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### Authors

Bansal, Nisha  
Vittinghoff, Eric  
Peralta, Carmen A  
et al.

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## Original Contribution

# Estimated Kidney Function Based on Serum Cystatin C and Risk of Subsequent Coronary Artery Calcium in Young and Middle-aged Adults With Preserved Kidney Function: Results From the CARDIA Study

**Nisha Bansal\***, Eric Vittinghoff, Carmen A. Peralta, Michael G. Shlipak, Vanessa Grubbs, David R. Jacobs, David Siscovick, Michael Steffes, John Jeffrey Carr, and Kirsten Bibbins-Domingo

\* Correspondence to Dr. Nisha Bansal, Division of Nephrology, University of California, San Francisco, 521 Parnassus Avenue, Box 0532, San Francisco, CA 94143 (e-mail: nisha.bansal@gmail.com).

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Whether kidney dysfunction is associated with coronary artery calcium (CAC) in young and middle-aged adults who have a cystatin C–derived estimated glomerular filtration rate (eGFR<sub>cys</sub>) greater than 60 mL/min/1.73 m<sup>2</sup> is unknown. In the Coronary Artery Risk Development in Young Adults (CARDIA) cohort (recruited in 1985 and 1986 in Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California), we examined 1) the association of eGFR<sub>cys</sub> at years 10 and 15 and detectable CAC over the subsequent 5 years and 2) the association of change in eGFR<sub>cys</sub> and subsequent CAC, comparing those with stable eGFR<sub>cys</sub> to those whose eGFR<sub>cys</sub> increased (>3% annually over 5 years), declined moderately (3%–5%), or declined rapidly (>5%). Generalized estimating equation Poisson models were used, with adjustment for age, sex, race, educational level, income, family history of coronary artery disease, diabetes, body mass index, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and tobacco use. Among 3,070 participants (mean age 35.6 (standard deviation, 4.1) years and mean eGFR<sub>cys</sub> 106.7 (standard deviation, 18.5) mL/min/1.73 m<sup>2</sup>), 529 had detectable CAC. Baseline eGFR<sub>cys</sub> was not associated with CAC. Moderate eGFR<sub>cys</sub> decline was associated with a 33% greater relative risk of subsequent CAC (95% confidence interval: 5, 68; *P* = 0.02), whereas rapid decline was associated with a 51% higher relative risk (95% confidence interval: 10, 208; *P* = 0.01) in adjusted models. In conclusion, among young and middle-aged adults with eGFR<sub>cys</sub> greater than 60 mL/min/1.73 m<sup>2</sup>, annual decline in eGFR<sub>cys</sub> is an independent risk factor for subsequent CAC.

calcification; cardiovascular diseases; chronic kidney insufficiency; coronary arteries; coronary disease; cystatin C; glomerular filtration rate; kidney

Abbreviations: CAC, coronary artery calcium; CARDIA, Coronary Artery Risk Development in Young Adults; CI, confidence interval; CKD, chronic kidney disease; CT, computed tomography; eGFR, estimated glomerular filtration rate; eGFR<sub>cys</sub>, glomerular filtration rate estimated by cystatin C; RR, relative risk.

Coronary artery calcium (CAC) has been shown to predict future cardiovascular disease and death in numerous prospective studies of the general population (1–5). Chronic kidney disease (CKD) is a known risk factor for cardiovascular disease (6, 7), and several studies have demonstrated a graded relation between lower estimated glomerular filtration rate (eGFR) and higher CAC (8–11).

Whether kidney dysfunction at earlier stages is associated with future CAC risk is unknown. Understanding this association and potential contributors to this association could be important in potentially elucidating mechanisms linking CKD and cardiovascular disease. Cystatin C is a novel biomarker that has been shown to be a highly sensitive measure of kidney function in healthy persons and a strong predictor

of adverse outcomes (12–15). We examined the association of glomerular filtration rate estimated by cystatin C (eGFR<sub>cys</sub>) and risk of subsequent CAC in young and middle-aged adults with preserved kidney function. Furthermore, we explored potential mediators that might link early kidney function decline to future CAC in young and middle-aged adults.

