Exposure to Secondhand Smoke and Biomarkers of Cardiovascular Disease Risk in Never-Smoking Adults

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Background—Exposure to secondhand smoke has been associated with a disproportionately high risk of coronary heart disease, thought to be mediated through inflammation, platelet aggregation, and/or endothelial dysfunction. The epidemiological association between objectively measured exposure to secondhand smoke and biomarkers of heart disease risk has not been investigated, however.

Methods and Results—We have investigated the cross-sectional relation between secondhand smoke exposure, measured objectively as cotinine, and recognized biomarkers of heart disease risk, namely C-reactive protein, homocysteine, fibrinogen, and white blood cell count, in 7599 never-smoking adults from the Third National Health and Nutrition Examination Survey. Compared with subjects with no detectable cotinine, those with detectable but low-level cotinine (range, 0.05 to 0.215 ng/mL) had significantly higher levels of both fibrinogen (adjusted mean difference, 8.9 mg/dL; 95% CI, 0.9 to 17.0; \( P = 0.03 \)) and homocysteine (0.8 \( \mu \)mol/L; 95% CI, 0.4 to 1.1; \( P < 0.001 \)) but not C-reactive protein or white blood cell count. Effect estimates of similar magnitude and significance were seen in subjects in the high category of cotinine exposure (>0.215 ng/mL). The increased levels of fibrinogen and homocysteine seen in relation to secondhand smoke exposure were equivalent to \( \sim 30\% \) to \( 45\% \) of those seen for active smoking.

Conclusions—Passive smokers appear to have disproportionately increased levels of 2 biomarkers of cardiovascular disease risk, fibrinogen and homocysteine. This finding provides further evidence to suggest that low-level exposure to secondhand smoke has a clinically important effect on susceptibility to cardiovascular disease. (Circulation. 2007;115:

Key Words: cardiovascular diseases ■ C-reactive protein ■ fibrinogen ■ homocysteine ■ tobacco smoke pollution

Active smoking is a major and well-established modifiable risk factor for coronary heart disease.\(^1\) Over recent years, interest has turned to the role of secondhand smoke (SHS) exposure in the origin of cardiovascular disease, and evidence is now suggesting a causal relationship.\(^2\) Interestingly, the magnitude of the increased risk associated with exposure to SHS has been estimated at about one-third that seen for active smokers, even though actual exposure to tobacco smoke is much smaller.\(^3,4\) Such a disproportionate effect might be explained by a number of mechanisms, including effects on inflammation, platelet aggregation, and endothelial dysfunction.\(^5\) If these are the mechanisms at play, then levels of biomarkers of these processes, including C-reactive protein (CRP), homocysteine, fibrinogen, and white blood cell (WBC) count, should be increased in relation to SHS exposure. The few epidemiological studies that have investigated these relations to date have reported positive associations, but all measured SHS exposure by self-report only.\(^6\)–\(^8\) The importance of using objective measures such as cotinine in epidemiological studies of cardiovascular disease was highlighted by a recent prospective study of cotinine and coronary heart disease events by Whincup et al\(^9\) in which effect estimates were larger than previously reported, possibly because earlier studies based on self-reported exposure had underestimated the true effect size.

Clinical Perspective p ●●●

Our present study investigates the association between serum cotinine as an objective measure of SHS exposure and levels of CRP, homocysteine, fibrinogen, and WBC count as biomarkers of cardiovascular disease risk in never-smoking adults participating in the Third National Health and Nutrition Examination Survey (NHANES III).

Methods

Study Sample

NHANES III is a nationally representative US survey carried out between 1988 and 1994, full details of which have been given elsewhere.\(^10\) In brief, the survey used a stratified, multistage probability design and was conducted in 2 phases, 1988 to 1991 and 1991 to 1994. Of the 39 695 individuals \( \geq 2 \) months of age selected for inclusion, questionnaire data were collected on 33 994 (86%) during a home interview. These subjects were then invited to a mobile examination center for a detailed medical examination; 78% (30 818) attended. At this examination, further questionnaires were
administered, a variety of tests were performed, and samples of blood (in those ≥1 year of age) and urine were collected for laboratory analysis. The survey was approved by the Institutional Review Board of the National Center for Health Statistics, and informed consent was obtained before participation.

