Ozone is a powerful oxidant associated with impairment of pulmonary function and increased airway inflammation. We recently reported that asthmatic children in Mexico City, an area with high ozone exposure, have significant ozone related decrements in the index of lung function, forced expiratory flow (FEF25–75), that were mitigated by supplementation with the antioxidant vitamins C and E in a double blind randomised trial.

Genetic factors probably contribute to the substantial variability between individuals in the effects of ozone on lung function and airway inflammation observed in humans. Glutathione transferases (GSTs) play a major role in protecting cells against damage from reactive oxygen species—such as those produced by ozone—by conjugating them with glutathione so that they can be rapidly eliminated. The glutathione S-transferase, GSTM1, may be especially important in response to oxidative stress, including ozone exposure. A common homozygous deletion polymorphism of the GSTM1 gene (GSTM1 null genotype) abolishes enzyme activity and may increase susceptibility to oxidative stress. We hypothesised that asthmatic children genetically lacking in GSTM1 activity might have greater susceptibility to reductions in FEF25–75 associated with ozone exposure and greater benefits from antioxidant supplementation. We tested this hypothesis by genotyping DNA samples from asthmatic children in our earlier randomised double blind trial of antioxidant supplementation in Mexico City.

METHODS
Study design
Details of the randomised double blind antioxidant supplementation study have recently been published. In brief, 158 asthmatic children were recruited through the allergy clinic of a large public hospital in Mexico City and followed over a 12 week period with two spirometric tests per week. At baseline, parents completed an interviewer administered questionnaire on diet and risk factors for asthma. Randomisation to receive the supplement (vitamin C 250 mg/day and vitamin E 50 mg/day) or placebo was conducted in a double blind manner. Children provided blood samples for measurements of vitamins C and E at baseline and at several points during the follow up period to verify compliance. Pulmonary function was measured at each visit with a spirometer (Medifacts Pneumotachograph, San Clemente, CA, USA) according to American Thoracic Society specifications. The best of three technically acceptable manoeuvres was used. Institutional review boards of the collaborating institutions approved the study and parents gave written informed consent for their child’s participation.

Measurements of ambient ozone and climate variables were obtained from the Mexican government’s air monitoring stations. All children resided within 5 km of a monitoring station whose values were assigned to that child. We also classified exposure to particles less than 10μm and nitrogen dioxide but we found no evidence for an effect of antioxidant intervention in response to these agents and thus they are not considered in this analysis. DNA was extracted from whole blood or Buffy coat using a standard Gentra Puregene protocol (Gentra Systems, Minneapolis, MN, USA). GSTM1 genotype was determined as previously described. The method distinguishes subjects with homozygous deletion of GSTM1 (GSTM1 null genotype) from those with either one or two copies of the gene (GSTM1 positive genotype).

Statistical analysis
The effect of ozone exposure on the day before spirometric tests was examined using the maximum 1 hour concentration. Generalised estimating equation models (GEEs) were used to assess the relation between ozone exposure and changes in lung function tests (FEF25–75) by GSTM1 genotype (null versus positive) within the two treatment groups (placebo and antioxidant supplemented). Confounding was evaluated as previously described.
We first compared the effect of ozone on lung function by GSTM1 genotype within the two supplementation groups, and then compared the effect of supplementation on the relation between ozone and lung function by genotype. Regression coefficients were compared using a $t$ test to estimate the modifying effect of GSTM1 polymorphisms/or supplementation groups on ozone mediated responses. We also tested the significance of interaction terms between genotypes, supplementation groups, and ozone. These analyses were conducted in all children and in the subsample of children with moderate and severe asthma. All analyses used Stata Release 7.0 (Stata Corporation, College Park, TX, USA).

**RESULTS**

The GSTM1 null genotype was present in 39% of children. Although more children with moderate and severe asthma had a GSTM1 null genotype (placebo 1039, supplement 1032), baseline pulmonary function did not differ and other characteristics were balanced between the two GSTM1 genotype groups (male 63.5% versus 66.1%; mean age 9.2 years in both groups; mother smoking 17.0% versus 18.3%). The number of spirometric tests per child was similar between the supplement and placebo groups (mean 22, range 12–28). All but three participants had at least one positive skin test for common antigens. The 1 hour maximum ozone level during the study period ranged from 12 to 309 ppb with a mean (SD) of 102 (47) ppb.

