Kidney Dysfunction, Inflammation, and Coronary Events: A Prospective Study

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Abstract. Kidney dysfunction and high C-reactive protein (CRP) levels are independently associated with coronary events. However, it is unclear whether the risk of coronary events associated with decreased kidney function is at least partially mediated by inflammation and whether the association between inflammatory biomarkers and coronary events is influenced by level of kidney function. With the use of a prospective, nested, case-control study design, the association among kidney function, inflammatory biomarker levels, and coronary events was studied. A total of 244 women who were participants in the Nurses' Health Study and had no history of cardiovascular disease received a diagnosis of an incident coronary event (defined as nonfatal myocardial infarction or death as a result of coronary disease) during the follow-up period from 1990 to 1998 and were matched to 486 control subjects. Serum creatinine and inflammatory biomarker levels were measured in blood samples collected in 1989. Creatinine clearance (CrCl) was estimated using creatinine, age, weight, and height. In multivariate analyses, the odds ratio (OR) for a coronary event in women with an estimated CrCl <60 ml/min was 2.33 (95% confidence interval [CI], 1.01 to 5.38) compared with those with a CrCl ≥ 90 ml/min. When soluble tumor necrosis factor receptor (sTNFR) I and II levels were added into this model individually, the observed OR for women with CrCl <60 ml/min was attenuated. In analyses stratified by estimated CrCl, higher high-sensitivity CRP (hs-CRP), IL-6, and sTNFR I and II levels each were significantly associated with an increased odds of coronary events in women with an estimated CrCl ≤ 74 ml/min but not in women with an estimated CrCl ≥ 75 ml/min. The OR per 5-mg/L unit increase in hs-CRP was 1.68 (95% CI, 1.13 to 2.52) for women with an estimated CrCl ≤ 74 ml/min, compared with 1.23 (95% CI, 0.86 to 1.76) and 0.99 (95% CI, 0.76 to 1.29) for women with an estimated CrCl 75 to 89 and ≥ 90 ml/min, respectively ($P = 0.004$ for interaction). In conclusion, kidney dysfunction is associated with an increased odds of coronary events, and inflammation, as assessed by higher sTNFR I and II levels, may mediate some of this risk. Higher inflammatory biomarkers levels, specifically, hs-CRP, IL-6, and sTNFR I and II, were significantly associated with coronary events only in women with reduced kidney function. These findings warrant further investigation in other populations.

Kidney dysfunction is a risk factor for cardiovascular disease (1,2); however, the pathophysiologic mechanisms underlying this relationship are unclear. Several theories have been proposed. Kidney dysfunction may be an early marker of micro- and macrovascular disease (3). Kidney dysfunction is also associated with specific pathophysiologic changes, such as nocturnal hypertension ("nondipping"), (4) cardiac remodeling (4), changes in lipid parameters (5), insulin resistance (6), and impaired endothelial vasodilation (7), all of which may lead to cardiovascular disease.

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The increased cardiovascular risk in individuals with kidney dysfunction may also be at least partially mediated through chronic inflammation (8,9). In a large cross-sectional study, Shlipak *et al.* (8) reported that C-reactive protein (CRP), fibrinogen, and IL-6 levels were higher in individuals with kidney dysfunction (serum creatinine ≥ 1.3 mg/dl in women and ≥ 1.5 mg/dl in men), and Muntner *et al.* (10) found that individuals with an estimated GFR <60 ml/min per 1.73 m^2 had higher CRP, fibrinogen, and homocysteine levels compared with individuals with an estimated GFR ≥ 90 ml/min per 1.73 m^2. Therefore, we hypothesized that the association between kidney dysfunction and coronary events may be partially mediated by inflammation. The inflammatory biomarkers that we examined included high-sensitivity C-reactive protein (hs-CRP), E-selectin, fibrinogen, intracellular adhesion molecule-1 (ICAM-1), IL-6, soluble tumor necrosis factors (sTNFR) I and II, and vascular cell adhesion molecule-1 (VCAM-1). We also examined homocysteine and pyridoxal 5'-phosphate (PLP; the active form of vitamin B_6), because higher homocysteine lev-

els have been associated with increased inflammation (11), and higher PLP levels have been associated with decreased inflammation independent of homocysteine levels (12). We have also shown that low vitamin B_6 intake is associated with an increased odds of myocardial infarction (MI) (13).

