ORIGINAL ARTICLE

Inflammation and Arterial Stiffness in Chronic Kidney Disease: Findings From the CRIC Study

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BACKGROUND
Chronic kidney disease (CKD) and arterial stiffness are associated with increased cardiovascular morbidity and mortality. Inflammation is proposed to have a role in the development of arterial stiffness, and CKD is recognized as a proinflammatory state. Arterial stiffness is increased in CKD, and cross-sectional data has suggested a link between increased inflammatory markers in CKD and higher measures of arterial stiffness. However, no large scale investigations have examined the impact of inflammation on the progression of arterial stiffness in CKD.

METHODS
We performed baseline assessments of 5 inflammatory markers in 3,939 participants from the chronic renal insufficiency cohort (CRIC), along with serial measurements of arterial stiffness at 0, 2, and 4 years of follow-up.

RESULTS
A total of 2,933 participants completed each of the follow-up stiffness measures. In cross-sectional analysis at enrollment, significant associations with at least 2 measures of stiffness were observed for fibrinogen, interleukin-6, high-sensitivity C-reactive protein, proteinuria, and composite inflammation score after adjustment for confounders. In longitudinal analyses, there were few meaningful correlations between baseline levels of inflammation and changes in metrics of arterial stiffness over time.

CONCLUSION
In a large cohort of CKD participants, we observed multiple significant correlations between initial markers of inflammation and metrics of arterial stiffness, but baseline inflammation did not predict changes in arterial stiffness over time. While well-described biologic mechanisms provide the basis for our understanding of the cross-sectional results, continued efforts to design longitudinal studies are necessary to fully elucidate the relationship between chronic inflammation and arterial stiffening.

Keywords: arterial stiffness; blood pressure; hypertension; inflammation; chronic kidney disease.

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Chronic kidney disease (CKD) is strongly associated with incident cardiovascular disease (CVD), and participants with end stage renal disease are 10–20 times more likely to die from CVD events than the general population.[1,2] Studies have demonstrated an incremental increase in CV risk even in milder CKD, with an inverse relationship between estimated glomerular filtration rate and CV events starting at glomerular filtration rate <90 ml/min/1.73 m^2.[3] While traditional CVD risk factors such as diabetes, hypertension (HTN), and dyslipidemia are more prevalent in the CKD population, their presence alone does not account for this excess risk of CV events and have historically performed poorly in predictive

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models.[3,4] The failure of traditional risk factors to explain the increased CVD risk of CKD participants has led to growing interest in nontraditional risk factors such as arterial stiffness, inflammation, and endothelial dysfunction.

Commonly used noninvasive measures of arterial stiffness including pulse wave velocity (PWV), augmentation index (AI), central pulse pressure (CPP), are higher, and peripheral pulse pressure amplification (PPA) is lower, in participants with CKD suggesting a greater degree of arterial stiffness compared with matched controls.[5] Arterial stiffness is an independent predictor of CVD morbidity and mortality,[6] and the relationship between increasing arterial stiffness and CVD events has been demonstrated in both high risk groups such as CKD or HTN[6] as well as in the general population without diagnosed risk factors.[7]

Endothelial dysfunction is a fundamental step in the pathogenesis of vascular disease, initiating a cascade of events that results in the development of stenoses, plaques, and aneurysms. Inflammation is the major underlying mechanism behind endothelial dysfunction,[8,9] and studies have demonstrated strong associations between endothelial dysfunction and increased arterial stiffness.[4,10,11] This suggests a potential causal relationship between inflammation and stiffness, but no study has been designed to examine this etiologic connection in a large prospective cohort of CKD participants. In this manuscript, we report findings from the chronic renal insufficiency cohort (CRIC) examining the relationship of inflammatory markers to arterial stiffness over a 4 year period.

METHODS

Details regarding the design and baseline participant characteristics of the CRIC study have been published.[12,13] Participating Clinical Centers obtained Institutional Review Board approval for all aspects of the protocol and written informed consent was obtained from all participants. Briefly, between 2003 and 2008 a total of 3,939 participants with CKD defined by age-specific levels of kidney function were enrolled in a longitudinal cohort study. Beginning at the second year follow up in the CRIC study, measures of arterial stiffness (carotid-femoral PWV) and biochemical analyses of a panel of representative markers of inflammation were incorporated into the protocol. Standard lab assays (blood and urine), and measures of body habitus, demographics, heart rate, and blood pressure were undertaken per the CRIC protocol as published previously.[12]

Arterial stiffness measurements

PWV was recorded supine using the Sphygmocor system (West Ryde, Australia). The distance from the sternal notch to the carotid in mm was subtracted from the distance from the sternal notch to the femoral site in mm for the pulse wave path length. A modified limb lead III electrocardiogram signal was used to time the onset of the pulse wave foot in each location, and the average of 10 seconds worth of data for each of the 2 sites was obtained. The average time to the carotid foot of the pulse wave was subtracted from the average time to the femoral foot of the pulse wave and divided into the path length distance to arrive at the PWV, and is reported as meters/second. Our PWV results on 2,564 participants in the CRIC population have been published.[14] Pulse wave analysis to determine CPP, AI, and the degree of PPA (brachial PP/central PP) was estimated from the supine radial waveform by tonometry as previously described.[15]

