Journal of the American Heart Association

ORIGINAL RESEARCH

# Early Contributors to Healthy Arterial Aging Versus Premature Atherosclerosis in Young Adults: The Bogalusa Heart Study

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**BACKGROUND:** Early identification of healthy arterial aging versus premature atherosclerosis is important for optimal atherosclerotic cardiovascular disease risk stratification and prevention. We sought to identify predictors for the long-term absence of carotid plaque among young adults.

**METHODS AND RESULTS:** We included 508 participants from the Bogalusa Heart Study without clinical atherosclerotic cardiovascular disease who were free of carotid plaque at baseline (2001–2002) and underwent ultrasound imaging at follow-up (2013–2016). Modified Poisson regression estimated the persistent absence of plaque over 12.8 years. Participants were on average age 36.2 years at baseline, 64% were women, and 29% were Black. Although nearly all participants (97%) had a 10-year atherosclerotic cardiovascular disease risk <7.5%, there were 162 people (32%) who developed premature atherosclerosis. Aside from younger age (risk ratio [RR], 1.21; 95% CI, 1.07–1.36, per 10 years) and a total cholesterol/high-density lipoprotein cholesterol ratio <3.5 (RR, 1.15; 95% CI, 1.01–1.30), normal values of traditional risk factors did not predict long-term absence of plaque. Independent from traditional markers including glomerular filtration rate, serum calcium-phosphate product (RR, 1.07; 95% CI, 1.01–1.14, per 1-SD lower), phosphate (RR, 1.15; 95% CI, 1.03–1.29, per 1 mg/dL lower), and dietary sodium <2300 mg/day (RR, 1.20; 95% CI, 1.02–1.41) were significantly associated with the non-development of plaque.

**CONCLUSIONS:** Nearly one third of young adults with a low burden of traditional risk factors developed premature atherosclerosis. Beyond younger age and an ideal lipoprotein profile, lower calcium-phosphate homeostasis and low sodium intake were associated with long-term absence of carotid plaque. These results suggest that dietary and intrinsic minerals are early contributors to the development of arterial aging phenotypes.

**Key Words:** aging ■ carotid artery plaque ■ dietary sodium ■ phosphate ■ premature atherosclerosis

See Editorial by Cheung and Cheung.

Approximately half of all adults living in the United States (121.5 million) have hypertension and/or clinical atherosclerotic cardiovascular disease (ASCVD), including coronary artery disease, stroke, or congestive heart failure.[1] While ASCVD remains the leading cause of morbidity and mortality both globally and nationally, the burden of disease still appears to be growing. In particular, deaths attributable to ASCVD have increased by nearly 7000 per year since 2008.[1] Aggressive risk factor modification in younger populations is key to reversing such trends, however, our current understanding of arterial aging phenotypes and long-term risk prediction of ASCVD remains incomplete. For example, there is significant heterogeneity between measured risk factors and atherosclerotic burden, as nearly 1 in 5 statin eligible

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Supplementary Material for this article is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.121.020774.

For Sources of Funding and Disclosures, see page 9.



*JAHA* is available at: www.ahajournals.org/journal/jaha

## CLINICAL PERSPECTIVE

### What is New?

- Normal values of traditional risk factors incompletely explain healthy arterial aging, as one third of young people with a low predicted 10-year risk of atherosclerotic cardiovascular disease developed premature carotid atherosclerosis.
- Independent of traditional risk factors, lower calcium-phosphate homeostasis and low dietary sodium consumption were associated with the long-term absence of carotid plaque throughout early adulthood.

### What are the Clinical Implications?

- Beyond age and an individual's lipoprotein profile, dietary and intrinsic minerals are early contributors to the development of arterial aging phenotypes.
- Future lifestyle intervention trials are required to determine the value of calcium-phosphate modulation and define the most optimal levels of these minerals for atherosclerotic cardiovascular disease prevention in younger people.

adults do not have substantial carotid atherosclerosis.[2] On the contrary, 13% of young individuals develop accelerated atherosclerosis in the absence of traditional risk factors, such as hypertension, hypercholesterolemia, and/or type 2 diabetes mellitus, and such markers do not strongly predict arterial lesion burden in this patient population.[3] These observations underline a need to further understand the predictors of healthy arterial aging versus premature atherosclerosis in young adults with low ASCVD risk to improve the timing and precision of primary prevention strategies.

The absence of atherosclerotic carotid plaque is associated with healthy arterial aging[4] and a low future risk of clinical ASCVD, even among statin eligible candidates.[2] However, the current forces in preventive medicine favor broad pharmacotherapy over precise lifestyle approaches for reducing ASCVD risk, even in the presence of data which demonstrate considerable heterogeneity in subclinical atherosclerosis prevalence and propensity among people who harbor traditional risk factors.[4,5] This discordance between treatment and true risk highlights an incomplete approach to primary prevention and may unnecessarily burden a substantial proportion of patients who have an overall low long-term risk of an ASCVD event with potential life-long lipid-lowering pharmacotherapy.

Studies that aim to identify upstream negative risk markers that predict the absence of atherosclerosis can thus help us shift towards a more precision-based approach to primary prevention. However, currently little is known about the factors that are most important for maintaining the absence of atherosclerosis, especially in younger diverse populations. Early identification of the factors associated with the long-term absence of carotid plaque in a racially diverse group of young adults can identify the predictors/pathways important in healthy arterial aging and help to devise the most optimal approaches for primary ASCVD prevention and risk stratification.

## METHODS

The authors declare that all supporting data are available within the article and its online supplementary files.

### Study Population

The Bogalusa Heart Study is an epidemiological study examining the natural history of ASCVD across the lifespan among residents of Bogalusa, Louisiana. As early as 1973, 9 surveys were conducted in children and adolescents aged 4 to 17 years, and 11 surveys were conducted among adults aged 18 to 51 years who had been examined previously as children.[6] There were 538 participants who were free of carotid plaque at the 2001 to 2002 baseline visit and underwent a second ultrasound examination at the 2013 to 2016 follow-up visit. Among this sample, 8 participants with missing covariable data, 18 participants with implausible values for measured lipids[7] (total cholesterol >300 mg/dL or <100 mg/dL, triglycerides >400 mg/dL or <25 mg/dL, and/or high-density lipoprotein cholesterol (HDL-C) >85 mg/dL or <20 mg/dL), and 4 participants who developed carotid plaque beyond premature atherosclerosis age cut-offs[8] (men aged >55 years and women aged >65 years) were excluded, resulting in a study sample of 508 individuals. All study participants provided written informed consent at each examination, and study protocols were approved by the Institutional Review Board of the Tulane University Health Sciences Center.

