PREVENTING CHRONIC DISEASE

PUBLIC HEALTH RESEARCH, PRACTICE, AND POLICY

STRONG HEART STUDY COLLECTION

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GUEST EDITORIAL

35 Years of Partnership to Advance Cardiovascular Health and Well-Being in American Indian Communities: The Strong Heart Study and Strong Heart Family Study

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PEER REVIEWED

The value of longitudinal studies in population health research cannot be overstated. Longitudinal studies, particularly prospective cohort studies, allow scientists to better understand associations of social, behavioral, clinical, and environmental factors with disease risk or progression over time — sometimes decades (1). These studies provide key sources of information needed to inform public health policy and interventions intended to maximize health outcomes for all people in the US.

The Strong Heart Study (SHS) and Strong Heart Family Study (SHFS) are 2 cohort studies funded by the National Heart, Lung, and Blood Institute (NHLBI) that have been ongoing since 1989 and 2001, respectively (2,3). The studies comprise 12 American Indian communities in Arizona, Oklahoma, North Dakota, and South Dakota. At the inception of the SHS, little was known about cardiovascular diseases in rural and American Indian communities because all the other large cohort studies that focused on cardiovascular diseases (eg, Cardiovascular Health Study, Framingham Heart Study, Coronary Artery Risk Development in Young Adults study) did not include American Indian people. The SHS was developed to better understand risk factors for cardiovascular diseases among American Indians in particular (2,4). In 2001, the study was expanded to include family members of participants from the original cohort. That study, called the SHFS, was designed to better understand the heritability of cardiovascular diseases (3). Over the past 35 years, scientists and participating communities have developed a better understanding of the major risk factors for cardiovascular diseases and worked together to develop, implement, and evaluate several interventions intended to improve cardiovascular health (5–10). These interventions have had meaningful impacts on the cardiovascular health of the participating communities, including a 47% reduction in urinary arsenic levels and increased self-reported use of arsenic-safe drinking and cooking water (5), significant reductions in systolic blood pressure, low-density lipoprotein cholesterol, and left ventricular mass (8), and increased awareness of the effect of physical activity and cholesterol on risk of cardiovascular diseases (10). Furthermore, data from the first 25 years of the SHS indicate a decrease in the incidence of cardiovascular diseases among both men and women and a decrease in deaths from cardiovascular diseases among men (11).

As part of the SHS and SHFS, participants completed in-person examinations every 3 to 14 years (2,3). Over time, the focus of the examinations shifted from collection of data on traditional risk factors for cardiovascular diseases (eg, smoking, diabetes, hypertension) to include novel risk factors of clinical, community, and public health importance (eg, environmental heavy metal exposures, metabolomics, food environment, resilience). These additional data collection components were driven by an evolving scientific landscape and changes in community interests. For instance, in the late 1990s, there was a strong interest in genetic factors that may contribute to the risk of cardiovascular diseases, and the inclusion of family members of original SHS participants enabled detailed genetic analyses (3). More recently, community members expressed concerns about potential heavy metals in groundwater and soil, and scientists have assessed associations of blood or urinary arsenic, uranium, and lead with several health outcomes by using data collected over the past 35 years (12–14).

This week *Preventing Chronic Disease* features 7 articles from the SHS and SHFS. The articles are an example of the wide breath of

hypotheses that can be tested with available data from the well-designed cohort studies and illustrate the importance of examining multifaceted social, behavioral, clinical, and environmental factors that may affect cardiovascular health. The articles deepen our knowledge of cardiovascular health in populations historically underrepresented in research and highlight 3 overarching themes: 1) the benefit of forging partnerships and sharing knowledge, 2) the importance of collecting data on multilevel and multidimensional risk factors using rigorous scientific methods, and 3) the value of stored biospecimens in longitudinal studies. Taken together, the collection features a successful 35-year partnership of scientists and tribes committed to promote optimal health outcomes in rural communities.

Forging Partnerships and Sharing Knowledge

The SHS (n = 3,500) and SHFS (n = 2,753) are the largest and longest ongoing cohort studies of cardiovascular diseases and risk factors among American Indians in the US, and a major strength of the SHS and SHFS is the involvement of multiple tribes across 4 states (4). The SHS and SHFS involve collaborations across participating tribes, academic institutions, and the NHLBI. These collaborations are bidirectional. As described by Howard et al, the tribes and academic partners work together at every stage of the study (15). This includes development of research goals based on community interests, strengths and priorities; creation or adaptation of data collection instruments; implementation of research protocols; interpretation of study results; dissemination of findings to tribal leadership, citizens, and local health-serving organizations; and exploring new research directions and next steps.

Some articles featured in this collection are direct products of community-initiated hypotheses. For instance, many participating tribes stress the importance of holistic health and the interconnections of physical, spiritual, emotional, and mental well-being. Because of community interest and concerns regarding the potential link between depression and cardiovascular diseases, Santori et al assessed the association of depressive symptoms with hypertension risk in the SHFS (16). Results indicate that participants who reported depressive symptoms at baseline had 54% higher odds of developing hypertension compared with participants with no depressive symptoms (16). Similarly, community concerns regarding exposure to heavy metals from groundwater and soil set the foundation for a deep dive into environmental determinants of health in the SHS and the SHFS. In their article, Patterson et al reported positive associations of urinary uranium levels with hypertension (17). Findings from these reports underscore the importance of aligning research goals with community interests and priorities to accelerate scientific discoveries of local public health importance.

Importance of Collection of Multilevel and Multidimensional Risk Factors by Using Rigorous Scientific Methods

The SHS and SHFS include comprehensive in-person examinations that use instruments with known validity and reliability (2,3). The data from these examinations enable scientists to conduct robust data analyses and adequately address confounding (1). Furthermore, the collection of data on a wide range of topics across several dimensions of health — sociodemographic, behavioral, clinical, and household factors; family history of chronic diseases; and the local environment — enables both scientists and the participating tribes to understand the complex interplay of multilevel factors on health outcomes.

A wide range of data are collected at the SHS and SHFS in-person examinations and through ongoing surveillance (ie, annual telephone calls and medical record reviews to ascertain major clinical events). Additionally, with permission from the Indian Health Service Institutional Review Boards, Tribal Research Review Boards, and study participants, the SHS and SHFS query several national health repositories and data sources to augment the type and precision of data available in the studies. The article by Fabsitz et al showcases an example of the use of combining data from the SHS and the Indian Health Service National Data Warehouse (18). The SHS and SHFS also use other sources, including the Centers for Medicare and Medicaid Services, state cancer registries, state vital records, and the National Death Index. Use of multiple data sources for event ascertainment ensures data quality and reliability of research findings and maximizes utility of the study.

Value of Stored Biospecimens in Longitudinal Studies

The COVID-19 pandemic taught us that it is not possible to anticipate what health will look like in the coming years. In this collection, 3 articles report on biomarkers that were not measured at the time of the in-person examinations and laboratory assessments but rather used stored specimens collected at past SHS and SHFS examinations (17,19,20). Freezing and storing biospecimens for future use is efficient for achieving medical advances in a timely fashion because it allows scientists to examine the relationship of exposures in the distance past (as measured using stored blood or urine) with current health outcomes. Without these historical biospecimens, scientists would need to design a study, collect biomarkers, and wait years for enough participants to develop the out-

come to assess if the biomarker is relevant to health. The use of stored biospecimens in research often leads to new interventions and therapies to improve health decades before it would be possible had these biospecimens not been available. For instance, at the time of the 2001–2003 examination, the use of mass spectrometry to measure the lipidome was largely unknown in large cohort studies. However, Chen et al were able to use stored samples from the 2001–2003 SHFS examination to measure lipidomics in 2017 (20).

Although the use of stored biospecimens in research studies is a time-, labor-, and cost-effective way to learn from the past to help ensure a healthier future for current and future generations, use of these samples also presents challenges. Strong academic–community partnerships based on trust, transparency, and ongoing dialogue are critical to ensure best practices for safeguarding biospecimens. There must be an upfront and clear understanding of where biospecimens will be stored, how long they will be stored, and how they will be used in advance of the study. In the SHS and SHFS, participants were asked permission to save biospecimens for future use (ie, opt-in or opt-out) and provided with a clear indication of what types of analyses the specimens may be used for. The Strong Heart Study Observational Study Monitoring Board, the Indian Health Service Institutional Review Boards, and Tribal Research Review Boards must review and approve all studies that use any SHS or SHFS data, including studies that propose to use stored biospecimens. Additionally, in the SHS and SHFS, every biological sample maintains a spiritual connection to those who donate it. The academic institutions and tribes have worked closely to determine the best way to honor these sacred samples, including blessings of the laboratories and biospecimens by tribal leaders. If at any time a study participant decides that they no longer wish to have their biospecimens used in research, the academic institutions and tribes work together to honor the tribe and participant's request in a culturally respectful way.

Future Directions

Looking ahead to the next 35 years of the SHS and SHFS, we hope to expand the study in several ways. Both scientists and tribes agree that early prevention of cardiovascular risk factors is critical to optimize health outcomes. The article by Reese et al, which focused solely on American Indian adolescents, demonstrated a high prevalence of cardiovascular risk factors (19). We hope to expand the study to adolescents and young adults to better understand early life risk factors for cardiovascular diseases and how best to maximize health throughout the life course. Additionally, we hope to continue to prioritize community capacity building. As part of the current SHS and SHFS cycle, the NHLBI provided funds for a handful of community members and organizations in the SHS and SHFS communities to design and lead pilot projects to fill scientific, policy, or community health needs to improve health status based on their interests or goals. These grants helped to center community voices and strengthen local research capacity. The SHS and SHFS are also working to design a user-friendly web interface that the tribes can use to better use SHS and SHFS data and leverage results to support internal programming. To encourage efficient data harmonization and spur research collaborations that support clinical and health policy decisions, scientists are also mapping SHS data to existing National Institutes of Health Common Data Elements (CDEs) and developing SHS- and SHFS-specific CDEs (21). Finally, findings from the SHS and SHFS have informed development of several community-based cardiovascular health promotion interventions over the past 35 years (5–7), and we hope to continue this work moving forward.

The SHS and SHFS are built on decades of trust, transparency, power-sharing, mutual learning, and a shared commitment to prevent cardiovascular diseases and promote longevity among American Indians. The goals of the study have evolved over time but demonstrate the value in academic–community partnerships to define health challenges and solutions to maximize health and well-being for all.

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ESSAY

Initiating Research in Indian Country: Lessons From the Strong Heart Study

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PEER REVIEWED

In the 1960s, publications from the Framingham Heart Study indicated that cardiovascular events could have preventable or treatable risk factors (1). Because the participants in that study were White men and women, the question naturally arose about risk factors in other racial and ethnic groups. Subsequently, the National Heart, Lung, and Blood Institute (NHLBI) funded studies to expand research to include Black, Hispanic, and Native Hawaiian populations. Advisors to the NHLBI noted that American Indian populations had not been included. In 1987, the NHLBI released grants that focused on conducting research to better understand cardiovascular disease in American Indian populations.

NHLBI senior staff were skeptical about this project because of the rural populations and the role of tribal sovereignty. Initial funding provided for a 3-year study in 3 groups of American Indians. Grants were awarded for 3 field centers 1) to recruit and examine a cohort of Northern Plains Indians in the Dakotas and establish a cardiology reading center, 2) to recruit and examine an American Indian cohort from the multiple tribes in southwestern Oklahoma and establish a data coordinating center, and 3) to recruit and examine an American Indian cohort in southwestern Arizona and establish a core laboratory. During the past 35 years, 7 phases of the Strong Heart Study (SHS) have been funded and 2 cohorts have been recruited and examined (2,3). The original cohort focused on adult men and women aged 45 to 74 years (1). The second cohort focused on large families with members aged 15 years or older (2). The objective of this essay is to assist health care advocates who are contemplating, or currently working on, research in Indian Country. Many of the points addressed may be applicable to studies conducted in other remote or isolated populations.

Challenges in Conducting Research in Indian Country

American Indian tribes are autonomous and vary in community size, approach to governance, cultural norms, English-speaking ability, and economic development. Conduct of a multicenter study must be prepared to deal with these differences and their potential effect on recruitment and scientific translation.

The NHLBI recognized some potential challenges and took actions to address them. For example, to ensure that selected grantees had an existing mutually respectful working relationship with the tribes, preselection site visits were made to potential grantees. These visits required a meeting that included leadership from the participating tribes so that the relationships could be evaluated.

Site visits also explored the challenges in transportation, logistics, facilities, and personnel in conducting clinical examinations at each center. Distances from grantee institutions to study sites and within study sites were unusually long, with sites ranging from no reservations in Oklahoma to reservations spanning more than 4,000 square miles in South Dakota. Transportation options were limited and often affected by severe weather conditions that could dramatically affect research budgets and timelines.

Examination facilities were limited and often required the cooperation of the Indian Health Service (IHS) and local tribes. Early support from the IHS director facilitated access to examination rooms and medical records. Tribes often provided meeting space for steering committee and community meetings. Hiring and training local personnel to recruit and examine study participants turned out to be win–win: we found dedicated excellent staff from the communities who could be trained for the study needs, and who, in turn, trained the outside staff on cultural norms, ways of life, and approaches to day-to-day challenges. A detailed study manual facilitated initial training, periodic retraining, and constant referencing. All interviewers and clinical staff were centrally trained in examination procedures and certified by qualified ex-

perts. Quality control measures were built into the examinations and study procedures.

Clinic space and examination equipment often were not available and had to be acquired or transported to the multiple examination sites at the study centers. Centers relied on mobile vans that were designed and equipped to support examinations. Finally, timely transport of samples was often constrained due to distances between examination sites and shipping facilities. As a result, the study protocol required modification to allow samples to be properly stored and periodically shipped to the central laboratory.

Key Areas and Important Steps in Conducting Research

Based on the SHS experience, we summarize key areas and important steps to conduct a research project in American Indian communities (Table).

Building trust

Establishing and maintaining trust and good working relationships with the communities is the most important step to conduct a research project in an American Indian community (4). Holding study meetings in the participating communities whenever possible builds awareness of the study within the community, promotes the relationship between study staff and study participants, and allows study staff to better understand the culture, opportunities, and challenges at each study site. Tribal leadership is often overburdened with the business of the tribe. The governance processes for approvals by the tribes are likely to vary. Working patiently and cooperatively with the tribe not only built support for the SHS but also promoted a long-term partnership providing mutual benefit.

SHS investigators communicated with tribal leaders, tribal health boards, and communities before, during, and after funding to inform them of the opportunity to participate in the study, to obtain approvals in every area where examinations would be performed, and to share what was learned. The tribes are most interested in what they can expect to gain from participating in the study. It is essential for investigators to align their interests with the tribes' interests. Taking every opportunity to explain the goals and methods of the project, what is to be gained, what are the risks, and how the community will benefit contributes to a successful project outcome.

Transparent conduct of the study

Hiring from within the community, when possible, is very important. Having staff who understand how to navigate challenges and address difficult situations is useful, and training staff from the community means that they become advocates for the research study. In the SHS, we used NHLBI training programs to hire and train people from high school students to postgraduates. The success of that approach is evident from the long list of students who have chosen to pursue medical careers or assume key roles in this and other research projects in Indian Country (4). Requirements, such as quality control procedures, are often considered a waste of time by community staff and participants and must be explained as a critical part of any research endeavor. Increasing understanding will increase community acceptance of the study procedures. One example is the storage and use of samples for future research that could not necessarily be envisioned at the time of the initial study. After discussions with communities, stored samples and data were blessed by a spiritual leader in a "cedaring" ceremony and provision was made for the return or proper disposal of unused samples at study's end. The value of stored samples is easily illustrated in a recent study (5) in which urine samples were used to address exposure to heavy metals that have now been linked to the risk of cardiovascular disease.

Sharing results and mutual feedback

Sharing individual and study results is essential for a respectful and lasting partnership with the study community. Ideally, dialogue with the community improves the process (6,7). A summary of examination findings is provided to each participant. Scientific manuscripts and abstracts are shared with the tribes before presentation. A periodic newsletter for participants, tribes, and others has been published since 1989. A website describing all aspects of the SHS is available online (https://strongheartstudy.org). In addition, educational brochures are published on various subjects (eg, diabetes, high blood pressure, diet) for participants, and tribes are assisted in preparing their reports and proposals for funding. For example, tribes were assisted in applying for a diabetes project funded by the IHS. In 2022, a successful program (Strongheart Tribal Approach to Research) to fund tribal research was established to provide funds for tribal members to initiate and conduct research on their own interests. This program provided research experience and built awareness and appreciation for the process of conducting studies in the community.

Finally, investigators must also be willing to listen to community feedback about the conduct of the study, including what is missing, ineffective, or unacceptable, and what the priorities of the community are. In SHS, we found that the communities were very interested in the next generation. They wanted their children to be included in the study so the children would have healthier lives. As a result, the investigators proposed a family study that opened new avenues for research, such as research on risk factors in the young, disease trends, generational differences, genetics, and oth-

er "omics" studies. SHS has demonstrated that listening and working closely across populations that have unique risk factor profiles, lifestyles, and environmental exposures can help investigators gain clinical insights and develop public health approaches to improving population health (4,5,7,8). SHS continues to progress with new explorations of environmental factors, social determinants of health, cognitive function, and additional omics. SHS has demonstrated that research conducted thoughtfully, respectfully, and in cooperation with the populations being studied offers substantial scientific opportunities.

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Table

Table. Important Considerations When Establishing and Implementing a Research Project in Diverse Communities: Reflections From the Strong Heart Study

Key area	Important consideration
Building trust	Meet with community leaders and community members before proposal is submitted and after funding
	Explain goals of the project and describe the importance in addressing needs in the community
	Describe the potential benefits to the individual participants and to the community
	Describe in detail the planned methods, including any potential impact or risks
Transparent implementation	Emphasize that community members will be employed for all possible roles
	Describe the procedures and how the members will be trained to perform them
	Explain the meaning of quality control procedures
Sharing results and mutual feedback	Describe how results will be provided to participants and their health care providers and also available to communities for funding opportunities
	Ask for suggestions at each step and implement as many as possible
	Provide a summary report to the community leaders at the end of the project to bring study closure but also promote continuing partnership

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ORIGINAL RESEARCH

Depression and Incident Hypertension: The Strong Heart Family Study

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PEER REVIEWED

Summary

What is already known on this topic?

Compared with White Americans, American Indian adults have disproportionately high rates of depression. Previous studies in non-American Indian populations report that depression is common in people with uncontrolled hypertension and may interfere with blood pressure control.

What is added by this report?

After adjustment, study participants with depressive symptoms at baseline had 54% higher odds of developing hypertension during the follow-up compared with those without depressive symptoms at baseline.

What are the implications for public health practice?

Mental health is a key determinant of cardiovascular disease risk, suggesting the need for mental health outreach programs focusing on depression prevention to mitigate downstream effects on hypertension.

Abstract

Introduction

Compared with White Americans, American Indian adults have disproportionately high depression rates. Previous studies in non-American Indian populations report depression as common among people with uncontrolled hypertension, potentially interfering with blood pressure control. Few studies have examined the association of depressive symptoms with hypertension development among American Indians despite that population's high burden of depression and hypertension. We examined the association of depressive symptoms with incident hypertension in a large cohort of American Indians.

Methods

We studied 1,408 American Indian participants in the Strong Heart Family Study, a longitudinal, ongoing, epidemiologic study assessing cardiovascular disease and its risk factors among American Indian populations. Depressive symptoms were assessed by using the Center for Epidemiological Studies-Depression (CES-D) scale, 2001–2003. At each study examination in 2001–2003 and 2007–2009, blood pressure was measured 3 times. The average of the last 2 measurements taken at baseline and follow-up examinations was used for analyses. Incident hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure of ≥90 mm Hg, or use of hypertension medications at follow-up. To account for within-family correlation, we used generalized estimating equations to examine the association of depressive symptoms with incident hypertension.

Results

During follow-up, 257 participants developed hypertension. Participants with symptoms consistent with depression (CES-D ≥16) at baseline had 54% higher odds of developing hypertension during follow-up (OR = 1.54; 95% CI, 1.06–2.23) compared with those without depression (CES-D <16) at baseline after adjustment for other risk factors.

Conclusion

These data suggest that participants who experienced symptoms consistent with depression were at increased odds of incident hypertension.

Introduction

Hypertension and depression are highly prevalent in the US (1,2). However, compared with White American adults, American Indian adults have disproportionately higher rates of cardiovascular risk factors and mental health issues, including hypertension and depression. In 2019, more than 18% (n = 260,000) of surveyed American Indians/Alaska Natives (AI/AN) aged 18 years or older experienced mental illness during the past year (3). Relatedly, in

2018, AI/AN adults were 50% more likely to be diagnosed with coronary heart disease than White adults. In that same year, AI/AN adults were 10% more likely than White adults to have high blood pressure (4). In the Strong Heart Family Study (SHFS), the largest cohort study of cardiometabolic health among American Indians in the US, nearly 50% of participants reported depressive symptoms (5) at the baseline study examination. Of these participants, approximately 30% experienced moderate to severe symptoms of depression.

Previous research has shown that depression is associated with hypertension, and a meta-analysis of prospective cohort studies report depression as an independent risk factor for hypertension (6). Previous studies have also shown that depression is common in people with uncontrolled hypertension and may interfere in blood pressure control (7). To our knowledge, few studies have examined the association of depression with development of hypertension among American Indians, despite the high burden of hypertension and depression in this population.

The purpose of our analysis was to examine the relationship of depressive symptoms with incident hypertension in a large cohort of American Indians. As a secondary exploratory analysis, we also examined whether self-reported experiences with social support affects this relationship in a subset of participants. We hypothesized that participants who reported a greater number of depressive symptoms were more likely to develop hypertension than participants who reported fewer depressive symptoms. Additionally, we hypothesized that the magnitude of the association of depressive symptoms with hypertension would be higher among participants who reported low levels of social support.

Methods

Setting and study population

SHFS is a family-based longitudinal study of cardiovascular disease (CVD) and its risk factors in 12 American Indian communities in Arizona, North Dakota, South Dakota, and Oklahoma. The study was designed to better understand genes that contribute to risk of CVD among American Indians (8). The study comprised 2,756 American Indian people aged 14 to 93 years from 92 multigenerational families. The cohort included 409 middle-aged or older participants (14.8%) from the original population-based Strong Heart Study and 3,145 of their spouses, offspring, offspring spouses, and grandchildren. For a family to be eligible to participate in SHFS, a participant of the original Strong Heart Study must have had a minimum of 4 full or half siblings and a total of 12 or more living offspring from the second generation who were aged 18 years or older. Smaller families were not eligible for participation. SHFS participants completed 2 examinations over an 8-year period: a baseline examination in 2001–2003 and a follow-up examination in 2006–2009. Surveillence for morbidity and mortality is ongoing. Each study examination included a personal interview, physical examination, medication review, and laboratory work-up. Data collection procedures have been described in detail in previous publications and are summarized and are summarized by North and colleagues and by Lee and colleagues (8,9). SHFS was approved by the institutional review board of each affected Indian Health Service, and written informed consent was obtained from study participants at each study examination.

Of the 2,756 participants who completed the baseline examination, we excluded SHFS participants who were pregnant (n = 3), because pregnancy may influence the risk of symptoms consistent with depression. Also, we excluded those who had prevalent hypertension (ie, use of antihypertensive medications, diuretics, or beta blockers; SBP ≥140 mm Hg; or DBP ≥90 mm Hg) (n = 713), did not complete the depression assessment (n = 195), or reported taking antidepressant or antipsychotic medications at baseline (n = 48). We excluded participants who reported taking antidepressant or antipsychotic medications at baseline because this may influence the risk of depressive symptoms, potentially clouding study results, and could attenuate the relationship between symptoms consistent with depression and incident hypertension toward the null. We also excluded participants who did not complete the follow-up exam (n = 389). Our total analytic sample comprised 1,408 participants.

Data collection

Assessment of depressive symptoms. The exposure of interest for our study was experiencing symptoms consistent with clinical depression (yes/no). We used the 20-item Center for Epidemiologic Studies Depression (CES-D) scale to assess depressive symptoms at baseline. The CES-D scale is a valid and reliable instrument used to assess depressive symptoms experienced during the past week (10). Symptoms assessed include, for example, feelings of guilt and hopelessness, feeling blue, experiencing insomnia, and the inability to focus. For each question, response options were captured by using a 4-point Likert scale ranging from 0 (none of the time/rarely) to 3 (most of the time). Responses to individual questions were summed after reverse coding of positively framed items per established CES-D guidelines (total possible score range: 0–60). A higher CES-D score is consistent with greater depressive symptomology, and scores of 16 or higher are consistent with diagnoses of major depressive disorder. As in previous SHFS analyses, CES-D scores were categorized as consistent with depression (CES-D ≥16) versus not consistent with depression (CES-D <16) (11).

Assessment of hypertension. Our primary outcome of interest was incident hypertension. At each study examination, blood pressure was measured 3 times on the right arm with a standard mercury sphygmomanometer after 5 minutes rest with the participant seated (12). The average of the last 2 measurements taken at both the baseline and follow-up examinations were used for these analyses. Incident hypertension was defined as SBP ≥140 mm Hg, DBP ≥90 mm Hg, or use of hypertension medications at follow-up.

Measurement of covariates. Detailed information on important confounding variables including demographic characteristics, diet and physical activity, and other CVD risk factors (eg, smoking status, prevalent diabetes) were collected at the baseline examination (2001–2003) by standardized interviews.

Past-year diet was assessed by using a Block 119-item food frequency questionnaire with an ethnic foods supplement (13). Diet quality was classified by using the Alternative Healthy Eating Index (AHEI) (14). Physical activity was captured using Accusplit AE120 pedometer (8). Average steps per day for each participant were estimated as the mean steps per day across the 3 to 7 days that the pedometer was worn.

Social support was categorized dichotomously based on participants' response to the question: "Can you count on anyone to provide you with emotional support (talking over problems or helping you make a difficult decision)?" Only a subset of the study sample (n = 332) completed the social support assessment.

Anthropometric measures were obtained with the participant wearing light clothing and no shoes. Bodyweight was measured with a Tanita BWB-800 5 adult digital scale. Height was measured with a vertical-mounted ruler. Body mass index (BMI) was calculated as body weight divided by height-squared (8,9).

Blood samples were collected after a 12-hour overnight fast and were stored at −70 °C. Plasma glucose, LDL cholesterol, and HDL cholesterol were measured by enzymatic methods (9). Diabetes was defined based on 2003 ADA criteria (15), including use of insulin or oral antidiabetic medication or a fasting plasma glucose level greater than or equal to 126 mg/dL.

Statistical analyses

Two sequential generalized estimating equation (GEE) models were run to assess the association of symptoms consistent with depression with incident hypertension. GEE was used to address potential familial correlation between participants within the data and were run with the assumption of an independent working correlation and specification of robust SEs. In total, 86 family clusters were included in the analysis with a mean of 16 participants per family cluster (range: 1–57 participants per family cluster). Model 1 (minimally adjusted model) adjusted for age (years), sex (male or female), and study site (Arizona, Oklahoma, North Dakota, South Dakota). Model 2 (primary model) further adjusted for CVD risk factors selected a priori based on potential associations with depression and blood pressure, including LDL cholesterol (mg/dL), HDL cholesterol (mg/dL), prevalent diabetes status (yes/no), smoking status (never/former/current), BMI, AHEI (score), and physical activity (steps per day).

In the exploratory analyses, we examined potential interaction of depression and social support with incidence of hypertension by inclusion of an interaction term (depression*social support) in the primary model and using a Wald test for significance. We used complete case analysis to conduct analyses. All statistical analyses were performed in R software (R Foundation).

Results

The median age of study participants was 33.5 years (range, 14.1 y–86.0 y) and 36.5% of the analytic cohort identified as male. Baseline characteristics of study participants were assessed according to CES-D (<16 or ≥16) (Table 1). At baseline, 27.3% (n = 385) of study participants reported symptoms consistent with depression (ie, CES-D score, ≥16). Participants who reported baseline symptoms consistent with depression were more likely to be female (74.0% vs 59.5%), were slightly younger (32.6 y vs 35.4 y), had less education (11.5 y vs 12.4 y), had a higher BMI (31.2 vs 29.9), and reported less physical activity (5,640 steps per day versus 6,690 steps per day) compared with participants with a CES-D score of <16. There was no difference in diet quality based on CES-D score.

During the follow-up period, 257 participants developed hypertension (Table 2). Participants who developed hypertension were more likely to be older (42.0 years old vs 33.0 years old), male (44.7% vs 34.7%), and have prevalent diabetes at baseline (21.8% vs 6.6%), reported fewer steps per day (5,730 steps per day vs 6,540 steps per day), and had higher BMI (32.4 vs 29.8) than participants who did not develop hypertension during follow-up. Additionally, participants who developed hypertension were more likely to smoke (43.2% vs 38.7%) than participants who did not develop hypertension. We observed no differences in education or diet quality based on hypertension status.

In multivariable GEE analyses, participants with baseline symptoms consistent with depression (CES-D ≥16) had 54% higher odds of developing hypertension during the 3- to 8-year follow-up (OR = 1.54; 95% CI, 1.06–2.23) compared with those with baseline symptoms not consistent with depression (CES-D <16)

after adjustment for age, demographic, behavioral, and dietary factors (Table 3).

We found no significant interaction of baseline depression status with social support on odds of hypertension in a model adjusted for age, sex, education, study center, baseline blood pressure measurement, HDL cholesterol, LDL cholesterol, smoking status, BMI, diabetes status, physical activity, diet index, sex, study site, and prevalent diabetes ($P = .35$).

Discussion

In our large cohort study of American Indian adults, participants who reported symptoms consistent with depression at baseline were more likely to develop hypertension when compared with participants who did not report symptoms consistent with depression. This finding supports the hypothesis that depression is associated with increased odds of incident hypertension.

Our findings are consistent with the findings from various prospective studies (17–19) and 1 meta-analysis of 9 (6) studies in non-American Indian populations that show a positive association between depressive symptoms and hypertension. Although these studies used a wide variety of instruments to assess depressive symptoms (eg, Depression Anxiety Stress Scale (DASS-21) [19], National Epidemiologic Survey on Alcohol and Related Conditions [17], 30-item General Health Questionnaire Depression subscale [18]), these findings highlight a positive association of depressive symptoms with hypertension in diverse populations across a wide range of ages and geographic contexts.