## MATERIALS AND METHODS

### Source population

Coronary Artery Risk Development in Young Adults (CARDIA) is a multicenter study of the development and determinants of cardiovascular risk factors in young adults who were 18 to 30 years of age at recruitment. The study design has been published previously in detail (16). In brief, black and white participants were recruited in 1985 and 1986 in 4 US cities (Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California). The cohort was designed to be balanced by age, sex, race, and educational level. Follow-up examinations were completed at years 2, 5, 7, 10, 15, and 20. Each examination protocol was approved by institutional review boards at each site, and informed consent was obtained at every examination.

### Cystatin C estimates of glomerular filtration rate

Cystatin C is a novel biomarker that has been shown to perform well in estimation of renal function in persons at higher ranges of glomerular filtration rate (13–15, 17) and might detect changes in kidney function earlier than creatinine (18). Therefore, for this analysis of healthy young and middle-aged adults with preserved kidney function, we chose to use eGFR<sub>cys</sub> as our primary measure of kidney function.

Cystatin C was originally measured in all CARDIA participants with stored frozen sera from years 10, 15, and 20 by nephelometer and the N Latex cystatin C kit (Dade Behring, now Siemens, Muenchen, Germany) (Figure 1). The coefficient of variation was 4.0%. Given the recent success in standardizing cystatin C assays (19), we randomly selected 93 samples from participants across all study years and remeasured the original samples with the Gentian assay (Moss, Norway). Recently, the International Federation for Clinical Chemists (IFCC) working group for serum cystatin C standardization and the Institute for Reference Materials and Measurements produced a certified reference material (ERM-DA471/IFCC) (20, 21), which was used to validate the accuracy of the Gentian assay. This recalibration yielded a mean correction factor of 12% from the original assays that was then applied to all cystatin C measures in the cohort. Renal function was then determined by cystatin C–derived eGFR (eGFR<sub>cys</sub>) according to the following formula:  $eGFR_{cys} = 76.7 \times \text{cystatin C}^{-1.19}$  (22).

### Coronary artery calcium

Computed tomography (CT)–measured CAC was reported as present or absent on the basis of a CAC score >0 or =0 in all CARDIA participants who had CT data available from the exams at years 15 and 20 (Figure 1). The CT protocol at these

exams consisted of 2 sequential noncontrast CT scans performed on electron beam CT scanners (at the Chicago and Oakland centers: Imatron C-150, GE Healthcare, Waukesha, Wisconsin) or multidetector CT scanners (at the Birmingham center: GE Lightspeed, GE Healthcare, Waukesha, Wisconsin; at the Minneapolis center: Volume Zoom, Siemens Medical Solutions, Erlangen, Germany). Experienced image analysts, masked to participant information and to prior measures of CAC, measured calcified plaque in the epicardial coronary arteries and used a modified Agatston method to account for slice thickness (23). As part of the reading protocol quality control, an expert in cardiovascular imaging reviewed CT scans that were on initial analysis discordant (one with and without calcified plaque), had a change in CAC status from positive to negative or negative to positive between year 15 and year 20, or had a possible surgical intervention (pace-maker, valve replacement, coronary stent, or bypass surgery) or a technical concern identified by the reader. The year 15 CT scans, which had been analyzed previously on different software, were reanalyzed to remove this potential bias. The year 15 CT scans were de-archived from media, and all originally positive (CAC > 0) cases and a random sample of negative (CAC = 0) cases were reanalyzed on the Aquarius Workstation with the same reading protocol as described above for year 20 CT scans. The accuracy, comparability, reproducibility, and robustness of the CAC score according to electron beam, helical, and multidetector CT systems have been published previously (24). The agreement between scans was excellent ( $\kappa = 0.79$ , 95% confidence interval (CI): 0.75, 0.83), with only 3.6% discordant (25).

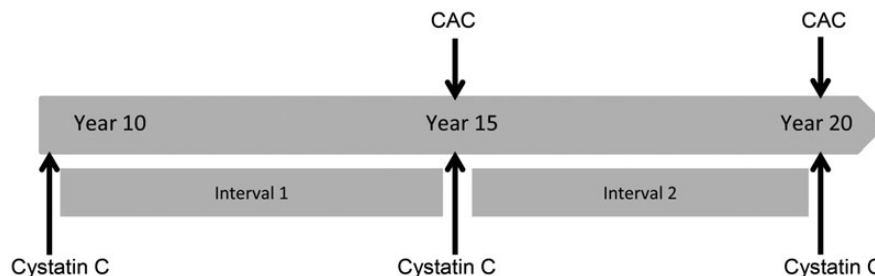
### Study population

For this analysis, we included only participants with at least 2 sequential measures of cystatin C at years 10, 15, or 20 and measurement of CAC at years 15 or 20. Baseline for this analysis was defined as the examination corresponding to first cystatin C measurement (examination year 10 or 15). Two 5-year intervals were studied: from examination years 10 to 15 and from years 15 to 20. Our outcome was detectable CAC (CAC score >0) at the end of the 5-year interval (Figure 1).