### General Clinical and Laboratory Variables

All covariate data were collected at baseline. Rigorous protocols were used to collect clinical and sociodemographic data on Bogalusa participants.[9] Validated questionnaires were used to obtain sociodemographic variables including, age, race, sex, and education status. Education status, post-high school educational attainment versus high school education or below, were represented as a 2-level categorical variable. Waist circumference was measured in triplicate from the lowest rib to the superior border of the iliac crest using a flexible tape.[10] Systolic and diastolic pressure were measured in triplicate using

mercury sphygmomanometers on the right arm of study participants in a seated position, and the average of the 3 readings was used.

Participants were instructed to undergo a 12-hour fast before measurement of blood lipids and glucose. Total cholesterol and triglycerides were assessed using enzymatic procedures (Abbott VP laboratories, Chicago, IL). Serum HDL-C, apolipoprotein-AI (apoAI), and apolipoprotein-B (apoB) were measured using agar-agarose gel electrophoresis and heparin-calcium precipitation procedures.[11] Serum values for low-density lipoprotein cholesterol (LDL-C) were calculated using the Friedewald equation.[12] Serum creatinine was measured using the Kinetic Jaffe method. Serum creatinine was used to calculate estimated glomerular filtration rate (eGFR) via the chronic kidney disease-epidemiology collaboration equation.[13] Serum phosphate and calcium were measured using standardized methods as part of a multiple chemistry profile on a multichannel Olympus Au-5000 Analyzer (Olympus, Lake Success, NY).[14] Fibrinogen was measured using a Technicon H6000 (Technicon Instrument Corp. Tarrytown, NY).[15] Plasma homocysteine was measured using an automated fluorescence polarization immunoassay (Abbott Diagnostics, Abbott Park, Illinois).[16]

## Lifestyle Variables

Dietary data were collected using the Youth/Adolescent Questionnaire, a semiquantitative, validated, 151-item food frequency questionnaire. Previous studies have demonstrated that the Youth/Adolescent Questionnaire can successfully capture dietary habits and patterns by this young adult population residing in Bogalusa, Louisiana.[17,18] Nutrient intake analysis, including dietary sodium, potassium, fiber, cholesterol, total fat, and total calorie intake was performed at the Channing Laboratory, Department of Medicine, Brigham and Women's Hospital at Harvard Medical School, Boston, MA. Cigarette smoking, alcohol drinking, and physical activity were measured via standardized questionnaires.[9,19]

## Carotid Ultrasonography

Experienced and trained technicians completed sonographic examinations on participants in the supine position and head rotated 10° away from the side examined. Images were captured using a Toshiba ultrasound instrument (Xario, SSA-660A, Toshiba America Medical Systems, Tustin, CA) with a linear array transducer of 7.5 mHz, following a detailed protocol.[14] Ultrasound B-mode measurements included maximum intima-media thickness at diastole from the far walls of the common carotid artery, carotid bulb and internal carotid artery segments bilaterally. The mean of the maximum carotid intima-media thickness readings of the 3 left and 3 right far walls of the common, bulb, and internal segments were used for analysis. The presence of a carotid artery atherosclerotic plaque was defined by a distinct focal wall thickening >1.5 mm.[20] There has been robust intra-observer reliability in blind duplicate carotid intima-media thickness measurements in the Bogalusa Heart Study, with an average correlation coefficient of 0.72 across all carotid reading sites.[21] Premature atherosclerosis was defined as the presence of carotid plaque in men aged ≤55 years and women aged ≤65 years.[8]

## Statistical Analysis

For study sample characteristics, continuous variables were presented as means and standard deviations and categorical variables were presented as percentages. Differences between categorical variables were evaluated through the Chi-square test. Differences in normally and non-normally distributed continuous variables were assessed through the Student *t*-test and Wilcoxon signed-rank test, respectively.

We categorized continuous predictor variables as ideal or non-ideal to assess their association with carotid plaque. For risk markers with a commonly designated clinical cut point we used categorical variables, and for variables without a clear ASCVD risk cut point we used a continuous scale. Categories included, waist circumference <40 inches for men and <35 inches for women, systolic blood pressure <120 and diastolic blood pressure <80 mm Hg, fasting blood glucose <100 mg/dL, fasting serum triglycerides <150 mg/dL, total cholesterol/HDL-C ratio <3.5, homocysteine <10 μg/dL, eGFR >90 mL/min/1.73 $m^2$, albuminuria <30 mg/L, dietary sodium <2300 mg/day, potassium >3600 mg/day for men and >2600 mg/day for women, fiber >30 g/day, cholesterol <300 mg/day, saturated fat <10% of total calories, ≤2 drinks/day in men, ≤1 drink/day in women, and ≥150 minutes/week of moderate activity or ≥75 minutes/week of vigorous activity.[8,22–24] Serum calcium, phosphate, and calcium-phosphate product were assessed on a continuous scale.[25] The Pooled Cohort Equations 10-year ASCVD risk[26] was calculated using the baseline age for each study participant, including the extension of risk calculation for participants aged <40 years.

Modified Poisson regression with robust standard error[27] estimated the absence of carotid plaque according to traditional and non-traditional ASCVD risk factors and lifestyle behaviors. In model 1, we adjusted for age, sex, race, waist circumference, total cholesterol/HDL-C ratio, fasting blood glucose, serum triglycerides, blood pressure, smoking status,

and lipid lowering-, glucose lowering-, and blood pressure-lowering medications. Each risk marker and lifestyle factor, including homocysteine, fibrinogen, eGFR, albuminuria, calcium, phosphate, calcium-phosphate product, dietary sodium, potassium, fiber, cholesterol, fat, alcohol drinking, and physical activity, was evaluated independently after adjusting for model 1 covariables. Given the varying strength of association between different lipoprotein ratios and carotid atherosclerosis,[28] we performed 2 a priori sensitivity analyses adjusting for LDL-C/HDL-C and apoB/apoA1 rather than total cholesterol/HDL-C. We performed 3 post hoc sensitivity analyses: (1) additionally adjusting significant mineral markers for continuous eGFR; (2) assessing the association of significant mineral markers with persistent absence of carotid plaque among individuals with an eGFR >90 mL/min/1.73 $m^2$, and a urinary albumin excretion <30 mg/L, and (3) assessing the cross-sectional association of significant mineral markers with total meat intake, including red meat, processed meat, poultry, and fish, after adjusting for baseline age, sex, race, and total calorie intake.

Statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC). All hypothesis tests were 2-sided. We used an alpha threshold of 0.05 for detecting differences in descriptive statistics and for detecting significant relative risk ratios in regression models.

## RESULTS

Participants were on average aged 36.2 years at baseline, 327 (64.4%) were women, and 149 (29.3%) were Black (Table 1). A total of 346 (68.1%) participants remained free of carotid plaque over 12.8 years follow-up. At baseline, people with persistent absence of carotid plaque were significantly younger and had a significantly lower burden of traditional ASCVD risk factors, except for fasting blood glucose, compared with those with premature carotid atherosclerosis. There were similar intakes of dietary cholesterol, saturated fat, fiber, sodium, and potassium, and a similar frequency of physical activity for individuals with persistent absence of plaque versus premature atherosclerosis. Individuals with long-term absence of plaque had lower values of serum calcium-phosphate product and phosphate compared with those with premature atherosclerosis, although both groups had similar baseline renal function.

Among groups with normal values of traditional risk factors, individuals with a total cholesterol/HDL-cholesterol ratio <3.5 (77.4%) and a fasting blood glucose <100 mg/dL (69.2%) had the highest and lowest proportion of participants with long-term absence of plaque, respectively (Figure 1). Although nearly all participants (97.0%) had a 10-year ASCVD risk <7.5%, there were 162 people (31.9%) who developed premature atherosclerosis (Figure 2).

In multivariable modeling, persistent absence of carotid plaque was observed for younger age (risk ratio [RR], 1.21; 95% CI, 1.07–1.36; *P*=0.003, per 10 years) and a total cholesterol/HDL-C ratio <3.5 (RR, 1.15; 95% CI, 1.01–1.30; *P*=0.03) (Table 2). Normal values of other individual traditional risk factors were not significantly associated with healthy arterial aging; however, people with ≥3 ideal traditional ASCVD factors were 29% more likely to maintain absence of carotid plaque compared with those with <3 ideal risk factors (RR, 1.28; 95% CI, 1.04–1.59; *P*=0.02). There were no significant associations of different lipoprotein ratios, LDL-C/HDL-C, or ApoB/ApoA1, with long-term absence of plaque (Tables S1 and S2).

Lower inflammatory risk factors, including homocysteine and fibrinogen, did not significantly predict persistent absence of carotid plaque (Table 3). On the contrary, mineral metabolism risk markers, including serum calcium-phosphate (RR, 1.07; 95% CI, 1.01–1.15; *P*=0.03) and phosphate (RR, 1.15; 95% CI, 1.03–1.29; *P*=0.01) were significantly associated with the long-term absence of carotid plaque, independent of traditional ASCVD risk factors. Relative risks and significance for phosphate and calcium-phosphate product were consistent after additionally adjusting for eGFR (RR, 1.15; 95% CI, 1.03–1.29; *P*=0.01; and RR, 1.08; 95% CI, 1.01–1.15; *P*=0.02) and among the 383 participants who had an eGFR >90 mL/min/1.73 $m^2$ and a urine albumin excretion <30 mg/L (RR, 1.19; 95% CI, 1.04–1.36; *P*=0.01; and RR, 1.10; 95% CI, 1.02–1.19; *P*=0.02). Post-hoc nutritional analyses demonstrated a significant linear trend towards higher serum phosphate and calcium-phosphate product (p-trend=0.04 for both) across higher tertiles of total meat intake (Table S3).

For lifestyle behaviors, participants consuming <2300 mg/day of sodium were 20% more likely to have long-term absence carotid plaque compared with those with a dietary sodium intake ≥2300 mg/day (RR=1.20, 95% CI: 1.02–1.41; *P*=0.03) (Table 3). No significant associations of other dietary or physical activity habits with long-term absence of plaque were observed. After adjusting for different lipoprotein ratio measures, including LDL-C/HDL-C and ApoB/ApoA1, similar parameter estimates were observed for serum phosphate, serum calcium-phosphate product, and dietary sodium (Tables S4 and S5).

## DISCUSSION

Although nearly all participants had a low 10-year ASCVD risk, approximately one third experienced premature atherosclerosis before middle-age. Beyond

