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Epidemiology. Author manuscript; available in PMC 2013 March 1.

Published in final edited form as:
Epidemiology. 2012 March ; 23(2): 332–340. doi:10.1097/EDE.0b013e31824523f0.

Air pollution and markers of coagulation, inflammation and endothelial function: Associations and epigene-environment interactions in an elderly cohort

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Abstract

BACKGROUND—Previous studies suggest that air pollution is related to thrombosis, inflammation, and endothelial dysfunction. Mechanisms and sources of susceptibility are still unclear. One possibility is that these associations can be modified by DNA methylation states.

METHODS—We conducted a cohort study with repeated measurements of fibrinogen, C-reactive protein, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) in 704 elderly men participating in the Veterans Administration Normative Aging Study (2000-2009). We investigated short- and intermediate-term air pollution effects on these blood markers, and epigene-environment interactions by DNA methylation of *Alu, LINE-1*, tissue factor (*F3*), Toll-Like Receptor 2 (*TLR-2*), and *ICAM-1*.

RESULTS—We found effects of particle number, black carbon, nitrogen dioxide (NO_2), and carbon monoxide (CO) on fibrinogen. Ozone was a significant predictor of C-reactive protein and ICAM-1. Particle number, black carbon, NO_2, CO, $PM_{2.5}$, and sulfates were associated with ICAM-1and VCAM-1. An interquartile range increase in 24-hour exposure for NO_2; was associated with a 1.7% (95% confidence interval = 0.2% to 3.3%) increase in fibrinogen for ozone a 10.8% (2.2% to 20.0%) increase in C-reactive protein for particle number, a 5.9% (3.6% to 8.3%) increase in ICAM-1; and for $PM_{2.5}$ a 3.7% (1.7% to 5.8%) increase in VCAM-1. The air pollution effect was stronger among subjects having higher *Alu*, lower *LINE-1*, tissue factor, or *TLR-2* methylation status.

CONCLUSION—We observed associations of traffic-related pollutants on fibrinogen, and both traffic and secondary particles on C-reactive protein, ICAM-1, and VCAM-1. There was effect modification by DNA methylation status, indicating that epigenetic states can convey susceptibility to air pollution.

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Particulate air pollution has been associated with triggering of myocardial infarctions and increased cardiovascular mortality.[1] Potential pathways for these effects include increased systemic cytokine-mediated inflammation, endothelial dysfunction, increased thrombosis, decreased plaque stability, and increased arrhythmias.[1-3] Previous studies have found that air pollution influences markers of coagulation (such as fibrinogen),[4,5] inflammation (such as C-reactive protein),[6] and endothelial function (such as ICAM-1 and VCAM-1).[7-9] Elevations in these blood markers, in turn, have been associated with increased risk of adverse cardiovascular events.[1,2,9-11] Fibrinogen plays a key role in the clotting cascade, where its conversion to fibrin stabilizes blood clots after injuries. Fibrinogen has procoagulant and proinflammatory properties, and promotes atherothrombosis. C-reactive protein is an acute-phase protein and a general marker for inflammation. ICAM-1 and VCAM-1 are transmembrane proteins that mediate endothelial transmigration of white blood cells in inflammatory response. Air pollution has also been linked to higher tissue factor (*F3*) and Toll-Like Receptor 2 (*TLR-2)* expression.[12,13] While TLR-2 is a member of the Toll-like receptor family, which plays an important role in pathogen recognition, tissue factor enables cells to initiate the blood coagulation cascade.

Air pollution effects on cardiovascular disease (CVD) are stronger among subjects with fibrinogen and IL-6 gene variants.[14] However, epigenetic modifications may be as important as genetic polymorphisms in CVD pathogenesis.[15] Epigenetics refers to potentially mutable marks on the chromosome that influence gene expression. These marks are established mostly in utero and are relatively stable over time. DNA methylation is an epigenetic modification that usually results in silencing of nearby genes. Most DNA methylation occurs in intergenic regions, such as in *Satellite, Alu*, and *LINE-1* repetitive elements. *LINE-1* hypomethylation seems to play an important and consistent role in diseases,[16,17] and has itself been associated with CVD and survival time.[18-20] Risk of stroke has also been related to *Alu* hypermethylation.[21]

We focused our investigation on the relative toxicity of various sources (traffic versus coal combustion) and components of air pollutants. We first hypothesized that increased air pollution is associated with changes in fibrinogen, C-reactive protein, ICAM-1, and VCAM-1 levels, and that various sources and components of air pollution affected these outcomes differentially. While epigenetic control of gene expression is important to DNA repair and cell proliferation, it may also play a key role in inflammatory gene expression.[15] Therefore, we also examined effect modification by methylation of *LINE-1, Alu, F3, TLR-2*, and *ICAM-1*. Methylation at specific positions within a gene's promoter region may be more important for gene expression than the average methylation of CpG sites in the promoter region.[22] Therefore, we investigated the epigene-environment interactions using average and position-specific methylation.

