Author's response to reviews

Title: The Relation of C-Reactive Protein to Chronic Kidney Disease in African Americans: The Jackson Heart Study

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Version: 4 Date: 29 August 2009

Author's response to reviews: see over
August 20, 2009

To the Editor:

Please see the revised version to the previously submitted manuscript entitled, “The Relation of C-Reactive Protein to Chronic Kidney Disease in African Americans: The Jackson Heart Study” by Ervin R. Fox, MD, MPH, Emelia J. Benjamin, MD, ScM, Harsha Nagarajarao, MD, Jason K. Taylor, MD, Daniel F. Sarpong, PhD, Michael W. Steffes, MD, PhD, Abdullah K. Salahudeen, MD, Ermeg L. Akylbekova, Caroline S. Fox, MD, MPH, Michael F. Flessner, MD, Robert J. Garrison, PhD, and Herman A. Taylor, Jr., MD. Also see the responses to the reviewers.

This manuscript is not under consideration by any other journal. All authors have reviewed the manuscript in its present form.

Ervin Fox, Emelia Benjamin, and Herman Taylor initiated the idea of the manuscript and participated in the development, analysis, write-up and final review. Also Harsha Nagarajarao and Jason Taylor participated heavily in the manuscript write-up and final review. Dr. Sarpong, Dr. Ermeg Akylbekova and Dr. Steffes contributed their expertise in C-reactive protein data in the Jackson Heart Study and contributed to the analysis and methods sections of the manuscript. Dr. Flessner, Dr. Carolyn Fox and Dr. Salahudeen contributed their knowledge base on chronic kidney disease and the methods and discussion section of the manuscript. Robert Garrison contributed greatly to the review and editorial changes to the final manuscript.

The authors feel the manuscript contributes by adding to current literature on the impact of systemic inflammation to chronic kidney disease in middle-aged African Americans using the large cohort of the Jackson Heart Study. C-reactive protein has been recently found to be a predictor of cardiovascular outcome in African Americans and there is interest toward its relation to subclinical disease in this group.

We thank you for your consideration.

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RESPONSES TO THE REVIEWERS

REVIEWER 1

Reviewer's report
Title: The Relation of C-reactive protein to Chronic Kidney Disease in African Americans: The Jackson Heart Study
Version: 1 Date: 10 July 2009
Reviewer: vecihi batuman

Reviewer's report:
This is a straightforward study demonstrating a significant association between CKD and CRP. The relation held after mutivariable-adjusted analysis. The authors address the limitations of their study adequately and emphasize that the study population consist of African Americans only. While a limitation, this may also be an interesting aspect of their study, and may point to a unique vulnerability in this population. The authors convincingly argue that higher CRP levels are unlikely to be caused by renal insufficiency citing data that CRP levels are independent of kidney function. This is plausible because CRP, mw ~ 115,000, is unlikely to be filterable in the glomerulus in significant quantities. The incidence of CKD (GFR < 60 ml/min/1.73 m²) was found at 5.9%, and this surprising because the prevalence of stage III CKD was estimated at 7.7% in the recent analysis by Coresh at al in the NHANES 1999-2004 population in a nationally representative sample of noninstitutionalized adults aged 20 years or older (JAMA.2007;298:2038-2047.) One would expect a higher incidence in this African American population. The authors may wish to elaborate on this apparent inconsistency.

Obviously, the conclusion that CKD is associated with inflammatory state would be more convincing had the investigators included additional markers of inflammation, such as IL-6, TNF-alpha, etc. If such data are available, it would significantly strengthen the manuscript.

Authors Response:

We agree with Dr. Batuman that it would be better to have additional markers of inflammation. Unfortunately, other inflammatory markers were not measured in the JHS Examination 1.
Reviewer Comment:

A minor point is that the authors point out to the association between CRP and graft failure. What kind of "graft" is meant here?

