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# Lipidomics profiling of biological aging in American Indians: the Strong Heart Family Study

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**Abstract** Telomeres shorten with age and shorter leukocyte telomere length (LTL) has been associated with various age-related diseases. Thus, LTL has been considered a biomarker of biological aging. Dyslipidemia is an established risk factor for most age-related metabolic disorders. However, little is known about the relationship between LTL and dyslipidemia. Lipidomics is a new biochemical technique that can simultaneously identify and quantify hundreds to thousands of small molecular lipid species. In a large population comprising 1843 well-characterized American Indians in the Strong Heart Family Study, we examined the lipidomic profile of biological aging assessed by LTL. Briefly, LTL was quantified by qPCR. Fasting plasma lipids were quantified by untargeted liquid chromatography–mass spectrometry. Lipids associated with LTL were identified by elastic net modeling. Of 1542 molecular lipids identified (518 known, 1024 unknown), 174 lipids (36 knowns) were significantly associated with LTL, independent of chronological age, sex, BMI, hypertension, diabetes status, smoking status, bulk HDL-C, and LDL-C. These findings suggest that altered lipid metabolism is associated with biological aging and provide novel insights that may enhance our understanding of the relationship between dyslipidemia, biological aging, and age-related diseases in American Indians.

**Keywords** Lipidomics · Biological aging · Telomere length · Biomarkers · American Indians · Strong Heart Study

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11357-022-00638-9.

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## Introduction

Telomeres are the tandem repeats of DNA sequences and their associated proteins at the end of each chromosome. While telomeres maintain DNA stability and protect DNA during cell division [1], some telomeric repeats are lost with each cell division, resulting in progressively shorter telomere length over the course of aging [2]. Thus, telomere length has been considered an indicator of biological aging. Shorter leukocyte telomere length (LTL) has been associated with accelerated biological aging and various age-related diseases such as obesity [3], diabetes [4–6], cardiovascular disease (CVD) [7, 8], and cancers [9, 10].

Dyslipidemia is an established risk factor for aging and age-related metabolic disorders [11, 12]. A few epidemiological studies have also reported associations of shorter LTL with dyslipidemia such as high levels of low-density lipoprotein cholesterol or low levels of high-density lipoprotein cholesterol [13–15]. Moreover, treatment with lipid-lowering drugs appeared to prevent the loss of telomeres in the endothelial progenitor cells among individuals diagnosed with coronary artery disease [15]. These findings suggest a relationship between dyslipidemia and biological aging as assessed by LTL.

Lipidomics is an emerging high-throughput biochemical technology that can identify and quantify hundreds to thousands of individual molecular lipid species in a biological sample. Because lipids are a subset of metabolites that represent the functional state of cells, identification and quantitation of all circulating lipid species (i.e., lipidome) may provide novel insight into the pathogenesis of aging and age-related metabolic disorders [16]. Indeed, lipidomic studies in different populations have reported associations of circulating molecular lipid species, such as triacylglycerols and phosphatidylethanolamine, with metabolic syndrome [17, 18], obesity [19, 20], diabetes [21], and cardiovascular diseases [22]. Moreover, previous studies have reported associations of various molecular lipids with LTL [23–25]. Specifically, among American Indians participating in the Strong Heart Family Study (SHFS), our group has shown that higher plasma levels of PE(O-18:0/13:0), PE (P-16:0/12:0), and PC(O-8:0/O-8:0) were significantly associated with longer LTL [24]. By contrast, higher plasma levels of MG(20:3), DG(18:2/14:1), and PG(20:4) were significantly associated with shorter LTL [24]. Another study including 3511 White women participating in the TwinsUK study showed that higher serum levels of glycerophospholipids (1-stearoylglycerophosphoinositol and 1-palmitoylglycerophosphoinositol) were significantly associated with shorter LTL [25]. Collectively, these findings suggest that altered individual lipid species may be involved in the regulation of telomere metabolism and thus the process of biological aging [26, 27].

