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Biomarkers of inflammation and progression of chronic kidney disease

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Background. Chronic kidney disease is associated with higher levels of inflammatory biomarkers. Statins have anti-inflammatory properties and may attenuate loss of kidney function. Although inflammation may mediate progressive renal injury, the relation between statin use, markers of inflammation, and the rate of kidney function loss has not been elucidated. We examined the association between pravastatin use, levels of C-reactive protein (CRP), soluble tumor necrosis factor receptor II (sTNFrii), and the rate of kidney function loss.

Methods. We performed a post hoc analysis of data from a randomized placebo controlled trial of pravastatin 40 mg daily in people with previous myocardial infarction. Glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease Study (MDRD) GFR equation. We studied 687 subjects with chronic kidney disease (GFR < 60 mL/min/1.73 m^2) who did not experience a cardiovascular event during follow-up. Multivariate linear regression was used to study the relation between baseline CRP and sTNFrii and the rate of kidney function loss in mL/min/1.73 m^2/year. Cross-product interaction terms were used to determine if these relations varied with pravastatin use.

Results. Median baseline GFR was 54.5 mL/min/1.73 m^2 (interquartile range 49.7, 57.8) and median duration of follow-up was 58 months. Higher baseline CRP level was independently associated with more rapid kidney function loss (highest tertile 0.6 mL/min/1.73 m^2 per year faster than lowest tertile) ($P = 0.001$). A similar independent relation was observed between tertile of sTNFrii and rate of kidney function loss (highest tertile 0.5 mL/min/1.73 m^2 per year faster than lowest tertile) ($P = 0.006$). Subjects with both CRP and sTNFrii in the highest tertile ("inflamed" status) appeared to derive more renal benefit from pravastatin than those without (P for interaction 0.047). In these 108 subjects, renal function loss in pravastatin recipients was 0.8 mL/min/1.73 m^2/year slower than placebo (95% CI 0 to 1.5 mL/min/1.73 m^2/year slower) ($P = 0.039$).

Conclusion. Higher CRP and sTNFrii are independently associated with faster rates of kidney function loss in chronic kidney disease. Pravastatin appears to prevent loss of kidney function to a greater extent in individuals with greater evidence of inflammation, although this was of borderline significance. These data suggest that inflammation may mediate the loss of kidney function among subjects with chronic kidney disease and concomitant coronary disease.

Characteristics predictive of progressive renal function loss in chronic kidney disease are similar to those associated with coronary disease in the general population, and include male gender, hypertension, diabetes mellitus, smoking status, dyslipidemia, and proteinuria [1–4]. As with cardiovascular outcomes, renal outcomes in chronic kidney disease are improved by blood pressure reduction [5], tight glycemic control [6], interruption of the renin-angiotensin system [7, 8], and possibly treatment of hypercholesterolemia [9, 10].

Evidence of systemic inflammation such as elevated C-reactive protein (CRP) levels correlates with higher coronary risk in the general population [11], and is associated with impaired kidney function [12, 13]. Elevated CRP is associated with endothelial injury and impaired vasodilation, both of which may lead to glomerular damage and progressive loss of kidney function [14, 15]. Tumor necrosis factor-α (TNF-α) is a central proinflammatory agonist mediator that is generated in a wide variety of innate

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and adaptive immune responses, including some forms of chronic kidney disease. TNF-α binds to cell surface receptors on target cells and induces expression of adhesion molecules, chemokines for leukocytes, and apoptosis in susceptible cells [16, 17]. Soluble TNF receptors are elevated in the setting of inflammation and chronic kidney disease [18–20]. Thus, TNF-α also appears to have multiple roles that could mediate progressive renal injury, and both soluble TNF receptor II (sTNFrii) and CRP may be used as markers of inflammation.

Given that risk factors for progressive renal disease and cardiovascular disease are similar, and since chronic kidney disease may constitute a chronic inflammatory state [12, 21], it is plausible that evidence of systemic inflammation might predict the rate of renal function loss in chronic kidney disease. Because elevated serum CRP levels predict decreased cardiovascular morbidity in response to 3-hydroxy-3-methylglutaryl coenzymeA (HMG-CoA) reductase inhibitors [22], markers of inflammation might also identify subjects who are likely to derive renal benefit from statins.

We analyzed data from a previously conducted randomized trial of pravastatin vs. placebo in subjects with hyperlipidemia and a history of myocardial infarction [23], considering only participants with chronic kidney disease [estimated glomerular filtration rate (GFR) <60 mL/min/1.73 m^2 body surface area (BSA)] [24]. We tested the hypotheses that two markers of inflammation (CRP and sTNFrii) would be associated with more rapid rates of renal function loss, and that pravastatin would reduce rates of kidney function loss to a greater extent in subjects with higher levels of these markers.

