Glutathione-S-Transferase M1, Obesity, Statins, and Autonomic Effects of Particles
Gene-by-Drug-by-Environment Interaction

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Rationale: Air pollution by particulate matter (PM) has been associated with cardiovascular deaths, although the mechanism of action is unclear. One proposed pathway is through disturbances of the autonomic control of the heart.

Objectives: We tested the hypothesis that such disturbances are mediated by PM increasing oxidative stress by examining the association between PM and the high-frequency (HF) component of heart rate variability as modified by the presence or absence of the allele for glutathione-S-transferase M1 (GSTM1) and the use of statins, obesity, high neutrophil counts, higher blood pressure, and older age.

Methods: We examined the association between particles less than 2.5 μm in aerodiameter (PM2.5) and HF in 497 participants in the Normative Aging Study, using linear regression controlling for covariates.

Main Results: A 10-μg/m3 increase in PM2.5 during the 48 h before HF measurement was associated with a 34% decrease in HF, 95% confidence interval (~8%, ~52%), in subjects without the allele, but had no effect in subjects with GSTM1 present. Among GSTM1-null subjects, the use of statins eliminated the effect of PM2.5. Obesity and high neutrophil counts also worsened the PM effects with or without GSTM1.

Conclusion: The effects of PM2.5 on HF appear to be mediated by reactive oxygen species. This may be a key pathway for the adverse effects of combustion particles.

Keywords: genetic polymorphisms; heart rate variability; oxidative stress; particles

A large body of evidence has demonstrated that particulate air pollution (PM) is associated with short-term changes in the risk of death (1–6). An early study showed the risk of dead-on-arrival deaths associated with particles was three times that for all deaths (7). This suggests a predominant effect on sudden deaths from arrhythmias and myocardial infarctions. Subsequent studies have confirmed that PM is associated with myocardial infarctions (8, 9), hospital admissions for cardiovascular disease (10, 13), discharges of implantable defibrillators (14), and electrocardiographic disturbances (15).

How airborne particles may be producing these responses in still unclear. However, particles have been linked with changes in intermediate markers, such as clotting factors (16, 17) and increased atherosclerosis (18).

Airborne particles have also been associated with changes in heart rate variability (HRV) (15, 19). The parasympathetic and sympathetic stimulation of the heart produces variations in the time intervals between normal heartbeats; analysis of this variability is therefore an estimate of cardiac autonomic regulation. HRV is a noninvasive measure that independently predicts cardiovascular mortality in patients with and without underlying cardiovascular disease (20, 21). Hence, disturbances in HRV may represent one pathway by which particles might be associated with sudden death.

Overall, studies have generally found significant associations with HRV in elderly subjects, but weaker associations in younger subjects (22), suggesting that age-related decreases in toxic defenses play a role in susceptibility. In a recent review (22), we found the only consistent PM association was with the high-frequency (HF) components of HRV, either HF in the frequency domain, or root mean squared differences between adjacent RR intervals (rMSSD) or proportion of adjacent NN intervals differing by more than 50 ms (PNN50) in the time domain. In contrast, low frequency was not associated with particles in four of five studies. This suggests a paramount effect on the parasympathetic nervous system. This may be because the vagus nerve innervates the lung.

Various mechanisms by which particles exert these effects have been proposed (23). Reactive oxygen species (ROS) have been mentioned as a potential pathway for the adverse effects of particles (24, 25). ROS have established importance in the pathogenesis of cardiovascular diseases (26). Exposure to urban particles increased ROS in a dose-dependent manner in the lung and heart of living animals (27). It is unclear what role ROS may play in explaining the effects of particles on autonomic endpoints, such as defibrillator discharge and HRV. This question may be addressed by examining the effects of particles on HRV in populations with different host defenses to oxidative stress challenge. Genetic polymorphisms have been linked to important differences in such defenses.

Glutathione pathways play a key role in cellular defenses against ROS (28). Glutathione-S-transferases (GSTs) are a family of enzymes involved in the metabolism of ROS and xenobiotic compounds.