Inflammation marker measurements

We used enzyme linked immunosorbent sandwich assays (ELISA) of high-sensitivity sandwich-type (Quantikine HS, R&D Systems, Minneapolis, MN) to measure plasma concentrations of interleukin 1-beta (IL-1β), IL-6, and tumor necrosis factor (TNF)-α levels. We used standard sandwich ELISAs (Quantikine, R&D Systems) to measure plasma IL 1-receptor antagonist (IL-1RA) and transforming growth factor-β levels. In our lab, the lower detection limit for assay of IL-1β was 0.06 pg/ml, for IL-6 it was 0.07 pg/ml, for TNF-α 0.11 pg/ml, for IL-RA 6.3 pg/ml, and for transforming growth factor-β it was 4.6 pg/ml. ELISAs were undertaken using a robotic liquid handling platform (Biomek FXp, Beckman Coulter, Brea, CA). The samples were stored at −80 °C after initial sample acquisition and assays were performed in duplicate at the time of initial thawing to prevent biomarker degradation.[16] Several samples had a concentration of IL-1β below the minimal level for detection to which we assigned a very low value for IL-1β (0.00001 pg/ml). In general, the coefficient of variation CV was <13% for all cytokines assays with the exceptions of TNF-α (15.2%) and transforming growth factor-β (21.5%). High-sensitivity C-reactive protein (hs-CRP) and fibrinogen were measured in plasma samples using specific laser-based immunonephelometric methods on the BNII (Siemens Healthcare Diagnostics, Deerfield, IL). The imprecision estimates for hs-CRP and fibrinogen were <5% for each. All these tests were performed in a single laboratory at the time of initial thawing.

Computing an inflammation score

An inflammation score predicts more accurately a phenotype of interest than does a single biomarker of inflammation.[17–19] In these reports, inflammation was said to be present in a subject if his/her serum level of any inflammatory biomarker exceeded its median value for the whole cohort. Since we have 5 representative biomarkers, we calculated a composite score for each participant that ranged from 0 to 5 based on a scoring system in which a "1" was assigned (a) hs-CRP >3 mg/l,[17] (b) fibrinogen >350 mg/dl,[18] (c) IL-6 ≥6 pg/ml,[19] (d) TNF-α ≥7 pg/ml, and (e) IL-1β≥ 0.39 pg/ml.[19,20] The cutoff values were chosen from published literature.

Statistical methods

Continuous variables were described using mean (SD) or median (interquartile range) as appropriate. Categorical variables were described using frequency and proportions.

The differences of baseline characteristics were compared across PWV tertiles. *P* values were calculated using chi-square test for categorical variables and analysis of variance for continuous variables. Linear regression models were fit to explore the cross-sectional associations between different measures of inflammatory markers (including total inflammatory score) and measures of stiffness at baseline. Variables that were adjusted in the model included demographics, mean arterial pressure, diabetes, smoking status, hemoglobin, total cholesterol, estimated glomerular filtration rate, and use of ACE inhibitor or angiotensin receptor blocker medication, all measured at baseline. To explore the associations of baseline inflammatory markers and longitudinal change in measures of stiffness, we employed linear mixed effects model which takes in account the correlated nature of the repeated measures from the same individual and allows estimation of individual intercept and slope terms. In addition to the main effect terms, the model included the interaction term between baseline inflammatory score and time, which represents its association with the change of stiffness measure and is of primary interest. We adjusted for the same covariates as were in the cross-sectional analyses. All analyses were done in SAS (version 9) and $P < 0.05$ was considered statistically significant.

RESULTS

Of 3,939 participants in the CRIC cohort, 2,933 participants completed follow-up assessments of PWV, AI, PPA, and CPP at both 2 and 4 years and were included in the final analysis (Table 1). The study population was majority male (56.6%) and racially diverse (44% non-Hispanic Black, 39.4% White, 12.9% Hispanic, 4.1% other). The vast majority had HTN (84.2%) and hyperlipidemia (81.2%), roughly half were obese or diabetic, 11% were active smokers, and more than two-thirds were on an ACE inhibitor or angiotensin receptor blocker. The mean PWV for the total population studied was 9.55 m/s, mean AI was 27.05, mean CPP was 46 mm Hg, and mean PPA was 1.29.

For further analysis of baseline characteristics, the population of the study was divided into tertiles of PWV. Table 1 shows the study population data by tertiles, along with measures of significance. Increasing tertiles of PWV were positively associated with age, male gender, Hispanic, and non-Hispanic Black ethnicity, HTN, diabetes, hyperlipidemia, CVD, congestive heart failure, current smoking, waist circumference, aspirin use, statin use, all antihypertensive medications use, 24-hour urine protein, serum creatinine, mean arterial pressure, baseline AI, and CPP, cystatin C level, total plasma homocysteine level, insulin level. Increasing tertiles of PWV were inversely proportional to level of education, exercise tolerance (in METs), hemoglobin level, serum albumin, total serum cholesterol, serum high-density lipoprotein, serum low-density lipoprotein, estimated glomerular filtration rate, and baseline PPA.