In the placebo group ozone levels were significantly and inversely associated with FEF$_{25-75}$ in children with the GSTM1 null genotype; no significant decrement was observed in GSTM1 positive children (table 1). Expressed as a percentage of baseline FEF$_{25-75}$, for an increase of 50 ppb in 1 h maximum ozone concentration, this decrement in children with the GSTM1 null genotype taking placebo corresponded to −2.9% (95% CI −5.2 to −0.6, $p = 0.01$). This effect was stronger in children with moderate to severe asthma (−4.7%, 95% CI −7.7 to −1.7; $p = 0.002$). The difference in the relation between ozone and FEF$_{25-75}$ by genotype among all asthmatic children receiving placebo was of borderline statistical significance ($p = 0.10$). Children receiving antioxidant supplementation had no statistically significant ozone related decrement in FEF$_{25-75}$ regardless of genotype. When we considered the effect of antioxidant supplementation by genotype, the beneficial effect was seen primarily in the GSTM1 null individuals (difference of 2.7% between the placebo and supplement groups, $p = 0.09$) rather

### Table 1 Difference in FEF$_{25-75}$ related to ozone concentration on the day before spirometric testing according to GSTM1 genotype in asthmatic children in Mexico City given antioxidant supplementation or placebo

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>Coefficient (95% CI)†, ml/s</th>
<th>Percentage change*</th>
<th>Coefficient (95% CI)†, ml/s</th>
<th>Percentage change*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50 ppb O$_3$</td>
<td></td>
<td>50 ppb O$_3$</td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>GSTM1 null</td>
<td>−50.5 (−90.1 to −10.9)</td>
<td>−2.9 (−5.2 to −0.6)</td>
<td>−50.5 (−90.1 to −10.9)</td>
<td>−2.9 (−5.2 to −0.6)</td>
</tr>
<tr>
<td></td>
<td>GSTM1 positive</td>
<td>−10.5 (−38.7 to 17.7)</td>
<td>0.6 (−2.1 to 0.9)</td>
<td>0.6 (−2.1 to 0.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Genotype effect</td>
<td>40.0 (−7.5 to 85.7)</td>
<td>2.3 (−0.2 to 4.9)</td>
<td>2.3 (−0.2 to 4.9)</td>
<td></td>
</tr>
<tr>
<td>Supplement</td>
<td>GSTM1 null</td>
<td>−3.0 (−40.4 to 37.4)</td>
<td>0.3 (−1.6 to 2.2)</td>
<td>−3.0 (−40.4 to 37.4)</td>
<td>0.3 (−1.6 to 2.2)</td>
</tr>
<tr>
<td></td>
<td>GSTM1 positive</td>
<td>5.1 (−29.8 to 40.1)</td>
<td>0.5 (−2.4 to 3.3)</td>
<td>0.5 (−2.4 to 3.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Genotype effect</td>
<td>8.1 (−44.1 to 60.4)</td>
<td>3.1 (−2.4 to 7.8)</td>
<td>3.1 (−2.4 to 7.8)</td>
<td></td>
</tr>
</tbody>
</table>

*The number of subjects in each group is given. The number of observations is: GSTM1 null (placebo 654, supplement 732), GSTM1 positive (placebo 1065, supplement 1039).
†Results and 95% confidence intervals from generalised estimating equation models adjusted for age, height, use of bronchodilator, respiratory symptoms on the day of the spirometric test, minimum temperature, and time since the beginning of the study. Ozone level is 1 hour maximum. During the observation period the mean (SD) 1 hour maximum ozone level was 102 (47) ppb (range 12–309). The Mexican standard is 110 ppb.
‡Percentage change from baseline FEF$_{25-75}$ for the individual stratum.
*‡p<0.01. All $p$ values were obtained by $t$ test comparing coefficients between the two groups.
*0.05<p<0.10

### Table 2 Difference in FEF$_{25-75}$ related to ozone concentration on the day before spirometric testing according to antioxidant supplementation groups within GSTM1 genotypes among asthmatic children in Mexico City