**Blood Measurements**

Details of the laboratory analysis of blood samples have been given elsewhere. Briefly, serum cotinine was measured by an isotope-dilution high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometric method and had a threshold of detection of 0.05 ng/mL. Serum CRP was measured with latex-enhanced nephelometry and had a threshold of detection of 0.3 mg/dL. Serum homocysteine was measured during phase 2 only with reverse-phase high-performance liquid chromatography and fluorescence detection. Fibrinogen was measured in the plasma of those ≥40 years of age through the use of a simple enzyme assay. A fully automated hematology analyzer (Coulter Model S-PLUS JR, Coulter Electronics, Hialeah, Fla) was used to provide a qualitative assessment of WBC count. The analytical methods used by each laboratory were consistent with the requirements of the Clinical Laboratory Improvement Act 1988, and rigorous quality control procedures were followed. Laboratory staff were blind to the active and passive smoking status of subjects.

**Statistical Analysis**

For our analysis, only adults ≥17 years of age (n = 20,050) were included; we restricted our analysis to the 9800 who gave a negative response when asked at the home interview whether they had ever smoked at least 100 cigarettes, 20 cigars, or 20 pipes of tobacco in their life. At the mobile examination center examination, participants were further asked about whether they had smoked any cigarettes, cigars, or pipes in the past 5 days; any who responded positively (n = 305) were excluded. Of these 9495 adults, 7828 (82%) had serum cotinine data available, and the 7599 with levels ≤15 ng/mL (consistent with their self-reported nonsmoking status) were included in subsequent analysis.

Statistical analyses were carried out in STATA version 8.0 (Stata Corp, College Station, Tex) using methods that accounted for the complex sampling design of NHANES III. Biochemical variables were analyzed in the original units provided in the NHANES III data sets. For the exposure variable serum cotinine, many individuals had levels below the threshold of detection; therefore, this variable was categorized for analysis into 3 groups using cut points of 0.05 ng/mL, the threshold level of detection, and 0.215 ng/mL, the median value among those with detectable levels. The groups were labeled no cotinine (<0.05 ng/mL), low cotinine (0.05 to 0.215 ng/mL), and high cotinine (>0.215 ng/mL). The associations between cotinine group and each of the response variables homocysteine, fibrinogen, and WBC count, with adjustment for potential confounders, were assessed using multiple linear regression. Because the response variables homocysteine and WBC count had a small number of high outlying values, model fitting based on both the original and log-transformed values was explored, and because both yielded similar results, those based on the original scale are presented.

For the response variable CRP, a high proportion of individuals had levels below the laboratory threshold of detection of 0.3 mg/dL; therefore, CRP was analyzed as a binary response variable in 2 ways: based on this cut point of 0.3 mg/dL and using a cut point of 1 mg/dL (representing clinically elevated CRP). The association between cotinine group and CRP, again with adjustment for potential confounders, was assessed using multiple logistic regression.

Effect estimates presented are adjusted for the main determinants of cardiovascular health: age, gender, race-ethnicity, social class, physical activity, and body mass index. Race-ethnicity was defined as non-Hispanic white, non-Hispanic black, Mexican American, or other. Social class was measured by the highest year or grade of regular school completed. Physical activity was classified as sedentary, lightly active, moderately active, and vigorously active and was derived from a series of questions asked at the home interview relating to type, intensity, and frequency of different activities and sports. Body mass index was computed by dividing the participant’s weight, as measured at the mobile examination center examination, by the measured height squared. A number of additional potential confounders were explored and included in the final model only if they altered the magnitude of the effect estimate by ≥10%. They included diet, alcohol consumption, history of diabetes, waist-to-hip ratio, frequency of aspirin use in the past month, triglycerides, systolic blood pressure, history of hypertension, and ratio of total to high-density lipoprotein cholesterol. Total fruit and vegetable consumption was used as a marker of diet and was derived by summing the number of occasions each month that the participant reported eating a fruit or vegetable. Total meat consumption was computed in a similar manner and was used as an alternative marker of diet. Alcohol consumption was assessed as self-reported total number of times per month that alcohol was consumed. Waist-to-hip ratio was computed as the participant’s waist circumference divided by the circumference of their buttocks, as measured at the mobile examination center examination. Triglyceride and cholesterol concentrations were quantified from the participant’s blood sample, and blood pressure was measured at the home interview. For each response variable, evidence of effect modification by age and gender was explored by fitting interaction terms, and stratified results were presented if any interactions were statistically significant (P < 0.05).

Our analyses are based on data collected by the US National Center for Health Statistics. Both authors had full access to this data and take full responsibility for the integrity of the data analysis. Both authors have read and agree to the manuscript as written.