While these results support a relationship between altered lipid metabolism and biological aging, existing studies were mainly conducted among White individuals, had a small sample size, and/or were limited by low coverage of the blood lipidome due to using targeted mass spectrometric approaches. To our knowledge, no study has examined the relationship of LTL with a full spectrum of blood lipidome in large-scale community-based populations in any racial/ethnic group. The objective of this study was to identify individual molecular lipids and lipidomic signatures associated with LTL in American Indians participating in the SHFS (2001–2003).

## Methods

### Study population

The Strong Heart Family Study (SHFS; 2001–ongoing) is a family-based prospective cohort study on cardiometabolic diseases and their risk factors among American Indians residing in Central Arizona (AZ), North and South Dakota (DK), and Southwestern Oklahoma (OK). The SHFS initially recruited 2786 participants from 94 families in 2001–2003 and re-examined all living participants every 4 to 5 years thereafter. At each visit, participants completed a personal interview and a physical examination. Fasting blood samples were collected for laboratory tests, including glucose, insulin, lipids, lipoproteins, creatinine, and biomarkers. Details on the study design, survey methods, and laboratory procedures have been reported previously [28, 29]. The SHFS protocol was approved by the Institutional Review Boards of the Indian Health Service, the participating institutions, and the participating tribes.

Of 1945 participants with available LTL and lipidomic data at baseline, we excluded participants with prevalent CVD ($n=19$) and those receiving lipid-lowering medications ($n=63$). We further excluded participants with outliers for lipids (±5 SD of the first five PCs in lipidomic data, $n=10$) or LTL (outside median±3 IQR, $n=10$). A total of 1843 individuals was included in the final data analysis.

### Lipidomics data acquisition by liquid chromatography–mass spectrometry (LC–MS)

Detailed methods for plasma sample collection, lipidomic data generation, pre-processing, and quality control in the SHFS have been reported elsewhere [30]. Briefly, a modified liquid–liquid extraction method (cold methanol/MTBE/water) [31, 32] was used to extract fasting plasma samples. Lipid species in the plasma were quantified using LC–MS analysis that was carried out in both positive electrospray (ESI (+)) mode on an Agilent 6530 QTOF mass spectrometer, and negative electrospray (ESI (-)) mode on an Agilent 6550 QTOF mass spectrometer. Standard methods were used to quantify the lipids species. False-negative features and peaks with 50% missing values across all samples were removed. The batch effect of the reformatted dataset was normalized by SERRF software (Systematic Error Removal using Random Forest) [33], which dramatically reduced the raw data variance coefficient by 23% in positive mode data and 25% in negative mode to less than 10% in result files. Finally, 1809 lipids were harvested from LC–MS, including 579 known and 1230 unknown lipids. After further removing internal standards ($n=24$) and lipids with a bimodal distribution ($n=37$) detected by the Hartigan's Dip test of unimodality (Hartigan and Hartigan 1985) ($P<0.05$), a total of 1542 lipids (518 known and 1024 unknown lipids) in 1843 individuals was used in the final data analysis.

### Measurement of LTL

Detailed methods for the measurement and QC of the telomere data in the SHFS have been reported previously [6]. Briefly, LTL was measured in genomic DNA isolated from peripheral blood using qPCR [34]. The telomeric product/single-copy gene (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. These 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, seven control DNA samples from various cancer cell lines were included in each assay plate and about 20% of the samples selected randomly were measured twice. Intra- and inter-assay %CV was 4.6% and 6.9%, respectively. We also categorized LTL into quartiles for downstream analysis.

### Assessments of covariates

Standard questionnaires were administered to collect information on sociodemographic characteristics (age, sex, and education), family health history, medical history, and lifestyle. Cigarette smoking was classified as current smoking, past smoking, and never smoking. Current smoking was defined as the participant having smoked at least 100 cigarettes in his/her entire life, having smoked cigarettes regularly, and smoking currently. Past smoking was defined as the participant having smoked at least 100 cigarettes in his/her entire life, having smoked cigarettes regularly in the past, and not smoking currently. Never smoking was defined as the participant having smoked fewer than 100 cigarettes or having never smoked in their lifetime or having quit smoking for > 12 months.