METHODS

Study population

This prospective cohort study included subjects from the Normative Aging Study, an investigation of aging established in 1963 by the US Veterans Administration and enrolling community-dwelling men from the Greater Boston area. Participants were confirmed to be free of known chronic medical conditions and visited the study center every three to five years to undergo physical examinations. Visits took place in the morning, after an overnight fast and smoking abstinence. Our analyses included 704 subjects whose levels of fibrinogen, C-reactive protein, ICAM-1, VCAM-1, and DNA methylation were measured at least once between 2000 and 2009. Because elevated C-reactive protein levels usually reflect an infection state, we restricted our analyses to participants having levels less than 10 mg/L. Attrition has been less than 1% per year, and is due predominantly to moving from the study

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area or death. This study was approved by the institutional review boards of all participating institutions.

Air pollution

Ambient particulate concentrations were measured at a central monitoring site (1 km from the medical center). We measured hourly ambient fine particle ($PM_{2.5}$) concentrations with a Tapered Element Oscillation Microbalance (Model 1400A, Rupprecht and Pastashnick, East Greenbush, NY), and hourly black carbon concentrations using an Aethalometer (Magee Scientific Co., Model AE-16, Berkeley, CA). Occasional missing daily values of black carbon and $PM_{2.5}$ were estimated using regression models that included previous and subsequent pollutant measurements, meteorologic data, seasonality, and long-term trend. We measured hourly particle number per cm^3 (0.007–3 μm) with a Condensation Particle Counter (TSI Inc, Model 3022A, Shoreview, MN). We determined daily sulfate (SO_4^{2-}) concentrations with a Sulfate Particulate Analyzer (Thermo. Electron Co., Model 5020, Franklin, MA) from 2000 to 2003. Subsequently, SO_4^{2-} levels were calculated from elemental sulfur, measured by X-Ray Fluorescence from particle filters. For the year of overlap we fit a calibration regression, which had an R^2 over 0.9, and a slope of 1. We obtained hourly measurements of carbon monoxide (CO), ozone (O_3) and nitrogen dioxide (NO_2) from local state monitors, taking the mean site values for each gas. Air pollution measured by the monitors is used as a surrogate for urban personal exposure. The median distance of the participant homes from the central site monitoring station was 19.0 km; the medians of the mean distances of the participant homes from the CO, O_3, and NO_2 monitors were 20.2 km, 22.3 km, and 21.4 km, respectively. The appropriate averaging time for the air pollution effect on coagulation, inflammation, and endothelial function is unclear. Previous studies suggest that the effect is spread overall several days,[23] months,[24] or even years.[25] For each pollutant concentration, we considered the short- and intermediate-exposure windows of 4 hours, 24 hours, and 3 to 28 days moving average (MA_3 to MA_{28}) preceding each subject's examination.

Epigenetics

We collected blood at every visit to measure *LINE-1, Alu*, and gene-specific methylation, following previous published methods.[19] We isolated bisulfite-treated DNA with the Wizard DNA Clean-Up System (Promega, Madison, WI) and measured DNA methylation using highly quantitative analysis based on PCR pyrosequencing. We determined levels of *F3, TLR-2*, and *ICAM-1* methylation at multiple CpG positions within each gene's promoter region and calculated the mean values of the position-specific measurements. The assay quantifying the gene-specific methylation levels was developed by locating the promoters using Genomatix Software (Genomatix Software Inc, Ann Arbor, MI, USA). The degree of methylation was expressed as the percentage of methylated cytosines over the sum of methylated and unmethylated cytosines.

Outcome blood markers

At each visit, we measured plasma fibrinogen using MDA Fibriquick, a thrombin reagent. We determined C-reactive protein concentrations with an immunoturbidimetric assay on the Hitachi 917 analyzer (Roche Diagnostics-Indianapolis, IN). We obtained plasma ICAM-1 and VCAM-1 levels using an enzyme-linked immunoabsorbent assay method (R&D Systems, Minneapolis, MN).

Statistical methods

Fibrinogen, C-reactive protein, ICAM-1, and VCAM-1 levels were log-transformed to guarantee a normal distribution of the residuals. Subjects had their blood marker levels

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measured on up to four visits. Because most participants had repeated measurements of the outcomes of interest, we analyzed our data using separate mixed models with a random intercept for each subject. This gives unbiased estimates of the fixed effects even when some subjects had only one measurement. We chose the following covariates a priori: age, seasonality, body mass index (BMI), temperature, relative humidity, smoking status, statin use, and diabetes. Seasonality was controlled using sine and cosine terms with a period of 365.24 days. Both sine and cosine were included to allow the regression to estimate both the amplitude of the seasonal cycle and its phase. Using interaction terms, we investigated air pollution effect modification by *LINE-1* and *Alu* methylation on every blood marker of interest, as well as air pollution interactions with *F3* modifying fibrinogen, *TLR-2* changing C-reactive protein, and *ICAM-1* modifying the ICAM-1 and VCAM-1 proteins' concentrations. We dichotomized methylation levels, using the 50th percentile of the distribution as a cut-off point. Because the pattern of 5-methylcytosine distribution in the genome of differentiated somatic cells varies according to cell type, we adjusted for percentages of neutrophil and lymphocyte in differential blood count. Our statistical model investigating epigene-environment interactions was:

$$Y_{ij}=\beta_1+\beta_2\, pollutant_{ij}+\beta_3\, methylation_{ij}+\beta_4\, pollutant*methylation_{ij}+\gamma Z_{ij}+b_i+\varepsilon_{ij}$$

- Y_{ij} : logarithm of blood marker for subject i at the jth measurement
- Z_{ij} : covariates for subject i at the jth measurement
- b_i : random intercept
- ε_{ij} : within-subject errors