**Results**

Table 1 shows the characteristics of the 7599 subjects analyzed; 2405 (32%) were men, 5194 (68%) were women, and their median age was 38 years (25th to 75th percentile, 26 to 61 years). Cotinine was undetectable in 1371 (18%) of participants (no cotinine group); in the other 6228 (82%), the median level was 0.215 ng/mL (25th to 75th percentile, 0.106 to 0.558 ng/mL). The distribution of cotinine among those with detectable levels is shown in the Figure. The overall proportion reporting that they lived with a smoker or were exposed to tobacco smoke at work was 31%, but this proportion increased in relation to cotinine group: 5%, 18%, and 56% in those with no, low, and high cotinine, respectively (P < 0.001 for χ² test).

Both the low- and high-cotinine groups had significantly higher levels of fibrinogen and homocysteine than the group with no detectable cotinine (Table 2). These higher levels were of similar magnitude in the low- and high-cotinine groups, with fibrinogen levels estimated to be ~9 to 10 mg/dL higher and homocysteine to be 0.8 μmol/L higher in those with compared with those without detectable cotinine. Those with detectable cotinine also had lower WBC, but not significantly so after adjustment for potential confounders (Table 2). There was no evidence that CRP was increased in relation to cotinine (Table 3). Further adjustment for the other potential confounders, including the diet variables (fruit and vegetable consumption and meat consumption), did not materially alter the results. The only exception was WBC count, for which estimates became stronger (ie, more negative) after adjustment for waist-to-hip ratio and weaker (ie, closer to unity) after adjustment for triglycerides. Inclusion of both variables in the model, however, resulted in estimates similar to those in Table 2.

Restriction of analysis to those ≤70 years of age with no history of a heart attack, heart failure, or stroke also made little
difference to the observed associations. Furthermore, there was no evidence of any effect modification by age or sex.

To put our observed effect estimates for SHS exposure into context, we compared them with those for active smoking by selecting all 4990 current smokers from the NHANES III data set (median cotinine, 208 ng/mL) and computing adjusted mean differences for this active smoking group relative to the same baseline group of never smokers with no detectable cotinine. The adjusted mean difference for fibrinogen was 29.2 mg/dL (compared with 9 to 10 mg/dL in relation to SHS exposure; Table 2) and for homocysteine was 1.8 μmol/L (compared with 0.8 μmol/L in relation to SHS exposure; Table 2).

**Discussion**

The results of the present study indicate that passive smokers have significantly elevated fibrinogen and homocysteine levels, both of which are important biomarkers of cardiovascular disease risk.\(^{13,14}\) These findings are generally consistent with those reported previously by Panagiotakos et al\(^{6}\) in a study based on self-reported exposure, but our effect sizes were approximately twice as high, perhaps because serum
cotinine is a more valid measure of SHS exposure than self-report. This would fit with previous evidence that self-reported exposure seems to underestimate the risks of exposure to SHS.9 In contrast to the study by Panagiotakos et al,6 we did not observe any associations with CRP or WBC count, although an association with CRP may have been missed because NHANES III did not use high-sensitivity laboratory methods for CRP. Panagiotakos et al6 also reported a significant association with oxidized low-density lipoprotein cholesterol,6 but we were unable to look at this because it was not measured in NHANES III. Our finding of an association with fibrinogen also is consistent with 2 other previous studies that looked at this biomarker only, again in relation to self-reported exposure.7,8 Only one of these, however, reported multivariable-adjusted effect estimates, and they were again slightly smaller than ours, except for that in relation to exposure both inside and outside the home, which was of a similar magnitude.7 It should be noted, however, that our effect estimates for fibrinogen lack precision because the 95% CIs are relatively wide.

The question arises as to whether the elevated levels of these biomarkers seen in passive smokers may be due to some other aspect of our participants’ lifestyles such as diet. We used self-reported fruit and vegetable consumption as a marker of diet because clinical trials point to this as one of the most influential aspects of diet in terms of cardiovascular health15 and because it is likely to be correlated with other aspects of a healthy diet. In addition, we considered self-reported meat consumption as an alternative marker of diet. Although we cannot completely rule out the possibility of residual confounding, the fact that our associations changed very little after adjustment for these variables and a large number of other lifestyle factors, including physical activity, social class, and obesity, suggests this is unlikely. It is also unlikely that our findings have arisen from selection bias because the overall response for NHANES III was high (86%)10 and the majority of participants (82%) provided a blood sample for which a valid cotinine could be measured. For homocysteine, 11% of our sample (phase 2 only) had a missing value because the homocysteine laboratory analysis was a later addition to the NHANES protocol and carried out in those with sufficient surplus sera; missing data for the other response variables were minimal (<3%).

