Oxidative Stress and Inflammation Are Associated with Adiposity in Moderate to Severe CKD

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ABSTRACT

Adiposity contributes to inflammation and oxidative stress in the general population, but this association has not been examined in the chronic kidney disease (CKD) population. We investigated the relationship between body mass index, body fat percentage, and markers of inflammation (C-reactive protein) and oxidative stress (F_2-isoprostanes and protein thiols) in 184 patients with stages III to IV CKD and 43 healthy controls. We found that, on average, patients with CKD had 62% higher F_2-isoprostanes, 7% lower protein thiols (a measure of endogenous anti-oxidant capacity, inversely related to protein oxidation), and 150% higher C-reactive protein levels than healthy controls (all unadjusted $P < 0.001$). In separate multivariable linear regression models, body mass index and body fat percentage each positively correlated with levels of F_2-isoprostanes and C-reactive protein and negatively correlated with levels of protein thiols among patients with CKD after adjusting for age, sex, race, hypertension, diabetes mellitus, smoking history, estimated glomerular filtration rate, total cholesterol, serum albumin, and study site. We conclude that increased adiposity may amplify the oxidative stress and inflammation that accompany moderate to severe CKD. Interventions focused on weight loss may decrease the inflammatory and oxidative burden in CKD, which may ultimately attenuate cardiovascular risk in this population.

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Evidence suggests that as many as 19 million Americans have chronic kidney disease (CKD), chiefly defined as a persistently reduced GFR.[1] Furthermore, moderate to severe CKD is strongly and independently associated with increased risk for hospitalizations, cardiovascular events, and mortality.[2] Conventional cardiovascular risk factors exemplified in the Framingham study do not fully account for cardiovascular risk in patients with CKD, suggesting that "nontraditional" risk factors influenced by the uremic metabolic milieu may be particularly important.[3] Oxidative stress and inflammation are known to contribute to the pathogenesis of atherosclerosis, and markers of these processes are predictive of cardiovascular events and mortality rates in the general population and patients with advanced CKD.[4,5] Although increased oxidative stress and inflammation have been identified in all stages of CKD,[6–8] the pathophysiology of increased oxidative stress and inflammation associated with the development of CKD is poorly understood.

There is also growing concern about the epidemic of obesity in the United States, because the overall health status of the general population is adversely affected by increasing adiposity. Recent studies indicated a significant relationship between increasing adiposity and CKD incidence.[9,10] Furthermore, the presence of an elevated body mass index (BMI) is an independent predictor for progression to ESRD, even after additional adjust-

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ments for baseline blood pressure and the presence or absence of diabetes.[11] Similar to CKD, obesity is associated with chronic inflammation and oxidative stress.[12,13]

Few studies have examined the potential relationship between obesity and the inflammatory and oxidative milieu in the CKD population. We hypothesized that BMI and body fat percentage would each be independently associated with levels of oxidative stress and inflammation in individuals with moderate to severe CKD (stages 3 to 4 as defined by the Kidney Disease Outcomes Quality Initiative [KDOQI]).[14] To test this hypothesis, we examined the relationship between estimated GFR (eGFR), BMI, and body fat percentage and markers of lipid peroxidation (F_2-isoprostane concentration), protein oxidation (protein thiol concentration), and inflammation (C-reactive protein [CRP]) in 184 patients with stages 3 to 4 CKD. We compared these results with a group of 43 healthy subjects who had normal kidney function and were frequency matched for gender, race, and BMI.

RESULTS

Baseline Characteristics

Table 1 depicts baseline characteristics for the cohort of the 184 patients with stages 3 to 4 CKD and 43 healthy, matched control subjects. There were 112 patients with stage 3 and 72 patients with stage 4 CKD. Patients in the CKD group were significantly older than matched healthy subjects (66.6 ± 12.4 *versus* 59.1 ± 10.0 yr; $P < 0.001$). As expected, serum creatinine was significantly higher and eGFR significantly lower in patients with CKD compared with control subjects (serum creatinine 2.1 ± 0.67 *versus* 1.0 ± 0.24 mg/dl [$P < 0.001$]; eGFR 34.1 ± 10.9 *versus* 79.9 ± 16.6 ml/min [$P < 0.001$]). Serum albumin was similar between groups. Table 2 shows the prevalence of cardiovascular disease events and interventions in the 184 patients with CKD.