Authors Response:
In the revision, we removed the statement on graft function as it is not relevant to the point being made. (Page 4, Paragraph 2)

We appreciate Dr. Batuman’s suggestions designed to improve the readability of the manuscript.
Reviewer's report

Title: The Relation of C-Reactive Protein to Chronic Kidney Disease in African Americans: The Jackson Heart Study

Version: 1 Date: 26 June 2009
Reviewer: Wilfried Karmaus

Reviewer's report:
- Major Compulsory Revisions

Reviewer Comment:

1. The role of the analyses linking CRP and albuminuria is not clear. Albuminuria may be considered as a marker of glomerular lesions. However, increased total urine protein (including albumin) is a marker of tubular lesions. I believe that the information on albuminuria is important. If no information on total urine protein is available, then please state this limitation, but indicate that the results not showing an association between urine albumin and CRP speaks against a glomerular involvement. Or discuss this.
   If you decide to include the information on albuminuria then also include the respective objective, methods, and results in the ABSTRACT.

Authors Response:
We thank Dr. Karmaus for helping us to improve the clarity of the manuscript. In the revision on page 9, we are clearer on the analysis linking CRP and albuminuria. We also now include information regarding albuminuria in the abstract. (page 2).

Statement on page 9

“CRP was not significantly associated with albuminuria in the multivariable adjusted model. This finding speaks against a glomerular involvement.”

Abstract:

“CRP was significantly associated with albuminuria in sex and age adjusted model however not in the multivariable adjusted model (p>0.05).”

Reviewer Comment:

2. Estimated GFRs (e-GFR), including Modified Diet in Renal Disease (MDRD) equation, are weak predictors of kidney problems (the specificity is OK, the sensitivity is low). Did the study collect other indicators of chronic kidney
disease? Please show the link between other interview/medical data and e-GFR. If not, discuss the limitation of MDRD.

**Authors Response:**
Our previous publication (now cited on page 6) investigated this and unfortunately there are no other markers for CKD available at present in the JHS. Limitations of MDRD is noted in the Study Limitation section on page 12.

Page 12:

“Fourth, renal function in the current analysis was estimated using the MDRD eGFR formula (a recommended means of estimating kidney function) rather than measured directly. Iothalamate measurement of GFR is not feasible in a population-based setting.”

**Reviewer Comment:**

3. Page 10, para 1: “Those with .. factors.” It is not clear what you want to say. I have some clues but do not understand this. If you relate to the predominant nephrological explanation that most kidney diseases are caused by diabetes mellitus, then state that your results emphasize another risk factor.

**Authors Response:**

In the revision, the authors make this statement clearer. (Page 11)

Page 11

“Those with end-stage renal disease but not on dialysis and those with mild renal insufficiency are at increased risk for the development of cardiovascular disease, which cannot be attributed entirely to traditional risk factors of diabetes, hypertension and obesity. Our results suggest that inflammation relates to CKD and may represent an additional (non-traditional) risk factor for cardiovascular events in those with CKD.”

**Reviewer Comment:**

4. Page 10, discussion section under “Mechanism linking CRP with renal function”: You may want to discuss, that others have shown that CRP is related to reduced kidney cortex width and both are reduced to increase blood pressure. (Dimitrov P et al. Increased blood pressure in adult offspring of families with Balkan endemic nephropathy: a prospective study. BMC Nephrology 2006;7:12. Karmaus W et al. Offspring of Parents with Balkan Endemic Nephropathy have higher C-reactive Protein Levels Indicative of Inflammatory Processes. BMC Nephrol. 2009;10:10.) Hence, CRP may be a marker of kidney inflammation with increased scarring in the kidney cortex, which may then relate to blood pressure. It may also be important for your investigations that urinary TGF-beta1 excretion
was significantly higher in chronic kidney patients than controls (Dukanović et al., 2009).

Authors Response:

Excellent points. In the revision, we have added information the Reviewer suggested regarding the mechanisms linking CRP with renal function. (Page 12 – last paragraph in section).

Page 12

“others have shown that higher CRP concentrations are related to reduced kidney cortex width and reduced renal cortex width is related to increased blood pressure. Hence, CRP may be a marker of kidney inflammation with increased scarring in the kidney cortex, which may then relate to blood pressure.”

Reviewer Comment:

5. I would suggest stratifying table 1 by age. The reason is that more women have increased CRP and also chronic kidney diseases. Please include the result of this stratification in the result and discussion sections.

