Arteriosclerosis, Thrombosis, and Vascular Biology

CLINICAL AND POPULATION STUDIES

Low-Carbohydrate Diet Score and Coronary Artery Calcium Progression

Results From the CARDIA Study

Jing-Wei Gao (高静伟),* Qing-Yun Hao (郝卿鋆),* Hai-Feng Zhang (张海峰),* Xiong-Zhi Li (李雄志), Zhi-Min Yuan (袁智敏), Ying Guo (郭颖), Jing-Feng Wang (王景峰), Shao-Ling Zhang (张少玲), Pin-Ming Liu (刘品明)

OBJECTIVE: To investigate whether low-carbohydrate diets (LCDs) were associated with coronary artery calcium (CAC) progression.

APPROACH AND RESULTS: We included the participants who completed computed tomography assessment of baseline CAC in 2000 to 2001 (year 15) and follow-up (year 20 or 25) and food frequency questionnaire (years 0, 7, and 20) in the CARDIA study (Coronary Artery Risk Development in Young Adults). CAC progression was defined as CAC >0 at follow-up among participants with baseline CAC of 0 and an annualized change of 10 or percent change of ≥10% for those with 0<baseline CAC<100 or baseline CAC≥100, respectively. Among 2226 included participants (age, 40.4±3.5 years; 45.4% men), the carbohydrate intake accounted for 47.8±6.5% of total energy, and 204 (9.2%) had CAC at baseline (year 15). Over a mean follow-up of 8.3 years, 591 (26.5%) participants had CAC progression. After adjustment for traditional cardiovascular risk factors and other dietary factors, carbohydrate intake as a percentage of total energy was inversely associated with the risk of CAC progression (hazard ratio, 0.731 [95% CI, 0.552–0.968]; *P*=0.029). Furthermore, the animal-based but not plant-based LCD score was significantly associated with a higher risk of CAC progression (animal-based LCD score: hazard ratio, 1.456 [95% CI, 1.015–2.089]; *P*=0.041; plant-based LCD score: hazard ratio, 1.016 [95% CI, 0.821–1.257]; *P*=0.884; both comparing extreme groups).

CONCLUSIONS: LCDs starting at a young age are associated with an increased risk of subsequent CAC progression, particularly when animal protein or fat are chosen to replace carbohydrates.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT00005130.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: cardiovascular diseases ■ diet, carbohydrate-restricted ■ follow-up studies ■ risk factors ■ young adult

Cardiovascular disease (CVD) is a major public health issue and the leading cause of death globally.[1,2] Healthy lifestyle choices are crucial for the prevention of CVD. Adopting a healthy lifestyle earlier in life holds the potential to have a greater impact on CVD risk reduction, whereas unhealthy lifestyle changes are associated with an increased risk for subclinical atherosclerosis in middle age.[3] Low-carbohydrate diets (LCDs), which restrict carbohydrates, in favor of increased protein or fat intake, have gained substantial popularity because LCD can induce at least short-term weight loss, which is considered to be beneficial for cardiovascular health.[4,5] However, due to relatively high levels of protein, fat, and cholesterol in the diets, long-term LCD may raise concerns over ketosis, abnormal

Correspondence to: Pin-Ming Liu, MD, PhD, Department of Cardiology, Sun Yat-sen Memorial Hospital of Sun Yat-sen University, Guangzhou 510120, China, Email liupm@mail.sysu.edu.cn; or Shao-Ling Zhang, MD, PhD, Department of Endocrinology, Sun Yat-sen Memorial Hospital of Sun Yat-sen University, Guangzhou 510120, China, Email zhshaol@mail.sysu.edu.cn

*These authors contributed equally to this article.

The Data Supplement is available with this article at https://www.ahajournals.org/doi/suppl/10.1161/ATVBAHA.120.314838.

For Sources of Funding and Disclosures, see page 499.

Nonstandard Abbreviations and Acronyms

CAC	coronary artery calcium
CARDIA	Coronary Artery Risk Development in Young Adults
CT	computed tomography
CVD	cardiovascular disease
HDL	high-density lipoprotein
LCD	low-carbohydrate diet
LDL	low-density lipoprotein
MESA	Multi-Ethnic Study of Atherosclerosis

lipid profiles, and renal effects,[5] which in turn counteracts the potential cardiovascular benefits on CVD.[6–8] Lagiou et al[9] found that low-carbohydrate, high-protein intake predicted higher total and cardiovascular mortality in relatively young Swedish women. More recently, results from a pooled analysis of the data from the US National Health and Nutrition Examination Survey 1999 to 2010 and 8 other relevant prospective studies (a total of 462934 participants, mean follow-up of 16.1 years) suggested a potentially unfavorable association of LCD with overall and cause-specific mortality, including CVD mortality.[10]

Coronary artery calcium (CAC) assessed by computed tomography (CT)—an established marker of atherosclerotic coronary artery disease—is a strong predictor of cardiovascular morbidity and mortality and a potent CVD risk stratification tool.[11,12] It is shown that nutrition and diet are of importance in the progression of vascular calcification.[13] Notably, the associations between dietary carbohydrates, CAC, and other atherosclerotic markers have been an area of scientific interest recently.[14] The CARDIA study (Coronary Artery Risk Development in Young Adults) is a prospective, multicenter cohort study sponsored by the National Heart, Lung, and Blood Institutes. It is designed to identify the influences of potentially risk factors began in young adulthood on CVD development in later life.[15] Findings from the CARDIA study have contributed substantially to the knowledge about the importance of lifestyle and environmental factors during young adulthood to the development of CAC through their midlife.[3,16–18]

In the present study, we aimed to provide new evidence about the contribution of LCD starting early in adulthood to future CVD risk. By using the longitudinal CARDIA study data, we examined the association of LCD patterns (including animal- and plant-based LCD score) with the risk of subsequent CAC progression.

MATERIALS AND METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Highlights

- Low-carbohydrate diets in young adults were associated with a higher risk of coronary artery calcium progression in middle age.
- Replacement of carbohydrates with predominantly animal but not plant protein or fat in low-carbohydrate diets has the potential of enhancing CAC progression.
- These data suggest that as a popular strategy for weight management, long-term animal-based low-carbohydrate diet should be advocated cautiously to avoid its potential impact on coronary atherosclerosis.

Subjects

Details of the CARDIA study design and examinations have been reported previously.[19] Briefly, it is a prospective cohort study designed to investigate the evolution of CVD risk. Participants were recruited from 4 field centers (Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California). In 1985 to 1986 (year 0 of the cohort), a total of 5115 Black and White men and women aged 18 to 30 years were enrolled in the study. Until now, 8 follow-up examinations have been conducted: years 2, 5, 7, 10, 15, 20, 25, and 30.

As CAC was first measured by CT scans at year 15 (2000–2001) in the CARDIA study, for the purpose of the present study, we included all the participants at year 15 (n=3671). As Figure 1 shows, participants were excluded if one of the following data could not be available: baseline CAC data (n=629), follow-up CAC data (n=271), covariate data (n=244), or validated dietary data (without complete dietary information or with implausible energy intake defined as <800 or >8000 kcal for men and <600 or >6000 kcal for women; n=301). The final study sample for this analysis was 2226 adults.