Table 2 shows cross-sectional data, comparing baseline measures of inflammation with initial measurements of arterial stiffness. In the unadjusted results, increasing PWV was significantly associated with increasing inflammation score, serum fibrinogen, IL-6, IL-10, IL-1RA, TNF-α, hs-CRP, and decreasing levels of serum albumin. In the adjusted model, increasing PWV was significantly associated only with fibrinogen and IL-10, though there was a trend suggesting association with inflammation score ($P = 0.079$).

In the unadjusted results, increasing tertiles of CPP were significantly associated with increasing inflammation score, serum fibrinogen, IL-6, IL-1RA, IL-1B, TNF-α, and with decreasing levels of serum albumin. In the adjusted model, increasing CPP had significant positive associations with serum fibrinogen and with hs-CRP.

Increasing tertiles of AI were associated with increasing TNF-α levels. In the adjusted model, AI was negatively associated with inflammation score, fibrinogen, IL-6, IL-1RA, and hs-CRP.

With regards to PPA, in the unadjusted results, there were no significant associations. In the adjusted model, PPA was associated with increasing inflammation score, serum fibrinogen, IL-6, and hs-CRP.

Table 3 describes the longitudinal data, comparing baseline inflammation with the changes in arterial stiffness during the 4 years follow-up. These data are notable for the relative lack of important correlations, with increasing PWV associated with higher albumin levels and increasing AI over time associated with higher IL-10 levels. There are several examples of correlations that approach the cutoff for statistical significance in the longitudinal data, with levels TNF-α positively correlated with increasing levels of PPA, and negatively correlated with increasing values of AI.

DISCUSSION

We observed several significant associations between systemic inflammation markers and baseline measurements of arterial stiffness. While several smaller studies have demonstrated similar findings using CRP, TNF-α, or IL-6[20,21] no prior work has examined this relationship with such a diverse panel of inflammatory markers, in a large CKD population with a wide range of kidney function, or prospectively with serial measurements of stiffness over years of follow-up. We found that CRP, fibrinogen, IL-6, and TNF-α were each associated with increased levels of at least 2 of the 4 different metrics of stiffness at baseline, suggesting a relationship worthy of further consideration. Although a robust predictive value of baseline inflammation was not seen with changes in arterial stiffness over time, these results have important implications for future research related to the mechanisms and treatment of arterial stiffness.

Increased plasma levels of TNF-α and IL-6 have been associated with increased arterial stiffness in prior studies.[20,21] TNF-α induces the production of IL-6 in myoblasts,[22] and upregulates CRP and other acute phase proteins in the liver. CRP exerts important effects on arterial wall physiology by promoting endothelial dysfunction through downregulation of endothelial nitric oxide synthase and upregulation of endothelin receptors on endothelial cells. This results in decreased nitric oxide production, increased endothelin binding, with increased vascular stiffness.[23–26] CRP also

Table 1. Total study population demographics and population demographics by tertile of PWV