Our findings are discordant with several studies that reported no (or inverse) associations betweem depressive symptoms and incident hypertension (20,21). Differences in study populations according to age and geography may account for contradictory findings across studies (22). For instance, although it has not been extensively studied, the etiology of depressive symptoms possibly may be different in old versus young populations (23). Additionally, access to quality health care (including mental health services) differs according to area of residence (eg, urban vs rural, US vs non-US, American Indian reservation vs nonreservation). Finally, the lived experiences of American Indians are different than those of non-American Indians, including the impact of multigenerational historical trauma and structural racism on mental and physical health.

To our knowledge, no studies to date have examined the relationship of depression with the development of hypertension in American Indians. In 1 cross-sectional study among 500 older AI/AN adults who resided in urban areas in the Pacific Northwest, clinic patients with prevalent hypertension were more likely to have depression than patients without hypertension (24). However, this cross-sectional study was unable to infer whether depression increased odds of hypertension or vice versa. Our work complements findings from the SHFS that reported that participants with severe depressive symptoms (ie, CES-D ≥16) have a 71% higher odds of developing CVD compared with participants who did not report symptoms consistent with depression (OR = 1.71; 95% CI, 1.01–2.91) (25).

Several studies of Indigenous populations point to high levels of depression and CVD risk factors and diseases (26–28). The high burden of depressive symptoms among American Indians may be due at least in part to generations of oppression and historical trauma, including forced migration to reservations, abuse and neglect of American Indian young people at government-operated boarding schools, and near-eradication of many tribal languages, spiritual practices, and cultures (29). Historical trauma and present-day socioeconomic factors may also affect the availability, accessibility, and use of mental and physical health services by American Indian communities. Because of historically poor interactions with the US government, present-day American Indian communities may have lost trust in many institutional sources, including some health care settings (30). Additionally, given the historical relationship between American Indian and US government authorities, many American Indians may prefer to seek care from American Indian mental and physical health care providers, who are scarce (31). The effects of a long history of oppression, colonization, and genocide have had lasting effects on the health of American Indians, which may explain in part the high rates of depression and CVD risk in many of their communities.

The mechanism by which depressive symptoms may influence odds of hypertension is multifaceted and includes stress, inflammation, and neurotransmission processes. Studies have shown that depression can increase the body's sympathetic tone and cortisol, which increase systemic inflammation, and lead to many cardiometabolic risk factors, including hypertension (32). Established research has linked dopamine to depression because this neurotransmitter plays a vital role in a person's ability to experience pleasure. Specifically, a dopamine deficit has been linked to anhedonia, the core feature of major depressive disorder (33). Recent studies have shown that lack of dopamine at key brain sites can increase blood pressure (7). Depression may also increase risk of key cardiovascular risk factors, including physical inactivity, obesity, poor dietary practices, and low social support (34).

We did not observe a significant effect of the interaction of social support with depression status on odds of incident hypertension. This may be due in part to our incomplete measurement of social support — we had only 1 question on social support — or the limited power of this measure, because this question was only asked

of a subset of SHFS participants (n = 332). Future studies are needed that include a comprehensive measure of social support and community and cultural engagement to better assess whether social support may mitigate the risk of symptoms consistent with depression on incident hypertension.

Our study has many strengths. To our knowledge, ours is the first to examine the association of depressive symptoms with incident hypertension in a well-characterized multitribal cohort of American Indians with detailed measures of depressive symptoms, hypertension, and key covariates. However, our study is not without limitations. Although the CES-D scale has been shown to be a valid and reliable measure of depressive symptoms in noninstitutionalized diverse populations (10), it is susceptible to social desirability bias. Residual confounding by unmeasured or poorly measured factors is possible. Finally, although these results are generalizable to American Indians from large families who reside in primarily rural communities in the Great Plains, Midwest, and Southwestern regions of the US, it is unclear whether findings are generalizable to other populations.

In conclusion, in this large study of American Indian adults, symptoms consistent with depression were found to be positively associated with incident hypertension. The study adds to a growing body of evidence identifying mental health as a key determinant of CVD risk and suggests the need for mental health outreach programs that focus on prevention of depression to mitigate downstream effects on hypertension.

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Tables

Table 1. Characteristics of Study Participants[a] (N = 1,408), by Baseline Depressive Symptoms, CES-D Scale[b]

Characteristic	CES-D score not consistent with depression[c] (N = 1,023)	CES-D score consistent with depression[d] (N = 385)	Total sample (N = 1,408)
Sex, n (%)			
Male	414 (40.5)	100 (26.0)	514 (36.5)
Female	609 (59.5)	285 (74.0)	894 (63.5)
Age, y, mean (SD)	35.4 (14.7)	32.6 (12.8)	34.6 (14.3)
Education, y, mean (SD)	12.4 (2.19)	11.5 (2.17)	12.2 (2.23)
BMI (kg/m^2), mean (SD)	29.9 (6.88)	31.2 (8.28)	30.2 (7.31)
Diabetes, n (%)			
Yes	89 (8.7)	43 (11.2)	132 (9.4)
No	929 (90.8)	339 (88.1)	1268 (90.1)
Current smoker, n (%)			
Yes	375 (36.7)	182 (47.3)	557 (39.6)
No	647 (63.2)	202 (52.5)	849 (60.3)
AHEI, mean (SD)	43.4 (9.06)	43.2 (8.76)	43.3 (8.98)
Physical activity (continuous, steps per day), mean (SD)	6,690 (3,980)	5,640 (3,510)	6,400 (3,880)

Abbreviations: AHEI, Alternative Healthy Eating Index; BMI, body mass index; CES-D, Center for Epidemiologic Studies Depression scale.
[a] The Strong Heart Family Study (8).
[b] Symptoms are scored per established CES-D guidelines (total possible score range: 0–60). A higher CES-D score is consistent with greater depressive symptomology, and scores of 16 or higher are consistent with diagnoses of major depressive disorder.
[c] CES-D <16. Missingness removed; therefore, not all columns total 100%.
[d] CES-D ≥16. Missingness removed; therefore, not all columns total 100%.

Table 2. Demographic and Health Characteristics by Hypertension[a] Status at Follow-Up, Participants[b] (N = 1,408)

Characteristic	No hypertension (n = 1,151)	Hypertension (n =257)	Total (n =1,408)
Depression, n (%)			
Not consistent with depression (CES-D <16)[c]	844 (73.3)	179 (69.6)	1023 (72.7)
Consistent with depression (CES-D ≥16)[b]	307 (26.7)	78 (30.4)	385 (27.3)
Sex, n (%)			
Male	399 (34.7)	115 (44.7)	514 (36.5)
Female	752 (65.3)	142 (55.3)	894 (63.5)
Age, y, mean (SD)	33.0 (13.6)	42.0 (14.9)	34.6 (14.3)
Education, y, mean (SD)	12.1 (2.23)	12.4 (2.22)	12.2 (2.23)
BMI (kg/m^2), mean (SD)	29.8 (7.15)	32.4 (7.63)	30.2 (7.31)
Diabetes, n (%)			
Yes	76 (6.6)	56 (21.8)	132 (9.4)
No	1068 (92.8)	200 (77.8)	1268 (90.1)
Current smoker, n (%)			
Yes	446 (38.7)	111 (43.2)	557 (39.6)
No	703 (61.1)	146 (56.8)	849 (60.3)
AHEI, mean (SD)	43.1 (9.09)	44.5 (8.40)	43.3 (8.98)
Physical activity (continuous, steps per day), mean (SD)	6,540 (3,870)	5,730 (3,870)	6,400 (3,880)

Abbreviations: AHEI, Alternative Healthy Eating Index; BMI, body mass index; CES-D, Center for Epidemiologic Studies Depression Scale.
[a] Hypertension defined as systolic blood pressure of ≥140 mm Hg, diastolic blood pressure of ≥90 mm Hg, or use of hypertension medications at follow-up.
[b] The Strong Heart Family Study (8).
[c] Symptoms are scored per established CES-D guidelines (total possible score range: 0–60). A higher CES-D score is consistent with greater depressive symptomology, and scores of 16 or higher are consistent with diagnoses of major depressive disorder.

Table 3. Odds of Incident Hypertension[a], by Depressive Symptom Exposure, All Study Centers, Participants[b] (N = 1,408)

Variable	Model 1[c], OR (95% CI) (n = 1,408)	Model 2[d], OR (95% CI) (n = 1,168)
Symptom category		
Not consistent with depression (CES-D <16[e])	1 [Reference]	1 [Reference]
Consistent with depression (CES-D ≥16[e])	1.54 (1.12–2.11)	1.54 (1.06 – 2.23)

Abbreviations: BMI, body mass index; CES-D, Center for Epidemiologic Studies Depression scale; OR, odds ratio.
[a] Hypertension defined as systolic blood pressure of ≥140 mm Hg, diastolic blood pressure of ≥90 mm Hg, or use of hypertension medications at follow-up.
[b] The Strong Heart Family Study (8).
[c] Model 1: Adjusted only for age, sex, education, study center.
[d] Model 2: Further adjusted for HDL cholesterol, LDL cholesterol, smoking status, BMI, diabetes status, physical activity, diet index.
[e] Symptoms are scored per established CES-D guidelines (total possible score range: 0–60). A higher CES-D score is consistent with greater depressive symptomology, and scores of 16 or higher are consistent with diagnoses of major depressive disorder.

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ORIGINAL RESEARCH

Uranium Exposure, Hypertension, and Blood Pressure in the Strong Heart Family Study

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PEER REVIEWED

Summary

What is already known on this topic?

American Indian communities disproportionately experience elevated exposure to uranium and a high prevalence of cardiovascular risk factors. Prior cross-sectional evidence suggests the two may be related but lacks sufficient representation from this population.

What is added by this report?

We leveraged the Strong Heart Family Study, the largest ongoing epidemiologic cohort of American Indians from the Great Plains and Southwest, to prospectively evaluate the associations between urinary uranium with hypertension and blood pressure measures. We found positive associations with increasing quartiles of urinary uranium levels.

What are the implications for public health practice?

Policy, primary, and secondary interventions should address inequities in uranium exposure via drinking water, diet, and dust, focusing on community education about relevant local environmental sources.

Abstract

Introduction

Uranium is common in drinking water, soil, and dust in American Indian communities. Hypertension is a cardiovascular risk factor affecting American Indians. We evaluated the association between uranium exposure and incident hypertension and changes in blood pressure among Strong Heart Family Study participants.

Methods

We included 1,453 participants ≥14 years with baseline visits in 1998–1999 or 2001–2003, and follow-up in 2001–2003 and/or 2006–2009. We estimated the association of urinary uranium with changes in systolic and diastolic blood pressure levels over time and hypertension incidence; we accounted for family clustering.

Results

Median (IQR) baseline urinary uranium levels were 0.029 (0.013–0.059) µg/g creatinine; 17.4% (n = 253) of participants developed hypertension. In the comparison of the urinary uranium quartile 4 (highest concentration) and quartile 1 (lowest concentration), the multi-adjusted risk ratio (95% CI) of incident hypertension was 1.44 (1.04–1.99). The associations between urinary uranium with changes in systolic and diastolic blood pressure were null and nonlinear, respectively. Both associations were modified by study site, and diastolic blood pressure showed a positive association beyond 5 µg/g creatinine. The association between urinary uranium and change in systolic blood pressure was inverse in Arizona and Oklahoma, and positive in North Dakota/South Dakota at higher ends of the uranium distribution.

Conclusion

Findings suggest a higher risk for hypertension at uranium levels typical of the Southwest and Great Plains than at levels in other regions (<0.01 µg/g creatinine); the associations with changes in systolic and diastolic blood pressure levels were consistent with a positive association with higher uranium exposure. Prospective research is critical to characterize the cardiovascular effects of uranium and develop preventive strategies for US Indigenous communities disproportionately exposed.

Introduction

Uranium is a naturally occurring toxic metal commonly found in the western United States. Populations from several American Indian communities in the Southwest and Great Plains have shown,

on average, higher metal levels in their urine compared with urban populations across the US (1–3). This disproportion might be explained by elevated levels of uranium in rocks and soil that lead to groundwater and surface water contamination in rural and suburban areas. Drinking water is a substantial source of uranium exposure in the US and is particularly relevant among rural and Native American populations, who rely more on private, unregulated water wells than on public sources in some areas (4). Both unregulated wells and public drinking water are major sources of total uranium exposure in American Indian communities (5). In many areas of the Southwest and Midwest, water wells exceed the US Environmental Protection Agency drinking water standard of 30 parts per billion (ppb) uranium in public drinking water supplies (6,7). Uranium groundwater contamination occurs naturally but is also exacerbated by a long history of uranium mining in these areas, with little to no clean-up (8). Most uranium mines are located on either federal or tribal land (9). For example, approximately 500 abandoned uranium mines are in the Navajo Nation, and an estimated 286,346 American Indians live less than 10 km from a mine (10). Climate change may also increase the mobilization of metals into groundwater (11,12), along with increased use of nitrate-containing fertilizer, which releases uranium stores (13).

The leading cause of death among American Indian people is cardiovascular disease (CVD), for which hypertension is a major risk factor (14). American Indian adults are 10% more likely than White adults to have high blood pressure (15). According to the Strong Heart Study (SHS), the prevalence of hypertension — defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or use of antihypertensive medication — among American Indians aged 45 to 74 years was close to 50% at the Oklahoma and Arizona study centers and approximately 25% at the North Dakota and South Dakota study center at baseline (1989–1991) (16).

Uranium is known to cause kidney damage and cancer, but it is unclear if it also has implications for CVD — in particular, hypertension. In a mixture analysis in the 2011–2016 National Health and Nutrition Examination Survey (NHANES), urinary uranium was significantly associated with prevalent hypertension, which supports evidence indicating that uranium exposure can be a risk factor for hypertension (17). Previous studies of uranium workers showed that uranium exposure may be associated with angina, increases in deaths due to circulatory system disease, and hypertension or risk factors for the development of hypertension (18–20). On the Navajo Nation, uranium mining exposure was associated with hypertension, and further molecular evidence showed that physical proximity to abandoned uranium mines predicted endothelial transcriptional response to serums that included biomarkers of inflammation chemokine (C–C motif) ligand, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 (21,22). However, little is known about the relationship between uranium exposure among people with chronic low-level uranium exposure and incidence of hypertension and change in blood pressure over time. More research is needed to understand the effects of chronic low-level uranium exposure on CVD (23).

In this study, we examined the association of differential uranium exposure across the 3 centers of the Strong Heart Family Study (SHFS) with the incidence of hypertension and with blood pressure change during the follow-up period. The SHFS is a family expansion of the original SHS cohort, and it provides data on urinary uranium. We used urinary uranium levels as a marker of internal uranium dose. We hypothesized that after controlling for relevant sociodemographic and blood pressure risk factors, higher uranium exposure versus lower exposure, as determined in urine, would be associated with increased systolic and diastolic blood pressure levels and an increased risk of hypertension.

Methods

The SHS is a population-based study of CVD in 12 participating American Indian communities in Arizona, Oklahoma, North Dakota, and South Dakota. Recruitment of men and women aged 45 to 74 years took place from 1989 to 1991. In 1998, the SHFS began; it was designed to study genetic and environmental determinants of diabetes and CVD among family members of the SHS (24). The investigators recruited 2,919 participants during 1998–1999 (Visit 3 pilot) and 2001–2003 (Visit 4), after excluding a community that declined participation in additional research. Participants recruited in 1998–1999 (n = 428) had follow-up visits in 2001–2003 and 2006–2009 (Visit 5). Participants recruited in 2001–2003 (n = 2,491) had a single follow-up visit in 2006–2009.

Hypertension is common among people with diabetes and is associated with renal dysfunction via mechanisms that include increased renal sodium reabsorption and endothelial cell dysfunction (25). We included young adult and adult participants who were free of diabetes at baseline and had sufficient urine available for uranium analyses (n = 1,948). We excluded 2 participants whose creatine-adjusted urinary uranium levels were greater than 10 times the 99th percentile. We also excluded participants missing information on relevant confounders at baseline, including education (n = 9), smoking status (n = 2), body mass index (BMI) (n = 7), systolic blood pressure (n = 1), diastolic blood pressure (n = 1), estimated glomerular filtration rate (eGFR) (n = 1), urinary cadmium (n = 25), or creatinine. We further excluded participants missing data on systolic and diastolic blood pressure at follow-up Visit 5 (n = 25) and prevalent hypertension cases at baseline (n = 422), making 1,453 participants available for this study. The SHS

and SHFS protocols were approved by participating tribal communities and all institutional review boards (IRBs), including the IRBs of the Indian Health Service. All participants provided written informed consent, and all participating communities reviewed and approved this article.

Urinary uranium measurements

Spot urine samples were frozen within 1 or 2 hours of collection and stored at −80 °C at Medstar Health Research Institute in Maryland. Detailed methods are described elsewhere (26). Urinary uranium concentrations were measured in spot urine collected at the baseline SHFS visit by using inductively coupled plasma-mass spectrometry with a multi-element protocol at the Trace Element Laboratory of Graz University, Austria (26).

To account for urine dilution, we divided urinary uranium concentrations by urinary creatinine concentrations (µg/g creatinine). The limit of detection (LOD) for uranium was 0.01 µg/L of urine (81.4% >LOD). All samples below the LOD were replaced by the LOD divided by the square root of 2. Urinary uranium was right-skewed and log-transformed for all analyses with a continuous predictor.

Hypertension measurements

Blood pressure was determined by measuring brachial artery blood pressure (first and fifth Korotkoff sounds) 3 consecutive times with a mercury sphygmomanometer (WA Baum Co, Inc). Participants were seated and rested for 5 minutes before blood pressure measurements. The cuff was placed on the right arm, pulse occlusion pressure was determined, and the cuff was inflated to 20 mm Hg above that pressure. To estimate blood pressure, the mean of the last 2 measurements was used. Hypertension was defined as the use of antihypertensive medication, or a systolic blood pressure of ≥140 mm Hg or a diastolic blood pressure of ≥90 mm Hg. At baseline, by design for this study, none of the participants were taking antihypertensive medication. At the follow-up visits, a constant (10 mm Hg for systolic blood pressure and 5 mm Hg for diastolic blood pressure) was added to the blood pressure of participants using antihypertensive medication to correct for the effect of treatment on blood pressure levels. This is an established method to adjust for medication use that has less bias and greater power than other methods (27).

Other variables

Participant sociodemographic and covariate information (age, sex, education, study center, BMI, smoking status, drinking status, prediabetes status, and eGFR) was obtained from SHFS baseline questionnaires that included standardized interviews, medication reviews, and physical examinations as detailed previously (28,29). Prediabetes was defined according to the 1997 American Diabetes Association criteria for impaired fasting glucose tolerance (blood glucose level 110–125 mg/dL) (30). eGFR was calculated by using the Chronic Kidney Disease Epidemiology Collaboration equation (31).

Statistical analysis

We compared participant baseline characteristics of those with and without incident hypertension and across quartiles of urinary uranium concentrations. We described baseline characteristics, including age (years; continuous), sex (male, female), study center (Arizona, Oklahoma, North Dakota, and South Dakota), education (<12, 12, >12 y), smoking status (never, ever, current), alcohol status (never, ever, current), BMI (continuous), prediabetes status (normal fasting glucose, impaired fasting glucose), systolic blood pressure (mm Hg; continuous), diastolic blood pressure (mm Hg; continuous), eGFR (mL/min; continuous) between those with and without incident hypertension and across quartiles of urinary uranium concentrations. To test group differences, we used Kruskal–Wallis tests for continuous values and χ^2 tests for categorical variables.

We jointly assessed the prospective association of baseline urinary uranium concentrations with incident hypertension by using a modified Poisson regression with robust variance and the prospective association between baseline urinary uranium concentrations and changes in blood pressure levels measured at follow-up versus baseline by using linear regression (32). To address the lack of independence among family members in the SHFS, we used generalized estimating equations (GEEs). In the main analysis, we estimated the risk ratio (RR) and 95% CIs for incident hypertension. As measured in urine, we calculated the association of uranium exposure with incident hypertension per interquartile range (IQR) increase, quartiles, and with restricted cubic splines. We determined the mean difference (95% CI) for the change in blood pressure levels between baseline and follow-up by baseline urinary uranium levels.

Urinary uranium was right-skewed and log-transformed for analysis. To assess normality assumptions, we used Q–Q plots and kernel density plots. We modeled urinary uranium concentrations as quartiles, continuous log-transformed (and reported per IQR), and restricted cubic splines (knots at 10th, 50th, and 90th percentiles) to allow for flexibility in the dose–response. We used a priori knowledge to make progressive adjustments for available variables associated with hypertension, blood pressure, and uranium. Model 1 was adjusted for age, sex, study center, and smoking status. Model 2 was further adjusted for eGFR, prediabetes status, and BMI. Model 3 was further adjusted for log-transformed urinary arsenic (µg/g creatinine) and cadmium (µg/g creatinine). We

analyzed possible effect modification by study center by stratifying by study center. Uranium exposure varied by study center, so we assessed possible effect modification for all main analyses, with adjustment for confounders included up to Model 3 (except study center). We obtained *P* values for interactions by using Wald tests for multiple coefficients. As a sensitivity analysis, we repeated our main models, adjusting for specific gravity instead of standardizing urinary uranium by urinary creatinine because creatinine is affected by kidney function and uranium is nephrotoxic.

Results

The median age of study participants was 34.1 years; 38.2% of participants were male (Table 1). Median (IQR) urinary uranium concentration was 0.029 µg/g (0.014–0.059). Of the 1,453 participants without hypertension at baseline, 253 (17.4%) developed hypertension during follow-up (mean age, 41.5 y). Compared with participants who did not develop hypertension during follow-up, those who developed hypertension were significantly more likely to be older, be male, self-report as an ever alcohol user, have a higher BMI, have impaired fasting glucose, and have lower eGFR. Median (IQR) levels of urinary uranium were higher among participants from Arizona (0.04 [0.02–0.07] µg/g), and North Dakota and South Dakota (0.04 [0.02–0.08] µg/g) than among participants from Oklahoma (0.02 [0.01–0.03] µg/g). Participants with lower education levels and those with higher eGFR levels were more likely to have higher urinary uranium levels (Table 2).

Incident hypertension

In the fully adjusted models, the RRs (95% CI) for incident hypertension for the second, third, and fourth quartiles of urinary uranium compared with the first quartile were 1.31 (0.96–1.78), 1.32 (0.95–1.83), and 1.44 (1.04–1.99) in the fully adjusted model (Table 3, Model 3), including adjustment for arsenic and cadmium. The RR (95% CI) for incident hypertension comparing the 25th and 75th percentiles was 1.15 (0.99–1.33) (Table 3). Uranium remained associated with incident hypertension in flexible dose–response models (Figure). In stratified models by study center, the RRs (95% CI) per IQR of urinary uranium were 1.01 (0.64–1.59) for Arizona, 1.25 (0.96–1.61) for Oklahoma, and 1.06 (0.88– 1.28) for North Dakota and South Dakota (*P* value for interaction = .55).

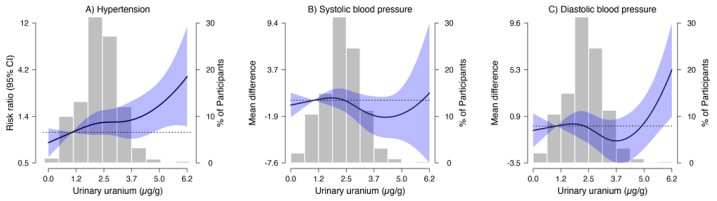

Figure. Risk ratio (RR) of hypertension (A) and mean difference (95% CI) for the change in systolic and diastolic blood pressure (mm Hg) levels at follow-up versus baseline (B, C) per log urinary uranium (µg/g creatinine) (N = 1,453), Strong Heart Family Study 1998–2009. The solid black line indicates adjusted effect estimate; shading indicates 95% CIs. Effect estimates were calculated by using restricted cubic splines for uranium with knots at the 10th (referent), 50th, and 90th percentiles of the urinary uranium (µg/g creatinine) distribution, and adjusted for sex, age, smoking status, study center, eGFR, prediabetes status, log urinary arsenic, and log urinary cadmium. Models include generalized estimating equations (GEEs) to account for the clustering of participants within families. Histograms indicate the distribution of log-transformed urinary uranium levels. Incident hypertension was defined as having a systolic blood pressure ≥140 mm Hg OR diastolic blood pressure ≥90 mm Hg OR taking hypertension medication. The horizontal dashed line indicates no association between urinary uranium and the outcomes.

Blood pressure

In the fully adjusted models, the mean difference (95% CI) for the change in systolic blood pressure at follow-up versus baseline per IQR of urinary uranium was −0.02 (−0.04 to 0.01) mm Hg, with evidence of nonlinearity for quartiles 2, 3 and 4 compared with the lowest quartile (Table 4, Model 3). We found no significant association between urinary uranium and diastolic blood pressure, and results followed a similar direction as for systolic blood pressure (Table 4). In models with flexible splines, the associations of urinary uranium with systolic blood pressure and diastolic blood pressure were nonlinear, with a potential increase in systolic blood pressure and particularly diastolic blood pressure at follow-up versus baseline at higher baseline urinary uranium levels (Figure).

We observed differences in the association between urinary uranium and blood pressure by study center. In stratified models by study center, the change in systolic blood pressure at follow-up versus baseline per IQR of urinary uranium was inverse in Arizona and Oklahoma and positive in North Dakota and South Dakota at higher ends of the uranium exposure distribution. The change in diastolic blood pressure at follow-up versus baseline per IQR of urinary uranium was inverse in Arizona and positive in Oklahoma and North Dakota and South Dakota at higher ends of the uranium exposure distribution. Corresponding fully adjusted mean differences (95% CI) for the change in systolic blood pressure at follow-up versus baseline per IQR of urinary uranium were −0.04 (−0.14 to 0.05) mm Hg for Arizona, 0.01 (−0.01 to 0.03) mm Hg for Oklahoma, and −0.04 (−0.07 to 0) mm Hg for North Dakota and South Dakota *(P* value for interaction = .03).

Sensitivity analyses

We observed similar and stronger findings when adjusting for specific gravity instead of standardizing urinary uranium for urinary creatinine. In fully adjusted models, the RR (95% CI) of hypertension comparing the 75th and 25th percentiles was 1.23 (1.05–1.44). The mean difference (95% CI) for the change in systolic blood pressure at follow-up versus baseline per IQR of urinary uranium was −0.01 (−0.05 to 0.02) mm Hg. Results were similar for urinary uranium and diastolic blood pressure compared with urinary creatinine standardization. In flexible spline dose–response plots, the association of urinary uranium with systolic blood pressure was linear and inverse at higher baseline urinary uranium levels.

Discussion

In the SHFS, conducted with American Indian communities in the Southwest and the Great Plains, urinary uranium was associated with a moderately increased risk for hypertension. The dose–response was linear for hypertension, null for the change in systolic blood pressure, and nonlinear for the change in diastolic blood pressure, which showed a positive association only beyond 5 µg U/g creatinine. We observed effect measure modification by study center. The association of urinary uranium with incident hypertension was stronger in Oklahoma than in Arizona or North Dakota and South Dakota. The change in systolic blood pressure levels at follow-up versus baseline per IQR of urinary uranium was inverse in Arizona and Oklahoma, and positive in North Dakota and South Dakota at the higher ends of the urinary uranium distribution.

Few studies have evaluated the relationship between uranium and hypertension or blood pressure. Prior literature evaluated uranium as a component of metal mixtures and suggested that uranium acts additively with lead and may affect waist circumference (33). Urinary uranium (> 0.028 µg/L) has also been associated with 30% higher odds of prevalent type 2 diabetes (34). Our results were consistent with a study that found a positive association between uranium and hypertension across 3 NHANES survey cycles (2012–2016) (17). Conversely, urinary uranium was not associated with hypertension in a larger study that used 9 survey cycles (1999–2016), although that study dichotomized urinary uranium into low and high categories without leveraging the full distribution, and models were unadjusted (34). Another study, which examined the health of residents living near an old uranium mine, found no association between uranium exposure, assessed via residential history, and hypertension (35). In a cohort of pregnant study participants in California, uranium in drinking water was inversely associated with hypertensive disorders in pregnancy (36). The current understanding of the mechanism by which uranium exerts its chemical toxicologic effects is limited (37). In a small study (N = 193 participants) in a community chronically exposed to low-to-moderate uranium levels in drinking water (median [IQR], 25 [5–148] µg/L) in Finland, higher urinary uranium levels were associated with higher systolic and diastolic blood pressure levels (38). To our knowledge, our study is novel in its prospective associations between urinary uranium with both hypertension and blood pressure and supports that uranium exposure is associated with a higher risk of hypertension and higher blood pressure levels.

The overall association between baseline urinary uranium and the change in blood pressure from baseline to follow-up differed across study centers. In stratified analyses, the association of continuous log-transformed urinary uranium with the change in systolic blood pressure was inverse in all 3 study centers at levels below 4.5 µg U/g creatinine. In North Dakota and South Dakota, the only study center where urinary uranium exceeded 4.5 µg U/g creatinine, associations were positive above 4.5 µg U/g creatinine. For the association of urinary uranium with the change in diastolic blood pressure, associations were positive in Oklahoma and in North Dakota and South Dakota at higher levels of the urinary uranium distribution. The positive association of urinary uranium with both hypertension and the change over time in diastolic blood pressure in the 3 SHS centers was strongest in Oklahoma, the center with overall lower levels of urinary uranium (39). A possible explanation could be related to regional differences in other environmental exposures that may either overwhelm or modify the association between uranium and hypertension that were not captured in our dataset. For example, arsenic and uranium frequently co-occur in both drinking water sources and urine in SHFS communities (1,7,40). Hence, our effect estimates for uranium with the change in blood pressure levels were attenuated with further adjustment for arsenic and cadmium, which are established risk factors for CVD (41). While self-reported dietary patterns and the association between food groups and urinary uranium differ across SHFS study sites, prior work indicates that diet explains relatively little variability in urinary uranium concentrations (dust exposure could also be relevant for some SHFS communities) (39). More research is needed to disentangle these inconsistencies between the association with blood pressure overall and by study center.

While this analysis provides useful insights into the associations of chronic uranium exposure with blood pressure and hypertension, there are several limitations. First, because most uranium is quickly excreted from the body, the measurement may not reflect the actual chronic exposure of participants to uranium (42). However, uranium levels in drinking water tend to be stable, so we expect that urinary uranium levels are likely to reflect chronic exposures, if there is no change in the source of drinking water. We

were unable to evaluate if drinking water source changed over time because drinking water source was not collected from SHFS participants. Furthermore, it is possible that those with the highest levels of uranium exposure may have already developed hypertension before baseline, and thus were excluded from analyses, resulting in selection bias. We were unable to adjust for lead exposure in this analysis because blood lead was not measured from samples collected at the baseline SHFS visit. Metal exposures are correlated in the SHFS, so future studies should explore complex metal mixtures to identify the effects of the most toxic metal components on blood pressure, hypertension, and other critical CVD risk factors, which was beyond the scope of this analysis. Additionally, there remains a critical need to evaluate uranium exposure with kidney disease events, subclinical measures, and risk factors, as these analyses were beyond the scope of this study.

