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Processed Meat, but Not Unprocessed Red Meat, Is Inversely Associated with Leukocyte Telomere Length in the Strong Heart Family Study[1–4]

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Abstract

Background: Telomeres are repetitive nucleotide sequences (TTAGGG) and their associated proteins at the end of eukaryote chromosomes. Telomere length shortens throughout the lifespan with each cell division, and leukocyte telomere length (LTL) is often used as a biomarker of cellular aging. LTL is related to many chronic diseases, including cardiovascular disease and diabetes. However, to our knowledge, the relation between LTL and risk factors for cardiovascular disease and diabetes, such as dietary intake of processed meat and unprocessed red meat, is largely unknown.

Objective: We examined the associations of processed meat intake and unprocessed red meat intake with LTL.

Methods: This cross-sectional study comprised 2846 American Indians from the Strong Heart Family Study who participated in the 2001–2003 examination. Dietary factors, including past-year consumption of processed meat and unprocessed red meat, were assessed with the use of a 119-item Block Food-Frequency Questionnaire. LTL was measured with the use of quantitative polymerase chain reaction. Generalized estimating equations were used to examine the associations of intake of processed meat and unprocessed red meat with LTL.

Results: Consumption of processed meat was negatively associated with LTL after adjustment for age, sex, site, education, smoking, alcohol use, physical activity, and other dietary factors. For every additional daily serving of processed meat, LTL was 0.021 units (telomeric product–to–single-copy gene ratio) shorter ($\beta \pm SE = -0.021 \pm 0.008$, $P = 0.009$). No association was observed between the intake of unprocessed red meat and LTL ($\beta \pm SE = 0.008 \pm 0.011$, $P = 0.46$).

Conclusions: In the Strong Heart Family Study, consumption of processed meat, but not unprocessed red meat, was associated with shorter LTL, a potential mediator for several age-related diseases. Further studies are needed to better understand the biological mechanism by which processed meat intake influences cellular aging. *J Nutr* 2016;146:2013–8.

Keywords: diet, processed meat, unprocessed red meat, telomeres, American Indians

Introduction

Telomeres are repetitive nucleotide sequences (TTAGGG) and their associated proteins at the end of eukaryote chromosomes. Telomere length progressively shortens throughout the lifespan with each cell division, and leukocyte telomere length (LTL)[13] is often used as a biomarker of cellular aging (1). Shorter LTL consistently has been shown to be associated with cardiovascular disease (CVD) (2) and diabetes (3, 4). However, the relation between LTL and cancer is less clear. Shorter LTL has been shown to be associated with some cancers (e.g., bladder, gastric,

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[3] The opinions expressed in this paper are those of the authors and do not necessarily reflect the views of the Indian Health Service.
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[4] Supplemental Tables 1–4 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
[13] Abbreviations used: AGE, advanced glycation end product; CVD, cardiovascular disease; GEE, generalized estimating equation; LTL, leukocyte telomere length; MESA, Multi-Ethnic Study of Atherosclerosis; SHFS, Strong Heart Family Study; T:S ratio, telomeric product–to–single-copy gene ratio.

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ovarian, esophagus, and head and neck) (5) and longer LTL has been shown to be associated with other cancers (e.g., melanoma) (6, 7), whereas other cancers (e.g., non-Hodgkin lymphoma and breast and lung cancer) have inconsistent or null relations with LTL (5). In the Strong Heart Family Study (SHFS), a study of CVD and its risk factors in 13 American Indian communities in Arizona, North Dakota, South Dakota, and Oklahoma, shorter LTL was associated with obesity (8), diabetes (9), and atherosclerosis (10). Because LTL has been shown to be associated with many cardiometabolic diseases and some cancers, it is of public health importance to identify potentially modifiable factors that influence LTL.

Although LTL is largely influenced by genetic factors, a growing body of evidence suggests that LTL may also be explained by lifestyle factors that affect oxidative stress and inflammation, including diet (11–13). The heating and processing of meat produces advanced glycation end products (AGEs), and AGEs influence both inflammation and oxidative stress (14, 15). Several studies have shown a positive association between processed meat intake and markers of inflammation and oxidative stress, including γ-glutamyltransferase (16, 17) and C-reactive protein (17, 18).