	Total (*n* = 2933)	PWV (<7.9, *n* = 968)	PWV (7.9–10.3, *n* = 997)	PWV (>10.3, *n* = 968)	*P* value[a]
Age	58.09 (SD: 10.93)	52.70 (11.92)	58.89 (9.86)	62.67 (8.34)	<0.0001
Female (%)	1272 (43.37%)	463 (47.83%)	422 (42.33%)	387 (39.98%)	0.0016
Race					
Non-Hispanic White	1290 (43.98%)	507 (52.38%)	418 (41.93%)	365 (37.71%)	<0.0001
Non-Hispanic Black	1156 (39.41%)	304 (31.40%)	409 (41.02%)	443 (45.76%)	<0.0001
Hispanic	367 (12.51%)	113 (11.67%)	127 (12.74%)	127 (13.12%)	<0.0001
Other	120 (4.09%)	44 (4.55%)	43 (4.31%)	33 (3.41%)	<0.0001
Education					
Less than high school	534 (18.21%)	126 (13.03%)	181 (18.15%)	227 (23.45%)	<0.0001
High school graduate	523 (17.84%)	142 (14.68%)	167 (16.75%)	214 (22.11%)	<0.0001
Some college	864 (29.47%)	273 (28.23%)	319 (32.00%)	272 (28.10%)	<0.0001
College grad or higher	1,011 (34.48%)	426 (44.05%)	330 (33.10%)	255 (26.34%)	<0.0001
Hypertension	2,469 (84.18%)	701 (72.42%)	871 (87.36%)	897 (92.67%)	<0.0001
Diabetes	1,329 (45.31%)	246 (25.41%)	432 (43.33%)	651 (67.25%)	<0.0001
Hyperlipidemia	2,381 (81.18%)	713 (73.66%)	819 (82.15%)	848 (87.60%)	<0.0001
Cardiovascular disease	873 (29.76%)	183 (18.90%)	297 (29.79%)	393 (40.60%)	<0.0001
CHF	209 (7.13%)	45 (4.65%)	71 (7.12%)	93 (9.61%)	0.0001
Current smoker	331 (11.29%)	90 (9.30%)	116 (11.63%)	125 (12.91%)	0.0387
BMI (kg/m^2)	31.35 (SD: 6.80)	31.23 (7.18)	31.15 (6.59)	31.67 (6.61)	0.189
Waist circumference (cm)	104.52 (SD: 16.43)	103.08 (16.91)	104.19 (16.52)	106.30 (15.68)	<0.0001
Exercise tolerance (Mets)	26.49 (SD: 45.42)	31.13 (46.74)	26.84 (47.76)	21.47 (40.90)	<0.0001
Mean arterial pressure	88.80 (SD: 13.61)	86.22 (12.04)	88.58 (13.08)	91.63 (15.04)	<0.0001
PWV	9.55 (SD: 3.01)	6.66 (0.90)	9.05 (0.70)	12.95 (2.47)	—
PPA	1.29 (SD: 0.22)	1.32 (0.23)	1.29 (0.22)	1.27 (0.21)	<0.0001
AI	27.05 (SD: 12.43)	24.67 (13.25)	27.82 (12.50)	28.65 (11.11)	<0.0001
CPP	45.97 (SD: 19.11)	37.51 (16.10)	44.84 (17.39)	55.53 (19.27)	<0.0001
Alpha 2 agonist (%)	235 (8.06%)	63 (6.56%)	74 (7.47%)	98 (10.17%)	0.0103
Alpha blocker (%)	401 (13.76%)	85 (8.84%)	145 (14.65%)	171 (17.74%)	<0.0001
Beta blocker (%)	1,357 (46.55%)	356 (37.04%)	476 (48.08%)	525 (54.46%)	<0.0001
CCB (%)	1,149 (39.42%)	285 (29.66%)	399 (40.30%)	465 (48.24%)	<0.0001
Aspirin (%)	1,246 (42.74%)	309 (32.15%)	424 (42.83%)	513 (53.22%)	<0.0001
ACE/ARB (%)	1,978 (67.86%)	581 (60.46%)	687 (69.39%)	710 (73.65%)	<0.0001
Statin (%)	1,581 (54.24%)	411 (42.77%)	522 (52.73%)	648 (67.22%)	<0.0001
Hemoglobin (g/dl)	12.70 (SD: 1.75)	13.04 (1.71)	12.76 (1.73)	12.31 (1.72)	<0.0001
Serum albumin (g/dl)	3.97 (SD: 0.46)	4.02 (0.44)	3.98 (0.48)	3.91 (0.45)	<0.0001
Triglycerides	153.6 (SD: 111.19)	149.02 (102.47)	156.28 (128.62)	155.30 (99.46)	0.2941
Total cholesterol	183.64 (SD: 43.45)	187.05 (42.07)	183.77 (44.29)	180.16 (43.71)	0.0022
LDL (mg/dl)	103.12 (SD: 34.53)	107.00 (35.82)	102.30 (33.55)	100.18 (33.93)	<0.0001

Table 1. Continued

	Total (n = 2933)	PWV (<7.9, n = 968)	PWV (7.9–10.3, n = 997)	PWV (>10.3, n = 968)	P value[a]
HDL (mg/dl)	47.84 (SD: 15.54)	48.64 (15.07)	48.42 (16.38)	46.47 (15.02)	0.0032
Serum creatinine (mg/dl)	1.71 (SD: 0.57)	1.61 (0.60)	1.70 (0.54)	1.81 (0.56)	<0.0001
eGFR (ml/min/1.73 m^2)	43.89 (SD: 13.58)	47.13 (14.68)	43.91 (13.15)	40.65 (12.03)	<0.0001
Calibrated cystatin C	1.45 (SD: 0.52)	1.34 (0.52)	1.44 (0.49)	1.58 (0.53)	<0.0001
Urine protein g/24h	0.94 (SD: 2.00)	0.74 (1.54)	0.92 (2.12)	1.16 (2.24)	<0.0001
Plasma insulin (uU/ml)	21.53 (SD: 21.48)	18.78 (15.36)	21.12 (21.50)	24.72 (25.90)	<0.0001
TPH (umol/l)	14.71 (SD: 5.90)	13.70 (5.66)	14.69 (5.99)	15.77 (5.85)	<0.0001

Abbreviations: AI, augmentation index; ACE/ARB, ACE inhibitor/angiotensin receptor blocker; BMI, body mass index; CCB, calcium channel blocker; CPP, central pulse pressure; eGFR, estimated glomerular filtration rate; Exercise tol, all intention exercise tolerance (METS); HDL, high-density lipoprotein; LDL, low-density lipoprotein; PPA, pulse pressure amplification; PWV, pulse wave velocity; TPH, total plasma homocysteine; Uprotein, urine protein.

[a]*P* values pertain to PWV tertiles.

promotes the over-production of cell-adhesion molecules on the endothelium, resulting in mononuclear cell and vascular smooth muscle cell recruitment.[21,23,24,27] Finally, CRP stimulates tissue factor production by endothelial cells, thereby initiating the clotting cascade and fibrinogen activation.[28] Because of its numerous vasoactive effects, CRP has not only been shown to predict higher levels of arterial stiffness in the Caerphilly prospective cohort,[29] but has also been recognized for significant associations with cardiovascular events.[30,31]