All participants provided informed consent, and the study was approved by the institutional review boards at each field center.

Assessment of the Diets

Dietary data were assessed by using a trained interviewer-administered CARDIA dietary history (years 0, 7, and 20 after inclusion in the cohort) as described previously.[20] Briefly, interviewers asked the participants open-ended questions about dietary consumption of 100 food categories that referenced 1609 separate food items within the past month. Foods consumed were assigned to 1 of 166 food groups devised by the Minnesota Nutrition Coordinating Center. Food group intake was assessed as servings per day of constituent foods based on their gram weight information. In the present study, we calculated the average of years 0 and 7 dietary data in analyses to reduce potential confounding from a single assessment only and to avoid the possibility that macronutrient consumption measured at year 20 may have been influenced by the measurement of CAC at year 15 as reported previously.[16,21] The macronutrients (carbohydrates, fat, and protein) were

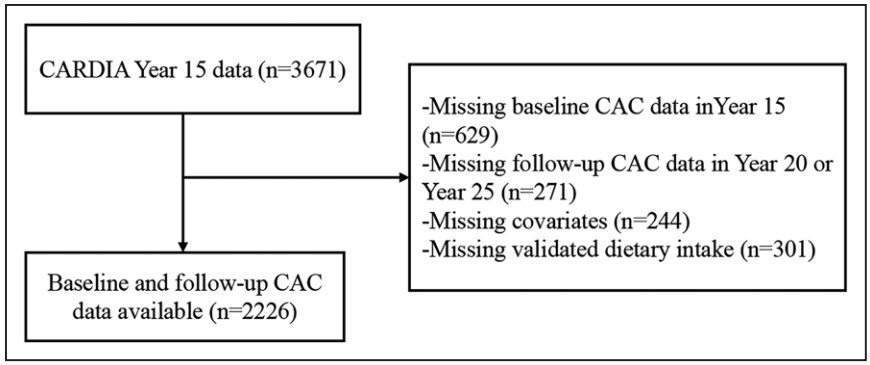

Figure 1. Flowchart for selecting the CARDIA (Coronary Artery Risk Development in Young Adults) participants for analysis.
CAC indicates coronary artery calcium.

expressed as the percentage of total energy, calculated as the daily calories derived from each macronutrient divided by the total number of calories for the day.

It is supposed that the effects of long-term carbohydrate restriction can be varied since different sources of protein or fat (animal or plant sources) in the diets are chosen to replace carbohydrates to provide enough energy. Therefore, we calculated animal- and plant-based LCD score, respectively, as reported previously.[6] As shown in Table 1, the points of each of the 3 macronutrients were summed to create the overall LCD score. Plant-based LCD score was calculated by summation of the points for carbohydrates, plant protein, and plant fat as a percentage of total energy; similarly, animal-based LCD score was calculated by summarizing carbohydrates, animal protein, and animal fat as a percentage of total energy. As a result, the highest score represented low-carbohydrate and high animal or plant protein or fat intake.

CAC Measurements

CAC was measured at years 15, 20, and 25 using a standardized protocol.[22] At years 15 and 20, the CARDIA study conducted either a cardiac-gated electron-beam CT or a multidetector CT depending on the study site to detect CAC. At year 25, given the high reproducibility at previous examinations, a single CT scan was performed using only multidetector CT scanners. The comparability of electron-beam CT and multidetector CT has been demonstrated previously.[23] Scans were read centrally by a trained reader who was blinded to the participant's characteristics.

CAC was scored using the Agatston method,[24] which was calculated for each calcified lesion; and the scores were summed across all lesions within a given artery and across all arteries (left anterior descending, left main, circumflex, and right coronary artery) to obtain the total calcium score. CAC progression was defined as one of the following: (1) CAC >0 at follow-up among participants with baseline CAC=0; (2) an annualized change of ≥10 at follow-up among those with 0<baseline CAC<100; (3) an annualized percentage change (annualized change in CAC score divided by the baseline CAC score) ≥10% among those with baseline CAC ≥100.[25]

Measurements of Other Covariates

The demographic, lifestyle, and education characteristics of participants (eg, age, race, sex, smoking status, alcohol consumption, levels of physical activity, and educational attainment) were assessed during interviews. Educational attainment was categorized as ≤12 years (representing ≤high school degree), 13 to 16 years (representing college degree or similar level of education), and ≥17 years (representing>college degree, such as MSc, MD, JD, and PhD). Smoking status was classified as current, former, or never. Participants rested in the sitting position for 5 minutes before resting systolic and diastolic blood pressures were measured 3× at 1-minute intervals with a random-zero sphygmomanometer on the right arm. The second and the third measurements were averaged and recorded. Laboratory tests were also performed. Briefly, overnight fasting blood samples were drawn using the EDTA vacutainers; plasma was isolated and frozen at −70 °C until

Table 1. Criteria Determining the Low-Carbohydrate-Diet Scores

Points	Carbohydrate intake	Animal fat intake	Plant fat intake	Animal protein intake	Plant protein intake
		Total energy, %			
0	≥56.3	<10.1	<16.3	<5.8	<3.1
1	53.1–56.3	10.1–11.2	16.3–17.7	5.8–7.0	3.1–3.6
2	50.8–53.1	11.2–12.0	17.7–18.8	7.0–7.9	3.6–4.0
3	49.0–50.8	12.0–12.7	18.8–19.7	7.9–8.6	4.0–4.4
4	47.6–49.0	12.7–13.2	19.7–20.5	8.6–9.3	4.4–4.7
5	46.1–47.6	13.2–13.8	20.5–21.3	9.3–10.0	4.7–5.2
6	44.4–46.1	13.8–14.5	21.3–22.2	10.0–10.8	5.2–5.6
7	42.4–44.4	14.5–15.2	22.2–23.2	10.8–11.9	5.6–6.2
8	39.9–42.4	15.2–16.3	23.2–24.8	11.9–13.7	6.2–7.2
9	<39.9	≥16.3	≥24.8	≥13.7	≥7.2

Table 2. Characteristics of Study Participants According to the Average of Carbohydrate Intake as a Percentage of Total Energy at Years 0 and 7

