At high cardiac output, diesel exhaust exposure increases pulmonary vascular resistance and decreases distensibility of pulmonary resistive vessels

**Brief title:** Air pollution and pulmonary vascular resistance

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ABSTRACT

Air pollution has recently been associated with development of acute decompensated heart failure but the underlying biological mechanisms remain unclear. A pulmonary vasoconstrictor effect of air pollution, combined with its systemic effects, may precipitate decompensated heart failure. The aim of this study was to investigate the effects of acute exposure to diesel exhaust on pulmonary vascular resistance (PVR) in resting and stress conditions, but also to determine whether air pollution may potentiate acquired pulmonary hypertension. Eighteen healthy male volunteers were exposed to ambient air (AA) or dilute diesel exhaust (DE) with a PM2.5 concentration of 300 μg/m³ for 2 hours in a randomized, crossover study design. The effects of DE on PVR, on the coefficient of distensibility of pulmonary vessels (α) and on right and left ventricular function were evaluated at rest (n=18), during dobutamine stress echocardiography (n=10), and during exercise stress echocardiography performed in hypoxia (n=8). Serum endothelin-1 (ET1) and exhaled nitric oxide (FeNO) were also measured. At rest, exposure to DE did not affect PVR. During dobutamine stress, the slope of the mean pulmonary artery pressure/cardiac output (mPpa/Q) relationship increased from 2.8±0.5 mmHg.min/l in AA to 3.9±0.5 mmHg.min/l in DE (p<0.05) and the α coefficient decreased from 0.96±0.15 to 0.64±0.12 %/mmHg (p<0.01). DE did not further enhance the hypoxia-related upper shift of the mPpa/Q relationship. Exposure to DE did not affect serum ET1 concentration or FeNO. In conclusion, acute exposure to DE increased pulmonary vasomotor tone by decreasing the distensibility of pulmonary resistive vessels at high cardiac output.

Keywords: pulmonary hemodynamics – pulmonary vascular resistance – hypoxic pulmonary vasoconstriction – air pollution – diesel exhaust
NEW AND NOTEWORTHY

This study demonstrates for the first time in human subjects exposed to diesel exhaust a pulmonary vasomotor tone impairment. A 2-hours exposure to diesel exhaust alters pulmonary vessel distensibility at high cardiac output and increases pulmonary vascular resistance.
INTRODUCTION

Air pollution is a growing public health issue, especially in developing countries, and is responsible for up to 7 million deaths/year worldwide. Air pollution is considered as an important and potentially modifiable cardiovascular risk factor (26, 38, 55). Epidemiological studies have demonstrated a consistent link between elevated ambient particulate matter concentrations and new onset cardiac athero-thrombotic events (43, 44). In addition to its vascular effects, air pollution also appears to be an important precipitating factor for heart failure. Results from a recent metaanalysis showed that increases in concentrations of particulate matter < 2.5 μm (PM2.5) were associated with heart failure hospitalizations and deaths (48).

There is now considerable evidence demonstrating the effects of polluted air on the cardiovascular system. Air pollution notably elicits peripheral vascular dysfunction through endothelial dysfunction and vasoconstriction (4, 33, 42, 52). It also induces a pro-thrombotic state, characterized by increased in vivo thrombus formation through platelet activation and decreased endogenous fibrinolytic capacity (28, 32, 53). A recent overview of the physiopathological mechanisms underlying the cardiovascular effect of air pollution, considered pulmonary tissue to be an important determinant of a systemic oxidative, inflammatory and neurohumoral reaction (38). Human data regarding the effects of dilute diesel exhaust (DE) on pulmonary hemodynamics are scarce. An echocardiographic study, in which the maximal velocity of the tricuspid regurgitation jet was assessed in resting conditions in 81 children, demonstrated an elevated mean pulmonary arterial pressure in polluted urban areas compared to rural areas (7). In 11 patients with heart failure, analysis of a continuous implantable heart monitoring system demonstrated that same-day mean PM2.5 concentration was associated with small but significant increases in estimated pulmonary
artery and right ventricular (RV) diastolic pressure, using a pacemaker algorithm-based calculation of vascular impedance (47).

