, plasma PAI-1 and LTL at baseline. Baseline plasma PAI-1 and LTL were adjusted to account for the longitudinal effect of their baseline values on telomere changes.

Sensitivity analysis—To examine whether diabetes influences our results, we performed separate analysis by excluding participants with overt diabetes at baseline. To examine whether antihypertensive medications affect our results, we excluded participants under antihypertensive medications. To examine whether smoking influences the association between plasma PAI-1 and LTL, we conducted stratification analysis by smoking status (smokers *vs.* non-smokers). A two-tailed *P* value less than 0.05 was considered statistically significant. All statistical analysis was conducted using SAS statistical software (version 9.4, Cary, North Carolina).

RESULTS

Characteristics of study participants

Characteristics of study participants are presented in Table 1. Mean age of the study participants was 38.9 ± 16.4 years. Women accounted for 60% of the study participants and had significantly longer age-adjusted LTL than men (*P*=0.009). Participants with higher plasma PAI-1 were more likely to be current smokers, current drinkers, and had higher BMI, waist circumference, fasting glucose, insulin, lipids, and hsCRP and shorter LTL than those with lower plasma PAI-1 (all *P*<0.05). No significant difference was detected for other variables listed in Table 1. Baseline characteristics of the 475 participants (39.9 ± 14.6 years) included in the longitudinal analysis was shown in Table 2.

Cross-sectional association between plasma PAI-1 and telomere length

Table 3 shows the cross-sectional association between plasma PAI-1 and age-adjusted LTL. Regression using plasma PAI-1 as a continuous variable demonstrated that a higher log-PAI-1 was significantly associated with a shorter age-adjusted LTL (β=−0.023, P<0.001). This association was substantially attenuated but remained statistically significant after adjusting for demographics, study site, lifestyle and metabolic factors (β=−0.015, P=0.029). Additional adjustment for log-hsCRP did not change this association (β=−0.015, P=0.032).

Regression analysis using categorical plasma PAI-1 (in quartiles) also detected an association between PAI-1 level and age-adjusted LTL in the same direction. Compared with participants in the lowest quartile of plasma PAI-1, those in the highest quartile had significantly shorter age-adjusted LTL (β=−0.041, P<0.001). This association was attenuated after adjusting for demographics, study site, lifestyle and metabolic factors (β=−0.027, P=0.040). Additional adjustment for log-hsCRP did not attenuate this association (β=−0.028, P=0.035).

Longitudinal association between plasma PAI-1 and telomere length

Of the 475 participants with repeated measurements for both LTL and plasma PAI-1, LTL was shortened in 383 participants (81%) during an average 5.3 years of follow-up. The other 92 participants gained LTL. Our statistical analysis included all of the 475 participants as telomere lengthening might be associated with PAI-1 change during the follow-up period. Table 4 presents the longitudinal association between change in plasma PAI-1 and change in LTL. It shows that change in plasma PAI-1 was significantly and inversely associated with change in telomere length after adjusting for demographics, study site, lifestyle, changes in metabolic factors during the same time period, follow-up years, and baseline plasma PAI-1 and LTL (β=−0.0005, P=0.017). We did not find an association between baseline plasma PAI-1 and change in LTL (β=0.0003, P=0.254).

Results of sensitivity analysis

After excluding participants with prevalent diabetes or those under antihypertensive medications, the cross-sectional association between plasma PAI-1 and age-adjusted LTL (Supplementary Tables S1 and S2) and the longitudinal association between change in plasma PAI-1 and change in LTL (Table 4) remained statistically significant. These results indicate that the observed associations between plasma PAI-1 and telomere length were unlikely to be attributed to prevalent diabetes and antihypertensive medications. The cross-sectional association between plasma PAI-1 and age-adjusted LTL (Supplementary Tables S3) and the longitudinal association between change in plasma PAI-1 and change in LTL (Supplementary Tables S4) appeared to be stronger in smokers than that in non-smokers.