Characteristics	Total	Low carbohydrate group	Moderate carbohydrate group	High carbohydrate group	*P* value
Total, n	2226	491	1309	426	
Carbohydrate, percentage of energy	47.8±6.5	39.5±2.9	47.9±2.8	57.5±3.9	<0.001
Baseline CAC at year 15, %	204 (9.2%)	58 (11.8%)	119 (9.1%)	27 (6.3%)	0.016
Baseline CAC score at year 15	7.4±93.5	11.2±107.5	7.8±102.5	1.8±13.8	0.012
Demographic characteristics*					
Age at year 15, y	40.4±3.5	40.7±3.5	40.3±3.5	40.3±3.6	0.102
Male at year 15, %	1011 (45.4%)	291 (59.3%)	604 (46.1%)	116 (27.2%)	<0.001
White at year 15, %	1317 (59.2%)	268 (54.6%)	797 (60.9%)	252 (59.2%)	0.053
BMI, kg/m^2	26.3±4.9	27.0±4.7	26.2±4.8	25.8±5.1	<0.001
SBP, mm Hg	110.0±10.3	112.1±10.5	109.9±10.1	108.0±10.0	<0.001
DBP, mm Hg	70.4±8.1	71.1±8.1	70.4±8.2	69.9±7.8	0.107
Hypertension at year 15, %	329 (14.8%)	81 (16.5%)	186 (14.2%)	62 (14.6%)	0.471
Diabetes at year 15, %	112 (5.0%)	30 (6.1%)	70 (5.3%)	12 (2.8%)	0.054
Smoke status at year 15, %					<0.001
Never	1369 (61.5%)	227 (46.2%)	831 (63.5%)	311 (73.0%)	
Current	454 (20.4%)	152 (31.0%)	255 (19.5%)	47 (11.0%)	
Former	403 (18.1%)	112 (22.8%)	223 (17.0%)	68 (16.0%)	
Alcohol consumption, drinks/wk	5.0 (0.8–14.3)	13.8 (4.8–29.3)	4.8 (0.8–12.0)	1.6 (0–6.1)	<0.001
Education level at year 15, %					<0.001
High school	421 (18.9%)	125 (25.5%)	233 (17.8%)	63 (14.8%)	
College	1287 (57.8%)	281 (57.2%)	754 (57.6%)	252 (59.2%)	
Graduate school	518 (23.3%)	85 (17.3%)	322 (24.6%)	111 (26.1%)	
Physical activity, exercise units	326.0 (196.3–495.8)	341.7 (213.3–485.0)	321.2 (192.0–502.4)	324.5 (190.8–495.3)	0.420
Biochemical characteristics*					
FPG, mg/dL	85.9±11.9	87.6±13.6	85.9±11.9	83.9±9.2	<0.001
TC, mg/dL	180.4±29.5	183.6±32.1	180.7±28.4	175.8±29.5	<0.001
TG, mg/dL	72.7 (54.9–101.1)	77.0 (58.3–109.0)	72.0 (55.0–100.7)	70.3 (52.7–94.0)	<0.001
HDL-C, mg/dL	51.8±12.3	52.1±13.7	51.5±12.0	51.9±11.1	0.355
LDL-C, mg/dL	110.9±27.5	111.9±29.4	111.6±26.7	107.6±27.5	0.009
Serum creatinine, mg/dL	1.0±0.3	1.0±0.2	1.0±0.3	1.0±0.2	<0.001
Dietary characteristics†					
Total energy intake, kcal	2716.3±1065.1	2994.9±1146.7	2738.8±1060.8	2322.8±843.0	<0.001
Total protein intake, percentage of energy	14.6±2.1	15.5±2.0	14.6±2.0	13.6±2.3	<0.001
Animal protein, percentage of energy	9.5±3.2	11.0±3.3	9.5±2.9	8.0±3.1	<0.001
Plant protein, percentage of energy	5.0±1.8	4.3±1.3	5.0±1.6	5.9±2.3	<0.001
Total fat intake, percentage of energy	36.4±5.3	41.3±4.3	36.7±3.6	29.8±3.8	<0.001
Animal fat, percentage of energy	13.2±2.5	15.0±2.1	13.4±2.0	10.5±1.9	<0.001
Plant fat, percentage of energy	20.5±3.4	23.3±3.2	20.7±2.6	16.9±2.6	<0.001
Dietary fiber intake, g/d	13.0 (9.6–17.2)	11.9 (8.9–15.9)	13.1 (9.7–17.4)	13.7 (10.1–18.6)	<0.001
Dietary magnesium intake, mg/d	379.8±150.9	392.6±153.1	383.3±151.1	354.7±146.3	<0.001
Dietary calcium intake, mg/d	1147.0±573.4	1214.3±682.6	1173.9±551.6	986.7±466.4	<0.001
Dietary vitamin D intake, mg/d	5.5 (3.5–8.4)	6.0 (3.8–9.1)	5.8 (3.7–8.6)	4.3 (2.8–6.8)	<0.001

Year 0: year of recruitment in the CARDIA cohort; years 7 and 15: 7 and 15 y after the recruitment in the cohort; year 15 (date of CAC first measurement was considered as the baseline in the present study). BMI indicates body mass index; CAC, coronary artery calcium; CARDIA, Coronary Artery Risk Development in Young Adults; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; and TG, triglycerides.

*Data are given as the average of the years 0, 7, and 15 measurements, unless otherwise indicated.

†Dietary data are given as the average of the years 0 and 7 measurements.

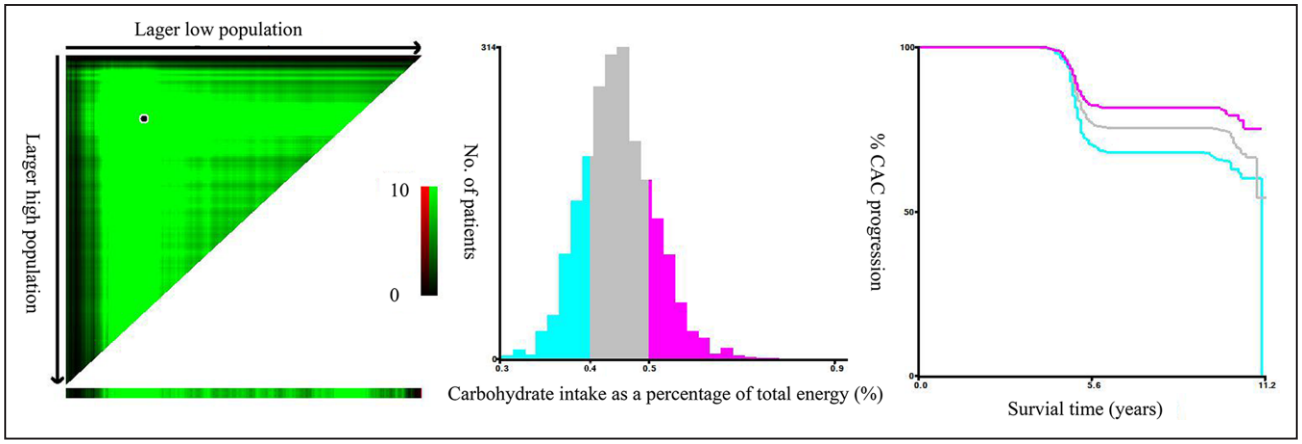

Figure 2. Determination of cutoff values of carbohydrate intake as a percentage of total energy for coronary artery calcium (CAC) progression by X-tile analysis in the CARDIA study (Coronary Artery Risk Development in Young Adults).
X-tile analysis was performed on dietary and CAC data from the CARDIA study, equally divided into training and validation sets. X-tile plots of training sets are shown in the **left**. The plot shows the χ^2 log-rank values produced when dividing the cohort with 2 cut points, producing high, middle, and low subsets. The *x* axis represents all potential cut points from low to high (**left** to **right**) that define a low subset, whereas the *y* axis represents cut points from high to low (**top** to **bottom**) that define a high subset. Green coloration of cut points indicates a direct association. The optimal cut point occurs at the brightest pixel. The cut point highlighted by the black dot in the **left** is shown on a histogram of the entire cohort (**middle**) and a Kaplan-Meier plot (**right**; low subset, blue; middle subset, gray; high subset, magenta). *P* was determined by using the cut point defined in the training set and applying it to the validation set. Finally, the cutoff points for the carbohydrate intake as a percentage of total energy were <43% (low carbohydrate intake group), 43% to 53% (moderate carbohydrate intake group), and ≥53% (high carbohydrate intake group).