We observed consistent, although stronger, effect estimates when adjusting for specific gravity compared with when standardizing urinary uranium for urinary creatinine. Uranium is nephrotoxic, as demonstrated in animal studies, and may influence the excretion of metals (including U) in urine (43). While we adjusted for eGFR to account for kidney function, it is unknown if models adjusting for specific gravity are more appropriate for studies of urinary uranium and cardiometabolic outcomes. Future studies can use environmental monitoring to avoid reverse causality concerns.

Prior SHS and SHFS work found arsenic, cadmium, and lead as risk factors for CVD, consistent with established evidence (41). For uranium, however, not enough research exists to make a comprehensive determination. Our findings, in one of the first prospective studies available, support an association consistent with previous cross-sectional studies, but more work is needed. Our research is especially relevant for American Indian and Alaska Native communities, in which disparities in uranium exposure, hypertension, and CVD are well-established. Future studies should evaluate the role of low-dose chronic uranium exposure on hypertension, elevated blood pressure levels, and other CVD risk factors, as well as clinical CVD in larger nationwide cohorts to better understand the relationship over time, including potential nonlinear patterns. Mechanistic and experimental research and understanding the processes by which uranium negatively affects biologic processes will also lend insight into how to prevent and treat diseases associated with uranium exposure. Furthermore, additional evidence could have implications for primary and secondary interventions. For example, community interventions and federal regulations (eg, the Final Arsenic Rule by the US Environmental Protection Agency in 2001 [44] reduced the maximum contaminant level from 50 µg/L to 10 µg/L) have been successful in reducing water arsenic exposure (45). Uranium exposure through drinking water, soil, and food is widespread in the US, particularly in western states. Recent evidence suggests drinking water is a substantial source of uranium exposure in SHS communities (5). Similar strategies as those developed for arsenic might be necessary to reduce uranium in drinking water. Clinical care settings could be used as an additional screening tool to identify patients who obtain drinking water from wells known to have high levels of uranium. Reducing uranium in drinking water can reduce disparities in exposure and related health outcomes.

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Supplemental material available. The following information is available on request from the corresponding author: 1) a figure enumerating inclusion and exclusion criteria for analysis of urinary uranium among participants in the Strong Heart Family Study (SHFS); 2) a histogram, a Q–Q plot, and a kernel density plot of urinary uranium (µg/g of creatine) in original scale and log scale among SHFS participants; 3) a table showing risk ratios (RRs) and 95% CIs of incident hypertension according to quartile increases in urinary uranium (µg/L) among SHFS participants; 4) a table showing mean differences (95% CIs) for the change in systolic and diastolic blood pressure (mm Hg) at follow-up versus baseline according to baseline urinary uranium (µg/L) among SHFS participants; 5) a figure showing RRs and 95% CIs for hypertension and mean differences (95% CIs) for the change in systolic and diastolic blood pressure (mm Hg) at follow-up versus baseline per log urinary uranium (µg/L) among SHFS participants; 6) a table show-

ing RRs and 95% CIs of incident hypertension according to quartile increases in urinary uranium (µg/g of creatinine) among SHFS participants in Arizona (n = 162); 7) a table showing RRs and 95% CIs of incident hypertension according to quartile increases in urinary uranium (µg/g of creatinine) among SHFS participants in Oklahoma (n = 557); 8) a table showing RRs and 95% CIs of incident hypertension according to quartile increases in urinary uranium (µg/g of creatinine) among SHFS participants in North Dakota and South Dakota (n = 734); 9) a table showing mean differences for the change in systolic and diastolic blood pressure (mm Hg) at follow-up versus baseline according to baseline urinary uranium (µg/g of creatinine) among SHFS participants in Arizona (n = 162); 10) a table showing mean differences (95% CIs) for the change in systolic and diastolic blood pressure (mm Hg) at follow-up versus baseline according to baseline urinary uranium (µg/g of creatinine) among SHFS participants in Oklahoma (n = 557); 11) a table showing mean differences for the change in systolic and diastolic blood pressure (mm Hg) at follow-up versus baseline according to baseline urinary uranium (µg/g of creatinine) among SHFS participants in North Dakota and South Dakota (n = 734); and 12) a figure showing mean differences (95% CIs) for changes in systolic and diastolic blood pressure (mm Hg) at follow-up versus baseline per log urinary uranium (µg/g of creatinine) among SHFS participants, stratified by study center.

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Tables

Table 1. Characteristics of Participants at Baseline (Visit 3 Pilot and Visit 4 Combined, 1998–2003), by Hypertension Status at Follow-Up (Visit 5, 2006–2009), Strong Heart Family Study

Characteristic	Overall at baseline[a]	Hypertension status at follow-up		
		No hypertension	Hypertension[b]	*P* value[c]
No. (%)	1,453 (100.0)	1,200 (82.6)	253 (17.4)	—
Age, mean (SD), y	34.1 (14.0)	32.6 (13.3)	41.5 (14.8)	<.001
Sex, no. (%)				
Female	898 (61.8)	771 (64.2)	127 (50.2)	<.001
Male	555 (38.2)	429 (35.8)	126 (49.8)	
Study center, no. (%)				
Arizona	162 (11.1)	135 (11.2)	27 (10.7)	.94
Oklahoma	557 (38.3)	461 (38.4)	96 (37.9)	
North Dakota and South Dakota	734 (50.5)	604 (50.3)	130 (51.4)	
Years of education, no. (%)				
<12	465 (32.0)	390 (32.5)	75 (29.6)	.66
12	519 (35.7)	424 (35.3)	95 (37.5)	
>12	469 (32.3)	386 (32.2)	83 (32.8)	
Smoking status, no. (%)				
Never smoker	589 (40.5)	499 (41.6)	90 (35.6)	.21
Ever smoker	275 (18.9)	223 (18.6)	52 (20.6)	
Current smoker	589 (40.5)	478 (39.8)	111 (43.9)	
Alcohol status, no. (%)[d]				
Never drinker	161 (11.1)	140 (11.7)	21 (8.3)	.006
Ever drinker	352 (24.3)	272 (22.7)	80 (31.7)	
Current drinker	938 (64.6)	787 (65.6)	151 (59.9)	
BMI[e]	30.1 (7.4)	29.6 (7.1)	32.3 (8.3)	<.001
Prediabetes status, no. (%)[f]				
Normal fasting glucose	1,130 (77.8)	961 (80.1)	169 (66.8)	<.001
Impaired fasting glucose	323 (22.2)	239 (19.9)	84 (33.2)	
Blood pressure, mean (SD), mm Hg				

Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate.
[a] People with prevalent hypertension at baseline were excluded from analysis.
[b] Meets criteria for hypertension: having systolic blood pressure 140 mm Hg OR diastolic blood pressure 90 mm Hg OR taking hypertension medication.
[c] *P* values were determined by Kruskal–Wallis test for continuous variables (age, BMI, blood pressure, urinary uranium, and eGFR) and χ^2 test for categorical variables (sex, study center, years of education, smoking status, alcohol status, and prediabetes status).
[d] Two participants did not answer the survey question.
[e] Calculated as weight in kilograms divided by height in meters squared.
[f] Normal fasting glucose defined as having fasting blood glucose <110 mg/dL AND no diabetes treatment; impaired fasting glucose defined as having blood glucose level 110–125 mg/dL (30).
[g] Calculated as sum of first and second measured systolic blood pressure divided by 2.
[h] Calculated as sum of first and second measured diastolic blood pressure divided by 2.

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Table 1. Characteristics of Participants at Baseline (Visit 3 Pilot and Visit 4 Combined, 1998–2003), by Hypertension Status at Follow-Up (Visit 5, 2006–2009), Strong Heart Family Study

Characteristic	Overall at baseline[a]	Hypertension status at follow-up		
		No hypertension	Hypertension[b]	*P* value[c]
Systolic[g]	116.0 (10.7)	114.4 (10.2)	123.5 (9.3)	<.001
Diastolic[h]	73.5 (8.9)	72.5 (8.8)	78.1 (7.9)	<.001
Urinary uranium, µg/g creatinine, median (IQR)	0.029 (0.014–0.059)	0.029 (0.014–0.058)	0.030 (0.016 vs. 0.066)	.17
eGFR	122.0 (16.9)	123.4 (16.5)	115.1 (17.3)	<.001

Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate.
[a] People with prevalent hypertension at baseline were excluded from analysis.
[b] Meets criteria for hypertension: having systolic blood pressure 140 mm Hg OR diastolic blood pressure 90 mm Hg OR taking hypertension medication.
[c] *P* values were determined by Kruskal–Wallis test for continuous variables (age, BMI, blood pressure, urinary uranium, and eGFR) and χ^2 test for categorical variables (sex, study center, years of education, smoking status, alcohol status, and prediabetes status).
[d] Two participants did not answer the survey question.
[e] Calculated as weight in kilograms divided by height in meters squared.
[f] Normal fasting glucose defined as having fasting blood glucose <110 mg/dL AND no diabetes treatment; impaired fasting glucose defined as having blood glucose level 110–125 mg/dL (30).
[g] Calculated as sum of first and second measured systolic blood pressure divided by 2.
[h] Calculated as sum of first and second measured diastolic blood pressure divided by 2.

Table 2. Characteristics of Participants Without Hypertension at Baseline (N = 1,453), by Quartile of Baseline Urinary Uranium Level, Strong Heart Family Study, 1998–2009

Characteristic	Overall: 0–6.2 µg/g	Quartile of urinary uranium, µg/g of creatinine				
		Quartile 1: <0.01	Quartile 2: 0.01–0.03	Quartile 3: 0.03–0.06	Quartile 4: >0.06	*P* value[a]
No. (%)	1,453 (100.0)	345 (23.7)	372 (25.6)	364 (25.0)	372 (25.6)	—
Age, mean (SD), y	34.1 (14.0)	34.5 (14.2)	33.9 (14.0)	33.9 (13.6)	34.2 (14.3)	.80
Sex, no. (%)						
Female	898 (61.8)	223 (64.6)	225 (60.5)	215 (59.1)	235 (63.2)	.41
Male	555 (38.2)	122 (35.4)	147 (39.5)	149 (40.9)	137 (36.8)	
Study center, no. (%)						
Arizona	162 (11.1)	19 (5.5)	35 (9.4)	56 (15.4)	52 (14.0)	<.001
Oklahoma	557 (38.3)	199 (57.7)	177 (47.6)	118 (32.4)	63 (16.9)	
North Dakota and South Dakota	734 (50.5)	127 (36.8)	160 (43.0)	190 (52.2)	257 (69.1)	
Years of education, no. (%)						
<12	465 (32.0)	91 (26.4)	109 (29.3)	129 (35.4)	136 (36.6)	.04
12	519 (35.7)	130 (37.7)	134 (36.0)	124 (34.1)	131 (35.2)	
>12	469 (32.3)	124 (35.9)	129 (34.7)	111 (30.5)	105 (28.2)	
Smoking status, no. (%)						
Never smoker	589 (40.5)	160 (46.4)	155 (41.7)	140 (38.5)	134 (36.0)	.09
Ever smoker	275 (18.9)	62 (18.0)	72 (19.4)	74 (20.3)	67 (18.0)	
Current smoker	589 (40.5)	123 (35.7)	145 (39.0)	150 (41.2)	171 (46.0)	
Alcohol status, no. (%)[b]						
Never drinker	161 (11.1)	34 (9.9)	40 (10.8)	48 (13.2)	39 (10.5)	.54
Ever drinker	352 (24.3)	94 (27.2)	92 (24.9)	84 (23.1)	82 (22.0)	
Current drinker	938 (64.6)	217 (62.9)	238 (64.3)	232 (63.7)	251 (67.5)	
BMI, mean (SD)[c]	30.1 (7.4)	30.1 (7.0)	30.1 (7.0)	30.3 (8.0)	29.9 (7.6)	.83
Prediabetes status[d]						
Normal fasting glucose	1,130 (77.8)	267 (77.4)	301 (80.9)	277 (76.1)	285 (76.6)	.37
Impaired fasting glucose	323 (22.2)	78 (22.6)	71 (19.1)	87 (23.9)	87 (23.4)	
eGFR, mean (SD)	122.0 (16.9)	121.1 (17.5)	120.5 (16.7)	123.3 (16.4)	123.0 (16.9)	.04
Blood pressure, mean (SD), mm Hg						
Systolic[e]	116.0 (10.7)	115.5 (10.6)	116.8 (10.8)	115.9 (10.7)	115.7 (10.5)	.94
Diastolic[f]	73.5 (8.9)	73.2 (9.3)	73.5 (9.0)	73.8 (9.0)	73.4 (8.3)	.68

Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate.
[a] *P* values were determined by Kruskal–Wallis test for continuous variables (age, BMI, blood pressure, urinary uranium, and eGFR) and χ^2 test for categorical variables (sex, study center, years of education, smoking status, alcohol status, and prediabetes status).
[b] Two participants did not answer the survey question.
[c] Calculated as weight in kilograms divided by height in meters squared.
[d] Normal fasting glucose defined as having fasting blood glucose <110 mg/dL AND no diabetes treatment; impaired fasting glucose defined as having blood glucose level 110–125 mg/dL (30).
[e] Calculated as sum of first and second measured systolic blood pressure divided by 2.
[f] Calculated as sum of first and second measured diastolic blood pressure divided by 2.

Table 3. Risk Ratios (RRs) for Incident Hypertension, by Quartile Increase in Urinary Uranium Among Participants (N = 1,453), Strong Heart Family Study, 1998–2009[a]

Model	Comparison of 25th and 75th percentiles (0.01 vs 0.06 µg/g)	Quartile increase in urinary uranium, µg/g of creatinine			
		Quartile 1: <0.01	Quartile 2: 0.01–0.03	Quartile 3: 0.03–0.06	Quartile 4: >0.06
No. of cases[b]/no. of noncases	253/1,200	52/312	66/297	66/297	69/294
Model 1, RR (95% CI)[c]	1.11 (0.96–1.28)	1 [Reference]	1.27 (0.94–1.72)	1.26 (0.92–1.74)	1.34 (0.96–1.86)
Model 2, RR (95% CI)[d]	1.13 (0.97–1.31)	1 [Reference]	1.28 (0.95–1.74)	1.29 (0.93–1.77)	1.38 (1.00–1.91)
Model 3, RR (95% CI)[e]	1.15 (0.99–1.33)	1 [Reference]	1.31 (0.96–1.78)	1.32 (0.95–1.83)	1.44 (1.04–1.99)

[a] Models were estimated by Poisson regression with robust error variance using generalized estimating equations with an independent covariance accounting for family clustering.
[b] Cases of hypertension were defined as systolic blood pressure ≥140 mm Hg OR diastolic blood pressure ≥90 mm Hg OR taking hypertension medication.
[c] Model 1 adjusted for age (continuous), sex (male/female), study center (Arizona/Oklahoma/South Dakota and North Dakota), and smoking status (never/former/current).
[d] Model 2 further adjusted for estimated glomerular filtration rate (continuous), prediabetes status (normal fasting glucose/impaired fasting glucose), and body mass index (continuous).
[e] Model 3 further adjusted for log-transformed arsenic (continuous) and log-transformed cadmium (continuous).

Table 4. Mean Differences in Change in Systolic and Diastolic Blood Pressure Levels at Follow-Up vs Baseline, by Quartile of Baseline Urinary Uranium Level Among Participants (N = 1,453), Strong Heart Family Study, 1998–2009[a]

Model	IQR (0.01 vs 0.06 µg/g)	Quartile of urinary uranium, µg/g of creatinine			
		Quartile 1: <0.01	Quartile 2: 0.01–0.03	Quartile 3: 0.03–0.06	Quartile 4: >0.06
No. (%)	1,453 (100.0)	364 (25.0)	363 (24.9)	363 (24.9)	363 (24.9)
Systolic blood pressure, mm Hg					
Model 1, β (95% CI)[b]	−0.02 (−0.05 to 0.00)	1 [Reference]	0.08 (−1.67 to 1.84)	−0.30 (−2.04 to 1.44)	−1.94 (−3.78 to −0.10)
Model 2, β (95% CI)[c]	−0.02 (−0.05 to 0.00)	1 [Reference]	0.08 (−1.70 to 1.85)	−0.35 (−2.08 to 1.38)	−2.00 (−3.83 to −0.16)
Model 3, β (95% CI)[d]	−0.02 (−0.04 to 0.01)	1 [Reference]	0.24 (−1.55 to 2.02)	−0.04 (−1.84 to 1.75)	−1.48 (−3.32 to 0.37)
Diastolic blood pressure, mm Hg					
Model 1, β (95% CI)[b]	−0.01 (−0.04 to 0.01)	1 [Reference]	0.50 (−0.99 to 1.99)	−0.66 (−2.39 to 1.07)	−1.49 (−3.33 to 0.35)
Model 2, β (95% CI)[c]	−0.02 (−0.04 to 0.01)	1 [Reference]	0.43 (−1.04 to 1.90)	−0.68 (−2.40 to 1.03)	−1.53 (−3.34 to 0.28)
Model 3, β (95% CI)[d]	−0.01 (−0.03 to 0.01)	1 [Reference]	0.39 (−1.02 to 1.80)	−0.60 (−2.25 to 1.06)	−1.25 (−2.95 to 0.44)

[a] Mean differences in blood pressure level were estimated by generalized estimating equations with an independent covariance accounting for family clustering.
[b] Model 1 adjusted for age (continuous), sex (male/female), study center (Arizona/Oklahoma/South Dakota and North Dakota), and smoking status (never/former/current).
[c] Model 2 further adjusted for estimated glomerular filtration rate (continuous), prediabetes status (normal fasting glucose/impaired fasting glucose), and body mass index (continuous).
[d] Model 3 further adjusted for log-transformed arsenic (continuous) and log-transformed cadmium (continuous).

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ORIGINAL RESEARCH

Visit-to-Visit Blood Pressure Variability as a Risk Factor for All-Cause Mortality, Cardiovascular Mortality, and Major Adverse Cardiovascular Events Among American Indians: the Strong Heart Study

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PEER REVIEWED

Summary

What is already known on the topic?

Blood pressure variability has been shown in multiple studies to be an independent risk factor for all-cause mortality, cardiovascular disease mortality, and major adverse cardiovascular events.

What is added by this report?

Ours is the first study to show the prognostic value of blood pressure variability in American Indians, a population with unique genetics, culture, lifestyle, and risk factors. The study expands the prognostic value of blood pressure variability to that population.

What are the implications for public health practice?

As electronic health record systems proliferate, the evidence to support the routine calculation of blood pressure variability offers a value-added proposition to those records and the impetus to advance therapies for blood pressure control.

Abstract

Introduction

Recent literature suggests blood pressure variability (BPV) is an independent risk factor for cardiovascular disease (CVD). Ours is the first study to assess the prognostic value of the intraindividual SD of systolic blood pressure (SBPSD) and diastolic blood pressure (DBPSD) in American Indians.

Methods

We computed BPV for 3,352 American Indians who had 8 nonurgent visit-to-visit blood pressure checks according to their electronic health records, and linked those measurements with Strong Heart Study cohort data. We used Cox proportional hazards models to determine whether the risk of all-cause mortality, CVD mortality, or major adverse cardiovascular events (MACE), was different for SBPSD and DBPSD quartiles, while controlling for covariates.

Results

Mean participant age was 54.5 years (SD = 17.3), 66% were female, mean SBPSD was 13.47 (SD = 5.71), and mean DBPSD was 8.05 (SD = 3.02). Over the 20-year follow-up, 45.4% died, 14.6% experienced CVD-related mortality, and 20.8% experienced MACE. Compared with the lowest SBPSD quartile (quartile 1), the risk of all-cause mortality was 35% higher for the highest quartile (quartile 4), while controlling for covariates (HR = 1.35; 95% CI, 1.13–1.61). The risk of CVD mortality and MACE was higher for quartile 4 SBPSD compared with quartile 1 (CVD mortality, HR = 1.81, 95% CI, 1.29–2.53; MACE HR = 1.39, 95 % CI, 1.07–1.80). The risk for quartile 4 DBPSD was not significant for these outcomes (all-cause mortality, HR = 1.15, 95% CI, 0.97–1.36; CVD mortality, HR=1.22, 95% CI, 0.91–1.65; MACE, HR = 1.11, 95% CI, 0.87–1.40).

Conclusion

Our study identified SBPSD as a significant risk factor for all-cause mortality, cardiovascular mortality, and MACE, whereas DBPSD in our cohort of American Indian subjects was not a significant risk factor after adjustment for covariates.

Introduction

Blood pressure level is a well-recognized risk factor for cardiovascular disease (CVD) (1). Over the last 2 decades, research and clinical interests in blood pressure have expanded to include blood pressure variability (BPV), here defined as the SD of 8 nonurgent, visit-to-visit blood pressure measurements. Interest in BPV as a risk factor independent of blood pressure level surged after Rothman and colleagues reported it as a risk factor for stroke independent of blood pressure (2). Since then, BPV has also been shown to be a risk factor, independent of blood pressure level, for all-cause mortality (3–13), CVD mortality (4,6,8,11–14), and CVD morbidity (2,4–7,9–12,14–17). In addition, BPV has been linked to cognitive decline (18), peripheral vascular disease (19), chronic kidney disease (5), decline in estimated glomerular filtration rate (eGFR) (15), type 2 diabetes (20), worsened cardiac structure and function (21), and progression of coronary artery calcification (22). Although BPV can be measured in several ways (23), the SD of visit-to-visit systolic blood pressures is the most common (11) and reproducible (24) measure.

Because BPV may be associated with genetic factors (25) and is likely to be influenced by cultural and lifestyle factors such as diet, alcohol and cigarette consumption, and exercise, it is important to investigate BPV in different populations. To date, none of the published investigations of BPV has focused on American Indians or had sufficient sample size to provide results specific to this population.

Our study is the first known effort to evaluate the prognostic value of BPV for all-cause and cardiovascular mortality and major adverse cardiovascular events (MACE) in a large, geographically diverse group of American Indians. We merged standardized data from cohort examinations with unstandardized blood pressure data collected in the "real world" from nonurgent clinic visits within a common medical care system to evaluate the prognostic value of clinical BPV for adjudicated mortality and morbidity endpoints over a 20-year follow-up.

Methods

Available data

The Strong Heart Study (SHS) is a longitudinal, observational study of CVD and its risk factors (26). It originally sampled 4,549 men and women aged 45 to 74 years from the general population of American Indians in Arizona, Oklahoma, and the Dakotas (27). The first examination was conducted in 1989–1992. The group was re-examined a second time in 1993–1995, and a third time in 1997–1999. Subsequently, the focus of the study changed, and we sampled large, 3-generation families (n = 3,665) with a first examination in 2001–2003. That examination was called the Strong Heart Family Study (SHFS). Its cohort was re-examined in 2006–2009. To incorporate longer follow-up, 825 overlapping individuals between the 2 cohorts were allocated to the SHS cohort. All tribal members aged 45 to 74 years were invited to be examined for the original study. For the SHFS, 120 families of 30 members or more aged 15 years or older were invited to participate. The study was approved by all relevant institutional review boards from the Indian Health Service (IHS), the various research institutions, and participating tribes at the initiation of each phase of the study. All participants provided written informed consent and access to their patient records.

For a subgroup of participants in the SHS and SHFS, we linked medical records for inpatient and outpatient visits to IHS facilities to gain access to routine blood pressure measurements collected as early as 1998. We obtained data from the National Data Warehouse (NDW) of the IHS, which houses the National Patient Information Reporting System (28). NDW requested that all service units (clinical care facilities) send data from all patient encounters dating back to October 1, 2000. Many, but not all, service units sent data as they were able. We requested NDW data for all SHS and SHFS participants. NDW provided data for only those identification numbers resulting in a match to available blood pressure records.

Extracted IHS NDW data available for each outpatient visit included systolic blood pressure (SBP), diastolic blood pressure (DBP), date of blood pressure measurement, and the clinic where blood pressure was measured. Blood pressures collected as part of hospitalizations, emergency department visits, urgent care visits, ambulance trips, or pregnancy clinical visits were excluded from the calculation to avoid measures taken at stressful times. Although blood pressure variability stabilizes at 6 blood pressure measurements (7), the investigators decided to use 8 measurements for added confidence in the data. Data were included for all SHS and SHFS cohort members with blood pressures recorded for at least 8 clinic visits during the first 5 years of available NDW records.

To maintain data integrity and consistency, analyses used the first measurement per day in each encounter (clinic visit) if there were multiple measures within the clinic visit on the same day, although most encounters provided a single measurement per visit. The first 8 eligible visits were used to calculate the standard deviation of the SBP (SBPSD) and DBP (DBPSD). For analysis, we divided SBPSD and DBPSD into quartiles. Blood pressures from clinic visits did not follow a strict standardized protocol and may be considered real world blood pressure as measured in routine visits across various clinics.

Covariates

We obtained covariate information from the SHS or SHFS examination that was closest to the time the blood pressures were measured. Generally, baseline cohort exams for this analysis were the third examination in the SHS (1997–1999) and the first examination in the SHFS (2001–2003). Potential covariates were drawn from personal interview, medical history, physical examination, and laboratory measurements at the closest examination. Potential covariates for this analysis were limited to those collected at the third examination of the original cohort and the first examination of the family cohort. Time-dependent covariates could not be obtained because the baseline examination for the original cohort was their last study examination, and the family cohort had only 1 additional study examination approximately 5 years later. Potential covariates for this analysis were age, sex, center, history of cardiovascular disease (myocardial infarction or stroke), diabetes, hypertension, kidney disease, SBP and DBP measured during the examination, ankle/brachial index (ABI), body mass index (weight in kilograms divided by height square meters) (BMI), low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides, current cigarette smoking, and current alcohol consumption. In addition, all statistical models included an indicator variable to account for cohort differences in disease and death rates.

Details of study design and methods are provided elsewhere for the SHS cohort (26) and the SHFS cohort (27). Interviews and physical examinations were conducted by trained personnel following strict protocols after informed consent was provided. For examination components included in both cohorts, protocols were maintained between SHS and SHFS. Prevalent morbidity was based on a positive response in the medical history interview.

Patients' SHS examination blood pressures were measured in the right arm after a 5-minute rest in a quiet room by using an appropriate-sized cuff and a mercury sphygmomanometer. Blood pressure was measured 3 times, and the average of the last 2 measurements was used for analysis.

Fasting blood samples from a 12-hour fast were obtained during the physical examination for laboratory measures. All variables were assayed at MedStar Research Institute, Washington, DC, and the University of Vermont by using standard laboratory methods as described previously (26,29).

Participants were considered hypertensive if they were taking antihypertension medication or if they had a systolic blood pressure greater than 130 mm Hg or a diastolic blood pressure greater than 80 mm Hg (1). Urinary albumin excretion was estimated by the ratio of albumin (mg) to creatinine (g). Microalbuminuria was defined as a ratio of urinary albumin (mg/mL) to creatinine (g/mL) of 30 to 299 mg/g and macroalbuminuria as a ratio at or above 300 mg/g. Estimated glomerular filtration rate (eGFR) was calculated by using the Modified Diet and Renal Disease equation (30). Participants reporting a history of end-stage renal disease, those found to have microalbuminuria or macroalbuminuria, and those with eGFR of less than 60 mL per minute per $1.73m^2$ were combined into a category of kidney disease.

Endpoints

We abstracted medical records for all participants for review for relevant endpoints by trained medical abstractors at each center each year since enrollment in the study. Two physicians reviewed endpoints, and a third reviewer adjudicated differences between reviewers as needed (26). The following 3 endpoints contributed to this analysis: all-cause mortality, CVD mortality, and MACE that included cardiovascular death, nonfatal myocardial infarction, and nonfatal stroke.

Annual endpoint surveillance for SHS cohort participants began following the initial examination in 1988–1989 and ended with the most recently released morbidity and mortality file ending December 31, 2021. Endpoint surveillance for the SHFS cohort began with the initial examination in 2001–2003 and ended December 31, 2021. Surveillance included an annual telephone call to determine vital status and recent hospitalizations, and an annual review and abstraction of medical records for potential endpoints. Follow-up for events for this analysis began after the baseline examination or the eighth blood pressure measurement, whichever came later. Thus, the baseline examination for this analysis was the third examination for the SHS (1997–1999) and the first examination for the SHFS (2001–2003). Follow-up for events extended approximately 20 years.

Statistical analysis

We used SAS (SAS Institute Inc) to conduct all analyses. Comparisons of baseline covariates for study participants between those included and excluded from analysis were done with independent sample *t* tests for normally distributed variables, Wilcoxon signed rank sum for skewed variables, and χ^2 for categorical variables. We created side-by-side box plots to present the distribution of SBPSD and DBPSD quartiles. We generated Kaplan–Meier survival curves with time to death as the outcome and used log rank tests to determine whether there were differences in time to death between the SBPSD and DBPSD quartiles. We conducted univariate and multivariate analyses of covariates and BPV by using shared frailty Cox proportional hazards models accounting for the correlation among family members. The models met the assumption of proportional hazards. Covariates were selected for adjustment in models based on literature review. Analyses were done in

2 steps of covariate adjustment after univariate analyses: first (Model 1), adjusting for cohort, center, age, sex, and SBP/DBP as appropriate, and second (Model 2), also adjusting for the remaining CVD covariates (hypertension treatment, diabetes, BMI, current smoking, current drinking, LDL, HDL, triglycerides, kidney disease, ankle/brachial index, prevalent myocardial infarction and stroke [not for all-cause mortality], and interaction of prevalent systolic blood pressure or diastolic blood pressure and hypertension treatment). To separate the therapeutic effect from the severity effect of blood pressure medications, one 2-way interaction term (blood pressure treatment × blood pressure level) was included in Model 2. Statistical tests of model components were assessed as significant at the $P < .05$ level. A sensitivity analysis was conducted to investigate whether the time interval to collect 8 blood pressure measurements was related to the results by dichotomizing the time interval to collect the 8 measures, breaking the 5-year maximum at 1 year.

Results

Of the original and family cohorts, 3,501 participants from SHS and 2,346 from SHFS were eligible for our study (Figure 1). Of these 2 cohorts, 1,940 of the original and 1,412 of the family cohorts, or a total of 3,352, had 8 or more blood pressure measurements within the first 5 years of available data.