METHODS

Study design and patients

The CARE Study was a randomized trial of pravastatin vs. placebo in 4159 individuals with hyperlipidemia and a history of myocardial infarction [23], and has been described in detail elsewhere [25]. Briefly, men and postmenopausal women were eligible if they had had an acute myocardial infarction between 3 and 20 months before randomization, were 21 to 75 years of age, and had low-density lipoprotein (LDL) cholesterol levels of 115 to 174 mg/dL (3.0 to 4.5 mmol/L), fasting glucose levels of no more than 220 mg/dL (12.2 mmol/L), left ventricular ejection fractions of no less than 25%, and no symptomatic congestive heart failure. Patients with serum creatinine levels >1.5 times the upper limit of normal for the central study laboratory were excluded from the CARE Study.

After stratification according to clinical center, eligible participants were assigned by computer-generated random order to receive either 40 mg of pravastatin (Pravachol) (Bristol-Myers-Squibb, Princeton, NJ, USA) once daily, or placebo. Treatment allocation was concealed using a centrally maintained code. Serum creatinine measurements using the alkaline picrate method of Jaffé were made annually at a central study laboratory. Quality assurance was maintained through use of standard Westgard quality control multirules [26], and external proficiency was provided through the Interlaboratory Survey Program of the American College of Pathologists (Northfield, IL, USA). Based on monthly averages of control materials analyzed several times daily, the percent coefficient of variation for creatinine measurement ranged from 0.8% at 9.0 mg/dL to 1.8% at 1.4 mg/dL. Internal and external quality assurance showed the measurement of creatinine to be stable and acceptably calibrated throughout the study. Treatment with pravastatin does not affect serum creatinine measurements [27], and pravastatin pharmacokinetics are not affected by renal insufficiency [28].

Definition of kidney disease

The primary index of renal function was estimated GFR, as calculated by the MDRD equation:

$$186 \times \text{plasma creatinine}^{-1.154} * \text{age in years}^{-0.203} * 1.210 \text{ (if black)} * 0.742 \text{ (if female)}$$

where plasma creatinine is in mg/dL. This formula correlates with iothalamate measurements of GFR [24]. Participants with GFR < 60 mL/min/1.73 m^2 BSA were considered to have chronic kidney disease [24] and were included in this analysis. Proteinuria was defined by trace or greater urinary protein on baseline dipstick urinalysis.

The primary end point was the rate of change in estimated GFR (mL/min/1.73 m^2 BSA/year). To minimize the potential for informative censoring, we included only those participants from the original CARE Study who did not experience a cardiovascular outcome during follow-up. To increase the precision of the estimates of rate of change in kidney function, we excluded subjects for whom < three estimates of GFR were available.

Measurement of inflammatory markers

Details of the blood collection and storage procedures used in the CARE Study have been outlined elsewhere [25]. Briefly, blood samples were collected in ethylenediaminetetraacetic acid (EDTA) at prerandomization clinic visits (during 1989 to 1991), shipped to a central collection site on cooled gel packs, and frozen at −80°C for future analyses. Stored frozen blood samples from baseline visits were analyzed specifically to perform the current analysis in 2004. CRP and sTNFrii assays were performed simultaneously according to methods described by the manufacturers. The concentration of CRP was determined using an immunoturbidimetric assay on the Hitachi 917 analyzer (Roche Diagnostics, Indianapolis, IN, USA), using reagents and calibrators from Denka Seiken (Niigata, Japan). sTNFrii was measured by