shipment on dry ice to the laboratory.[26] Detailed descriptions of measurements of total cholesterol, LDL (low-density lipoprotein) cholesterol, HDL (high-density lipoprotein) cholesterol, triglycerides, serum creatinine, and fasting plasma glucose of the participants have been published previously.[26] Diabetes was defined as fasting plasma glucose ≥126 mg/dL or a history of diabetes medication use. Hypertension was defined as taking antihypertensive medications, having a diagnosis of hypertension, or having 3 consecutive systolic blood pressure readings ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg. Body mass index was calculated as weight divided by the square of height (kg/m^2).

Statistical Analysis

Normally distributed continuous data were expressed as mean±SD, and the non-normally distributed continuous data, otherwise, were expressed as the median (interquartile range). Categorical data were expressed as numbers (percentage). Since we evaluated baseline CAC at year 15, all continuous characteristics reflect the average of years 0, 7, and 15, except age (year 15) and dietary data (the average of years 0 and 7). Differences among groups were evaluated using ANOVA or Kruskal-Wallis *h* test when appropriate for the continuous variables and the χ^2 test for the categorical variables. The follow-up period was defined as the time from 2000 to 2001 (year 15) to the incidence of CAC progression or loss to follow-up, whichever occurred first. X-tile (version 3.6.1; Yale University School of Medicine) software was used to determine the optimal cutoff points for carbohydrate intake as a percentage of total energy according to the highest χ^2 value defined by the Kaplan-Meier survival analysis.[27] Kaplan-Meier estimates were used to compute cumulative incidence of CAC progression by carbohydrate intake groups, and the differences in estimates were compared using the log-rank procedure. Cox regression was used to compute hazard ratios and 95% CIs for the association between carbohydrate intake and CAC progression. Covariates of known established or suspected risk factors for CAC progression were considered in the multivariable models. As reported in the literature,[6] because LCD may decrease subsequent energy intake, we did not control for total energy intake in multivariate models. Likewise, we did not perform adjustment for intake of other macronutrient components, as LCD

Table 3. Risk of CAC Progression for Carbohydrate Intake as a Percentage of Total Energy

Carbohydrate intake groups (percentage of total energy)	Events/ No. at risk	Model 1 h (95% CI)	*P* value	Model 2 h (95% CI)	*P* value	Model 3 h (95% CI)	*P* value
Low carbohydrate intake group (<43%)	170/491	1.0	…	1.0	…	1.0	…
Moderate carbohydrate intake group (43%–53%)	337/1309	0.728 (0.606–0.876)	0.001	0.732 (0.608–0.880)	0.001	0.841 (0.694–1.020)	0.079
High carbohydrate intake group (≥53%)	84/426	0.527 (0.406–0.685)	<0.001	0.536 (0.413–0.697)	<0.001	0.731 (0.552–0.968)	0.029

Model 1: unadjusted. Model 2: adjusted for age and race. Model 3: adjusted for model 2 covariates plus alcohol consumption, baseline CAC score, BMI, diabetes, diastolic blood pressure, dietary calcium intake, dietary fiber intake, dietary magnesium intake, dietary vitamin D intake, education levels (high school, college, and graduate school), fasting plasma glucose, high-density lipoprotein cholesterol, hypertension, low-density lipoprotein cholesterol, physical activity, serum creatinine, smoking status (current, former, and never), systolic blood pressure, and triglycerides. BMI indicates body mass index; and CAC, coronary artery calcium.

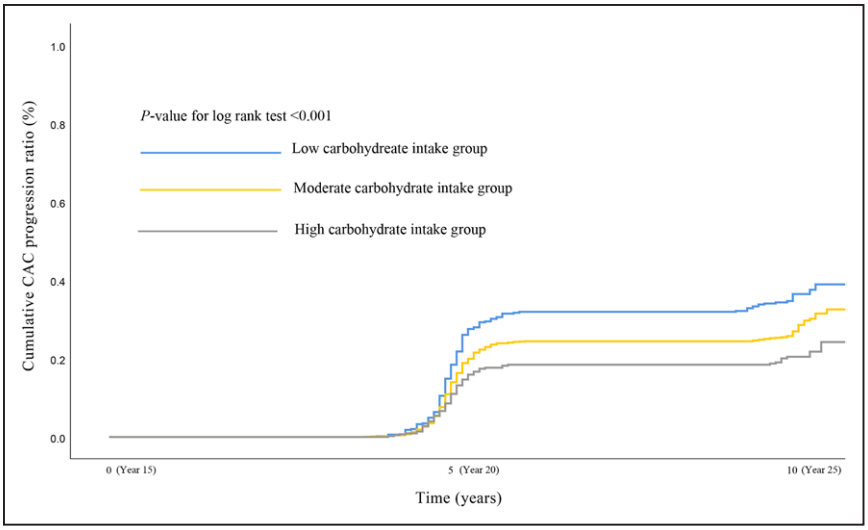

Figure 3. Cumulative incidence of coronary artery calcium (CAC) progression by carbohydrate intake as a percentage of total energy. Cumulative incidence curves are statistically different (log-rank $P<0.001$).

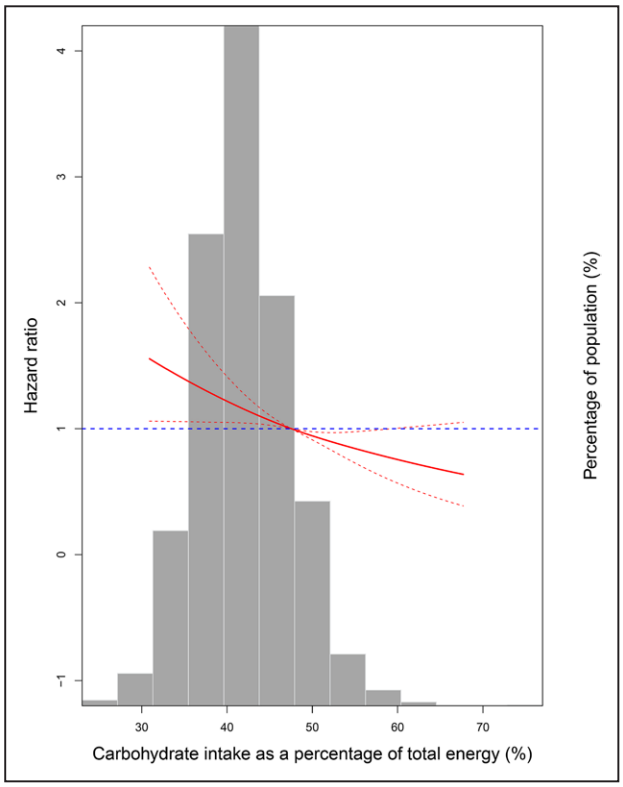

Figure 4. Hazard ratios of coronary artery calcium progression by carbohydrate intake as a percentage of total energy. Each hazard ratio was computed with a carbohydrate intake level of 47.5% as the reference. Red solid line represents the hazard ratio of carbohydrate intake across the whole range. Red dotted lines represent the 95% CI. Black dotted line is the reference line as hazard ratio=1. Histograms represent the frequency distribution of carbohydrate intake as a percentage of total energy.