Of the 4,376 participants with a visit at year 10, 15, or 20, a total of 4,311 (98.5%) had at least 1 cystatin C measurement. Of these, 3,361 (78%) had 2 consecutive measurements of cystatin C at years 10 and 15 or years 15 or 20, allowing us to define percent change in eGFR<sub>cys</sub> for at least one 5-year interval. Of these, 3,108 (92.5%) had a CAC study at the end of the 5-year interval. Finally, we excluded 11 (0.4%) with prevalent CKD, defined as eGFR<sub>cys</sub> <60 mL/min/1.73 m<sup>2</sup>, at examination year 10. Of the remaining 3,097 remaining participants, 3,070 (99.1%) had complete covariate data, and these comprised our final study sample.

### Covariates

A common protocol and common quality control procedures were used at all examinations. Participants were asked to fast for 12 hours and to avoid smoking and heavy physical activity for 2 hours before their examinations (26). Age, sex,



**Figure 1.** Schematic of study measurements in the Coronary Artery Risk Development in Young Adults (CARDIA) Study cohort, recruited in 1985 and 1986 in Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California. CAC indicates coronary artery calcium.

race, educational level, and smoking habits were ascertained through questionnaires. Educational level has been shown to be an important risk factor for CAC in this cohort and was thus considered as a cardiovascular risk factor (27). We used covariates ascertained from the year 10 or year 15 examination, which corresponded to the first cystatin C measurement used for the analyses.

Other covariates included plasma high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, diabetes status (defined as elevated fasting glucose or use of diabetic medications), and body mass index. High-density lipoprotein cholesterol was determined with an enzymatic assay by Northwest Lipids Research Laboratory (Seattle, Washington) at all time periods; reanalysis at each examination of stored samples from the previous examination indicated that measures over the course of time were comparable (26). Low-density lipoprotein cholesterol was derived by the Friedewald equation (28). Serum glucose was measured by the hexokinase assay (hexokinase coupled to glucose-6-phosphate dehydrogenase; Linco Research, St. Louis, Missouri). Body weight with light clothing was measured to the nearest 0.2 pound (0.09 kg), body height without shoes was measured to the nearest 0.5 cm, and body mass index was calculated from these measures (weight (kg)/height (m)<sup>2</sup>).

To further understand the association between eGFR<sub>cys</sub> and subsequent CAC, we studied 2 potential mediators in our models—systolic blood pressure and microalbuminuria—comorbid conditions that often occur as a result of kidney dysfunction, have also been strongly associated with CAC (29–31), and thus could be in the causal pathway between kidney function and CAC. As in prior CARDIA studies, we computed overall blood pressure trajectories and named this variable “cumulative exposure of systolic blood pressure” (32). Three seated blood pressure measurements were obtained with a random-zero sphygmomanometer; the mean of the second and third readings was used. Mixed models were used to estimate a blood pressure trajectory for each participant from first eGFR<sub>cys</sub> measurement to the time of his or her CAC measurement (32). We assumed that the trajectory for each participant had a constant slope within each decade of life (ages 20–30, 30–40, and 40–50 years). Using these individual blood pressure trajectories, we calculated an integrated measure of years of exposure to blood pressure elevation

from first cystatin C measurement to CAC measurement for each participant (expressed in mm Hg-years) by calculating the area under the trajectory for each participant (32). Albuminuria (urine albumin/creatinine ratio) was measured from a single, untimed (spot) urine sample collected at examinations at years 10, 15, and 20 (33); urine albumin concentrations were measured by a nephelometric procedure with a specific anti-albumin monoclonal antibody, and creatinine was assessed with the Jaffe method (33). All urine albumin-to-creatinine ratios were standardized to sex and race and expressed in milligrams per gram creatinine (34). Microalbuminuria was defined as a urine albumin-to-creatinine ratio  $\geq 30$  mg/g.