RESULTS

Descriptive statistics

We present the subjects' baseline characteristics and the weather distributions in the Table. The study population was elderly, with a mean age of 73.2 years. Air pollution characteristics are reported in eAppendices 1 to 4 (http://links.lww.com). There were four sites for NO_2 and CO, and two for O_3. Spearman correlations between monitors were 0.84 for O_3, and ranged from 0.52 to 0.75 for NO_2 and from 0.35 to 0.57 for CO. We observed correlation coefficients of 0.72 between black carbon and $PM_{2.5}$, 0.83 between $PM_{2.5}$ and SO_4^{2-}, and -0.43 between particle number and O_3. We described the different positions within the promoter region of the *F3, TLR-2*, and *ICAM-1* genes in eAppendix 5 (http://links.lww.com).

Air pollution main effects

We observed positive effects of traffic-related pollutants on fibrinogen (Figure 1). An interquartile range-increase in exposure (three-day moving average) was associated with a 2.4% (95% confidence interval [CI]= 0.1 % to 4.81%) increase in fibrinogen for particle number, a 2.6% (0.9% to 4.3%) increase for black carbon, a 4.5% (2.6% to 6.4%) increase for NO_2, and a 2.5% (0.9% to 4.0%) increase for CO. In contrast, $PM_{2.5}$, SO_4^{2-} and O_3 (which are mostly secondary pollutants) were not associated with fibrinogen. O_3 was, however, a predictor of C-reactive protein and ICAM-1 (Figures 2 and 3). A 24-hour interquartile range increase in O_3 was associated with a 10.8% (2.2% to 20.5%) increase in C-reactive protein. We found higher ICAM-1 levels after short- and intermediate-term exposures to particle number, $PM_{2.5}$, and SO_4^{2-}, and only after intermediate-term exposure to black carbon, NO_2, and CO (Figure 3). We obtained similar associations for VCAM-1 (Figure 4). ICAM-1 and VCAM-1 were negatively related to intermediate-term ozone

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exposure. However, after adding particle number to the model, these associations were weakened.

Effect modification by DNA methylation status

Repetitive elements methylation—We observed stronger effects of traffic-related pollutants on fibrinogen and C-reactive protein, among subjects with lower *LINE-1*or higher *Alu* methylation status (Figure 5). While among subjects with higher *Alu* methylation, an interquartile range increase in NO_2 (14 day moving average) was associated with a 6.4% (3.9% to 8.5%) increase in fibrinogen, we observed only a 2.4% (-0.04% to 5.0%) fibrinogen increase among participants with lower methylation. *LINE-1* also increased the effect on fibrinogen of SO_4^{2-}, after 1 to 4 weeks of exposure. The air-pollution effect on ICAM-1 and VCAM-1 was not modified by methylation of repetitive elements status.

Gene-specific methylation—The air-pollution effect on fibrinogen was not modified by the mean tissue factor (*F3*) methylation states. However, when we examined effect modification by position within the promoter region, we observed longer short- and intermediate-term effects on fibrinogen of particle number, black carbon, NO_2, and CO among subjects with low *F3* methylation status for Position 1 or 5 (Figure 6). For instance, among the low *F3* methylation group for Position 1, an interquartile range increase in NO_2 (3-day moving average) was associated with a 6.2% (3.8% to 8.5%) increase in fibrinogen, but only with a 2.9% (0.5% to 5.5%) increase among participants with higher methylation levels. When examining interaction between air pollution and *TLR-2* methylation on C-reactive protein, we found greater intermediate-term effects of particle number and NO_2, among the low *TLR-2* methylation group (Figure 6). We examined effect modification by *ICAM-1* methylation status on ICAM-1 and VCAM-1 levels and did not find any consistent interactions.

Sensitivity analyses

As sensitivity analysis, we investigated the epigene-environment interactions using continuous methylation variables; the direction and magnitude of the results did not change. Air pollution and smoking (pack-years or history) were not correlated in our data set. We also examined effect modification by smoking and found no evidence of interactions (results not shown). Since air pollution and methylation are correlated,[26] the epigene-air pollution interactions could be the result of a non-linear effect of air pollution on the blood markers of interest. We checked this possibility by regressing methylation on air pollution and relevant covariates. Because the resulting residuals reflected the methylation variability independent of air pollution, dichotomizing them into low versus high avoids the ambiguity due to effects of air pollution on methylation. When we used them for the interaction term with air pollution, we obtained similar interactions.

DISCUSSION

Our results suggest that current concentrations of traffic-related air pollutants increase levels of intermediary CVD-related blood markers (fibrinogen, C-reactive protein, ICAM-1, and VCAM-1) after short- and intermediate-term exposures to air pollution (up to one month), and that those responses vary with a person's baseline DNA methylation pattern. Our results support previous findings that showed influence of air pollutants on markers of coagulation, inflammation, and endothelial function.[5,7-9,23,27]

We observed short- and intermediate-term effects of particle number on ICAM-1 and VCAM-1. This short time window is consistent with previous experimental studies. After 1 hour of diesel particles inhalation in a chamber, volunteers had increased ICAM-1 and

