ARIC Manuscript Proposal #1862

PC Reviewed: 11/8/11 Status: A Priority: 2
SC Reviewed: _________ Status: _____ Priority: ____

1.a. Full Title: The sex-specific impact of smoking on markers of inflammation and hemostasis and its contribution to the greater relative risk of coronary heart disease associated with smoking in women: The Atherosclerosis Risk in Communities Study

b. Abbreviated Title (Length 26 characters): Sex-specific impact of smoking

2. Writing Group:
Rachel R. Huxley, Hiroshi Yatsuya, Pamela L. Lutsey, Derek Smolenski, Aaron R. Folsom, others welcome

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _RH__ [please confirm with your initials electronically or in writing]

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ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).

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3. Timeline:
Data analysis – 3 months
First draft of the manuscript – 3 months

4. Rationale:
Currently, it is estimated that 23% and 18% of American men and women smoke, respectively, placing themselves at substantially increased risk of a wide range of
chronic conditions including cardiovascular disease, respiratory disease and cancer (1). Cigarette smoking is associated with a doubling of the risk of coronary heart disease (2) and is a leading cause of heart attacks in the United States (3). Despite smoking fewer cigarettes per day, women have a larger relative risk of coronary heart disease related to smoking compared with males, an interaction that persists even after adjustment for differences in other major vascular risk factors (2). The mechanisms that may mediate this interaction are unknown but may include a combination of physiological, behavioral or biological risk factors.

The exact pathophysiological pathways by which smoking causes coronary heart disease are unclear but endothelial dysfunction has been suggested to be a key mechanism (4,5). Previous studies have shown that cigarette smoking promotes oxidative stress (6), increased blood thrombogenicity (7) and inflammation (8), which are all components of endothelial dysfunction. However, to the best of our knowledge no previous study has investigated whether there is a sex differential in the association of smoking with biomarkers of hemostasis and inflammation. If smoking was shown to have a more deleterious effect on these biomarkers in women as compared with men, then this may help to explain why women who smoke are at greater coronary risk compared with their male counterparts.

The ARIC study represents a novel opportunity to study in the first instance, whether there are sex-interactions in the cross sectional associations between smoking and biomarkers of inflammation and hemostasis. If so, we intend to prospectively examine whether markers of inflammation and hemostasis mediate the known interaction between sex and smoking behavior (including duration and smoking intensity) on CHD risk. ARIC's large sample size and longitudinal study design combined with detailed smoking records and information on a wide number of covariates will enable reliable investigation of a possible sex-differential in the impact of smoking on biomarkers of hemostasis and inflammation.

5. **Main Hypothesis/Study Questions:**
We hypothesize that (1) the association of smoking with biomarkers of hemostasis and inflammation and major cardiovascular risk factors will vary by sex (effect modification). (2) The sex-smoking interaction observed for CHD will be partly or wholly eliminated after adjustment for sex differences in markers of inflammation and hemostatic function.

The objectives of the proposal are as follows;

i. Compare sex differences in the adjusted levels of inflammatory and hemostasis biomarkers (as a proxy for endothelial dysfunction) in current and never smokers.
ii. Determine whether adjustment for any adjusted sex differences in the level of the biomarkers between smokers and never smokers attenuates the sex-smoking interaction for coronary heart disease

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

This will be a two-part analysis. First, we will test for effect modification by sex in the associations of smoking with biomarkers of hemostasis and inflammation and major cardiovascular risk factors in a sample consisting of smokers and never smokers using a cross-sectional approach. Second, using a cohort approach, any associations between smoking and biomarkers and other risk factors that were significantly modified by sex will be adjusted for in an analysis (together with other important covariates) of the association between smoking and risk of CHD. We have previously shown that there are no significant differences in smoking and cardiovascular risk between whites and African-Americans, and hence, for the purpose of these analyses the sample will be combined and the analyses will be adjusted by race (9).

Exposure

Study participants will be grouped as ‘current’ smokers if they reported smoking at study baseline and ‘never’ smoker if they reported never having smoked at baseline. Individuals who reported that they had previously smoked but were no longer currently smoking at baseline will be excluded from this analysis. For current smokers the number of cigarettes smoked per day (CPD) will be classified into three approximately equal sized groups: <15, 15-24, and > 25 CPD. Information on age of initiation of smoking will also be obtained. Pack-years of smoking will be calculated by calculating the average number of cigarettes smoked per day multiplied by the years of smoking divided by 20. In the longitudinal analysis, only current and never smokers will also be included; individuals who quit smoking during follow up will be censored at their previous study visit. The rationale for excluding former smokers is two-fold; first, there is no evidence to suggest that there is effect modification by sex on the association between former smoking and risk of CHD. Second, any dilution of effect due to misclassification of individuals who quit smoking sometime during follow up as current smokers, will be avoided.