### Statistical methods

We had 2 predictors of interest: 1) baseline eGFR<sub>cys</sub> and 2) annual change in eGFR<sub>cys</sub> before measurement of CAC at examination years 15 and 20. In exploratory analyses, we found that the distribution of change in eGFR<sub>cys</sub> was skewed. Spline analyses revealed that the association of change in eGFR<sub>cys</sub> with CAC appeared to have a J-shaped distribution. Therefore, we categorized the predictor eGFR<sub>cys</sub> change into 4 categories: 1) increase in eGFR<sub>cys</sub>, defined as more than 3% per year; 2) stable eGFR<sub>cys</sub>, defined as  $\pm 3\%$  annual change; 3) moderate eGFR<sub>cys</sub> decline, defined as 3%–5% per year, and 4) rapid eGFR<sub>cys</sub> decline, defined as more than 5% per year. This categorical definition of our predictor was less sensitive to outlier values of eGFR<sub>cys</sub> change. We defined stable eGFR<sub>cys</sub> as  $\pm 3\%$  change per year on the basis of a commonly used threshold for defining rapid change of eGFR<sub>cys</sub> that has been used in several studies (35–37). A total of 5,006 person-intervals were included, with a maximum of 2 intervals for each participant. For example, a participant with all 3 measures of cystatin C who did not develop CAC at year 15 would contribute 2 intervals (years 10–15 and years 15–20) to the analyses.

We first examined the characteristics of the study participants in each eGFR<sub>cys</sub> change category. Wald  $\chi^2$  tests were used to assess heterogeneity across eGFR change categories.

We used continuation ratio models to study the association between baseline eGFR<sub>cys</sub> and annual change in eGFR<sub>cys</sub> and detectable CAC. A continuation ratio model (38) is a

discrete-time survival model, in which each participant contributes 1 or 2 observations to the analysis, with binary outcomes defined as (respectively) detection of CAC (yes/no) at year 15 and year 20. The second observation was used only for participants who did not have CAC at year 15 (or have missing values) and who had a year 20 visit. This is analogous to a Cox model, in which participants are omitted from the so-called risk sets after the outcome event has occurred, or they are censored. To provide estimates of risk ratios rather than odds ratios, the continuation ratio models were implemented by using generalized estimating equations Poisson models (39) with robust standard errors to account for clustering by participant.

Baseline eGFRcys was defined as the first measured eGFRcys in the 5-year interval (i.e., examination year 10 or 15). We examined the association of baseline eGFRcys (continuous and categorical) with detectable CAC in models adjusted for age, sex, and race. We then adjusted for possible confounders: educational level; income level; family history of coronary heart disease; and time-varying body mass index, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, diabetes status, and tobacco use.

Our primary predictor was annual change in eGFRcys over 5 years (i.e., interval from examination years 10–15 and years 15–20). We examined detectable CAC in each of the 4 eGFR change categories. We used continuation ratio models to study change in eGFRcys (categorical) and detectable CAC in models

adjusted for demographic variables (age, sex, and race) and in models fully adjusted for the aforementioned potential confounders. In these analyses, we further adjusted for baseline eGFRcys. We also tested for a possible mediation effect of cumulative exposure to systolic blood pressure and microalbuminuria.

In all models, we tested for interaction of eGFRcys change first with race, then with sex, and finally with a 4-level variable defined by both factors. We further tested for interactions between baseline eGFRcys and eGFRcys change categories for the outcome of detectable CAC.

In a sensitivity analysis, we calculated eGFRcys using the recently published CKD-EPI cystatin C equation (17), which includes age and sex as coefficients. This was a secondary analysis in our paper because this equation was published after we had completed our analyses.

All analyses were performed in Stata version 12 (StataCorp LP, College Station, Texas).

## RESULTS

Among 3,070 participants, the mean age was 35.6 (standard deviation, 4.1) years, 55% were women, 44% were black, the mean eGFRcys was 106.7 (standard deviation, 18.5) mL/min/1.73 m<sup>2</sup>, mean systolic blood pressure was 109.9 (standard deviation, 12.6) mm Hg, and 4% had microalbuminuria at their baseline visit. Among the entire study

