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Exposure to Diesel Exhaust and Plasma Cortisol Response: A Randomized Double-Blind Crossover Study

Errol M. Thomson,[1,2] Alain Filiatreault,[1] Andrew Williams,[1] Christopher F. Rider,[3] and Chris Carlsten[3,4]

[1]Environmental Health Science and Research Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario, Canada
[2]Department of Biochemistry, Microbiology, and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada
[3]Department of Medicine, Faculty of Medicine, University of British Columbia, Vancouver, British Columbia, Canada
[4]School of Population and Public Health, Faculty of Medicine, University of British Columbia, Vancouver, British Columbia, Canada

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Introduction

Traffic-related air pollution (TRAP) is associated with a variety of adverse health effects. Although a central role has been proposed for oxidative stress, elucidating underlying mechanisms remains an area of active investigation. Experimental work has demonstrated that glucocorticoid stress hormones are potential mediators of pulmonary and systemic pollutant effects of both particulate and gaseous pollutants (Thomson 2019), but direct evidence of TRAP-dependent stress axis activation is lacking. Moreover, although oxidative stress is a regulator of glucocorticoid signaling (Okamoto et al. 1999), involvement in pollutant-induced stress axis activation is unknown. In population studies, nitrogen dioxide was associated with a flattened salivary cortisol profile in adolescents (Wing et al. 2018) and higher awakening cortisol and flattened profile in 45 to 85-y-old adults (Hajat et al. 2019), suggesting potential TRAP-related impacts. The present study evaluated whether short-term exposure to diesel exhaust increases plasma cortisol levels and considered effect modification by sex, asthma status, antioxidant gene variants, and antioxidant treatment.

Methods

The study was approved by the University of British Columbia, Vancouver Coastal Health Research Institute, and Health Canada ethics review boards, and registered at clinicaltrials.gov (trial NCT01699204). Each participant provided written informed consent. Study details, including exclusion criteria, diet and medications, questionnaires, assessment of airway hyperresponsiveness, and genotyping, are provided elsewhere (Carlsten et al. 2014). In brief, study participants [$n = 19$; mean age 28 (range 19–46), 14 with doctor-diagnosed asthma (7 females, 7 males), and 5 without (3 females, 2 males); all free from current use of inhaled corticosteroids or long-acting β_2 agonists, from regular use of bronchodilators, and from vitamin A, C, or E supplementation] took *N*-acetylcysteine (NAC; 600 mg) or placebo capsules three times daily for 6 d. On day 6, participants were exposed for 2 h to filtered air or diesel exhaust, producing three experimental conditions [filtered air with placebo (FAP), diesel exhaust with placebo (DEP), and diesel exhaust with NAC (DEN)], using a randomized, double-blind, crossover design that included a minimum 2-wk washout period between exposures. The protocol is nearly identical to another in which analysis of questionnaires demonstrated that participants were effectively blinded to exposures (Carlsten et al. 2013). Diesel exhaust was generated using a 6.0-kW diesel generator operated to simulate on-road emissions and diluted to maintain a nominal particulate concentration of 300 $\mu g/m^3$ [fine particulate matter (PM) with aerodynamic diameter less than or equal to 2.5 μm ($PM_{2.5}$); mass median aerodynamic diameter of 102.5 ± 14 nm)]. Blood was collected by venipuncture into EDTA tubes 18 h prior to exposure, immediately before exposure (0 h), and at 2, 6, and 30 h. Plasma was assessed in duplicate using Arbor Assays cortisol enzyme immunoassay kits (Cedarlane Laboratories Canada). Blood samples from each volunteer underwent polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis to measure the variant status of two present/null alleles (GSTM1, GSTT1; coded as 0 or 1 for null), and three single-nucleotide polymorphisms [SNPs; (GSTP1 rs1695; NFκB1 rs28362491; NQO1 rs1800566; coded as 0, 1, or 2 for homozygous risk variant)]. Genetic risk scores were generated using unweighted summation of null and risk alleles. Data were divided into high (4–6) and low (0–3) risk scores based on the median value. A generalized estimating equation approach was used to analyze the data using geepack library (version 1.3-1) in the R software (R Development Core Team) environment (Højsgaard et al. 2005). Cortisol levels were logarithmically transformed and analyzed, assuming a normal distribution and an exchangeable correlation structure. Results were back-transformed, and the delta method was used to approximate the standard error for the estimates, with Holm-Sidak adjustment to control the family-wise error rate.