Genetic polymorphisms of the GSTs are common, and have been shown to modify the response to air pollutants (29). The GSTM1 gene is deleted in approximately half of the white population (the polymorphic “null” genotype), and lack of the GSTM1 protein has been associated an enhanced nasal allergic

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response to diesel exhaust particles (30). Children who are GSTM1-null and are exposed to environmental tobacco smoke had elevated odds of developing asthma (31), and the GSTM1-null genotype interacts with tobacco smoke in increasing the risk of coronary disease (32).

Drugs that modify oxidant defenses may also influence susceptibility to particle-induced ROS. Statins are a widely prescribed class of drugs originally developed for their lipid-lowering properties, but they have been shown to have substantial antiinflammatory and antioxidant activity as well (33). In addition to lowering C-reactive protein concentrations (34), statins have been shown to decrease superoxide production (35), increase release of nitric oxide (NO) (36), which is an antioxidant as well as a vasodilator, and reduce markers of oxidative stress in APOE_{-/-} mice (37).

We examined the association of fine-particle air pollution of less than 2.5 μM in aerodynamic diameter (PM_{2.5}) on the HF HRV of elderly subjects living in the Boston metropolitan area, and how that association varied by GSTM1 genotype or statin use. In addition, because statins have important effects beyond their antioxidant properties, we examined whether obesity or elevated neutrophil count, which are associated with inflammation and oxidative stress, also modified the association. Finally, we examined two more generic markers of susceptibility, older age or higher blood pressure, as modifiers of the PM effect. This study was reviewed and approved by the institutional review boards of all of the participating institutions.

METHODS

Study Population

The Normative Aging Study is a longitudinal study established by the Veterans Administration in 1961, enrolling 2,280 men from the greater Boston area who were free of known chronic medical conditions (38). Beginning in 2000, during each participant’s regularly scheduled evaluation, HRV was measured. Further details have been described previously (22). That study examined a range of air pollutants and measures of HRV, but did not look at genetic or other factors related to oxidative stress. It found the most consistent associations with PM_{2.5} and with HRV measures indicative of a parasympathetic effect (HF, rMSSD).

HRV Measurement

HRV was measured for 7 min in a sitting position using a two-channel (five-lead) ECG monitor (Trillium 3000; Forest Medical, East Syracuse, NY). Only normal-to-normal (NN) beat intervals were included in the analysis. We used the best 4-consecutive-min intervals for the HRV calculations, and computed the HF (0.15–0.4 Hz) component of HRV using software complying with guidelines (39). Subjects with irregular ECG patterns that interfere with HRV estimation were excluded.

GSTM1 Genotyping. The assay consists of polymerase chain reaction amplification of exons 4 and 5 of the GSTM1 allele. Because this polymorphism is a gene deletion, polymerase chain reaction product indicates the presence of one or more copies of the gene. Further details are in the online supplement.

Air Pollution and Weather Data

Continuous PM_{2.5} was measured at a monitoring site 1 km from the exam site, using the Tapered Element Oscillating Microbalance (TEOM, model 1400A; Rupprecht & Patashnick, Albany, NY), with a season-specific correction to compensate for the loss of semivolatile mass (40). Weather measurements were obtained from the airport weather station.

To control for outdoor weather, we used apparent temperature, defined as a person’s perceived air temperature, given the humidity (41). We used the average of PM_{2.5} concentrations in the 48 h before examination as our exposure index, because that exposure period has been most consistently associated with sudden death (4, 5).

Statistical Methods

HRV was log_{10}-transformed to improve normality and stabilize the variance. The following variables were chosen a priori and included in the linear regression analysis: age, cigarette smoking, body mass index, diastolic blood pressure, fasting blood glucose, alcohol consumption (>= 2 drinks/d), use of β-blockers, angiotensin-converting enzyme inhibitors, and/or calcium channel blockers, season, room temperature, and average apparent temperature 48 h before the HRV measurement. We used a spline with 3 degrees of freedom to account for potential nonlinearity in the relationship between apparent temperature and HRV. After 14 subjects with missing values of covariates were excluded, 497 subjects were available for the analyses.

Stratified regression models examined subjects with and without the GSTM1 gene, with and without statin use, and by the four possible combinations of genotype and statin use. Stratified analyses were also done, in turn, by the four possible combinations of GSTM1 gene and by whether or not the subjects were in the most adverse quartile of neutrophil count, blood pressure, or age, or whether they had a body mass index above 30.