In view of the small numbers of subjects involved in these retrospective studies and potential bias from post-capillary increases in pulmonary pressure, these data are not sufficient to draw definitive conclusions on a possible pulmonary vasoconstrictor effect of air pollution. Because pulmonary vascular resistance (PVR) is the main determinant of RV afterload, an acute pulmonary vasoconstrictive response to PM exposure may favor acute decompensated heart failure through increased RV afterload and limited left ventricular (LV) filling. If DE toxicity primarily affects pulmonary tissue, an air pollution-related pulmonary vasoconstrictive effect could thus be considered as an explanation for the epidemiological relationship between air pollution and heart failure (48). Interventional studies performed in animal models have confirmed the ability of air pollution to elicit pulmonary vasoconstriction, which seemed to be related to an impaired NO pathway (11, 29). Models in which animals are chronically exposed to polluted urban air showed marked pulmonary arterial remodeling with a decrease in the lumen/wall ratio (5, 12). Moreover, chronic exposure induced pulmonary arterial vasoconstriction. This vascular effect is coupled to overexpression of the endothelin receptor and local, but not systemic, inflammation (12, 30).

By contrast, increase in pulmonary tone may also reflect a response to increased vasoconstrictive factors. Indeed, several studies reported that exposure to PM consistently increased the circulating levels of endothelin-1 (ET-1), a potent pulmonary vasoconstrictor, and others demonstrated increased peripheral vascular sensitivity to ET-1 (7, 22, 42).

We designed our study to test the hypothesis that standardized exposure of healthy subjects to dilute DE would acutely increase PVR, and may potentiate acquired pulmonary hypertension. We used a standardized exposure protocol that has been widely used in previous experiments in human subjects (28, 32, 33). Consistent with previous
echocardiographic research in the same field, we studied PVR at rest and the mPpa/Q relationship during dobutamine or exercise stress (24, 35). To produce an external increase in pulmonary pressure, we used an experimental model of hypoxia-mediated pulmonary hypertension (13, 18, 35). Finally, we explored the effect of DE exposure on exhaled NO and circulating ET-1 concentrations. By using this approach to assess both resting and dynamic changes in pulmonary pressure following air pollution exposure, we believed that this study improves our understanding of the acute effects of air pollution on pulmonary vascular function.
MATERIAL AND METHODS

Subjects

Thirty-three healthy, male non-smokers with normal physical examination were screened and 18 with an easily interpretable tricuspid regurgitation jet were selected for the study (mean age 22.2±0.5 years, body mass index [BMI] 21.7±0.5 kg/m²). The Ethical Committee of Erasme Hospital approved the study protocol (reference P2010/086) and informed written consent was obtained from all subjects.

Study design

The 18 subjects were exposed to either non-filtered ambient air (AA) or DE for 120 minutes using a randomized, crossover, double-blinded design (fig.1) with the different exposures occurring at least 1 week apart. Pulmonary hemodynamic parameters were calculated using echocardiography, which was initiated 2 hours after exposure. Ten subjects performed the dobutamine stress protocol under AA and DE conditions. Eight subjects performed the exercise in acute hypoxia stress protocol, which consisted of three different exercise sessions, one in normoxia during AA exposure, one in hypoxia during AA and one in hypoxia during DE exposure.

Diesel exhaust exposure

Exposure sessions were performed in a dedicated room (2.65 m x 2.65 m x 2.65 m) (52). Semi-automated valves controlled gas arrival and extraction from the room to insure a stable concentration of pollutants throughout the experimental session. The DE was generated by a PSA DW10 engine using a common ultra low sulfur diesel, and diluted with AA in the conduction system. DE was delivered to achieve a PM2.5 concentration of 300 μg/m³ as previously described (23, 32, 52). The PM concentration was measured by photometry using
a GRIMM Laser Aerosol Spectrometer 1109 (GRIMM Aerosol Technik GmbH & Co, Ainring, Germany). The subject’s blood pressure, heart rate (HR), oxygen saturation (Compaq, Datex-Ohmeda, Helsinki, Finland) and minute ventilation (Pneumotrace, Medical Electronic Construction, Brussels, Belgium) were recorded during each exposure. The Department for Protection and Prevention at Work of our University (SIPP-ULB) approved the experimental setting, the reliability of the technical equipment and the accuracy of all safety procedures.

