Online Supplement

Acute exposure to diesel exhaust impairs NO-mediated endothelial vasomotor function by increasing endothelial oxidative stress

Running title: Endothelial dysfunction in polluted environment.

Aurélien Wauters¹, MD; Céline Dreyfuss¹, MD;

Stéphanie Pochet², PhD; Patrick Hendrick³, PhD; Guy Berkenboom¹, MD, PhD;

Philippe van de Borne¹, MD, PhD; and Jean François Argacha¹, MD, PhD.

¹: Department of Cardiology, Erasme Hospital, Université Libre de Bruxelles (ULB), Belgium

²: Laboratory of Physiology and Pharmacology, Faculty of Pharmacy, Université Libre de Bruxelles (ULB), Belgium

³: Laboratory of Aero-Thermo-Mechanics, Université Libre de Bruxelles (ULB), Belgium

Address for correspondence and reprint requests:

Aurélien Wauters

Department of Cardiology, Erasme Hospital, 808 Lennik Street, 1070 Brussels, Belgium.

E-mail: Aurelien.Wauters@erasme.ulb.ac.be Phone: +32-2-555 39 07. Fax: +32-2-5556713.
MATERIALS AND METHODS

Study design

The two different exposure sessions were separated by an interval of at least 7 days. Subjects were exposed to ambient air and polluted air while wearing a nose clip and covered glasses to prevent unblinding. The same experimental conditions were reproduced in all sessions and the diesel engine was turned on each time. The exhaust air was added to the air of the experimental chamber only during the polluted air sessions. Subjects thus heard the loud diesel engine noise during all experimental sessions but were not aware whether the exhaust gases were or were not present in the chamber. The investigators were unaware of the experimental session since exposure to polluted and ambient air did not occur at the same location than the LDI measures.

All subjects abstained from meals for 12 hours and from alcohol and coffee beverages for at least 48 hours prior to each exposure session. The volunteers did not wash their forearms on the morning of the experiment and had to avoid non-steroidal anti-inflammatory drugs for at least 3 days before each visit.

Microcirculatory vasomotor function evaluation

The measurements were performed in a quiet room, in the supine position under carefully standardized conditions. The ambient temperature achieved by the air conditioner in the room was 23+/−1°C.

Heating and Ach and SNP iontophoresis were continued for 26 minutes in order to achieve maximal skin vasodilation. Ach and SNP solutions were prepared to obtain a final concentration of 2 g/100 ml in deionized water; 2.5 ml of these solutions was introduced into the cathode (Ach electrode) and the anode (SNP electrode) chambers. Electric current was generated by an iontophoresis controller (MIC 2, Moor Instruments Ltd, Axminster, United Kingdom), which was set to apply a current of 100 µA.

Superoxide anion measurement

Briefly, after the pre-incubation, the cells were detached with trypsin/EDTA and centrifuged at 1500 g. The cell pellet was resuspended in 500 µl Dulbecco's Modified Eagle's medium (DMEM, Gibco, Paisley, United Kingdom) containing 5 µM lucigenin (Sigma, Saint Louis, USA) and 100 µM NADPH was added. Lucigenin chemiluminescence was recorded for 1 hour and the maximal value (at 40 min) was expressed in units per minute as a percentage of the control sample. Experiments were performed in triplicate.

Diesel exhaust exposure

The PM concentration was measured by photometry using a GRIMM Laser Aerosol Spectrometer 1109 (GRIMM Aerosol Technik GmbH & Co, Ainring, Germany). NO, NO₂, NOx, and CO concentrations and temperature were monitored by electrochemical sensors (Multilyzer NG, KWE Technologies Group, Waterloo, Ontario, Canada). We obtained recordings of blood pressure (BP), heart rate (HR), oxygen saturation (SpO₂) (Compaq, Datex Ohmeda, Helsinki, Finland) and minute ventilation (VE) (Pneumotrace, Medical Electronic Construction, Brussels, Belgium). The experimental setting as well as the reliability of the technical equipment and the accuracy of all safety procedures were repeatedly inspected and approved by the Department for Protection and Prevention at Work of our University (SIPP-ULB).

Data analysis

Diesel exhaust exposure was expressed as the total amount of inhaled PM2.5 calculated from the mean VE and the mean PM2.5 concentration in the exposure room. All
measurements were analyzed in a blinded fashion. Physiological parameters (BP, HR, SpO₂ and VE) were expressed as the mean of all measurements performed during each session. Skin blood flow was measured automatically (LDI version 5.3D software, Moor Instruments Ltd, Axminster, United Kingdom) and was expressed in Perfusion Units, PU (arbitrary units of blood flow). The skin blood flow values during baseline scans and during hyperemia tests were calculated and expressed as a percentage of the mean baseline scan, after insuring that there were no differences in the baseline values for the different exposures. The response to stimulation was quantified as the absolute unit change of every scan from the baseline skin blood flow value. The area under the curve (AUC) was calculated by summing each of the 11 measures of skin vasodilation in response to local heating. The NO-mediated skin vasodilatory response to local heating is presented separately (fig.2). We analyzed the effect of L-NAME iontophoresis in both ambient air and polluted air conditions. As in our previous studies, we looked more closely at the effect of these interventions on the late phase of the skin reaction to heating (1). The late phase AUC during heating-induced vasodilation was defined as the AUC observed after the initial peak of the hyperemic reaction, i.e., after Scan5. The delta AUC, representing NO-mediated skin thermal hyperemia, was then calculated as the difference between the Control and L-NAME AUCs during the heating-induced hyperemia (fig.S1). The T50 expressed the time needed to reach 50% of the maximal vasodilation. The Ach-AUC to SNP-AUC ratio (Ach/SNP ratio) estimated the relative contributions of endothelium-dependent and -independent vasodilation (2). Superoxide anion production by HUVEC was expressed as the percentage of production increase compared to a control sample without serum incubation.
REFERENCES


PM10: particulate matter < 10 µm; PM2.5: particulate matter < 2.5 µm; PM1: particulate matter < 1 µm; NO: nitric oxide; NO₂: nitric dioxide; NOx: nitrous oxides; CO: carbon monoxide; T: temperature.

**TABLE S1**
Pollution parameters during exposure

<table>
<thead>
<tr>
<th>Pollution parameters</th>
<th>Ambient Air</th>
<th>Polluted Air</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM10 (µg/m³)</td>
<td>21±0</td>
<td>311±2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PM2.5 (µg/m³)</td>
<td>11±0</td>
<td>306±2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PM1 (µg/m³)</td>
<td>9±0</td>
<td>303±2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PM2.5 inhaled (µg)</td>
<td>11±1</td>
<td>325±22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NO (ppb)</td>
<td>69±5</td>
<td>1240±41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NO₂ (ppb)</td>
<td>41±1</td>
<td>2906±94</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NOx (ppb)</td>
<td>110±6</td>
<td>4147±134</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CO (ppm)</td>
<td>0±0</td>
<td>18±1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T (°C)</td>
<td>21.3±0.2</td>
<td>25.5±0.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Figure S1: Illustrative example of LDI recordings
Panel A: Suppression of increased blood flow after heating (CTL, 20 min skin heating) by inhibition of eNOS by skin pre-treatment with L-NAME iontophoresis (L-NAME, 20 min skin heating). Panel B: Recording over time in perfusion units (PU) of skin heating-induced hyperemia in saline (continuous line) and L-NAME pre-treated skin (dotted line).