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Effect of *GST* variants on lung function following diesel exhaust and allergen co-exposure in a controlled human crossover study

Xin Zhang [a,b], Jeremy A. Hirota [b,c], Chenxi Yang [d], Chris Carlsten [b,c,*]

[a] Institute of Environmental Science, Shanxi University, Taiyuan, China
[b] Department of Medicine, Division of Respiratory Medicine, Chan-Yeung Centre for Occupational and Environmental Respiratory Disease, Vancouver Coastal Health Research Institute, University of British Columbia, Vancouver, Canada
[c] Institute for Heart and Lung Health, University of British Columbia, Vancouver, Canada
[d] Center for Heart Lung Innovation, University of British Columbia, Vancouver, Canada

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ABSTRACT

Background: Isolated exposure to diesel exhaust (DE) or allergen can cause decrements in lung function that are impacted by the presence of genetic variants in the glutathione-S-transferase (*GST*) family but the effect of *GST* interactions with DE-allergen co-exposure on lung function is unknown. We aimed to assess the impact of DE and allergen co-exposure on lung function and the influence of *GSTM1* or *GSTT1* variation

Methods: We used a blinded crossover study design with 17 atopic subjects exposed to filtered air (FA; the control for DE) or DE for 2 h. One hour following each exposure to DE or FA, bronchoscopy was performed to deliver a diluent-controlled segmental allergen challenge (SAC). Methacholine challenge and forced expiratory volume in 1 s (FEV_1) was performed pre-exposure (baseline airway responsiveness) and 24 h post-exposure (effect of co-exposure). Additionally, FEV_1 was performed hourly after DE/FA exposure and protein carbonyl content was measured in plasma as an oxidative stress marker.

Results: Changes in FEV_1 from baseline were dependent on time following allergen exposure. DE, as opposed to FA, led to a significant change in FEV_1 at 2 h post-allergen exposure in *GSTT1* variants only ($24.5 \pm 19.6\%$ reduction in *GSTT1* null individuals vs. $9.2 \pm 7.3\%$ reduction in *GSTT1* present individuals). Moreover, plasma protein carbonyl level 4 h after co-exposure was higher in the individuals who have the *GSTT1* null genotype.

Conclusions: This suggests a gene-environment interaction that endangers susceptible populations co-exposed to DE and allergen.

1. Background

Exposure to ambient air pollution is associated with adverse health effects ranging from increased symptoms of respiratory disease to increased premature mortality [1–3]. Studies have demonstrated an association between short-term exposure to particulate matter (PM), a component of air pollution, and the occurrence of asthma [4–6]. Importantly, $PM_{2.5}$ (PM with an aerodynamic diameter < 2.5 μm) reflects the smaller size fraction of traffic-related air pollution that can penetrate deeply into the lung and may drive adverse respiratory health effects [7,8]. $PM_{2.5}$ is a complex mixture of compounds that includes road dust, tire wear, and combustion related products including diesel exhaust (DE) [9].

DE is a major component of traffic pollution that has been used experimentally in a number of controlled human exposure studies [10–14]. DE can cause respiratory inflammation associated with decrements of lung function, measured as forced expiratory volume in 1 s (FEV_1) [15–17]. Controlled allergen exposure by inhalation or segmental allergen challenge (SAC) also induces decreases in FEV_1 [18,19]. It has been reported that PM can act as a carrier for allergens resulting in simultaneous co-exposure to air pollution and allergens [20]. Importantly, changes in FEV_1 are not universally observed following single exposures in controlled human exposure studies, suggesting that there may be underlying

Abbreviations: BMI, body mass index; DE, diesel exhaust; DeltaDEA, delta diesel exhaust with allergen; DRS, dose-response slope; FA, filtered air; DeltaFAA, delta filtered air with allergen; FEV_1, forced expiratory volume in one second; *GST*, glutathione S-transferases; *GSTM1*, glutathione S-transferase M1; *GSTT1*, glutathione S-transferase T1; PC_{20}, concentration of methacholine that induces a 20% fall in FEV_1; PM, particulate matter; $PM_{2.5}$, PM less than 2.5 μm in aerodynamic diameter; SAC, segmental allergen challenge

* Correspondence to: 7th floor The Lung Center, Vancouver General Hospital (VGH) – Gordon and Leslie Diamond Health Care Centre, University of British Columbia, 2775 Laurel Street, V5Z1M9 Vancouver, BC, Canada.
E-mail address: carlsten@mail.ubc.ca (C. Carlsten).

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genetic components that predispose certain populations [15] or due to unappreciated effects of co-exposures. To explore the impact of complex exposures on lung function, we performed a controlled human exposure study to assess the combination of DE and allergen in the lung.