Decreased renal function may also identify individuals who may be more susceptible to the adverse effects of inflammation on the development of coronary artery plaques and physical disruption of these plaques (14,15). This theory is supported by the observation that a combination of elevated CRP and reduced GFR predicted a fivefold increase in the risk of death or MI (16). Thus, we hypothesized that elevated inflammatory biomarker levels may be more likely to predict acute coronary events in individuals with reduced kidney function compared with individuals with normal kidney function.

Materials and Methods

Participants

The Nurses' Health study began in 1976, when 121,700 female nurses 30 to 55 yr of age completed a detailed questionnaire regarding health-related information. Since then, questionnaires ascertaining lifestyle factors and new medical diagnoses have been sent biennially. In 1989, 32,826 participants provided blood samples that were shipped on ice *via* overnight delivery and stored at −130°C. From these women, we identified 244 who were free of cardiovascular disease in 1989 and had a coronary event during follow-up from 1990 through 1998. We selected 486 control subjects, matched by age, smoking status, and month of blood draw, who did not have a coronary event during the same follow-up period. Because of a laboratory problem, two samples did not have all of the biomarker values measured, so these subjects were excluded from the analyses.

Assessment of Inflammatory Biomarker and Creatinine Levels

Levels of inflammatory biomarkers and creatinine were measured from the above blood samples. hs-CRP, E-selectin, fibrinogen, ICAM-1, sTNFR I and II, and VCAM-1 were measured at the Boston Children's Hospital Laboratory. The specific methods for these assays were reported previously (17). Creatinine was measured using a modified Jaffe method. PLP and homocysteine levels were measured at the Tufts University Nutrition Laboratory. PLP levels were assayed by an enzymatic procedure using radioactive tyrosine and the apoenzyme tyrosine carboxylase (18). Homocysteine levels were assayed using HPLC with fluorescence detection (19). The coefficient of variation for each assay was <10%.

The assessment of short-term stability of these inflammatory biomarkers was reported previously (17). In brief, in pilot testing in 17 women, these biomarkers showed good short-term stability. For example, the mean hs-CRP (mg/L) and sTNFR I and II levels (pg/ml) were 0.58, 963, and 2201 at time 0, respectively, and 0.59, 1074, and 2233 at 36 h, respectively. We also examined the 1-yr stability of these inflammatory biomarkers in 100 women who provided a blood sample in 1989 and a second sample 1 yr later. These biomarkers also showed good 1-yr stability. For example, the geometric mean hs-CRP (mg/L) and sTNFR I and II levels (pg/ml) in 1989 were 1.2, 1194, and 2437, respectively, and in 1990 were 1.4, 1179, and 2308, respectively. On similar analyses for creatinine, the geometric mean level was 0.73 mg/dl in 1989 and 0.75 in 1990.

Only women with measured creatinine levels were included in this study. Of the 730 women who were selected as cases and controls, the following number of individuals were missing the specified inflammatory biomarker: hs-CRP ($n = 18$), homocysteine ($n = 4$), IL-6 ($n = 33$), PLP ($n = 4$), VCAM-1 ($n = 3$), sTNFR I ($n = 3$), and sTNFR II ($n = 3$).

Assessment of Kidney Function

In accordance with national guidelines, for our primary analyses, we used a modified version of the Cockcroft-Gault formula to estimate creatinine clearance (20–22). This formula is based on fat-free body mass and has the advantage of attenuating the overestimation of creatinine clearance (CrCl) in obese individuals with the Cockcroft-Gault equation while providing similar results in average-weight women (22). The formula for women is $(146 - \text{age}) \times [(0.287 \times \text{weight}) + (9.74 \times \text{height}^2)]/(60 \times \text{creatinine})$, where age is measured in years, weight is measured in kilograms, height is measured in meters, and creatinine is measured in mg/dl; the units for CrCl are ml/min. This formula has been validated compared with measured CrCl (22). For secondary analyses, we used the simplified formula from the Modification of Diet in Renal Disease (MDRD) study to estimate GFR (20). This formula was empirically derived from 1070 individuals with kidney dysfunction using iothalamate GFR measurements and was subsequently validated in 558 individuals in the same study (23). The simplified formula is $186 \times \text{creatinine}^{-1.154} \times \text{age}^{-0.203} \times 0.742$ (multiplied by a factor of 1.210 for black race), where creatinine is measured in mg/dl, age is measured in years, and the GFR units are ml/min per 1.73 m^2 (20). We classified kidney function on the basis of categories recommended by the National Kidney Foundation (20). Specifically, estimated CrCl and GFR were initially classified as ≥90, 60 to 89, and <60 ml/min. Then, because a large proportion of women fell into the middle category, we created two separate categories within the 60 to 89 ml/min stratum: 60 to 74 and 75 to 89 ml/min. This left four categories of estimated CrCl and GFR: ≥90, 75 to 89, 60 to 74, and <60 ml/min.