Table 1. Baseline characteristics between CKD and control groups

Characteristic	Control (*n* = 43)	CKD (*n* = 184)
Male gender (*n* [%])	20 (47)	107 (58)
Black race (*n* [%])	6 (14)	24 (13)
Age (yr; mean ± SD)	59.1 ± 10.0	66.6 ± 12.4[a]
Diabetes (*n* [%])	–	82 (45)
Weight (kg; mean ± SD)	83.2 ± 18.4	87.4 ± 21.9
BMI (kg/m^2; mean ± SD)	28.9 ± 4.2	30.6 ± 7.6
Body fat (%; mean ± SD)	31.5 ± 11.4	30.5 ± 13.1
Blood urea nitrogen (mg/dl; mean ± SD)	16.7 ± 4.2	39.1 ± 16.9[a]
Serum creatinine (mg/dl; mean ± SD)	1.0 ± 0.24	2.1 ± 0.67[a]
eGFR (ml/min; mean ± SD)	79.9 ± 16.6	34.1 ± 10.9[a]
Serum albumin (g/dl; mean ± SD)	4.4 ± 0.22	4.3 ± 0.38

[a] $P < 0.001$ between patients with CKD and control subjects.

Markers of Oxidation and Inflammation in Patients with CKD Compared with Control Subjects

Table 3 shows differences in F_2-isoprostanes, protein thiols, and CRP between CKD and control groups. Concentrations of F_2-isoprostanes were significantly elevated in patients with CKD compared with control subjects (0.081 ± 0.049 *versus* 0.050 ± 0.033 ng/ml; $P < 0.001$). Protein-reduced thiol content (a measure of endogenous antioxidant capacity inversely related to protein oxidation) was significantly decreased in the CKD group compared with control subjects (304.0 ± 55.2 *versus* 328.4 ± 33.3 μmol/L; $P < 0.001$). Levels of CRP were significantly higher in the CKD group than in control subjects (5.5 ± 7.6 *versus* 2.2 ± 1.9 mg/L; $P < 0.001$). After adjustment for age, gender, race, BMI, eGFR, history of diabetes, systolic BP, history of smoking, total cholesterol, and serum albumin with multivariable linear regression, the observed differences remained significant. Because recruitment of participants occurred at two different study sites, we further adjusted for study site location in the same regression model. After adjustment for study site in the model, F_2-isoprostanes ($P < 0.001$) remained significantly different between patients with CKD and healthy control subjects; however, CRP ($P = 0.06$) and protein thiols ($P = 0.80$) were not significantly different between groups after adjustment for study site.

Association between BMI and Markers of Oxidative Stress and Inflammation in Patients with CKD and Control Subjects

The associations of BMI with markers of oxidative stress and inflammation in both the CKD and control groups are represented in Figure 1. When participants were categorized by conventional BMI groups (lean <25.0, overweight 25 to 29.9, and obese ≥30), F_2-isoprostanes ($P = 0.01$), protein thiols ($P = 0.05$), and CRP ($P = 0.03$) were significantly different within the CKD group by Kruskal-Wallis test. CRP ($P = 0.01$) was the only biomarker significantly different within the control group. Interaction analysis may indicate a potential effect modification by disease status on the effect of BMI and CRP (P for interaction: F_2-isoprostanes = 0.21; protein thiols = 0.75; CRP = 0.10; Figure 1).

The potential relationships between BMI and F_2-isoprostanes, protein thiols, and CRP are depicted in Table 4. In unadjusted analysis, there was a significant positive correlation between BMI and F_2-isoprostanes ($r_s = 0.30$, $P < 0.001$) and CRP ($r_s = 0.24$, $P = 0.001$) in the CKD group. A significant negative correlation between BMI and protein thiols ($r_s = -0.18$, $P = 0.02$) was also seen in the CKD group. Controlling for age, eGFR, and history of diabetes with multivariable linear regression, the observed associations remained significant for the CKD group. Adjusted analysis showed significant associations between BMI and protein thiols and CRP in the BMI-matched control group. Among the CKD group, the associations also remained significant with further adjustment for gender, race, hypertension, smoking history, total cholesterol,