Bodyweight (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 ($kg/m^2$). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice by trained staff using a standard mercury sphygmomanometer after the participants had been resting for at least 5 min and the mean of the two measurements was used in statistical analysis. Fasting glucose, insulin, HbA1C, and blood lipids, including total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and HDL-C, were measured using standard laboratory methods [28].

Reproductive history and hormone use data were based on self-report. Menopause was recorded as whether menstrual cycles have stopped or not. Oral contraceptive use was recorded as ever having taking birth control pills; when participants answered "yes," they were further asked about how many years they

had been taking them. Estrogen use was also recorded for reasons other than as birth control pills, including after hysterectomy, as a relief of menopause symptoms and to prevent bone loss.

Statistical analysis

Prior to statistical analysis, lipidomics data were log-transformed (base 10) to improve normality.

**Lipid selection by elastic net** Given the high dimension and the interrelationships between plasma lipids, we used an elastic net model to identify lipids associated with LTL. The analytic plan for lipids selection is illustrated in Fig. 1. Briefly, we first split the sample into training and testing datasets (6:4) using a random binomial variable. In the training dataset (60% of the sample), we selected lipids associated with LTL using regularized regression (elastic net) implemented in the R package *glmnet* [35]. Elastic net is a mix between ridge and lasso regression that implements similar sparsity of representation as lasso but also encourages a grouping effect and can successfully model high-dimensional correlated data [36]. The elastic net model was controlled by two parameters: $\alpha$ and $\lambda$. $\alpha=0$ corresponds to ridge regression and $\alpha=1$ corresponds to lasso regression. In this study, we set $\alpha=0.05$ which was commonly used in genomic data analysis [37]. $\lambda$ was optimized by tenfold cross-validation based on the criteria of minimum mean squared error [36]. The model included all 1542 lipids simultaneously along with clinical factors, including age, sex, study site, BMI, hypertension, diabetes status, smoking status, bulk HDL-C, and LDL-C. Relatedness among family members was accounted for by including family ID in the model. To examine to what extent the selected lipids explain the variability in LTL over clinical factors, we constructed two mixed-effect linear models in the testing dataset (40% of the sample), with one model including clinical factors only (age,