In addition to the effects described above, IL-6 also promotes synthesis and release of fibrinogen from the liver,[27,32,33] with associated increases in arterial stiffness.[32] Fibrinogen is the only clotting factor for which there are compelling data supporting an association between plasma levels and increasing risk of vascular events.[27] Fibrinogen may migrate into the intima of arteries under increased mechanical and inflammatory stress where it forms cross-linked fibrin, mural thrombi, and fibrin degradation products. These by-products reduce arterial wall elasticity, and promote vascular smooth muscle cell and macrophage proliferation further propagating the inflammatory cascade.[27,32]

A notable feature of the cross-sectional results presented in this study is the relative absence of meaningful significant associations between inflammatory markers and AI or PPA. Although statistical significance was achieved for certain markers, the standardized coefficients β are predominantly negative for AI suggesting counterintuitive inverse relationships between inflammation and stiffness. Similarly, the standardized coefficient β is positive for most of the associations between inflammation and PPA, a metric that is classically described as decreasing with increased central arterial stiffness. This discordance between measures of wave reflection (AI and PPA) with the more direct measures of central stiffness (PWV and CPP) with regards to the effects of inflammation has been observed before,[34] and may represent a limitation of the wave reflections as a useful marker of stiffness in this pathophysiologic context.

While inflammation likely alters central stiffness through the mechanisms discussed above, inflammatory cytokines have long been recognized for their ability to decrease peripheral tone in the small muscular arteries and arterioles. This occurs *via* alterations of the nitric oxide balance in the microvasculature, as most dramatically demonstrated in conditions like sepsis.[35–37] This mechanism is supported by prior research in which discordant values of AI and PWV in the setting of a vaccine-induced inflammatory state correlated with a measured decrease in peripheral vascular tone and markedly diminished reflected wave amplitude.[34] Ultimately, when attempting to understand the impact of inflammation on arterial stiffness, the competing effects of central arterial remodeling and peripheral vasodilatation may limit the utility of metrics like AI and PPA.

While plausible mechanisms underlie associations between inflammation and arterial stiffness, the failure of some of these markers to endure adjustment for confounders and their further failure to predict progression of stiffness over time suggest shortcomings either in study methodology or in understanding the underlying pathophysiology. Besides CKD, many of our participants had HTN, diabetes, hyperlipidemia, and coronary artery disease. Each of these conditions has been associated with increased arterial stiffness and chronic inflammation in previous research, and are likely the primary reason for the markedly higher PWVs seen at baseline in our population (mean 9.6) compared to age-matched individuals without these risk factors (mean 8.1).[38] Given the clustering of risk factors and elevated baseline PWVs in this study, it's possible that our participants already experienced the augmentation of normal vascular biology that results from chronic inflammation and had thus reached a flattened portion of the arterial stiffness curve from which further increases would be relatively insignificant. Alternatively, it is possible that chronic low-grade inflammation influences stiffness in such a gradual fashion that 4 years was too short a time to appreciate the impact. It is also possible that prior cross-sectional research has misunderstood the relationship between inflammation and stiffness, assuming that inflammation begets stiffness when in actuality, the association is due to stiffness causing chronic low-grade inflammation as

Table 2. Associations between baseline measures of stiffness and inflammatory markers (cross-sectional)

Variable	PWV		CPP		AI		PPA	
	Unadjusted *P* value (β)	Adjusted *P* value (β)	Unadjusted *P* value (β)	Adjusted *P* value (β)	Unadjusted *P* value (β)	Adjusted *P* value (β)	Unadjusted *P* value (β)	Adjusted *P* value (β)
Fibrinogen	**<0.001** (0.411)	**0.044** (0.095)	**<0.001** (3.171)	**0.020** (0.688)	0.066 (0.376)	**0.001** (−0.660)	0.935 (0.000)	**0.001** (0.012)
Albumin	**<0.001** (−0.566)	0.490 (−0.084)	**<0.001** (−6.177)	0.169 (−1.060)	0.319 (–0.514)	0.715 (0.194)	0.604 (−0.005)	0.484 (−0.007)
IL-6	**<0.001** (0.676)	0.530 (0.052)	**<0.001** (3.852)	0.191 (−0.686)	0.060 (0.697)	**0.025** (−0.816)	0.775 (0.002)	**0.002** (0.021)
IL-10	**0.004** (0.268)	**0.024** (0.182)	0.492 (0.410)	0.817 (−0.119)	0.718 (−0.140)	0.221 (−0.433)	0.671 (−0.003)	0.990 (0.000)
IL-1RA	**0.026** (0.127)	0.210 (0.067)	**0.016** (0.883)	0.793 (−0.089)	0.424 (−0.191)	0.048 (−0.463)	0.338 (0.004)	0.201 (0.006)
IL-1 β	0.288 (0.091)	0.682 (0.034)	**<0.001** (2.016)	0.270 (0.578)	0.941 (−0.026)	0.619 (−0.181)	0.937 (−0.001)	0.506 (−0.005)
TGF-β	0.886 (0.012)	0.361 (–0.072)	0.804 (0.132)	0.921 (−0.050)	0.334 (−0.334)	0.443 (−0.267)	0.526 (0.004)	0.793 (0.002)
TNF-α	**<0.001** (0.644)	0.240 (0.132)	**<0.001** (5.075)	0.556 (0.419)	**0.005** (1.363)	0.196 (0.636)	0.461 (−0.006)	0.937 (0.001)
hs-CRP	**0.002** (0.210)	0.591 (0.032)	0.063 (0.800)	**0.019** (0.894)	0.884 (−0.041)	**<0.001** (−1.046)	0.092 (0.008)	**<0.001** (0.020)
Inflam Score	**<0.001** (0.329)	0.079 (0.087)	**<0.001** (2.113)	0.272 (−0.345)	0.236 (0.257)	**0.002** (−0.678)	0.393 (0.003)	**<0.001** (0.014)