in turn may increase intake of protein or fat. However, we performed adjustment for dietary intake of micronutrients, which have been reported to be associated with the risk of CAC progression in multivariate nutrient density models. We further used a restricted cubic spline regression model with 3 knots to assess the nonlinear dose-response association between carbohydrate intake and CAC progression. We did a time-varying sensitivity analysis for participants who were identified with CAC progression before year 20; carbohydrate intake was calculated on the basis of the mean of years 0 and 7 food frequency questionnaire responses. From year 20 onward, carbohydrate intake was calculated on the basis of the mean of years 0, 7, and 20 food frequency questionnaire results. As a total of 327 participants did not have dietary information at year 20, we first did the sensitivity analysis excluding these people with missing data, and again, we used average values of dietary information at years 0 and 7 to replace missing data to conduct the sensitivity analysis for all our included participants. In addition, we performed specified subgroup analysis by age, sex, race, body mass index, presence of hypertension, and baseline CAC and tested for potential interactions of these covariates with carbohydrate intake separately. All analyses were conducted in SPSS, version 23 (SPSS, Inc, Chicago, IL). A 2-sided P of <0.05 was considered statistically significant.

RESULTS

Characteristics According to Carbohydrate Intake

The characteristics of all the included participants were summarized in Table 2. The average age at the first CT scans was 40.4±3.5 years (year 15), 1011 (45.4%) participants were men, 454 (20.4%) were current smokers, and 329 (14.8%) were hypertensive. In addition, 204 (9.2%) participants exhibited CAC at baseline, and the mean carbohydrate intake as a percentage of total energy was 47.8±6.5%. Here, according to results of X-tile analysis (Figure 2), individuals were categorized into 3 groups by the percentage of total energy from carbohydrates: low (<43%), moderate (43%–53%), and high (≥53%) carbohydrate intake group. Participants with lower energy from carbohydrate intake were more likely to be men, current smokers; to have a lower education level; to drink more; and to have higher levels

of body mass index, systolic blood pressure, fasting plasma glucose, total cholesterol, LDL cholesterol, and triglycerides. With regard to dietary patterns, participants with lower energy from carbohydrates tended to have higher intake of total energy, protein, fat (both animal and plant sources), and higher dietary magnesium, calcium, and vitamin D, along with lower dietary fiber intake. However, there were no significant differences regarding race, hypertension and diabetes prevalence, physical activity, and serum HDL cholesterol levels among the 3 groups.

Association Between Dietary Carbohydrate Intake and CAC Progression

During a follow-up period of 8.3±2.3 years, 591 (26.5%) cases exhibited CAC progression. As Table 3 shows, the percentage of CAC progression decreased as the carbohydrates in diets increased (low versus moderate versus high carbohydrate intake group: 170 [34.6%] versus 337 [25.7%] versus 84 [19.7%]). As results yielded from both unadjusted and adjusted models, the most significant CAC progression was found in participants with the lowest carbohydrate intake ($P<0.05$; Table 3). In the final

Subgroups	Events/no. at risk	Groups*	HR (95% CI)	*P* value	Interaction *P* value
Age, years					
<40	169/858	Moderate carbohydrate intake group	0.716 (0.490, 1.049)	0.086	0.172
		High carbohydrate intake group	0.688 (0.397, 1.193)	0.183	
≥40	422/1368	Moderate carbohydrate intake group	0.909 (0.723, 1.143)	0.415	
		High carbohydrate intake group	0.761 (0.546, 1.062)	0.109	
Sex					
Male	394/1011	Moderate carbohydrate intake group	1.016 (0.707, 1.461)	0.931	<0.001
		High carbohydrate intake group	0.904 (0.650, 1.257)	0.549	
Female	197/1215	Moderate carbohydrate intake group	0.900 (0.619, 1.308)	0.581	
		High carbohydrate intake group	0.782 (0.479,1.275)	0.324	
Ethnicity					
White	376/1317	Moderate carbohydrate intake group	0.779 (0.608, 0.997)	0.047	0.030
		High carbohydrate intake group	0.646 (0.448, 0.932)	0.019	
Black	215/909	Moderate carbohydrate intake group	0.903 (0.659, 1.238)	0.526	
		High carbohydrate intake group	0.853 (0.539, 1.350)	0.496	
BMI,kg/m^2					
<30	437/1788	Moderate carbohydrate intake group	0.823 (0.657, 1.030)	0.088	0.640
		High carbohydrate intake group	0.642 (0.459, 0.897)	0.010	
≥30	154/438	Moderate carbohydrate intake group	0.957 (0.642, 1.426)	0.827	
		High carbohydrate intake group	1.192 (0.666, 2.133)	0.554	
Hypertension					
Yes	130/329	Moderate carbohydrate intake group	1.232 (0.798, 1.902)	0.347	0.723
		High carbohydrate intake group	0.995 (0.524, 1.889)	0.988	
No	461/1897	Moderate carbohydrate intake group	0.751 (0.605, 0.932)	0.009	
		High carbohydrate intake group	0.687 (0.498, 0.946)	0.022	
Baseline CAC					
Yes	136/204	Moderate carbohydrate intake group	0.703 (0.458, 1.080)	0.108	0.312
		High carbohydrate intake group	0.787 (0.413, 1.501)	0.467	
No	455/2022	Moderate carbohydrate intake group	0.877 (0.703, 1.094)	0.246	
		High carbohydrate intake group	0.731 (0.531, 1.007)	0.055	

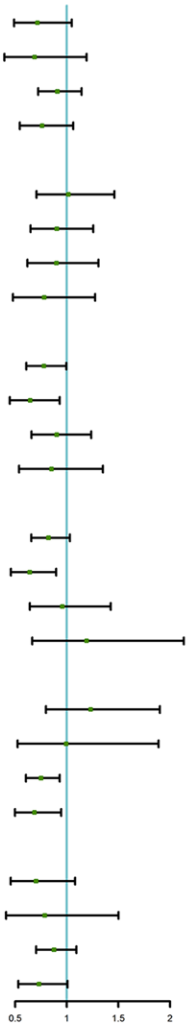

Figure 5. Subgroup analysis of the association between dietary carbohydrate intake and coronary artery calcium (CAC) progression.