**Table 1.** Baseline<sup>a</sup> Characteristics of Study Population by Annual Change in Glomerular Filtration Rate Estimated by Cystatin C Over a 5-Year Interval (*n* = 3,070), Coronary Artery Risk Development in Young Adults Study,<sup>b</sup> 1985–1991

| Characteristic                      | Annual eGFRcys Change Over 5-Year Interval |    |  |    |                                    |    |                                  |    | P Value <sup>d</sup> |
|-------------------------------------|--|----|--|----|------------------------------------|----|----------------------------------|----|----------------------|
|                                     | Increase >3%<br>( <i>n</i> = 249)          |    | Stable <sup>c</sup><br>( <i>n</i> = 2,471) |    | Decline 3%–5%<br>( <i>n</i> = 240) |    | Decline >5%<br>( <i>n</i> = 110) |    |                      |
|                                     | Mean (SD)                                  | %  | Mean (SD)                                  | %  | Mean (SD)                          | %  | Mean (SD)                        | %  |                      |
| Age, years                          | 35.3 (3.7)                                 |    | 35.5 (4.0)                                 |    | 36.5 (4.6)                         |    | 36.9 (5.5)                       |    | <0.001               |
| Female                              |  | 57 |  | 53 |                                    | 70 |                                  | 60 | <0.001               |
| Black                               |  | 46 |  | 43 |                                    | 49 |                                  | 62 | <0.001               |
| Less than 12 years of education     |  | 5  |  | 4  |                                    | 4  |                                  | 5  | 0.9                  |
| Current tobacco use                 |  | 24 |  | 23 |                                    | 23 |                                  | 28 | 0.5                  |
| History of diabetes                 |  | 11 |  | 6  |                                    | 5  |                                  | 21 | <0.001               |
| History of hypertension             |  | 9  |  | 7  |                                    | 10 |                                  | 23 | <0.001               |
| Cystatin C, mL/L                    | 0.81 (0.13)                                |    | 0.78 (0.11)                                |    | 0.73 (0.11)                        |    | 0.75 (0.16)                      |    | <0.001               |
| eGFRcys, mL/min/1.73 m <sup>2</sup> | 101.6 (18.8)                               |    | 106.1 (17.2)                               |    | 114.3 (20.1)                       |    | 115.9 (31.4)                     |    | <0.001               |
| Microalbuminuria                    |  | 6  |  | 4  |                                    | 3  |                                  | 21 | <0.001               |
| Systolic blood pressure, mm Hg      | 108.6 (12.9)                               |    | 109.6 (12.0)                               |    | 111.9 (13.9)                       |    | 116.5 (17.1)                     |    | <0.001               |
| Body mass index <sup>e</sup>        | 26.3 (6.1)                                 |    | 26.5 (5.5)                                 |    | 27.2 (5.0)                         |    | 27.9 (6.0)                       |    | 0.02                 |
| LDL cholesterol, mg/dL              | 108.8 (30.5)                               |    | 109.5 (28.2)                               |    | 113.0 (29.8)                       |    | 108.2 (36.8)                     |    | 0.3                  |
| HDL cholesterol, mg/dL              | 51.9 (14.6)                                |    | 51.7 (12.6)                                |    | 52.3 (13.4)                        |    | 50.8 (13.7)                      |    | 0.8                  |

Abbreviations: eGFRcys, glomerular filtration rate estimated by cystatin C; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation.

<sup>a</sup> Characteristics from visit corresponding to first eGFRcys measurement (year 10 or 15) of first 5-year interval.

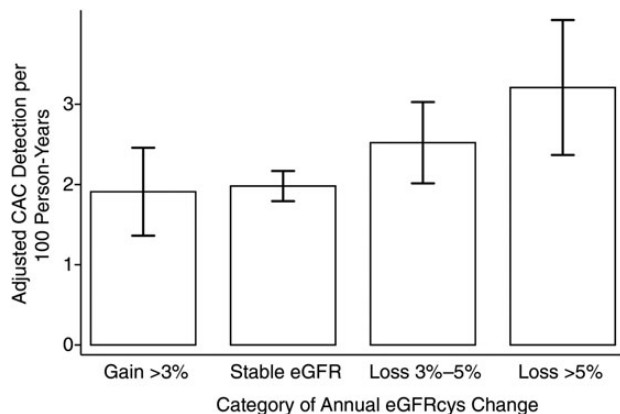
<sup>b</sup> Participants were recruited from Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California.

<sup>c</sup> Defined as  $\pm 3\%$  per year change in eGFRcys.

<sup>d</sup> P value for heterogeneity.

<sup>e</sup> Weight (kg)/height (m)<sup>2</sup>.