DISCUSSION

In a large cohort of American Indians free of overt CVD, we demonstrated for the first time that plasma PAI-1 was significantly and inversely associated with leukocyte telomere length in both cross-sectional and longitudinal analyses, independent of many known risk factors and inflammation assessed by hsCRP. Our findings suggest that plasma PAI-1 may play a

critical role in biological aging, probably through pathways beyond known risk factors, though the precise molecular mechanisms through which PAI-1 influences telomeric aging remain to be determined.

The observed inverse association between plasma PAI-1 and biological aging is in line with previous experimental studies showing that PAI-1 advanced cellular senescence both in *vitro* and in *vivo*.[16, 17] This association was also supported by previous experimental research which showed that PAI-1 deficiency (PAI-1$^{-/-}$ mice) attenuated cellular senescence and prolonged survival,[38] and pharmacologic inhibition of PAI-1 promoted telomere maintenance in mice.[17] Further, PAI-1 expression and plasma PAI-1 concentration were increased in the *klotho* mouse model (a premature aging model widely used in aging research).[39] Although the relationship between PAI-1 and biological aging has been evidenced by experimental [16, 17] and animal [17, 38, 39] studies, no large-scale epidemiological studies have investigated this association in any ethnic groups including American Indians. So far, we are aware of two human studies with rather small sample sizes examining the association between plasma PAI-1 and leukocyte telomere length.[24, 25] One examined several hemostatic factors including PAI-1, fibrinogen, von Willebrand factor, and D-dimer in 171 South Africans and 182 European descendants but no association was identified between plasma PAI-1 and telomere length after adjusting for metabolic factors. [24] Another study comprising of 272 elderly Europeans with diabetes and myocardial infarction examined the relationship of LTL to fibrinogen, hsCRP, and PAI-1 but found that telomere length was not correlated to any of these biomarkers.[25] Herein we identified a significant association between plasma PAI-1 and leukocyte telomere length in a large sample of American Indians (N=2,560) in the SHFS. Of note, our analysis showed that, the association between PAI-1 and LTL was largely attenuated (~50%) after adjusting for metabolic factors, indicating that the observed association could be modulated by these metabolic factors. This emphasizes the importance and necessity of adjustment for metabolic factors in examining the relationship between PAI-1 and LTL.

In a subsample including 475 participants with repeated measurements of both PAI-1 and LTL (average follow-up of 5.3 years), we examined the longitudinal association between PAI-1 and LTL. Results showed that a larger increase in plasma PAI-1 was associated with a larger reduction in telomere length. Although this observational association does not establish the causal role of PAI-1 in biological aging, this finding provides a hypothesis that is worth of further investigation in future research.

PAI-1 is secreted in response to inflammatory reactions.[40] As such, an elevated level of plasma PAI-1 was almost always accompanied by increased inflammatory responses.[26] Because inflammation has also been associated with shorter telomere length,[27–29] it may modulate the association between plasma PAI-1 and telomere length. To test this hypothesis, we conducted additional analysis by further adjusting for hsCRP in the multivariate GEE model. It shows that additional adjustment for hsCRP did not materially alter the observed association between plasma PAI-1 and telomere length. This suggests that plasma PAI-1 may play a role in biological aging through pathways beyond inflammation.

Some anti-hypertensive drugs e.g., angiotensin-converting enzyme inhibitors, can reduce plasma level of PAI-1 [41] and thus may influence our results. As such, we conducted sensitivity analysis by excluding participants who self-reported receiving anti-hypertensive medications. It shows that the observed associations, both cross-sectional and longitudinal, between plasma PAI-1 and telomere length persisted. Similarly, excluding participants with prevalent diabetes did not change the association between plasma PAI-1 and telomere length either. These results suggested that the observed association between plasma PAI-1 and biological aging may not be attributed to anti-hypertensive or hypoglycemic drugs. Both cross-sectional and longitudinal associations between plasma PAI-1 and telomere length appeared to be stronger in smokers than that in non-smokers.