Cox regression after adjustment for alcohol consumption, diastolic blood pressure, diabetes, dietary calcium intake, dietary fiber intake, dietary magnesium intake, dietary vitamin D intake, education levels (high school, college, and graduate school), fasting plasma glucose, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, physical activity, serum creatinine, systolic blood pressure, smoking status (current, former, and never), systolic blood pressure, and triglycerides was performed in subgroups according to age (<40 or ≥40 y), sex (male or female), race (White or Black), body mass index (BMI; <30 or ≥30 kg/m^2), hypertension (yes or no), and baseline CAC (yes or no). *Hazard ratio (HR) and 95% CI were derived from Cox regression models, and the low carbohydrate intake group was used as the reference in each subgroup analysis.

Table 4. Risk of CAC Progression for Animal-Based LCD Score

LCD score groups	Model 1 h (95% CI)	*P* value	Model 2 h (95% CI)	*P* value	Model 3 h (95% CI)	*P* value
Low LCD score group (≤4)	1.0	…	1.0	…	1.0	…
Moderate LCD score group (5–15)	1.577 (1.124–2.212)	0.008	1.605 (1.144–2.252)	0.006	1.397 (0.986–1.981)	0.060
High LCD score group (16–27)	1.836 (1.307–2.579)	<0.001	1.873 (1.333–2.631)	<0.001	1.456 (1.015–2.089)	0.041

Model 1: unadjusted. Model 2: adjusted for age and race. Model 3: adjusted for model 2 covariates plus alcohol consumption, baseline CAC score, BMI, diabetes, diastolic blood pressure, dietary calcium intake, dietary fiber intake, dietary magnesium intake, dietary vitamin D intake, education levels (high school, college, and graduate school), fasting plasma glucose, high-density lipoprotein cholesterol, hypertension, low-density lipoprotein cholesterol, physical activity, serum creatinine, smoking status (current, former, and never), systolic blood pressure, and triglycerides. BMI indicates body mass index; CAC, coronary artery calcium; and LCD, low-carbohydrate diet.

model, the hazard ratios (95% CI) for CAC progression comparing the moderate and the highest carbohydrate intake group with the lowest carbohydrate intake group were 0.841 (95% CI, 0.694–1.020) and 0.731 (95% CI, 0.552–0.968), respectively (Table 3; Figure 3). Figure 4 shows the restricted cubic splines of the risk of CAC progression across levels of carbohydrates as a percentage of total energy. Consistent with the above analysis, spline regression analysis confirmed that carbohydrate intake was inversely and nonlinearly associated with the risk of CAC progression, compared with the reference level of 47.5% (Figure 4). Similar results were found in the time-varying sensitivity analyses when we combined dietary data at years 0 and 7 with those at year 20 (Tables I and II in the Data Supplement).

When participants were stratified by age (<40 or ≥40 years), sex (male or female), race (White or Black), body mass index (<30 or ≥30 kg/m^2), hypertension (yes or no), and baseline CAC (yes or no), the association between carbohydrate intake and CAC progression remained similar (Figure 5). However, a differential association was observed if subgroups divided by race, showing a stronger inverse association between carbohydrate intake and CAC in Whites than in Blacks (*P* for interaction, 0.030; Figure 5).

Association of Animal- or Plant-Based LCD Score With CAC Progression

To further explore the effects of the specific sources of fat and protein alternatives to low-carbohydrate intake, we analyzed the association of animal- or plant-based LCD score with CAC progression. As shown in Table 4, after adjustment for all covariates, the animal-based LCD score was significantly associated with more severe CAC progression (hazard ratio, 1.456 [95% CI, 1.015–2.089]; *P*=0.041). However, there was no such significant relationship between plant-based LCD score and CAC progression in the fully adjusted model (hazard ratio, 1.016 [95% CI, 0.821–1.257]; *P*=0.884; Table 5).

DISCUSSION

In this large prospective cohort study, we found that lower carbohydrate intake as a percentage of total energy during young adulthood was associated with an increased risk of CAC progression later in life. Moreover, replacement of carbohydrates with predominantly animal but not plant sources of protein or fat exerts potent effects on CAC progression.

To our knowledge, this study presents the first evidence that long-term LCDs beginning at a young age were associated with a higher risk of subsequent CAC progression. This is distinct from the results of the MESA study (Multi-Ethnic Study of Atherosclerosis).[14] Several plausible explanations could at least, in part, account for this discordance. The participants included in the present study are much younger than those enrolled in the MESA study (mean age, 40.4 versus 62.5 years). As CAC is an age-related pathological process characterized by mineral deposition within the coronary arteries, CAC severity increases with age[28]; the prevalence of CAC at baseline was lower in our analysis than in the MESA study (9.2% versus 48.5%). Thus, the potential role of LCD on CAC progression may be overwhelmed among the relatively older adults enrolled in the MESA study. Indeed, our findings confirmed an attenuated tendency between LCD and CAC progression in older participants. On the other hand, the different carbohydrate intake as a percentage of total energy across the study population was also a possible confounder. Since participants in the MESA data generally consumed moderate-to-high levels of carbohydrates, the percentage of participants

Table 5. Risk of CAC Progression for Plant-Based LCD Score

LCD score groups	Model 1 h (95% CI)	*P* value	Model 2 h (95% CI)	*P* value	Model 3 h (95% CI)	*P* value
Low LCD score group (≤9)	1.0	…	1.0	…	1.0	…
Moderate LCD score group (10–13)	1.155 (0.910–1.466)	0.236	1.119 (0.881–1.420)	0.357	1.031 (0.811–1.311)	0.803
High LCD score group (14–27)	1.235 (1.003–1.520)	0.047	1.190 (0.966–1.467)	0.102	1.016 (0.821–1.257)	0.884

Model 1: unadjusted. Model 2: adjusted for age and race. Model 3: adjusted for model 2 covariates plus alcohol consumption, baseline CAC score, BMI, diabetes, diastolic blood pressure, dietary calcium intake, dietary fiber, dietary magnesium intake, dietary vitamin D intake, education level (high school, college, and graduate school), fasting plasma glucose, high-density lipoprotein cholesterol, hypertension, low-density lipoprotein cholesterol, physical activity, serum creatinine, smoking status (current, former, and never), systolic blood pressure, and triglycerides. BMI indicates body mass index; CAC, coronary artery calcium; and LCD, low-carbohydrate diet.

consuming LCD defined as ≤45% of total energy intake from carbohydrates[29] in our sample was 2.3-fold greater than in MESA data (34.9% versus 15.2%).[14] Additionally, the interaction was observed when the analysis was stratified by race, indicating the stronger association between LCD and CAC progression among Whites compared with Blacks. It was reported that compared with Blacks, Whites consumed less carbohydrates,[8] and they exhibited higher CAC levels at younger ages.[28] This implies that there might be a possible link between LCD and a higher risk of CVD among Whites in this cohort.