**Figure 2.** Rates of detectable coronary artery calcium (CAC) by annual change in cystatin C–derived estimated glomerular filtration rate (eGFRcys) in the Coronary Artery Risk Development in Young Adults (CARDIA) Study cohort, recruited in 1985 and 1986 in Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California. Adjusted for age, sex, race, educational level, income level, family history of coronary heart disease, diabetes, body mass index, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and tobacco use.

population, 7% had diabetes and 8% had hypertension at baseline. Over the 10 years of follow-up, there were 529 cases of detectable CAC (263 first detected at year 15 and 266 first detected at year 20).

The 3,070 participants represented a total of 5,006 person-intervals (each interval being 5 years). At the time of the first eGFRcys measurement (of the first interval if applicable), participants with moderate or rapid annual eGFRcys decline were older; more likely to be female and black; and more likely to have hypertension and diabetes, higher body mass index, microalbuminuria, and higher eGFRcys at baseline (Table 1).

Baseline eGFRcys was associated with detectable CAC in models adjusted for age, sex, and race (relative risk (RR) = 1.08, 95% CI: 1.02, 1.14; per every 10% decrease in eGFRcys).

However, after adjustment for other cardiovascular risk factors (educational level, income level, family history of coronary heart disease, diabetes, body mass index, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and tobacco use), this association was attenuated to null.

Persons with declining eGFRcys had higher adjusted rates (per 100 person-years) of detectable CAC (Figure 2). Participants with annual increases in eGFRcys were not at increased risk for detectable CAC in demographic-adjusted or fully adjusted models (Table 2). In analyses adjusted for age, sex, and race, both groups of eGFRcys decline were associated with higher risk for detectable CAC than that of the stable eGFR group. Among participants with moderate annual decline in eGFRcys, there was a 33% increased risk of detectable CAC after adjustment for demographics, cardiovascular risk factors, and baseline eGFRcys. Participants with rapid annual eGFRcys decline had a 48% higher risk of detectable CAC in models fully adjusted for demographics and cardiovascular risk factors (Table 3). This association was unchanged after additional adjustment for baseline eGFRcys (RR = 1.51, 95% CI: 1.10, 2.08;  $P = 0.01$ ). In all models, interactions of eGFRcys change with sex, race, and sex and race were not significant. Additionally, there was no interaction between baseline eGFRcys and eGFRcys change categories for the outcome of CAC ( $P = 0.95$ ). In a mediation analysis, among participants with moderate and rapid decline of eGFRcys, addition of cumulative exposure to systolic blood pressure and microalbuminuria to the models attenuated the association with detectable CAC by 25% (Table 3).

In our sensitivity analysis in which we used the recently published CKD-EPI cystatin C equation, our results were qualitatively similar. There was no association between baseline eGFRcys and detectable CAC in multivariable models. Participants with rapid annual eGFRcys decline had a 50% increased relative risk (RR = 1.50, 95% CI: 1.07, 2.11) of CAC in models adjusted for age, sex, and race. This association was slightly attenuated after adjustment for cardiovascular risk factors and baseline eGFRcys (RR = 1.39, 95% CI: 1.00, 1.93). The association between moderate annual eGFRcys decline and CAC was not statistically significant (RR = 1.05, 95% CI: 0.82, 1.36).

**Table 2.** Change in Glomerular Filtration Rate Estimated by Cystatin C and Relative Risk for Detectable Coronary Artery Calcium, Coronary Artery Risk Development in Young Adults Study,<sup>a</sup> 1985–1991

| eGFRcys Change               | Adjusted for Age, Sex, and Race |            | Additional Adjustment for Cardiovascular Risk Factors <sup>b</sup> |            | Additional Adjustment for Baseline eGFRcys <sup>b</sup> |            |
|------------------------------|---------------------------------|------------|--|------------|---|------------|
|                              | Relative Risk                   | 95% CI     | Relative Risk  | 95% CI     | Relative Risk   | 95% CI     |
| Increase >3% per year        | 0.98                            | 0.68, 1.39 | 0.92   | 0.65, 1.32 | 0.91  | 0.64, 1.30 |
| Stable ( $\pm 3\%$ per year) | Referent                        |            | Referent   |            | Referent  |            |
| Decline 3%–5% per year       | 1.33*                           | 1.05, 1.68 | 1.31*  | 1.04, 1.66 | 1.33*   | 1.05, 1.68 |
| Decline >5% per year         | 1.52*                           | 1.10, 2.09 | 1.48*  | 1.08, 2.03 | 1.51*   | 1.10, 2.08 |

Abbreviations: CI, confidence interval; eGFRcys, glomerular filtration rate estimated by cystatin C.