**Table 1. Characteristics of 508 BHS Participants Free of Carotid Plaque at the Baseline Visit**

| Variable | All (n = 508) | Persistent Absence of Carotid Plaque (n = 346) | Premature Carotid Atherosclerosis (n = 162) | *P* Value‡ |
|---|---|---|---|---|
| Sociodemographic and lifestyle | | | | |
| Age, y | 36.2 ± 4.4 | 35.8 ± 4.6 | 37.2 ± 3.9 | <0.001 |
| Women, % | 64.4 | 67.1 | 58.6 | 0.07 |
| Black, % | 29.3 | 29.5 | 29.0 | 0.91 |
| Post-high school education, % | 62.4 | 65.6 | 55.6 | 0.03 |
| Never smokers, % | 60.0 | 64.2 | 51.9 | 0.008 |
| Dietary cholesterol, g/day | 254.5 (182.1–335.8) | 254.5 (181.8–334.4) | 252.3 (187.3–338.4) | 0.70 |
| Dietary saturated fat, g/day | 23.5 (17.1–31.3) | 23.3 (16.9–30.6) | 23.9 (17.3–32.1) | 0.41 |
| Dietary fat, g/day | 65.75 (49.1–87.5) | 65.3 (48.3–85.7) | 68.4 (49.8–87.9) | 0.48 |
| Dietary fiber, g/day | 13.6 (9.7–18.3) | 13.3 (9.7–18.4) | 13.8 (9.8–18.1) | 0.68 |
| Dietary sodium, mg/day | 2216.1 (1655.0–2914.7) | 2137.0 (1646.9–2921.6) | 2301.6 (1726.5–2906.9) | 0.57 |
| Dietary potassium, mg/day | 2087.5 (1562.6–2771.4) | 2076.8 (1545.1–2795.9) | 2109.6 (1613.2–2729.3) | 0.86 |
| Alcohol drinking, drinks/day | 0.0 (0.0–0.4) | 0.0 (0.0–0.4) | 0.0 (0.0–0.6) | 0.06 |
| Physical activity, min/week | 45.0 (12.5–90.0) | 45.0 (15.0–80.0) | 45.0 (7.0–120.0) | 0.39 |
| Cardiovascular imaging | | | | |
| Baseline carotid intima-media thickness, mm | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.1 | <0.001 |
| Established ASCVD risk factors | | | | |
| 10-y ASCVD risk, %* | 0.7 (0.3, 1.7) | 0.5 (0.2, 1.3) | 1.0 (0.5, 2.6) | <0.001 |
| Systolic blood pressure, mm Hg | 114.7 ± 12.7 | 112.8 ± 12.0 | 118.7 ± 13.3 | <0.001 |
| Diastolic blood pressure, mm Hg | 77.6 ± 9.3 | 76.4 ± 8.9 | 80.2 ± 9.4 | <0.001 |
| Antihypertensive medication, % | 6.3 | 4.3 | 10.5 | 0.008 |
| Total cholesterol, mg/dL | 188.1 ± 34.2 | 184.7 ± 33.2 | 195.2 ± 35.3 | 0.001 |
| HDL cholesterol, mg/dL | 48.3 ± 12.1 | 49.0 ± 12.2 | 46.9 ± 11.7 | 0.07 |
| Apolipoprotein A1, mg/dL | 169.6 ± 31.3 | 170.1 ± 30.9 | 168.7 ± 32.2 | 0.66 |
| LDL cholesterol, mg/dL | 122.7 ± 30.7 | 119.5 ± 30.4 | 129.7 ± 30.3 | <0.001 |
| Apolipoprotein B, mg/dL | 83.8 ± 20.4 | 80.9 ± 18.7 | 90.0 ± 22.5 | <0.001 |
| Total cholesterol/HDL cholesterol | 4.1 ± 1.2 | 4.0 ± 1.2 | 4.4 ± 1.3 | <0.001 |
| LDL cholesterol/HDL cholesterol | 2.7 ± 1.0 | 2.6 ± 1.0 | 2.9 ± 1.1 | <0.001 |
| Apolipoprotein B/Apolipoprotein A1 | 0.5 ± 0.2 | 0.5 ± 0.1 | 0.6 ± 0.2 | <0.001 |
| Serum triglycerides, mg/dL* | 99.0 (72.0, 141.5) | 95.0 (71.0, 136.0) | 114.0 (75.0, 158.0) | 0.01 |
| Lipid-lowering medication, % | 3.2 | 2.0 | 5.6 | 0.03 |
| Fasting blood glucose, mg/dL | 84.9 ± 19.5 | 84.1 ± 19.4 | 86.7 ± 19.7 | 0.18 |
| Glucose-lowering medication, % | 1.4 | 1.2 | 1.9 | 0.69 |
| Waist circumference, cm | 92.3 ± 17.3 | 91.1 ± 17.3 | 94.6 ± 17.2 | 0.03 |
| Mineral metabolism | | | | |
| eGFR, mL/min/1.73m$^2$ | 105.2 ± 16.4 | 105.0 ± 16.2 | 105.5 ± 16.8 | 0.78 |
| Urine microalbumin, mg/L* | 7.0 (6.0, 13.0) | 7.0 (6.0, 12.0) | 9.0 (6.0, 14.0) | 0.11 |
| Serum calcium, mg/dL | 9.5 ± 0.4 | 9.5 ± 0.3 | 9.5 ± 0.4 | 0.30 |
| Serum phosphate, mg/dL | 3.4 ± 0.6 | 3.4 ± 0.6 | 3.5 ± 0.6 | 0.02 |
| Calcium-phosphate product, mg$^2$/dL$^2$ | 32.7 ± 5.6 | 32.3 ± 5.7 | 33.6 ± 5.4 | 0.02 |
| Inflammatory markers | | | | |
| Homocysteine, μmol/L*† | 7.4 (6.2, 8.6) | 7.2 (6.2, 8.8) | 7.6 (6.3, 8.6) | 0.30 |
| Fibrinogen, mg/dL*† | 255.0 (236.0, 272.0) | 255.0 (235.0, 270.0) | 254.0 (237.0, 276.0) | 0.65 |

All variables presented are baseline values unless otherwise noted.
Continuous variables are mean (SD) unless otherwise noted.
ASCVD indicates atherosclerotic cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein cholesterol; and LDL, low-density lipoprotein cholesterol.
*Median (Q1–Q3).
†Measured in subset of sample (homocysteine, 501; fibrinogen, 504).
‡Difference between long-term absence of carotid plaque vspremature carotid atherosclerosis.

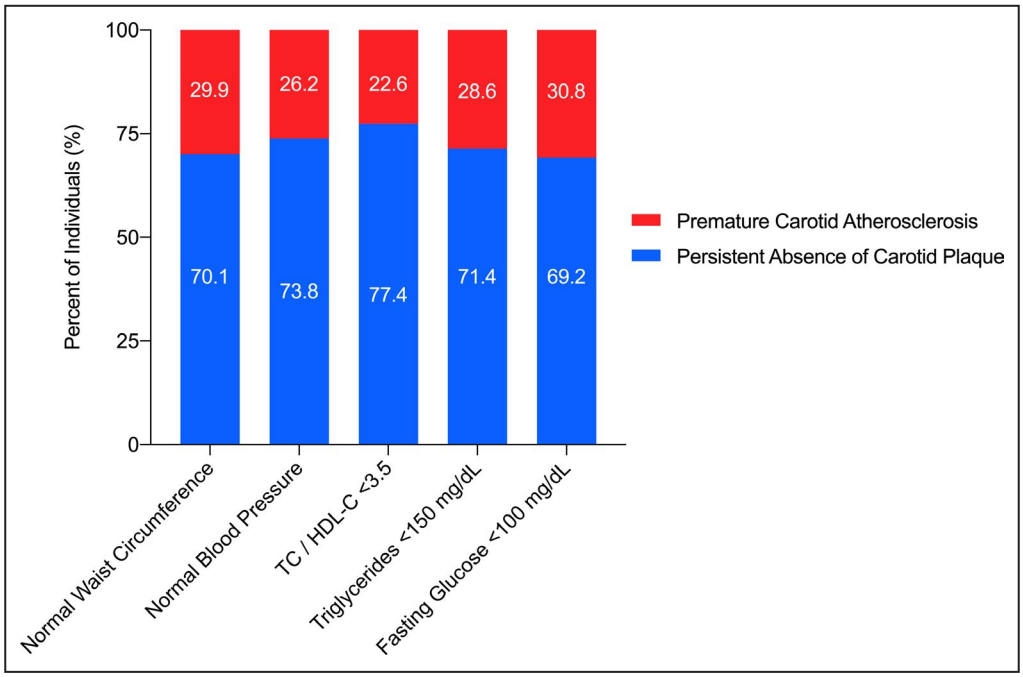


**Figure 1. Arterial aging phenotypes among people with normal values of traditional risk factors**
TC indicates total cholesterol; and HDL-C, high-density lipoprotein cholesterol.

younger age and a total cholesterol/HDL-C ratio <3.5, normal values of traditional risk factors were not associated with the persistent absence of plaque. Contrastingly, people with lower calcium-phosphate homeostasis and low sodium intake were more likely to have long-term absence of carotid plaque, independent of traditional risk factors. These results suggest that both dietary and intrinsic minerals are early contributors to the development of arterial aging phenotypes.