Echocardiographic measures

Based on our previous experiments that peripheral effects of DE on microvascular function were observed up to 2 hours after exposure, we decided to start the echocardiographic measures also 2 hours after the exposure (52). These measurements were performed in the left lateral decubitus position in a quiet and temperature-controlled environment. As conventionally recommended, we followed a classic transthoracic echocardiographic protocol. We used a short axis parasternal view for acquisition of the LV outflow tract diameter and a 4-5 chamber apical view for acquisition of other parameters. Echocardiographic data were collected using CX 50 (Philips Healthcare, Best, Netherlands) and Vivid 7 Dimension (GE Healthcare, Little Chalfont, United Kingdom) by experienced echocardiographists blinded to the randomization group. Post-acquisition analysis was performed by the same investigator using a dedicated viewer (Xcelera, Philips Healthcare, Best, Netherlands). Measurements were calculated from an average of three cardiac cycles. The reproducibility of these measurements has been assessed previously in our echolab (14).

Echocardiographic data analysis

The primary end-point of this study was the PVR at rest and the mPpa/Q relationship
during cardiac stress tests. PVR was defined as the ratio of mPpa/Q. Cardiac output (Q) was calculated as the product of the velocity-time integral of the pulsed-Doppler tracing in the LV outflow tract, the cross-sectional area of the LV outflow tract, and the HR (fig.2A). Systolic pulmonary pressure (sPpa) was estimated from the transtricuspid pressure gradient calculated from the maximum velocity of continuous Doppler tricuspid regurgitation (fig.2A). Because the diameter of the inferior vena cava was < 20 mm with a collapse into inspiration, the right atrial pressure was considered as equal to 5 mmHg (56). We then calculated mean pulmonary pressure (mPpa) as 0.61 × sPpa + 2 mmHg (9). During stress tests, the slope of the mPpa/Q relationship was determined by linear regression for each subject (fig.2B).

We also measured the coefficient of vessel distensibility, $\alpha$, expressed in %/mmHg, during stress tests and according to the equation (27):

$$mPpa = \frac{\left[ \left(1+\alpha Pla\right)^5 + 5\alpha PVR_0 Q \right]^{1/5} - 1}{\alpha}$$

where Pla is the left atrial pressure, estimated at 8 mmHg using the Nagueh formula (37), and PVR$_0$ the pulmonary vascular resistance at rest. Pulsed Doppler of the mitral inflow allowed to calculate the maximal early (E) and late (A) velocities in diastole. Left diastolic function was evaluated by mitral E/A ratios. Using tissue Doppler analysis of the velocity of the mitral annulus, left atrial pressure was estimated from the mitral E/E’ ratio. This ratio provides a more reliable evaluation of left atrial pressure than E/A ratio, which is affected by diastolic function (41). Tricuspid annular plane systolic excursion (TAPSE) and the tissue Doppler tricuspid S wave, which evaluate RV systolic function, were also measured.

**Dobutamine stress protocol**

Dobutamine was administered into a brachial vein at an initial dose of 2 $\mu$g/kg/min and progressively increased by 2 $\mu$g/kg/min every 5 min. Echocardiographic measurements (CX 50, Philips Healthcare, Best, Netherlands) were performed at rest, to determine the baseline
parameters, and during the infusion of 4, 6 and 8 μg/kg/min of dobutamine. We simultaneously recorded the subject’s blood pressure, HR, and a 3-lead electrocardiogram.

Exercise in acute hypoxia stress protocol

Before echocardiographic measures, subjects were exposed to normoxic conditions or to hypoxia with an inspired fraction of O₂ of 12%, for 90 minutes using a dedicated airtight mask (Rüsch 4vent, Teleflex Medical, Athlone, Ireland). This level of hypoxia was previously demonstrated to be optimal to produce a maximal hypoxic pulmonary pressure increase in healthy humans with minimal changes in arterial PCO₂ (17). Hypoxia was maintained during the echocardiographic measurements. Symptomatic tolerance to hypoxia was assessed using the Lake Louise Score.