Whether intake of processed meat or unprocessed red meat is associated with LTL is largely unknown. To date, to our knowledge, only one study has assessed the relation of meat intake with LTL. In that study, processed meat, but not unprocessed red meat, was associated with LTL (19). The purpose of this analysis was to examine the relation between dietary intake of processed meat and unprocessed red meat and LTL in participants of the SHFS. We hypothesized that participants who reported diets high in processed meats would have a shorter LTL than participants who consumed fewer processed meats. Because unprocessed red meats are not a major source of oxidative stress and inflammation, and because the consumption of unprocessed red meats has not been associated consistently with the risk of chronic diseases, such as diabetes and CVD, we did not expect to find an association between the intake of unprocessed red meat and LTL.

Methods

Study population. The SHFS is a longitudinal study designed to identify genetic factors that influence risk of CVD in American Indians. In total, 3665 individuals from 94 multigenerational families selected from a population-based cohort had a baseline examination in 2001–2003 and a follow-up examination 5 y later (2006–2009). Each examination included a detailed personal interview, physical exam, laboratory measures, medication review, and 1-wk pedometer log. LTL was assessed with the use of blood samples from the baseline exam only. Details on the SHFS design and methods have been described previously (20). The SHFS protocol was approved by the institutional review boards from each Indian Health Service region and the participating communities, and written informed consent was obtained from all participants at each examination. All procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983.

The current analysis included 2846 SHFS participants who completed the baseline examination with available diet, covariate, and LTL measures. Participants with missing (n = 210) or unreliable (n = 237) dietary data were excluded. This included participants who skipped >10% of the questions on the FFQ or who reported an extreme caloric intake (<600 or >6000 kcal/d for women and <600 or >8000 kcal/d for men) (21). Participants with missing values for LTL (n = 88), smoking (n = 2), alcohol consumption (n = 2), education (n = 12), BMI (n = 20), physical activity (n = 235), or dietary covariates (n = 13) also were excluded. Participants excluded from analyses because of missing data reported slightly less education (mean ± SD: 11 ± 2 compared with 12 ± 2 y) and had higher fibrinogen concentrations (mean ± SD: 400 ± 94 compared with 387 ± 89 mg/dL), but otherwise were similar to participants included in the analyses.

Dietary assessment. In order to estimate past-year diet, participants were administered a 119-item Block FFQ at baseline. The Block FFQ is a widely used FFQ, and it has demonstrated reliability and validity (22). In addition to food items on the standard Block FFQ, participants were asked about intake of foods commonly consumed in the participating American Indian communities, such as menudo (pork or beef stomach with red chili), pozole (hominy soup), guysava (roasted corn, beef, and chili), red or green chili, Indian taco, fry bread, corn tortilla, flour tortilla, and canned meat. For some ethnic groups, the inclusion of an ethnic foods section on the FFQ produces more accurate nutrient estimates (23). Past-year diet was estimated with the use of measures of consumption frequency (i.e., seasonally, never, a few times/y, 1 time/mo, 2–3 times/mo, 1 time/wk, 2 times/wk, 3–4 times/wk, 5–6 times/wk, or daily) and portion size (small, medium, or large). Mean daily energy and macronutrient intake was calculated for each study participant with the use of the Block database (Block Dietary Systems) by multiplying the frequency response for each food on the FFQ and American Indian supplementary foods questionnaire by the nutrient content of the documented portion size of the food, and then summing for all foods.

Measurement of LTL. Genomic DNA was isolated from peripheral blood leukocytes collected at the baseline examination, and LTL was measured by qPCR under the guidance of the EH Blackburn lab, Department of Biochemistry and Biophysics, University of California, San Francisco. The LTL was quantified as the telomeric product–to–single-copy gene ratio (T:S ratio). Details of the laboratory methods and quality control procedures have been reported previously (9). All samples were assessed in triplicate, and the mean LTL was used in statistical analyses. Laboratory personnel were blinded to the clinical characteristics of the study participants. Approximately 4% of samples were assayed twice for quality control. The Pearson correlation for the duplicate samples was high (r = 0.95). The mean interassay and intraassay CVs were 6.9% and 4.6%, respectively.