Strengths of this study included the high quality data of telomere length, the longitudinal follow-up, the large sample size, and the extensive adjustments for known risk factors. However, several limitations should also be noted. First, similar to all other observational studies, we cannot establish the causal relationship between plasma PAI-1 and telomere length. A prospective study is needed to assess the temporal sequence between PAI-1 and biological aging. Second, our findings were derived from American Indians whose metabolic health profiles could be different from other ethnic groups. Thus, the generalizability of our results to other populations is unknown. However, our study may serve as a model for analysis of other ethnic groups with high rates of diabetes and obesity. Third, as hsCRP was not available in the clinical exam in 1998–1999, we were unable to assess whether the longitudinal relationship between PAI-1 and telomere length was independent of inflammation in our analysis.

In conclusion, a higher level of plasma PAI-1 was significantly associated with shorter telomere length in American Indians, independent of many known covariates including metabolic factors and inflammation. This finding suggests that PAI-1 may play a critical role in cellular aging and aging-related disorders, although its causal impact on biological aging warranted further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ESSENTIALS

- Plasminogen activator inhibitor-1 (PAI-1) advanced cellular senescence in experiment studies.

- No population study exists on the association between PAI-1 and biological aging in American Indians.

- We found cross-sectional and longitudinal associations between higher PAI-1 and shorter telomere length.

- Our findings suggest a pathway linking PAI-1 with biological aging beyond of metabolic factors.
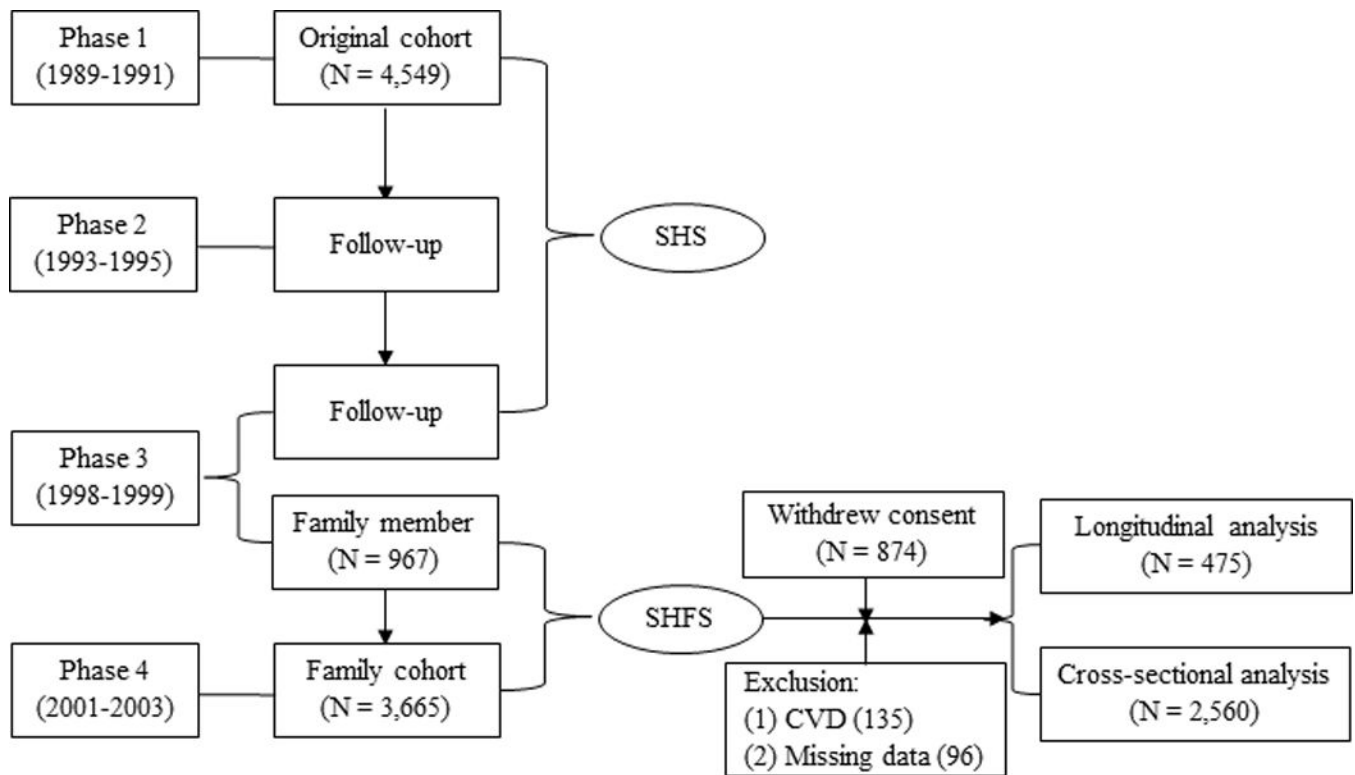
Figure 1.
Flowchart illustrating the study design of SHFS and the selection of study participants. The Strong Heart Family Study (SHFS) was initiated in 1997 by enrolling 967 family members of the original cohort, and then expanded in 2001–2003 by enrolling a total of 3,665 participants from 94 families including the re-examination of most of the 967 family members. The current analysis excluded 874 participants because one community withdrew consent for continuing participating in the study. After further excluding participants with overt CVD and missing data on either PAI-1 or LTL, a total of 2,560 participants were included in the cross-sectional analysis and 475 participants were included in the longitudinal analysis.