Table 2. Cardiovascular history in 184 patients with stages 3 and 4 CKD

Parameter	Stage 3 CKD (*n* [%]; *n* = 112)	Stage 4 CKD (*n* [%]; *n* = 72)	Combined CKD Stages (*n* [%]; *n* = 184)
Unstable angina	25 (22.3)	16 (22.2)	41 (22.3)
Acute myocardial infarction	25 (22.3)	10 (13.9)	35 (19.0)
Cerebrovascular accident	14 (12.5)	11 (15.3)	25 (13.6)
Congestive heart failure	10 (8.9)	9 (12.5)	19 (10.3)
Peripheral artery disease	22 (19.6)	15 (20.8)	37 (20.1)
Percutaneous coronary intervention	8 (7.1)	9 (12.5)	17 (9.2)
Coronary artery bypass graft surgery	18 (16.1)	11 (15.3)	29 (15.8)
Carotid endarterectomy	9 (8.0)	4 (5.6)	13 (7.1)
Peripheral artery disease surgery	9 (8.0)	8 (11.1)	17 (9.2)

Table 3. Differences in Markers of Oxidative Stress and Inflammation Between CKD and Controls Group

Inflammatory/Oxidative Biomarkers	Control (mean ± SD; *n* = 43)	CKD (mean ± SD; *n* = 184)	Unadjusted *P*[a]	Adjusted *P*[b]
Plasma F_2-isoprostanes (ng/ml)[c]	0.050 ± 0.033	0.081 ± 0.049	<0.001	<0.001
Protein thiols (μ mol/L)[c]	328.4 ± 33.3	304.0 ± 55.2	<0.001	0.050
CRP (mg/L)[c]	2.2 ± 1.9	5.5 ± 7.6	<0.001	0.010

[a]Mann-Whitney ***U*** test for unadjusted analysis between groups.
[b]***P*** values were obtained using multivariable linear regression adjusted for age, gender, race, BMI, eGFR, history of diabetes, systolic BP, history of smoking, total cholesterol, and serum albumin.
[c]Log_{10} transformed dependent variable in multivariable linear regression model.

serum albumin, and study site when the independent contribution of BMI on F_2-isoprostanes ($P < 0.001$,), protein thiols ($P = 0.01$), and CRP ($P = 0.001$) was assessed.

Similar correlation and regression analyses were conducted to assess the effect of body fat percentage on F_2-isoprostanes, protein thiols, and CRP (Table 5). In the CKD group, there was a strong positive correlation between body fat percentage and F_2-isoprostanes ($r_s = 0.45$, $P < 0.001$) and CRP ($r_s = 0.19$, $P = 0.01$). These associations remained significant with adjustment for age, eGFR, and history of diabetes using multivariable linear regression in the CKD group. Using the same multivariable linear regression model revealed associations between body fat percentage and F_2-isoprostanes in the BMI-matched control group. Among the CKD group, the associations were significant with further adjustment for gender, race, hypertension, smoking history, total cholesterol, serum albumin, and study site when the independent contribution of body fat percentage on F_2-isoprostanes ($P < 0.001$), protein thiols ($P = 0.02$), and CRP ($P = 0.001$) was assessed.

Correlation analyses were also conducted between two other bioimpedance parameters—phase angle and reactance—and measured biomarkers. Phase angle was significantly associated with F_2-isoprostanes ($r_s = -0.204$, $P = 0.01$), thiols ($r_s = 0.219$, $P = 0.004$), and CRP ($r_s = -0.194$, $P = 0.01$) in the CKD group but not in the control group. Using multiple linear regression analysis, phase angle was independently associated with F_2-isoprostanes ($P = 0.03$) but not with thiols or CRP in the CKD group. Reactance was not correlated with measured biomarkers in either the CKD or the control group.

DISCUSSION

Recent data indicate that CKD is associated with inflammation and oxidative stress, and this metabolic milieu may contribute significantly to the excessive cardiovascular disease seen in these patients. Multiple investigations have shown that obesity is an independent risk factor for incident CKD and progression to ESRD.[9–11,15,16] In this study, we hypothesized that BMI and body fat percentage are independently associated with increased oxidative stress and inflammation in stages 3 to 4 CKD. Our results indicate that patients with moderate to severe CKD have significantly higher F_2-isoprostanes and CRP and significantly lower protein thiols compared with healthy control subjects. BMI and body fat percentage were highly associated with the measured markers of inflammation and oxidative stress in the CKD group. In addition to body fat percentage, phase angle, a bioimpedance parameter estimated by bioelectrical impedance analysis (BIA), was associated with oxidative stress and inflammation in the CKD group. In the control group, BMI and body fat percentage were also associated with the measured markers, highlighting the contribution of adiposity to the metabolic milieu regardless of kidney function. Our interaction analysis indicated that there may be a potential effect modification by disease status on the effect of BMI and CRP (Figure 1). In separate multivariable linear regression models, BMI and body fat percentage each were independently associated with oxidative stress and inflammation in patients with CKD. Previous studies have shown significant associations between obesity and inflammation in early stages of CKD and those who had ESRD and were on hemodialysis.[17–21] To our knowledge, this is