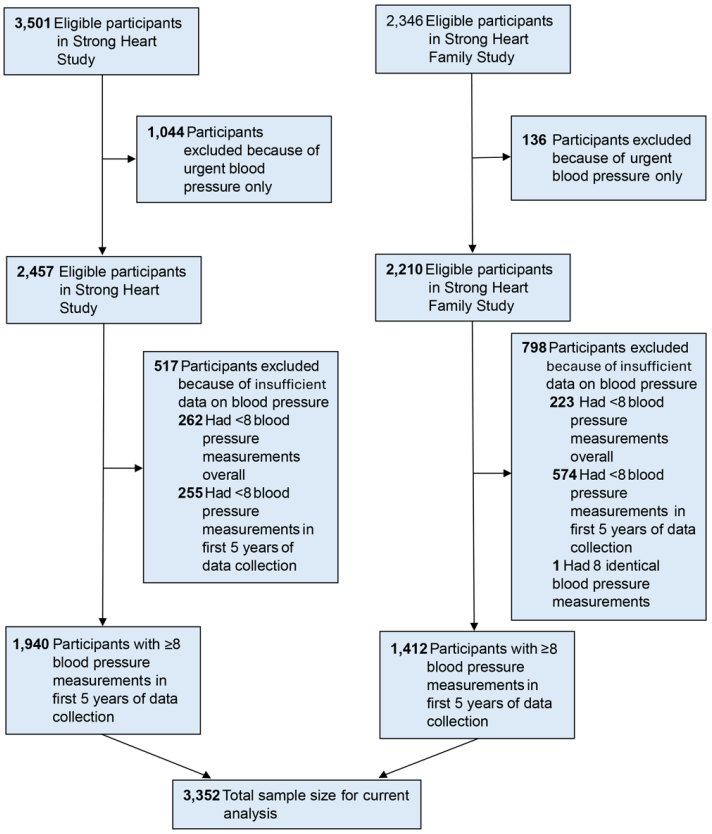

Figure 1. CONSORT Diagram for participants with 8 nonurgent blood pressure measurements in the first 5 years of available medical records from the National Data Warehouse of the Indian Health Service. Study participants were drawn from the Strong Heart Study (26) and the Strong Heart Family Study (27) of American Indians residing in Arizona, Oklahoma, North Dakota, and South Dakota (1997–2003).

Deaths included 1,523 all-cause deaths (45.4%) and 566 (16.9%) cardiovascular deaths among the cohort members during the 20-year follow-up period. In addition, participants experienced 249 nonfatal myocardial infarctions and 172 nonfatal strokes during the follow-up period. After exclusion of those with prior myocardial infarctions or strokes, 697 MACE events were available for analysis.

We compared the demographic, risk factor, and prevalent morbidity measures for those meeting the inclusion criteria versus those excluded for our current analyses (Table 1). Notably, those excluded had a higher SBP level (125.72 vs 123.49, $P < .001$) but were not statistically different for DBP or history of hypertension.

We calculated the SBPSD and DBPSD quartiles and the mean, median, and ranges of the SBPSD and DBPSD for each quartile (Figure 2). For example, the medians of successive quartiles for

SBPSD differ by 3.3, 3.2, and 6.0, and quartile 4 for SBPSD had a median value of 20.1 and a range of 16.4 to 41.4 mm Hg. We also conducted an initial analysis of the relationship of SBPSD and DBPSD as Kaplan–Meier Curves (Figure 3). The curves show a clear dose–response relationship between reduced survival from all-cause mortality with increasing quartiles of SBPSD. For DBPSD, quartiles 3 and 4 have significantly reduced survival versus quartile 1.

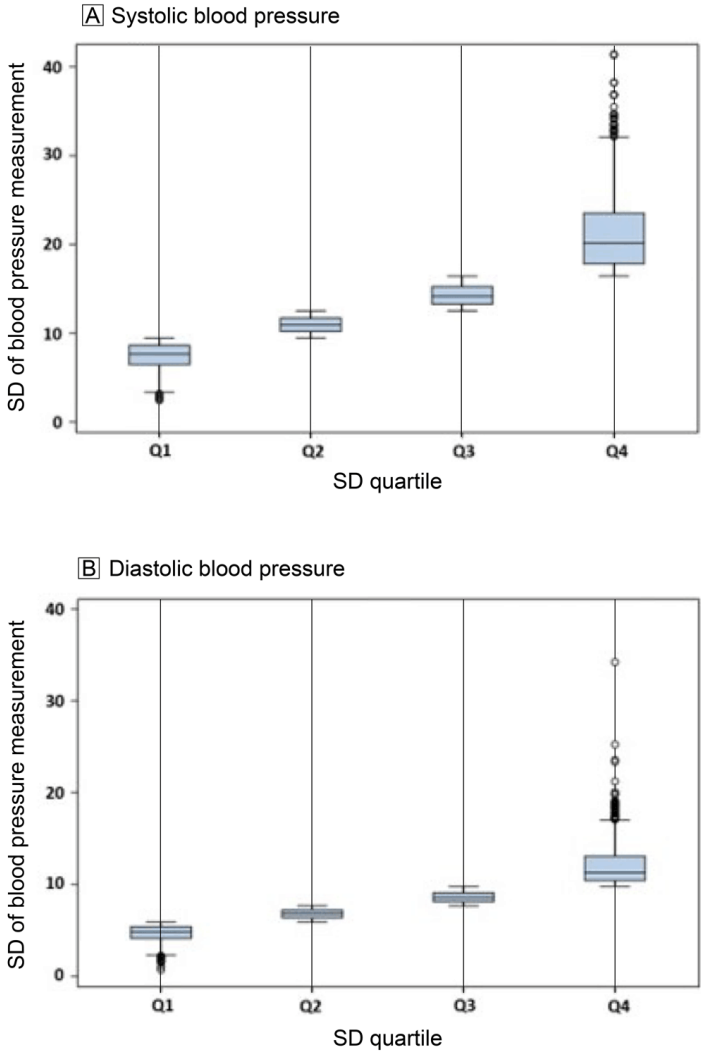

Figure 2. Box plots of the quartiles of the SD of systolic blood pressure and diastolic blood pressure for 8 nonurgent blood pressure measurements taken at clinic visits (urgent visits were defined as hospitalizations, emergency department visits, urgent care visits, ambulance trips, and pregnancy clinical visits and excluded) within a 5-year period closest to the dates of the Strong Heart Study (26) examination 3 (1997–1999) or the Strong Heart Family Study (27) examination 1 (2001–2003) of American Indians residing in in Arizona, Oklahoma, North Dakota, and South Dakota.

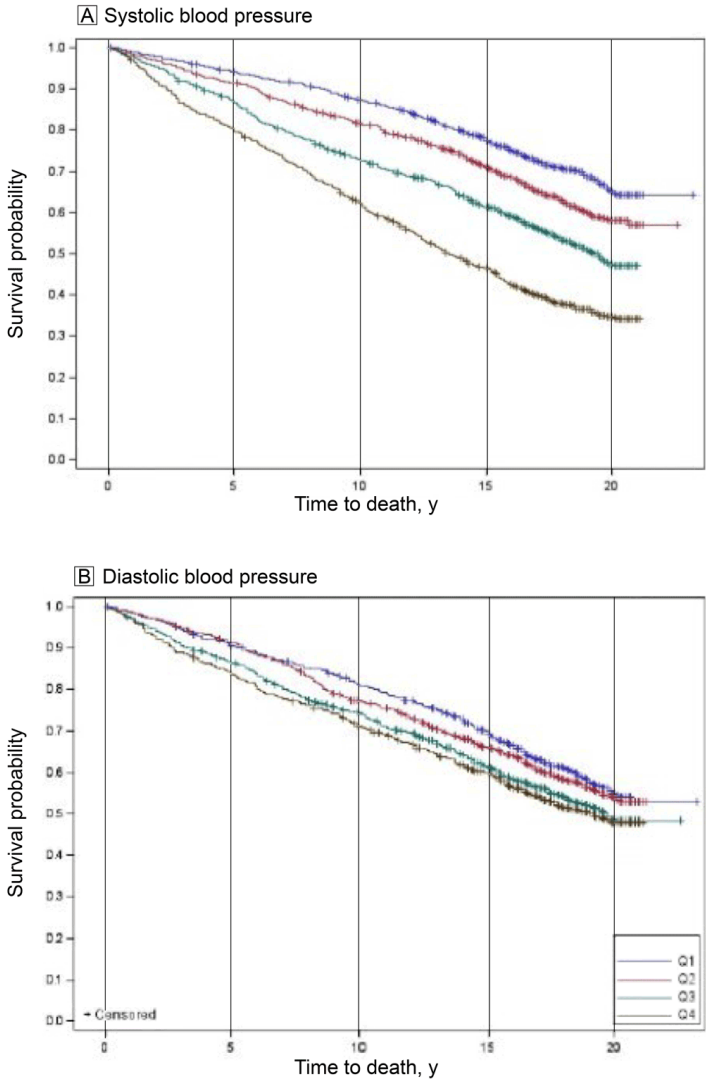

Figure 3. Survival curves for all-cause mortality, by quartiles, of the SD of systolic blood pressure (A) and diastolic blood pressure (B) for 8 blood pressure measurements taken at nonurgent clinic visits (urgent visits were defined as hospitalizations, emergency department visits, urgent care visits, ambulance trips, and pregnancy clinical visits and excluded) within 5 years, over 23 years of follow-up (1999–2022) for American Indians residing in Arizona, Oklahoma, North Dakota, and South Dakota.

We summarized the effects of covariate adjustment on the hazard ratios (HRs) for SBPSD and DBPSD with additional covariates under Models 1 and 2 for all-cause mortality, CVD mortality, and MACE (Table 2). Details of the analyses for all covariates for both

models are available (Appendix, Table 1A for SBPSD all-cause mortality and Appendix, Table 1B for DBPSD all-cause mortality; Appendix, tables 2A and 2B for CVD mortality; and Appendix, tables 3A and 3B for MACE).

Unadjusted analyses for all-cause mortality confirm the results of the Kaplan–Meier analysis of stronger relationships with each quartile of SBPSD (Table 2). Adjustment in Model 1 reduced the HRs but maintained significant effects for quartiles 3 and 4. Adjustment under Model 2 further reduced the HRs so that only quartile 4 remained significant (HR = 1.35; 95% CI, 1.13–1.61).

For DBPSD, unadjusted data show significant relationships for quartiles 3 and 4 with all-cause mortality. Model 1 results provided HRs that remained significant for quartile 3 (HR = 1.18; 95% CI, 1.01–1.38) and quartile 4 (HR = 1.26; 95% CI, 1.08–1.47). Adjustment under Model 2 showed attenuation of the HR point estimates, and they were no longer significant.

Similar analyses for CVD mortality showed results that were significant for quartile 2 and quartile 4 for SBPSD under Model 1. Results remained significant and substantial for quartile 4 under Model 2 (HR = 1.81; 95% CI, 1.29–2.53). For DBPSD, quartile 4 was significant under Model 1 (HR = 1.30; 95% CI, 1.00–1.69) but was no longer significant under Model 2.

Finally, the results for SBPSD for MACE show significant HRs for quartile 2, quartile 3, and quartile 4 in unadjusted analyses, remain significant for quartile 2 and quartile 4 under Model 1, and are significant for quartile 2 (HR=1.36; 95% CI, 1.05–1.77) and quartile 4 (HR = 1.39; 95% CI, 1.07–1.80) under Model 2. Results for DBPSD show no significant relationship with MACE before or after adjustment.

It is worth noting that all the *P* values for diabetes, renal disease, and prevalent MI and stroke are $P<.05$ and for ABI are $P<.07$ for all 3 endpoints for both SBPSD and DBPSD (Appendix, Tables 1A–3B) suggesting these morbidity measures play a significant role in reducing the HRs and their significance for BPV for these endpoints (Table 2, Appendix, Tables 1A–3B).

We performed a sensitivity analysis to investigate potential differences in results for individuals who have nonurgent clinic visits clustered over a shorter interval. The time interval for collection of 8 blood pressures was dichotomized at less than or equal to 1 year (n = 1,232 [36.8%]) versus 1-to-5 years (n = 2,120 [63.2%]). We found similar trends in both groups but stronger results for those in the 1-to-5-year group, particularly for Model 2 results for SBPSD. Findings were also significant for quartiles 3 and 4 for all-cause mortality, for quartiles 2, 3, and 4 for cardiovascular mortality, and for MACE. Additional stratified analyses related to the covariates in the model were assessed by testing interactions in the model, and none was found to be significant.

Discussion

Ours is the first study to assess the relationship of BPV to all-cause mortality, CVD mortality, and MACE in American Indians. Our study confirmed significant prognostic value for SBPSD with all-cause mortality, CVD mortality, and MACE, primarily for the highest quartile of SBPSD. Like many previous analyses of non-American Indian samples, results for DBPSD were less compelling, with significant results for quartile 4 under Model 1 for all-cause mortality and CVD mortality that did not survive the adjustment for CVD risk factors under Model 2. Findings were strongest for all-cause mortality and CVD mortality, then MACE, and were probably affected by the number of events available for analysis and the exclusions of those with prevalent disease in the case of the MACE analyses. The differences in the medians of SBPSD between quartile 1 and quartile 2, quartile 2 and quartile 3, and quartile 3 and quartile 4 were 3.3, 3.2, and 6.0 mm Hg, respectively. Thus, it is not surprising that significant findings were primarily in quartiles 3 and 4, given that the pooled results for 1 standardized log hazard ratio of SBPSD would represent 5.7 mm Hg for this study as described in the methods of Stevens and colleagues (31). These findings support the conclusion that there is little evidence of racial- and ethnicity-specific differences in the effects of BPV on the outcomes addressed.

The mechanisms linking BPV to all-cause mortality, CVD mortality, and MACE are autonomic dysfunction, endothelial dysfunction, atherosclerosis, vascular stiffness, aortic distensibility, diastolic dysfunction, subclinical inflammation, and cognitive decline. BPV may also reflect seasonal effects, measurement errors, antihypertensive treatment effects, and medication adherence (21,24,32).

It might be argued that much of the effect of BPV on all-cause and CVD morbidity and mortality may be via a wide array of end organ damage (33). In this analysis, end organ damage of BPV may be captured more proximally by the covariates used for adjustment in Model 2: history of CVD, peripheral vascular disease, diabetes, or kidney disease. As noted in our introduction, the literature is extensive linking SD and other measures of BPV to clinical cardiovascular disease (2,4–17) and subclinical CVD (19,21,22). In relation to diabetes, visit-to-visit BPV measured by average real variability for both SBP and DBP was a significant prognostic indicator for the development of type 2 diabetes in a Chinese cohort over a 16-year follow-up (20). In regard to kidney disease, in a large sample of US veterans, SBPSD based on 8 or more outpa-

tient blood pressure measurements was significantly associated with end stage renal disease in a dose responsive way for quartiles 2, 3, and 4 compared with quartile 1 of SBPSD (5). Multiple studies have shown BPV is a prognostic indicator for albuminuria (34,35). Thus, our Model 2 adjustments for prevalent CVD, ABI, diabetes, and kidney disease may represent over-adjustment, and the results for BPV after adjustment in Model 1 may be more representative of the underlying prognostic effects of BPV on the endpoints. In this interpretation, DBPSD would be significantly related to both all-cause mortality (quartiles 3 and 4) and CVD mortality (quartile 4). The HRs for the above covariates were highly significant in Model 2 adjustments for SBPSD and DBPSD for all-cause mortality, cardiovascular mortality, and MACE. The decline and reduced significance in HRs, particularly for SBPSD, from unadjusted to Model 1 to Model 2 are certainly compatible with the foregoing interpretation. However, the fact that SBPSD endures these adjustments, particularly for quartile 4 for all-cause mortality and cardiovascular mortality, suggests the potential strength of this measure as a prognostic indicator. The use of causal mediation analysis to investigate the potential for indirect effects of BPV on outcomes may make more targeted mechanistic investigations possible.

Results of the sensitivity analysis dichotomizing the sample at 1 year strengthened the results for the two-thirds of the sample in the 1-to 5-year group, suggesting that those with 8 blood pressures taken in nonurgent clinic visits in less than 1 year may be different from those requiring a longer time to reach this frequency. Further research is needed to understand the nuances of the group differences.

We have attempted to address many of the statistical and methodological challenges described in the literature. First, raw blood pressure data used for these analyses were limited to nonurgent visits to a wide array of medical care providers without the benefit of standardized blood pressure protocols normally found in epidemiological studies. As such, they represent real-world blood pressure levels and variability as might be found in most large medical care systems. Second, the number of blood pressures required for eligibility was set at 8 based on a tradeoff between a larger sample size, longer follow-up for events, and reduced secular blood pressure effects associated with fewer, rather than more visits. The literature suggests stability occurs with a minimum of 6 measurements (7). Third, the calculation of BPV focused on the SD as the measure of variability of visit-to-visit blood pressures because it is the most frequently cited measure of blood pressure variability (31). Fourth, the time interval over which the blood pressure measures were taken was limited to 5 years as in other studies (8,10,13), and most measures for this analysis were taken within a 2-year interval to minimize secular changes in blood pressure and consequently blood pressure variability. Fifth, analyses for each endpoint included the level of blood pressure in both adjusted models to account for its effect on blood pressure variability. Sixth, the follow-up for events extended to 2 decades to minimize the influence of short-term effects. Finally, only the first blood pressure taken on each visit was used to maintain comparability of the measures across participants and visits.

This study is not without limitations. First, we used blood pressure recorded in a variety of clinic visits, which may therefore reflect greater variability than those collected in a single setting, or following standardized measurement protocols. However, these measurements are likely to be representative of the range of blood pressures that would be found in real-world clinical settings, including electronic health records data from large health care facilities. Also, this type of variability would reduce the observability of associations (Type II error), which would not affect any detected or reported associations. Second, this study was conducted in a ell-characterized cohort of participants, many of whom reside in a rural setting and with a unique risk factor profile; results should be interpreted with caution for generalizability to other populations. As evidence of BPV as a potential risk factor continues to mount, we need to determine which measures of BPV are associated with adverse events and settle on their definitions. In a world of significant progress in data mining, the incorporation of these measures, once defined, into large electronic health record systems appears relatively straightforward. The next challenge will be to determine whether it is possible to modify BPV with existing or new treatments, and finally, whether reduction of BPV will affect subsequent morbidity and/or mortality.

Our analysis offers the first look at the prognostic value of blood pressure variability for all-cause mortality, cardiovascular mortality, and MACE in American Indians. It demonstrates clear and significant differences in survival in a dose responsive way for quartiles of SBPSD, and for quartiles of DBPSD (beginning in quartile 3). After adjustment for cardiovascular risk factors, significant differences for BPV with all-cause mortality were persistent for quartile 4 for SBPSD. Finally, the results for CVD mortality and MACE are not as consistent in dose response or in final significance in adjusted models, particularly for DBPSD, and may reflect lack of effect, smaller numbers of events, the vagaries of sample variability, or missing covariates.

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Tables

Table 1. Demographic Characteristics and Medical History of Eligible Participants by Inclusion Status, Strong Heart Study Examination 3 (1997–1999) and the Strong Heart Family Study Examination 1 (2001–2003)[a]

Inclusion status of eligible participants	Overall (n = 5,847)	Included (n = 3,352)	Excluded (n = 2,495)	*P* value[b]
Age at enrollment,				
Mean (SD)	48.39 (14.76)	47.77 (13.77)	49.21 (15.96)	<.001
Range	14.10–93.30	15.00–90.80	14.10-93.30	
Sex, n (%)				
Female	3,422 (59.9)	2,226 (66.4)	1,196 (47.9)	<.001
Male	2,425 (40.1)	1,126 (33.6)	1,299 (52.1)	
Strong Heart Study cohort, n (%)				
Strong Heart Study	3,501 (59.9)	1,940 (57.9)	1,561 (62.6)	<.001
Strong Heart Family Study	2,346 (40.1)	1,412 (42.1)	934 (37.4)	
Strong Heart Study data collection center, n (%)				
Arizona	743 (12.7)	474 (14.1)	269 (10.8)	<.001
Oklahoma	2,504 (2.8)	1,267 (37.8)	1,237 (49.6)	
Dakotas	2,600 (44.5)	1,611 (48.1)	989 (39.6)	
Systolic blood pressure, nearest visit[c], mm Hg				
Mean (SD)	124.44 (18.20)	123.49 (17.11)	125.72 (19.29)	<.001
Range	73–224	84–223	73–224	
Diastolic blood pressure, nearest visit[c], mm Hg				
Mean (SD)	76.50 (10.57)	76.55 (10.42)	76.42 (10.77)	.64
Range	38–133	42–133	38–118	
Ankle-brachial index				
Mean (SD)	1.18 (0.14)	1.18 (0.13)	1.18 (0.15)	.04
Range	0.52–2.83	0.59–2.83	0.52–2.35	
Ankle-brachial index category, n (%)				
Low (≤0.9)	77 (1.4)	30 (0.9)	47 (2.0)	<.001
Normal (0.9–1.4)	5,269 (94.1)	3,074 (95.3)	2,195 (92.4)	
High (>1.4)	255 (4.6)	121 (3.8)	134 (5.6)	
Body mass index, kg/m^2				
Mean (SD)	30.76 (6.77)	31.49 (6.73)	29.77 (6.68)	.004
Range	15.40–91.43	15.62–74.36	15.40–91.43	
LDL, mg/dL				
Mean (SD)	106.05 (31.73)	106.53 (31.21)	105.40 (32.41)	.18
Range	9.00–288.00	9.00–274.00	10.00–288.00	
HDL, mg/dL				

Abbreviations: HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.
[a] Strong Heart Study (26), Strong Heart Family Study (27).
[b] *t* tests used for continuous variables and χ^2 used for categorical variables.
[c] The nearest visit is the study exam closest in time from the date of the 8th clinic blood pressure measurement.
[d] Wilcoxon test used.

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Table 1. Demographic Characteristics and Medical History of Eligible Participants by Inclusion Status, Strong Heart Study Examination 3 (1997–1999) and the Strong Heart Family Study Examination 1 (2001–2003)[a]

Inclusion status of eligible participants	Overall (n = 5,847)	Included (n = 3,352)	Excluded (n = 2,495)	*P* value[b]
Mean +/– SD	48.38 (14.50)	48.19 (14.11)	48.64 (15.02)	.25
Range	12.00–146.00	16.00–138.00	12.00–146.00	
Triglycerides, mg/dL[d]				
Median	123.00	126.00	117.00	<.001
Range	2.00–5,323.00	2.00–5,323.00	7.00–1,757.00	
Medical history, n (%)				
History of myocardial infarction	103 (1.8)	49 (1.5)	54 (2.2)	.04
History of stroke	33 (0.6)	10 (0.3)	23 (0.9)	.002
Hypertension	1,916 (32.8)	1,082 (32.3)	834 (33.4)	.34
Diabetes	1,697 (29.0)	963 (28.7)	734 (29.4)	.49
Kidney disease	1,598 (27.3)	867 (25.9)	731 (29.3)	.004
Smoking status, n (%)				
Never smoked	1,941 (33.2)	1,099 (32.8)	842 (33.8)	.06
Previous smoker	1,668 (28.5)	997 (29.8)	671 (26.9)	
Current smoke	2,231 (38.2)	1,253 (37.4)	978 (39.3)	
Alcohol consumption, n (%)				
Never drank	764 (13.1)	429 (12.8)	335 (13.5)	.005
Previous drinker	2,111 (36.1)	1,271 (38.0)	840 (33.8)	
Current drinker	2,958 (50.6)	1,648 (49.2)	1,310 (52.7)	

Abbreviations: HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.
[a] Strong Heart Study (26), Strong Heart Family Study (27).
[b] *t* tests used for continuous variables and χ^2 used for categorical variables.
[c] The nearest visit is the study exam closest in time from the date of the 8th clinic blood pressure measurement.
[d] Wilcoxon test used.

Table 2. Blood Pressure Variation as a Prognostic Factor for All-Cause Mortality, Cardiovascular Disease Mortality, and Major Adverse Cardiovascular Events, With 8 Nonurgent[a] Clinic Blood Pressure Measurements Within a 5-Year Period, by SD of Systolic Blood Pressure and Diastolic Blood Pressure Quartile, Combined Strong Heart Study Examination 3 (1997–1999) and Strong Heart Family Study Examination 1 (2001–2003)[b]

Event	Quartile	Unadjusted		Model 1[c]		Model 2[d]	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
All-cause mortality							
SBPSD	2	1.30 (1.07–1.58)	.009	1.09 (0.92–1.30)	.32	1.02 (0.85–1.23)	.81
	3	1.72 (1.42–2.09)	<.001	1.23 (1.04–1.51)	.02	1.17 (0.98–1.40)	.08
	4	2.51 (2.08–3.04)	<.001	1.51 (1.27–1.78)	<.001	1.35 (1.13 –1.61)	.001
DBPSD	2	1.07 (0.89–1.29)	.47	1.06 (0.91–1.24)	.46	1.01 (0.85–1.19)	.94
	3	1.32 (1.09–1.58)	.003	1.18 (1.01–1.38)	.04	1.17 (0.99–1.38)	.06
	4	1.40 (1.16–1.68)	<.001	1.26 (1.08–1.47)	.004	1.15 (0.97–1.36)	.10
Cardiovascular disease mortality							
SBPSD	2	1.68 (1.20–2.37)	.003	1.39 (1.01–1.91)	.05	1.24 (0.87–1.76)	.24
	3	1.82 (1.30–2.57)	<.001	1.28 (0.93–1.76)	.13	1.17 (0.82–1.66)	.38
	4	3.73 (2.70–5.13)	<.001	2.09 (1.55–2.83)	<.001	1.81 (1.29–2.53)	<.001
DBPSD	2	0.93 (0.69–1.26)	.65	0.96 (0.73–1.26)	.76	0.92 (0.68–1.26)	.62
	3	1.27 (0.95–1.71)	.11	1.19 (0.91–1.56)	.20	1.13 (0.84–1.52)	.43
	4	1.39 (1.04–1.86)	.03	1.30 (1.00–1.69)	.05	1.22 (0.91 –1.65)	.18
Major adverse cardiovascular events							
SBPSD	2	1.59 (1.22–2.07)	<.001	1.41 (1.11–1.78)	.004	1.36 (1.05–1.77)	.02
	3	1.49 (1.13–1.95)	.004	1.09 (0.86–1.39)	.47	1.06 (0.81–1.39)	.65
	4	2.53 (1.95–3.28)	<.001	1.49 (1.18–1.88)	<.001	1.39 (1.07–1.80)	.01
DBPSD	2	0.99 (0.77–1.28)	.97	1.02 (0.82–1.27)	.84	0.98 (0.78–1.25)	.90
	3	1.22 (0.96–1.57)	.11	1.19 (0.96–1.47)	.11	1.10 (0.87–1.39)	.45
	4	1.24 (0.97–1.60)	.08	1.18 (0.95–1.46)	.13	1.11 (0.87–1.40)	.41

Abbreviations: DBPSD, SD of diastolic blood pressure; HDL, high-density lipoprotein cholesterol; HR, hazard ratio; LDL, low-density lipoprotein cholesterol; SBPSD, SD of systolic blood pressure.

[a] Excluded hospitalizations, emergency room visits, urgent care visits, ambulance trips, and pregnancy clinical visits.

[b] Strong Heart Study (26), Strong Heart Family Study (27).

[c] Shared frailty Cox proportional hazards model adjusted for cohort, center, age, sex, and systolic blood pressure/diastolic blood pressure, as appropriate.

[d] Shared frailty Cox proportional hazards model adjusted for Model 1 and hypertension treatment, diabetes, body mass index, current smoking, current drinking, LDL, HDL, triglycerides kidney disease, ankle/brachial index, prevalent myocardial infarction and stroke (not for all-cause mortality), and interaction of prevalent systolic blood pressure or diastolic blood pressure and hypertension treatment.

Appendix

Appendix Table 1A. Association Between All-Cause Mortality and Systolic Blood Pressure SD Quartiles of 8 Nonurgent[a] Clinic Visits Within a 5-Year Period

Variable	Unadjusted			Model 1 adjusted[b]			Model 2 adjusted[c]		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Systolic blood pressure SD quartiles (reference, quartile 1)									
2	1.30	1.07–1.58	<.001	1.09	0.92–1.30	.32	1.02	0.85–1.23	.81
3	1.72	1.42–2.09	<.001	1.23	1.04–1.46	.02	1.17	0.98–1.40	.08
4	2.51	2.08–3.04	<.001	1.51	1.27–1.78	<.001	1.35	1.13–1.61	.001
Strong Heart Study (26) cohort (reference, Strong Heart Family Study [27])									
Strong Heart Study	4.72	4.14–5.39	<.001	1.29	1.04–1.60	.02	1.27	1.00–1.63	.05
Sex (reference, female)									
Male	1.45	1.26–1.65	<.001	0.72	0.64–0.81	<.001	0.74	0.65–0.84	<.001
Strong Heart Study site (reference, Oklahoma)									
Arizona	0.87	0.69–1.10	0.25	1.15	0.96–1.37	0.13	1.10	0.89–1.35	.38
South Dakota	1.33	1.12–1.57	<.001	1.38	1.22–1.57	<.001	1.34	1.17–1.54	<.001
Age at 8th blood pressure measurement, y	1.06	1.06–1.07	<.001	1.06	1.05–1.06	<.001	1.06	1.05–1.07	<.001
Baseline systolic blood pressure, mm Hg (continuous)	1.02	1.01–1.02	<.001	1.01	1.00–1.01	.004	1.00	0.99–1.01	.94
Hypertension treatment (reference, no)									
Yes	1.86	1.62–2.13	<.001	NA	NA	NA	0.51	0.21–1.22	.13
Diabetes (reference, no)									
Yes	2.15	1.89–2.45	<.001	NA	NA	NA	1.66	1.46–1.88	<.001
Body mass index (kg/m^2) baseline (continuous)	1.80	1.50–2.61	<.001	NA	NA	NA	1.00	0.99–1.01	.98
Current smoker (reference, no)									
Yes	1.15	1.01–1.31	.03	NA	NA	NA	1.28	1.12–1.45	<.001
Current drinker (reference, no)									
Yes	0.82	0.72–0.93	.003	NA	NA	NA	1.12	0.98–1.27	.10
LDL, mg/dL (reference, <100 mg/dL)									
100-129	0.91	0.78–1.06	.24	NA	NA	NA	0.84	0.73–0.97	.02
130-159	0.95	0.78–1.14	.57	NA	NA	NA	0.83	0.70–0.98	.03
160-189	0.96	0.71–1.30	.82	NA	NA	NA	0.74	0.57–0.95	.02
≥190	1.04	0.63–1.73	.87	NA	NA	NA	1.01	0.66–1.54	.97

Abbreviations: NA, not applicable; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.
[a] Excluded hospitalizations, emergency room visits, urgent care visits, ambulance trips, and pregnancy clinical visits.
[b] Shared frailty Cox proportional hazards model adjusted for cohort, center, age, sex, and systolic blood pressure/diastolic blood pressure, as appropriate.
[c] Shared frailty Cox proportional hazards model adjusted for cohort, sex, center, age, baseline systolic blood pressure, hypertension treatment, diabetes mellitus, body mass index, current smoking, current drinking, LDL, HDL, triglycerides, kidney disease, ankle/brachial index, prevalent myocardial infarction and stroke interaction of systolic blood pressure, and hypertension treatment.