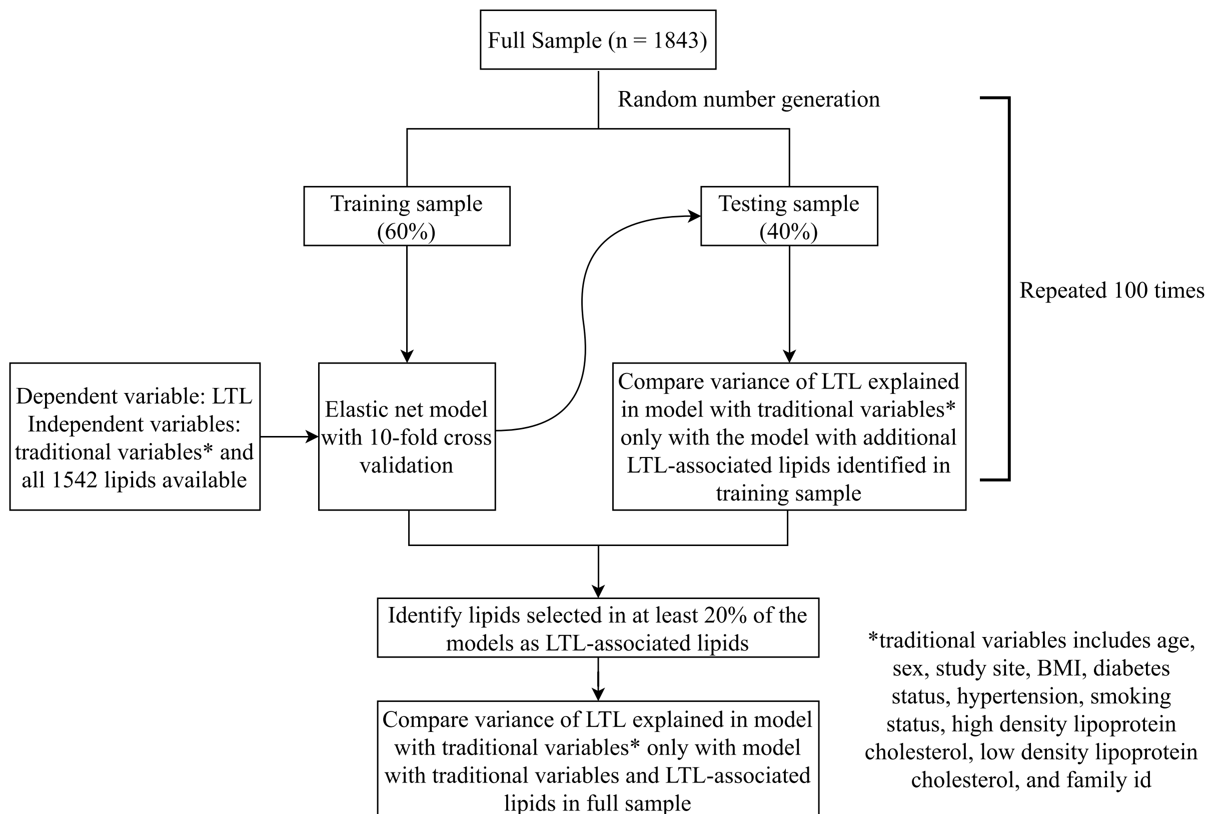


**Fig. 1** Schematic illustration of study design

sex, study site, BMI, hypertension, diabetes status, smoking status, bulk HDL-C, and LDL-C) and the other model including clinical factors plus the lipids selected by the elastic model. The incremental value of plasma lipids over clinical factors was assessed by comparing the multivariable-adjusted $R^2$ obtained from the two models in the testing dataset. We repeated this process 100 times and obtained the frequency of selection for each lipid over the 100 iterations. Lipids selected ≥ 20 times over 100 runs were considered top correlates of LTL. To examine whether the top ranked lipids explained additional variance in LTL over standard clinical factors in the full dataset (100% of the sample), we additionally constructed two mixed-effects linear models as stated above. We compared multivariable-adjusted $R^2$ obtained from the model with clinical factors only to the model including clinical factors plus the top-ranked lipids. To examine whether and how physical activity and diet influence our results, we conducted sensitivity analysis by additionally adjusting for physical activity and alternate healthy eating index (AHEI) in the elastic net model as described above.

While all lipids selected by the elastic net model (i.e., those with non-zero coefficients) were by definition statistically significantly associated with LTL, the coefficients obtained from the model were biased due to the regularization of the model on the coefficients. We thus conducted additional analysis by generalized estimating equation (GEE) using the R package *geepack* [38] to estimate the individual effect of each selected lipid on LTL. In this model, LTL was the dependent variable, and each selected lipid was the independent variable, adjusting for age, sex, study site, BMI, hypertension, diabetes status, smoking status, HDL-C, and LDL-C. The family correlation was included as a random effect in the model.

**Sensitivity analysis** To examine the potential effect of sex on the relationship between lipids and LTL, we performed male- and female-specific analyses. Furthermore, years of oral contraceptive use, menopause, and hormone therapy (estrogen administration for reasons other than birth control) were included as covariates in an additional female-specific analysis.

## Results

The mean age of participants was 39.7 years (18 to 75 years old, SD: 13.8 years; Online Resource 1). Women accounted for 62.6% of the study population. Around 40% of participants were current smokers. The prevalence of hypertension and diabetes was 28% and 16%, respectively. Compared to participants with longer LTL (Q4), those with shorter LTL (Q1) were significantly older, more likely to have hypertension and diabetes, and had higher BMI, SBP, fasting plasma glucose, fasting insulin, and LDL. Table 1 shows the clinical characteristics of participants according to LTL quartiles.

### Fasting plasma lipids associated with LTL

Over 100 iterations of the elastic net modeling, age was selected every time. Study center ($n=99$), BMI ($n=88$), and diabetes status ($n=87$) were selected in majority of the runs, whereas LDL-C ($n=21$) was selected in less than a third of the 100 iterations. Smoking ($n=7$), sex ($n=5$), family ID ($n=4$), hypertension ($n=2$), and HDL-C ($n=1$) were selected in only a few of the 100 iterations.