An interesting result of our study was that only animal protein or fat chosen to replace carbohydrates in LCD promoted CAC, which was supported by the notion that animal sources of protein and fat in LCD may have an impact on CVD-related mortality.[9,30] For example, a large prospective cohort study documented that LCDs with high intake of animal protein and fat were significantly associated with higher long-term CVD mortality.[30] It was speculated that increased inflammatory cytokines, oxidative stress, and reduced endothelial progenitor cells under LCD, together with high-fat diet (Western diet) or high animal protein,[31–33] are possible mechanisms for enhancing CAC development.[34] Thus, it is crucial to incorporate quality and types of macronutrients when investigating the associations of LCD with CAC progression.

Some limitations of this study need, nevertheless, to be acknowledged. First, dietary intake is not perfectly measured by the validated food frequency questionnaire, which typically has fewer random errors but may produce systematic errors.[35] Second, the analysis of the current study was based on dietary information with cumulative average dietary consumption from years 0 and 7. We have conducted the time-varying sensitivity analysis spanning over 10 years, which showed consistent conclusions. However, the potential influences of dietary pattern changes during the follow-up period could not be completely excluded. Third, despite our careful adjustment for the known and suspected confounders, residual confounding cannot be completely ruled out because of the observational nature of prospective cohort studies.

Taken together, we found that LCDs beginning at early adulthood were associated with an increased risk of subsequent CAC progression in middle age, particularly when animal protein or fat in LCD are increased. As a popular strategy for weight management, long-term animal-based LCD should be advocated with great caution to avoid its potential impact on coronary atherosclerosis.

ARTICLE INFORMATION

Received June 8, 2020; accepted October 16, 2020.

Affiliations

Department of Cardiology (J.-W.G., Q.-Y.H., H.-F.Z., X.-Z.L., J.-F.W., P.-M.L.), Department of Clinical Nutrition (Z.-M.Y.), and Department of Endocrinology (Y.G., S.-L.Z.), Sun Yat-sen Memorial Hospital of Sun Yat-sen University, Guangzhou, China.

Acknowledgments

We thank the investigators, the staffs, and participants of the CARDIA study (Coronary Artery Risk Development in Young Adults) for their highly valued contributions. We are grateful to Dominique Vuitton, Professor Emeritus from Université Bourgogne Franche-Comté, Besançon, France, for her assistance with the work.

Sources of Funding

This work was supported by grants from the National Natural Science Foundation of China (81870315, 81900379, 81970683, and 81870334). The CARDIA study (Coronary Artery Risk Development in Young Adults) was funded by contracts N01-HC-48047 through N01-HC-48050 and N01-HC-95095 from the National Heart, Lung, and Blood Institute, National Institutes of Health, of the United States.

Disclosures

None.

Supplemental Materials

Tables I and II

REFERENCES

1. Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, Ahmed M, Aksut B, Alam T, Alam K, et al. Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. *J Am Coll Cardiol.* 2017;70:1–25. doi: 10.1016/j.jacc.2017.04.052
2. Virani SS, Alonso A, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Delling FN, et al; American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics-2020 update: a report from the American Heart Association. *Circulation.* 2020;141:e139–e596. doi: 10.1161/CIR.0000000000000757
3. Spring B, Moller AC, Colangelo LA, Siddique J, Roehrig M, Daviglus ML, Polak JF, Reis JP, Sidney S, Liu K. Healthy lifestyle change and subclinical atherosclerosis in young adults: Coronary Artery Risk Development in Young Adults (CARDIA) study. *Circulation.* 2014;130:10–17. doi: 10.1161/CIRCULATIONAHA.113.005445
4. Nordmann AJ, Nordmann A, Briel M, Keller U, Yancy WS Jr, Brehm BJ, Bucher HC. Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: a meta-analysis of randomized controlled trials. *Arch Intern Med.* 2006;166:285–293. doi: 10.1001/archinte.166.3.285
5. Kirkpatrick CF, Bolick JP, Kris-Etherton PM, Sikand G, Aspry KE, Soffer DE, Willard KE, Maki KC. Review of current evidence and clinical recommendations on the effects of low-carbohydrate and very-low-carbohydrate (including ketogenic) diets for the management of body weight and other cardiometabolic risk factors: a scientific statement from the National Lipid Association Nutrition and Lifestyle Task Force. *J Clin Lipidol.* 2019;13:689.e1–711.e1. doi: 10.1016/j.jacl.2019.08.003
6. Halton TL, Willett WC, Liu S, Manson JE, Albert CM, Rexrode K, Hu FB. Low-carbohydrate-diet score and the risk of coronary heart disease in women. *N Engl J Med.* 2006;355:1991–2002. doi: 10.1056/NEJMoa055317
7. Dehghan M, Mente A, Zhang X, Swaminathan S, Li W, Mohan V, Iqbal R, Kumar R, Wentzel-Viljoen E, Rosengren A, et al; Prospective Urban Rural Epidemiology (PURE) Study Investigators. Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): a prospective cohort study. *Lancet.* 2017;390:2050–2062. doi: 10.1016/S0140-6736(17)32252-3
8. Seidelmann SB, Claggett B, Cheng S, Henglin M, Shah A, Steffen LM, Folsom AR, Rimm EB, Willett WC, Solomon SD. Dietary carbohydrate intake and mortality: a prospective cohort study and meta-analysis. *Lancet Public Health.* 2018;3:e419–e428. doi: 10.1016/S2468-2667(18)30135-X
9. Lagiou P, Sandin S, Lof M, Trichopoulos D, Adami HO, Weiderpass E. Low carbohydrate-high protein diet and incidence of cardiovascular diseases in Swedish women: prospective cohort study. *BMJ.* 2012;344:e4026. doi: 10.1136/bmj.e4026
10. Mazidi M, Katsiki N, Mikhailidis DP, Sattar N, Banach M. Lower carbohydrate diets and all-cause and cause-specific mortality: a population-based cohort study and pooling of prospective studies. *Eur Heart J.* 2019;40:2870–2879. doi: 10.1093/eurheartj/ehz174