To minimize the inclusion of misclassified active smokers in our sample, we excluded anyone with a value of serum cotinine >15 ng/mL, the cut point used to distinguish current smokers from nonsmokers,12 and as the Figure shows, the

<table>
<thead>
<tr>
<th>TABLE 2. Associations Between Cotinine Level and Fibrinogen, Homocysteine, and WBC Count</th>
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<tbody>
<tr>
<td><strong>Fibrinogen,</strong>† <strong>mg/dL</strong></td>
</tr>
<tr>
<td>No cotinine‡</td>
</tr>
<tr>
<td>Low cotinine</td>
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<tr>
<td>High cotinine</td>
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<tr>
<td><strong>Homocysteine,</strong>§ <strong>µmol/L</strong></td>
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<tr>
<td>No cotinine</td>
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<td>Low cotinine</td>
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<tr>
<td>High cotinine</td>
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<td><strong>WBC, 10³ cells/µL</strong></td>
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<tr>
<td>No cotinine</td>
</tr>
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<td>Low cotinine</td>
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<td>High cotinine</td>
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*Mean differences adjusted for age, sex, race-ethnicity, body mass index, physical activity index, and education.
†Fibrinogen measured in subset =40 years of age only.
‡No cotinine, <0.05 ng/mL; low cotinine, 0.05 to 0.215 ng/mL; high cotinine, 0.215 to 15 ng/mL.
§Homocysteine measured in phase 2 of survey only.

Distribution of cotinine in study subjects with detectable cotinine (n=6228).
vast majority of our sample had values well below this cut point. Although we can be less confident about the exclusion of misclassified former smokers from our sample, the association between former smoking and fibrinogen and homocysteine in the NHANES III population is relatively weak; therefore, such misclassification is unlikely to explain our findings.

Our finding of similar-sized effect estimates in the low-cotinine group, of whom only 18% lived with a smoker or were exposed at work, and those with high exposure indicates that even very low levels of exposure may be associated with appreciable increases in cardiovascular risk. Cotinine levels were only ≤0.1% of those in active smokers, but the apparent effects of passive smoking on the biomarkers we measured were approximately one-third to one-half those for active smoking. These disproportionate associations not only are consistent with the previous studies of fibrinogen and homocysteine but also fit with the epidemiological evidence of a similar disproportionate association with coronary heart disease risk. Furthermore, they are biologically plausible because both fibrinogen and homocysteine are markers of inflammation and platelet activation, 2 important processes of the cardiovascular system thought to be very sensitive to the toxins in SHS. It is likely that multiple mechanisms play a role in the association between SHS and cardiovascular disease, however, including others that we have been unable to consider in this study, and that they interact with each other.

Meta-analyses of the relation between fibrinogen and homocysteine and cardiovascular disease risk shed light on the clinical meaningfulness of our findings. They provide strong evidence that these biomarkers are causally related to disease risk and indicate that the magnitude of each of the associations with fibrinogen and homocysteine observed in our study should translate into increases in disease risk of the order of 5%, although their combined effect is likely to be greater than this. This is lower than the excess risk of 30% estimated from case-control studies using cardiovascular disease as the main outcome, perhaps because other mechanisms are playing a role.

Conclusions
Passive smokers have disproportionately increased levels of fibrinogen and homocysteine, 2 important biomarkers of cardiovascular disease risk. These findings lend support to existing evidence that SHS has a clinically important effect on susceptibility to cardiovascular disease and indicate further that exposure to SHS is likely to be an important avoidable cause of cardiovascular disease in the population.

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Disclosures
None.

References
Secondhand smoke contains the same spectrum of toxins as mainstream smoke and thus is likely to have the same spectrum of harmful effects as active smoking. Because the level of exposure of nonsmokers who live or work with smokers is typically \( \approx 1\% \) of that of an active smoker, the absolute magnitude of the risk posed by secondhand smoke exposure might be expected to be much lower than that of active smoking. Recent evidence suggests, however, that this may not be the case for cardiovascular disease, for which the risk in nonsmokers exposed to secondhand smoke appears to be \( \approx 30\% \) of that of active smoking. The present study of the relation between secondhand smoke exposure, measured objectively as serum cotinine, and blood levels of several biological markers of cardiovascular pathology demonstrates that plasma levels of fibrinogen and homocysteine are significantly increased in nonsmokers even with low levels of cotinine in a representative sample of US adults. These findings may suggest a possible mechanism for the finding that very low levels of cigarette smoke have a disproportionately large effect in promoting the pathogenesis of cardiovascular disease. The data provide further evidence that secondhand smoke exposure is an important public health hazard. Policies to protect the public even from modest secondhand smoke exposure are therefore important.
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