Assessment of Other Factors

Potentially important confounders that we included were age, race (black or other), body mass index (BMI), estimated lean body mass, physical activity level, hemoglobin A1C level, total cholesterol level, HDL level, LDL level, diabetes, hypertension, smoking status, parental history of MI at age 60 or earlier, systolic BP, diastolic BP, aspirin use, nonsteroidal anti-inflammatory use, month of the blood draw, and fasting status at the time of the blood draw.

Age, race, weight, and height were obtained from the Nurses' Health Study biennial questionnaires. Weight was assessed on the 1990 questionnaire and, along with height, was used to calculate BMI [weight (kg)/(height (m))^2]. Reported weight has been previously validated in this cohort compared with measured weights ($r = 0.96$) (24). Lean body mass was estimated using the following formula: [weight (kg) × 0.287] + [9.74 × (height (m))^2] (25). Physical activity information was obtained from a validated questionnaire (26) administered in 1988, which asked about time spent per week walking, jogging, running, bicycling, swimming, playing tennis, and participating in other aerobic exercise. Using this information, we calculated a weekly metabolic-equivalent (MET) score for total physical activity. One MET is the caloric need per kilogram of body weight per hour of activity, divided by the caloric need per kilogram per hour at rest. Participants were classified as having diabetes or hypertension when a woman self-reported these conditions on any questionnaire from 1976 through 1990. Self-reported diabetes and hypertension have been previously validated in this cohort (27,28). Hemoglobin A1C was used to assess glucose control. Cigarette smoking status was

assessed on the 1990 questionnaire, and this information was used to classify participants as current, past, or never smokers. Parental history of MI was assessed on the 1984 questionnaire, and a positive response was recorded when either parent had an MI at age 60 or earlier. Self-reported systolic and diastolic BP were obtained from the 1990 questionnaire. Both aspirin and nonsteroidal anti-inflammatory use were also assessed on the 1990 questionnaire and were classified as days of use per month: none, 1 to 4, 5 to 14, 15 to 21, and 22 or more.

Assessment of Coronary Events

We included as cases individuals who had a validated nonfatal MI or death as a result of coronary disease from 1990 through 1998. Physicians with no knowledge of the individual's inflammatory biomarker or creatinine levels reviewed the medical records. The diagnosis of nonfatal MI was confirmed using World Health Organization criteria: symptoms in addition to either diagnostic electrocardiographic changes or elevated cardiac enzyme levels (29). Deaths were reported by family members or the U.S. Postal Service or identified from state vital records and the National Death Index. Fatal coronary disease (codes 410 through 414 of the *International Classification of Disease, Eighth Revision*) (30) was confirmed by review of the hospital or medical autopsy records or by review of the death certificate when coronary disease was the stated cause of death and evidence of previous coronary disease was available. The Brigham and Women's Hospital Institutional Review Board approved the study protocol.

Statistical Analyses

The mean and SD were calculated for all continuous variables stratified by level of CrCl. We compared inflammatory biomarker levels stratified by levels of estimated CrCl using the Wilcoxon signed-rank test, where an estimated CrCl <60 ml/min was the referent group. We also examined the Spearman correlation between each pair of inflammatory biomarkers.

For multivariate analyses, we used unconditional logistic regression and controlled for the matching and other factors. The following prespecified covariates were included in all models: age (continuous), black race (yes or no), BMI (continuous), lean body mass (continuous), physical activity level (continuous), hemoglobin A1C level (continuous), total cholesterol level (continuous), HDL level (continuous), diabetes (yes or no), hypertension (yes or no), smoking status (current, past, or never), and family history of MI at age 60 or earlier (yes or no). We did not include LDL level, systolic BP, diastolic BP, aspirin use, nonsteroidal anti-inflammatory use, month of blood draw, or fasting status at time of blood draw in our final multivariate analyses because they did not affect the primary associations or the interaction terms.