Adjusted model controls for mean arterial pressure, age, race, sex, diabetes, active smoking, hemoglobin, estimated glomerular filtration rate, and angiotensin converting enzyme inhibitor/angiotensin receptor blocker use. Bolded *P* values are statistically significant. Abbreviations: AI, augmentation index; CPP, central pulse pressure; hs-CRP, high-sensitivity C-reactive protein; Inflam Score, inflammation score; IL, interleukin; PPA, pulse pressure amplification; PWV, pulse wave velocity; TGF, transforming growth factor; TNF, tumor necrosis factor; Uprot, 24-hour urine protein.

Table 3. Associations between measures of stiffness and inflammatory markers over time (longitudinal)

	PWV *P* value (β)	CPP *P* value (β)	AI *P* value (β)	PPA *P* value (β)
Fibrinogen	0.125 (0.039)	0.225 (−0.194)	0.697 (0.045)	0.952 (0.000)
Albumin	**0.002 (0.202)**	0.233 (0.490)	0.180 (0.402)	0.131 (−0.009)
IL-6	0.428 (0.035)	0.739 (−0.094)	0.379 (−0.181)	0.356 (−0.004)
IL-10	0.431 (0.032)	0.399 (−0.224)	**0.021 (0.450)**	0.521 (−0.002)
IL-1RA	0.255 (−0.034)	0.414 (−0.153)	0.092 (−0.231)	0.865 (−0.000)
IL-1 β	0.921 (0.005)	0.899 (0.037)	0.230 (−0.258)	0.411 (0.004)
TGF-β	0.479 (0.028)	0.684 (0.102)	0.724 (−0.065)	0.683 (−0.001)
TNF-α	0.391 (0.050)	0.162 (−0.518)	0.066 (−0.497)	0.069 (0.010)
hs-CRP	0.837 (0.006)	0.903 (0.024)	0.788 (−0.038)	0.753 (−0.001)
Inflam Score	0.346 (0.024)	0.340 (−0.151)	0.157 (−0.165)	0.975 (0.000)

Adjusted model controls for mean arterial pressure, age, race, sex, diabetes, active smoking, hemoglobin, estimated glomerular filtration rate, and angiotensin converting enzyme inhibitor/angiotensin receptor blocker use. Abbreviations: AI, augmentation index; CPP, central pulse pressure; hs-CRP, high-sensitivity C-reactive protein; Inflam Score = inflammation score; IL, interleukin; PPA, pulse pressure amplification; PWV, pulse wave velocity; TGF, transforming growth factor; TNF, tumor necrosis factor; Uprot, 24-hour urine protein.

pointed out recently.[39] In this scenario, baseline inflammation levels would be the result of already stiffened vasculature, and would not be expected to drive or predict further stiffening longitudinally.

Other limitations may have impacted the ability to demonstrate significant longitudinal associations that merit further discussion. Our participants, although similar with regards to basic demographics and comorbidities, are not wholly representative of the populations in which prior cross-sectional studies had shown associations between inflammation and stiffness. Cross-sectional studies by definition collect data on participants at one point in time, often involving same day enrollment and sampling, thus requiring little commitment from those enrolled. Patients included in our analysis were selected from the CRIC cohort, which per protocol involves frequent follow-up with expert practitioners at large academic hospitals and may not be generalizable to other CKD populations. Progression of arterial stiffness may be abrogated by careful management of medical conditions such as blood pressure[25] which was reasonably well controlled in CRIC suggesting another possible explanation for the modest absolute increases in PWV observed over the study period. In this regard, most of the participants (67.9%) were on ACE inhibitor or angiotensin receptor blocker medications at the time of enrollment, a higher number than seen in other studies. This is especially relevant as these agents produce inhibitory effects on the renin–angiotensin–aldosterone system and have been shown to modify stiffness above and beyond their effects on blood pressure alone due to proposed impacts on vascular inflammation.[25,39,40]

Finally, it is possible that inflammation, despite plausible biological mechanisms, may not be as important in the pathogenesis of arterial stiffness as many other investigators have hypothesized. While there is an abundance of cross-sectional data suggesting a connection between inflammation and pathological stiffness, the existence of a causal relationship can only be determined through more rigorous investigations. It may be that in CKD, chronic inflammation is a less important exposure for the development of pathological stiffening, with factors such as age, time-under-stress, chronic renin–angiotensin–aldosterone system activation, and the buildup of other vasoactive substances playing a more primary role. In the future, further prospective research along with Mendelian randomization trials will be required to more conclusively establish whether the apparent association between inflammation and stiffness is on the basis of causation or confounding.