Measurement of covariates. Detailed information on demographic and behavioral factors, including education, smoking and alcohol use, and medical history, were collected as part of the personal interview at the baseline examination (20). Body weight was measured with the use of a Tanita BWB-800–5 digital scale with participants wearing light clothing and no shoes. Height was measured with the use of a vertical mounted ruler. BMI was calculated as body weight in kilograms divided by height in meters squared (20). Blood pressure was measured 3 times on the right arm with the use of standard mercury sphygmomanometers after 5 min rest while seated, and the mean of the second and third systolic and diastolic measurements was used in this analysis (24, 25). Blood samples were collected after a 12-h overnight fast and were stored at −70°C. Plasma glucose was measured with the use of enzymatic methods. Prevalent diabetes was defined with the use of 2003 American Diabetes Association criteria, including use of insulin or oral antidiabetes medication or fasting plasma glucose ≥126 mg/dL at baseline. LDL cholesterol and HDL cholesterol were measured with the use of standard procedures, as described previously (25). Fibrinogen was measured with the use of the Clauss method (26). Accusplit AE120 pedometers were used to measure the number of steps taken per day during a 7-d period; the primary measure of physical activity used in the present analysis was the mean number of steps taken per day (27).

Statistical analyses. We examined the cross-sectional associations of consumption of processed meat (i.e., breakfast sausage, canned meat, hotdogs, and lunch meat) and unprocessed red meat [i.e., ribs, hamburger, cheeseburger, roast beef, steak, pork (excluding pork-based luncheon meats), veal, lamb, deer, and liver] with LTL with the use of generalized estimating equations (GEEs), using an independence working correlation and robust SEs. A GEE was used to account for the potential

familial correlation within the data that may have resulted from the family-based sampling of the SHFS. All statistical analyses were conducted with the use of STATA version 13.0.

Both meat intake (servings per day) and LTL (T:S ratio) were assessed continuously. Consistent with previous studies (28), we considered 50 g and 100 g to be 1 serving of processed meat and unprocessed red meat, respectively. Covariates were selected a priori on the basis of their potential association with meat intake and LTL. Three models were fitted to examine the associations of processed meat or unprocessed red meat intake with LTL. Model 1 (the minimally adjusted model) adjusted for age, sex, site, and total energy intake. Model 2A was adjusted in addition for a priori confounders known to be associated with diet and LTL from previous studies, including education, smoking status (never, former, or current), alcohol consumption (grams per day), and physical activity. Model 2B (primary model) included in addition the dietary intake of monounsaturated fat, polyunsaturated fat, saturated fat, *trans* fat, fiber, protein, servings of fruits and vegetables, sugar-sweetened beverages, unprocessed red meat (for analyses of processed meat and LTL) and processed meat (for analyses of unprocessed red meat and LTL). Model 2 comprised 2 parts so that the reader could better distinguish the impact of dietary adjustments on risk estimates. Because it was possible that *1*) BMI, systolic blood pressure, LDL cholesterol, and fibrinogen may influence LTL; and either *2*) BMI, systolic blood pressure, LDL cholesterol, and fibrinogen may influence diet choices and meat intake; or *3*) meat intake may influence values for BMI, systolic blood pressure, LDL cholesterol, and fibrinogen, these factors may have either confounded (i.e., if conditions 1 and 2 were met) or mediated (i.e., if conditions 1 and 3 were met) the association of meat intake with LTL. Model 3 was adjusted in addition for BMI, systolic blood pressure, LDL cholesterol, and fibrinogen to better determine whether these factors affected the relation of meat intake with LTL. Multicollinearity of models 2 and 3 were assessed with the use of variance inflation factor testing, and no collinearity was observed.

To better understand whether prevalent CVD (i.e., myocardial infarction, heart failure, or stroke), diabetes, and self-reported cancer influenced the association of meat intake and LTL, we performed sensitivity analyses that *1*) further adjusted for prevalent CVD, diabetes, and cancer; and *2*) restricted analyses to participants without prevalent CVD, diabetes, or cancer. In addition, we reran all analyses without adjustment for monounsaturated fat, polyunsaturated fat, and saturated fat to better understand how these factors may influence the association of meat intake and LTL. In secondary analyses, all analyses were repeated with the use of quartiles of meat intake as the exposure of interest.

We examined the potential interaction of processed meat and unprocessed red meat intake with age, sex, smoking, and BMI to determine whether these factors modify the relation of meat intake with LTL (29–31). Wald tests were used to evaluate the statistical significance of each interaction term.

Values in the text are means (ranges) or β ± SE unless otherwise indicated. Because telomere length (T:S ratio) values are small, meaningful differences can be obscured by rounding results to 2 figures past the decimal. As such, for GEE analyses, data are provided to 3 figures past the decimal to illustrate the effect of adjustments across models.