Table 1

Characteristics of SHFS participants examined in 2001–2003 according to quartiles of plasma PAI-1

Characteristics	Quartiles of plasma PAI-1 (ng/mL)				Trend P*
	Q1 (28)	Q2 (29 – 47)	Q3 (48 – 73)	Q4 (74)	–
N	675	634	623	628	
Age, year	39.1±17.8	40.2±17.7	41.3±15.9	38.3±14.6	0.784
Males, n (%)	241 (35.70)	262 (41.32)	263 (42.22)	251 (39.37)	0.129
Current smoking, n (%)	210 (31.11)	235 (37.07)	235 (37.72)	266 (42.36)	<0.001
Current drinking, n (%)	365 (54.07)	372 (58.68)	366 (58.75)	409 (65.13)	0.001
Education, years	12.1±2.4	12.2±2.3	12.4±2.3	12.1±2.2	0.718
Physical activity, steps/day	6796±4390	6244±4053	5725±3852	4978±3044	<0.001
Body mass index, kg/m²	27.1±5.8	30.2±7.0	32.4±7.4	35.4±7.5	<0.001
Waist circumference, cm	91.6±14.9	99.8±17.7	105.7±17.4	111.9±17.2	<0.001
SBP, mmHg	119.9±18.0	122.0±16.5	124.7±16.6	124.0±15.0	<0.001
DBP, mmHg	73.8±10.8	75.4±10.6	77.6±11.1	78.5±10.9	<0.001
FPG, mg/dL	100.8±40.8	103.3±36.4	110.7±46.5	119.9±54.2	<0.001
Insulin, uU/mL	11.7±11.8	15.5±17.6	19.7±23.8	23.6±19.5	<0.001
Total cholesterol, mg/dL	176.1±33.1	180.5±35.5	185.8±35.7	189.2±44.4	<0.001
Triglycerides, mg/dL	125.2±71.3	140.7±82.1	171.0±105.2	224.3±326.7	<0.001
LDL-C, mg/dL	94.1±28.2	101.1±30.1	102.3±29.4	102.5±32.3	<0.001
HDL-C, mg/dL	57.2±15.9	51.9±13.6	50.2±13.5	47.1±13.2	<0.001
eGFR, mL/min/1.73 m²	95.9±26.6	95.9±23.6	97.5±25.8	101.2±25.0	0.001
Log-hsCRP, mg/L	0.8±1.3	1.0±1.3	1.4±1.1	1.6±1.1	<0.001
LTL, T/S ratio	1.01±0.25	0.99±0.23	0.98±0.22	0.97±0.22	<0.001

*
Sex-adjusted using GEEs to account for family relatedness of participants.