In conclusion, using a longitudinal approach in a large CKD population with extensive biomarker collection, we observed only weak associations between markers of inflammation and the development of arterial stiffening. While well-described biologic mechanisms continue to provide the basis for our understanding of these results, continued efforts to design longitudinal studies are necessary to fully elucidate the relationship between chronic inflammation and arterial stiffening. We anticipate that the findings described in this study will promote further research in this important field and inspire new approaches to reach this end.

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DISCLOSURE

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REFERENCES

1. Parfrey PS, Foley RN. The clinical epidemiology of cardiac disease in chronic renal failure. *J Am Soc Nephrol* 1999; 10:1606–1615.
2. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004; 351:1296–1305.
3. Henry RM, Kostense PJ, Bos G, Dekker JM, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD. Mild renal insufficiency is associated with increased cardiovascular mortality: The Hoorn Study. *Kidney Int* 2002; 62:1402–1407.
4. Moody WE, Edwards NC, Madhani M, Chue CD, Steeds RP, Ferro CJ, Townend JN. Endothelial dysfunction and cardiovascular disease in early-stage chronic kidney disease: cause or association? *Atherosclerosis* 2012; 223:86–94.
5. Covic A, Gusbeth-Tatomir P, Goldsmith DJ. Arterial stiffness in renal patients: an update. *Am J Kidney Dis* 2005; 45:965–977.
6. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J Am Coll Cardiol* 2010; 55:1318–1327.
7. Willum-Hansen T, Staessen JA, Torp-Pedersen C, Rasmussen S, Thijs L, Ibsen H, Jeppesen J. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. *Circulation* 2006; 113:664–670.
8. Cleland SJ, Sattar N, Petrie JR, Forouhi NG, Elliott HL, Connell JM. Endothelial dysfunction as a possible link between C-reactive protein levels and cardiovascular disease. *Clin Sci (Lond)* 2000; 98:531–535.
9. Verma S, Wang CH, Li SH, Dumont AS, Fedak PW, Badiwala MV, Dhillon B, Weisel RD, Li RK, Mickle DA, Stewart DJ. A self-fulfilling prophecy: C-reactive protein attenuates nitric oxide production and inhibits angiogenesis. *Circulation* 2002; 106:913–919.
10. Nigam A, Mitchell GF, Lambert J, Tardif JC. Relation between conduit vessel stiffness (assessed by tonometry) and endothelial function (assessed by flow-mediated dilatation) in patients with and without coronary heart disease. *Am J Cardiol* 2003; 92:395–399.
11. McEniery CM, Wallace S, Mackenzie IS, McDonnell B, Yasmin, Newby DE, Cockcroft JR, Wilkinson IB. Endothelial function is associated with pulse pressure, pulse wave velocity, and augmentation index in healthy humans. *Hypertension* 2006; 48:602–608.
12. Feldman HI, Appel LJ, Chertow GM, Cifelli D, Cizman B, Daugirdas J, Fink JC, Franklin-Becker ED, Go AS, Hamm LL, He J, Hostetter T, Hsu CY, Jamerson K, Joffe M, Kusek JW, Landis JR, Lash JP, Miller ER, Mohler ER 3rd, Muntner P, Ojo AO, Rahman M, Townsend RR, Wright JT; Chronic Renal Insufficiency Cohort (CRIC) Study Investigators. The Chronic Renal Insufficiency Cohort (CRIC) Study: design and methods. *J Am Soc Nephrol* 2003; 14:S148–S153.
13. Lash JP, Go AS, Appel LJ, He J, Ojo A, Rahman M, Townsend RR, Xie D, Cifelli D, Cohan J, Fink JC, Fischer MJ, Gadegbeku C, Hamm LL, Kusek JW, Landis JR, Narva A, Robinson N, Teal V, Feldman HI; Chronic Renal Insufficiency Cohort (CRIC) Study Group. Chronic Renal Insufficiency Cohort (CRIC) Study: baseline characteristics and associations with kidney function. *Clin J Am Soc Nephrol* 2009; 4:1302–1311.
14. Townsend RR, Wimmer NJ, Chirinos JA, Parsa A, Weir M, Perumal K, Lash JP, Chen J, Steigerwalt SP, Flack J, Go AS, Rafey M, Rahman M, Sheridan A, Gadegbeku CA, Robinson NA, Joffe M. Aortic PWV in chronic kidney disease: a CRIC ancillary study. *Am J Hypertens* 2010; 23:282–289.
15. Townsend RR, Chirinos JA, Parsa A, Weir MA, Sozio SM, Lash JP, Chen J, Steigerwalt SP, Go AS, Hsu CY, Rafey M, Wright JT Jr, Duckworth MJ, Gadegbeku CA, Joffe MP; Chronic Renal Insufficiency Cohort Investigators. Central pulse pressure in chronic kidney disease: a chronic renal insufficiency cohort ancillary study. *Hypertension* 2010; 56:518–524.
16. de Jager W, Bourcier K, Rijkers GT, Prakken BJ, Seyfert-Margolis V. Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays. *BMC Immunol* 2009; 10:52.
17. Duncan BB, Schmidt MI, Pankow JS, Ballantyne CM, Couper D, Vigo A, Hoogeveen R, Folsom AR, Heiss G; Atherosclerosis Risk in Communities Study. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes* 2003; 52:1799–1805.
18. Recasens M, López-Bermejo A, Ricart W, Vendrell J, Casamitjana R, Fernández-Real JM. An inflammation score is better associated with basal than stimulated surrogate indexes of insulin resistance. *J Clin Endocrinol Metab* 2005; 90:112–116.
19. Molnar MZ, Czira ME, Rudas A, Ujszaszi A, Haromszeki B, Kosa JP, Lakatos P, Beko G, Sarvary E, Varga M, Fornadi K, Novak M, Rosivall L, Kiss I, Remport A, Goldsmith DJ, Kovesdy CP, Mucsi I. Association between the malnutrition-inflammation score and post-transplant anaemia. *Nephrol Dial Transplant* 2011; 26:2000–2006.
20. Schnabel R, Larson MG, Dupuis J, Lunetta KL, Lipinska I, Meigs JB, Yin X, Rong J, Vita JA, Newton-Cheh C, Levy D, Keaney JF Jr, Vasan RS, Mitchell GF, Benjamin EJ. Relations of inflammatory biomarkers and common genetic variants with arterial stiffness and wave reflection. *Hypertension* 2008; 51:1651–1657.
21. Duprez DA, Somasundaram PE, Sigurdsson G, Hoke L, Florea N, Cohn JN. Relationship between C-reactive protein and arterial stiffness in an asymptomatic population. *J Hum Hypertens* 2005; 19:515–519.
22. Tanabe K, Matsushima-Nishiwaki R, Yamaguchi S, Iida H, Dohi S, Kozawa O. Mechanisms of tumor necrosis factor-alpha-induced interleukin-6 synthesis in glioma cells. *J Neuroinflammation* 2010; 7:16.
23. Bassuk SS, Rifai N, Ridker PM. High-sensitivity C-reactive protein: clinical importance. *Curr Probl Cardiol* 2004; 29:439–493.
24. Park S, Lakatta EG. Role of inflammation in the pathogenesis of arterial stiffness. *Yonsei Med J* 2012; 53:258–261.
25. London GM, Marchais SJ, Guerin AP, Metivier F, Adda H, Pannier B. Inflammation, arteriosclerosis, and cardiovascular therapy in hemodialysis patients. *Kidney Int Suppl* 2003; 63:S88–S93.
26. Yasmin, McEniery CM, Wallace S, Mackenzie IS, Cockcroft JR, Wilkinson IB. C-reactive protein is associated with arterial stiffness in apparently healthy individuals. *Arterioscler Thromb Vasc Biol* 2004; 24:969–974.
27. Feinbloom D, Bauer KA. Assessment of hemostatic risk factors in predicting arterial thrombotic events. *Arterioscler Thromb Vasc Biol* 2005; 25:2043–2053.
28. Danenberg HD, Szalai AJ, Swaminathan RV, Peng L, Chen Z, Seifert P, Fay WP, Simon DI, Edelman ER. Increased thrombosis after arterial injury in human C-reactive protein-transgenic mice. *Circulation* 2003; 108:512–515.
29. Schumacher W, Cockcroft J, Timpson NJ, McEniery CM, Gallacher J, Rumley A, Lowe G, Smith GD, Wilkinson IB, Ben-Shlomo Y. Association between C-reactive protein genotype, circulating levels, and aortic pulse wave velocity. *Hypertension* 2009; 53:150–157.
30. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC Jr, Taubert K, Tracy RP, Vinicor F; Centers for Disease Control and Prevention; American Heart Association. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the centers for disease control and prevention and the American Heart Association. *Circulation* 2003; 107:499–511.
31. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997; 336:973–979.
32. Vlachopoulos C, Pietri P, Aznaouridis K, Vyssoulis G, Vasiliadou C, Bratsas A, Tousoulis D, Xaplanteris P, Stefanadi E, Stefanadis C.