RESULTS

Table 1 shows the demographic and clinical characteristics and HRV measurements of the subjects, as well as environmental variables. The study participants were all male, and their average age was 72.7 yr (SD, 6.6 yr). The correlation between temperature and particle concentrations was modest (0.35).

In a model including all subjects, and the covariates listed above, a 10-μg/m³ increase in PM_{2.5} was associated with a 27% decrease in HF (95% confidence interval, −8%, −42%). Note that the interquartile range for PM_{2.5} in these data was 7 μg/m³.

When stratified by GSTM1 status, no relationship of PM_{2.5} and HF was seen in persons with the gene, whereas a significant association (34% decrease; 95% confidence interval, −9%, −52%) was seen in non-GSTM1 heterozygotes (23% decrease; 95% confidence interval, −21%, −36%). When stratified by GSTM1 status and by statin use, the PM by statin interaction was significant (28% decrease; 95% confidence interval, −12%, −42%). In a model including all subjects, and the covariates listed above, a 10-μg/m³ increase in PM_{2.5} was associated with a 27% decrease in HF (95% confidence interval, −8%, −42%). Note that the interquartile range for PM_{2.5} in these data was 7 μg/m³.

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Table 1. Descriptive Statistics of the Variables (Mean [SD] or Number [%])

<table>
<thead>
<tr>
<th>Variable</th>
<th>All Subjects (n = 497)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>72.7 (6.6)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.3 (4.1)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>75.7 (9.4)</td>
</tr>
<tr>
<td>Heart rate, beat/min</td>
<td>70.7 (6.7)</td>
</tr>
<tr>
<td>Fasting blood glucose, mg/dl</td>
<td>108.0 (29.0)</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>197.0 (37.6)</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>49.7 (13.5)</td>
</tr>
<tr>
<td>Smoking status, n (%)</td>
<td>160 (32.2)</td>
</tr>
<tr>
<td>Never smoker</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Former smoker</td>
<td>311 (62.6)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>26 (5.2)</td>
</tr>
<tr>
<td>Alcohol intake (&gt;= 2/day), n (%)</td>
<td>96 (19.3)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>335 (67.4)</td>
</tr>
<tr>
<td>Use of β-blocker, n (%)</td>
<td>163 (32.8)</td>
</tr>
<tr>
<td>Use of Ca-channel blocker, n (%)</td>
<td>70 (14.1)</td>
</tr>
<tr>
<td>Use of ACE inhibitor, n (%)</td>
<td>100 (20.1)</td>
</tr>
<tr>
<td>Use of statins, n (%)</td>
<td>179 (36)</td>
</tr>
<tr>
<td>Neutrophil count (% of cells)</td>
<td>62 (8.8)</td>
</tr>
<tr>
<td>Heart rate variability</td>
<td>24.5 (1.4)</td>
</tr>
</tbody>
</table>

Definition of abbreviations: ACE = angiotensin-converting enzyme; HDL = high-density lipoprotein; HF = high frequency; PM_{2.5} = particulate matter less than 2.5 μM in aerodynamic mass.

Values are listed as mean (SD) or numbers (%).
We found that the association between PM₂.₅ and reduced HF is only evident in persons missing the allele for GSTMI or in persons likely to have greater than average baseline systemic inflammation and oxidative stress, such as in obese individuals. Furthermore, among GSTMI-null subjects, statins were protective against the effects of PM₂.₅. In nonobese subjects, we saw effect modification by GSTMI, but the response was almost doubled in obese subjects who were GSTMI null. Being in the upper quartile of neutrophil counts also substantially increased the PM₂.₅ effect in subjects who were GSTMI null.

These results suggest that the confuence of two factors resulting in increased levels of or impaired defenses against oxidative stress results in even greater response to particles.

Although GSTMI, obesity, increased neutrophils, and statins involve several physiologic pathways, the striking observation that statins counter the susceptibility to PM₂.₅-associated reductions in HF conferred by the GSTMI deletion, and that the genotype interacts with obesity and increased neutrophil counts, suggests there exists a common mechanism of action. It seems likely that ROS (a common mechanism of action of these modifiers) plays an important role in this response. Obesity is known to increase systemic inflammation and oxidative stress, and increased neutrophil count is also a marker of systemic inflammation and oxidative stress, such as in obese individuals. Furthermore, among subjects with those conditions had a response to PM₂.₅ even in the presence of GSTMI, but an enhanced response in its absence, also suggests a central role of inflammation and oxidative stress in the autonomic effects of PM₂.₅.