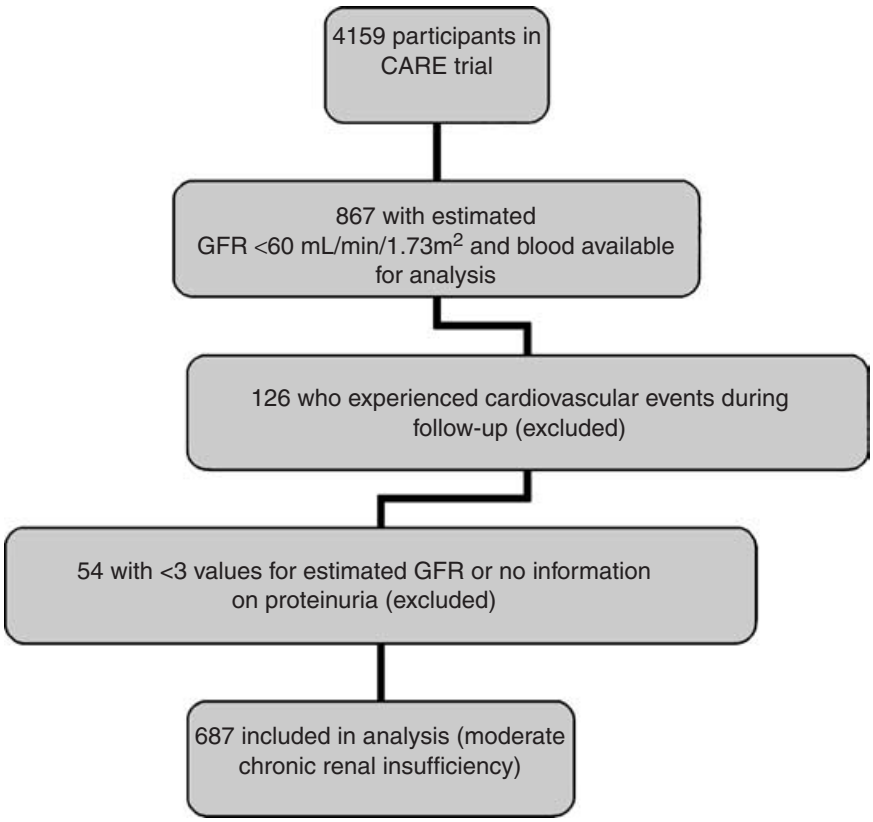

Fig. 1. Flow of participants. GFR is glomerular filtration rate.

an enzyme-linked immunosorbent assay (ELISA) assay from R&D Systems (Minneapolis, MN, USA). All assays were performed without knowledge of treatment assignment.

Statistical analysis

The distribution of CRP values was right-skewed, but the distribution of sTNFrii was approximately normal. To facilitate statistical analyses while retaining interpretable regression coefficients, each of these inflammatory markers was divided into tertiles. To avoid bias related to autocorrelation, the annual GFRs were computed and regressed for each subject onto time, resulting in a single measure of the rate of change in kidney function per subject. These GFR slopes were then used as dependent variables in univariate and multivariate least squares regression models, which were built using purposeful selection of covariates [29]. Covariates were considered for inclusion in the multivariable model if they were significant at the $P < 0.25$ level in univariate analyses. They were retained in the multivariable model if they were significant at the $P < 0.1$ level, or if they resulted in an important (>10%) change in the coefficient of interest (usually CRP and/or sTNFrii). Covariates considered in the multivariate models included age, race, gender, baseline renal function, systolic blood pressure, baseline high-density lipoprotein (HDL) cholesterol level, smoking status, diabetic status, use of angiotensin-converting enzyme (ACE) inhibitors, and baseline proteinuria. For face validity, baseline proteinuria and systolic blood pressure were retained in all models. After the final model was built, use of ACE inhibitors, use of pravastatin, smoking status, and diabetic status were individually forced back into the model to ensure that these key factors did not confound the relation of interest. Tests for interaction were performed using cross-product terms in the regression models. The regression models were inspected for outliers and influential observations using leverage vs. residual^2 and residual vs. fitted value plots. Values are reported as mean ± SD or percentages; 95% CIs are provided where appropriate. All analyses were intention-to-treat, and P values are two-sided. Analyses were performed with SAS 8.2 and Stata 8 SE software.

Role of the funding source

The CARE Study and this substudy on kidney disease were investigator-initiated studies funded by Bristol-Myers-Squibb. The authors had unlimited access to the data used in this analysis. The sponsor was entitled to comment on manuscripts before submission, and the authors could consider these comments, but the rights to publication reside contractually with the investigators. The sponsor maintained information on adverse events and other trial data, as required by federal regulation.

Table 1. Baseline characteristics of participants with chronic kidney disease, by treatment group