Of 1542 lipids included in the elastic net model, 809 lipids were selected at least once over 100 runs in the training set (Online Resource 2). In the testing data set ($n=737$), the average multivariable-adjusted $R^2$ slightly increased from 0.28 to 0.29 units when LTL-related lipids identified in the training set were added to the models with clinical factors only (Online Resource 3). Of the 809 lipids selected at least once, 174 lipids (36 known, 138 unknown) were selected ≥ 20 times over the 100 iterations, and we considered these lipids to be associated with LTL. Of these, palmitic acid [FA (16:0)] and one unknown lipid were selected in each run. The known lipids associated with LTL are presented in Table 2. A full list of 174 lipids associated with LTL is presented in Online Resource 4. Of the 36 known lipids selected by the elastic net, 9 lipids belong to fatty acyls, 10 belong to sphingolipids, 14 belong to glycerophospholipids, 2 belong to glycerolipids, and 1 belongs to sterol lipid species. In the full sample, adding these 174 lipids to the model with clinical variables resulted in an increase of adjusted $R^2$ from 0.29 to 0.38, suggesting that these lipids can explain the variability in telomere length in addition to the clinical

**Table 1** Baseline characteristics of SHFS participants according to quartiles of LTL (2001–2003)

| Characteristics | Total ($n$ = 1843) | Q1 ($n$ = 461) | Q2 ($n$ = 461) | Q3 ($n$ = 460) | Q4 ($n$ = 461) | $P$ |
|---|---|---|---|---|---|---|
| Age, years | 39.7 (13.8) | 44.5 (12.9) | 42.2 (13.6) | 38.4 (13.2) | 33.9 (12.9) | < 0.001 |
| Male, $n$ (%) | 690 (37.4) | 173 (37.5) | 172 (37.4) | 172 (37.4) | 173 (37.5) | 0.999 |
| Study center | | | | | | < 0.001 |
| Arizona | 220 (11.9) | 64 (13.9) | 65 (14.1) | 46 (10.0) | 45 (9.8) | |
| Oklahoma | 805 (43.7) | 212 (46.0) | 221 (47.9) | 201 (43.7) | 171 (37.1) | |
| South Dakota | 818 (44.4) | 185 (40.1) | 175 (38.0) | 213 (46.3) | 245 (52.1) | |
| Smoking, $n$ (%) | | | | | | 0.237 |
| Current | 744 (40.4) | 165 (35.8) | 184 (39.9) | 192 (41.7) | 203 (44.0) | |
| Former | 422 (22.9) | 117 (25.4) | 111 (24.1) | 100 (21.7) | 94 (20.4) | |
| Body mass index, kg/m$^2$ | 31.8 (7.6) | 32.8 (7.8) | 32.2 (8.0) | 31.9 (7.4) | 30.4 (7.0) | < 0.001 |
| Systolic blood pressure, mmHg | 122.2 (15.4) | 122.4 (15.1) | 123.1 (16.1) | 123.3 (16.2) | 199.8 (13.9) | 0.002 |
| Diastolic blood pressure | 77.2 (10.7) | 77.1 (10.2) | 77.2 (11.2) | 77.9 (11.1) | 76.6 (10.0) | 0.311 |
| Hypertension, $n$ (%) | 510 (27.7) | 142 (30.8) | 151 (32.8) | 134 (29.1) | 83 (5.6) | < 0.001 |
| Fasting plasma glucose, mg/dL | 108.2 (44.9) | 115.7 (53.1) | 111.9 (46.8) | 105.4 (44.1) | 99.74 (31.3) | < 0.001 |
| Fasting insulin, mIU/L | 18.1 (20.7) | 20.4 (25.4) | 18.1 (19.7) | 17.2 (17.9) | 16.7 (18.6) | 0.031 |
| Diabetes status, $n$ (%) | | | | | | < 0.001 |
| Impaired fasting glucose | 404 (21.8) | 105 (22.8) | 109 (23.6) | 105 (22.8) | 85 (18.4) | |
| Diabetes | 300 (16.3) | 111 (24.1) | 93 (20.2) | 56 (12.2) | 40 (8.7) | |
| LDL-C, mg/dL | 101.4 (29.8) | 102.8 (31.2) | 101.5 (29.5) | 103.5 (29.9) | 97.9 (28.2) | 0.022 |
| HDL-C, mg/dL | 51.9 (14.6) | 52.3 (15.0) | 52.0 (14.4) | 51.0 (14.6) | 52.1 (14.5) | 0.539 |