Results

Baseline characteristics of the study participants are shown in **Table 1**. The mean age of the 2846 study participants was 39.6 y (14.1–93.3 y), and 60% of the analytic cohort was female. Mean BMI (in kg/m^2) was 32, and 34% of study participants reported smoking. In total, 22% of participants had diabetes and 6% had CVD. Participants consumed a mean of 0.71 servings of processed meat/d (0–4.72 servings) and 0.57 servings of unprocessed red meat/d (0–7.62 servings). An inverse correlation was observed between age and LTL (Spearman's ρ = −0.36, $P < 0.0001$).

The intake of processed meat was associated with a shorter LTL. For every additional serving of processed meat, LTL was 0.021 units (T:S ratio) shorter (−0.021 ± 0.008, $P = 0.009$) after adjustment for age and demographic, behavioral, and dietary factors (**Table 2**). Further adjustment for potential mediators, including systolic blood pressure, LDL cholesterol, fibrinogen, and BMI, did not materially change the observed associations (Table 2). Although the association of total processed meat intake and LTL may appear modest, the magnitude of the risk estimate corresponds to a 4-y difference in age. That is, in the SHFS, a 1-y difference in age was associated with 0.005 units (T:S ratio) shorter in LTL (−0.005 ± 0.0004, $P < 0.0001$).

There was no significant association of unprocessed red meat consumption with LTL (0.008 ± 0.011, $P = 0.46$) (**Table 3**). There were also no statistically significant interactions of processed meat or unprocessed red meat intake with age, sex, smoking, or BMI on LTL (smallest *P*-interaction = 0.20) (data not shown). Sensitivity analyses that *1*) further adjusted model 2 for prevalent CVD, diabetes, and cancer, or *2*) removed adjustment for monounsaturated fat, polyunsaturated fat, and saturated fat from analyses did not materially alter results (data not shown). In addition, analyses restricted to participants without prevalent CVD, diabetes, or cancer produced similar findings (**Supplemental Table 1**). Results of analyses in which meat intake was assessed in quartiles are shown in Tables 2 and 3. Finally, baseline characteristics of study participants according to quartile of processed meat, unprocessed red meat, and LTL are shown in **Supplemental Tables 2–4**.

TABLE 1 Baseline characteristics of SHFS participants[1]

Characteristic	Value
Age, y	39.6 ± 16.4
Female, %	60.2
Education, y	12 ± 2
Current smoking, %	34.1
Current alcohol consumption, %	58.6
BMI, kg/m^2	32 ± 8
Waist circumference, cm	104 ± 18
Systolic blood pressure, mm Hg	122 ± 16
HDL cholesterol,[2] mg/dL	51 ± 15
LDL cholesterol,[3] mg/dL	99 ± 29
TGs,[4] mg/dL	168 ± 174
Fibrinogen,[5] mg/dL	387 ± 89
Prevalent diabetes, %	22.1
Prevalent CVD, %	6.0
Activity, steps/d	5652 ± 3870
Saturated fat, en%	11.6 ± 2.4
Protein, en%	13.2 ± 2.8
Fiber, g/kcal	7.6 ± 2.6
Fruits and vegetables,[6] servings/d	3.5 ± 2.5
Sugar-sweetened beverages, kcal/d	213 ± 227
Total energy, kcal/d	2460 ± 1325

[1] Values are means ± SDs or percentages, $n = 2846$. CVD, cardiovascular disease; en%, percentage of energy; SHFS, Strong Heart Family Study.
[2] CV = 28.4.
[3] CV = 29.8.
[4] CV = 103.9.
[5] CV = 22.9.
[6] One serving of fruits and vegetables is equivalent to 80 g.

Discussion

In this large, cross-sectional study of American Indians, the intake of processed meat, but not unprocessed red meat, was

TABLE 2 Association of reported daily processed meat intake with LTL in SHFS participants[1]

	Per 50-g serving		Quartiles				
	Value	*P*	1	2	3	4	*P*-trend
n			712	711	712	711	
Model 1[2]	−0.015 ± 0.008	0.07	1.00 (Ref)	−0.013 ± 0.013	−0.017 ± 0.014	−0.021 ± 0.017	0.12
Model 2a[3]	−0.015 ± 0.008	0.07	1.00 (Ref)	−0.015 ± 0.013	−0.016 ± 0.017	−0.020 ± 0.017	0.08
Model 2b[4]	−0.021 ± 0.008	0.009	1.00 (Ref)	−0.012 ± 0.013	−0.015 ± 0.014	−0.026 ± 0.017	0.038
Model 3[5]	−0.022 ± 0.008	0.007	1.00 (Ref)	−0.012 ± 0.010	−0.015 ± 0.014	−0.026 ± 0.017	0.048