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VCAM-1 levels in their bronchial lining fluid and tissue.[27] After concentrated fine-particle exposure, ICAM-1 levels measured in venous blood increased after 4 hours and 22 hours.[28] A similar study showed that after hours of diesel particles exposure, healthy subjects had higher VCAM-1 concentrations in the bronchial mucosa.[29] For the other traffic-related pollutants, we observed only intermediate-term effects on fibrinogen, ICAM-1, and VCAM-1. Zeka et al.[5] also found lagged exposure effects of traffic-related particles (1 week) and black carbon (1 month) on fibrinogen. Higher VCAM-1 have been reported after days of black carbon exposures and higher ICAM-1 after weeks of exposure.[7,8] A key finding in our study was the associations with secondary pollutants as well as primary pollutants. ICAM-1 and VCAM-1 were also higher after short- and intermediate-term exposure to $PM_{2.5}$ and sulfates, which is consistent with a previous study reporting similar $PM_{2.5}$ and sulfate effects (5-day moving average).[30] We also found a short-term ozone effect on ICAM-1 and C-reactive protein. A positive association between C-reactive protein and ozone was previously obtained after three days of exposure.[6]

We found the strongest associations with particle number, which is an assessment of fresh traffic emissions. Black carbon is, however, a mix of fresh local emissions and some aged transported traffic particles. In Boston, black carbon shows daily patterns peaking at 6 a.m. and a lower peak in the afternoon rush hour, suggesting a predominance of local traffic. However, transported black carbon is also important, as its concentrations were higher when the back trajectory of the air mass over Boston originated in New York,[31] indicating some secondary contribution as well. NO_2 is a secondary pollutant that reflects both local sources and transported pollution. These differences might explain why the time pattern of the effects of particle number appears to be distinct from that of black carbon and NO_2. Even though particle number, black carbon, and NO_2 are traffic-related pollutants, they may be markers of something more complex, including the freshness of the particles. $PM_{2.5}$ signals on fibrinogen were weak, which is concordant with previous studies.[5,32] Fine particles are too small to easily settle out by gravity and too large to coagulate into larger particles, which makes them able to stay in the atmosphere for weeks and travel thousands of kilometers before returning to Earth. In Boston, sulfate particles and secondary organic aerosols constitute a large fraction of $PM_{2.5}$ mass, with a lesser role for traffic particles. SO_4^{2-} and O_3 (which are other secondary pollutants) were not associated with fibrinogen. Hence, our findings on fibrinogen clearly point toward local traffic particles.

We also observed short- and intermediate-term effects of traffic-related particles, $PM_{2.5}$, and SO_4^{2-} on ICAM-1 and VCAM-1. These results on adhesion molecules point toward both primary and secondary pollutants. We reported short-term ozone effects on C-reactive protein and ICAM-1. Ozone is a powerful oxidizing agent and is created with high concentration of pollution and daylight UV rays at the Earth's surface. Ozone exposure has been linked to lung dysfunction and respiratory system irritation,[33] and the C-reactive protein and ICAM-1 increases indicate acute inflammatory and endothelial responses to short-term exposure.

Our main air pollution findings suggest some plausible biological mechanisms that could explain some of the exacerbation of CVD morbidity and mortality.[2,27] Air pollution exposure may increase systemic cytokine-mediated inflammation and prothrombotic activity. In susceptible people, ultrafine particles were able to provoke alveolar inflammation, with release of mediators capable of increasing blood coagulability.[34] Increased plasma viscosity is a potential mechanism explaining why high fibrinogen levels are related to increased CVD risk.[3] Similarly, elevated C-reactive protein, ICAM-1, and VCAM-1 levels have been associated with inflammation and cardiovascular risk.[10,35] Increase in C-reactive protein may reflect arterial damage from white blood cell invasion and inflammation within the wall due to air pollution exposure, thus inducing cardiovascular

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events. In normal human arterial tissues, ICAM-1 levels were lower than in atherosclerotic lesions.[11] ICAM-1 plays a role in general inflammation and upregulation in non-endothelial cells, while VCAM-1 may be more involved in distribution in the vascular system.[9] This could explain why the ICAM-1 and VCAM-1 signals have slightly different time windows.

We found greater air pollution effects among subjects with high *Alu*, but low *LINE-1, F3*, or *TLR-2* methylation status. To our knowledge, this is the first study to report evidence of epigene-environment interactions. Our results suggest that DNA methylation may play an important role in inflammatory gene expression and CVD. Knock-out mice for methylation enzymes develop atherosclerotic fatty streaks.[36] In the same cohort, *LINE-1* hypomethylation was associated with higher VCAM-1 protein levels,[19] as well as higher CVD incidence and mortality.[20] *LINE-1* elements, as retrotransposable sequences, have enhanced activity when they are demethylated, which may induce gene transcription dysfunction, and alterations of the genome by insertion or homologous recombination.[37] *LINE-1* expression has been identified as a mediator of ischemic heart damage.[18] Repetitive elements become activated during oxidative and cellular stress conditions.[38] Interestingly, recent studies have reported a higher risk of cardiovascular events among subjects with low *LINE-1*, but also with high *Alu* methylation in peripheral blood leukocytes.[20,21] *Alu* and *LINE-1* have also showed opposite changes according to season.[26] Therefore, *LINE-1* hypomethylation and Alu hypermethylation may be distinct responses to oxidative stress and inflammation, and therefore might identify a subset of subjects who have stronger molecular responses to air pollution.