	Subjects excluded from analysis ($N = 180$)	All subjects included in analysis ($N = 687$)	Included placebo recipients ($N = 344$)	Included pravastatin recipients ($N = 343$)
Demographic variables				
Age *years*	64.8 ± 6.8	63.5 ± 7.5	63.7 ± 7.4	63.3 ± 7.6
Male (%)	136 (75.6)	523 (76.1)	262 (76.2)	261 (76.1)
Black race (%)	7 (3.9)	10 (1.5)	8 (2.3)	2 (0.6)
Body surface area m^2	1.92 ± 0.20	1.91 ± 0.19	1.91 ± 0.19	1.90 ± 0.19
History of hypertension (%)	76 (42.2)	364 (53)	192 (55.8)	172 (50.1)
Current smoker (%)	21 (11.7)	68 (9.9)	32 (9.3)	36 (10.5)
History of diabetes (%)	44 (24.4)	98 (14.3)	55 (16)	43 (12.5)
Medication use				
Calcium antagonists (%)	80 (44.4)	292 (42.5)	139 (40.4)	153 (44.6)
ACE inhibitor (%)	49 (27.2)	119 (17.3)	56 (16.3)	63 (18.4)
Aspirin (%)	135 (75)	566 (82.4)	275 (79.9)	291 (84.8)
Beta adrenergic antagonist (%)	67 (37.2)	296 (43.1)	155 (45)	141 (41.1)
Lipid status				
Total cholesterol *mg/dL*	208.1 ± 18.1	209.9 ± 17.1	210.2 ± 16.8	209.7 ± 17.4
LDL cholesterol *mg/dL*	138.4 ± 14.4	138.5 ± 13.7	138.5 ± 13.7	139.2 ± 14.7
HDL cholesterol *mg/dL*	40.0 ± 10.8	39.4 ± 9.7	39.8 ± 9.7	39.1 ± 9.6
Triglycerides *mg/dL*	148.3 ± 52.7	158.4 ± 62.6	159.2 ± 63.2	157.6 ± 62.2
Renal function and blood pressure				
Glomerular filtration rate *mL/min/1.73 m^2*[a]	53.6 (48.6, 57.2)	54.5 (49.7, 57.8)	54.4 (49.5, 57.5)	54.9 (50.0, 57.9)
Serum creatinine *mg/dL*	1.41 ± 0.24	1.38 ± 0.21	1.39 ± 0.23	1.37 ± 0.20
Proteinuria (dipstick positive)	55 (30.6)	118 (17.2)	59 (17.2)	59 (17.2)
Systolic blood pressure *mm Hg*	132.7 ± 19.6	132.7 ± 18.9	132.4 ± 19.6	132.5 ± 19.2
Diastolic blood pressure *mm Hg*	77.6 ± 10.9	78.5 ± 10.5	79.4 ± 10.1	78.9 ± 10.3
Mean arterial pressure *mm Hg*	96.0 ± 12.5	96.6 ± 11.7	97.0 ± 11.8	96.8 11.7
Inflammatory markers				
C-reactive protein *mg/L*[a]	—	2.6 (1.4, 5.3)	2.7 (1.4, 5.4)	2.4 (1.4, 5.1)
Proportion with C-reactive protein in highest tertile (%)	—	229 (33.3)	115 (33.4)	114 (33)
sTNFRii *pg/mL*	—	3719 ± 1143	3711 ± 1208	3715 ± 1176
Proportion with sTNFRii level in highest tertile (%)	—	229 (33.3)	116 (33.7)	113 (33)
Proportion with "inflamed" status (%)	—	108 (15.7)	50 (14.5)	58 (16.9)

Abbreviations are: ACE, angiotensin-converting enzyme; LDL, low-density lipoprotein; HDL, high-density lipoprotein; sTNFRii, soluble tumor necrosis factor receptor II.

Mean ± SD or N (%) except [a]Median (25th%, 75th%). Tertiles of C-reactive protein were 0.11 to 1.76, 1.77 to 4.10, and 4.2 to 68.3 mg/L, Tertiles of tumor necrosis factor receptor II were 16.8 to 3089, 3097 to 3930, and 3931 to 8828 pg/mL. "Inflamed" status was defined by both C-reactive protein and soluble tumor necrosis factor receptor II in the highest tertile.

RESULTS

Baseline characteristics

Trial flow is shown in Figure 1. There were 687 CARE participants who were eligible for this analysis and 180 who were excluded (Table 1). Median baseline GFR was 54.5 mL/min/1.73 m^2 (interquartile range 49.7, 57.8) and median duration of follow-up was 58 months in study subjects. The use of ACE inhibitors was similar in pravastatin and placebo groups at baseline and during follow-up. Bivariate analysis showed that baseline GFR was associated with baseline log CRP and sTNFrii concentrations (both $P < 0.0001$), as well as the baseline tertile of CRP and sTNFrii (both $P < 0.0001$) (Fig. 2). Results were similar after multivariate adjustment (all $P \leq 0.0002$), indicating that levels of CRP and sTNFrii in the highest tertile were both more common at lower levels of kidney function. The mean GFR of individuals with both CRP and sTNFrii in the highest tertile ("inflamed" status) ($N = 108$) was 48.5 ± 7.8 mL/min/1.73 m^2, significantly lower than individuals with one or neither of these characteristics in the highest tertile (53.7 ± 0.22 mL/min/1.73 m^2) ($P < 0.001$).

Kidney function loss in study subjects

Among the 687 subjects, mean rate of decline in estimated GFR was 0.7 ± 1.8 mL/min/1.73 m^2/year. Rates of GFR decline were similar in individuals with proteinuria at baseline (0.9 ± 2.2 mL/min/1.73 m^2/year) compared to those without (0.6 ± 2. 2 mL/min/1.73 m^2/year) ($P = 0.13$). As previously noted, pravastatin use was not significantly associated with kidney function loss when all subjects with GFR < 60 mL/min/1.73 m^2 were considered ($P = 0.53$) [9].