Results and Discussion

Short-term exposure to diesel exhaust rapidly and transiently increased plasma cortisol levels (*Exposure* × *Time* interaction; $p < 0.0001$; DEP vs. FAP at 2 h; $p = 0.04$; Figure 1). The relative increase in cortisol during exposure to diesel exhaust compared with filtered air was similar in males and females and was observed primarily in those diagnosed with asthma or with gene deletions or polymorphisms in antioxidant genes (Figure 2).

The data show that exposure to diesel exhaust affects levels of an important stress hormone with wide-ranging systemic effects, substantiating a role for cortisol as a potential mediator of TRAP-dependent health effects and providing support under controlled conditions for epidemiological associations between TRAP and cortisol. The variation in cortisol response to diesel exhaust across participants was in line with known variation in response to acute stressors within the population according to factors including age, genetic variability, disease, and chronic stress (Rohleder et al. 2003). Although genetic variability in antioxidant genes was associated with the magnitude of cortisol response, treatment with the antioxidant NAC tended to reduce, but did not eliminate, the diesel exhaust-dependent increase. It is unclear whether this reflects insufficient antioxidant supplementation to block effects, lack of involvement of oxidative stress in the response, or inadequate

Address correspondence to Errol Thomson, Inhalation Toxicology Laboratory, Environmental Health Science and Research Bureau, Health Canada, 2203B Tunney's Pasture, Ottawa, Ontario, K1A 0K9, Canada. Telephone: (613) 614-2994. Email: errol.thomson@canada.ca

The authors declare they have no actual or potential competing financial interests.

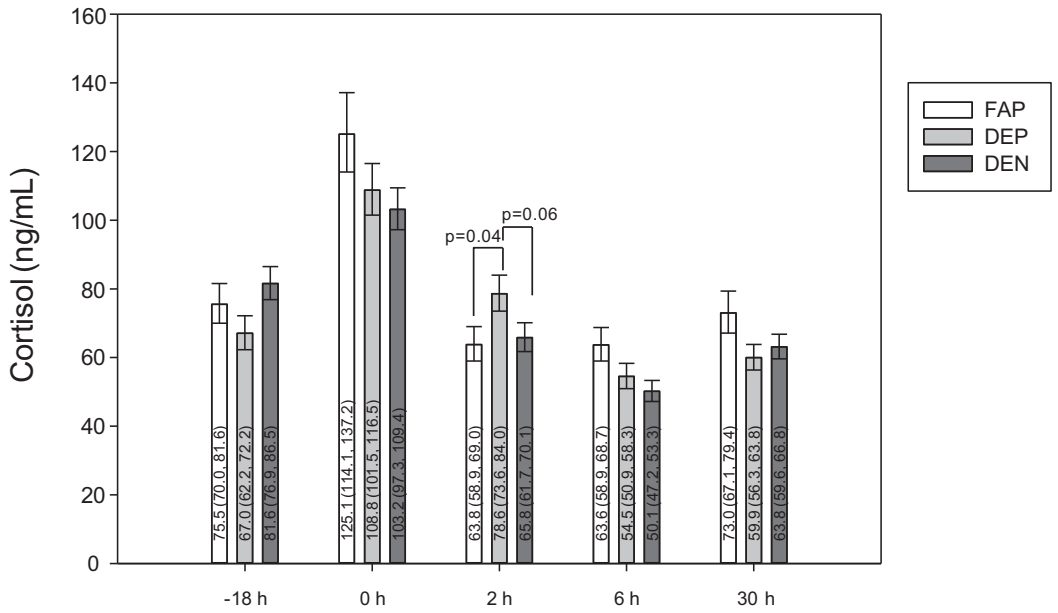

Figure 1. Time course of plasma cortisol response to exposure. Participants ($n = 19$) took the antioxidant NAC or placebo capsules three times daily for 6 d, followed by a 2-h inhalation exposure, leading to the following three experimental conditions: FAP, DEP, and DEN. Plasma cortisol was repeatedly measured pre- and post exposure at the times indicated. Data were assessed using a generalized estimating equation and adjusted according to the Holm-Sidak approach. Results are presented with 95% confidence intervals. Statistical significance is displayed for comparisons of treatments within a given time point. Note: DEN, diesel exhaust with NAC; DEP, diesel exhaust with placebo; FAP, filtered air with placebo; NAC, *N*-acetylcysteine.