[1] Values are β ± SE, *n* = 2846. Data were analyzed with the use of generalized estimating equations. LTL, leukocyte telomere length; Ref, reference; SHFS, Strong Heart Family Study.
[2] Adjusted for age, sex, site, and total caloric intake.
[3] Adjusted in addition for education, smoking (never, former, or current), alcohol use (never, former, or current; grams per day), and physical activity (steps per day).
[4] Adjusted for dietary intake of monounsaturated, polyunsaturated, saturated, and *trans* fat; fiber; protein; fruits and vegetables; sugar-sweetened beverages; and unprocessed red meat.
[5] Adjusted for model 1 and model 2 covariates, in addition to systolic blood pressure, LDL cholesterol, fibrinogen, and BMI.

associated with a shorter LTL. These findings suggest that the relation between meat intake and LTL differs according to processing characteristics. Moreover, these findings support the hypothesis that dietary and other lifestyle factors may influence LTL, a potential mediator for several age-related chronic diseases, such as CVD and diabetes.

Studies suggest that telomere attrition is related to cardiometabolic diseases (2) and cancer (32). Few studies have assessed the relation of LTL with diet (12, 33–36), and, to our knowledge, only one published study has investigated the association of meat intake with LTL (19). The study reported an inverse association of processed meat intake with LTL, and no association of red meat intake with LTL in 840 white, black, and Hispanic adults who participated in the Multi-Ethnic Study of Atherosclerosis (MESA). The magnitude of the reported risk estimate in the MESA was slightly larger than our findings. In the MESA, each additional serving of processed meat per day was associated with a 0.07 ± 0.03-lower LTL. The difference in the magnitude of the risk estimates between the MESA and the SHFS may be explained at least in part by underlying differences between the populations studied, including differences in race/ethnicity, demographic factors, health behaviors, and morbidity. For instance, MESA participants were older (mean age across telomere quintiles: MESA, 58–65 y; SHFS, 31–47 y), had a lower BMI (mean BMI across telomere quintiles: MESA, 28–29; SHFS, 31–33), and reported consuming fewer servings or processed meat per day (mean processed meat intake across telomere quintiles: MESA, 0.11–0.19 servings/d; SHFS, 0.67–0.76 servings/d) than did participants in the SHFS.

The findings reported herein are supported by several studies that indicate differential health effects of processed meat compared with unprocessed red meat on the risk of several health outcomes, including coronary artery disease, type 2 diabetes, and mortality. Specifically, processed meat intake is associated with a higher risk of coronary artery disease, type 2 diabetes, and mortality, whereas there are modest associations (or no associations) of unprocessed red meat intake with these outcomes (28, 37). In the SHFS, we previously reported a positive association of processed meat intake with incident diabetes, and no association of unprocessed red meat intake with incident diabetes (21). Because of the findings reported herein, we reran previous analyses of processed meat intake and incident diabetes to evaluate the magnitude of potential mediation of LTL on this relation. The magnitude of the reported risk estimate when comparing extreme quartiles of processed meat intake and incident diabetes was attenuated by 5% when LTL was added to the model. This suggests that LTL only modestly affects the relation between processed meat intake and incident diabetes in the SHFS, and that the processed meat and diabetes relation must be explained by other mechanisms.

There are several possible mechanisms that may explain the observed association of processed meat intake and LTL. Processed meats contain high concentrations of AGEs. AGEs are formed in the heating and processing of meats and have been

TABLE 3 Association of reported daily unprocessed meat intake with LTL in SHFS participants[1]

	Per 100-g serving		Quartiles				
	Value	*P*	1	2	3	4	*P*-trend
n			712	711	712	711	
Model 1[2]	0.004 ± 0.010	0.67	1.00 (Ref)	−0.024 ± 0.012	−0.023 ± 0.012	−0.010 ± 0.012	0.44
Model 2a[3]	0.008 ± 0.010	0.40	1.00 (Ref)	−0.021 ± 0.012	−0.020 ± 0.013	−0.006 ± 0.016	0.62
Model 2b[4]	0.008 ± 0.011	0.46	1.00 (Ref)	−0.022 ± 0.012	−0.023 ± 0.013	−0.012 ± 0.017	0.33
Model 3[5]	0.009 ± 0.011	0.41	1.00 (Ref)	−0.021 ± 0.012	−0.018 ± 0.013	−0.007 ± 0.018	0.53