Authors Response:

In the revision we age stratify table 1 by age > 60 and < 60 years The results are described in the Results section and in the Discussion section.

Reviewer Comment:

- Minor Essential Revisions
  1. Page 8, para 2: Delete on full stop (.)

Authors Response:

Thanks, this is corrected in the revision.

Reviewer Comment:

2. Figure 2 and 3: In the left column, do not use “adjustment” as a column header but put it on top of the rows with “age and sex” and “multivariate”.

Authors Response:

This is corrected in the revision.
Reviewer's report
Title: The Relation of C-Reactive Protein to Chronic Kidney Disease in African Americans: The Jackson Heart Study
Version: 1 Date: 12 July 2009
Reviewer: Peter Barany
Reviewer's report:
This is a cross-sectional observational study of African Americans and associations between CKD and CRP levels. A few comments and criticism of the study:

Reviewer Comment:

1. The selection of participants is made in four different ways; from the ARIC study (driving licence registry, from the Acudata list, from volunteers and from the JHS family study. How well balanced is the final population, i.e. is there a risk that sick individuals are underrepresented? You may compare your population's GFR distribution with the NHANES study.

Authors Response:

We agree that this population is community based and partially volunteer and family-based; this is mentioned in the manuscript. Also we cited the prevalence paper that discussed in detail the issues related to CKD in the Jackson Heart Study and its comparison to NHANES. (Page 9 and 10)

Page 9 and 10

“This result is somewhat surprising given the prevalence of stage III CKD was estimated at 7.7% in the recent analysis by Coresh et.al in the NHANES 1999-2004 population (a nationally representative sample of non-institutionalized adults aged 20 years or older).13, 17 This may be affected by a number of factors ……differences in sample recruitment between our cohort and that in NHANES may favor a lower percentage of unhealthy individuals and subsequently lower prevalence of CKD in the Jackson Heart Study. “

Reviewer Comment:

2. The MDRD formula is not particularly suited for population surveys since it is validated in a US CKD population with GFR <60 ml/min. When using a cut-off of 60 ml/min there is great uncertainty if the participants are correctly classified. However, I accept its use since more precise estimation of GFR is not available.

Authors Response:
We agree there may be some limitation with the MDRD however this is the best current measure of CKD that was available in Examination 1 for this cohort. We stress this as a limitation in the study.

Reviewer Comment:

3. You may subdivide the population further; Without CKD is GFR>90 and no albuminuria, CKD 1 is GFR>90 and albuminuria, CKD 2 GFR 60-90 ml/min.

Authors Response

We reevaluated this group using GFR>90 with albuminuria and GFR>90 without albuminuria and found significant relation of CRP with CKD based on p-trend for the age and sex adjusted model. (p-trend=0.0099) The p-trend for the multivariable adjusted was 0.0734. Coresh and others in the epidemiology world have looked at this and found an error of ~10-20% at these higher clearances (>60). We included this table as an electronic supplement to the manuscript.

<table>
<thead>
<tr>
<th>Stages of CKD</th>
<th>p-trend</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
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<tr>
<td>2</td>
<td></td>
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<tr>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

**, pooled signify both men and women included in the analysis

CKD, chronic kidney disease; GFR, glomerular filtration rate; GM = geometric mean; SE = Standard Error

Normal (no chronic kidney disease) and Stages 1, 2 and 3 of chronic kidney disease are defined as follows: No chronic kidney disease = glomerular filtration rate >90 without albuminuria, Stage 1 chronic kidney disease = glomerular filtration rate >90 with albuminuria, Stage 2 chronic kidney disease = glomerular filtration rate between 89 and 60 with albuminuria, and Stage 3 chronic kidney disease between 59 and 30.

Reviewer Comment:

4. Overall, I think the headings of the Tables do not sufficiently describe what is shown. The Tables (and Figures) should be possible to read without further information from the text.

Authors Response:
We appreciate the Reviewers feedback designed to improve the clarity of the manuscript.

In the revision, we revised the headings of some of the Tables so that they sufficiently describe what is shown.