### DISCUSSION

We found that the association between PM₂.₅ and reduced HF is only evident in persons missing the allele for GSTMI or in persons likely to have greater than average baseline systemic inflammation and oxidative stress, such as in obese individuals. Furthermore, among GSTMI-null subjects, statins were protective against the effects of PM₂.₅. In nonobese subjects, we saw effect modification by GSTMI, but the response was almost doubled in obese subjects who were GSTMI null. Being in the upper quartile of neutrophil counts also substantially increased the PM₂.₅ effect in subjects who were GSTMI null.

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Although GSTMI, obesity, increased neutrophils, and statins involve several physiologic pathways, the striking observation that statins counter the susceptibility to PM₂.₅-associated reductions in HF conferred by the GSTMI deletion, and that the genotype interacts with obesity and increased neutrophil counts, suggests there exists a common mechanism of action. It seems likely that ROS (a common mechanism of action of these modifiers) plays an important role in this response. Obesity is known to increase systemic inflammation and oxidative stress, and increased neutrophil count is also a marker of systemic inflammation. That subjects with those conditions had a response to PM₂.₅ even in the presence of GSTMI, but an enhanced response in its absence, also suggests a central role of inflammation and oxidative stress in the autonomic effects of PM₂.₅.

### TABLE 2. EFFECT OF A 10-μg/m³ INCREASE IN PM₂.₅ ON HIGH FREQUENCY BY STRATA OF GSTMI AND OTHER POTENTIAL EFFECT MODIFIERS

<table>
<thead>
<tr>
<th>Category</th>
<th>Change (%)</th>
<th>95% Confidence Interval</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTMI null, no statin</td>
<td>−34.0</td>
<td>−53.0, −7.20</td>
<td>162</td>
</tr>
<tr>
<td>GSTMI null, statin</td>
<td>−6.4</td>
<td>−66.5, 161.9</td>
<td>81</td>
</tr>
<tr>
<td>GSTMI present, no statin</td>
<td>−3.6</td>
<td>−40.5, 56.2</td>
<td>117</td>
</tr>
<tr>
<td>GSTMI present, statin</td>
<td>−3.2</td>
<td>−50.0, 87.2</td>
<td>81</td>
</tr>
<tr>
<td>GSTMI null, high neutrophils*</td>
<td>−55.7</td>
<td>−88.0, 63.1</td>
<td>64</td>
</tr>
<tr>
<td>GSTMI null, normal neutrophils</td>
<td>−36.1</td>
<td>−55.2, −8.7</td>
<td>179</td>
</tr>
<tr>
<td>GSTMI present, high neutrophils*</td>
<td>−49.6</td>
<td>−86.4, 86.1</td>
<td>51</td>
</tr>
<tr>
<td>GSTMI present, normal neutrophils</td>
<td>17.6</td>
<td>−20.0, 73.3</td>
<td>147</td>
</tr>
<tr>
<td>GSTMI null, obese§</td>
<td>−57.3</td>
<td>−88.0, 52.0</td>
<td>61</td>
</tr>
<tr>
<td>GSTMI null, not obese§</td>
<td>−31.0</td>
<td>−50.6, −3.6</td>
<td>182</td>
</tr>
<tr>
<td>GSTMI present, obese§</td>
<td>−34.2</td>
<td>−77.9, 96.5</td>
<td>54</td>
</tr>
<tr>
<td>GSTMI present, not obese§</td>
<td>7.5</td>
<td>−29.7, 64.3</td>
<td>144</td>
</tr>
<tr>
<td>GSTMI null, older*</td>
<td>−37.0</td>
<td>−64.9, 13.0</td>
<td>63</td>
</tr>
<tr>
<td>GSTMI null, younger*§</td>
<td>−33.1</td>
<td>−55.3, 0.3</td>
<td>180</td>
</tr>
<tr>
<td>GSTMI present, older*§</td>
<td>−7.6</td>
<td>−57.1, 98.8</td>
<td>49</td>
</tr>
<tr>
<td>GSTMI present, younger§</td>
<td>−0.3</td>
<td>−41.6, 70.1</td>
<td>149</td>
</tr>
<tr>
<td>GSTMI null, higher blood pressure*</td>
<td>−47.3</td>
<td>−81.0, 45.7</td>
<td>59</td>
</tr>
<tr>
<td>GSTMI null, lower blood pressure§</td>
<td>−34.9</td>
<td>−54.7, −6.5</td>
<td>184</td>
</tr>
<tr>
<td>GSTMI present, higher blood pressure*</td>
<td>−21.1</td>
<td>−76.4, 164.4</td>
<td>48</td>
</tr>
<tr>
<td>GSTMI present, lower blood pressure§</td>
<td>−8.7</td>
<td>−40.0, 38.9</td>
<td>150</td>
</tr>
</tbody>
</table>