First, we examined the multivariate association between different levels of estimated CrCl and estimated GFR and coronary events. Then we included each inflammatory biomarker individually as a continuous variable in separate multivariate models to examine the impact of each biomarker on the parameter estimate for each level of CrCl. We also examined interaction terms between CrCl as a continuous variable and each inflammatory biomarker as a continuous variable. Next, we repeated these analyses using the natural logarithm of hs-CRP, because its distribution was right skewed. Finally, we repeated these interaction analyses using estimated GFR and serum creatinine instead of estimated CrCl.

To examine further the interaction between kidney function and specific inflammatory biomarkers, we performed additional multivariate logistic regression analyses stratified by three levels of estimated CrCl: ≥ 90, 75 to 89, and ≤74 ml/min. There were too few participants in the ≤60 ml/min subgroup to perform multivariate analyses in this group alone. We repeated these stratified analyses using the similar categories of estimated GFR.

We tested the goodness of fit of all of our reported models using the method of Lemeshow and Hosmer (31), and there was no evidence of lack of goodness of fit for any of the models. All analyses were performed using SAS software, version 8.2 (SAS Institute, Cary, NC).

Results

Baseline characteristics by level of CrCl are presented in Table 1. As expected, participants in the lowest estimated CrCl category (<60 ml/min) were older, had higher serum creatinine levels, had a higher prevalence of diabetes and hypertension, and were more likely to have a parental history of

Table 1. Baseline characteristics by category of estimated creatinine clearance[a]

	Estimated CrCl (ml/min)			
	≥90 (N = 349)	75–89 (N = 222)	60–74 (N = 125)	<60 (N = 34)
Age (yr)[b]	57.6 ± 6.8	61.7 ± 5.5	63.7 ± 4.4	65.4 ± 3.3
Creatinine (mg/dl)	0.6 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	1.2 ± 0.3
BMI (kg/m^2)	27.1 ± 5.5	25.0 ± 4.0	24.4 ± 3.9	24.1 ± 5.1
Physical activity (metabolic h/wk)	14 ± 15	15 ± 17	16 ± 18	14 ± 17
Hemoglobin A1C (g/dl)	5.9 ± 1.2	5.9 ± 0.9	5.9 ± 1.1	5.9 ± 0.9
Total cholesterol (mg/dl)	226 ± 41	228 ± 39	239 ± 39	233 ± 44
HDL (mg/dl)	56 ± 17	60 ± 16	59 ± 17	55 ± 22
Diabetes (%)	11%	12%	7%	21%
Hypertension (%)	37%	36%	45%	50%
Current smoker (%)	35%	29%	30%	26%
Parental history of MI at age 60 or less (%)	17%	14%	11%	24%

[a] CrCl, creatinine clearance; BMI, body mass index; MI, myocardial infarction.
[b] Continuous variables are presented as means ± SD.

premature MI. These participants also had lower BMI and were less likely to smoke. Overall, the racial distribution of the cohort was a follows: 599 white, 3 black, 3 Asian, and 125 unknown or not reported.

The mean and SD for each inflammatory biomarker level stratified by estimated CrCl are presented in Table 2. sTNFR I and II, ICAM, and VCAM levels each were significantly higher in the estimated CrCl <60 ml/min group compared with the other subgroups of estimated CrCl. In addition, homocysteine and IL-6 levels each were significantly higher in the <60 ml/min subgroup compared with the 75 to 89 and ≥90 ml/min subgroups (Table 2).

Compared with women with an estimated CrCl ≥90 ml/min, the age-, BMI-, and smoking-adjusted odds ratio (OR) for coronary events for participants with an estimated CrCl <60 ml/min was 3.33 (95% confidence interval [CI], 1.55 to 7.15). For participants with an estimated CrCl of 60 to 74 ml/min, the OR was 1.30 (95% CI, 0.81 to 2.10); for those with an estimated CrCl of 75 to 89 ml/min, the OR was 0.98 (95% CI, 0.66 to 1.45). After we adjusted for other factors, the point estimate for an estimated CrCl <60 ml/min was attenuated but remained statistically significant (Table 3). Our results were unchanged when we examined similar categories of estimated GFR. Specifically, the multivariate OR for coronary events was 2.31 (95% CI, 1.05 to 5.10) for an estimated GFR <60 ml/min per 1.73 m^2 compared with an estimated GFR ≥90 ml/min per 1.73 m^2. Because the proportion of individuals with the outcome is >10%, the OR will be higher than the relative risk.