Air pollution effects on fibrinogen were stronger in subjects with lower *F3* methylation. *F3* expression and fibrinogen increase in response to proinflammatory stimuli, such as those caused by air pollution.[4,12] Tissue factor upregulation is found in inflammatory and hypercoagulable states, which are accompanied by some elevations of clotting signals, including fibrin overproduction.[39] Tissue factor hypomethylation may participate in pathways leading to increased tissue factor expression and fibrinogen in response to air pollution and therefore reflects a proinflammatory state leading to stronger coagulation responses.

We observed greater air pollution effects on C-reactive protein among participants with lower *TLR-2* methylation. *TLR-2* plays an important role in activation of innate immunity. Several cytokines and interleukins, including IL-6, assist in this immunity process. Compared with knockout mice for *TLR-2*, wildtype mice had greater $PM_{2.5}$ effects on IL-6.[13] IL-6 rise has been associated with increase in C-reactive protein, which also plays a role in innate immunity as an early defense system against infections. Our findings suggest that *TLR-2* hypomethylation may silence the *TLR-2* gene and participate in biologic processes enhancing IL-6 and C-reactive protein after air pollution exposure, and therefore induces a proinflammatory state leading to stronger inflammatory responses.

Our results suggest that *Alu, LINE-1, F3*, and *TLR-2* methylation status interact with several air pollutants. While tissue factor methylation status seems to modify the effects of all traffic pollutants on fibrinogen, we observed effect modification by *LINE-1* methylation only with black carbon and sulfate. *Alu* and *TLR-2* methylation status interacted with NO_2 and particle number. Particle number represents freshly generated ultrafine traffic particles, while black carbon captures both fresh and aged traffic particles. The finding that the freshest and smallest traffic particles interact with *Alu, TLR-2*, and tissue factor suggests a different mechanism, perhaps movement of the ultrafine particles into the blood stream. The interactions of *LINE-1* methylation with sulfates and black carbon, capturing fewer fresh particles, are probably related to a different pathway. A recent study supports this hypothesis.[40] After 4 hours of ultrafine particles exposure, mice had increased thrombin

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formation, related to tissue factor (extrinsic coagulation pathway). Alternatively, the procoagulant chronic effects of particulate matter could be mediated via factor XII activation (intrinsic coagulation pathway).

While *Alu* methylation state modified the particle-number effect on fibrinogen and C-reactive protein after the first hours and days of exposure, it increased the NO_2 effect on fibrinogen after 2 to 4 weeks. Similarly, *F3* methylation interacted with all traffic-related pollutants over the first hours or days, while *LINE-1* interacted with black carbon or SO_4^{2-} after a longer period (1 to 4 weeks) to modify fibrinogen. These time differences indicate possible distinctions in the properties of air pollutants— especially between primary and secondary pollutants, for which a longer averaging time is required to see an interaction, suggesting different biological mechanisms.

Strengths and limitations

We prospectively investigated the role of air pollution from various sources on repeated measures of intermediary CVD-related markers. Our findings were not the result of confounding by CVD risk factors, such as age, seasonality, BMI, temperature, relative humidity, diabetes, statin, and smoking status. Smoking is an important predictor of methylation. However, we found no correlation between air pollution and any smoking variable (category or pack-years). Therefore, we believe that smoking is not confounding the air pollution effects seen in our study. With regard to the possibility that the interaction with methylation is really an interaction with past smoking that produced stable changes in methylation patterns, we examined this question using interaction terms with former and current smoking rather than with methylation. None of these associations explained our results. The observed epigene-air pollution interactions were also not the result of a non-linear effect of air pollution on the blood markers of interest. Our analysis is, however, limited to five methylation variables. Methylation of other genes and histone modifications might be important variables to evaluate. To be confounding variables, they would have to be correlated with *Alu, LINE-1, F3*, or *TLR-2* methylation. There are few data to indicate how likely this is. In addition, DNA methylation is more stable than mRNA or protein expression, and therefore represents a somewhat longer time window in system behavior. We also identified positions within promoter regions of *F3* and *TLR-2* that may play an epigenetic role in coagulation and inflammation.

We were not able to assess personal air pollution exposure, which is likely to be non-differentially misclassified. Therefore, exposure misclassification for using ambient monitors presumably attenuates our associations. This attenuation should be larger for short-term exposures and for pollution components that have the greatest spatial variability, which are the traffic pollutants. Exposure assignments for ozone and particularly for sulfates are expected to be fairly accurate and reinforce the null effects found on fibrinogen. Fibrinogen, C-reactive protein, ICAM-1, VCAM-1, and DNA methylation are measured directly from blood. Even though these measurements are likely to be accurate, we also expect some non-differential misclassification of the underlying biological function. Our cohort consists of elderly men that may be less susceptible than the population they are representing. Loss to-follow-up may also make this study population healthier than the initial population. Furthermore, our results may not be generalizible to the general population if the biologic mechanisms are modified by race, sex, and age.

Supplementary Material

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Acknowledgments

Normative Aging Study participants, Tania Kotlov, Johanna Lepeule

Sources of financial support

This study was supported by the U.S. Environmental Protection Agency grants EPA R827353 and R832416; National Institute of Environmental Health Sciences (NIEHS) grants RO1-ES015172, 2RO1-ES015172, ES014663, ES00002, PO1_ES008925. The VA Normative Aging Study is supported by the Cooperative Studies Program/ Epidemiology Research and Information Center of the U.S. Department of Veterans Affairs and is a component of the Massachusetts Veterans Epidemiology Research and Information Center, Boston, Massachusetts.