Association between inflammatory markers and kidney function loss

After multivariate adjustment, a significant association was noted between log-transformed CRP level and the

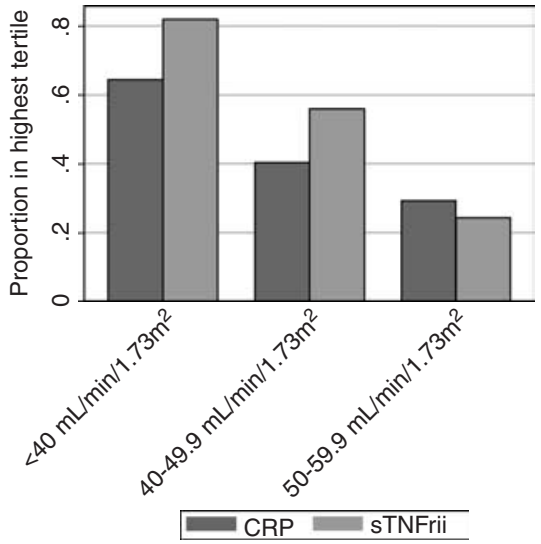

Fig. 2. Unadjusted relation between markers of inflammation and baseline kidney function. The occurrence of C-reactive protein (CRP) in the highest tertile and the occurrence of tumor necrosis factor receptor II (TNFrii) in the highest tertile were both more frequent at lower levels of kidney function. Tertiles of CRP were 0.11-1.76, 1.77-4.10, and 4.2-68.3 mg/L. Tertiles of TNFrii were 16.8-3089, 3097-3930, and 3931-8828 pg/mL.

rate of GFR decline ($P = 0.002$). A similar association was noted between sTNFrii level and rate of GFR decline ($P = 0.002$) (Table 2).

The tertile of CRP level was significantly associated with the rate of kidney function loss, with higher levels having more rapid loss after adjustment for the tertile of sTNFRii level and other potential confounders ($P = 0.003$) (Table 2). A similar relation was observed between tertile of sTNFrii and the adjusted rate of kidney function loss ($P = 0.008$) (Table 2). Subjects with CRP or sTNFrii in the highest tertile had significantly more rapid rates of kidney function loss than those in the lowest tertile [0.6 mL/min/1.73 m^2/year faster ($P = 0.001$) and 0.5 mL/min/1.73 m^2/year faster ($P = 0.006$), respectively] (Table 2) (Fig. 3). Subjects with both CRP and sTNFrii in the highest tertile had significantly more rapid rates of kidney function loss than those with one or neither characteristic in the highest tertile (0.8 mL mL/min/1.73 m^2/year faster) ($P < 0.0001$).

Interaction between inflammatory markers and effect of pravastatin on kidney function loss

When CRP and sTNFrii were considered individually, there was no significant interaction between the highest tertile of CRP level and the effect of pravastatin on kidney function ($P = 0.18$), or between the highest tertile of sTNFrii level and the effect of pravastatin on kidney function ($P = 0.63$). However, when both CRP and TNFrii levels were considered, the 108 subjects with "inflamed" status appeared to derive more renal benefit from pravastatin than those without (P for interaction 0.047) (Fig. 4). Specifically, the adjusted rate of kidney function loss in pravastatin recipients with both CRP and sTNFrii in the highest tertile was 0.76 mL/min/1.73 m^2/year slower than placebo (95% CI 0.04 to 1.48 mL mL/min/1.73 m^2/year slower) ($P = 0.039$) (Table 3). In contrast, the rate of kidney function loss in pravastatin recipients without such evidence of inflammation was no different than placebo (Table 3).

Because of the previously reported interaction between proteinuria and renal benefit from pravastatin [9], we performed an exploratory examination of the effect of pravastatin on kidney function loss in subjects with both proteinuria and "inflamed" status ($N = 30$). We found that renal benefit appeared particularly favorable in subjects with both characteristics (P for interaction <0.001 compared with subjects who had one or neither characteristic). Renal function loss in pravastatin recipients was 2.25 mL/min/1.73 m^2/year slower than placebo in this subgroup (95% CI 0.86 to 3.63 mL/min/1.73 m^2/year slower) ($P = 0.003$), compared to no change in the remaining 657 subjects.

DISCUSSION

We found that higher levels of CRP and sTNFrii were more common at lower levels of kidney function, and were independently associated with faster rates of kidney function loss. When considered individually, neither CRP nor sTNFrii level was associated with the magnitude of renal benefit from pravastatin. However, pravastatin appeared to reduce rates of kidney function loss predominantly among subjects in whom levels of both markers were in the highest tertile, especially those who also had proteinuria. This apparent interaction between inflammation and renal benefit from pravastatin persisted after controlling for other variables which might affect rates of renal function loss.