Reviewer Comment:

4a Table 1; Low and High CRP are not defined and the values (<5.6 and >5.6 mg/dL) are not correct. CKD should be defined in the table. Albuminuria could be included

Authors Response:

In the revision, we state that for assessing demographics in those with CRP we in the upper 25th and lower 75th percentile of CRP for our study population. The table and text reflect this change and explanation.

Reviewer Comment:

4b Table 2-3 It is not clear which analyses have been done and what you show in these tables. As I understand you have adjusted the CRP values with data obtained from multiple regression calculations, please explain how this calculation has been done. Calculation of hazard ratios with Cox regression analysis may be another way to describe the association between CRP and GFR<60 (or albuminuria). Then you need to define either a CRP (or albuminuria) cut-off or use CRP/albuminuria as a continuous variable (absolute or log CRP). Compare e.g. analyses in the paper by Astor et al (Am J Epidemiol 2008;167:1226–1234).

Authors Response:

Given that the data for this paper is cross-sectional with only prevalent cases of CKD defined by estimated GFR or albuminuria, the use of the general linear modeling approach (linear regression) is appropriate. Though in some instances the use of Poisson regression or Cox proportional hazard models will be a better alternative, the underline condition for these two methods is not quite for this case. Cox proportional hazard models are generally used with survival like analyses, where the primary outcome is mortality or the occurrence of incident events was the case in Astor et al paper (Am J. Epidemiol 2008; 167:1226-1234).

Reviewer Comment:

Authors Response:

In the revision, we mention the paper by Astor et al as suggested by the reviewer.

Page 4

“a10-ml/minute/1.73 m² lower estimated GFR [(eGFR) among persons with eGFR <60 ml/minute/1.73 m²)] has been associated with an incidence rate ratio of 1.29 (95% confidence interval: 1.06, 1.55) for cardiovascular mortality and a doubling of albuminuric has been associated with an incidence rate ratio of 1.06 (95% confidence interval: 1.04, 1.08) for cardiovascular mortality.” — Finding from Astor et al
Reviewer's report
Title: The Relation of C-Reactive Protein to Chronic Kidney Disease in African Americans: The Jackson Heart Study
Version: 1 Date: 2 July 2009
Reviewer: Kosaku Nitta
Reviewer's report:
The manuscript by Fox et al. describes the important relation between CRP and CKD in African Americans in the US. The authors insist that there is significant correlation between CRP and CKD in the population. It is interesting to investigate contribution of inflammatory status to CKD among various races. However, at this moment, the manuscript is too much premature for publication. Although correlates analyses show significant differences between CRP high and low groups, there are several problems as mentioned below.

Major Points

Reviewer Comment:
1. The authors divided the participants into two groups according to serum CRP levels. But they totally ignored existence of inflammatory disease, collagen disease, and malignancy. These factors always and strongly affect CRP levels in various degrees. In the study, the participants are divided into two groups without considering the factors. Further, these factors are known strong contributors for CKD. Taken together, there is no meaning to consider correlation between CRP and CKD without considering these factors. We strongly recommend that causes to elevated CRP levels should be clearly mentioned and that the two groups should be adjusted with the factors.

Authors Response:
In the manuscript, we excluded individuals with malignancy and other causes of elevated CRP (that is those with WBC >12, those on rheumatologic medications).

Reviewer Comment:
2. It is difficult to understand the conclusion about the study. Do the authors conclude that measurement of circulating CRP levels should be as beneficial for assessing renal dysfunction as serum creatinine or urea nitrogen levels?

Authors Response:
We regret if we left the impression that we were conveying CRP concentrations were beneficial for assessing renal dysfunction.

We conclude that CRP elevation is related to renal function and that this supports that inflammation may play a role in renal disease. We also state that knowing
this may be beneficial if in future longitudinal studies we can elucidate whether CRP as a marker of inflammation is also related to progression of CKD and whether management/treatment of inflammation using lowering CRP concentration as an indicator of successful treatment may reverse the progression of kidney disease.

Minor Points

Reviewer Comment:

1. What are current hormones? Why the authors added this in criteria?

Authors Response:

In the revision, the authors placed hormone replacement therapy in the Table rather than the term “current hormones”. We included hormone replacement therapy because prior studies have demonstrated that estrogen therapy increases CRP concentrations.