11. Detrano R, Guerci AD, Carr JJ, Bild DE, Burke G, Folsom AR, Liu K, Shea S, Szklo M, Bluemke DA, et al. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. *N Engl J Med.* 2008;358:1336–1345. doi: 10.1056/NEJMoa072100
12. Budoff MJ, Young R, Burke G, Jeffrey Carr J, Detrano RC, Folsom AR, Kronmal R, Lima JAC, Liu KJ, McClelland RL, et al. Ten-year association of coronary artery calcium with Atherosclerotic Cardiovascular Disease (ASCVD) events: the Multi-Ethnic Study of Atherosclerosis (MESA). *Eur Heart J.* 2018;39:2401–2408. doi: 10.1093/eurheartj/ehy217
13. Nicoll R, Howard JM, Henein MY. A review of the effect of diet on cardiovascular calcification. *Int J Mol Sci.* 2015;16:8861–8883. doi: 10.3390/ijms16048861
14. Hu T, Jacobs DR, Bazzano LA, Bertoni AG, Steffen LM. Low-carbohydrate diets and prevalence, incidence and progression of coronary artery calcium in the Multi-Ethnic Study of Atherosclerosis (MESA). *Br J Nutr.* 2019;121:461–468. doi: 10.1017/S0007114518003513
15. Cutter GR, Burke GL, Dyer AR, Friedman GD, Hilner JE, Hughes GH, Hulley SB, Jacobs DR Jr, Liu K, Manolio TA. Cardiovascular risk factors in young adults. The CARDIA baseline monograph. *Control Clin Trials.* 1991;12(1 suppl):1S–77S. doi: 10.1016/0197-2456(91)90002-4
16. Reis JP, Loria CM, Steffen LM, Zhou X, van Horn L, Siscovick DS, Jacobs DR Jr, Carr JJ. Coffee, decaffeinated coffee, caffeine, and tea consumption in young adulthood and atherosclerosis later in life: the CARDIA study. *Arterioscler Thromb Vasc Biol.* 2010;30:2059–2066. doi: 10.1161/ATVBAHA.110.208280
17. Loria CM, Liu K, Lewis CE, Hulley SB, Sidney S, Schreiner PJ, Williams OD, Bild DE, Detrano R. Early adult risk factor levels and subsequent coronary artery calcification: the CARDIA Study. *J Am Coll Cardiol.* 2007;49:2013–2020. doi: 10.1016/j.jacc.2007.03.009
18. Miedema MD, Petrone A, Shikany JM, Greenland P, Lewis CE, Pletcher MJ, Gaziano JM, Djousse L. Association of fruit and vegetable consumption during early adulthood with the prevalence of coronary artery calcium after 20 years of follow-up: the Coronary Artery Risk Development in Young Adults (CARDIA) study. *Circulation.* 2015;132:1990–1998. doi: 10.1161/CIRCULATIONAHA.114.012562
19. Friedman GD, Cutter GR, Donahue RP, Hughes GH, Hulley SB, Jacobs DR Jr, Liu K, Savage PJ. CARDIA: study design, recruitment, and some characteristics of the examined subjects. *J Clin Epidemiol.* 1988;41:1105–1116. doi: 10.1016/0895-4356(88)90080-7
20. McDonald A, Van Horn L, Slattery M, Hilner J, Bragg C, Caan B, Jacobs D Jr, Liu K, Hubert H, Gernhofer N, et al. The CARDIA dietary history: development, implementation, and evaluation. *J Am Diet Assoc.* 1991;91:1104–1112.
21. Hirahatake KM, Jacobs DR, Shikany JM, Jiang L, Wong ND, Steffen LM, Odegaard AO. Cumulative intake of artificially sweetened and sugar-sweetened beverages and risk of incident type 2 diabetes in young adults: the Coronary Artery Risk Development In Young Adults (CARDIA) study. *Am J Clin Nutr.* 2019;110:733–741. doi: 10.1093/ajcn/nqz154
22. Carr JJ, Nelson JC, Wong ND, McNitt-Gray M, Arad Y, Jacobs DR Jr, Sidney S, Bild DE, Williams OD, Detrano RC. Calcified coronary artery plaque measurement with cardiac CT in population-based studies: standardized protocol of Multi-Ethnic Study of Atherosclerosis (MESA) and Coronary Artery Risk Development in Young Adults (CARDIA) study. *Radiology.* 2005;234:35–43. doi: 10.1148/radiol.2341040439
23. Mao SS, Pal RS, McKay CR, Gao YG, Gopal A, Ahmadi N, Child J, Carson S, Takasu J, Sarlak B, et al. Comparison of coronary artery calcium scores between electron beam computed tomography and 64-multidetector computed tomographic scanner. *J Comput Assist Tomogr.* 2009;33:175–178. doi: 10.1097/RCT.0b013e31817579ee
24. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol.* 1990;15:827–832. doi: 10.1016/0735-1097(90)90282-t
25. Okwuosa TM, Greenland P, Burke GL, Eng J, Cushman M, Michos ED, Ning H, Lloyd-Jones DM. Prediction of coronary artery calcium progression in individuals with low Framingham Risk Score: the Multi-Ethnic Study of Atherosclerosis. *JACC Cardiovasc Imaging.* 2012;5:144–153. doi: 10.1016/j.jcmg.2011.11.008
26. Meyer KA, Benton TZ, Bennett BJ, Jacobs DR Jr, Lloyd-Jones DM, Gross MD, Carr JJ, Gordon-Larsen P, Zeisel SH. Microbiota-dependent metabolite trimethylamine N-oxide and coronary artery calcium in the Coronary Artery Risk Development in Young Adults study (CARDIA). *J Am Heart Assoc.* 2016;5:e003970.
27. Camp RL, Dolled-Filhart M, Rimm DL. X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. *Clin Cancer Res.* 2004;10:7252–7259. doi: 10.1158/1078-0432.CCR-04-0713
28. McClelland RL, Chung H, Detrano R, Post W, Kronmal RA. Distribution of coronary artery calcium by race, gender, and age: results from the Multi-Ethnic Study of Atherosclerosis (MESA). *Circulation.* 2006;113:30–37. doi: 10.1161/CIRCULATIONAHA.105.580696
29. U.S. Department of Health and Human Services and U.S. Department of Agriculture. *2015–2020 Dietary Guidelines for Americans.* 8th ed; 2015. Accessed June 8, 2020. http://health.gov/dietaryguidelines/2015/guidelines/.
30. Fung TT, van Dam RM, Hankinson SE, Stampfer M, Willett WC, Hu FB. Low-carbohydrate diets and all-cause and cause-specific mortality: two cohort studies. *Ann Intern Med.* 2010;153:289–298. doi: 10.7326/0003-4819-153-5-201009070-00003
31. Kostogrys RB, Franczyk-Żarów M, Maślak E, Gajda M, Mateuszuk L, Jackson CL, Chłopicki S. Low carbohydrate, high protein diet promotes atherosclerosis in apolipoprotein E/low-density lipoprotein receptor double knockout mice (apoE/LDLR(-/-)). *Atherosclerosis.* 2012;223:327–331. doi: 10.1016/j.atherosclerosis.2012.05.024
32. Foo SY, Heller ER, Wykrzykowska J, Sullivan CJ, Manning-Tobin JJ, Moore KJ, Gerszten RE, Rosenzweig A. Vascular effects of a low-carbohydrate high-protein diet. *Proc Natl Acad Sci USA.* 2009;106:15418–15423. doi: 10.1073/pnas.0907995106
33. Wycherley TP, Brinkworth GD, Keogh JB, Noakes M, Buckley JD, Clifton PM. Long-term effects of weight loss with a very low carbohydrate and low fat diet on vascular function in overweight and obese patients. *J Intern Med.* 2010;267:452–461. doi: 10.1111/j.1365-2796.2009.02174.x
34. Demer LL, Tintut Y. Inflammatory, metabolic, and genetic mechanisms of vascular calcification. *Arterioscler Thromb Vasc Biol.* 2014;34:715–723. doi: 10.1161/ATVBAHA.113.302070
35. Pan A, Lin X, Hemler E, Hu FB. Diet and cardiovascular disease: advances and challenges in population-based studies. *Cell Metab.* 2018;27:489–496. doi: 10.1016/j.cmet.2018.02.017