\*  $P < 0.05$ .

<sup>a</sup> Participants were recruited from Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California.

<sup>b</sup> Adjusted for age, sex, race, educational level, income level, family history of coronary heart disease, diabetes, body mass index, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and tobacco use.

**Table 3.** Mediators in the Association Between Change in Estimated Glomerular Filtration Rate and Detectable Coronary Artery Calcium, Coronary Artery Risk Development in Young Adults Study,<sup>a</sup> 1985–1991

| eGFRcys Change              | Multivariable Model With Additional Adjustment Cumulative Exposure to Systolic Blood Pressure <sup>b,c</sup> |            | Additional Adjustment for Microalbuminuria <sup>b,c,d</sup> |            |
|-----------------------------|--|------------|---|------------|
|                             | Relative Risk  | 95% CI     | Relative Risk   | 95% CI     |
| Increase >3% per year       | 0.91   | 0.64, 1.30 | 0.90  | 0.63, 1.28 |
| Stable ( $\pm$ 3% per year) | Referent   |            | Referent  |            |
| Decline 3%–5% per year      | 1.30*  | 1.02, 1.65 | 1.28*   | 1.01, 1.63 |
| Decline >5% per year        | 1.44*  | 1.05, 1.98 | 1.38*   | 1.0, 1.90  |

Abbreviations: CI, confidence interval; eGFRcys, glomerular filtration rate estimated by cystatin C.

\*  $P < 0.05$ .

<sup>a</sup> Participants were recruited from Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California.

<sup>b</sup> Adjusted for age, sex, race, educational level, income level, family history of coronary heart disease, diabetes, body mass index, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, tobacco use, and baseline eGFRcys.

<sup>c</sup> Mixed models were used to estimate a blood pressure trajectory for each participant from first eGFRcys measurement to the time of his or her CAC measurement. Using these individual blood pressure trajectories, we calculated an integrated measure of years of exposure to blood pressure elevation from first measure of cystatin C for each participant (mm Hg-years) to CAC measurement by calculating the area under the trajectory for each participant.

<sup>d</sup> Defined as urine albumin-to-creatinine ratio  $\geq 30$  mg.

## DISCUSSION

In this study, we examined the association between kidney function and risk of detectable CAC in a healthy cohort of young and middle-aged adults with an eGFRcys greater than 60 mL/min/1.73 m<sup>2</sup>. We found that in this healthy cohort, participants with a 3%–5% annual decline in eGFRcys had a 33% greater risk of detectable CAC in the subsequent 5 years, whereas persons with a greater than 5% annual decline in eGFRcys had a 51% greater risk, even after adjustment for cardiovascular risk factors and baseline eGFRcys. These associations appeared to be partially mediated by higher systolic blood pressure and microalbuminuria. These results highlight that longitudinal decline in kidney function, even within the normal range of eGFRcys ( $\geq 60$  mL/min/1.73 m<sup>2</sup>), could be an important contributor to future cardiovascular disease in young and middle-aged adults and that this association might be explained in part by elevated blood pressure and microalbuminuria associated with early kidney dysfunction.

We found that moderate and rapid decline in kidney function (3%–5% and >5% per year) was strongly associated with detectable CAC, whereas baseline kidney function was not. Our results suggest that in adults with eGFRcys greater than 60 mL/min/1.73 m<sup>2</sup>, single measures of kidney function during

young and middle age might be less informative than longitudinal measures in predicting future cardiovascular risk. eGFR decline has been an important predictor of adverse outcomes in several other studies, as well (36, 37). In a study of elderly patients, rapid eGFR decline was a strong predictor of cardiovascular events in patients with and without CKD at baseline (36). Nevertheless, even in those studies, baseline kidney function was an important risk factor for adverse events. In contrast to those studies, our present results show that in young and middle-aged adults with preserved kidney function, rapid decline of eGFRcys, rather than single measures of eGFRcys, was a strong risk factor for subclinical cardiovascular disease. This observation could reflect true biological risk associated with early declines in kidney function. It is also possible that within this range of normal eGFR, the greater heterogeneity in single measures of eGFRcys between individuals could mean that repeated, longitudinal measures of eGFRcys are more informative for capturing dynamic, potentially deleterious changes in kidney function.