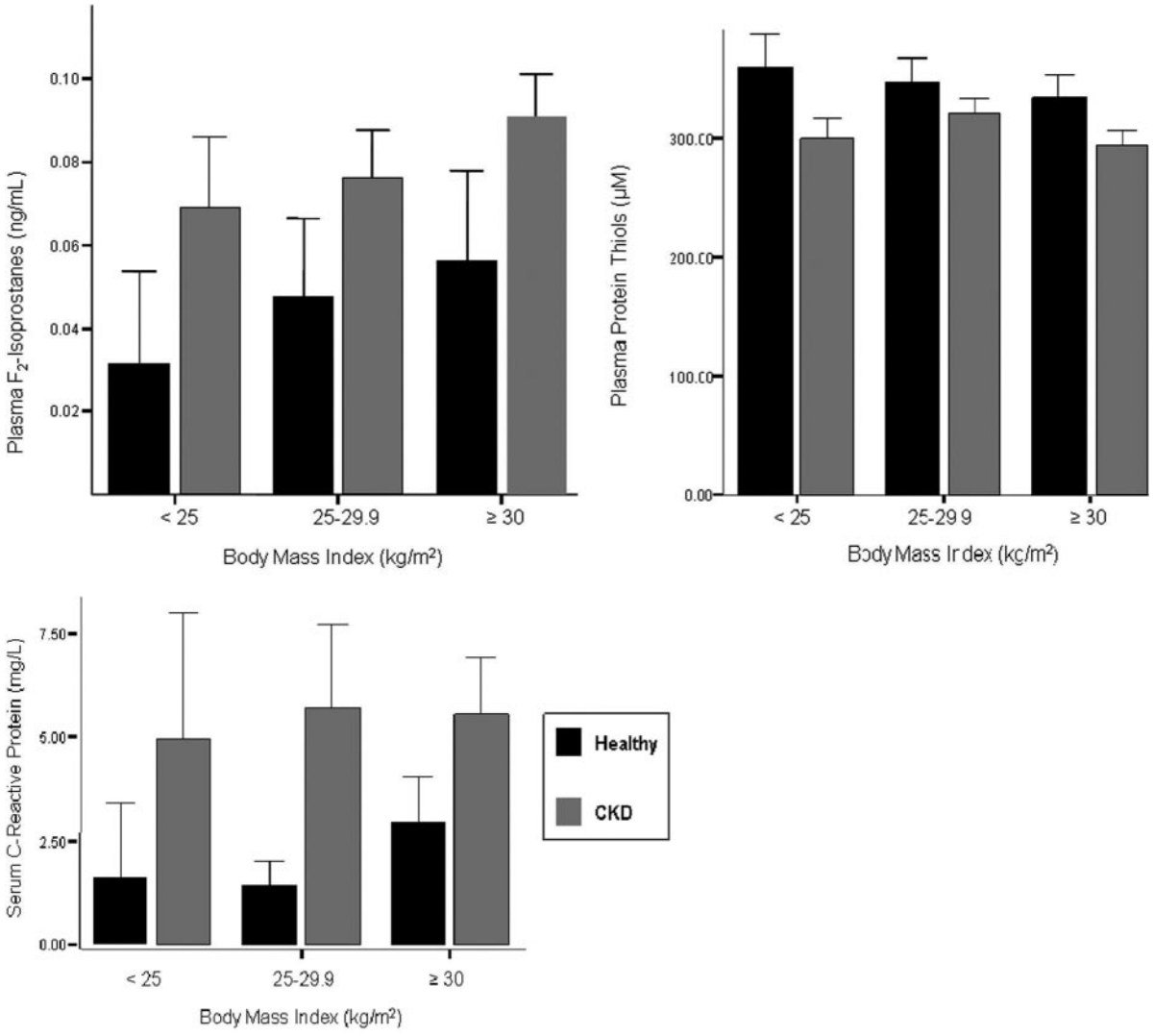

Figure 1. Relationship between BMI and biomarkers of inflammation and oxidative stress in 184 patients with CKD and 43 control subjects. The effect of BMI on F_2-isoprostanes ($P = 0.21$ for interaction) and protein thiols ($P = 0.75$ for interaction) was similar in CKD and control groups. There may be a potential effect modification by disease status on the effect of BMI and CRP ($P = 0.10$ for interaction).