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Appendix Table 1A. Association Between All-Cause Mortality and Systolic Blood Pressure SD Quartiles of 8 Nonurgent[a] Clinic Visits Within a 5-Year Period

Variable	Unadjusted			Model 1 adjusted[b]			Model 2 adjusted[c]		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
HDL, mg/dL (reference: men, <40 mg/dL; women, <50 mg/dL)									
Men, 40-59, women 51-59	0.73	0.64–0.83	<.001	NA	NA	NA	1.01	0.87–1.18	.88
Men ≥60, women ≥60	0.60	0.52–0.71	<.001	NA	NA	NA	1.08	0.89–1.31	.44
Triglycerides, mg/dL (reference, <150 mg/dL)									
150–199	1.03	0.87–1.23	.71	NA	NA	NA	0.96	0.82–1.12	.61
200–499	1.06	0.90–1.25	.51	NA	NA	NA	0.83	0.71–0.97	.02
≥500	1.10	0.70–1.71	.60	NA	NA	NA	0.85	0.57–1.27	.42
Kidney disease (reference, no)									
Yes	2.37	1.99–2.82	<.001	NA	NA	NA	1.27	1.08–1.50	.005
Ankle/brachial index (reference, >0.9 to≤1.4)									
≤0.9 or >1.4	2.01	1.57–2.56	<.001	NA	NA	NA	1.22	0.99–1.51	.06
Prevalent myocardial infarction or stroke (reference, no)									
Yes	3.06	2.32–4.04	<.001	NA	NA	NA	1.55	1.22–1.99	<.001

Abbreviations: NA, not applicable; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.
[a] Excluded hospitalizations, emergency room visits, urgent care visits, ambulance trips, and pregnancy clinical visits.
[b] Shared frailty Cox proportional hazards model adjusted for cohort, center, age, sex, and systolic blood pressure/diastolic blood pressure, as appropriate.
[c] Shared frailty Cox proportional hazards model adjusted for cohort, sex, center, age, baseline systolic blood pressure, hypertension treatment, diabetes mellitus, body mass index, current smoking, current drinking, LDL, HDL, triglycerides, kidney disease, ankle/brachial index, prevalent myocardial infarction and stroke interaction of systolic blood pressure, and hypertension treatment.

Appendix Table 1B. Association Between Diastolic Blood Pressure SD Quartiles of 8 Nonurgent[a] Clinic Visits Within a 5-Year Period and All-Cause Mortality

Variable	Unadjusted			Model 1 Adjusted[b]			Model 2 Adjusted[c]		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Diastolic blood pressure SD quartiles (reference, quartile 1)									
2	1.07	0.89–1.29	.72	1.06	0.91–1.24	.46	1.01	0.85–1.19	.94
3	1.32	1.09–1.58	.003	1.18	1.01–1.38	.04	1.17	0.99–1.38	.06
4	1.40	1.16–1.68	<.001	1.26	1.08–1.47	.004	1.15	0.97–1.36	.10
Strong Heart Study (26) cohort (reference, Strong Heart Family Study [27])	4.72	4.14–5.39	<.001	1.29	1.05–1.60	.02	1.27	1.00–1.62	.05
Sex (reference, female)									
Male	1.45	1.26–1.65	<.001	0.71	0.63–0.80	<.001	0.74	0.65–0.84	<.001
Strong Heart Study site (reference, Oklahoma)									
Arizona	0.87	0.69–1.10	.25	1.13	0.94–1.35	.19	1.06	0.86–1.30	.58
South Dakota	1.33	1.12–1.57	<.001	1.36	1.20–1.55	<.001	1.33	1.16–1.52	<.001
Age at eighth blood pressure measurement, y (continuous)	1.06	1.06–1.07	<.001	1.06	1.05–1.07	<.001	1.06	1.05–1.07	<.001
Baseline diastolic blood pressure, mm Hg (continuous)	1.02	1.01–1.02	<.001	1.00	0.99 – 1.00	.13	1.00	0.99–1.01	.23
Hypertension treatment (reference, no)									
Yes	1.86	1.62–2.13	<.001	NA	NA	NA	0.77	0.32–1.88	.57
Diabetes (reference, no)									
Yes	2.15	1.89–2.45	<.001	NA	NA	NA	1.68	1.48–1.91	<.001
Body mass index (kg/m^2) baseline (continuous)	1.80	1.50–2.61	<.001	NA	NA	NA	1.00	0.99–1.01	.90
Current smoker (reference, no)									
Yes	1.15	1.01–1.31	.03	NA	NA	NA	1.28	1.12–1.45	<.001
Current drinker (reference, no)									
Yes	0.82	0.72–0.93	.003	NA	NA	NA	1.12	0.99–1.28	.07
LDL, mg/dL (reference, ≤100 mg/dL)									
100–129	0.91	0.78–1.06	.24	NA	NA	NA	0.84	0.73–0.97	.02
130–159	0.95	0.78–1.14	.57	NA	NA	NA	0.83	0.70–0.99	.04
160–189	0.96	0.71–1.30	.82	NA	NA	NA	0.77	0.60–0.98	.04
≥190 mg/dL	1.04	0.63–1.73	.87	NA	NA	NA	1.04	0.68–1.58	.87

Abbreviations: NA, not applicable; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.
[a] Excluded hospitalizations, emergency room visits, urgent care visits, ambulance trips, and pregnancy clinical visits.
[b] Shared frailty Cox proportional hazards model adjusted for cohort, center, age, sex, and systolic blood pressure/diastolic blood pressure, as appropriate.
[c] Shared frailty Cox proportional hazards model adjusted for cohort, sex, center, age, baseline systolic blood pressure, hypertension treatment, diabetes, body mass index, current smoking, current drinking, LDL, HDL, triglycerides, kidney disease, ankle/brachial index, prevalent myocardial infarction and stroke, interaction of systolic blood pressure, and hypertension treatment.

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Appendix Table 1B. Association Between Diastolic Blood Pressure SD Quartiles of 8 Nonurgent[a] Clinic Visits Within a 5-Year Period and All-Cause Mortality

Variable	Unadjusted			Model 1 Adjusted[b]			Model 2 Adjusted[c]		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
HDL (reference, men, <40; women, <50)									
Men 40–59, omen 51–59	0.73	0.64–0.83	<.001	NA	NA	NA	1.01	0.87–1.18	.89
Men, ≥60; women, ≥60	0.60	0.52–0.71	<.001	NA	NA	NA	1.09	0.90–1.32	.40
Triglycerides, mg/dL (reference, ≥150 mg/dL)									
150–199 mg/dL	1.03	0.87–1.23	.71	NA	NA	NA	0.96	0.82–1.13	.64
200–499 mg/dL	1.06	0.90–1.25	.51	NA	NA	NA	0.84	0.72–0.98	.03
≥500 mg/dL	1.10	0.70–1.71	.69	NA	NA	NA	0.88	0.59–1.31	.53
Kidney disease (reference, no)									
Yes	2.37	1.99–2.82	<.001	NA	NA	NA	1.30	1.10–1.53	.002
Ankle/brachial index (reference≥0.9 to ≤1.4)									
≤0.9 or >1.4	2.01	1.57–2.56	<.001	NA	NA	NA	1.23	0.99–1.51	.06
Prevalent myocardial infarction or stroke (reference, no)									
Yes	3.06	2.32–4.04	<.001	NA	NA	NA	1.52	1.19–1.94	<.001

Abbreviations: NA, not applicable; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.
[a] Excluded hospitalizations, emergency room visits, urgent care visits, ambulance trips, and pregnancy clinical visits.
[b] Shared frailty Cox proportional hazards model adjusted for cohort, center, age, sex, and systolic blood pressure/diastolic blood pressure, as appropriate.
[c] Shared frailty Cox proportional hazards model adjusted for cohort, sex, center, age, baseline systolic blood pressure, hypertension treatment, diabetes, body mass index, current smoking, current drinking, LDL, HDL, triglycerides, kidney disease, ankle/brachial index, prevalent myocardial infarction and stroke, interaction of systolic blood pressure, and hypertension treatment.

Appendix Table 2A. Association Between Systolic Blood Pressure SD Quartiles of 8 Nonurgent[a] Clinic Visits Within a 5-Year Period and Cardiovascular Mortality

Variable	Unadjusted			Model 1 adjusted[b]			Model 2 adjusted[c]		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Systolic blood pressure SD quartiles (reference, quarter 1)									
2	1.68	1.20–2.37	.003	1.39	1.01–1.91	.05	1.24	0.87–1.76	.24
3	1.82	1.30–2.57	<.001	1.28	0.93–1.76	.13	1.17	0.82–1.66	.38
4	3.73	2.70–5.13	<.001	2.09	1.55–2.83	<.001	1.81	1.29–2.53	<.001
Strong Heart Study (26) cohort (reference, Strong Heart Family Study (27)									
Strong Heart Study	7.24	5.52–9.49	<.001	1.60	1.12–2.30	.01	1.81	1.15–2.85	.01
Sex (reference, female)									
Male	1.57	1.27–1.94	<.001	0.66	0.54–0.80	<.001	0.6	0.51–0.81	<.001
Strong Heart Study site (reference, Oklahoma)									
Arizona	0.61	0.41–0.90	0.01	0.82	0.58–1.13	.23	0.79	0.52–1.19	.25
South Dakota	1.35	1.05–1.73	0.02	1.41	1.15–1.73	.001	1.44	1.14–1.83	.002
Age at eighth blood pressure measurement, y (continuous)	1.08	1.07–1.09	<.001	1.07	1.05–1.08	<.001	1.06	1.05–1.07	<.001
Baseline systolic blood pressure, mm Hg (continuous)	1.02	1.02–1.03	<.001	1.01	1.00–1.01	.01	1.00	0.99–1.01	.63
Hypertension treatment (reference, no)									
Yes	2.67	2.16–3.30	<.001	NA	NA	NA	0.94	0.12–2.68	.48
Diabetes (reference, no)									
Yes	3.24	2.61–4.02	<.001	NA	NA	NA	2.49	1.97–3.13	<.001
Body mass index (kg/m^2) baseline (continuous)	0.99	0.97–1.00	.03	NA	NA	NA	0.99	0.97–1.01	.46
Current smoker (reference, no)									
Yes	1.08	0.88–1.34	.46	NA	NA	NA	1.30	1.03–1.63	.03
Current drinker (reference, no)									
Yes	0.62	0.50–0.76	<.001	NA	NA	NA	0.89	0.70–1.12	.30
LDL l, mg/dL (reference, ≥100 mg/dL)									
100–129	1.10	0.85–1.42	.48	NA	NA	NA	0.95	0.73–1.24	.72
130–159	1.26	0.94–1.70	.12	NA	NA	NA	1.02	0.75–1.38	.90
160–189	1.35	0.86–2.11	.19	NA	NA	NA	0.82	0.53–1.27	.38

Abbreviations: NA, not applicable; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol.
[a] Excluded hospitalizations, emergency room visits, urgent care visits, ambulance trips, and pregnancy clinical visits.
[b] Shared frailty Cox proportional hazards model adjusted for cohort, center, age, sex, and systolic blood pressure/diastolic blood pressure, as appropriate.
[c] Shared frailty Cox proportional hazards model adjusted for cohort, sex, center, age, baseline systolic blood pressure, hypertension treatment, diabetes, body mass index, current smoking, current drinking, LDL, HDL, triglycerides, kidney disease, ankle/brachial index, prevalent myocardial infarction and stroke, interaction of systolic blood pressure, and hypertension treatment.

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Appendix Table 2A. Association Between Systolic Blood Pressure SD Quartiles of 8 Nonurgent[a] Clinic Visits Within a 5-Year Period and Cardiovascular Mortality

Variable	Unadjusted			Model 1 adjusted[b]			Model 2 adjusted[c]		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
≥190	1.56	0.75–3.23	.23	NA	NA	NA	1.33	0.65–2.76	.44
HDL mg/dL(reference, men <40; women <50 mg/dL)									
Men 40–59, women 51–59	0.72	0.58–0.90	.003	NA	NA	NA	1.16	0.88–1.52	.30
Men ≥60, women ≥60	0.51	0.38–0.68	<.001	NA	NA	NA	1.26	0.87–1.83	.21
Triglycerides (reference, <150 mg/dL)									
150–199 mg/dL	1.33	1.01–1.75	.04	NA	NA	NA	1.17	0.88–1.55	.28
200–499 mg/dL	1.51	1.17–1.95	.002	NA	NA	NA	1.07	0.82–1.41	.61
≥500 mg/dL	1.09	0.51–2.30	.83	NA	NA	NA	0.93	0.45–1.90	.84
Kidney disease (reference, no)									
Yes	3.40	2.62–4.40	<.001	NA	NA	NA	1.51	1.15–1.99	.003
Ankle/brachial index (reference, >0.9 to ≤1.4)									
≤0.9 or >1.4	2.68	1.87–3.85	<.001	NA	NA	NA	1.49	1.05–2.10	.03
Prevalent myocardial infarction or stroke (reference, no)									
Yes	5.73	3.99–8.22	<.001	NA	NA	NA	2.47	1.73–3.52	<.001

Abbreviations: NA, not applicable; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol.
[a] Excluded hospitalizations, emergency room visits, urgent care visits, ambulance trips, and pregnancy clinical visits.
[b] Shared frailty Cox proportional hazards model adjusted for cohort, center, age, sex, and systolic blood pressure/diastolic blood pressure, as appropriate.
[c] Shared frailty Cox proportional hazards model adjusted for cohort, sex, center, age, baseline systolic blood pressure, hypertension treatment, diabetes, body mass index, current smoking, current drinking, LDL, HDL, triglycerides, kidney disease, ankle/brachial index, prevalent myocardial infarction and stroke, interaction of systolic blood pressure, and hypertension treatment.

Appendix Table 2B. Association Between Diastolic Blood Pressure SD Quartiles of 8 Nonurgent[a] Clinic Visits Within a 5-Year Period and Cardiovascular Mortality

Variable	Unadjusted			Model 1, adjusted[b]			Model 2, adjusted[c]		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Diastolic blood pressure SD quartiles (reference, quartile 1)									
2	0.93	0.69–1.26	.65	0.96	0.73–1.26	.76	0.92	0.68–1.26	.62
3	1.27	0.95–1.71	.11	1.19	0.91–1.56	.20	1.13	0.84–1.52	.43
4	1.39	1.04–1.86	.03	1.30	1.00–1.69	.05	1.22	0.91–1.65	.18
Strong Heart Study (26) cohort (reference, Strong Heart Family Study (27)									
Strong Heart Study	7.24	5.52–9.49	<.001	1.63	1.14–2.34	.007	1.85	1.18–2.90	.007
Sex (reference, female)									
Male	1.57	1.27–1.94	<.001	0.66	0.55–0.81	<.001	0.64	0.51–0.81	<.001
Strong Heart Study site (reference, Oklahoma)									
Arizona	0.61	0.41–0.90	.01	0.79	0.56–1.09	.15	0.74	0.49–1.11	.15
South Dakota	1.35	1.05–1.73	.02	1.37	1.12–1.69	.002	1.44	1.14–1.82	.002
Age at eighth blood pressure measurement, y	1.08	1.07–1.09	<.001	1.07	1.06–1.08	<.001	1.06	1.05–1.08	<.001
Baseline diastolic blood pressure, mm Hg (continuous)	1.02	1.02–1.03	<.001	0.99	0.98 – 1.00	0.15	0.99	0.97–1.00	.12
Hypertension treatment (reference, no)									
Yes	2.67	2.16–3.30	<.001	NA	NA	NA	0.98	0.16–3.98	.79
Diabetes (reference, no)									
Yes	3.24	2.61–4.02	<.001	NA	NA	NA	2.50	1.98–3.14	<.001
Body mass index (kg/m^2) baseline (continuous)	0.99	0.97–1.00	.03	NA	NA	NA	0.99	0.98–1.01	.58
Current smoker (reference, no)									
Yes	1.08	0.88–1.34	.46	NA	NA	NA	1.31	1.04–1.64	.02
Current drinker (reference, no)									
Yes	0.62	0.50–0.76	<.001	NA	NA	NA	0.91	0.73–1.14	.41
LDL (reference, 100 mg/dL)									
100–129	1.10	0.85–1.42	.48	NA	NA	NA	0.96	0.74–1.25	.76
130–159	1.26	0.94–1.70	.12	NA	NA	NA	1.05	0.77–1.42	.76
160–189	1.35	0.86–2.11	.19	NA	NA	NA	0.88	0.57–1.36	.57

Abbreviations: NA, not applicable; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol.
[a] Excluded hospitalizations, emergency room visits, urgent care visits, ambulance trips, and pregnancy clinical visits.
[b] Shared frailty Cox proportional hazards model adjusted for cohort, center, age, sex, and systolic blood pressure/diastolic blood pressure, as appropriate.
[c] Shared frailty Cox proportional hazards model adjusted for cohort, sex, center, age, baseline diastolic blood pressure, hypertension treatment, diabetes, body mass index, current smoking, current drinking, LDL, HDL, triglycerides, kidney disease, ankle/brachial index, prevalent myocardial infarction and stroke, interaction of systolic blood pressure, and hypertension treatment.

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Appendix Table 2B. Association Between Diastolic Blood Pressure SD Quartiles of 8 Nonurgent[a] Clinic Visits Within a 5-Year Period and Cardiovascular Mortality

Variable	Unadjusted			Model 1, adjusted[b]			Model 2, adjusted[c]		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
≥190	1.56	0.75–3.23	.23	NA	NA	NA	1.39	0.67–2.87	.37
HDL (reference, men, <40; women, <50)									
Men 40–59, women 51–59	0.72	0.58–0.90	.003	NA	NA	NA	1.15	0.87–1.52	.31
Men ≥60, Women ≥60	0.51	0.38–0.68	<.001	NA	NA	NA	1.28	0.88–1.84	.19
Triglycerides (reference, ≥150 mg/dL)									
150–199 mg/dL	1.33	1.01–1.75	.04	NA	NA	NA	1.17	0.88–1.54	.28
200–499 mg/dL	1.51	1.17–1.95	.002	NA	NA	NA	1.10	0.84–1.45	.48
≥500 mg/dL	1.09	0.51–2.30	.83	NA	NA	NA	1.00	0.49–2.04	.99
Kidney disease (reference, no)									
Yes	3.40	2.62–4.40	<.001	NA	NA	NA	1.58	1.21–2.08	.001
Ankle/brachial index (reference, >0.9 to ≤1.4)									
≤0.9 or >1.4	2.68	1.87–3.85	<.001	NA	NA	NA	1.51	1.07–2.13	.02
Prevalent myocardial infarction or stroke (reference, no)									
Yes	5.73	3.99–8.22	<.001	NA	NA	NA	2.39	1.68–3.39	<.001

Abbreviations: NA, not applicable; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol.
[a] Excluded hospitalizations, emergency room visits, urgent care visits, ambulance trips, and pregnancy clinical visits.
[b] Shared frailty Cox proportional hazards model adjusted for cohort, center, age, sex, and systolic blood pressure/diastolic blood pressure, as appropriate.
[c] Shared frailty Cox proportional hazards model adjusted for cohort, sex, center, age, baseline diastolic blood pressure, hypertension treatment, diabetes, body mass index, current smoking, current drinking, LDL, HDL, triglycerides, kidney disease, ankle/brachial index, prevalent myocardial infarction and stroke, interaction of systolic blood pressure, and hypertension treatment.

Appendix Table 3A. Association Between Systolic Blood Pressure SD Quartiles of 8 Nonurgent[a] Clinic Visits Within a 5-Year Period and Major Adverse Cardiovascular Events

Variable	Unadjusted			Model 1, adjusted[b]			Model 2, adjusted[c]		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Diastolic blood pressure SD quartiles (reference, quartile 1)									
2	1.59	1.22–2.07	<.001	1.41	1.11–1.78	.004	1.36	1.05–1.77	.02
3	1.49	1.13–1.95	.004	1.09	0.86–1.39	.47	1.06	0.81–1.39	.65
4	2.53	1.95–3.28	<.001	1.49	1.18–1.88	<.001	1.39	1.07–1.80	.01
Strong Heart Study (26) cohort (reference, Strong Heart Family Study (27)									
Strong Heart Study	4.84	3.99–5.86	<.001	1.35	1.05–1.74	.02	1.38	0.98–1.95	.06
Sex (reference, female)									
Male	1.69	1.41–2.03	<.001	0.64	0.55–0.75	<.001	0.62	0.52–0.75	<.001
Strong Heart Study site (reference, Oklahoma)									
Arizona	0.75	0.53–1.07	.11	0.92	0.69–1.22	.55	0.91	0.65–1.28	.60
South Dakota	2.34	1.87–2.94	<.001	2.25	1.90–2.67	<.001	2.41	1.98–2.94	<.001
Age at eighth blood pressure, y (continuous)	1.06	1.05–1.07	<.001	1.05	1.05–1.06	<.001	1.05	1.04–1.06	<.001
Baseline systolic blood pressure, mm Hg (continuous)	1.02	1.01–1.02	<.001	1.01	1.00–1.01	<.001	1.00	1.00–1.01	.20
Hypertension treatment (reference, no)									
Yes	2.22	1.84–2.67	<.001	NA	NA	NA	0.95	0.27–3.28	.93
Diabetes (reference, no)									
Yes	2.64	2.20–3.16	<.001	NA	NA	NA	1.97	1.64–2.36	<.001
Body mass index (kg/m^2) baseline (continuous)	0.99	0.99–1.00	.02	NA	NA	NA	0.99	0.98–1.01	.48
Current smoker (reference, no)									
Yes	1.30	1.09–1.56	.003	NA	NA	NA	1.41	1.18–1.69	.001
Current drinker (reference, no)									
Yes	0.69	0.58–0.83	<.001	NA	NA	NA	0.84	0.70–1.02	.07
LDL (reference,<100 mg/dL)									
100–129	1.18	0.94–1.46	.15	NA	NA	NA	1.08	0.87–1.33	.51
130–159	1.73	1.35–2.22	<.001	NA	NA	NA	1.32	1.04–1.68	.02
160–189	1.60	1.08–2.36	.02	NA	NA	NA	0.95	0.67–1.36	.79
≥190	1.18	0.59–2.36	.63	NA	NA	NA	0.90	0.47–1.74	.75

Abbreviations: NA, not applicable; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol.
[a] Excluded hospitalizations, emergency room visits, urgent care visits, ambulance trips, and pregnancy clinical visits.
[b] Shared frailty Cox proportional hazards model adjusted for cohort, center, age, sex, and systolic blood pressure/diastolic blood pressure, as appropriate.
[c] Shared frailty Cox proportional hazards model adjusted for cohort, sex, center, age, baseline systolic blood pressure, hypertension treatment, diabetes, body mass index, current smoking, current drinking, LDL, HDL, triglycerides, kidney disease, ankle/brachial index, interaction of systolic blood pressure, and hypertension treatment.

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Appendix Table 3A. Association Between Systolic Blood Pressure SD Quartiles of 8 Nonurgent[a] Clinic Visits Within a 5-Year Period and Major Adverse Cardiovascular Events

Variable	Unadjusted			Model 1, adjusted[b]			Model 2, adjusted[c]		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
HDL (reference, men <40; women <50)									
Men 40–59, women 51–59	0.72	0.58–0.90	.003	NA	NA	NA	1.05	0.84–1.30	.68
Men ≥60, women ≥60	0.51	0.38–0.68	<.001	NA	NA	NA	0.99	0.74–1.32	.95
Triglycerides (reference, <150 mg/dL)									
150–199	1.31	1.04–1.65	.02	NA	NA	NA	1.10	0.88–1.38	.40
200–499	1.37	1.10–1.72	.005	NA	NA	NA	0.98	0.78–1.22	.85
≥500	1.63	0.91–2.91	.10	NA	NA	NA	1.24	0.71–2.16	.46
Kidney disease (reference, no)									
Yes	2.68	2.12–3.39	<.001	NA	NA	NA	1.42	1.12–1.79	.003
Ankle/brachia index (reference, >0.9 to ≤1.4)									
≤0.9 or >1.4	2.11	1.51–2.95	<.001	NA	NA	NA	1.33	0.98–1.82	.07

Abbreviations: NA, not applicable; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol.
[a] Excluded hospitalizations, emergency room visits, urgent care visits, ambulance trips, and pregnancy clinical visits.
[b] Shared frailty Cox proportional hazards model adjusted for cohort, center, age, sex, and systolic blood pressure/diastolic blood pressure, as appropriate.
[c] Shared frailty Cox proportional hazards model adjusted for cohort, sex, center, age, baseline systolic blood pressure, hypertension treatment, diabetes, body mass index, current smoking, current drinking, LDL, HDL, triglycerides, kidney disease, ankle/brachial index, interaction of systolic blood pressure, and hypertension treatment.

Appendix Table 3B. Association Between Diastolic Blood Pressure SD Quartiles of 8 Nonurgent[a] Clinic Visits Within a 5-Year Period and Major Adverse Cardiovascular Events

Variable	Unadjusted			Model 1, adjusted[b]			Model 2, adjusted[c]		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Diastolic blood pressure SD quartiles (reference, quartile 1)									
2	0.99	0.77–1.28	.97	1.02	0.82–1.27	.84	0.98	0.78–1.25	.90
3	1.22	0.96–1.57	.11	1.19	0.96–1.47	.11	1.10	0.87–1.39	.45
4	1.24	0.97–1.6	.08	1.18	0.95–1.46	.13	1.11	0.87–1.40	.41
Strong Heart Study (26) cohort (reference, Strong Heart Family Study [27])									
Strong Heart Study	4.84	3.99–5.86	<.001	1.32	1.03–1.69	.03	1.36	0.97–1.90	.07
Sex (reference, female)									
Male	1.69	1.41–2.03	<.001	0.66	0.56–0.77	<.001	0.65	0.54–0.78	<.001
Strong Heart Study site (reference, Oklahoma)									
Arizona	0.75	0.53–1.07	.11	0.90	0.68–1.19	.46	0.88	0.62–1.23	.44
South Dakota	2.34	1.87–2.94	<.001	2.13	1.80–2.53	<.001	2.29	1.88–2.79	<.001
Age at 8th blood pressure, y (continuous)	1.06	1.05–1.07	<.001	1.06	1.05–1.07	<.001	1.06	1.04–1.07	<.001
Baseline diastolic blood pressure, mm Hg (continuous)	1.02	1.01–1.02	<.001	1.01	1.00–1.01	.19	1.01	0.99–1.02	.34
Hypertension treatment (reference, no)									
Yes	2.22	1.84–2.67	<.001	NA	NA	NA	1.50	0.42–5.43	.53
Diabetes (reference, no)									
Yes	2.64	2.20–3.16	<.001	NA	NA	NA	2.00	1.67–2.40	<.001
Body mass index (kg/m^2) baseline (continuous)	0.99	0.99–1.00	.02	NA	NA	NA	1.00	0.98–1.01	.50
Current smoker (reference, no)									
Yes	1.30	1.09–1.56	.003	NA	NA	NA	1.41	1.18–1.69	<.001
Current drinker (reference, no)									
Yes	0.69	0.58–0.83	<.001	NA	NA	NA	0.85	0.71–1.03	.09
LDL (reference, <100 mg/dL)									
100–129 mg/dL	1.18	0.94–1.46	.15	NA	NA	NA	1.05	0.85–1.30	.63
130–159 mg/dL	1.73	1.35–2.22	<.001	NA	NA	NA	1.31	1.03–1.66	.03
160–189 mg/dL	1.60	1.08–2.36	.02	NA	NA	NA	1.01	0.71–1.43	.97
≥190 mg/dL	1.18	0.59–2.36	.63	NA	NA	NA	0.90	0.47–1.74	.76

Abbreviations: NA, not applicable; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.
[a] Excluded hospitalizations, emergency room visits, urgent care visits, ambulance trips, and pregnancy clinical visits.
[b] Shared frailty Cox proportional hazards model adjusted for cohort, center, age, sex, and systolic blood pressure/diastolic blood pressure as appropriate.
[c] Shared frailty Cox proportional hazards model adjusted for cohort, sex, center, age, baseline diastolic blood pressure, hypertension treatment, diabetes, body mass index, current smoking, current drinking, LDL, HDL, triglycerides, kidney disease, ankle/brachial index, interaction of systolic blood pressure, and hypertension treatment.

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Appendix Table 3B. Association Between Diastolic Blood Pressure SD Quartiles of 8 Nonurgent[a] Clinic Visits Within a 5-Year Period and Major Adverse Cardiovascular Events

Variable	Unadjusted			Model 1, adjusted[b]			Model 2, adjusted[c]		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
HDL (reference, men <40; women <50)									
Men 40–59 (Women 51–59	0.72	0.58–0.90	.003	NA	NA	NA	1.06	0.85–1.32	.60
Men ≥60 (Women ≥60	0.51	0.38–0.68	<.001	NA	NA	NA	1.00	0.75–1.33	.98
Triglycerides (reference, <150 mg/dL)									
150–199	1.31	1.04–1.65	.02	NA	NA	NA	1.09	0.87–1.36	.45
200–499	1.37	1.10–1.72	.005	NA	NA	NA	0.99	0.79–1.23	.91
≥500	1.63	0.91–2.91	.10	NA	NA	NA	1.26	0.73–2.19	.41
Kidney disease (reference, no)									
Yes	2.68	2.12–3.39	<.001	NA	NA	NA	1.49	1.19–1.88	<.001
Ankle/brachial index (reference, >0.9 to ≤1.4)									
≤0.9 or >1.4	2.11	1.51–2.95	<.001	NA	NA	NA	1.34	0.98–1.82	.06

Abbreviations: NA, not applicable; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol.
[a] Excluded hospitalizations, emergency room visits, urgent care visits, ambulance trips, and pregnancy clinical visits.
[b] Shared frailty Cox proportional hazards model adjusted for cohort, center, age, sex, and systolic blood pressure/diastolic blood pressure as appropriate.
[c] Shared frailty Cox proportional hazards model adjusted for cohort, sex, center, age, baseline diastolic blood pressure, hypertension treatment, diabetes, body mass index, current smoking, current drinking, LDL, HDL, triglycerides, kidney disease, ankle/brachial index, interaction of systolic blood pressure, and hypertension treatment.