Results are expressed as mean (SD) unless otherwise noted. *Q*, quartile; *LDL-C*, low-density lipoprotein cholesterol; *HDL-C*, high-density lipoprotein cholesterol. Statistical differences across LTL quartiles were calculated via ANOVA for continuous variables and chi-squared for categorical variables

factors. In our sensitivity analysis that additionally included physical activity and AHEI, 166 lipids (30 knowns) were selected. Among the 30 known lipids identified in this model, 24 were associated with LTL in our main model (Online Resource 5).

Sex-specific analysis showed that 1052 and 935 lipids were selected at least once for females and males, respectively. Of the 1052 lipids in females only, 211 lipids (44 known, 167 unknown) were selected ≥ 20 times over the 100 iterations. A full list of these 211 lipids associated with LTL in female participants is presented in Online Resource 6. Of the 935 lipids in males only, 138 lipids (23 known, 115 unknown) were selected ≥ 20 times over the 100 iterations. A full list of 138 lipids associated with LTL in male participants is presented in Online Resource 7. Further adjusting for menopause, estrogen use and years of birth control use in the females-only model resulted in 1107 lipids (selected at least once). Of those, 238 lipids (47 known, 191 unknown) were selected ≥ 20 times over the 100 iterations. A full list of these 238 lipids associated with LTL in female participants with further adjustment for sex hormone variables is presented in Online Resource 8. A total of 190 unique lipids were selected in female-only analyses (i.e., before and after adjustment for sex hormone variables).

## Discussion

In this large-scale lipidome profiling of 1843 community-dwelling American Indians, we identified 174 fasting plasma lipids associated with LTL independent of clinical factors including age, sex, smoking, BMI, diabetes status, hypertension, LDL-C, and HDL-C. Of these, 36 were known lipids, including fatty acyls, sphingolipids, glycerophospholipids, glycerolipids, and sterol lipid species. To our knowledge, this is the first study examining the relationship between a full spectrum of plasma lipidome and LTL in a large sample of community-dwelling individuals in any racial/ethnic group. Our findings suggest that altered lipid metabolism may underlie biological

**Table 2** Fasting plasma lipids significantly associated with LTL (selected ≥ 20 times in elastic net). Only known lipids are shown