Table 4. Unadjusted and adjusted correlation of BMI ***versus*** markers of oxidative stress and inflammation

Parameter	CKD ($n = 184$)			Controls ($n = 43$)		
	r_s	P [a]	Adjusted P [b]	r_s	P [a]	Adjusted P [b]
F_2-isoprostanes[c]	0.30	<0.001	<0.001	0.45	0.020	0.600
Protein thiols	−0.18	0.020	0.003	−0.18	0.270	0.040
CRP[c]	0.24	0.001	<0.001	0.47	0.002	0.010

[a]Spearman rank correlation for unadjusted analysis between BMI and markers of oxidative stress and inflammation.
[b]Multivariable linear regression was used to control for age, eGFR, and history of diabetes.
[c]Log_{10} transformed dependent variable in multivariable linear regression model.

the first study to examine a potential relationship between adiposity and markers of lipid peroxidation, protein oxidation, and inflammation in patients with moderate to severe CKD.

Inflammation and oxidative stress are known to be prevalent in patients with CKD.[22] We previously showed that increased markers of oxidative stress and inflammation are present in stages 3 to 5 CKD before initiation of maintenance dialysis but that levels did not correlate with eGFR.[6] These findings suggest that the mere presence of CKD, regardless of the level of GFR, is an important driver of the inflammatory and oxidative process. Similarly, adiposity is highly correlated with inflammation and oxidative stress.[13,23–25] Visceral fat secretes proinflammatory cytokines that attract macrophages to infiltrate adipocytes, leading to further release of cytokines and

Table 5. Unadjusted and adjusted correlation of body fat percentage ***versus*** markers of oxidative stress and inflammation

Parameter	CKD ($n = 184$)			Controls ($n = 43$)		
	r_s	P [a]	Adjusted P [b]	r_s	P [a]	Adjusted P [b]
F_2-isoprostanes[c]	0.45	<0.001	<0.001	0.66	<0.001	0.002
Protein thiols	−0.09	0.230	0.060	−0.06	0.700	0.640
CRP[c]	0.19	0.010	0.003	0.36	0.020	0.090

[a]Spearman rank correlation coefficients for unadjusted analysis between body fat percentage and markers of oxidative stress and inflammation.
[b]Multivariable linear regression was used to control for age, eGFR, and history of diabetes.
[c]Log_{10} transformed dependent variable in multivariable linear regression model.

oxygen free radicals, which may ultimately cause oxidative damage and atherosclerosis.[26,27] Our findings suggest that adiposity may be a potent, independent amplifier to the inflammatory and oxidative milieu already present in CKD.

Our study has important implications for the CKD population. These findings suggest that obesity, *via* vascular damage induced by oxidative stress and inflammation, may be an additional nontraditional cardiac risk factor responsible for the accelerated atherosclerosis and high cardiovascular mortality in the earlier stages of CKD. The potential contribution of the inflammatory and oxidative state to atherogenesis is clinically significant when considering that the majority of patients with CKD, approximately 19 million in the United States alone, will succumb to atherosclerotic disease before requiring renal replacement therapy.[28] In addition to contributing to cardiovascular risk, obesity is an independent predictor of incident CKD and progression to ESRD.[9–11,15,16] Thus, it is logical to hypothesize that weight loss may be an effective therapeutic strategy that not only prevents the development of CKD but also mitigates the inflammatory and oxidative burden that may lead to accelerated atherosclerosis in renal disease.

There are limitations to our study. BMI is an insensitive marker of adiposity. Abdominal waist circumference, which is a better measure of visceral adiposity, was not measured in our study. We recognize that body composition analysis by BIA may be confounded by factors such as extracellular volume, diet, and physical inactivity. None of the study patients with CKD had significant volume overload, malnutrition, or physical inactivity because potential participants with these comorbidities were excluded from the study. In addition, the recruitment of research participants from two different medical institutions may be a potential source of bias; however, after adjustment for study site in the multivariable linear regression models, BMI and body fat percentage each were independently associated with oxidative stress and inflammation. The cross-sectional study design does not prove a causal relationship between BMI and markers of oxidation and inflammation. There is no evidence from this investigation that the association among adiposity, inflammation, and oxidative stress is predictive of cardiovascular morbidity and mortality or will translate into improved cardiovascular end points if patients with stages 3 to 4 CKD were to lose significant fat weight.