The pathophysiology of some forms of progressive kidney function loss (such as glomerulosclerosis) and atherosclerosis is similar. For example, both are accompanied by local upregulation of proinflammatory molecules [30–32]. Recent work has found that CRP localizes to sites of vascular injury and potentiates atherosclerotic lesions in CRP transgenic mice [33, 34]. Inflammatory molecules may mediate glomerular injury by promoting an influx of monocytes and macrophages, triggering proliferation of mesangial cells, and facilitating fibrosis [31, 35–39]. In experimental models, TNF-α causes direct glomerular injury, and glomerular injury is markedly attenuated in mice lacking TNF-α [37, 40–42]. In addition, serum TNF-α levels are associated with the severity of proteinuria in diabetic nephropathy [43, 44], and reductions in TNF-α appear to be temporally associated with decreases

Table 2. Multivariate adjusted association between markers of inflammation and rate of renal function loss (mL/min/1.73 m^2/year)

	β	95% CI	*P* value
Continuous			
Age (per year)	−0.045	−0.062, −0.026	<0.0001
Female	−0.68	−1.01, −0.36	<0.0001
Systolic blood pressure (10 mm Hg)	−0.015	−0.08, 0.05	0.67
Baseline glomerular filtration rate (10 mL/min/1.73 m^2)	−0.66	−0.90, −0.42	<0.0001
Presence of proteinuria	−0.17	−0.52, 0.17	0.33
HDL cholesterol mg/dL	0.018	0.004, 0.033	0.01
Log C-reactive protein [log 9 mg/L)]	−0.20	−0.33, −0.072	0.002
Soluble tumor necrosis factor receptor II *pg/mL*	−0.0002	−0.0003, −0.00008	0.002
Tertiles			
Age (per year)	−0.045	−0.064, −0.027	<0.0001
Female	−0.67	−1.00, −0.35	<0.0001
Systolic blood pressure (10 mm Hg)	−0.016	−0.09, 0.05	0.65
Baseline glomerular filtration rate (mL/min/1.73 m^2)	−0.061	−0.085, −0.038	<0.0001
Presence of proteinuria	−0.20	−0.55, 0.14	0.25
HDL cholesterol (mg/dL)	0.019	0.004, 0.033	0.01
C-reactive protein tertile 2 (vs. tertile 1)[a]	−0.25	−0.57, 0.071	0.13
C-reactive protein tertile 3 (vs. tertile 1)	−0.57	−0.90, −0.24	0.001
Soluble tumor necrosis factor receptor II tertile 2 (vs. tertile 1)[b]	−0.03	−0.35, 0.29	0.85
Soluble tumor necrosis factor receptor II tertile 3 (vs. tertile 1)	−0.49	−0.84, −0.14	0.006

Abbreviations are: LDL, low-density lipoprotein; HDL, high-density lipoprotein.

In analysis of continuous, log C-reactive protein and soluble tumor necrosis factor receptor II were entered into the model as continuous variables, and in analysis of tertiles, the tertile of C-reactive protein and soluble tumor necrosis factor receptor II were entered into the model as categoric variables. Variables considered for inclusion in the model were age, race, gender, baseline renal function, systolic blood pressure, baseline HDL cholesterol level, smoking status, diabetic status, use of angiotensin-converting enzyme inhibitors and baseline proteinuria.