Relationship of fibrinogen with arterial stiffness and wave reflections. *J Hypertens* 2007; 25:2110–2116.
33. Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. *Biochem J* 1990; 265:621–636.
34. Vlachopoulos C, Dima I, Aznaouridis K, Vasiliadou C, Ioakeimidis N, Aggeli C, Toutouza M, Stefanadis C. Acute systemic inflammation increases arterial stiffness and decreases wave reflections in healthy individuals. *Circulation* 2005; 112:2193–2200.
35. Naziri W, Joshua IG. Role of tumor necrosis factor-alpha in small intestinal microcirculation. *Am Surg* 1998; 64:203–209; discussion 209.
36. Tyagi A, Sethi AK, Girotra G, Mohta M. The microcirculation in sepsis. *Indian J Anaesth* 2009; 53:281–293.
37. Matsuda N, Hattori Y. Vascular biology in sepsis: pathophysiological and therapeutic significance of vascular dysfunction. *J Smooth Muscle Res* 2007; 43:117–137.
38. Vermeersch SJ, Dynamics B, Society L. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: "establishing normal and reference values". *Eur Heart J* 2010; 31:2338–2350.
39. Hashimoto J, O'Rourke MF. C-reactive protein and cardiovascular disease: are we looking in the right direction? *J Hypertens* 2006; 24:2115.
40. Takami T, Shigemasa M. Efficacy of various antihypertensive agents as evaluated by indices of vascular stiffness in elderly hypertensive patients. *Hypertens Res* 2003; 26:609–614.