[1] Values are β ± SE, *n* = 2846. Data were analyzed with the use of generalized estimating equations. LTL, leukocyte telomere length; Ref, reference; SHFS, Strong Heart Family Study.
[2] Adjusted for age, sex, site, and total caloric intake.
[3] Adjusted in addition for education, smoking (never, former, or current), alcohol use (never, former, or current; grams per day), and physical activity (steps per day).
[4] Adjusted in addition for dietary intake of monounsaturated, polyunsaturated, saturated, and *trans* fat; fiber; protein; fruits and vegetables; sugar-sweetened beverages; and processed meat.
[5] Adjusted for model 1 and model 2 covariates, in addition to systolic blood pressure, LDL cholesterol, fibrinogen, and BMI.

shown to influence inflammation and oxidative stress (38, 39). Oxidative stress and inflammation promote the production of reactive oxygen species, which impede telomerase activity and promote telomere exhaustion (1). Over time, lower telomerase activity and exhaustion increase base oxidation, damage to telomeres, and subsequent telomere attrition (40). In addition, telomeres are high in guanines—a nucleobase known to be particularly sensitive to injury because of oxidative stress (40). Oxidative stress also promotes the formation of 8-oxo-2′-deoxyguanosine, which may stimulate single-strand breaks in DNA (40, 41). Unlike other parts of genomic DNA that easily repair chain breaks or oxidative lesions, telomeric DNA are unable to recover efficiently from damage (40, 42). Chronic inflammation also promotes cell turnover and senescence (43), and several studies show a strong association of markers of inflammation, such as TNF-α and IL-6, with telomere attrition (43–47).

Nonbiological mechanisms may also explain the observed association of processed meat intake with LTL. In the present analyses, participants who consumed the most processed meat had less education than those who consumed the least processed meat. Because education may be a marker of social disadvantage, and a handful of studies have shown a positive relation between social disadvantage and telomere attrition (48–50), residual confounding may account for the observed association of processed meat and LTL if processed meat intake is a marker for unmeasured social adversity. More studies are needed to better characterize this relation.

This study has some limitations. First, this is a cross-sectional analysis, and it is not possible to infer the temporality of the observed association, because both exposure and outcome were measured at a single time. Although we adjusted for several demographic, behavioral, and dietary factors that may be related to both diet and LTL, we cannot disregard the possibility of residual confounding from unmeasured or poorly measured factors. In the SHFS, pedometers were used to capture physical activity. These devices are designed only to capture ambulatory movement, such as walking and running, and activities such as swimming, biking, pushing, or lifting were not captured. Dietary data were collected with the use of an FFQ, and some participants might not have accurately recalled type, frequency, or portion size of foods consumed (over- or under-reporting), thereby limiting our ability to accurately measure processed meat and unprocessed red meat intake. Finally, the analytic cohort comprised American Indians from 4 states, and results may not be generalizable to other populations. However, previous published manuscripts from the SHFS have demonstrated that reported diet and lifestyle patterns among SHFS participants are similar to that of the general US population (51).

This study also has several strengths. The SHFS is a large multisite study of CVD and its risk factors in an underserved population. The available detailed data on demographic, behavioral, and health factors maximized our capacity to control for potential confounders. The availability of a Block FFQ and a supplemental ethnic foods questionnaire allowed us to more accurately characterize the typical diet of study participants than with the use of an FFQ alone.

In conclusion, the consumption of processed meat, but not unprocessed red meat, was associated with a shorter LTL in a large cohort of men and women over a wide age range. These findings suggest that lifestyle factors such as diet are associated with LTL, a biomarker of cellular aging, and may explain at least in part the associations of LTL with multiple chronic diseases. Further studies are needed to better understand the biological mechanism by which processed meat intake influences cellular aging.

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AMF, BVH, and JZ designed the research; BVH, LGB, and JZ provided the essential materials necessary for the research; MM was the statistician on the project and supervised the statistical methods; BVH, DSS, LGB, SAAB, MM, SE-A, NS, and JZ participated in all analyses and revised drafts of the paper; and AMF analyzed the data, wrote the paper, and had primary responsibility for the final content. All authors read and approved the final manuscript.

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