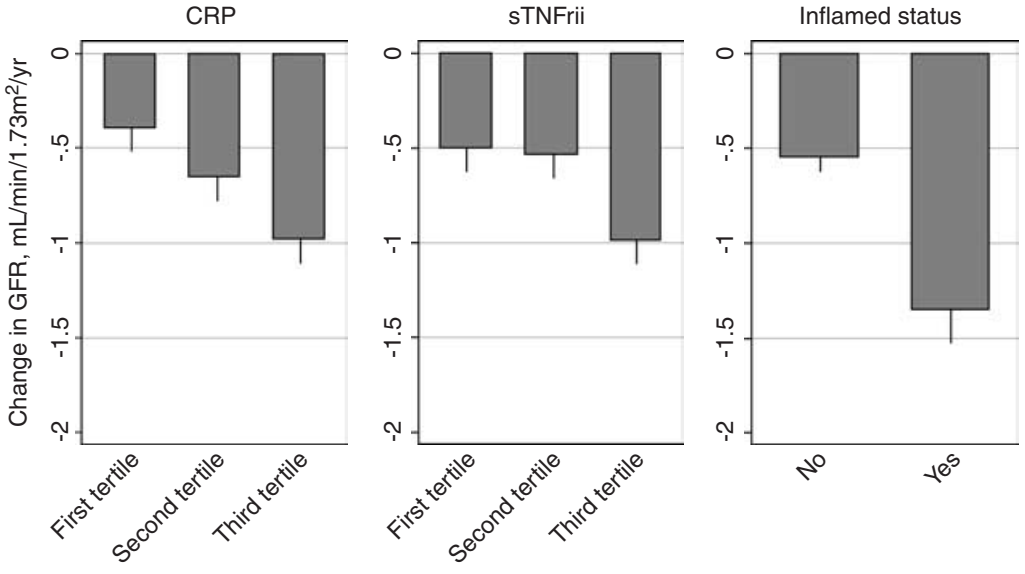

Fig. 3. Adjusted relation between markers of inflammation and the rate of kidney function loss. Error bars denote standard error (SE). Inflamed status was defined by both C-reactive protein (CRP) and soluble tumor necrosis factor receptor II (sTNFrii) levels in the highest tertile. Multivariate *P* values for relation with change in kidney function loss: CRP tertile ($P = 0.003$), sTNFrii tertile ($P = 0.008$), and inflamed status ($P < 0.0001$). Subjects with inflamed status ($N = 108$)

in urinary protein excretion [43], perhaps due to altered glomerular hemodynamics mediated by reduced prostaglandin levels [45] or reductions in glomerular permeability [46]. Thus, it seems plausible that TNF-α plays a pathogenic role in progressive nephropathy, especially when proteinuria is present.

Several small cross-sectional studies demonstrate an association between impaired kidney function and tumor necrosis factor or its receptors [18, 19, 47, 48]. To our knowledge, no previous studies examine the relation between sTNFrii and the rate of kidney function loss in humans.

Multiple cross-sectional studies document an association between CRP and impaired kidney function, including data from the third National Health and Nutrition Examination [18, 19, 49–53]. However, studies examining

Table 3. Multivariate adjusted effect of pravastatin treatment on adjusted rate of renal function loss (mL/min/1.73 m^2/year) in subjects with and without "inflamed" status

	β	95% CI	*P* value
With "inflamed" status ($N = 108$)			
Systolic blood pressure (10 mm Hg)	−0.19	−0.36, 0.02	0.03
Baseline glomerular filtration rate (10 mL/min/1.73 m^2)	−0.57	−1.06, −0.08	0.02
Presence of proteinuria	−0.42	−1.25, 0.41	0.32
Pravastatin use	0.76	0.04, 1.48	0.039
Without "inflamed" status ($N = 579$)			
Age (per year)	−0.056	−0.07, −0.04	<0.0001
Systolic blood pressure (10 mm Hg)	0.01	−0.07, 0.09	0.82
Baseline glomerular filtration rate (10 mL/min/1.73 m^2)	−0.055	−0.082, −0.028	<0.0001
Presence of proteinuria	−0.18	−0.57, 0.21	0.36
High-density lipoprotein cholesterol *mg/dL*	0.017	0.003, 0.031	0.02
Pravastatin use	0.02	−0.26, 0.30	0.89

Subjects with both C-reactive protein and tumor necrosis factor receptor II in the highest tertile were considered to have "inflamed" status. The coefficient for pravastatin use represents the benefit of pravastatin treatment on the rate of kidney function loss in mL/min/1.73 m^2 per year (positive values represent rate of loss which is slower than placebo). Variables considered for inclusion in the model were age, race, gender, baseline renal function, systolic blood pressure, baseline high-density lipoprotein cholesterol level, smoking status, diabetic status, use of angiotensin-converting enzyme inhibitors, and baseline proteinuria.

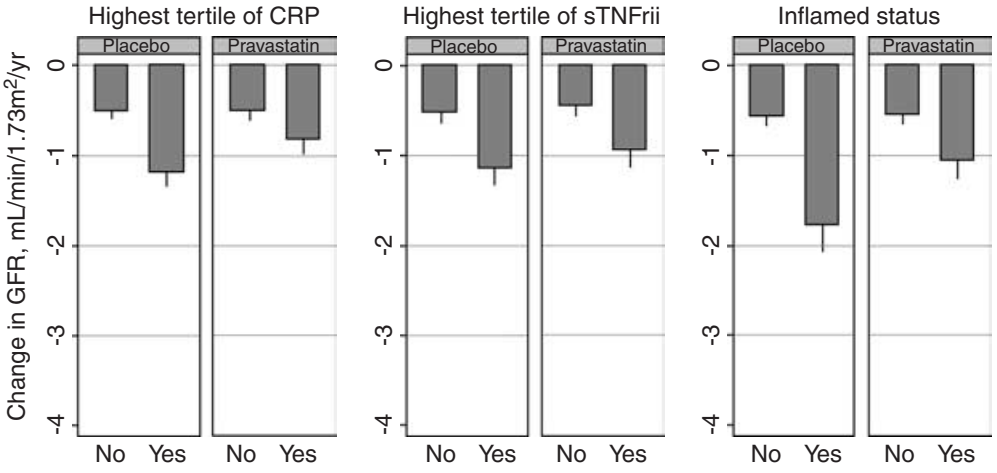

Fig. 4. Adjusted effect of treatment on the rate of kidney function loss. Error bars denote standard error (SE). Inflamed status was defined by both C-reactive protein (CRP) and soluble tumor necrosis factor receptor II (sTNFrii) levels in the highest tertile. Tests for interaction with pravastatin use: CRP ($P = 0.18$), sTNFrii ($P = 0.63$),and inflamed status ($P = 0.047$). Subjects in highest tertile of CRP ($N = 229$), subjects in highest tertile of sTNFrii ($N = 229$), and subjects with inflamed status ($N = 108$).