SBP: systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting plasma glucose; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; eGFR: estimated glomerular filtration rate; log-hsCRP: log-transformed high sensitivity C-reactive protein; LTL: leukocyte telomere length.

Table 2

Characteristics of SHFS participants with available PAI-1 and LTL in both examinations conducted in 1998–1999 and 2001–2003 (N = 475)

Characteristics	Mean/percentage
Age, year	39.9±14.6
Males, n (%)	179 (37.68)
Current smoking, n (%)	184 (38.98)
Current drinking, n (%)	293 (62.08)
Education, years	12.4±2.0
Body mass index, kg/m^2	31.3±7.4
Waist circumference, cm	102.4±18.6
SBP, mmHg	123.2±15.4
DBP, mmHg	76.7±9.3
FPG, mg/dL	112.3±55.4
Insulin, uU/mL	23.1±25.7
Total cholesterol, mg/dL	183.6±34.2
Triglycerides, mg/dL	132.8±125.3
LDL-C, mg/dL	117.6±28.8
HDL-C, mg/dL	44.4±12.8
eGFR, mL/min/1.73 m^2	94.6±23.6
LTL, T/S ratio	1.19±0.54
Log-PAI-1	3.64±0.90

SBP: systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting plasma glucose; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; eGFR: estimated glomerular filtration rate; Log-PAI-1: log-transformed plasminogen activator inhibitor 1; LTL: leukocyte telomere length.

Table 3

Cross-sectional association between plasma PAI-1 and age-adjusted LTL among SHFS participants

PAI-1 level	Unadjusted		Model 1[*]		Model 2[†]	
	β (SE)	P	β (SE)	P	β (SE)	P
Continuous						
Log-PAI-1	−0.023 (0.006)	<0.001	−0.015 (0.007)	0.029	−0.015 (0.007)	0.032
Quartiles						
Quartile 1	reference	–	reference	–	reference	–
Quartile 2	−0.011 (0.011)	0.300	−0.015 (0.011)	0.160	−0.016 (0.011)	0.145
Quartile 3	−0.023 (0.010)	0.020	−0.024 (0.011)	0.033	−0.025 (0.011)	0.027
Quartile 4	−0.041 (0.011)	<0.001	−0.027 (0.013)	0.040	−0.028 (0.013)	0.035
P for trend		<0.001		0.035		0.030

LTL: leukocyte telomere length; PAI-1: plasminogen activator inhibitor-1; Log-PAI-1: log-transformed plasminogen activator inhibitor-1.

[*]
Model 1 adjusted for demographics (sex, education level), study site, lifestyle factors (current smoking, current drinking, physical activity), and metabolic factors (waist circumference, systolic blood pressure, fasting glucose, insulin, low- and high- density lipoprotein cholesterol, estimated glomerular filtration rate).

[†]
Model 2 adjusted for covariates in model 1 plus log-transformed high sensitivity C-reactive protein.

Table 4

Longitudinal association between change in plasma PAI-1 and change in LTL among SHFS participants

Sub-populations	N	Multivariate adjusted[*]	
		β[†] (SE)	P
Total participants	475	−0.0005 (0.0002)	0.017
Excluding diabetic participants	392	−0.0005 (0.0002)	0.014
Excluding participants under antihypertensive treatment	424	−0.0005 (0.0002)	0.017

LTL: leukocyte telomere length; PAI-1: plasminogen activator inhibitor-1.

[*] Adjusted for age, sex, education level, study site, lifestyle (smoking, drinking), changes in metabolic factors (waist circumference, systolic blood pressure, fasting glucose, insulin, low- and high- density lipoprotein cholesterol, estimated glomerular filtration rate), follow-up years, and baseline plasma PAI-1 and LTL.

[†] Change in LTL (T/S ratio) associated with per unit of change in plasma PAI-1.