power to detect effect modification. Although contrasting with the hyporesponsiveness to psychosocial stressors of individuals with asthma, the heightened cortisol response to diesel exhaust in participants with asthma is similar to the greater cortisol response to inhaled allergen challenge produced in people with asthma (Peebles et al. 2000). Notwithstanding the association of asthma diagnosis with increased cortisol response, degree of airway hyperresponsiveness was not significantly associated with differential cortisol response to diesel exhaust ($r = -0.083$; $p = 0.77$). The variability in cortisol responses to diesel exhaust seen here in

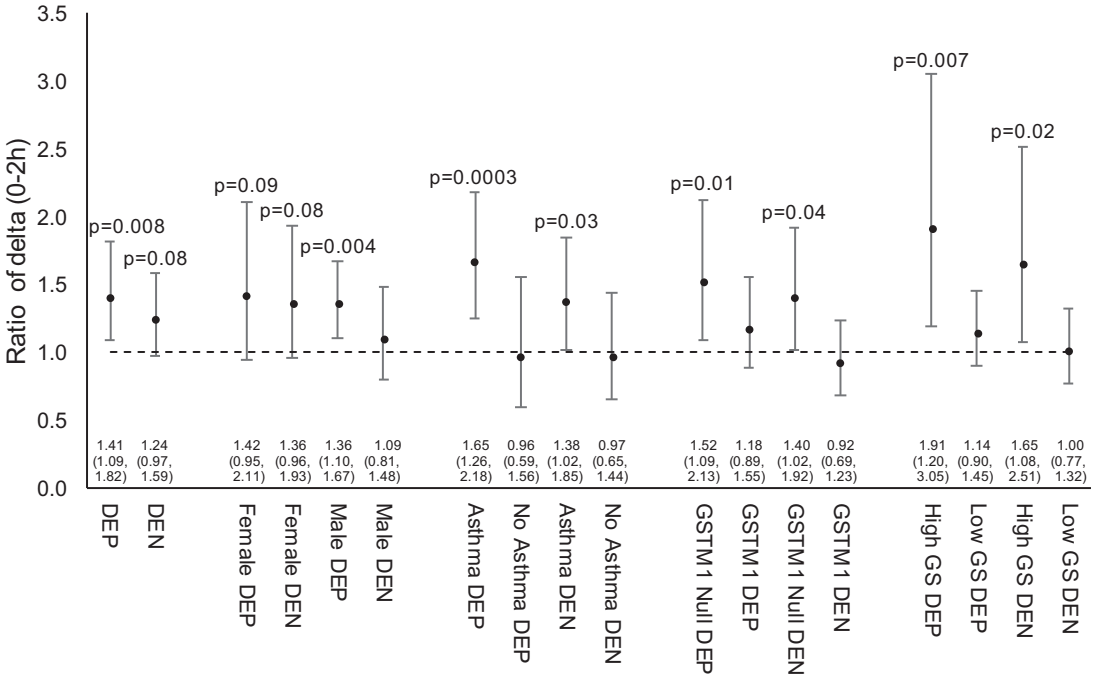

Figure 2. Cortisol response to exposure according to sex, asthma diagnosis, GSTM1 status (present or null) and GS (high or low). The change (delta) in log cortisol levels from 0 to 2-h time points for DEP or DEN exposures was compared to the delta over that same period for the FAP exposure. Analyses were conducted using the generalized estimating equation approach and adjusted according to the Holm-Sidak multiple comparison procedure. Results are presented with 95% confidence intervals. Statistical significance is indicated relative to the FAP exposure. DEN vs. DEP comparisons were all nonsignificant and for simplicity are not shown here. Note: DEN, diesel exhaust with NAC; DEP, diesel exhaust with placebo; FAP, filtered air with placebo; GS, genetic risk score; NAC, *N*-acetylcysteine.

relation to individual characteristics may have relevance to individual risk of local and systemic health effects of air pollutants (Thomas et al. 2018; Thomson 2019).

Limitations

Although the crossover design is statistically powerful, the study may nevertheless be underpowered to detect effect modification. Participants were predominantly young and had asthma; it is unknown whether effects would manifest similarly in other populations. Despite the washout period between exposures, participants will have been exposed to TRAP elsewhere, which could condition responses. Finally, although we assessed antioxidant genes, other genetic variation could also influence cortisol responses.

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