Individuals who had a total cholesterol/HDL-C ratio <3.5 were 16% more likely to maintain healthy arterial aging compared with those with a total cholesterol/HDL-C ratio ≥3.5. Total cholesterol/HDL-C ratio is a marker of atherogenic risk and has consistently been

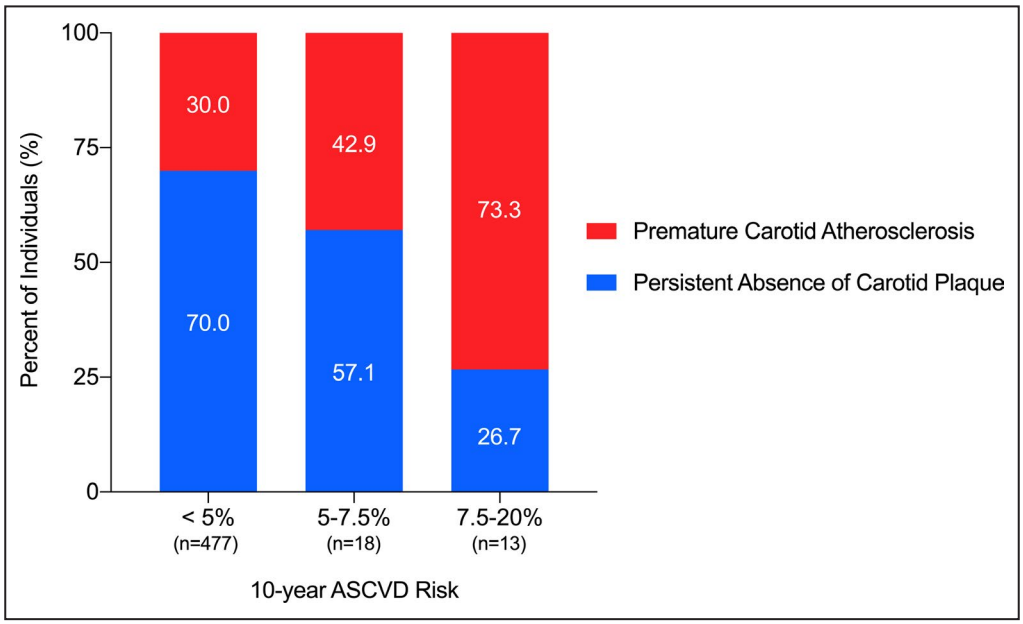


**Figure 2. Arterial aging phenotypes according to baseline 10-year atherosclerotic cardiovascular disease risk**
ASCVD indicates atherosclerotic cardiovascular disease.

**Table 2. Association of Demographics and Normal Values of Traditional ASCVD Risk Factors With the Persistent Absence of Carotid Plaque**

| Variable | Relative Risk (95% CI) | *P* Value |
|---|---|---|
| Demographic | | |
| Female sex | 1.01 (0.88–1.16) | 0.92 |
| Black | 1.01 (0.88–1.17) | 0.86 |
| Age, per 10 y younger | 1.21 (1.07–1.36) | 0.003 |
| ASCVD risk factors | | |
| Never cigarette smoker | 1.12 (0.99–1.28) | 0.08 |
| Waist circumference <40 in (men), <35 in (women) | 0.97 (0.85–1.11) | 0.64 |
| Systolic BP <120 mm Hg and diastolic BP <80 mm Hg | 1.13 (0.98–1.30) | 0.10 |
| Fasting blood glucose <100 mg/dL | 1.05 (0.78–1.41) | 0.76 |
| Triglycerides <150 mg/dL | 1.10 (0.92–1.32) | 0.29 |
| Total cholesterol/HDL-C < 3.5 | 1.15 (1.01–1.30) | 0.03 |
| >3 ideal ASCVD risk factors* | 1.29 (1.04–1.59) | 0.02 |

Adjusted for age, sex, race, education, antihypertensive medication, lipid-lowering medication, glucose-lowering medication, cigarette smoking, waist circumference, blood pressure, fasting blood glucose, fasting serum triglycerides, and total cholesterol/high-density lipoprotein cholesterol ratio.

ASCVD indicates atherosclerotic cardiovascular disease; BP, blood pressure; and HDL-C, high-density lipoprotein cholesterol.

*>3 ideal atherosclerotic cardiovascular disease risk factors vs <3 ideal risk factors.

**Table 3. Association of Normal Values of Novel ASCVD Risk Factors and Lifestyle Behaviors with the Persistent Absence of Carotid Plaque**

| Variable* | Relative Risk (95% CI) | *P* Value |
|---|---|---|
| Inflammatory | | |
| Homocysteine <10 μmol/L | 0.98 (0.82–1.16) | 0.79 |
| Fibrinogen, per 1-SD lower | 1.00 (0.94–1.06) | 0.99 |
| Renal and mineral metabolism | | |
| eGFR >90 mL/min/1.73 $m^2$ | 0.98 (0.84–1.15) | 0.83 |
| Urinary albumin <30 mg/L | 1.03 (0.84–1.26) | 0.78 |
| Serum calcium, per 1 mg/dL lower | 1.04 (0.88, 1.24) | 0.66 |
| Calcium-phosphate product, per 1-SD lower | 1.07 (1.01–1.15) | 0.03 |
| Serum phosphate, per 1 mg/dL lower | 1.15 (1.03–1.29) | 0.01 |
| Lifestyle* | | |
| Dietary sodium <2300 mg/day | 1.20 (1.02–1.41) | 0.03 |
| Dietary potassium >3400 mg/day in men, >2600 mg/day in women | 0.99 (0.82–1.19) | 0.88 |
| Dietary fiber >30 g/day | 1.20 (0.91–1.58) | 0.20 |
| Dietary cholesterol <300 mg/day | 1.05 (0.89–1.25) | 0.54 |
| Dietary saturated fat <10% of total calories | 1.04 (0.91–1.19) | 0.59 |
| Moderate alcohol drinking† | 1.17 (0.91–1.51) | 0.23 |
| Adequate physical activity† | 0.95 (0.79–1.15) | 0.61 |
| >3 ideal lifestyle behaviors‡ | 1.12 (0.98–1.28) | 0.10 |

Adjusted for age, sex, race, education, antihypertensive medication, lipid-lowering medication, glucose-lowering medication, cigarette smoking, waist circumference, blood pressure, fasting blood glucose, fasting serum triglycerides, and total cholesterol/high-density lipoprotein cholesterol ratio.