* Upper 25th percentile of the distribution in the study population.
§ Lower 75% of the distribution in the study population.
† Obesity: body mass index of 30 kg/m² or greater.
‡ Body mass index < 30 kg/m².
Particles increase ROS production, perhaps in a catalytic fashion via redox cycling (24, 25, 27). A recent follow-up to the study that showed particles induced ROS in the lung and heart (27) found that administration of N-acetyl cysteine, a glutathione precursor, blunted that effect (42). Those results suggest an important role of the glutathione pathway in the defense against urban particles.

Particles induce proinflammatory mediators such as cytokines in the lungs (24, 43), and increase extracellular calcium influx, possibly through activation of calcium channels in the plasma membrane (44). Recently, particle exposure has been shown to increase circulating levels of asymmetric dimethylarginine, an endogenous inhibitor of NO synthase that is associated with impaired vascular function and increased risk for cardiovascular events (45). This suggests that NO concentrations may be impaired after particle exposure. This fits in well with the observation that statins, which blocked the effects of PM$_{2.5}$ in this study, enhance NO release. In general, all three PM-associated impairments have been linked with an increase in sympathetic and a reduction in vagal tone (46–48).

Nevertheless, we cannot rule out the importance of other pathways in the modification of the PM$_{2.5}$ effects. Statins are associated with lower risk of arrhythmic events (49) and increased HF component of HRV (50). The mechanisms of this antiarrhythmic properties are unclear, but may include enhanced NO synthase (51), decreased endothelin-1 (52), or other pathways not yet understood. Similarly, obesity affects many metabolic pathways, and does not merely increase inflammation.

We have also demonstrated that questions of mechanism of action of environmental agents, often considered the domain of toxicology, can also be addressed in humans using gene by environment, gene by drug by environment, and gene by phenotype by environment interactions. Although there are limitations to this approach, the ability to study the species of interest in the exposure range of interest makes it a valuable tool for examining mechanisms of environmental toxins.

There are a number of limitations to this analysis. First, we have used PM$_{2.5}$ concentrations at a single monitoring site as a surrogate for recent exposure to PM$_{2.5}$. A recent study comparing personal exposures to monitoring at the same site, in several panels of subjects, reported a high longitudinal correlation between the monitor’s readings and personal exposure (53). In addition, PM$_{2.5}$ concentrations have been shown to be spatially heterogeneous over the Boston area, suggesting that this is a reasonable approximation, and the error is likely to be non-differential (53). Other genes affect responses to ROS, and our findings suggest that these also may play a role in individual response to air pollution–induced morbidity and/or mortality. We believe our current findings provide further evidence that ROS are an important pathway for particle toxicity.

**Conflict of Interest Statement**: J.S. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.K.P. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. M.S.O. has no financial relationship with a commercial entity that has no interest in the subject of this manuscript. D.S. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.W. received a grant for $900,065, Asthma Policy Modeling Study, from AstraZeneca from 1997–2003. He was a coinvestigator on a grant from Boehringer Ingelheim, which began in 2001, to investigate a chronic obstructive pulmonary disease natural history model. He has received no funds for his involvement in this project. He has been an advisor to the TENOR study for Genentech and has received $5,000 for 2003–2004. He received a grant from Glaxo-Wellcome for $300,000 for genomic equipment from 2000–2003. He was a consultant for Roche Pharmaceuticals in 2000 and received no financial remuneration for this consultancy. K.K. has no financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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**References**