Outcome

1. Cross-sectional analysis
   a. Levels of inflammatory and hemostasis biomarkers in smokers and never smokers testing effect modification by sex:
      i. White blood cell count
      ii. Factor VIII
      iii. Activated PTT
iv. Fibrinogen
v. Von Willebrand Factor
vi. CRP (from visit 4 only)

b. Levels of major cardiovascular risk factors in smokers and never smokers stratified by sex:
   i. Systolic blood pressure
   ii. Body mass index
   iii. Heart rate
   iv. Type 2 diabetes and fasting glucose
   v. Lipids (TC, HDL-c, LDL-c and TG.)
   vi. Physical activity
   vii. Alcohol consumption
   viii. Medication usage
   ix. Socioeconomic indicators (i.e. income, education)

Longitudinal analysis
Incidence of CHD will be ascertained through December 2008. Events will be ascertained by annual follow-up interview and surveillance of hospital discharges in the ARIC study areas. Events will be validated by abstraction of hospital discharge records and death certificates, followed by classification according to ARIC study criteria including trained physician reviewers. Out-of-hospital deaths will be ascertained through death certificates and, when available, coroner or autopsy reports. CHD will be defined as a validated or definite or probable hospitalized MI, a definite CHD death, an unrecognized MI defined by electrocardiographic reading or coronary revascularization.

Exclusions
Study participants will be excluded from the analysis if they fulfill at least one of the following criteria:

- ethnicity other than Black or White
- missing data on smoking, biomarkers and physiological risk factors at baseline
- prevalent cardiovascular disease at study baseline
- former smokers at study baseline

Statistical analysis
Cross-sectional analysis
Linearity of the biomarkers will be assessed, and skewed biomarkers will be log transformed, accordingly. General linear regression models will be adjusted for age, race, study site, education, income, alcohol and physical activity. To evaluate cross-sectional interactions between sex and smoking status, we will include cross-product terms in the models. As outlined in the table below, we will also report
adjusted means (or geometric means) stratified by sex and smoking status (current/never). Additionally we will compute, by sex, the difference in adjusted biomarker levels between current and never smokers.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men</th>
<th>Women</th>
<th>P-value for interaction between sexes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Current smoker</td>
<td>Never smoker</td>
<td>Adjusted* difference</td>
</tr>
<tr>
<td>WBCx 10^9/L</td>
<td>8.0 +/- 0.3</td>
<td>6.8 +/- 0.2</td>
<td>1.1 +/- 0.3</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for age, race, study site, education, income, alcohol, physical activity and associated cardiovascular co-morbidities including, BMI, blood pressure and use of blood pressure lowering medication, dyslipidemia and lipid lowering medication, and type-2 diabetes and diabetic medication

**Additionally adjusted for smoking duration and pack years of smoking

Longitudinal analysis

Previous work indicates that there is a significant interaction with sex in the relationship between smoking and risk of CHD. As a first step, we will test whether this interaction is present in ARIC via Cox proportional hazard models which include a cross-product term between sex and smoking in the model (Model 1). This model will be adjusted for age, race, BMI, study site, education, income, smoking duration and intensity, alcohol and physical activity.

Next, we will assess whether differences in the impact of smoking between men and women on levels of physiological, hemostatic and inflammatory markers (i.e. biomarkers) mediate the sex-interaction between smoking and risk of CHD by adding these markers to Model 1 (Model 2). Finally, to determine whether sex-interactions between smoking and the aforementioned markers attenuates the sex-interaction between smoking and CHD we will add sex-biomarker interaction terms to Model 2 (Model 3). Thus to summarize, we will construct the following three models to test our hypothesis:

A) Sex*smoking
B) Sex*smoking + physiological/biomarkers
C) Sex*smoking + physiological / biomarkers + physiological/biomarkers *sex

We will explore the assumption of proportional hazards adding to the model an interaction term between follow-up time and exposure of interest, computing Schoenfeld residuals, and by inspection of the log (-log[survival function]) curves (10).
Limitations
In our primary analysis, we will be reliant upon self-reported smoking habits as there is no biochemical measure of smoking status in ARIC. However, as we are excluding former smokers from the analysis at baseline or censoring them during follow up, this should reduce any error introduced through misclassification of former smokers as current smokers potentially avoiding any dilution of the magnitude of the observed associations.

7.a. Will the data be used for non-CVD analysis in this manuscript?  ___ Yes  ____ x No

b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES_OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES_DNA = “CVD Research” would be used?  ___ x Yes  ____ No
(This file ICTDER03 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript?  ___ Yes  ____ x No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = “No use/storage DNA”?  ___ Yes  ____ No

8.c. If yes, is the author aware that the participants with RES_DNA = ‘not for profit’ restriction must be excluded if the data are used by a for profit group?  ___ Yes  ____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.csc.unc.edu/ARIC/search.php

___ X  Yes  _____ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?  ___ x Yes  ____ No
11.b. If yes, is the proposal
   A. primarily the result of an ancillary study (list number*)
   2008.09
   ___  B. primarily based on ARIC data with ancillary data playing a
   minor role (usually control variables; list number(s)* __________
   __________)

*ancillary studies are listed by number at http://www.cscu.unc.edu/aric/forms/

12. Manuscript preparation is expected to be completed in one to three years. If a
    manuscript is not submitted for ARIC review at the end of the 3-years from the date
    of the approval, the manuscript proposal will expire.

References

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