ASCVD indicates atherosclerotic cardiovascular disease; and eGFR, estimated glomerular filtration rate.

*Dietary risk markers and the cumulative lifestyle behavior risk score were also adjusted for total caloric intake.

†<2 drinks/day in men, <1 drink/day in women, >150 min/wk of moderate activity or >75 min/wk of vigorous activity.

‡>3 ideal lifestyle behaviors vs <3 ideal lifestyle behaviors.

shown to be a robust predictor of incident ASCVD, beyond that of individual lipoprotein subfractions.[29,30] Ideal measures of other lipoprotein ratios, including LDL-C/HDL-C and ApoB/ApoA1, however, did not significantly associate with healthy arterial aging independent from adjacent ASCVD risk factors. These results indicate that ideal lipid values in and of themselves do not fully explain the non-development of atherosclerosis over the life course. Furthermore, our findings add to earlier studies that show how a global optimal cardiovascular health profile is more strongly associated with the absence of ASCVD compared with optimal values of individual risk factors alone.[4,31]

While a majority of ideal traditional risk factors were not individually associated with long-term absence of carotid plaque, lower serum phosphate and calcium-phosphate product were among protective markers for arterial aging. Our results are among the first to show that lower calcium-phosphate homeostasis may be beneficial for healthy arterial aging and the persistent absence of significant atherosclerosis in younger individuals. While previous studies have shown that higher phosphate and calcium-phosphate product associate with subclinical atherosclerosis and ASCVD events, these studies have been limited to participants that are older, have underlying comorbidities, or have only reported cross-sectional relationships.[14,32,33] Chronic kidney disease is a key underlying comorbidity that predisposes people to hyperphosphatemia and accelerated atherosclerosis, as phosphate can combine with calcium in serum to promote both vascular and valvular calcification via osteoblastic pathways.[34] Yet we show that lower serum phosphate and calcium-phosphate associate with healthy arterial aging among individuals who on average had *preserved* kidney function, suggesting that calcium-phosphate elevations still within standard range may have adverse vascular consequences in young adults. Furthermore, these results remained consistent in a subgroup analysis including only individuals with an eGFR >90 mL/min/1.73 $m^2$ and urinary albumin excretion <30 mg/L. Additional follow-up studies are required to further understand the potential interaction between subclinical

renal injury and mineral metabolism on arterial aging phenotypes in younger people.

Serum calcium and phosphate are maintained within a relatively narrow physiological range, yet there may be a benefit for maintaining low-normal concentrations of these minerals to preserve optimal cardiovascular health. Lower serum phosphate has been identified among individuals who consume more vegetarian-style diets,[35] while preclinical research indicates that high dietary phosphate load may be a precipitant for physical inactivity by inducing exercise intolerance and early fatigue.[36] In a post hoc exploratory analysis, we found that total meat intake, including red meat, processed meat, poultry, and fish, significantly associated with higher serum values of both phosphate and the calcium-phosphate product independently of age, sex, race, and total calories.

In addition to intrinsic minerals, we observed a strong association between sodium, a key dietary mineral, with healthy arterial aging over a 12-year period. Individuals who consumed <2300 mg of sodium per day were 20% more likely to sustain long-term absence of carotid plaque versus those with ≥2300 mg/day of sodium intake. Excess sodium intake has direct effects on arterial smooth muscle cells, ultimately leading to increased intracellular calcium, smooth muscle contraction, and essential hypertension,[37] primary factors for vascular injury and the subsequent atherosclerotic cascade. On the other hand, lower sodium intake has been shown to slow age-related increases in systolic blood pressure across the life course[38] and diets that restrict sodium, including the dietary approaches to stop hypertension diet, confer independent reductions in blood pressure compared with a traditional Western dietary pattern.[39] There may be several different pathways by which dietary sodium influences vascular aging complimentary to blood pressure. For example, earlier cell-based models have indicated that excess sodium influences expression of up to 84 genes, including vascular cell adhesion protein1, selectin E, and, chemokine ligand 1, that are involved in endothelial cell adhesion, proliferation, and lymphocyte activation.[40]

A majority of ideal traditional risk factors were not individually strongly associated with healthy arterial aging. These results further underline the discordance between optimal risk factor burden and the absence of subclinical atherosclerosis on imaging for ASCVD risk stratification. Carotid ultrasonography is a widely available imaging modality for risk reclassification and a finding of absent carotid plaque has a downward net reclassification index of 4.4 in participants with a 10-year intermediate ASCVD risk or higher.[41] Furthermore, absence of carotid plaque is associated with a low risk of incident ASCVD (<2%) among statin eligible candidates through older age.[2] Thus, our findings provide upstream insights that may help to explain such earlier observations and suggest that mineral metabolism may be important early on in the differentiation between healthy arterial aging and premature atherosclerosis.

We found that one third of young adults who had a median 10-year ASCVD risk of 0.7% developed premature atherosclerosis and at least 1 in every 5 individuals with normal values of traditional risk factors had incident carotid atherosclerosis during follow-up. Such findings are larger than the previously reported prevalence of premature atherosclerosis, which is estimated to affect ≈11% of young adults.[42] Premature atherosclerosis remains a largely unexplained phenomenon among individuals with a low burden of traditional ASCVD factors[3] and further research is needed to better understand the pathophysiology of premature atherosclerosis in younger people.