There is large variation between individuals in their response to air pollution. Individuals with impaired antioxidant capacity may be at increased risk for development of asthma as well as exacerbations [21,22]. Several small molecules and proteins are involved in airway antioxidant defenses that might mediate sensitivity to DE [23]. The glutathione S-transferases (*GST*) family of antioxidant and detoxifying enzymes plays an important role in respiratory health [24]. Common *GSTM1* and *GSTT1* variants have been associated with lung function deficits in asthmatics and people with respiratory symptoms [25,26]. In addition, *GSTM1* and *GSTT1* variants may impact nasal allergen responsiveness, bronchial hyperactivity, respiratory inflammation and might explain variation in responses to DE [27,28]. Currently it is unknown how these common *GST* polymorphisms impact lung function following controlled co-exposure to DE and allergen.

To address this knowledge gap, we performed a blinded crossover study in atopic individuals who were exposed to allergen in combination with freshly generated DE or filtered air (FA; the control for DE). A recent study from our laboratory, which used the same subjects, has demonstrated that DE augmented allergic inflammation in the lower airways of atopic individuals and the *GSTT1* genotype enhanced this response [29]. This study was aimed at examining the impact of these exposures and genotypes on lung function, as measured by FEV_1 and methacholine challenge. We hypothesized that null *GSTM1* and/or *GSTT1* genotypes would be associated with decreased airflow and increased airway hyperresponsiveness, particularly with co-exposure to allergen and DE (relative to allergen alone).

2. Materials and methods

The ethics review boards of the University of British Columbia and the Vancouver Coastal Health Research Institute approved the study. Written informed consent was obtained from each participant.

2.1. Study design

We performed a randomized double-blinded crossover study with two exposures (DE or FA) separated by four weeks between exposures, the order of which was randomized and counter-balanced. One hour following each exposure to DE or FA, a bronchoscopy was performed to deliver a diluent-controlled SAC. Following an approximately four weeks washout period, subjects returned and received the second two hours exposure, followed by a second bronchoscopy during which allergen and saline were administered to opposite lungs and different segments than those during the first exposure. A validated common cold questionnaire [30] was used to confirm that participants were free of viral infection for at least four weeks before each experimental condition. Notably, the protocol allows for effective blinding [31] to both the subject and those analyzing all material and data. Table 1 summarizes the experimental time-course and outcome measurements.

2.2. Diesel exhaust and filtered air exposure

DE exposure was nominally at 300 micrograms per cubic meter of particulate matter of 2.5 μm or less in aerodynamic diameter (300 μg $PM_{2.5}/m^3$), using a validated system (as previously described [32] using ultra-low sulfur commercial diesel fuel and modified only in that we used a constant 2.5 kW load) that excludes potential contamination with lipopolysaccharide.

Table 1
Experimental time-course and outcome measurements.

	Screening visit	Day 1								Day 2
Time point designation (h)		−3	−2	−1	0	1	2	3	4	24
FA or DE exposure		⟷								
Segmental allergen challenge					•					
Spirometry	•	•	•	•		•	•	•	•	•
Methacholine challenge	•									•

Subjects were exposed to either filtered air (FA) or diesel exhaust (DE) for 2 h and allergen was performed 1 h following each exposure on Day 1.

2.3. Segmental allergen challenge

The SAC was instilled into the right middle lobe segment (5 mL solution of the positive skin prick allergen extract at a concentration 10-fold lower than the dose producing a positive wheal) with a 5 mL diluent control instilled into the lingular segment [33]. DE exposure preceded allergen instillation to avoid the possibility that starting with SAC would lead to acute segmental bronchoconstriction and thus decreased deposition of DE within that segment [29]. We used local allergen monitoring data, both historical and updated for the year of study, to ensure that subjects with birch or grass were studied outside of the respective season (before the start of the season and at least one month after the end of the season).

2.4. Subject characteristics

Eighteen subjects were recruited for our study by local advertising and referral/solicitation of clinic patients (consistent with the University of British Columbia Research Ethics Board). One subject did not consent for genotyping, resulting in all subsequent analyses being performed on 17 subjects. The following inclusion criteria were used: 19–49 years old, non-smoking, able to provide informed consent and positive skin-prick to one of our three study allergens: birch, Pacific grasses and house dust mite (*Dermatophagoides pteronyssinus* group). The following exclusion criteria were used: (1) pregnant/breastfeeding, (2) using inhaled corticosteroids, (3) regularly using bronchodilator medications (i.e., use of bronchodilators more than 3 times per week), (4) unstable asthma symptoms, (5) using vitamins A, C, E or antioxidant supplements, (6) co-morbid conditions judged by the investigators to increase risk of dropout, or (7) having worked in an industrial setting or other setting of significant inhaled exposures.

2.5. Lung function measurements

FEV_1 was determined by standard spirometry performed according to American Thoracic Society and European Respiratory Society guidelines [34]. Airway responsiveness was evaluated by methacholine challenge using the 2-min tidal breathing method [35]. Starting at 0.5 mg/ml, methacholine concentrations were increased in doubling doses to a maximum of 128 mg/ml. Airway responsiveness is described by the provocative concentration of methacholine inducing a 20% fall in FEV_1 (PC_{20}), and the dose-response slope (DRS=log [(% fall in FEV_1/cumulative dose methacholine)+1]), which allowed determination of a numerical value even when a FEV_1 dropped less than 20% following inhalation of the final dose of methacholine [36].