Echocardiographic measurements (Vivid 7 Dimension, GE Healthcare, Little Chalfont, United Kingdom) were performed first at rest, to determine the baseline parameters, and then during an incremental exercise schedule. Exercise echocardiography was performed in a semi-recumbent position using a supine ergometer (Ergoselect 1000, Ergoline, Bitz, Germany) with the table tilted up to 30° in the left lateral decubitus position. The exercise test was started at 10 W for 3 min, followed by a 10 W increase every 3 min. Echocardiographic measurements were performed at each step until the subject became uncomfortable or there was a loss of quality in the echographic images because of increased ventilation. We standardized the within subject workload in our exercise in hypoxia protocol. The achieved workload was 57±3 W. This schedule enabled us to perform 4.5±0.2 sets of measures for each of the three tests.

Simultaneously with echocardiographic measurements, we recorded the blood pressure, HR, oxygen saturation (Nellcor Puritan Bennett Inc, Pleasanton, CA, USA) and 3-lead electrocardiogram of the subjects.
ET-1 and fractional exhaled nitric oxide measures

Blood samples were collected immediately after each exposure (n=10). Sera were isolated after centrifugation at 2000 g for 15 minutes at 20°C and stored at -20°C until analysis. Serum concentrations of ET-1 were obtained using commercially available ELISA kits (R&D Systems®, Abingdon, United Kingdom). Fractional exhaled nitric oxide (FeNO, n=10) values were obtained before and immediately after exposure session using a nitric oxide monitor (Niox Mino, Aerocrine, Solna, Sweden) according to 2005 ATS/ERS guidelines (57).

Statistical analysis

Data are expressed as mean±SEM. Statistical analyses were performed using SPSS (SPSS 18.0, Chicago, IL, USA). Exposure pollution parameters were compared using a Student’s t-test. Physiological parameters, echocardiographic measurements and α coefficients were compared using a Student’s t-test or a one-way ANOVA test. Two-way repeated-measures ANOVAs were used to detect significant changes in PVR during the stress test. p values for the intervention effect and the time to intervention interaction are presented when the intervention effect p value alone was <0.05. Bonferroni corrections were applied for between group comparisons. Based on previous data from our echolab, our study was designed to identify a 20% difference in the slope of the mPpa/Q relationship with a statistical power of 80% with a two-sided alpha error of 0.05 (13). Statistical significance was assumed when p was <0.05.
RESULTS

Parameters prior to cardiac stress test

The concentration of PM2.5 increased from 21±1 in AA to 304±1 μg/m³ in DE (p<0.001, table 1). Blood pressure, HR, oxygen saturation and minute ventilation were similar in DE and AA. Serum ET-1 concentrations and FeNO were also similar in the two exposure groups.

DE exposure did not modify the mPpa or PVR at rest compared to AA. The mitral E/A or mitral E/E’ ratios, the TAPSE and the tricuspid S wave were also not altered.

Pulmonary hemodynamics during the dobutamine stress protocol

Compared to resting conditions, dobutamine induced maximal increases in Q of 4.05±0.47 in AA and 3.98±0.63 l/min in DE (p=NS). The slope of the mPpa/Q relationship was higher with DE than with AA (3.9±0.5 vs 2.8±0.5 mmHg.min/l) during the dobutamine stress test (fig.3A; p<0.05) and the a coefficient was lower (0.64±0.12 vs 0.96±0.15%/mmHg, p<0.01).

DE exposure did not modify the mitral E/A or mitral E/E’ ratios, the TAPSE or the tricuspid S wave (table 2). The BP and HR responses to the dobutamine stress test were similar under AA and DE conditions.