In summary, we have shown that elevated BMI and body fat percentage independently predict oxidative stress and inflammation in moderate to severe CKD. Obesity and CKD each are responsible for a disproportionate degree of cardiovascular disease morbidity and mortality, and the medical and financial impacts of both diseases pose significant public health challenges for the US health care system. Randomized clinical studies investigating whether weight loss by dietary or exercise interventions mitigate the oxidative and inflammatory burden in the obese, stages 3 to 4 CKD population are warranted.

CONCISE METHODS

Patients

This was a cross-sectional study of 184 patients with moderate to severe CKD. Patients were recruited from the outpatient nephrology clinics at Maine Medical Center (Portland, ME) and Vanderbilt University Medical Center (Nashville, TN). Criteria for study participation included patients who had CKD of any cause and were followed in one of the previously mentioned nephrology clinics, the presence of stages 3 to 4 CKD (as defined by an eGFR 30 to 59 ml/min for stage 3 and eGFR 15 to 29 ml/min for stage 4) as measured by the Modification of Diet in Renal Disease (MDRD) formula,[14] were age >18 yr, and could provide informed consent for study participation. Patients who had acute inflammatory illnesses, hospitalization for cardiac or infection-related morbidity within 6 wk before study, severe comorbid complications, or previous kidney transplantation; were on experimental drug protocols; were pregnant; or were otherwise vulnerable were excluded from the study. Control subjects ($n = 43$) between the ages of 45 and 80 yr were frequency matched for BMI, race, and gender to the CKD group. Control subjects were recruited from both Maine Medical Center and Vanderbilt University Medical Center *via* e-mail communication. All control subjects had a normal eGFR and no previous diagnosis of diabetes or hypertension. The study was approved by each center's respective institutional review board, and all patients provided written informed consent before study enrollment.

History and Physical Information

Patient history including demographic data, cardiovascular events, diabetic status, and smoking history were collected for all participants. Unstable angina; acute myocardial infarction; acute cerebrovascular accident; congestive heart disease; peripheral vascular disease; percutaneous intervention with angioplasty with or without stent placement; and revascularization by coronary artery bypass, carotid endarterectomy, or femoral/aorto-iliac bypass were defined as cardiovascular events. Physical examinations were performed and included BP, heart rate, weight, height, and BMI assessments. Body fat percentage, measured by BIA, was also obtained for all participants.

Analytical Procedures

Blood Samples.

All blood draws were performed at the General Clinical Research Center (Vanderbilt) or Research Core Laboratory (Maine Medical Center Research Institute) of the participating institutions. Blood samples included routine chemistries, nutritional markers (albumin, prealbumin, lipid panel, glucose, glycosylated hemoglobin), serum CRP, plasma F_2-isoprostanes, and plasma protein thiols. Venous blood was drawn into Vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ) containing EDTA supplemented with 1000 U/ml catalase and serum separator tubes containing clot activator for plasma and serum separation. Samples for plasma collection were transported on ice and immediately centrifuged at 4°C at 1700 × *g* for 15 min, whereas the samples for serum collection were allowed to clot at room temperature before centrifugation. Supernatants were stored in aliquots at −70°C until further use.

Serum CRP.

Serum CRP levels were measured using the high-sensitivity particle-enhanced immunoturbidimetric assay by Roche Modular System (Indianapolis, IN). Data are expressed in milligrams per liter.

Plasma F_2-Isoprostane Concentration.

Plasma F_2-isoprostanes were measured by gas chromatography/negative-ion chemical ionization mass spectrometry as described by Morrow *et al.*[29] The precision of the assay was ±6%, with an accuracy of 96%. Data are expressed in nanograms per milliliter.

Plasma Protein Thiol Concentration.