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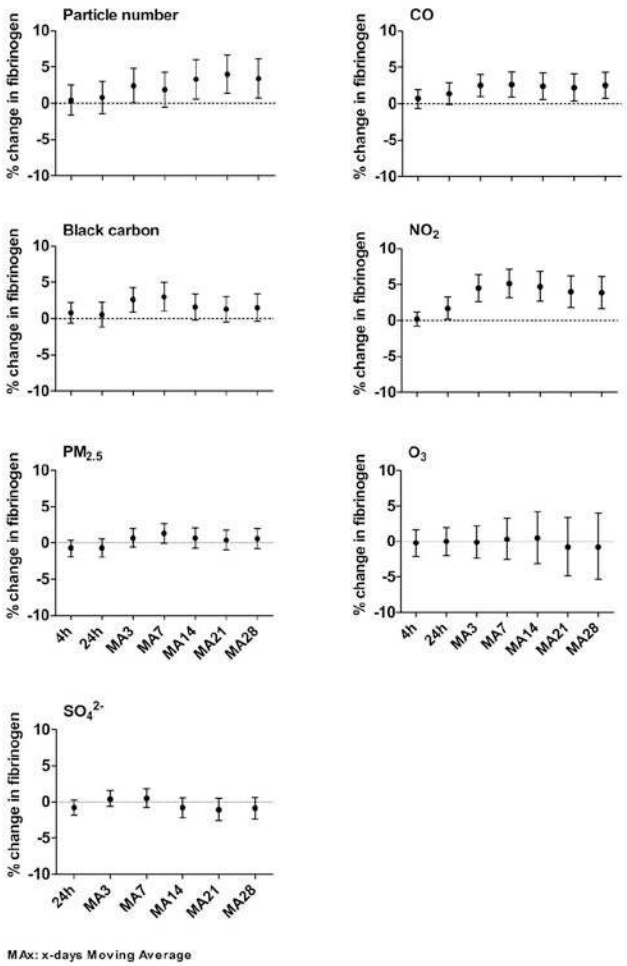

Figure 1.
Effects of one interquartile range increase of air pollutants on fibrinogen for exposures 4 hours, 24 hours, and 3-28 days moving average (MA_3 to MA_{28}) preceding each subject's physical examination

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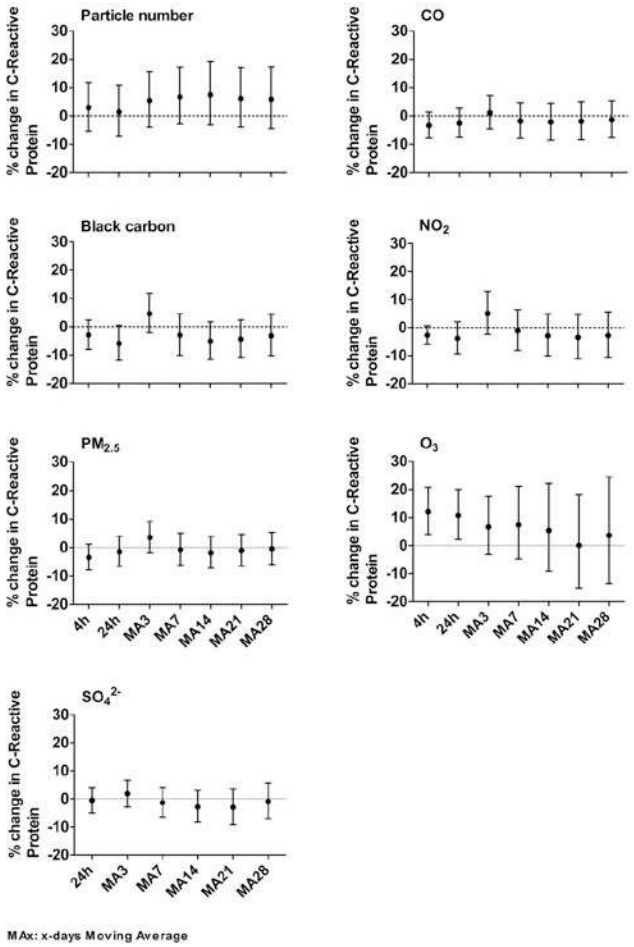

Figure 2.
Effects of one interquartile range increase of air pollutants on C-reactive protein for exposures 4 hours, 24 hours, and 3-28 days moving average (MA_3 to MA_{28}) preceding each subject's physical examination