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ORIGINAL RESEARCH

Vitamin D Deficiency and Cardiovascular Disease Risk Factors Among American Indian Adolescents: The Strong Heart Family Study

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PEER REVIEWED

Summary

What is already known on this topic?

In non-Native populations in the US, vitamin D deficiency is associated with obesity prevalence and may be amenable to interventions through changes in diet and vitamin supplementation.

What is added by this report?

This first report of vitamin D deficiency prevalence among American Indian adolescents and its association with cardiovascular disease risk factors demonstrated an independent association between the prevalence of metabolic syndrome and vitamin D deficiency. Thirteen years after baseline, the incidence rate of diabetes was significantly higher among American Indian adolescents with (vs without) vitamin D deficiency.

What are the implications for public health practice?

These results may provide a path for developing measures to reduce cardiovascular disease risk factors at an early age in American Indians.

Abstract

Introduction

We aimed to describe the prevalence of vitamin D deficiency among American Indian adolescents and determine its association with cardiovascular disease (CVD) risk factors.

Methods

Our study population consisted of 307 adolescents (aged ≤20 years) participating in the Strong Heart Family Study with serum 25-hydroxyvitamin D (25[OH]D) measured on samples collected during baseline examinations (2001–2003). We defined baseline prevalence of vitamin D deficiency as 25(OH)D ≤20 ng/mL. We evaluated outcomes related to obesity (BMI, waist circumference, wait-to-hip ratio, and body fat percentage), diabetes, cholesterol, and metabolic syndrome. We used generalized estimating equations to determine whether the prevalence of the outcomes differed according to vitamin D deficiency status, while controlling for covariates. To determine incidence, we conducted a follow-up examination a median 5.8 years after baseline (2006–2009) and a second follow-up a median of 13.3 years after baseline (2014–2018). We calculated incidence rates (IR) per 100 person-years for the total group and stratified by vitamin D deficiency status at baseline. Finally we used shared frailty cox proportional hazards models to determine if the risk of the outcomes differed according to vitamin D deficiency status, while controlling for covariates.

Results

The prevalence of vitamin D deficiency was 50.8% at baseline, and it was associated with the prevalence of obesity, low HDL-C, and metabolic syndrome, while controlling for covariates. By the first follow-up, the IRs per 100 person-years were the following: obesity (5.03), diabetes (1.07), any dyslipidemia (10.80), and metabolic syndrome (3.31). By the second follow-up, the IR of diabetes was significantly higher among those with (vs without) baseline vitamin D deficiency (1.32 vs 0.68 per 100 person-years; $P = .02$), although the association was not significant after adjusting for covariates.

Conclusion

Vitamin D deficiency in adolescence may be associated with the CVD risk factors obesity, low HDL-C, and metabolic syndrome

and may also contribute to the development of diabetes later in life.

Introduction

According to National Health and Nutrition Examination Survey (NHANES) data for 2001 through 2018, only 25.5% to 27.5% of US adolescents have sufficient serum 25-hydroxyvitamin D (25[OH]D) (1). Racial and ethnic differences in 25(OH)D levels exist, with the prevalence of deficiency higher among non-Hispanic Black and Hispanic groups than non-Hispanic White groups (1,2). However, information on 25(OH)D levels among American Indian adolescents is limited to a single study, which reported a mean (SD) 25(OH)D level of 17.8 (0.4) ng/mL (deficient) in a population of American Indian children and adolescents (aged 5–18 y) in Nebraska (3).

Vitamin D deficiency may be associated with obesity and other cardiovascular disease (CVD) risk factors, such as dyslipidemia and diabetes among adults (4,5). Associations have been observed between vitamin D deficiency and obesity, elevated hypertension, low high-density lipoprotein cholesterol (HDL-C), and diabetes among children, adolescents, and young adults (aged 1–21 y) (6). However, these associations are largely from cross-sectional studies; the temporal relationship between vitamin D deficiency and CVD risk factors remains to be determined. Additionally, no current studies have addressed associations between vitamin D deficiency and CVD risk factors in the American Indian adolescent population (7).

This study aimed to address this gap by using Strong Heart Family Study (SHFS) data to establish the baseline prevalence of vitamin D deficiency among American Indian adolescents. Because low levels of 25(OH)D are amenable to interventions through diet, vitamin supplementation, and lifestyle modifications, if temporal relationships between vitamin D deficiency and CVD risk factors exist, there is potential to reduce and control obesity and other CVD-related factors at a young age (8). Therefore, the objectives of this study were to evaluate the cross-sectional associations between vitamin D deficiency and CVD risk factors, as well as associations between vitamin D deficiency and incident obesity, diabetes, dyslipidemia, and metabolic syndrome, among American Indian adolescents who participated in SHFS.

Methods

All data were collected, analyzed, and reported under agreements made with the sovereign tribal nations that partnered in this research; the agreements preclude commonly accepted modes of data sharing. Requests to access the data set from qualified researchers trained in human subject confidentiality protocols may be sent to the Strong Heart Study Coordinating Center at https://strongheartstudy.org. Requests will be reviewed by tribal research partners before data can be released. This policy is consistent with the NIH Policy for Data Management and Sharing: Responsible Management and Sharing of American Indian/Alaska Native Participant Data (9).

Study population

SHFS is a multicentered, family-based, prospective cohort study of CVD among American Indians (10,11). It includes 12 American Indian communities and tribes living in central Arizona, southwestern Oklahoma, and North and South Dakota (10). Participants include the original Strong Heart Study cohort members, their extended family members, and additional families from the same regions and communities. For this analysis, we included adolescents who participated in the baseline examination (2001–2003) of the SHFS (11,12). We used information collected at baseline to determine prevalence measures of obesity, diabetes, any dyslipidemia, metabolic syndrome, and covariates (13,14). We invited all baseline participants to participate in a follow-up examination (2006–2009; median [range] years after baseline = 5.8 [3.0–8.5]) (11). We also performed a second follow-up (2014–2018; median [range] years after baseline = 13.3 [11.1–15.5]). This second follow-up was limited in that it included only collection of survey data (demographic and medical history questionnaire) and a medical record review for selected variables, including diabetes. We did not assess obesity, dyslipidemia, or metabolic syndrome at the second follow-up because we did not perform a physical examination. Information collected at baseline and follow-up was used to determine the incidence of obesity, diabetes, any dyslipidemia, and metabolic syndrome (13,14).

25(OH)D assessment

At the time of baseline recruitment, we collected and stored blood samples in a −80° C freezer. During an SHFS ancillary study in 2014, we used tandem mass spectrometry to measure the predominant circulating form of vitamin D, 25(OH)D, on blood collected during the baseline examination; this measurement took place 11 to 13 years after baseline data collection. We defined vitamin D deficiency according to the Institute of Medicine–recommended serum cut points for 25(OH)D: deficient is defined as ≤20 ng/mL (≤50 nmol/L) and sufficient as >20 ng/mL (>50 nmol/L) (15).

Obesity assessment

At both baseline and follow-up, we assessed height, weight, waist, and hip circumference during the physical examination. We measured weight with a Tanita BWB-800 5 adult digital scale and height with a vertical mounted ruler (16). We calculated body

mass index (BMI) by dividing weight in kilograms by height in meters squared (kg/m^2) (17). At baseline, because all participants were adolescents, we defined obesity as the 95th percentile and overweight as the 85th percentile of BMI based on age, definitions developed by the National Center for Health Statistics (Table 1) (18). At follow-up, when all participants were adults, we defined obesity as BMI ≥30 kg/m^2 and overweight as BMI equal to 25.0–29.9 kg/m^2 (17).

Similarly, at baseline we defined high waist circumference on the basis of age- and sex-specific cutoffs for adolescents (19) and at follow-up as >40 in for men or >35 in for women (20). We calculated waist-to-hip ratio by dividing the waist circumference by the hip circumference. We defined high waist-to-hip ratio as ≥0.90 for males and ≥0.85 for females at baseline and follow-up. We used an impedance meter (model B14101, RJL Equipment Co) to estimate body mass and used equations based on total body water validated in American Indian populations (21). We defined high body fat as ≥25% for males and ≥35% for females at baseline and follow-up (21). We defined incident high BMI, high waist circumference, high waist-to-hip ratio, or high body fat as the development by the first follow-up examination among participants who did not have these conditions at baseline.

Diabetes assessment

We defined diabetes as taking diabetes medication, and/or having a fasting plasma glucose (FPG) level ≥126 mg/dL (22). We defined impaired fasting glucose (IFG) as an FPG from 110 mg/dL to <126 mg/dL (16,22). To measure FPG, we drew blood after a 12-hour fast at baseline and first follow-up (23). In addition, to determine the use of medications for diabetes at baseline and the first follow-up, we asked participants to bring their medications to the physical examination and to recall (with assistance from an adult for minors) additional medications (24). At second follow-up, based on medical record review, we classified a participant as having diabetes if FPG ≥126 mg/dL, hemoglobin A_{1c} ≥6.5%, 2-hour plasma glucose during an oral glucose tolerance test ≥200 mg/dL, or the participant was using insulin, oral agents, or diet and/or exercise for diabetes treatment. We defined incident diabetes as the development of diabetes by the first or second follow-up among participants who did not have diabetes at baseline.

Dyslipidemia assessment

To measure lipids, we drew blood after a 12-hour fast at baseline and first follow-up examination (14,23). At baseline, abnormal cholesterol was based on age- and sex-specific cutoffs for adolescents (19) and at follow-up based on sex-specific cutoffs for adults (Table 1). We defined any dyslipidemia as high total cholesterol, high low-density lipoprotein cholesterol (LDL-C), low HDL-C, high non-HDL-C, high triglycerides, or taking lipid-lowering medication (Table 1) (14,25). We defined dyslipidemia incidence as the development of any dyslipidemia by the first follow-up examination among participants who did not have dyslipidemia at baseline.

Metabolic syndrome assessment

We defined metabolic syndrome as having at least 3 of the 5 components for the syndrome: high waist circumference, high blood pressure, high triglycerides, high FPG, or low HDL-C. At baseline, we used age- and sex-specific cutoffs, and at follow-up, we used adult cutoffs (Table 1) (13,14). We measured blood pressure in the right arm while the participant was in the seated position after 5 minutes of rest, and we used the average of the second and third measurements for analysis (16,24). We defined metabolic syndrome incidence as the development of metabolic syndrome by the first follow-up examination among participants who did not have it at baseline.

Covariate assessment

We selected several covariates, which investigations previously reported to be associated with both vitamin D deficiency and the CVD risk factor outcomes (7,24,26). During the baseline and follow-up examinations, we collected self-reported data on demographic and clinical characteristics (age, sex, and current smoking) (14). At both baseline and first follow-up, we defined hypertension as having high blood pressure (Table 1) and/or taking antihypertension medication. We estimated renal function by using the urinary albumin-creatinine ratio and defined albuminuria as ≥30 mg/g.

To determine the amount of vitamin D intake at baseline, we administered a Block 119-item food frequency questionnaire (FFQ) (27). In addition to the questions on the standard Block FFQ, we included supplemental questions about consumption of common American Indian foods, such as menudo, pozole, guava, red or green chili, Indian taco, fry bread, corn tortilla, flour tortilla, and Spam (16,28). For each standard and supplemental food item listed on the FFQ, participants reported how often they consumed each in the previous year, consumption frequency (never, a few times per year, once per month, 2 or 3 times per month, once per week, twice per week, 2 or 3 times per week, 5 or 6 times per week, or daily), and portion size (small, medium, or large) (7,16). We used the Block database (Block Dietary Systems) to calculate micronutrient intakes, including vitamin D (28).

Statistical analysis

We used descriptive statistics to summarize the prevalence of 25(OH)D according to the standardized cut point (20 ng/mL). For

the baseline cross-sectional analysis, we reported the mean (SD) for normally distributed continuous variables, the median (IQR) for skewed variables, or the frequency and percentage for categorical variables. Because of the familial sampling design, our data were correlated. Therefore, we used generalized estimating equation (GEE) methods to determine whether risk factors at baseline differed between participants with and without vitamin D deficiency, while accounting for clustering between families. Since vitamin D deficiency may vary according to sunlight exposure, we used study center as a surrogate for sunlight exposure. We summarized the prevalence of vitamin D deficiency at each study location and for all 3 centers combined. In addition, we evaluated the season of serum collection as a surrogate for sunlight exposure.

To evaluate the cross-sectional association while controlling for covariates and accounting for the clustered family sampling study design, we used GEE methods to estimate multivariable logistic regression models and calculate prevalence odds ratios (PORs) and 95% CIs. We selected covariates (age, sex, study center, current smoking, hypertension, BMI percentile, diabetes, or any dyslipidemia) on the basis of previously reported associations with vitamin D deficiency and outcomes (7,24,26). All selected covariates were simultaneously entered in the multivariable models. Because metabolic syndrome is a combined outcome containing measures of obesity, lipids, blood pressure, and FPG, we adjusted the metabolic syndrome model for age, sex, study center, and current smoking.

To explore how baseline 25(OH)D levels may influence future CVD risk factors, we analyzed the incidence of CVD risk factors. We defined the incidence of CVD risk factors as the development of the risk factor, based on age- and sex-specific cut points, among participants who did not have the risk factor at baseline. After we made baseline exclusions for each outcome, we calculated the incidence rate (IR) per 100 person-years for the total group and stratified by vitamin D deficiency status at baseline. We used Kaplan–Meier curves and log-rank tests to determine whether the probabilities of each CVD risk factor outcome differed between participants with or without baseline vitamin D deficiency (29).

We assessed the multivariable relationship between vitamin D deficiency and incident CVD risk factors by using a shared frailty Cox model based on proportional hazards to account for the relatedness among participants (29). We used this method to calculate hazard ratios (HRs) and 95% CIs of associations between vitamin D deficiency and time to each incident CVD risk factor. Each reported analysis met the assumption of proportional hazards. We used similar model-building procedures as we used in the cross-sectional analyses. Interaction between covariates and any dyslipidemia was evaluated by including appropriate cross-product terms in the model, and no significant interactions were found. We also considered models with season of blood draw as a covariate (spring/summer vs fall/winter), waist circumference instead of BMI as a covariate (in the diabetes and dyslipidemia models), and continuous outcomes instead of categorical outcomes. The outcomes of these analyses were not meaningfully different than those presented. We used a significance level of .05 for hypothesis tests and performed statistical analyses in SAS version 9.4 (SAS Institute Inc).

Results

Population characteristics and prevalence of vitamin D deficiency

At baseline, 320 participants met the inclusion criteria for being aged 20 years or younger. Of these, 307 (95.9%) had valid 25(OH)D measurements. Of these 307, 38 (12.4%) did not participate in the first follow-up examination. The mean (SD) age at baseline was 17.4 (1.5) years; 52.1% were female, 25.5% were smokers, 10.0% had hypertension, and none had albuminuria (Table 2). Median (IQR) vitamin D intake was 127.8 (61.9–267.3) IU, and 9.8% were taking vitamin D supplements. Of the 307 participants at baseline, 156 (50.8%) had vitamin D deficiency. When stratified by study center, the prevalence of vitamin D deficiency was 80.0% in Arizona, 40.6% in Oklahoma, and 48.4% in the Dakotas ($P < .001$). The prevalence of vitamin D deficiency was significantly higher when data were collected in fall or winter than when collected in spring or summer (59.5% [50 of 84] vs 47.5% [106 of 223]; $P = .03$).

Cross-sectional associations between vitamin D deficiency and CVD risk factors

In the cross-sectional analysis at baseline of associations between vitamin D deficiency and measures of obesity, the frequencies of all outcome measures of obesity were higher among participants with vitamin D deficiency than among participants with sufficient 25(OH)D levels (all P values $< .01$, Table 2). These outcome measures included the prevalence of obesity, overweight or obesity percentile, high waist circumference, high waist-to-hip ratio, and body fat percentage. We found no significant difference in diabetes measures between participants with (vs without) vitamin D deficiency. Finally, the prevalence of the following outcome measures was significantly higher among participants with vitamin D deficiency than among participants without the deficiency: low HDL-C (66.1% vs 33.9%, $P < .001$), high triglycerides (63.6% vs 36.4%, $P = .04$), any dyslipidemia (60.5% vs 39.5%, $P < .001$), and metabolic syndrome (65.4% vs 34.6%, $P = .02$).

In the assessment of the multivariable relationship between vitamin D deficiency and prevalence of CVD risk factors, controlling

for age, sex, study center, smoking, hypertension, diabetes, and any dyslipidemia, the odds of all measures of obesity were higher among American Indian adolescents with vitamin D deficiency than among those without the deficiency (*P* value for all outcomes < .05). The odds of prevalent low HDL-C were twice as high (POR = 2.02, 95% CI, 1.19–3.44), controlling for age, sex, study center, current smoking, hypertension, BMI ≥95th percentile, and diabetes (Figure 1). Finally, the odds of prevalent metabolic syndrome were a little over twice as high (POR = 2.19, 95% CI, 1.12–4.28), controlling for age, sex, study center, and smoking (Figure 1).

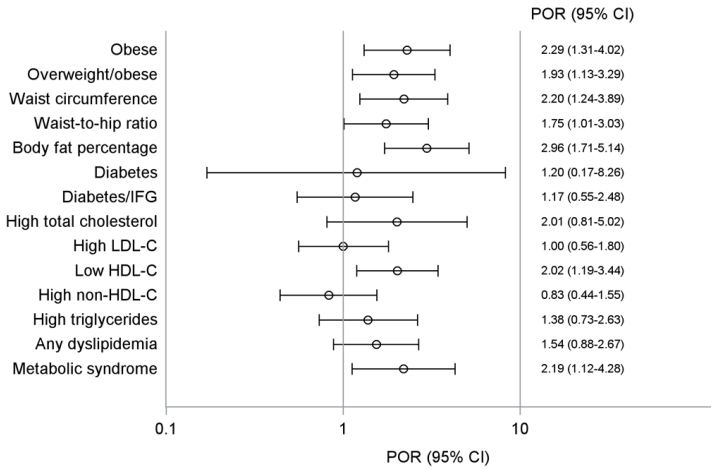

Figure 1. Baseline cross-sectional association between prevalence of vitamin D deficiency and prevalence of CVD risk factors among American Indian adolescents, Strong Heart Family Study. All models accounted for the correlated family structure; see text for definitions of risk factors and details on how models were adjusted. Abbreviations: HDL-C, high-density lipoprotein cholesterol; IFG, impaired fasting glucose; LDL-C, low-density lipoprotein cholesterol; POR, prevalence odds ratio.

Associations between vitamin D deficiency and incidence of CVD risk factors

Because we made exclusions at baseline for each outcome and the prevalence of each factor at baseline differed, the sample size differed for each outcome (Table 3). At first follow-up, sample sizes ranged from 115 participants for any dyslipidemia to 257 participants for diabetes. Likewise, the IR per 100 person-years of each outcome ranged from 1.02 for diabetes at second follow-up (median 13.3 y after baseline) to 10.80 for any dyslipidemia at first follow-up (median 5.8 y after baseline). When stratifying by vitamin D deficiency status at baseline, the IRs for CVD risk factors were higher (but not significant) for participants with vitamin D deficiency at baseline, except for obesity, high body fat percentage, and any dyslipidemia, where the IR was slightly lower for participant vitamin D deficiency (but also not significant). Finally, the univariate IR per 100 person-years of diabetes at second follow-up was significantly higher among American Indian adolescents with vitamin D deficiency at baseline compared with those without vitamin D deficiency (1.32 vs 0.68; *P* = .02) (Table 3). In the multivariable analysis controlling for covariates, the risk of developing CVD risk factors was not significantly different among American Indian adolescents stratified by vitamin D deficiency status (Figure 2).

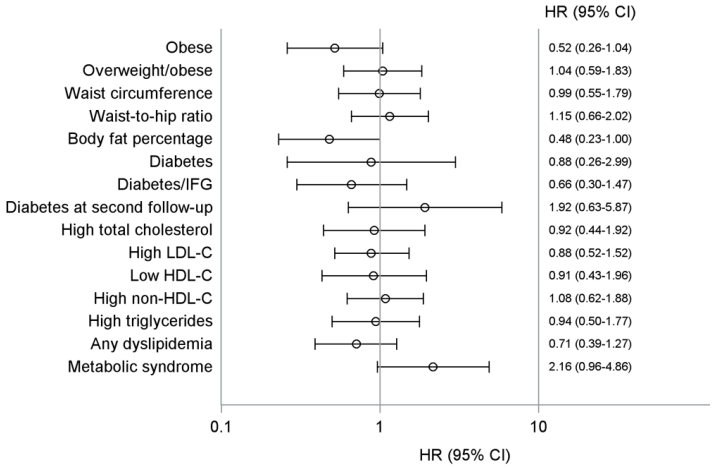

Figure 2. Association between vitamin D deficiency and development of cardiovascular disease risk factors among American Indian adolescents, Strong Heart Family Study. For each model, those who had the risk factor at baseline were excluded, and all outcomes were directly measured at the first follow-up (except for diabetes) at second follow-up. All models accounted for the correlated family structure; see text for definitions of risk factors and details on how models were adjusted. Abbreviations: HR, hazard ratio; HDL-C, high-density lipoprotein cholesterol; IFG, impaired fasting glucose; LDL-C, low-density lipoprotein cholesterol.

Discussion

This is the first study to evaluate the potential association between vitamin D deficiency and the prevalence and incidence of CVD risk factors among American Indian adolescents. Half (50.8%) of American Indians that made up the study population had vitamin D deficiency in adolescence, which is more than twice that of non-Hispanic White adolescents from NHANES (1). Additionally, various indicators of obesity and adiposity, low HDL-C, and metabolic syndrome were more prevalent among participants with versus without vitamin D deficiency, although the prevalence of diabetes was similar between vitamin D deficiency groups at baseline. When adolescents without risk factors were followed prospectively for incident outcomes, we observed no evidence of associations between baseline vitamin D deficiency and subsequent development of obesity, dyslipidemia, diabetes, or metabolic syndrome after a median follow-up of 5.8 years. However, at

13-year follow-up for diabetes, the unadjusted IR was significantly higher for participants with baseline vitamin D deficiency versus without; however, the HR was not significant after adjusting for covariates. This finding may be due to a smaller sample size after making baseline exclusions and the relatively short follow-up time.

Inverse associations between 25(OH)D and CVD risk factors are well documented, although the causal mechanisms underlying these associations are not fully elucidated (30). Experimental evidence indicates that low levels of 25(OH)D may play a role in regulating gene expression or altering leptin and parathyroid hormones to influence obesity via adipose tissue differentiation and growth (30). Alternative hypotheses suggest that the causal relationship may be reversed such that the state of obesity alters circulating 25(OH)D concentrations. These mechanisms may include volumetric dilution over greater mass (31), greater storage of 25(OH)D in adipose tissue, which reduces circulating levels (32), or decreased hepatic 25-hydroxylase activity that reduces bioactivity by suppressing 25-hydroxylation (33). 25(OH)D receptor polymorphisms have been associated with increased cholesterol and triglycerides, with the proposed pathway involving regulation of the synthesis of bile acid (34); 25(OH)D effects on lipid metabolism may also occur via its role in regulating calcium and parathyroid hormone (34). Furthermore, evidence suggests that 25(OH)D contributes to the regulation of pancreatic β-cell function through the expression of a calcium-binding protein. The latter protects against cytokine-mediated cell death, which is consistent with in vivo findings that link vitamin D deficiency to impaired insulin secretion and glucose tolerance (35).

Strengths and limitations

A strength of our project is that it was conducted in a prospectively followed cohort of American Indian populations in 3 regions of the US, with assessment of risk factor incidence at 6 and 13 years of follow-up. However, the cohort contained relatively few adolescents, so power to assess associations was limited; larger studies with young American Indians are needed to assess the potential relationship. In addition, data for the 13-year follow-up were not available for incident obesity, any dyslipidemia, and metabolic syndrome. Diabetes at second follow-up was not directly measured by clinical assessment but defined according to medical record confirmation of self-reported diagnoses; thus, potential exists for nondifferential misclassification. In addition, 25(OH)D measurements were conducted on samples that were stored for 11 to 13 years before measurement. However, previous investigators have determined that 25(OH)D is stable under usual storage conditions of −80°C (36,37). Furthermore, if samples were degraded due to long-term storage, we would likely observe fewer participants with vitamin D deficiency and therefore the result would be biased toward the null (36). The larger SHFS and this substudy were designed to fill a gap in the literature on heart disease and its risk factors by including American Indians; thus, the generalizability of study results is limited to American Indian adolescents from SHFS communities. Also, because no clinically relevant definition of vitamin D deficiency has been established for optimal cardiovascular health, we used 25(OH)D ≤ 20 ng/mL, which is recommended by the Institute of Medicine for optimal bone health (15). Some literature suggests that levels should be at least 25 ng/mL to 30 ng/mL for extra skeletal benefit (1,15); however, increasing the serum cut point to 25 ng/mL to 30 ng/mL would have increased the prevalence of vitamin D deficiency, thus making our analysis involving 20 ng/mL more conservative. Finally, the prevalence of vitamin D deficiency was higher in participants from Arizona, which was unexpected because, compared with participants from Oklahoma and the Dakotas, Arizona participants theoretically may receive more sunlight due to the climate in Arizona. However, perhaps people in Arizona spend more time inside due to the high temperatures. More studies are needed with direct measurements of sunlight exposure.

Conclusions and implications for public heath practice

We demonstrated that vitamin D deficiency in adolescence is related to measures of obesity, low HDL-C, and metabolic syndrome in American Indian populations, which is consistent with other US populations (4,5). Furthermore, vitamin D deficiency early in life may be associated with the development of diabetes later in life; however, larger studies with longer follow-up are needed to confirm this observation. Because of these observations and because vitamin D deficiency is amenable to changes in diet and vitamin supplementation, the results of this study could provide evidence for public health strategies designed to reduce vitamin D deficiency. These could include community health programs targeting vitamin D supplementation among American Indian adolescents. In addition, community education programs on the benefits of vitamin D supplementation, consuming foods high in vitamin D, and getting adequate amounts of sun exposure may reduce the high levels of vitamin D deficiency that we observed among American Indian adolescents.

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Tables

Table 1. Definitions of Baseline and Follow-Up Cardiovascular Disease Risk Factors Used in the Strong Heart Family Study[a]

Risk factor	Baseline definition	Follow-up definition
Overweight and obesity		
Obese	BMI ≥95th percentile based on age	BMI ≥30kg/m^2
Overweight or obese	BMI ≥85th percentile based on age	BMI ≥25 kg/m^2
High waist circumference		
Male	≥39.2 in for age 15 y	≥40 in
	≥39.6 in for age 16 y	
	≥39.9 in for age 17 y	
	≥40.0 in for age 18 y	
	≥40.0 in for age 19 y	
Female	≥33.1 in for age 15 y	≥35 in
	≥33.5 in for age 16 y	
	≥33.9 in for age 17 y	
	≥34.3 in for age 18 y	
	≥34.5 in for age19 y	
High waist-to-hip ratio		
Male	≥0.90	≥0.90
Female	≥0.85	≥0.85
High body fat percentage		
Male	≥25%	≥25%
Female	≥35%	≥35%
Impaired FPG	≥110 to <126 mg/dL	≥110 to <126 mg/dL
Diabetes	FPG ≥126 mg/dL and/or taking diabetes medication	FPG ≥126 mg/dL and/or taking diabetes medication
High total cholesterol	≥200 mg/dL	≥200 mg/dL
High LDL-C	≥100 mg/dL	≥100 mg/dL
Low HDL-C		
Male	≤40.2 mg/dL for age 15 y	≤40 mg/dL
	≤39.8 mg/dL for age 16–20 y	
Female	≤48.7 mg/dL for age 15 y	≤50 mg/dL
	≤49.1 mg/dL for age 16–17 y	
	≤49.5 mg/dL for age 18 y	
	≤49.9 mg/dL for age19 y	
High non-HDL-C	≥130 mg/dL	≥130 mg/dL

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
[a] At baseline, because all participants were adolescents, we used age- and sex-specific cutoffs when they existed; otherwise, we used adult cutoffs. Because all participants were adults at follow-up, we used adult cutoffs.

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Table 1. Definitions of Baseline and Follow-Up Cardiovascular Disease Risk Factors Used in the Strong Heart Family Study[a]

Risk factor	Baseline definition	Follow-up definition
High triglycerides		
Male	≥138 mg/dL for age 15 y	≥150 mg/dL
	≥141 mg/dL for age 16 y	
	≥143 mg/dL for age 17 y	
	≥146 mg/dL for age 18 y	
	≥149 mg/dL for age 19 y	
Female	≥127 mg/dL for age 15 y	≥150 mg/dL
	≥129 mg/dL for age 16 y	
	≥135 mg/dL for age 17 y	
	≥142 mg/dL for age 18 y	
	≥149 mg/dL for age19 y	
Any dyslipidemia	Any abnormal value of total cholesterol, LDL-C, HDL-C, non-HDL-C, or triglycerides, listed above, and/or taking lipid medication	Any abnormal value of total cholesterol, LDL-C, HDL-C, non-HDL-C, or triglycerides, listed above, and/or taking lipid medication
High blood pressure, mm Hg		
Male	>126/81 for age 15 y	>140/90 mm Hg
	>128/82 for age 16 y	
	>128/83 for age 17 y	
	>129/84 for age 18 y	
	>130/85 for age 19 y	
Female	>126/84 for age 15 y	
	>128/84 for age 16 y	
	>128/85 for age 17 y	
	>129/85 for age 18 y	
	>130/85 for age 19 y	
Hypertension	High blood pressure based on the above criteria, and/or taking antihypertensive medication	High blood pressure based on the above criteria, and/or taking antihypertensive medication
Albuminuria	Albumin-creatinine ratio ≥30 mg/g	Albumin-creatinine ratio ≥30 mg/g
Metabolic syndrome	Any 3 of the following: high waist circumference, high blood pressure, high triglycerides, or low HDL-C, based on the criteria above, or FPG ≥100 mg/dL	Any 3 of the following: high waist circumference, high blood pressure, high triglycerides, or low HDL-C, based on the criteria above, or FPG ≥100 mg/dL

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
[a] At baseline, because all participants were adolescents, we used age- and sex-specific cutoffs when they existed; otherwise, we used adult cutoffs. Because all participants were adults at follow-up, we used adult cutoffs.