After we included each inflammatory biomarker level individually into separate multivariate models, there was no material impact on the point estimate for coronary events for the groups with an estimated CrCl of 75 to 89 or 60 to 74 ml/min (Table 3). However, after sTNFR I and II were entered individually into separate multivariate models, we observed a diminution in the OR for coronary events for the group with estimated CrCl <60 ml/min (Table 3). There was a statistically significant interaction between estimated CrCl and hs-CRP, IL-6, and PLP (Table 4) for the odds of coronary events. The interaction between estimated CrCl and the natural logarithm of hs-CRP level was also statistically significant ($P = 0.02$). We did not observe a statistically significant interaction between estimated CrCl and E-selectin ($P = 0.43$), fibrinogen ($P = 0.61$), homocysteine ($P = 0.27$), ICAM-1 ($P = 0.12$), sTNFR I ($P = 0.08$), sTNFR II ($P = 0.06$), and VCAM-1 ($P = 0.79$) levels. We obtained similar results when we examined the interactions between estimated GFR and serum creatinine and these inflammatory biomarkers.

To explore these interactions further, we examined the association between inflammatory biomarkers and coronary events stratified by level of estimated CrCl. hs-CRP, IL-6, PLP, and sTNFR I and II levels each were significantly associated with coronary events in the estimated CrCl <74 ml/min stratum but not in the other strata (Table 4). Our results were qualitatively unchanged when we examined similar categories of estimated GFR. For example, the OR for a 5-mg/L increase in hs-CRP was 1.75 (95% CI, 1.17–2.61) in women with an estimated GFR <75 ml/min per 1.73 m^2, 1.22 (95% CI, 0.87–1.71) in women with an estimated GFR of 75 to 89 ml/min per 1.73 m^2, and 0.93 (95% CI, 0.70–1.23) in women with an estimated GFR ≥90 ml/min per 1.73 m^2.

Discussion

We found that an estimated CrCl <60 ml/min was associated with a significantly increased odds of having a coronary event compared with an estimated CrCl ≥90 ml/min after adjusting for multiple established cardiovascular risk factors. We did not observe a significant association between an estimated CrCl of 60 to 74 or 75 to 89 ml/min and increased odds of having a coronary event. In addition, adjusting for sTNFR I

Table 2. Unadjusted mean and SD of inflammatory biomarkers by category of estimated CrCl[a]

Biomarker	Estimated CrCl (ml/min)			
	≥90 ($N = 349$)	75–89 ($N = 222$)	60–74 ($N = 125$)	<60 ($N = 34$)
hs-CRP (mg/l)	4.5 ± 5.6	4.0 ± 4.6	4.5 ± 5.5	6.5 ± 7.8
E-selectin (ng/ml)	50.4 ± 24.4	47.4 ± 24.7	46.0 ± 20	50.7 ± 17.2
Fibrinogen (mg/dl)	349 ± 88	351 ± 98	367 ± 99	364 ± 104
Homocysteine (μmol/l)	10.3 ± 3.3[b]	11.3 ± 7.5[b]	11.9 ± 4.2	13.6 ± 5.8
ICAM-1 (ng/ml)	283 ± 89[b]	278 ± 111[b]	280 ± 89[b]	319 ± 84
IL-6 (pg/ml)	2.4 ± 2.8[b]	2.6 ± 3.5[b]	2.6 ± 2.4	4.3 ± 6.2
Pyridoxal 5′-phosphate (ng/ml)	60.9 ± 87.4	80.4 ± 168.0	67.1 ± 80.4	60.6 ± 68.7
sTNFR I (pg/ml)	1230 ± 343[b]	1284 ± 378[b]	1478 ± 419[b]	2104 ± 1004
sTNFR II (pg/ml)	2414 ± 691[b]	2539 ± 664[b]	2797 ± 785[b]	4024 ± 1553
VCAM-1 (ng/ml)	668 ± 163[b]	665 ± 143[b]	708 ± 153[b]	816 ± 193

To convert mg/L to mg/dl, divide by 10.

[a] hs-CRP, high-sensitivity C-reactive protein; sTNFR, soluble tumor necrosis factor receptor; ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion molecule.