the relation between CRP and the rate of kidney function loss have reached conflicting results. A small study in humans with IgA nephropathy found that CRP levels predicted more rapid loss of kidney function, but these authors did not control for other potential mediators of renal loss such as proteinuria and hypertension [54]. On the other hand, a larger study of 804 humans with moderate to severe chronic kidney disease found no association between CRP and renal function loss [55]. The latter study had several important strengths, including a range of different underlying renal diseases, direct measurement of GFR (rather than estimation as in the current study), and rigorous methodology. However, follow-up was short (mean 2.2 years), there was limited variability in observed CRP values, and coronary disease was infrequent among participants in that study (<10%). These differences in study design and population may explain the discrepant findings compared with the current study.

Since statins reduce CRP and interfere with multiple other inflammatory pathways [19–25], the interaction between "inflamed" status and the renal benefit of pravastatin would support a pathophysiologic role for inflammation in at least some subjects with chronic kidney disease, and suggest that other therapies which reduce or prevent inflammation might be of benefit. This hypothesis is supported by recent data which suggest that pravastatin reduces TNF-α and sTNFrii [56, 57]. An alternative possibility is that oxidative stress may have been partially responsible for the apparent interaction between inflammation, pravastatin use, and kidney function loss. This hypothesis is supported by observations that markers of oxidative stress are correlated with markers of inflammation in chronic kidney disease [58], and that statins reduce markers of oxidative stress. However, the role of oxidative stress in progressive chronic kidney disease is yet to be conclusively determined, and unfortunately, we

did not have access to markers of oxidative stress in the current analysis.

Our study has limitations that should be considered. First, renal function in the current analysis was estimated using the MDRD GFR formula (a recommended means of estimating kidney function) rather than measured directly. Although iothalamate measurement of GFR would have been preferable, the error associated with use of the MDRD equation was probably reduced because of the central study laboratory. Second, although the serum creatinine assay used in the current study was not calibrated against a reference laboratory, lack of calibration would presumably affect all serum creatinine measurements equally, and would thus not affect the estimation of rates of change in kidney function. Third, since the cause of renal disease in study subjects is unknown, and since all had coronary disease at baseline without recurrent events during follow-up, the generalizability of these findings is uncertain. However, given the frequent coexistence of renal and cardiovascular disease, such individuals are common in the general population [59]. Fourth, the number of subjects in some subgroups was small, and thus these findings must be viewed as hypothesis-generating, especially since *P* values for some analyses were of marginal significance. Fifth, we used an arbitrary definition of inflammation that was based on the distribution of values observed in our population (tertiles) rather than an objective definition. Sixth, although we attempted to reduce bias due to informative censoring by excluding subjects who experienced a cardiovascular event during follow-up, it may have been preferable to include all participants in this analysis. Although we cannot rule out the possibility that this exclusion resulted in bias, we believe that it is more likely to have reduced generalizability instead (i.e., applicability to participants who experienced recurrent cardiovascular events). Seventh, the blood specimens we used to measure the markers of inflammation were collected as long as 15 years before the assays were performed. Previous work found that levels of CRP and sTNFrii appeared to remain stable in frozen specimens over an interval of 4 years [60]. While we cannot exclude the possibility that the longer-term storage of specimens in the current analysis influenced our results, this would be expected to introduce random error, suggesting that performing the assays immediately after blood collection may have strengthened our results. Finally, there was no significant interaction between the effect of pravastatin and inflammation as defined in terms of either CRP alone or sTNFrii alone, in contrast with the findings when inflammation was defined in terms of both biomarkers (both CRP and sTNFrii in the highest tertiles). It is possible that the latter definition was more specific for clinically relevant inflammation (thus increasing statistical power to demonstrate the interaction), or that the interaction depends on inflammatory pathways which involve simultaneous elevation of CRP and sTNFrii. Since this was a post hoc analysis, our findings require confirmation by additional studies.

CONCLUSION

Levels of CRP and sTNFrii were independently associated with the severity of renal insufficiency in people with coronary disease, and elevated levels of both markers appeared to be associated with increased renal benefit from pravastatin. These findings support the hypotheses that inflammation may mediate some types of progressive renal disease, and that statins and possibly other anti-inflammatory therapies may be of clinical benefit.

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