Plasma protein–reduced thiol group content was assayed according to the method of Ellman[30] as modified by Hu *et al.*[31] as we have previously described.[7] Briefly, 1 ml of buffer containing 0.1 mol/L Tris, 10 mmol/L EDTA (pH 8.2), and 50 μl of plasma was added to cuvettes, followed by 50 μl of 10 mmol/L 5′5′dithio-bis (2-nitrobenzoic acid) in methanol. Blanks were run for each sample, prepared as described previously, with the exception that there was no 5′5′dithio-bis (2-nitrobenzoic acid) in the methanol. After incubation for 15 min at room temperature, sample absorbance was read at 412 nm on a Lambda 2 spectrophotometer (Perkin Elmer, Norwalk, CT). Sample and reagent blanks were also subtracted. The concentration of sulfhydryl group was determined using the 5-thio-2-nitrobenzoic acid molar extinction coefficient of 14,100 M/cm. The coefficient of variation for this assay was 2.67%. Data are reported as micromoles per liter.

Calculations

GFR was estimated using the abbreviated equation described in the MDRD study: $\text{eGFR} = 186.3 \times \text{serum creatinine}^{-1.154} \times \text{age}^{-0.203} \times 0.742$ (if female) × 1.21 (if black).[14] BMI was defined as the individual's body weight in kilograms divided by the square of the height.

Bioelectrical Impedence Analysis

Body fat percentage, phase angle, and reactance were determined using a bioelectrical impedance analyzer (RJL Systems, Clinton Twp, MI). The participants were placed in the supine position with their arms at but not touching their sides and with their legs apart. Disposable impedance plethysmography source electrodes were positioned on the dorsal surface of the wrist on the right side and the anterior surface of the ipsilateral ankle. The proximal detector electrodes were placed between the distal prominences of the radius and ulna and between the malleoli of the ankle. A current of 800 μA at 50 kHz was applied to the participant at the distal electrodes. A voltage drop detected through the proximal electrodes records impedance. Resistance and reactance were measured as current flows through the compartments of the body and used to determine overall fat percentage.[32]

Statistical Analyses

To assess whether the oxidative stress and inflammatory state were associated with advanced CKD, we first compared the level of oxidative stress and inflammatory markers between the patients with CKD and control subjects. Baseline characteristics and markers of oxidative stress and inflammation were compared between the CKD and control groups using the Mann-Whitney *U* test for continuous variables. χ^2 test was used to assess differences between categorical variables. Multivariable linear regression analysis was performed to adjust for *a priori* chosen confounding variables (age, gender, race, BMI, eGFR, history of diabetes, systolic BP, history of smoking, total cholesterol, and serum albumin) because they were thought to be associated with both oxidative stress and inflammatory markers. For graphic presentation, participants were categorized using conventional BMI groups (lean <25.0, overweight 25 to 29.9, and obese ≥30) to illustrate BMI's association with F_2-isoprostanes, protein thiols, and CRP values separately among patients with CKD and control subjects. None of the participants was considered underweight (BMI <18.5). Kruskal-Wallis tests were conducted to assess the equivalence in measured biomarkers among BMI categories.

To study the pathophysiology of increased oxidative stress and inflammation associated with CKD, we assessed the effect of BMI and body fat percentage on F_2-isoprostanes, protein thiols, and CRP separately for patients with CKD and control subjects using Spearman correlation coefficients (r_s). Multivariable linear regression was used to assess the independent effect of BMI on F_2-isoprostanes, protein thiols, and CRP controlling for age, eGFR, and history of diabetes separately in the CKD and control groups. For assessment of whether the effects of BMI on biomarkers are modified between patients with CKD and control subjects, a cross-product term between BMI and disease status was included in the multivariable linear regression analysis. To investigate the contribution of adiposity, rather than body size estimated by BMI, we substituted body fat percentage for BMI in separate linear regression models. For all analyses, BMI and body fat percentage were analyzed as continuous variables.

Because the analysis of the CKD group included a large number of participants, we further conducted analyses adjusting for a set of covariates including gender, race, hypertension, smoking history, total cholesterol, serum albumin, and study site in addition to age, eGFR, and history of diabetes. The variables were selected *a priori* on the basis of their clinical significance and possible effect on inflammation and oxidative stress. Similar multivariable linear regression models were performed substituting body fat percentage for BMI.

For all analyses using linear regressions, residual assumptions were verified for normality. When residual analysis did not fulfill the assumptions of normality, $\log_{10}$ transformation of the dependent vari-

able was performed. The statistical software package SPSS 14.0 (SPSS, Chicago, IL) was used for analyses, and two-sided $P < 0.05$ was required to reject the null hypothesis. Two-sided $P < 0.20$ was required to indicate a potential interaction.

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DISCLOSURES

None.

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