[b] $P \leq 0.05$ compared with estimated CrCl <60 subgroup.

Table 3. Multivariate odds ratios for coronary events according to estimated CrCl and the impact of adjustment for individual inflammatory markers[a]

Biomarker	Estimated CrCl (ml/min)			
	≥90 (Cases = 117, Controls = 232)	75–89 (Cases = 65, Controls = 157)	60–74 (Cases = 43, Controls = 82)	<60 (Cases = 19, Controls = 15)
Base model[b]	1.00 (referent)	0.95 (0.62–1.46)	1.07 (0.63–1.83)	2.33 (1.01–5.38)
+hs-CRP (mg/l)	1.00 (referent)	0.98 (0.63–1.51)	1.17 (0.68–2.02)	2.17 (0.92–5.12)
+E-selectin (ng/ml)	1.00 (referent)	0.95 (0.61–1.46)	1.07 (0.63–1.83)	2.37 (1.02–5.48)
+Fibrinogen (mg/dl)	1.00 (referent)	0.95 (0.62–1.46)	1.08 (0.63–1.85)	2.34 (1.01–5.41)
+Homocysteine (μmol/L)	1.00 (referent)	0.98 (0.63–1.51)	1.16 (0.67–2.01)	2.52 (1.08–5.89)
+IL-6 (pg/ml)	1.00 (referent)	0.90 (0.57–1.40)	0.94 (0.54–1.65)	2.43 (1.02–5.81)
+ICAM-1 (ng/ml)	1.00 (referent)	0.95 (0.67–1.46)	1.07 (0.63–1.83)	2.31 (1.00–5.35)
+Pyridoxal 5′-phosphate (ng/ml)	1.00 (referent)	0.95 (0.61–1.47)	1.03 (0.60–1.77)	2.25 (0.97–5.21)
+sTNFR I (pg/ml)	1.00 (referent)	0.92 (0.59–1.43)	0.98 (0.56–1.72)	1.73 (0.67–4.45)
+sTNFR II (pg/ml)	1.00 (referent)	0.93 (0.60–1.44)	1.02 (0.58–1.77)	1.82 (0.71–4.67)
+VCAM-1 (ng/ml)	1.00 (referent)	0.96 (0.62–1.48)	1.10 (0.64–1.88)	2.39 (1.02–5.61)

[a] To convert mg/L to mg/dl, divide by 10.
[b] Adjusted for age, race, BMI, lean body mass, physical activity level, hemoglobin A1C level, total cholesterol level, HDL level, diabetes, hypertension, smoking status, and family history of MI.

Table 4. Multivariate[a] odds ratios for coronary events for hs-CRP, IL-6, pyridoxal 5′-phosphate, and sTNFR I and II levels as continuous variables stratified by estimated CrCl Level

Biomarker	Estimated CrCl (ml/min)			*P* Value for Interaction
	≥90 (Cases = 117, Controls = 232)	75–89 (Cases = 65, Controls = 157)	<75 (Cases = 62, Controls = 97)	
hs-CRP (per 5 mg/L)	0.99 (0.76–1.29)	1.23 (0.86–1.76)	1.68 (1.13–2.52)	0.004
IL-6 (per 10 pg/ml)	0.34 (0.07–1.53)	1.01 (0.38–2.71)	3.89 (1.18–12.85)	0.02
Pyridoxal 5′-phosphate (per 100 ng/ml)	0.96 (0.70–1.33)	0.92 (0.67–1.24)	0.54 (0.29–0.98)	0.05
sTNFR I (per 1000 pg/ml)	1.05 (0.43–2.57)	0.81 (0.28–2.32)	2.86 (1.25–6.55)	0.08
sTNFR II (per 1000 pg/ml)	0.85 (0.55–1.32)	1.09 (0.62–1.93)	1.64 (1.08–2.49)	0.06

[a] Adjusted for age, race, BMI, lean body mass, physical activity level, hemoglobin A1C level, total cholesterol level, HDL level, diabetes, hypertension, smoking status, and family history of MI. Note, the lowest cut-point of estimated CrCl is <74 ml/min, because there were too few participants in the <60 ml/min category to analyze this subgroup separately. To convert mg/L to mg/dl, divide by 10.

and II levels attenuated the association between an estimated CrCl <60 ml/min and coronary events, but these results must be interpreted with caution given the small number of individuals ($n = 34$) in this subgroup. Elevated levels of these inflammatory biomarkers may reflect inflammatory processes that may mediate some of the association between kidney dysfunction and coronary events. We also found that higher hs-CRP, IL-6, and sTNFR I and II levels and lower PLP levels each were significantly related to greater odds of coronary events in women with an estimated CrCl <75 ml/min but not in women with an estimated CrCl ≥75 ml/min.