The association between eGFRcys decline and detectable CAC remained significant even after adjustment for well-recognized cardiovascular risk factors. Novel, kidney-specific risk factors contribute to the development of premature and accelerated cardiovascular disease in patients with known CKD (40). It is possible that alterations in these novel vascular toxins could be initiated by (and accumulate with) small changes in kidney function, even in ranges of normal eGFR. For example, phosphorus has been found to be an important contributor to vascular calcification in populations with CKD (41) and even in healthy populations (42, 43). Early alterations in phosphorus (and other mineral metabolism markers) can occur with rapid eGFR decline even within the “normal” range and could contribute to development of atherosclerosis. Future studies of novel cardiovascular risk factors in healthy young adults are needed to further elucidate the complex relation between early declines in kidney function and development of subclinical cardiovascular disease.

Both systolic blood pressure and microalbuminuria were found to be partial mediators in the causal pathway between early declines in kidney function and detectable CAC. These important risk factors attenuated the risk of detectable CAC by 25% in participants with rapid eGFRcys decline. These results are consistent with previous studies in healthy populations and in populations with CKD. Elevated blood pressure and albuminuria are common manifestations of abnormal kidney function. As such, hypertension and microalbuminuria are independent predictors of future CAC, regardless of the level of kidney function (29–31). Our study suggests that elevated systolic blood pressure and microalbuminuria could explain in part the association between early declines in kidney function and future CAC, and thus they might be important clinical targets for modifying future risk of CAC.

Our study is the first to identify early decline in kidney function as a risk factor for CAC in healthy adults with otherwise low to intermediate risk for atherosclerosis. Earlier recognition of risk factors that can contribute to kidney function decline and vigilant longitudinal monitoring of kidney function could be important early primary interventions in young adulthood to decrease the burden of cardiovascular disease later in life.

Our study had several strengths. Our analyses were based on a large, racially diverse, well-characterized longitudinal cohort of young adults with more than 10 years of follow-up. We used serial measures of cystatin C, a novel biomarker that has been shown to be a highly sensitive marker of renal function at higher ranges of eGFR and a strong predictor of adverse outcomes (12, 44, 45). Our study had some limitations, as well. We did not have direct measures of glomerular filtration rate and therefore could not assess whether the changes we observed in eGFRcys were truly reflective of changes in kidney function, although studies have suggested that eGFR might perform better than measured glomerular filtration rate in predicting clinical outcomes (46, 47). Although less likely in this young and relatively healthy population ages 18–30 years at the time of enrollment, it is possible that a participant could have had detectable CAC before year 10, the time of first cardiac CT. Although cystatin C appears to be a very sensitive marker of kidney function and a strong predictor of outcomes, there might be nonrenal determinants of cystatin C for which we did not adjust (48). Despite adjustment for traditional cardiovascular risk factors and microalbuminuria, we cannot exclude the possibility of residual confounding. Finally, in an observation study, we cannot determine whether the association of kidney function decline with detectable CAC is causal or rather is a marker of atherosclerotic physiological processes affecting both organs in parallel.

In conclusion, our results suggest that decline in eGFRcys is strongly associated with detectable CAC in young and middle-aged adults with an eGFR greater than 60 mL/min/1.73 m<sup>2</sup>, whereas baseline eGFRcys is not. Additional studies are needed to further elucidate the pathological mechanisms involved in acceleration of cardiovascular disease at this normal range of eGFR and to study the association between CAC and future cardiovascular events in persons with rapid eGFR decline.

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Author affiliations: Department of Medicine, School of Medicine, University of California, San Francisco, San Francisco, California (Nisha Bansal, Carmen A. Peralta, Michael G. Shlipak, Vanessa Grubbs, Kirsten Bibbins-Domingo); Department of Epidemiology and Biostatistics, School of Medicine, University of California, San Francisco, California (Eric Vittinghoff); Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, Minnesota (David R. Jacobs); Department of Epidemiology, School of Public Health, University of Washington, Seattle, Washington (David Siscovick); Department of Medicine, University of Washington, Seattle, Washington (David Siscovick); Department of Laboratory Medicine and Pathology, Medical School, University of Minnesota, Minneapolis, Minnesota (Michael Steffes); Department of Radiology, School of Medicine, Wake Forest University, Winston-Salem, North Carolina (John Jeffrey Carr).

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