| Lipids | Category | Coefficients ($\beta$) by GEE | Selection frequency (%) |
|---|---|---|---|
| FA(16:0) | Fatty acyls | −0.003 | 100 |
| LPI(20:4) | Glycerophospholipids | −0.012 | 86 |
| SM(d42:3) A | Sphingolipids | 0.013 | 81 |
| FA(10:0) | Fatty acyls | 0.005 | 71 |
| PC(36:3) A | Glycerophospholipids | 0.014 | 70 |
| PC(p-32:0)/PC(o-32:1) | Glycerophospholipids | 0.004 | 70 |
| PI(16:0/18:0) | Glycerophospholipids | −0.006 | 67 |
| SM(d18:0/24:1) | Sphingolipids | −0.005 | 62 |
| FAHFA(18:0/3:0) | Fatty acyls | −0.005 | 57 |
| CER(d41:1) | Sphingolipids | −0.001 | 50 |
| CER(d43:1) | Sphingolipids | 0.0005 | 50 |
| PC(p-16:0/22:6)/PC(o-16:1/22:6) | Glycerophospholipids | 0.012 | 49 |
| TG(56:9) | Glycerolipids | −0.008 | 49 |
| PC(20:2/20:2) | Glycerophospholipids | 0.011 | 47 |
| FA(20:5) B | Fatty acyls | −0.005 | 46 |
| SM(d32:0) B | Sphingolipids | −0.029 | 38 |
| SM(d34:2) B | Sphingolipids | 0.011 | 37 |
| CER(d42:2) A | Sphingolipids | 0.013 | 36 |
| PE(p-18:2/20:4)/PE(o-18:3/20:4) | Glycerophospholipids | −0.010 | 34 |
| SM(d42:2) B | Sphingolipids | 0.017 | 32 |
| AC(13:1) | Fatty acyls | 0.008 | 31 |
| Cholesterol | Sterol lipids | −0.041 | 31 |
| LPC(p-18:0)/LPC(o-18:1) | Glycerophospholipids | −0.006 | 31 |
| FA(18:2) | Fatty acyls | −0.004 | 29 |
| PE(p-18:0/22:5)/PE(o-18:1/22:5) | Glycerophospholipids | 0.014 | 28 |
| TG(40:0) | Glycerolipids | 0.004 | 28 |
| PC(p-14:0/2:0)/PC(o-14:0/2:0) | Glycerophospholipids | −0.003 | 27 |
| AC(18:1) B | Fatty acyls | −0.014 | 26 |
| PC(34:3) A | Glycerophospholipids | −0.016 | 24 |
| FA(26:0) | Fatty acyls | 0.031 | 24 |
| SM(d36:0) A | Sphingolipids | −0.017 | 24 |
| PC(p-16:0/2:0)/PC(o-16:0/2:0) | Glycerophospholipids | 0.044 | 23 |
| LPE(22:5) | Glycerophospholipids | 0.005 | 22 |
| FAHFA(16:0/3:0) | Fatty acyls | −0.009 | 21 |
| SM(d32:2) A | Sphingolipids | 0.012 | 20 |
| LPE(18:0) | Glycerophospholipids | −0.017 | 20 |

The elastic net models and the GEE models were adjusted for age, sex, study site, BMI, hypertension, diabetes status, smoking status, HDL-C, LDL-C, and family ID

aging assessed by LTL and shed light on the mechanisms through which dyslipidemia may affect biological aging and age-related diseases.

Fatty acyls (FAs) are molecules synthesized by chain elongation of an acetyl-CoA primer with malonyl-CoA (or methylmalonyl-CoA) groups [39]. The altered composition of fatty acids in cellular membranes plays an important role in the aging process, probably through their effects on oxidative stress [40]. In this study, we found that an elevated level of plasma palmitic acid [FA (16:0)] was associated with shorter LTL. This finding is in agreement with

previous studies demonstrating that increased levels of palmitate initiated cellular senescence or apoptosis [41]. Moreover, elevated serum or plasma level of palmitic acid has been associated with various age-related chronic conditions such as inflammation, metabolic syndrome, insulin resistance, diabetes, and cardiovascular diseases in human populations [42–45]. In one study, patients with diabetes exhibited at least three times higher levels of palmitic acid compared to those without diabetes [42]. Besides palmitic acid, we also found that increased plasma levels of five other fatty acids, including FAHFA (18:0/3:0), FA (20:5), AC (18:1), FA (18:2), and FAHFA (16:0/3:0), were associated with shorter LTL. By contrast, increased plasma levels of three saturated fatty acids including capric acid [FA (10:0)], cerotic acid [FA (26:0)], and AC (13:1) were associated with longer LTL.

We found that altered levels of AC (18:1) and AC (13:1) were significantly associated with LTL. These results are consistent with previous research demonstrating that an increased level of acylcarnitines was associated with insulin resistance [46–49]. For instance, the accumulation of short- or long-chain acylcarnitines in muscle cells reduced insulin sensitivity by 20–30% [46]. Reduction in acylcarnitines resulted in increased glucose and insulin tolerance in mice under a high-fat diet [47]. Moreover, patients with obesity or diabetes exhibited higher plasma levels of acylcarnitines compared to controls [48].