The primary strength of this study was the longitudinal assessment of carotid atherosclerosis in a diverse cohort of young adults over a 12-year follow-up period. We examined the natural history of arterial aging while including a high proportion of Black participants (29%) and women (64%), demographic groups traditionally underrepresented in research and whom also appear to be disproportionately affected by premature atherosclerosis.[43,44] In addition to these timely findings and study design, we also systematically assessed traditional, novel, and lifestyle risk factors, including markers associated with inflammation and mineral metabolism that may help to explain non-development of carotid atherosclerosis and inform future interventional studies for ASCVD risk reduction.

Limitations of the analysis primarily relate to an inability to more comprehensively characterize arterial function. Our discovered relationship between mineral markers and arterial aging phenotypes suggests that a majority of participants who developed significant carotid atherosclerosis potentially had a certain degree of calcified plaque morphology. However, the Bogalusa carotid ultrasound protocol did not differentiate between calcified versus non-calcified plaques, and we did not have measures of broader vascular territories. Nevertheless, the absence of carotid plaque independently predicts long-term absence of coronary artery calcification,[4] suggesting that the non-development of atherosclerosis across different vascular sites is correlated. Furthermore, because of the young average age of the sample, we may have had a limited ability to detect prevalent and/or more severe manifestations of carotid atherosclerosis. Another limitation of our analysis was the relatively small sample size because of our interests in comprehensively assessing the predictors of healthy cardiovascular aging in a young cohort of the Bogalusa Heart Study. This may have hindered our ability to detect significant

associations and larger studies with rigorous assessment of exposure variables are required to build on our findings. For example, the one-time food frequency assessment of dietary variables, such as sodium and potassium, could be improved in future studies by measuring 24-hour urine sodium and potassium excretion values over several study visits. Lastly, although our study included a wide variety of exposure variables, there was overall limited information about lifestyle habits and residual confounding may have still influenced our results given the observational design of the study. Because of this lack of direct information on diet and lifestyle, our identified associations of lower dietary sodium, lower serum phosphate and calcium-phosphate product, and a normal total cholesterol/HDL-C ratio with persistent absence of carotid plaque may reflect surrogate relationships of diet and physical activity with healthy arterial aging.

To conclude, nearly one third of young adults with a relatively low burden of ASCVD risk factors developed premature atherosclerosis over 12 years. Though younger age and a total cholesterol/HDL-C ratio <3.5 predicted healthy arterial aging, normal values of blood pressure, fasting glucose, triglycerides, and waist circumference were not significantly associated with persistent absence of carotid plaque, suggesting that traditional risk factors incompletely explain healthy arterial aging. Beyond traditional markers, lower calcium-phosphate homeostasis and low sodium intake associated with the long-term absence of carotid plaque. These results suggest that dietary and intrinsic minerals are early contributors to the development of arterial aging phenotypes. Future dietary and/or pharmaceutical intervention trials are required to determine the value of calcium-phosphate modulation and define the most optimal levels of these minerals for ASCVD prevention.

## ARTICLE INFORMATION

Received January 4, 2021; accepted March 31, 2021.

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### Acknowledgments

We thank all staff members and study personnel who help conduct, sustain, and continue the Bogalusa Heart Study. We are especially grateful to the Bogalusa Heart Study participants.

### Sources of Funding

Researchers contributing to this article research were supported by the National Institute on Aging as well as the National Heart, Lung, and Blood Institute of the National Institutes of Health under grant numbers: 2R01AG041200 (Principal Investigator, Lydia A. Bazzano), P20GM109036 (Principal Investigators, Jiang He and Marie Krousel-Wood).

### Disclosures

None.

### Supplementary Material

Tables S1–S5

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# Supplemental Material

**Table S1. Association of demographics and normal values of traditional ASCVD risk factors with the persistent absence of carotid plaque, adjusting for LDL-C/HDL-C ratio.**

| Variable | Relative Risk (95% CI) | P-value |
|---|---|---|
| ***Demographic*** | | |
| Female Sex | 1.03 (0.90, 1.19) | 0.63 |
| African American | 1.02 (0.89, 1.18) | 0.78 |
| Age, per 10 years younger | 1.19 (1.06, 1.34) | 0.004 |
| ***ASCVD Risk Factors*** | | |
| Never Cigarette Smoker | 1.12 (0.99, 1.28) | 0.08 |
| Waist circumference <40 inches in men, <35 inches in women | 0.98 (0.86, 1.12) | 0.76 |
| Systolic BP <120 mmHg and Diastolic BP <80 mmHg | 1.13 (0.98, 1.31) | 0.09 |
| Fasting Blood Glucose <100 mg/dL | 1.05 (0.78, 1.41) | 0.75 |
| Triglycerides <150 mg/dL | 1.12 (0.94, 1.34) | 0.21 |
| LDL-C/HDL-C <3 in men, <2.5 in women | 1.08 (0.95, 1.22) | 0.23 |
| $\geq$3 Ideal ASCVD Risk Factors * | 1.25 (1.00, 1.57) | 0.05 |

Adjusted for: age, sex, race, education, antihypertensive medication, lipid-lowering medication, glucose-lowering medication, cigarette smoking, waist circumference, blood pressure, fasting blood glucose, fasting serum triglycerides, and LDL-C/HDL-C

* = $\geq$3 ideal ASCVD risk factors versus <3 ideal risk factors

**Table S2. Association of demographics and normal values of traditional ASCVD risk factors with the persistent absence of carotid plaque, adjusting for ApoB/ApoAI ratio.**

| Variable | Relative Risk (95% CI) | P-value |
|---|---|---|
| ***Demographic*** | | |
| Female Sex | 1.04 (0.91, 1.19) | 0.57 |
| African American | 1.03 (0.89, 1.18) | 0.71 |
| Age, per 10 years younger | 1.18 (1.05, 1.33) | 0.006 |
| ***ASCVD Risk Factors*** | | |
| Never Cigarette Smoker | 1.12 (0.98, 1.27) | 0.09 |
| Waist circumference <40 inches in men, <35 inches in women | 0.98 (0.86, 1.12) | 0.80 |
| Systolic BP <120 mmHg and Diastolic BP <80 mmHg | 1.14 (0.99, 1.32) | 0.06 |
| Fasting Blood Glucose <100 mg/dL | 1.06 (0.78, 1.44) | 0.69 |
| Triglycerides <150 mg/dL | 1.10 (0.92, 1.31) | 0.31 |
| ApoB/ApoAI <0.9 in men, <0.8 in women | 1.73 (0.86, 3.49) | 0.13 |
| ≥3 Ideal ASCVD Risk Factors * | 1.21 (0.86, 1.70) | 0.28 |