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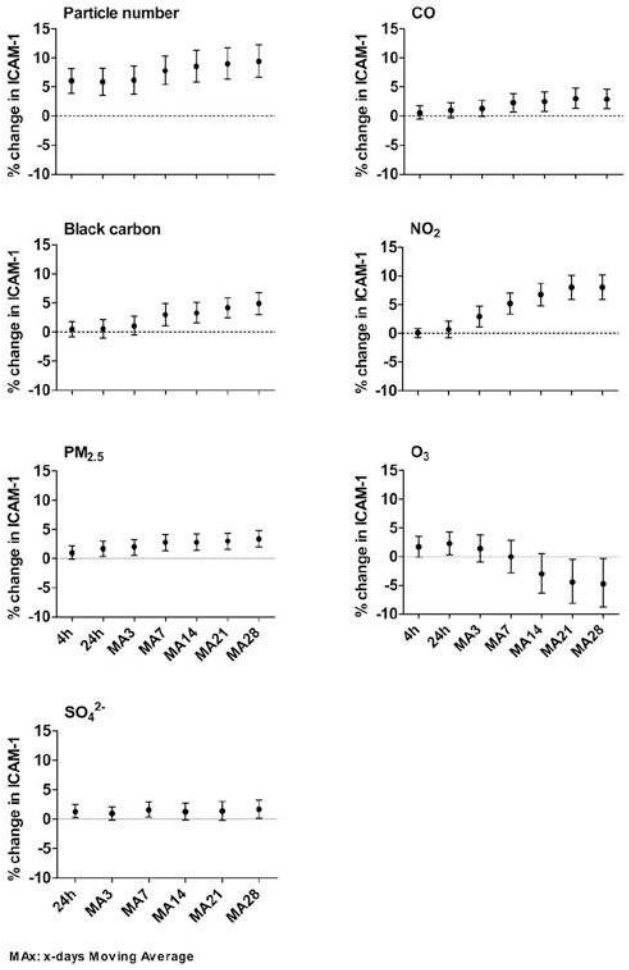

Figure 3.

Effects of one interquartile range increase of air pollutants on ICAM-1 for exposures 4 hours, 24 hours, and 3-28 days moving average (MA_3 to MA_{28}) preceding each subject's physical examination

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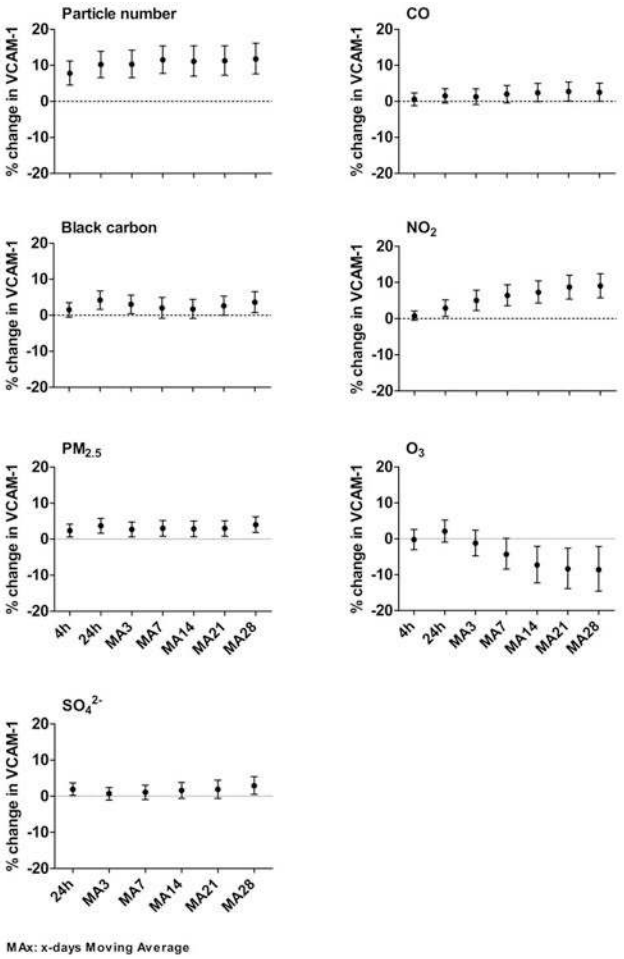

Figure 4.
Effects of one interquartile range increase of air pollutants on VCAM-1 for exposures 4 hours, 24 hours, and 3-28 days moving average (MA_3 to MA_{28}) preceding each subject's physical examination