Table 2. Comparison of Baseline Demographic and Cardiovascular Disease Risk Factors Among American Indian Adolescents, Stratified by Vitamin D Deficiency Status,[a] Strong Heart Family Study, 2001–2003[b]

Variable at baseline	Total (N = 307)	Vitamin D deficiency status		
		Deficient (n = 156)	Not deficient (n = 151)	*P* value[c]
Age, mean (SD), y	17.4 (1.5)	17.6 (1.5)	17.2 (1.4)	.06
Sex, no. (%)				
Female	160/307 (52.1)	99 (61.9)	61 (38.1)	<.001
Male	147/307 (47.9)	57 (38.8)	90 (61.2)	
Center, no. (%)				
Arizona	49/307 (16.0)	39 (80.0)	10 (20.0)	<.001
Oklahoma	101/307 (32.9)	41 (40.6)	60 (59.4)	
North and South Dakota	157/307 (51.1)	76 (48.4)	81 (51.6)	
Smokes, no. (%)	78/306 (25.5)	48 (61.5)	30 (38.5)	.01
Hypertension				
Has hypertension, no. (%)[d]	31/307 (10.1)	18 (58.1)	13 (41.9)	.34
Takes hypertension medication, no. (%)	1/307 (0.3)	0	1 (100.0)	—[e]
Blood pressure, mean (SD), mm Hg				
Systolic	113.0 (10.9)	113.8 (10.5)	112.8 (11.2)	.79
Diastolic	69.0 (9.9)	70.9 (9.3)	67.8 (10.3)	.03
Albuminuria, no. (%)	0	0	0	—[e]
Plasma creatinine, mean (SD), mg/dL	0.8 (0.1)	0.7 (0.1)	0.8 (0.1)	<.001
Vitamin D				
Intake, median (IQR), IU	127.8 (61.9–267.3)	110.3 (52.8–207.8)	142.2 (84.1–349.9)	.007
Takes vitamin D supplements, no. (%)	29/296 (9.8)	14 (48.3)	15 (51.7)	.60
Data collected in fall or winter, no. (%)	84/307 (27.4)	50 (59.5)	34 (40.5)	.03
Obesity				
Overweight and obesity[d]				
BMI ≥ 85th percentile (overweight or obese)	161/306 (52.6)	99 (61.5)	62 (38.5)	.001
BMI ≥ 95th percentile (obese)	103/306 (33.7)	70 (68.0)	33 (32.0)	<.001
Waist circumference				
Measurement, mean (SD), inches	36.0 (7.1)	37.9 (7.2)	34.0 (6.3)	<.001
Has high waist circumference, no. (%)[d]	136/305 (44.6)	93 (68.4)	43 (31.6)	<.001
Waist-to-hip ratio				
Ratio, mean (SD)	0.9 (0.1)	0.9 (0.1)	0.8 (0.1)	.005
Has high ratio, no. (%)[d]	128/304 (42.1)	84 (65.6)	44 (34.4)	<.001

Abbreviations: 25(OH)D, serum 25-hydroxyvitamin D; BMI, body mass index; HDL-C, high density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
[a] Vitamin D deficiency defined as 25(OH)D ≤20 ng/mL.
[b] Baseline measurements were taken during 2001–2003.
[c] Determined from generalized estimating equations, controlling for familial clustering; *P* <.05 considered significant.
[d] Based on age-specific and sex-specific cut points for adolescents.
[e] Sample size not adequate to generate a *P* value.

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Table 2. Comparison of Baseline Demographic and Cardiovascular Disease Risk Factors Among American Indian Adolescents, Stratified by Vitamin D Deficiency Status,[a] Strong Heart Family Study, 2001–2003[b]

Variable at baseline	Total (N = 307)	Vitamin D deficiency status		
		Deficient (n = 156)	Not deficient (n = 151)	*P* value[c]
Body fat				
Body fat percentage, mean (SD)	31.9 (11.6)	36.3 (11.6)	27.3 (9.7)	<.001
Has high body fat percentage, no. (%)[d]	162/303 (53.4)	108 (66.7)	54 (33.3)	<.001
Diabetes				
Fasting plasma glucose, mean (SD), mg/dL	91.7 (17.1)	91.7 (15.9)	91.6 (18.2)	.97
Has diabetes, no. (%)	6/307 (2.0)	4 (66.7)	2 (33.3)	.38
Has diabetes or impaired fasting glucose, no. (%)	42/307 (13.7)	21 (50.0)	21 (50.0)	.78
Takes diabetes medication, no. (%)	3/307 (1.0)	2 (66.7)	1 (33.3)	.38
Lipids				
Total cholesterol, mean (SD), mg/dL	154.4 (30.0)	153.8 (30.6)	155.1 (29.4)	.34
Has high total cholesterol, no. (%)	25/307 (8.1)	16 (64.0)	9 (36.0)	.34
LDL-C, mean (SD), mg/dL	82.9 (24.6)	82.6 (25.6)	83.1 (23.7)	.50
Has high LDL-C, no. (%)	70/307 (22.8)	38 (54.3)	32 (45.7)	.86
HDL-C, mean (SD), mg/dL	49.4 (13.0)	47.9 (12.5)	51.0 (13.4)	.11
Has low HDL-C, no. (%)[d]	124/307 (40.4)	82 (66.1)	42 (33.9)	<.001
Non-HDL-C, no. (%), mg/dL	105.0 (30.9)	105.8 (31.7)	104.1 (30.2)	.80
Has high non-HDL-C, no. (%)	60/307 (19.5)	32 (53.3)	28 (46.7)	.66
Triglycerides, median (IQR), mg/dL	93.0 (73.0–133.0)	97.0 (77.0–141.5)	89.0 (70.0–119.0)	.26
Has high triglycerides, no. (%)[d]	66/307 (21.5)	42 (63.6)	24 (36.4)	.04
Takes lipid-lowering medication, no. (%)	0/307	0	0	—[e]
Has any dyslipidemia, no. (%)	172/307 (56.0)	104 (60.5)	68 (39.5)	<.001
Composite cardiovascular disease risk factor				
Has metabolic syndrome	52/305 (17.0)	34 (65.4)	18 (34.6)	.02

Abbreviations: 25(OH)D, serum 25-hydroxyvitamin D; BMI, body mass index; HDL-C, high density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
[a] Vitamin D deficiency defined as 25(OH)D ≤20 ng/mL.
[b] Baseline measurements were taken during 2001–2003.
[c] Determined from generalized estimating equations, controlling for familial clustering; $P < .05$ considered significant.
[d] Based on age-specific and sex-specific cut points for adolescents.
[e] Sample size not adequate to generate a *P* value.

Table 3. Incidence of Cardiovascular Disease Risk Factors at First Follow-Up Among American Indian Adolescents, Stratified by Baseline Vitamin D Deficiency Status,[a] Strong Heart Family Study[b]

Risk factor	No. of participants without risk factor at baseline[c]	Incidence rate per 100 person-years			*P* value[d]
		Total	Deficient	Not deficient	
Obesity					
Obese (BMI ≥30 kg/m^2)[e]	178	5.03	4.82	5.19	.46
Overweight or obese (BMI ≥25 kg/m^2)[e]	126	9.14	10.09	8.53	.50
High waist circumference (>40 in for males, >35 in for females)[e]	146	6.63	7.45	6.16	.84
High waist-to-hip ratio (≥0.9 for males, ≥0.85 for females)	153	10.07	11.69	8.93	.52
High body fat percentage (≥25% males, ≥35% females)	123	9.10	8.11	9.63	.31
Diabetes					
Has diabetes	257	1.07	1.34	0.80	.34
Has diabetes or impaired fasting glucose	228	3.30	3.41	3.19	.91
Has diabetes at second follow-up	242[f]	1.02	1.32	0.68	.02
Lipids					
Has high total cholesterol (≥200 mg/dL)	243	2.97	3.02	2.91	.91
Has high LDL-C (≥100 mg/dL)	203	6.24	6.28	6.19	.68
Has low HDL-C (≤40 mg/dL for males, ≤50 for females)[e]	154	4.60	5.22	4.17	.57
Has high non-HDL-C (≥130 mg/dL)	212	6.03	6.35	5.72	.82
Has high triglycerides (≥150 mg/dL)[e]	205	4.63	5.03	4.28	.97
Any dyslipidemia	115	10.80	9.44	11.65	.27
Composite cardiovascular disease risk factor					
Metabolic syndrome	214	3.31	6.42	3.86	.13

Abbreviations: 25(OH)D, serum 25-hydroxyvitamin D; BMI, body mass index, LDL-C, low-density lipoprotein cholesterol, HDL-C, high-density lipoprotein cholesterol.

[a] Vitamin D deficiency defined as 25(OH)D ≤20 ng/mL.

[b] Baseline variables were measured during 2001–2003, the first follow-up variables were measured during 2006-2009, and the second follow-up variable was measured 2014-2018. All variables presented were measured during the first follow-up using direct measurements during physical examinations. Diabetes was the only variable measured during the second follow-up, which was assessed through medical record review.

[c] Includes participants without the risk factor at baseline who were not missing at follow-up.

[d] *P* values generated from univariate log-rank tests; $P < .05$ considered significant.

[e] Baseline measures for BMI, high waist circumference, low HDL-C, and high triglycerides are based on age-specific and sex-specific cut points for adolescents. Otherwise, categories are based on adult standards.

[f] Includes participants without the risk factor at baseline or first follow-up who were not missing at the second follow-up.

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ORIGINAL RESEARCH

Longitudinal Lipidomic Profile of Subclinical Peripheral Artery Disease in American Indians: The Strong Heart Family Study

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PEER REVIEWED

Summary

What is already known on this topic?

Peripheral artery disease (PAD) and dyslipidemia are both independent predictors of cardiovascular disease (CVD). Lipidomics can identify and quantify individual lipid species associated with subclinical PAD.

What is added by this report?

This is the first longitudinal lipidomic study of subclinical PAD in a large community-based cohort of American Indians. Altered baseline levels of multiple individual lipid species and their changes were associated with subclinical PAD, with some lipids also associated with coronary heart disease risk beyond traditional risk factors.

What are the implications for public health practice?

Given the high prevalence of CVD risk factors among American Indians, early screening for PAD at younger ages is essential.

Abstract

Introduction

Peripheral artery disease (PAD) and dyslipidemia are both independent predictors of cardiovascular disease, but the association between individual lipid species and subclinical PAD, assessed by ankle-brachial index (ABI), is lacking in large-scale longitudinal studies.

Methods

We used liquid chromatography-mass spectrometry to repeatedly measure 1,542 lipid species from 1,886 American Indian adults attending 2 clinical examinations (mean ~5 years apart) in the Strong Heart Family Study. We used generalized estimating equation models to identify baseline lipid species associated with change in ABI and the Cox frailty regression to examine whether lipids associated with change in ABI were also associated with incident coronary heart disease (CHD). We also examined the longitudinal association between change in lipid species and change in ABI and the cross-sectional association of individual lipids with ABI. All models were adjusted for age, sex, body mass index, smoking, alcohol use, hypertension, estimated glomerular filtration rate, diabetes, and lipid-lowering medication.

Results

Baseline levels of 120 lipid species, including glycerophospholipids, glycerolipids, fatty acids, and sphingomyelins, were associated with change in ABI. Among these, higher baseline levels of 3 known lipids (phosphatidylinositol[16:0/20:4], triacylglycerol[48:2], triacylglycerol[55:1]) were associated with a lower risk of CHD (hazard ratios [95% CIs] ranged from 0.67 [0.46–0.99] to 0.76 [0.58–0.99]), while cholesterol was associated with a higher risk of CHD (hazard ratio [95% CI] = 1.37 [1.00–1.87]). Longitudinal changes in 32 lipids were significantly associated with change in ABI during 5-year follow-up. Plasma levels of glycerophospholipids, triacylglycerols, and glycosylceramides were significantly associated with ABI in the cross-sectional analysis.

Conclusion

Altered plasma lipidome is significantly associated with subclinical PAD in American Indians beyond traditional risk factors. If validated, the identified lipid species may serve as novel biomarkers for PAD in this high-risk but understudied population.

Introduction

Lower extremity peripheral artery disease (PAD) is characterized by a partial or complete obstruction of lower limb arteries by atherosclerotic blockages. PAD affects more than 200 million adults globally and poses a substantial burden to public health (1). Patients with atherosclerotic PAD are at increased risk of myocardial infarction, stroke, and death. American Indians have a higher prevalence than non-Hispanic White people of inpatient PAD and chronic limb-threatening ischemia (2). Dyslipidemia, defined as high levels of total or low-density lipoprotein (LDL) cholesterol or low levels of high-density lipoprotein (HDL) cholesterol, is associated with PAD (3). Traditional lipid panels measure only bulk lipoproteins and fail to reflect the diverse molecular lipid species in a blood sample. A comprehensive profiling of all individual lipid species in our blood (ie, blood lipidome) is required to identify novel biomarkers and enhance our understanding of the mechanism through which dyslipidemia may contribute to atherosclerotic PAD.

Lipidomics is an emerging high-throughput biochemical technique that can identify and quantify hundreds to thousands of molecular lipid species in biofluids or tissues. Epidemiologic studies have used lipidomics to describe associations of altered lipid species, such as ceramides (CERs), cholesterol, phospholipids, and fatty acids (FAs) with PAD in human populations (4,5). However, these studies were largely cross-sectional, had small sample sizes, and/or had low coverage of the blood lipidome. To our knowledge, no large-scale longitudinal lipidomic study has investigated the association between longitudinal change in blood lipidome and change in ankle-brachial index (ABI), a sensitive and cost-effective tool for PAD screening, in any racial or ethnic group. The normal range for ABI is generally 0.9 to 1.4; an ABI <0.9 typically indicates occlusive PAD related to atherosclerosis, while an ABI >1.4 reflects noncompressible vessels, which may still suggest underlying occlusive disease (6). Although the prevalence of ABI >1.4 among adults aged 40 years or older (mean age, 56.9 y) is relatively low (approximately 1.4%) in the general US population (7), data from the Strong Heart Study cohort (8) showed a higher prevalence of ABI >1.4 (9.2%) among American Indians of similar age (mean age, 57.1 y). The higher prevalence among American Indians may be largely attributed to the higher rates of obesity and diabetes.

The objective of our study was to investigate 1) whether changes in individual lipid species are associated with change in ABI over an average of 5-year follow-up among American Indians, independent of baseline lipids and traditional risk factors; and 2) whether ABI-related lipid species are associated with incident CHD during an average of 18-year follow-up over traditional risk factors. We also analyzed the cross-sectional association of each lipid species with ABI at baseline and 5-year follow-up.

Methods

We conducted the first large-scale longitudinal lipidomic profiling of 3,645 fasting plasma samples from 1,886 unique American Indians attending 2 clinical examinations (1,886 at baseline; 1,759 at follow-up) approximately 5 years apart on average in the Strong Heart Family Study. By including participants with an ABI <0.9 or an ABI >1.4, we aimed to capture a broad spectrum of lipid changes associated with cardiovascular health, rather than focusing only on people with ABI values within the normal range.

Study population

The Strong Heart Family Study (2001–ongoing) is a family-based prospective study designed to identify genetic, metabolic, and behavioral factors for cardiovascular disease (CVD) and CVD risk factors among American Indians (9). Briefly, 2,786 tribal members (aged ≥14 y) residing in Arizona, North Dakota, South Dakota, and Oklahoma were recruited and examined at baseline (2001–2003) and re-examined after 5-year follow-up (2006–2009). Details of the study design, laboratory protocols, and phenotype collection are available elsewhere (9). Participants were interviewed and had a physical examination at each visit, during which fasting blood samples were collected for laboratory tests. Laboratory methods were described previously (9). We included in analysis 1,886 individuals (62.2% females; mean age, 40.1 y) who were free of overt CVD at baseline and had complete clinical and lipidomic data. All study participants provided informed consent, and protocols were approved by the institutional review boards of participating institutions and the American Indian tribes.

Measurement of ABI

At baseline and at 5-year follow-up, blood pressure was measured in the right arm and bilateral ankles (both left and right posterior tibial artery) by using a handheld Doppler (Imex Medical Limited) while the participant was in a supine position. Each measurement was taken twice in immediate succession, and the average of the 2 readings was used. If no pulse was detected either by palpation or Doppler, a second examiner was asked to confirm the absence and ankle blood pressure was obtained from the dorsalis pedis artery. The ABI for each leg was then calculated by dividing the average systolic blood pressure in the ankle (posterior tibial or dorsalis pedis) by the average systolic blood pressure in the right arm (brachial artery). The worse of the 2 ABI values (ie, the lower value for ABI <0.9 or the higher value for ABI >1.4) was used to define ABI for each person. Change in ABI was calculated as the differ-

ence in ABI between baseline and 5-year follow-up. Symptoms indicative of PAD, such as intermittent claudication, were evaluated at both baseline and follow-up by using the Rose Angina Questionnaire (10), which asks about symptoms such as leg pain during walking or resting and other signs of vascular insufficiency. PAD was defined as participants who had an ABI of either <0.9 or >1.4 in at least 1 leg during the clinical examination (6). Incident PAD was defined as not having PAD at baseline but having PAD at 5-year follow-up.

Assessment of clinical covariates

Information on demographic characteristics (age, sex), lifestyle (smoking, alcohol use, physical activity), medical history, and use of prescription medications was collected via standard questionnaires (11). Anthropometric measures (height, weight, waist circumference) and fasting blood samples were collected at each visit. Smoking status was categorized as current, former, or never smokers, and alcohol use as current versus noncurrent drinkers. Hypertension was defined as blood pressure ≥140/90 mm Hg or use of antihypertensive medication, and type 2 diabetes as fasting glucose ≥126 mg/dL or use of hypoglycemic medication. CVD events included fatal and nonfatal myocardial infarction, CHD, sudden cardiac death, heart failure, and stroke. Use of lipid-lowering and antihypertensive medication was recorded at each visit.

Ascertainment of incident coronary heart disease (CHD)

Details on the ascertainment of incident CHD are available elsewhere (12). In brief, CHD included definite CHD (fatal or nonfatal), definite myocardial infarction (fatal or nonfatal), and sudden death due to CHD. CHD events were ascertained by annual review of hospitalizations, death records, and self-reports (with subsequent medical record verification) during follow-up visits. Time to event was recorded based on the date of baseline examination (2001–2003) to either the date of the first CHD event or the last follow-up (December 31, 2020). For participants who experienced more than 1 CHD event during an average 18-year follow-up period, we used the earliest event date in our analysis.

Lipidomic data acquisition, preprocessing, and quality control

Methods for blood sample collection, lipidomic data acquisition, processing, and normalization are described elsewhere (13). Briefly, relative abundance of molecular lipid species in fasting plasma samples at 2 time points (~5 years apart) was quantified by untargeted liquid chromatography–mass spectrometry. After preprocessing and quality control, we obtained 1,542 lipids (518 known) in 3,950 samples (1,970 at baseline; 1,980 at 5-year follow-up). After further excluding outlier samples (n = 2 at baseline; n = 3 at follow-up) and people with prevalent CVD (n = 12 at baseline; n = 87 at follow-up) or missing covariates (n = 70 at baseline; n = 131 at follow-up), we included 1,886 participants (1,886 at baseline; 1,759 at 5-year follow-up) with complete clinical and lipidomic data. We observed no clear batches in our lipidomic data.

Statistical analysis

Continuous variables, including lipid levels, were standardized to zero mean and unit variance. Multiple testing was controlled by false discovery rate by using the Storey *Q*-value method (14); $Q < .05$ was used to determine significance.

Prospective association analysis

To identify baseline plasma lipids that can predict change in ABI (ie, the difference in ABI between baseline and 5-year follow-up), we constructed a generalized estimating equation (GEE) model, which accounted for the relatedness among family members. In this model, the baseline level of each lipid was the predictor, and change in ABI was the outcome, adjusting for age, sex, body mass index (BMI), smoking status (current smoker vs ever smoker vs nonsmoker), alcohol use (yes/no), hypertension (yes/no), diabetes (yes/no), estimated glomerular filtration rate (eGFR), use of lipid-lowering medication (yes/no) at baseline, and baseline ABI. We excluded participants with prevalent PAD at baseline from this analysis.

To assess whether the identified lipids improved the prediction of PAD risk beyond known clinical factors, we used data from 2 study centers (North and South Dakota and Arizona) as the training set (n = 995; 32 cases), and 1 center (Oklahoma) as the testing set (n = 788; 65 cases). We then compared a base model including traditional risk factors only (age, sex, BMI, smoking status, alcohol use, hypertension, diabetes, eGFR, and use of lipid-lowering medication) with a model that included both traditional risk factors and the lipids associated with change in ABI. We assessed the incremental predictive value of lipids over known risk factors by area under the receiver operating characteristic curve (AUROC) (15).

To further examine whether plasma lipids associated with change in ABI were also associated with incident CHD during an average of 18-year follow-up, we constructed a frailty Cox proportional hazards model. In this model, the baseline level of each ABI-associated lipid was the predictor, and the time to incident CHD event was the outcome, adjusting for the same covariates as described above, plus LDL cholesterol, HDL cholesterol, and physical activity at baseline. The frailty term was used to account for the relatedness among family members.

Repeated measurement analysis

For the 1,459 participants free of prevalent CVD and PAD at baseline and 5-year follow-up, we constructed GEE models to examine the longitudinal association between changes in lipid species (difference in the relative abundance of each lipid) and change in ABI between 5-year follow-up and baseline. In the model, change in ABI was the outcome, and change in the relative abundance of a lipid was the predictor. The model adjusted for age, sex, BMI, smoking status (current smoker vs ever smoker vs nonsmoker), alcohol use (yes/no), hypertension (yes/no), eGFR, diabetes (yes/no), and use of lipid-lowering medication (yes/no) at baseline, as well as changes in continuous variables (ie, age, BMI, eGFR) and baseline lipid. The associations between changes in lipids and change in cardiometabolic factors, including BMI, systolic and diastolic blood pressure, eGFR, fasting blood plasma glucose, insulin, and insulin resistance, were similarly examined.

Cross-sectional association analysis

To identify lipids that are cross-sectionally associated with ABI at each point (baseline or 5-year follow-up), we constructed GEE models in which ABI was the outcome and the plasma level of each lipid was the predictor, adjusting for age, sex, BMI, smoking status (current smoker vs ever smoker vs nonsmoker), alcohol use (yes/no), hypertension (yes/no), eGFR, diabetes (yes/no), and lipid-lowering medication (yes/no). This analysis was conducted separately by using data collected at baseline or 5-year follow-up. Results from both points were then combined by fixed-effects meta-analysis.

Sensitivity analysis

To evaluate the robustness of our results, we conducted the following sensitivity analyses. First, to examine the potential effect of bulk lipids (ie, HDL cholesterol, triglycerides) and physical activity on our results for change in ABI or ABI at each point, we additionally adjusted for these variables in the models. Second, to examine whether sex modulated the association between lipid species and ABI or change in ABI, we further included an interaction term (lipid × sex) in the model. Third, to examine whether the inclusion of symptomatic participants or the potential effect of PAD symptoms affected our results, we performed additional analyses. First, we excluded participants who reported symptoms indicative of PAD from the models. We excluded 423 symptomatic participants from the prospective association analysis, 338 from the repeated measurement analysis, and 460 from the cross-sectional analysis. Second, we adjusted for PAD symptoms in the models.

Results

The mean age of participants was 40.1 years at baseline and 44.9 years at follow-up (Table). The median ABI was 1.1 at baseline and 1.2 at follow-up, respectively. Most participants had an ABI within the normal range ($0.9 \leq \text{ABI} \leq 1.4$) at both baseline (1,828 of 1,886; 96.9%) and follow-up (1,652 of 1,759; 93.9%). Among the 1,828 participants free of PAD at baseline, 97 participants (5.3%) developed incident PAD during an average 5-year follow-up.

Baseline lipids predict change in ABI beyond known clinical factors

We identified 358 lipids (143 known) significantly associated with change in ABI at $P < .05$. After correction for multiple testing, 120 lipids (46 known: 13 triacylglycerols [TAGs], 10 phosphatidylcholines [PCs], 9 phosphatidylethanolamines [PEs], 6 phosphatidylinositols [PIs], 3 sphingomyelins [SMs], 2 diacylglycerols [DAGs], fatty acid [FA{22:0}], fatty acid ester of hydroxy fatty acid [FAHFA {18:0/3:0}], and cholesterol, were significantly associated with change in ABI at $Q < .05$. Of the 46 known lipids, higher baseline levels of the following 37 lipids — 13 TAGs, 8 PCs, 6 PIs, 6 PEs, 2 DAGs, FA(22:0), and FAHFA(18:0/3:0) — were positively associated with change in ABI (regression coefficient [β] = 0.03–0.08). In contrast, higher baseline levels of 9 lipids (3 PEs, 3 SMs, 2 PCs, and cholesterol) were inversely associated with change in ABI (β, −0.05 to −0.07) (Figure 1).

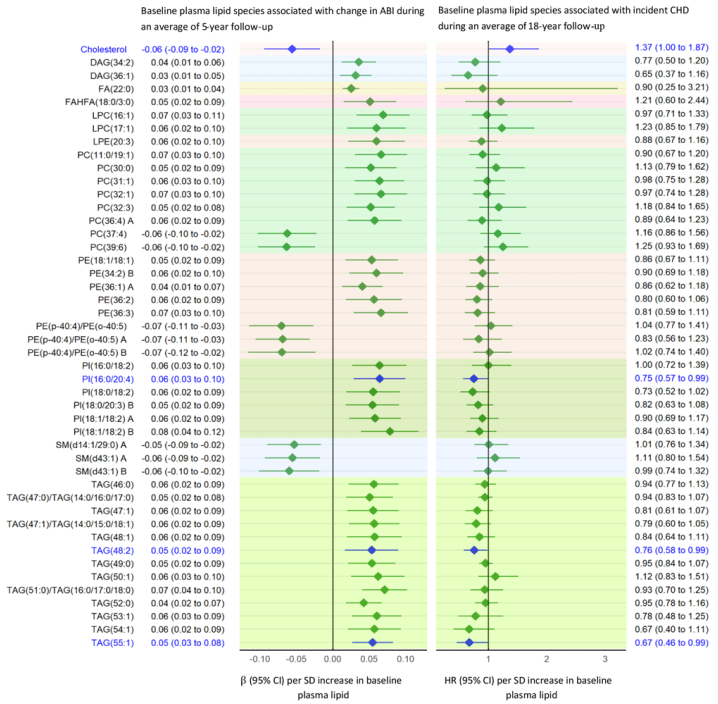

Figure 1. Baseline plasma lipid species associated with change in ABI ($Q < .05$). Lipids significantly associated with incident CHD are highlighted in blue. "A" or "B" in name of lipids indicates isomers. Abbreviations: ABI, ankle-brachial index; CHD, coronary heart disease; DAG, diacylglycerol;

eGFR, estimate glomerular filtration rate; HR, hazard ratio; FA, fatty acid; FAHFA, fatty acid ester of hydroxy fatty acid; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; SM, sphingomyelin; TAG, triacylglycerol.

Addition of the top 9 of 46 lipids associated with change in ABI, namely FA(22:0), FAHFA(18:0/3:0), LPC(16:1), PI(16:0/20:4), PI(18:1/18:2) B, PI(18:0/18:2), PI(18:0/20:3) B, SM(d43:1) B, and LPE(20:3), significantly improved risk prediction for PAD over clinical factors (AUROC increased from 0.529 to 0.568, $P = .04$) (Figure 2).

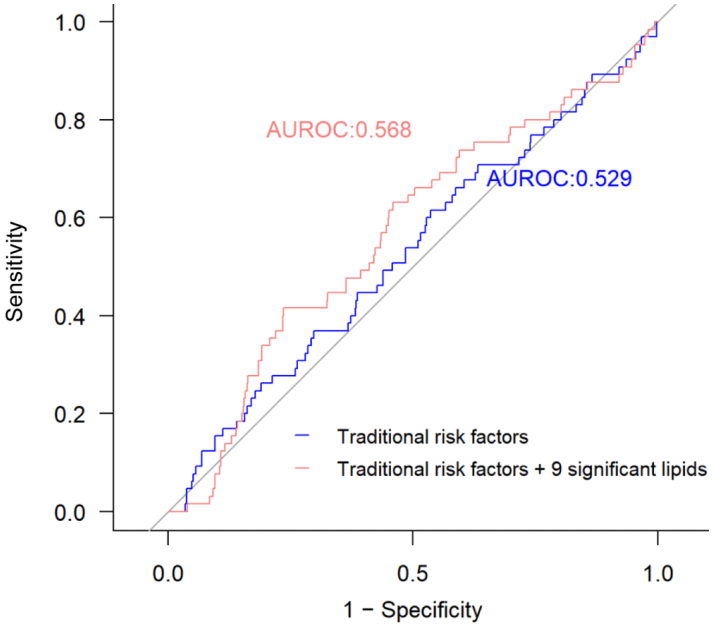

Figure 2. Incremental value of the identified plasma lipids associated with change in ABI for PAD risk prediction. Data used from 2 study centers (North and South Dakota and Arizona) as training set (n = 995, 32 cases), used for model training, and 1 center (Oklahoma) as the testing set (n = 788, 65 cases), used to test classification performance. Model 1 (blue line): traditional risk factors only, including age, sex, body mass index, smoking status, alcohol use, hypertension, diabetes, eGFR, and lipid-lowering medication use at baseline. Model 2 (red line): clinical factors plus 9 lipids significantly associated with change in ABI. Compared with Model 1, additional inclusion of plasma lipids (Model 2) significantly increased risk prediction for PAD; *P* value for increase in AUROC = .04. Abbreviations: ABI, ankle-brachial index; AUROC, area under the receiver operating characteristic curve; eGFR, estimated glomerular filtration rate, PAD, peripheral artery disease.

ABI-related lipids associated with incident CHD

Ninety study participants developed incident CHD during an average of 18 years of follow-up. Of the 46 known lipids whose baseline levels are associated with change in ABI during 5-year follow-up, baseline levels of 4 lipids were also significantly associated with risk of CHD at $P < .05$, after adjusting for age, sex, BMI, smoking, alcohol use, diabetes, hypertension, eGFR, LDL cholesterol, HDL cholesterol, physical activity, and use of lipid-lowering medication at baseline. Specifically, higher baseline levels of 3 known lipids, TAG(48:2), TAG(55:1), and PI(16:0/20:4), were associated with a decreased risk of CHD (hazard ratio [95% CI] ranged from 0.67 [0.46–0.99] to 0.76 [0.58–0.99]), while a higher baseline level of cholesterol was associated with an increased risk of CHD (hazard ratio [95% CI] = 1.37 [1.00–1.87]) during an average of 18-years follow-up.