Adjusted for: age, sex, race, education, antihypertensive medication, lipid-lowering medication, glucose-lowering medication, cigarette smoking, waist circumference, blood pressure, fasting blood glucose, fasting serum triglycerides, and ApoB/ApoAI

* = ≥3 ideal ASCVD risk factors versus <3 ideal risk factors

**Table S3**. Cross-sectional association of total meat intake with serum phosphate and calcium-phosphate product.

| | Beta (SE) | P-Value | P-Value for Linear Trend |
|---|---|---|---|
| ***Serum Phosphate (mg/dL)*** | | | |
| First Tertile of Total Meat Intake | Ref | - | |
| Second Tertile of Total Meat Intake | 0.01 (0.06) | 0.95 | 0.04 |
| Third Tertile of Total Meat Intake | 0.15 (0.07) | 0.03 | |
| ***Calcium-Phosphate Product ($mg^2/dL^2$)*** | | | |
| First Tertile of Total Meat Intake | Ref | - | |
| Second Tertile of Total Meat Intake | 0.01 (0.62) | 0.99 | 0.04 |
| Third Tertile of Total Meat Intake | 1.54 (0.71) | 0.03 | |

Models adjusted for: age, sex, race, and total calorie intake

**Table S4.** Association of normal values of novel ASCVD risk factors and lifestyle behaviors with the persistent absence of carotid plaque, adjusting for LDL-C/HDL-C ratio.

| Variable* | Relative Risk (95% CI) | P-value |
|---|---|---|
| ***Inflammatory*** | | |
| Homocysteine <10 μmol/L | 0.98 (0.83, 1.17) | 0.85 |
| Fibrinogen, per 1-SD lower | 1.00 (0.94, 1.07) | 0.93 |
| ***Renal and Mineral Metabolism*** | | |
| eGFR >90 mL/min/1.73m$^2$ | 0.98 (0.84, 1.15) | 0.82 |
| Urinary Albumin <30 mg/L | 1.03 (0.84, 1.26) | 0.81 |
| Serum Calcium, per 1 mg/dL lower | 1.03 (0.87, 1.22) | 0.73 |
| Calcium-Phosphate Product, per 1-SD lower | 1.15 (1.03, 1.28) | 0.02 |
| Serum Phosphate, per 1 mg/dL lower | 1.07 (1.01, 1.14) | 0.03 |
| ***Lifestyle **** | | |
| Dietary Sodium <2300 mg/day | 1.20 (1.02, 1.41) | 0.03 |
| Dietary Potassium >3400 mg/day in men, >2600 mg/day in women | 0.98 (0.81, 1.18) | 0.85 |
| Dietary Fiber >30 g/day | 1.21 (0.92, 1.59) | 0.18 |
| Dietary Cholesterol <300 mg/day | 1.07 (0.90, 1.26) | 0.45 |
| Dietary Saturated Fat <10% of total calories | 1.03 (0.90, 1.18) | 0.65 |
| Moderate Alcohol Drinking † | 1.17 (0.90, 1.51) | 0.24 |
| Adequate Physical Activity † | 0.94 (0.78, 1.14) | 0.54 |
| ≥3 Ideal Lifestyle Behaviors ‡ | 1.12 (0.98, 1.29) | 0.09 |

Adjusted for: age, sex, race, education, antihypertensive medication, lipid-lowering medication, glucose-lowering medication, cigarette smoking, waist circumference, blood pressure, fasting blood glucose, fasting serum triglycerides, and LDL-C/HDL-C ratio

* = dietary risk markers and the cumulative lifestyle behavior risk score were also adjusted for total caloric intake

† = ≤2 drinks/day in men, ≤1 drink/day in women, ≥150 minutes/week of moderate activity or ≥75 minutes/week of vigorous activity

‡ = ≥3 ideal lifestyle behaviors versus <3 ideal lifestyle behaviors

**Table S5. Association of normal values of novel ASCVD risk factors and lifestyle behaviors with the persistent absence of carotid plaque, adjusting for ApoB/ApoAI ratio.**

| Variable* | Relative Risk (95% CI) | P-value |
|---|---|---|
| ***Inflammatory*** | | |
| Homocysteine <10 μmol/L | 0.97 (0.82, 1.16) | 0.75 |
| Fibrinogen, per 1-SD lower | 1.01 (0.95, 1.07) | 0.80 |
| ***Renal and Mineral Metabolism*** | | |
| eGFR >90 mL/min/1.73m$^2$ | 0.99 (0.85, 1.16) | 0.92 |
| Urinary Albumin <30 mg/L | 1.01 (0.82, 1.24) | 0.90 |
| Serum Calcium, per 1 mg/dL lower | 1.03 (0.87, 1.22) | 0.76 |
| Calcium-Phosphate Product, per 1-SD lower | 1.15 (1.03, 1.29) | 0.01 |
| Serum Phosphate, per 1 mg/dL lower | 1.07 (1.01, 1.14) | 0.02 |
| ***Lifestyle** * | | |
| Dietary Sodium <2300 mg/day | 1.19 (1.02, 1.40) | 0.03 |
| Dietary Potassium >3400 mg/day in men, >2600 mg/day in women | 0.98 (0.81, 1.18) | 0.81 |
| Dietary Fiber >30 g/day | 1.20 (0.91, 1.57) | 0.20 |
| Dietary Cholesterol <300 mg/day | 1.07 (0.91, 1.27) | 0.42 |
| Dietary Saturated Fat <10% of total calories | 1.03 (0.90, 1.18) | 0.63 |
| Moderate Alcohol Drinking † | 1.16 (0.90, 1.50) | 0.26 |
| Adequate Physical Activity † | 0.94 (0.78, 1.14) | 0.53 |
| ≥3 Ideal Lifestyle Behaviors ‡ | 1.13 (0.99, 1.30) | 0.07 |

Adjusted for: age, sex, race, education, antihypertensive medication, lipid-lowering medication, glucose-lowering medication, cigarette smoking, waist circumference, blood pressure, fasting blood glucose, fasting serum triglycerides, and ApoB/ApoAI ratio

* = dietary risk markers and the cumulative lifestyle behavior risk score were also adjusted for total caloric intake

† = ≤2 drinks/day in men, ≤1 drink/day in women, ≥150 minutes/week of moderate activity or ≥75 minutes/week of vigorous activity

‡ = ≥3 ideal lifestyle behaviors versus <3 ideal lifestyle behaviors