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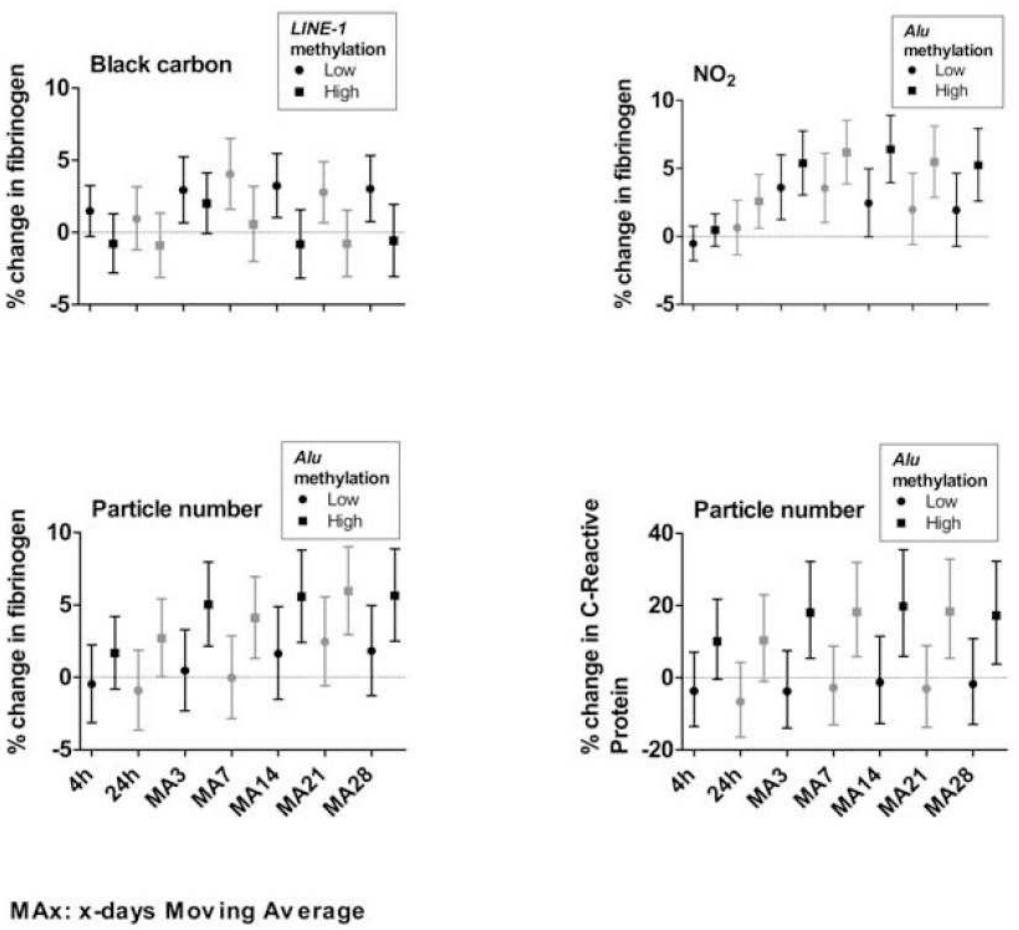

Figure 5.
Effects of one interquartile range increase of air pollutants on blood markers according to the methylation status of repetitive elements

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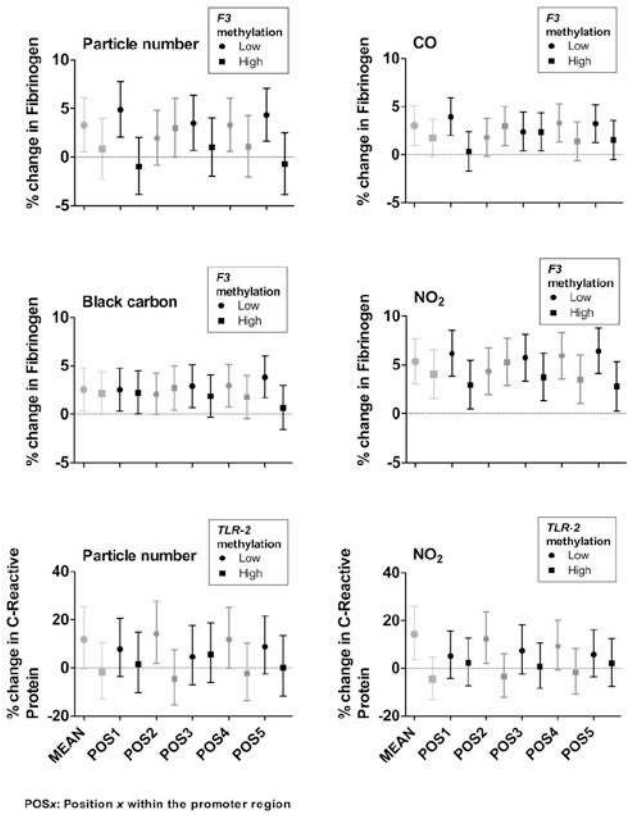

Figure 6.

Effects of one interquartile range increase of air pollutants (3-days moving average) on blood markers according to the gene-specific methylation status and the position within the promoter region

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Epidemiology. Author manuscript; available in PMC 2013 March 1.

Table

Baseline characteristics of the VA Normative Aging Study subjects and weather distributions between 2000 and 2009

Characteristics					
Diabetes; %	13.9				
Obesity; %	26.1				
Smoking; %					
Never	29.0				
Current	4.2				
Former	66.8				
Statin; %	39.8				
			Percentiles		
	Mean	**(SD)**	**5%**	**50%**	**95%**
Age (years)	73.2	(6.8)	62	73	85
BMI (kg/m^2)	28.2	(4.1)	22.5	27.7	35.2
Inflammatory markers[a]					
Fibrinogen (mg/dL)	341.8	(77.2)	240	331	494
CRP (mg/dL)	2.27	(1.99)	0.33	1.63	6.56
ICAM-1 (ng/mL)	311.2	(79.0)	211.5	304.9	422.8
VCAM-1 (ng/mL)	1087.3	(381.2)	632.3	1024.6	1674.0
Methylation (%)					
ALU	25.8	(1.31)	23.5	25.9	27.7
LINE-1	79.2	(3.16)	74.6	78.6	84.0
F3	2.5	(1.25)	1.0	2.3	4.5
TLR-2	2.9	(1.30)	1.2	2.7	5.3
ICAM-1	4.2	(1.55)	2.2	4.0	6.8
Meteorology					
Temperature (°C)[b]	12.4	(8.85)	-2.9	12.7	25.7
Relative humidity (%)[b]	67.9	(15.49)	43.7	67.8	92.7

[a] 35.2% of the participants had one visit, 35.4% had two visits, 29.3% had three visits, and 0.1% had four visits

[b] Based on repeated data