Pulmonary hemodynamics during exercise in acute hypoxia stress protocol:

There were no differences in the decrease in SpO₂ with hypoxia between AA and DE conditions. The SpO₂ at peak exercise in hypoxia was 66±2.8 in AA conditions compared to 68.6±2.8% in DE conditions (p=NS). At the end of the hypoxia, subjects had a Lake Louise Score of 1.0±0.5 with no difference between the exposure conditions. At rest, mPpa increased from 18.8±0.8 mmHg in normoxia to to 25.9±1.1 mmHg in hypoxia (p<0.001) and Q
increased from 4.9±0.3 to 5.8±0.4 l/min (p<0.05). PVR increased from 3.9±0.2 in normoxia to 4.6±0.2 mmHg.min/l in hypoxia (p<0.05).

Compared to resting conditions, exercise induced comparable maximal increases in Q of 7.6±0.3 in normoxia, 7.7±0.5 in hypoxia under AA, and 6.4±0.7 l/min in hypoxia under DE (p NS). During exercise stress, hypoxia induced an upper shift of the mPpa/Q relationship to a higher pressure regimen (hypoxia effect p value <0.05; fig.3B). Hypoxia under AA or DE exposure did not alter the slope of the mPpa/Q relationship compared to AA in normoxia (respectively 2.1±0.3 and 1.9±0.5 mmHg.min/l vs.2.5±0.3 mmHg.min/l, ANOVA p value NS).

The α coefficient, the mitral E/A or mitral E/E’ ratios, the TAPSE and the tricuspid S wave were not affected by exposure to DE (table 2). BP, HR and hypoxemic responses to the exercise stress test in hypoxia were similar with exposure to AA and DE.
DISCUSSION

The main new findings of our study can be summarized as follows. Recordings made 2 hours after a 120 minutes exposure to DE reveal that DE: 1) did not modify PVR in resting conditions; 2) increased the slope of the Ppa/Q relationship measured during cardiac stress, and this was associated with decreased vessel distensibility at high cardiac output; 3) did not enhance hypoxic-mediated pulmonary vasoconstriction. The cardiac output and pulmonary pressure measures we used during transthoracic echocardiography are now part of daily clinical practice and are recommended in cardiology but also in critically ill patients (8, 31). 4) DE did not modify FeNO or serum ET_1 levels, immediately after exposure.

We used a highly standardized method of DE exposure which enabled us to generate a level of exposure of 300 µg/m³ for 2 hours (52, 53). This level has been used by other authors to study the biological and physiological effects of DE exposure in humans (23, 32). Although rare, our exposure level has been encountered during recent pollution peaks in industrialized countries (51). Although the PM concentration was high, this occurred for a short period of time. If we extrapolate our exposure to a daily basis, as suggested by air pollution guidelines, it would represent an additive exposure of 12.5 µg/m³/hour for 24 hours. This is a common daily variation of PM2.5 observed in western countries (2). The increase in cardiac output we observed with dobutamine or exercise corresponds to moderate physical activity (14). We have previously shown that exercise in a polluted environment enhances the thrombogenic effect of air pollution (53). Our study illustrates, for the first time, a change in pulmonary hemodynamics after DE exposure at high cardiac output, which may, therefore, occur in real life scenarios.

PVR was measured non-invasively using a previously described methodology (24, 36). To uncover early stages of vascular dysfunction, we applied a multipoint pulmonary coordinate Ppa/Q analysis during a cardiac stress test (13–15, 35). Indeed, as shown in our
study, a single point measurement in resting conditions is not sufficient to identify subtle hemodynamic changes in the low pressure, high flow pulmonary circuit. Our results confirm accumulating evidence suggesting that the assessment of the pulmonary circulation under dynamic stress provides important clinical information in addition to resting measurements, as in “early” pulmonary arterial hypertension (10, 21, 24, 45).