Longitudinal changes in lipid species associated with change in ABI during 5-year follow-up

After adjusting for clinical covariates, baseline ABI, and baseline lipids, longitudinal changes in 188 lipids (61 known) were significantly associated with change in ABI at $P < .05$. After correction for multiple testing, changes in 32 lipids (7 known) remained significant at $Q < .05$. Of the 7 known lipids, 6 lipids, including 3 PIs, AC(18:2), CE(18:3), and LPE(22:5), were positively associated with change in ABI at $Q < .05$, whereas change in LPC(p-18:0)/LPC(o-18:1) was inversely associated (Figure 3). Among lipid species associated with change in ABI, changes in most were also associated with changes in cardiovascular risk factors.

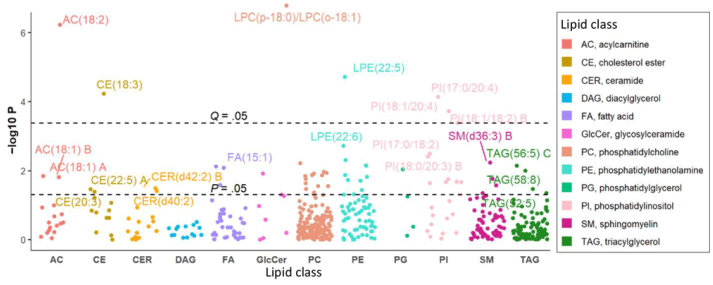

Figure 3. Manhattan plot displaying the longitudinal associations between change in plasma lipids and change in ABI during an average of 5-years follow-up. The dashed lines represent significance level at $P = .05$ and $Q = .05$. Abbreviation: ABI, ankle-brachial index.

Lipids cross-sectionally associated with ABI

At baseline, 310 lipids (128 known) were associated with ABI at $P < .05$. Of the 128 known lipids, 123 lipids, including 37 TAGs, 24 PCs, 17 PEs, 14 PIs, 10 DAGs, 8 acylcarnitine (ACs), 6 FAs, 2 CERs, SM(d32:2) A, GlcCer(d14:1(4E)/20:0(2OH)) and CE(22:5) B were inversely associated with ABI, whereas 5 lipids, including 2 PCs (LPC[20:0], LPC[o-16:0]), 2 PEs (PE[p-18:0/22:4]/PE[o-18:1/22:4], PE[p-40:4]/PE[o-40:5] A), and PS(18:0/20:4), were positively associated. Of these, 51 (23 known) lipids remained significant at $Q < .05$.

At follow-up, 185 lipids (53 known) were associated with ABI at $P < .05$. Of the 53 known lipids, 22 lipids, including 13 PCs, 3

TAGs, 2 PEs, FA(15:1), AC(11:1), CER(d33:1), GlcCer(d14:1[4E]/20:0[2OH]), and CE(22:5) B were inversely associated with ABI at $P<.05$, whereas 31 lipids, including 9 PEs, 5 PIs, 4 GlcCers, 3 ACs, 2 CEs, 3 SMs, 2 FAs, and CER(d42:2) B, were positively associated. Of these, 39 (9 known) lipids remained significant at $Q<.05$.

Meta-analysis combining results from both time points showed that 14 lipids, including 7 TAGs, 3 FAs, and LPC(p-18:0)/LPC(o-18:1), were inversely associated with ABI, whereas 2 CEs and LPC(o-16:0) were positively associated at $Q<.05$ (Figure 4).

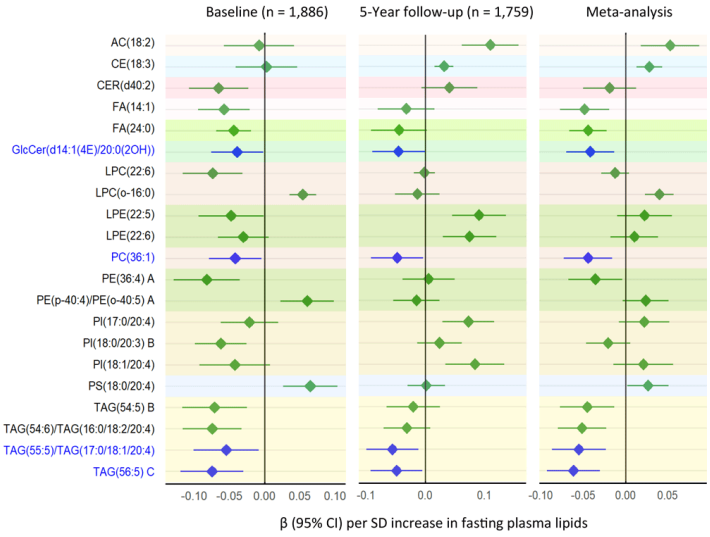

Figure 4. Top-ranked plasma lipids associated with ABI at $P < .05$ identified at baseline or 5-year follow-up. Lipids significantly associated with ABI ($P < .05$) at baseline, at follow-up, and in the meta-analysis are highlighted in blue. "A," "B," or "C" in name of lipids indicates isomers. Abbreviations: ABI, ankle-brachial index; AC; acylcarnitine; CE, cholesterol ester; CER, ceramide; eGFR, estimated glomerular filtration rate; FA, fatty acid; GlcCer, glycosylceramide; HR, hazard ratio; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; SM, sphingomyelin; TAG, triacylglycerol.

Results from sensitivity analyses

Additional adjustments for clinical lipids (ie, HDL cholesterol, triglycerides) and physical activity did not attenuate the observed associations. We did not observe significant sex difference in the associations of identified lipids with ABI or change in ABI. In addition, most lipids associated with change in ABI or ABI remained significant even after excluding symptomatic participants or adjusting for symptoms.

Discussion

In this first large-scale longitudinal lipidomic profiling of subclinical PAD, assessed by change in ABI, among American Indians, we had several significant findings. First, we found that baseline levels of multiple lipid species (eg, glycerophospholipids, glycerolipids, FAs, and SMs) were significantly associated with change in ABI beyond traditional risk factors. Some identified lipids (ie, TAG(48:2), TAG(55:1), PI(16:0/20:4), and cholesterol) were also significantly associated with risk of CHD during an average of 18-years follow-up. Second, our repeated measurement analysis showed, for the first time, that longitudinal changes in several lipid species (ie, ACs, CEs, glycerophospholipids) were significantly associated with change in ABI, independent of clinical factors, baseline ABI, and baseline lipids. Third, cross-sectional analysis showed that altered levels of ACs, FAs, glycerophospholipids (ie, PCs, PEs, PIs), and TAGs were significantly associated with ABI among American Indians. Together, our results could shed light on lipidomic markers associated with subclinical PAD and deepen our understanding of how dyslipidemia may contribute to the development of PAD.

We observed that higher baseline levels of most glycerophospholipids (ie, PCs, PEs, PIs) were positively associated with change in ABI in American Indians. Moreover, longitudinal changes in glycerophospholipids were significantly associated with cardiometabolic traits such as BMI, blood pressure, fasting blood plasma glucose, and insulin resistance. These findings are in line with previous epidemiologic studies demonstrating that some glycerophospholipids, such as PC(32:1), PC(32:3), PC(36:4), and LPC(16:1), were inversely associated with risk of PAD (16) and CHD (17) in non-Hispanic White and Asian people.

Glycerophospholipids, such as PCs, PEs, and PIs, are key components of apolipoprotein B (ApoB)-containing lipoproteins. These lipids are essential in maintaining membrane structure, fluidity, and cell signaling, and they regulate pathways involved in inflammation and oxidative stress (18). Alterations in the levels of PCs, PEs, and PIs can influence the size, density, and atherogenic potential of lipoproteins such as LDL cholesterol and lipoprotein(a) (Lp[a]), contributing to increased CVD risk. Specifically, PCs can be hydrolyzed by lipoprotenin-associated phospholipase A2 (Lp-PLA2), producing lysophosphatidylcholine (LPC) and oxidized FAs (19). LPC is further converted into lysophosphatidic acid (LPA) by autotaxin, which is often elevated in people with high Lp(a) levels (20). Elevated Lp(a) levels are associated with increased LPA levels (21), promoting vascular inflammation and oxidative stress, contributing to atherosclerotic plaques and PAD (20). Disrupted PE metabolism can induce oxidative

stress and endothelial dysfunction in arterial walls, exacerbating vascular pathologies (18). Dysregulation of the PI signaling pathway can result in endothelial dysfunction and increased vascular inflammation, both of which are key factors in the development of PAD (22).

Statin therapy, the most widely recognized cholesterol-lowering treatment for managing CVD, including PAD, can influence glycerophospholipid levels. For instance, previous studies (23,24) showed that statins altered key lipid species such as PC(36:4) and PI(18:0/18:2), both of which are involved in lipoprotein metabolism and cardiovascular risk. Notably, the ratio of PI(18:0/18:2) to PC(38:4) explained 58% of the relative CVD risk reduction associated with pravastatin during a 12-month follow-up, independent of change in LDL cholesterol (23). Our study supports this finding, as we observed that higher baseline level of PI(18:0/18:2) was positively associated with change in ABI. This finding aligns with a previous study (25) identifying PI(18:0/18:2) as a predictor of statin response in patients with familial hypercholesterolemia. These results highlight the potential of monitoring specific lipid species, such as PCs and PIs, as biomarkers for statin efficacy in the management of PAD.

Besides glycerophospholipids, we also found that higher baseline levels of certain long- chain glycerolipids (eg, TAGs, DAGs), which have a higher number of carbon atoms and fewer double bonds, were positively associated with change in ABI, suggesting a protective role against subclinical PAD. This aligns with previous research demonstrating that elevated levels of long-chain unsaturated glycerolipids (eg, TAG [48:2], TAG[55:1], DAG[36:1]) were associated with a reduced risk of CHD (17) in multiple populations. Moreover, we observed novel associations, including 2 lipids (ie, TAG[48:2], TAG[55:1]) that demonstrated a protective effect on subclinical PAD and were also associated with a decreased risk of CHD. Conversely, our cross-sectional analysis found that some TAGs with a higher number of carbon atoms and double bonds (eg, TAG[56:5] C, TAG[55:5]/TAG[17:0/18:1/20:4]) showed an inverse association with ABI. This observation is supported by previous studies reporting that 2 TAGs (ie, TAG[56:5] C and TAG[55:5]) were positively associated with the risk of diabetes in Asian (26) and non-Hispanic White people (27). These results suggest that the composition of specific TAG subtypes may have differential associations with PAD. Further investigation is warranted to validate these findings and deepen our understanding of these associations.

Our findings that baseline levels of SMs were inversely associated with change in ABI corroborate previous studies reporting that higher plasma levels of SMs were associated with an increased risk of atherosclerotic plaque (28) and CHD (29). As the predominant sphingolipids in mammalian cell membranes, SMs are crucial for signal transduction, apoptosis, regulation of inflammation, and the oxidative stress response (30). The transformation of sphingomyelin to ceramide in LDL cholesterol by sphingomyelinase, which triggers ceramide aggregation, could represent an early stage in the development of atherosclerosis (31).

Our repeated measurement analysis revealed, for the first time, the association between longitudinal changes in plasma lipidome and change in ABI, independent of clinical covariates, baseline ABI, and baseline lipids. Specifically, changes in ACs, CEs, and glycerophospholipids (eg, PCs, PEs, PIs) were associated with change in ABI as well as changes in cardiovascular risk factors. Besides the contributions of glycerophospholipids to the change in ABI, changes in several lipid classes, such as ACs and CEs, were also associated with changes in multiple cardiometabolic traits. ACs, which are esters formed by carnitine and fatty acids, serve as transporters that move activated long-chain FAs into mitochondria for β-oxidation, an essential process for cellular energy production (32). An excess of ACs may indicate a bottleneck in the β-oxidation pathway, suggesting potential mitochondrial dysfunction that could contribute to the development of PAD (33). Previous studies (34) showed that plasma concentrations of unsaturated cholesterol esters, such as CE(18:2) and CE(17:1), are elevated in patients with intermittent claudication compared with controls. Our study extends these findings by showing that changes in CE(18:3) were positively associated with changes in ABI among participants without intermittent claudication, suggesting its potential role as an early biomarker for subclinical PAD. Interestingly, no such association with CE(18:3) was observed in symptomatic participants in the prior studies (34). CEs serve as a storage form of cholesterol and are the primary neutral lipids found in lipid droplets. Their accumulation in macrophage foam cells, characterized by cholesterol ester-rich lipid droplets, is a key feature of atherosclerosis (35). This differential association across symptomatic and asymptomatic stages suggests the importance of exploring lipid biomarkers like CE(18:3) to enhance early detection and prevention strategies for PAD across its clinical spectrum.

Although younger than the typical screening age for clinical PAD (around 50 years for smokers and 70 years for nonsmokers) (1), the American Indian population has a disproportionate share of PAD, with nearly twice the prevalence compared with the non-Hispanic White population (36). This elevated prevalence is largely attributed to a higher prevalence of cardiovascular risk factors, including high rates of smoking (>40% were current smokers), obesity (55% at baseline, 60% at follow-up), and diabetes (17.9% at baseline, 23.4% at follow-up). These factors accelerate the onset of subclinical PAD, suggesting the need for early screening and prevention strategies in young, high-risk populations, especially in American Indians.

Strengths and limitations

Our study has several strengths. The major strength is the longitudinal profiling of plasma lipidome in a large, community-based prospective cohort. To our knowledge, our study is the first and by far the largest longitudinal study examining the relationship between change in plasma lipidome and change in ABI across any racial or ethnic group. Second, we used an untargeted lipidomic approach, quantifying more than 1,500 distinct lipid species across 14 known lipid classes in a large prospective cohort of American Indians. While existing biomarkers such as LDL-C, Lp(a), and ApoB are crucial, these markers, combined with other risk factors such as hypertension and tobacco use, still explain only a small proportion of CVD-related outcomes, including PAD, suggesting that additional unmeasured or unknown factors could improve early detection and prevention efforts. Lipidomics can offer a nuanced view by identifying individual lipid species that may not be identified by standard lipid panels. These newly discovered lipids offer supplementary value and could improve risk prediction beyond established risk factors. Third, our statistical models adjusted for a wide range of traditional PAD risk factors, ensuring that the lipids identified in our study are independent of these risk factors. If validated, these newly identified lipid species may serve as novel biomarkers for PAD prediction and risk stratification. Finally, we performed comprehensive statistical analyses, including cross-sectional, prospective, and repeated measurement analyses, allowing us to thoroughly investigate the association between lipid metabolism and ABI in this understudied population.

However, our study has several limitations. First, although our research identified numerous lipid species, many lipids are unknown. More research is needed to further characterize these unknown lipids if they are deemed of interest. Second, although our statistical models adjusted for many traditional risk factors, we cannot entirely rule out potential confounding by unknown or unmeasured factors. Third, our study included only American Indians, a population with high rates of dyslipidemia (78% among adolescents and young adults vs 30% in in the same age groups in the US overall) (37), diabetes (3-fold higher age-adjusted rate than among the non-Hispanic White population) (38), and smoking (>40% of adults vs 27.4% of non-Hispanic White adults) (39). Due to these unique characteristics, our findings may not be generalizable to other racial or ethnic groups with different genetic backgrounds or environmental exposures. Fourth, due to lack of an external cohort with a similar study design and longitudinal lipidomic data, we could not replicate our findings in an independent cohort. However, the large sample size of the study cohort and the identification of multiple lipid species associated with ABI using different statistical models lend credence to our findings. Fifth, while our study identifies novel lipid biomarkers that may enhance early prevention of PAD, the clinical application of these methods is limited by the emerging nature of lipidomics technology. Broader implementation in clinical practice, particularly in resource-limited settings, will require further validation, cost-effectiveness analyses, and technologic advancements. Finally, the observational nature of our study precludes any inference about the causal role of altered lipid metabolism in PAD pathogenesis.

Conclusion

In this large-scale longitudinal lipidomic analysis, we have, for the first time, reported associations of multiple individual lipid species with subclinical PAD, independent of traditional risk factors. These findings enhance our understanding of the mechanism through which dyslipidemia may contribute to PAD and provide potential novel biomarkers for early prediction and risk stratification in American Indians, an important but traditionally understudied racial minority population.

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The phenotype data used in this study can be requested through the Strong Heart Study (https://strongheartstudy.org). The lipidomic data can be obtained from the corresponding author upon request.

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Table

Table. Characteristics of Study Participants in the Strong Heart Family Study at Baseline (2001–2003) and 5-Year Follow-Up (2006–2009)[a]

Characteristics	Baseline (n = 1,886)	5-Year follow-up (n = 1,759)
Age, mean (SD), y	40.1 (13.9)	44.9 (13.4)
Female, no. (%)	1,175 (62.2)	1,103 (62.7)
Body mass index,[b] mean (SD)	31.8 (7.5)	32.7 (7.7)
Current smoking, no. (%)	757 (40.1)	670 (38.3)
Current drinking, no. (%)	1,182 (62.6)	1,032 (58.9)
Type 2 diabetes, no. (%)	338 (17.9)	412 (23.4)
Systolic blood pressure, mean (SD), mm Hg	122.3 (15.3)	122.6 (16.3)
Diastolic blood pressure, mean (SD), mm Hg	77.3 (10.6)	74.9 (11.1)
eGFR, mean (SD), mL/min/1.73m^2	114.9 (17.5)	108.6 (19.9)
High-density lipoprotein cholesterol, median (IQR), mg/dL	49.0 (42.0–59.0)	48.0 (40.0–58.3)
Low-density lipoprotein cholesterol, mean (SD), mg/dL	101.7 (29.9)	106.2 (30.7)
Triglycerides, median (IQR), mg/dL	138.0 (99.0–195.0)	133.0 (96.5–188.0)
Total cholesterol, median (IQR), mg/dL	182.0 (162.0–205.0)	186.3 (161.0–210.0)
Ankle brachial index, median (IQR)[c]	1.1 (1.1–1.2)	1.2 (1.1–1.3)
Physical activity, median (IQR), steps per day	5,147.5 (3,325.2–7,516.9)	5,841.0 (3,838.0–8,087.0)
Lipid-lowering medication, no. (%)	61 (3.2)	172 (9.8)
Antihypertensive medication, no. (%)	199 (10.6)	353 (20.1)

Abbreviations: eGFR, estimated glomerular filtration rate.
[a] Values are as mean (SD) for normally distributed data or median (IQR) for nonnormally distributed data. Categorical variables are expressed as number (percentage).
[b] Measured as weight in kilograms divided by height in meters squared.
[c] Ankle brachial index is the ratio of the systolic blood pressure in the ankle to the systolic blood pressure in the arm; the normal range is 0.9 to 1.4.

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RESEARCH BRIEF

Two Modeling Strategies in Analyzing Clustered Time-to-Event Data: the Strong Heart Family Study

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PEER REVIEWED

Summary

What is already known on this topic?

The shared frailty model has been a popular way of analyzing clustered survival data, though other methods, like the marginal Cox model, handle this data.

What is added by this report?

We used data on leukocyte telomere length and stroke to demonstrate that the marginal Cox model produces very similar results to the shared frailty model.

What are the implications for public health practice?

The marginal Cox model adds to the toolbox for analyzing clustered survival data in population genetic studies, which investigate the hereditary component of human diseases. Researchers may choose the marginal Cox model when the model will be interpreted at the population level and a robust covariance estimator is required.

Abstract

Researchers need applicable tools to analyze and account for familial relatedness when working with family study data. In this brief article, we describe the application of 2 modeling strategies for studying the association between leukocyte telomere length and incident stroke based on data collected in the Strong Heart Family Study: the shared frailty model and the marginal Cox proportional hazards model. Although these modeling strategies are based on different theoretical frameworks, their results were similar. Future simulation study may help us to better understand the limitations and performance of each strategy in a controlled environment.

Objective

The Strong Heart Study (SHS) is a cohort study of cardiovascular diseases (CVD) among American Indians living in Arizona, Oklahoma, North Dakota, and South Dakota. In Phase IV of the SHS (also called the Strong Heart Family Study [SHFS]), members of 91 families from 12 tribal communities were recruited and assessed for demographic, clinical, and behavioral characteristics (1,2). Participants have been followed for CVD outcomes to the present day. When analyzing data from the SHFS, we must address relatedness among family members.

The shared frailty model is one approach for analyzing clustered time-to-event data (3). We used it previously to determine the association between leukocyte telomere length (LTL) and cardiometabolic outcomes, such as stroke (4), carotid atherosclerosis (5), and diabetes (6). The marginal Cox proportional hazards model provides another approach to account for familial relatedness in survival data analyses (7). However, its application is less demonstrated in family studies.

In this report, we used both the shared frailty and the marginal Cox proportional hazards models to study the association between LTL and time-to-incident stroke. We hypothesized that results generated by both approaches would be similar. We aimed to illustrate the use of multiple tools for researchers to appropriately analyze family study data.

Methods

The Cox proportional hazards model (Cox model) is commonly used to identify risk factors that affect survival time among independent participants. To analyze clustered data, the shared frailty model adds a random frailty term to the Cox model, which models the effect of cluster membership on the outcome risk (3). Conversely, the marginal Cox model (7–9) accounts for family relatedness by using a robust sandwich covariance estimator, which makes no distributional assumptions about the model parameters

and is consistent even when model assumptions (eg, independence) are violated (10,11).

Full details about the study design were published previously (1,2). We included data from 4,635 people from the original and family cohorts who were stroke-free at the time of their baseline examinations (1989–1991 and 2001–2003, respectively) and had LTL measurements. Participants were followed through December 31, 2018, for fatal and nonfatal stroke events (12,13), and they all gave informed consent. This study was approved by the institutional review boards of the participating institutions, the participating tribes, and area offices of the Indian Health Service (4).

Summary statistics were generated, and *P* values were obtained by using the χ^2 test or Mann–Whitney test. Four shared frailty and marginal models were built in the same manner with time to first stroke as the outcome. We first studied the univariable association between age-adjusted LTL (in log quartile) and stroke. We then built 3 multivariable models with demographic (Model 2), behavioral (Model 3), and clinical (Model 4) covariates added to the models sequentially to create our final model. Covariates were chosen based on our previous work and literature review (4). Hazard ratios for each log LTL quartile were obtained. Type III tests assessed the significance of the frailty term. All models were created in SAS, version 9.4 (SAS).

Results

Among our 4,635 participants, 2,645 belonged to 87 families, and 1,990 were independent individuals considered as single-member families. Family sizes ranged from 1 to 109 (median, 31). In total, 301 participants experienced incident stroke with a median follow-up time of 16.8 years (interquartile range: 15.0–20.3) (Table 1). Those who had a stroke event were older, had higher blood pressure, and had worse lipid profiles (higher triglyceride, higher total and LDL cholesterol, and lower HDL-cholesterol) than participants free from stroke event during the follow up. The prevalence of atrial fibrillation, diabetes mellitus, and smoking was higher in those with a stroke event than those without a stroke event.

Across both the shared frailty and marginal models, point estimates, CIs, and *P* values are almost the same, except for the univariate models that showed about 5%–10% differences (eg, hazards ratio of 0.88 and 0.90 from the frailty model vs 0.83 and 0.98 from the marginal model) (Table 2). For the shared frailty model, the frailty term was significant for all models except Model 1 ($P = .06$), though results for all models were similar to independent Cox models. Both methods showed that after adjustment for demographic, behavioral, and clinical covariates, participants whose LTL was in the third quartile had significantly lower risk of developing a stroke event during the 17-year follow-up period with a hazard ratio of 0.66 (95% CI, 0.46–0.94; *P* value, .02) compared with participants with LTL in the first quartile. Participants with LTL in the second or fourth quartiles did not have significantly different risks of developing a stroke compared with participants with LTL in the first quartile. The shared frailty model and the marginal model generated similar estimates on the same set of data collected in the SHFS.

Discussion

Two modeling strategies, the shared frailty model and the marginal Cox proportional hazards model, generated similar estimates in studying the association between LTL and incident stroke based on the same data collected in the SHFS. Although previous studies have used the shared frailty model (4–6), our results show that the less complex marginal Cox model could be considered as a viable alternative for clustered data, such as family or panel data. However, we must consider the advantages and disadvantages of each model when choosing the best model for a situation.

The shared frailty model accounts for the relatedness between family members by introducing a random variable called a frailty to a Cox proportional hazards model (3). Each family is treated as a cluster, and each individual family member is treated as a randomly selected individual from that cluster. One advantage of this model is that the differences between each of the clusters can be easily described (14). In addition, if the frailty term is found to be insignificant, we can reduce our model to an independent Cox model. The shared frailty model yields more efficient estimation when the distribution of the frailty term is modeled correctly. However, this is prone to misspecification because choices for this distribution are limited by software. Coefficients from the shared frailty model should be interpreted as conditional on the unobserved frailty term (7). In contrast, the marginal Cox proportional hazards model uses a robust sandwich covariance estimator to account for the relatedness between family members. A benefit of this model is that the dependence between related observations is unspecified, which allows for greater flexibility in practice because we are not limited by our ability to correctly specify a frailty model (7). However, this model is still somewhat reliant on the specified model and can be affected if the coefficients are heavily biased by unobserved covariates. The marginal model can be interpreted at the population level (7). Both models are useful tools for analyzing survival data from family studies, such as the SHFS. A simulation study of the 2 modeling strategies would be helpful for us to better understand their limitations and performance under a controlled environment. In addition, future studies may consider comparing methods for clustered competing risks data. However, it is beyond the scope of this brief article aiming to demonstrate

the application of both methods in analyzing clustered survival data collected from family studies.

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Tables

Table 1. Baseline Characteristics by Incident Stroke Status[a]

Variables	Total (N = 4,635)	Incident stroke (n = 301)	Stroke-free (n = 4,334)	*P* value[b]
Leukocyte telomere length (LTL)	1.0 (0.9–1.2)	1.0 (0.8–1.4)	1.0 (0.9–1.2)	.85
Age, y	48.2 (36.8–56.5)	56.2 (50.0–63.1)	47.7 (35.7–55.8)	<.001
Sex, male, n (%)	1,900 (41)	120 (40)	1,780 (41)	.68
Phase I Cohort, yes, n (%)	2,369 (51)	237 (79)	2,132 (49)	<.001
Field sites, n (%)				<.001
Arizona	499 (11)	13 (4)	486 (11)	
Oklahoma	1,889 (41)	103 (34)	1,786 (41)	
Dakotas	2,247 (48)	185 (61)	2,062 (48)	
Education, y	12.0 (10.0–14.0)	12.0 (10.0–13.0)	12.0 (10.0–14.0)	<.001
Smoking, yes, n (%)	3,089 (67)	223 (74)	2,866 (66)	.005
Body mass index, kg/m^2	29.9 (26.2–34.5)	30.0 (26.5–34.3)	29.9 (26.1–34.5)	.77
Atrial fibrillation, yes, n (%)	270 (6)	46 (15)	224 (5)	<.001
Diabetes mellitus, yes, n (%)	1,197 (26)	137 (46)	1,060 (25)	<.001
Systolic blood pressure, mmHg	121.0 (111.0–132.0)	128.0 (117.0–140.0)	121.0 (111.0–132.0)	<.001
Diastolic blood pressure, mmHg	76.0 (69.0–83.0)	77.0 (71.0–84.0)	76.0 (69.0–83.0)	.03
Total cholesterol, mg/dL	186.0 (162.0–211.0)	192.0 (169.0–216.0)	185.0 (162.0–210.0)	<.001
LDL cholesterol, mg/dL	102.0 (83.0–124.0)	106.0 (88.0–130.0)	102.0 (83.0–124.0)	.006
HDL cholesterol, mg/dL	46.0 (39.0–56.0)	44.0 (37.0–54.0)	46.0 (39.0–56.0)	.002
Triglycerides, mg/dL	123 (87.0–179.0)	130.0 (96.0–180.0)	122.0 (86.0–179.0)	.05

Abbreviations: LTL, leukocyte telomere length; LDL, low-density lipoprotein; HDL, high-density lipoprotein.
[a] Continuous variables are described by using the median (first quartile, third quartile). Categorical variables are described by using as count (percentage). Participants were monitored for stroke events for a median follow-up time of 16.8 years (interquartile range, 15.0–20.3). Values are median (interquartile range) unless otherwise noted.
[b] Calculated by using the χ^2 test for categorical variables and the Mann–Whitney test for continuous variables.

Table 2. The Association Between Log LTL and Time to Incident Stroke, From the Frailty and Marginal Models[a]

Model	Log LTL quartile	Frailty model		Marginal model	
		Hazard ratio (95% CI)	*P* value[b]	Hazard ratio (95% CI)	*P* value[c]
Model 1, univariable model	2 vs 1	0.88 (0.64–1.23)	0.46	0.83 (0.61–1.13)	.24
	3 vs 1	0.53 (0.37–0.77)	<.001	0.54 (0.38–0.75)	<.001
	4 vs 1	0.90 (0.66–1.23)	0.50	0.98 (0.73–1.30)	.87
Model 2, adjusted for demographic covariates[d]	2 vs 1	0.95 (0.69–1.30)	.75	0.95 (0.70–1.30)	.75
	3 vs 1	0.61 (0.43–0.87)	.007	0.61 (0.43–0.86)	.005
	4 vs 1	0.90 (0.67–1.21)	.49	0.90 (0.68–1.19)	.47
Model 3, adjusted for covariates in model 2 plus behavioral covariates[e]	2 vs 1	0.97 (0.71–1.33)	.86	0.97 (0.71–1.33)	.86
	3 vs 1	0.62 (0.44–0.89)	.01	0.62 (0.44–0.88)	.007
	4 vs 1	0.92 (0.69–1.24)	.59	0.92 (0.70–1.22)	.57
Model 4, adjusted for covariates in models 2 and 3 plus clinical covariates[f]	2 vs 1	0.95 (0.69–1.32)	0.77	0.95 (0.69–1.31)	.77
	3 vs 1	0.66 (0.46–0.94)	.02	0.66 (0.46–0.93)	.02
	4 vs 1	0.94 (0.69–1.26)	.67	0.94 (0.70–1.25)	.66

Abbreviations: LTL, leukocyte telomere length.
[a] For each frailty model, the significance of the frailty term was assessed using type III tests. The frailty term was significant for Model 2 ($P < .001$), Model 3 ($P < .001$), and Model 4 ($P < .001$) but insignificant for Model 1 (P .06).
[b] P values calculated by using the Wald test. Significant at $P < .05$.
[c] P values calculated by using the robust Wald test. Significant at $P < .05$.
[d] Demographic covariates: study site, cohort, and education.
[e] Behavioral covariates: smoking status and body mass index.
[f] Clinical covariates: atrial fibrillation, diabetes, systolic and diastolic blood pressure, total, LDL and HDL cholesterol.