Reduced kidney function may result from and cause a state of chronic inflammation (32), and inflammation may lead to coronary artery disease (33). In terms of the relation between kidney function and inflammatory biomarkers, our results are consistent with previous reports that CRP, IL-6, and sTNFR levels are higher in individuals with reduced kidney function (4,8,9,34–36). These levels may be higher because of increased production in individuals with kidney disease. For example, experimental kidney injury increases TNF-α mRNA expression (37). Angiotensin II levels may also be elevated in chronic kidney disease, and angiotensin II activates NF-κB, which activates the TNF gene, resulting in upregulation of sTNFR (32). Then TNF-α binds to these receptors, which stimulates the production of inflammatory cytokines (38). Some of these inflammatory biomarkers may also be elevated in individuals with lower renal function because of reduced clearance (39).

The reasons that hs-CRP, IL-6, PLP, and sTNFR I and II levels may selectively predict coronary event risk in individuals with reduced kidney function are unclear, and there is limited literature on this topic. One plausible hypothesis is that

the pathophysiologic changes associated with kidney dysfunction, such as endothelial dysfunction and increased hemodynamic stress, may make these individuals more vulnerable to the development and rupture of coronary artery plaques in the setting of increased chronic inflammation (15,16). Other pathophysiologic mechanisms also may play a role in increased sensitivity to chronic inflammation. For example, in individuals with ESRD, there is an increased prevalence of small-sized LDL subclass B, and these small-sized LDL particles sensitize vascular cells to inflammatory signals more than normal-sized LDL (40). Clearly, the pathophysiologic interrelationships between renal function, inflammation, and coronary events merit further study.

We cannot draw definitive conclusions about the relative and independent importance of increased hs-CRP, IL-6, PLP, and sTNFR I and II levels for predicting coronary event risk in women with reduced kidney function. The levels of these biomarkers were highly correlated, and only 159 women in the study (62 cases, 97 controls) had an estimated CrCl <75 ml/min.

Many prediction equations have been proposed to quantify kidney function, and there are multiple methods to classify kidney function. We used the prediction formulas and classification that were recommended by an expert panel of nephrologists under the auspices of the National Kidney Foundation (20). This panel recommended using the Cockcroft-Gault or the MDRD formula to estimate renal function (21,23). We chose to use a modified version of the Cockcroft-Gault formula, because the original formula overestimates CrCl in obese women (21,22). We did not use the MDRD formula as our primary estimate of kidney function because it has not been validated in a large group of individuals with normal or mildly reduced kidney function (20,23).

Several limitations of these analyses deserve discussion. First, this cohort was almost exclusively white women. Second, this population had only 34 subjects with CrCl <60 ml/min; thus, very few women had moderate or advanced chronic kidney disease. Therefore, our findings may not necessarily apply to other racial and ethnic groups and women with more advanced chronic kidney disease. Third, because we did not have a direct measurement of kidney function, such as inulin clearance, we may have misclassified kidney function. However, the primary formula that we used to estimate CrCl has been validated in a similar population using measured 24-h CrCl, and we obtained consistent results when we repeated our analyses using estimated GFR. Fourth, for parameters such as weight and hypertension, we relied on self-report, but previous Nurses' Health Study investigators have validated most of the self-reported variables that we used for these analyses (24,28). Fifth, we analyzed relatively large increments in biomarker levels across levels of renal function because of the limited number of cases.

In summary, the current study suggests that the association between renal function and coronary events may be partially but not completely mediated by inflammation, as measured by hs-CRP and sTNFR levels. In addition, in this population, higher hs-CRP, IL-6, and sTNFR I and II levels each were significantly associated with coronary events only in participants with reduced renal function. If these results are confirmed in other populations, then randomized trials will be needed to determine whether individuals with kidney dysfunction and elevated levels of these inflammatory biomarker levels may selectively benefit from preventive strategies to reduce inflammation and, consequently, coronary event risk (41).

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