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>Coefficient (95% CI)†, ml/s</th>
<th>Percentage change*</th>
<th>Coefficient (95% CI)†, ml/s</th>
<th>Percentage change*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50 ppb O$_3$</td>
<td></td>
<td>50 ppb O$_3$</td>
<td></td>
</tr>
<tr>
<td>GSTM1 null</td>
<td>Placebo</td>
<td>29</td>
<td>50.5 (−90.1 to −10.9)</td>
<td>−2.9 (−5.2 to −0.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Supplement</td>
<td>33</td>
<td>−3.0 (−40.4 to 37.4)</td>
<td>0.3 (−1.6 to 2.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Supplement effect</td>
<td>47.5 (−7.2 to 102.2)**</td>
<td>2.1 (−0.2 to 5.8)**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTM1 positive</td>
<td>Placebo</td>
<td>49</td>
<td>−10.5 (−38.7 to 17.7)</td>
<td>0.1 (−1.6 to 3.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Supplement</td>
<td>47</td>
<td>5.1 (−29.8 to 40.1)</td>
<td>0.9 (−1.6 to 3.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Supplement effect</td>
<td>15.6 (−29.2 to 60.4)</td>
<td>0.9 (−1.6 to 3.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The number of subjects in each group is given. The number of observations is: GSTM1 null (placebo 654, supplement 732), GSTM1 positive (placebo 1065, supplement 1039).
†Results and 95% confidence intervals from generalised estimating equation models adjusted for age, height, use of bronchodilator, respiratory symptoms on the day of the spirometric test, minimum temperature, and time since the beginning of the study. Ozone level is 1 hour maximum. During the observation period the mean (SD) 1 hour maximum ozone level was 102 (47) ppb (range 12–309). The Mexican standard is 110 ppb.
‡Percentage change from baseline FEF$_{25-75}$ for the individual stratum.
*‡p<0.01. All $p$ values were obtained by $t$ test comparing coefficients between the two groups.
*0.05<p<0.10
than in the GSTM1 positive children (difference of 0.9% between placebo and supplement groups, p = 0.49). Among GSTM1 null children with moderate and severe asthma, the effect of supplementation was enhanced (4.4%, p = 0.04; table 2). In a general model, interactions between genotypes and ozone and supplement group and ozone were marginally significant (p = 0.14 and p = 0.093, respectively).

**DISCUSSION**

The findings are consistent with the role of GSTM1 in oxidative stress. GSTM1, along with other GSTs, appears to be critical in protecting against oxidative stress by catalysing the conjugation of glutathione to reactive oxygen species. GSTM1 null genotype appears to contribute to increased levels of biomarkers of oxidative stress after ozone exposure. In our population, as in other populations of Mexican descent, nearly 40% of the children had a GSTM1 null genotype. However, given our sample size, we could not stratify by other genotypes that might interact with GSTM1 in relation to the response to ozone. A larger intervention study would be necessary for this type of analysis.

Our data provide preliminary evidence that asthmatic children who may be genetically impaired in their ability to handle oxidative stress, by virtue of deletion of the GSTM1 gene, are more susceptible to the impact of ozone exposure on small airways function. Furthermore, supplementation with the antioxidants vitamins C and E above the minimum daily requirement might compensate for this genetic susceptibility.

**ACKNOWLEDGEMENTS**

The authors acknowledge the Monitoring Network of Mexico City for providing data on air pollutants, the Roche Laboratory for providing the supplements and placebos used in the study, Gary Hatch from the US EPA for vitamin C and E measurements, and Bioserve Biotechnologies, Laurel, MD for contract genotyping services.

**REFERENCES**


**LUNG ALERT**

Environmental nitrogen dioxide causes a pro-allergic response in the bronchial epithelium of normal subjects in vivo


High levels of nitrogen dioxide (NO₂) are associated with asthma related morbidity and mortality although the precise mechanisms surrounding this remain unclear. This study evaluated the effects of in vivo NO₂ exposure on expression of a variety of biomarkers.

Twelve non-asthmatic subjects were randomly exposed, with an intervening 3 week period, to 2 ppm NO₂ or filtered air on four successive days. Bronchial biopsies were taken after exposure and subsequently immunostained and their expression quantified using computerised image analysis. Expression of IL-5, IL-10, IL-13, and ICAM-1 were significantly greater (p ≤ 0.05) in subjects exposed to NO₂ than in those exposed to filtered air.

The study showed that NO₂ exposure results in an upregulation of pro-allergic Th2 cytokines. This suggests a mechanism by which environmental agents may trigger changes in bronchial epithelium which are associated with the asthmatic inflammatory process. In turn, this raises the possibility of future targets for disease prevention and treatment. Larger studies in asthmatic subjects incorporating the effects of more long term NO₂ exposure on cytokines in conjunction with parameters such as lung function, airway hyperresponsiveness, and inflammatory surrogates are required.

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Genetic polymorphism of GSTM1 and antioxidant supplementation influence lung function in relation to ozone exposure in asthmatic children in Mexico City


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