Sphingolipids play important roles in multiple biological processes, such as senescence, apoptosis, stress response, inflammation, and immune system [50–53]. In the present study, we found that higher plasma levels of CER (d41:1), SM (d32:0), SM (d18:0/24:1), and SM (d36:0) were associated with shorter LTL. These results appear to be consistent with previous research demonstrating that ceramides induced senescence and apoptosis in human cells [54] as well as findings that senescent cells exhibited higher levels of ceramides than younger cells [54–56]. In human population studies, sphingolipids, especially ceramides, have been associated with age-related diseases such as metabolic disorders, cancer, neurodegeneration, and CVD [50, 53, 57, 58]. We also found that higher plasma levels of two ceramides (CER (d43:1), CER (d42:2)) and four sphingomyelins (SM (d42:3), SM (d34:2), SM (d42:2), and SM (d32:2)) were significantly associated with longer LTL. These results seem to be consistent with a previous study demonstrating that very-long-chain ceramides (e.g., 18:1/20:0 and 18:1/24:0) were negatively correlated with insulin resistance or glucose levels in rats with pre-diabetes [59]. The observed positive associations of very-long-chain unsaturated SMs with LTL corroborate findings from our own group in the same study population showing that some specific SMs (e.g., d40:3) were associated with decreased risk of diabetes [30] and chronic kidney disease [60].

In addition to fatty acids and sphingolipids, we also detected significant associations of 14 glycerophospholipids with LTL. Among these, higher plasma levels of multiple ether phospholipids such as PC(p-32:0)/PC(o-32:1), LPE(22:5), PC(20:2/20:2), PC(p-16:0/22:6)/PC(o-16:1/22:6), PE(p-18:0/22.5)/PE(o-18:1/22:5), PC(36:3) A, and PC(p-16:0/20:0)/PC(o-16:0/2:0), were associated with longer LTL, while elevated levels of other lipids such as PC(34:3) A, LPI(20:4), LPE(18:0), PE(p-18:2/20:4)/PE(o-18:3/20:4), LPC(p-18:0)/LPC(o-18:1), PI(16:0/18:0), and PC(p-14:0/2:0)/PC(o-14:0/2:0) were associated with shorter LTL. These findings are in agreement with previous studies demonstrating that altered levels of glycerophospholipids in plasma [61] or cerebrospinal fluid [62, 63] were associated with Alzheimer's disease.

We identified sex-specific lipidomic signatures of LTL. For instance, several lipids, such as SM(d32:0) B, LacCer(18:1/24:1(15Z)), and AC(10:0), were associated with LTL in women but not in men. In contrast, some lipids, such as AC(13:1) and DAG(38:6), were associated with LTL in men but not in women. These observations appear to be in line with previous studies demonstrating sex-specific differences in lipids [64] and/or LTL [65] in human population studies.

To the best of our knowledge, this is the first large-scale lipidomic study examining the relationship between a full spectrum of fasting plasma lipidome and LTL among community-dwelling individuals in any racial/ethnic group. Strengths of this study include a large sample size, high coverage of plasma lipidome, and extensive adjustments for known clinical factors including bulk lipids and use of lipid-lowering drugs and estrogens. Several limitations should also be noted. First, the current analysis only included American Indians, and the mean BMI of the study population is relatively high. Thus, the generalizability of our findings to other

ethnic groups or population settings should be cautious. Second, we did not include an external replication due to the lack of population cohorts where both LTL and lipidomics data are available. Yet, we employed a machine learning approach (e.g., elastic net) by splitting the entire sample into training and testing sets, which may serve as an internal validation. In addition, the elastic net selects statistically most significant lipids, which are not necessarily biologically relevant to LTL. Third, although we controlled for many clinical factors known to be associated with aging, we cannot exclude the possibility of potential confounding by unknown or unmeasured factors. Fourth, while our untargeted lipidomic platform provides high coverage of the plasma lipidome, the majority of the lipids detected are unknowns and we cannot ascertain their structures or distinguish isomers. Additional experiments are required to identify these molecules of interest in future research. Finally, the observational nature of our study precludes any causal inference regarding the role of altered lipids in telomere metabolism.

In summary, we identified significant associations between altered plasma lipidome and biological aging in a large sample of community-dwelling American Indians. Our results provide initial evidence that altered lipid metabolism may underlie biological aging assessed by LTL. Such information will enhance our understanding of the molecular processes through which dyslipidemia affects aging and age-related diseases.

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**Declarations**

**Ethical approval** All procedures performed in this study were in accordance with the ethical standards of the Indian Health Service Institutional Review Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Conflict of interest** The authors declare no competing interests.

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