In our study, we evaluated pulmonary hemodynamics during dobutamine stress echocardiography, which has the advantage of providing high quality acoustic images without a marked increase in ventilation (24). By using a dose limited of 10 μg/kg/min, we insured that dobutamine had no flow-independent vasoactive effects which could interfere with the pulmonary vascular effects of air pollution (40). The slope of the mPpa/Q relationship and the α coefficient values observed in our control conditions are concordant with previous studies, and within normal ranges (35). We demonstrated that for each 1 l/min increase in cardiac output, pulmonary pressure increased by 1 additional mmHg under DE exposure than under AA. This represents a relative PVR increase of 35% after DE exposure. Multipoint mPpa/Q plot analysis also showed a slightly curvilinear relationship generated by vascular distensibility at high cardiac output, which can be addressed using α coefficient calculation (27). We observed a reduction in the distensibility of pulmonary resistive vessels at high cardiac output after DE exposure. Loss of pulmonary vessel distensibility at high cardiac output appears to be a new pathophysiological pathway that may explain the effect of DE exposure on pulmonary hemodynamics (46). Hence, using standardized exposure and a state of the art measure of PVR, we have demonstrated, for the first time in humans, a change in pulmonary vascular tone after DE exposure, characterized by increased PVR and decreased vessel distensibility at high cardiac output.

In accordance with previous studies, acute hypoxia increased mPpa but had no effect on the slope of the mPpa/Q relationship (14, 15). Moreover, we found that DE exposure did
not enhance the hypoxic modification of the mPpa/Q relationship. However, this result may not be sufficient to rule out a potential change in pulmonary hemodynamics secondary to DE in the pre-existing situation of pulmonary hypertension. Indeed, both DE and hypoxemic-related pulmonary vasoconstriction share some common mechanisms and sites of actions on small resistive arteries (6, 49). Thus, similar end pathways of DE and hypoxia may explain why our hypoxic pulmonary vasoconstrictive stimulus masked the effects of DE. Nevertheless, the mPpa/Q relationship and vessel distensibility changes we observed in our healthy subjects did also not affect RV systolic function. However, in heart failure patients, an acute vasoconstrictive pulmonary response to DE exposure might lead to a worsen RV function and alter LV filling. Large epidemiological studies are needed to further confirm if DE-related changes in pulmonary hemodynamics can exacerbate heart failure.

The vasomotor tone of pulmonary vessels is under complex regulatory control. The pulmonary endothelium releases vasoactive agents, such as ET-1, NO and prostacyclin (16). However, the hemodynamic effects of DE observed in our study seem to be independent of the ET-1 pathway. Compared to another study, which demonstrated an increase in ET-1 in a chronic DE exposure model, the fact that our blood samples were collected immediately after acute exposure may explain the lack of effect observed (7). Decreased NO bioavailability is a major player in the toxicity of DE, responsible for part of the pollution-induced systemic vascular dysfunction (20, 39, 52). Contrary to animal studies, DE did not alter FeNO in our subjects. This suggests that the pulmonary endothelial production of NO did not change (11, 29). However, FeNO is not specific and despite the very short timeline between exposure and measurements, NO produced in the DE or an acute lung inflammatory reaction might have confounded FeNO measurements (34). Finally, DE particles, similar to other airborne pollutants, stimulate afferent sensitive C-fibers, which modulate sympathetic drive towards the pulmonary circulation (1, 25, 54). Further studies are required to confirm the involvement
of an adrenergic vasoconstrictive reflex mechanism in the pulmonary hemodynamic effects of DE exposure.

Study limitations

The diesel fuel and engine setting used in our protocol are similar to those previously reported and correspond to a classic motor vehicle. Inference to other types of fuel, such as biodiesel, could not be made because the type of fuel may influence the health effect associated with exposure (19). We used two modalities for the cardiac stress test in our study. First, dobutamine in normoxia to guarantee the best image acquisition and the best measurement accuracy and, second, exercise in hypoxia, which is the best validated and most widely described modality in the literature (13, 15, 35). Reproducibility between these two techniques of cardiac stress testing in the pulmonary circulation evaluation has been demonstrated previously (24). Although not significant, we observed a difference in baseline PVR in our two populations, which may be related to differences in the experimental setting. Experimental conditions were standardized inside each protocol. However, the presence of an airtight respiratory mask and a supine ergometer in the exercise protocol explain why these results were not compared to the dobutamine protocol.

Our study was designed to test the effects of DE exposure on PVR. However, the power of the study may not be sufficient to provide definitive conclusions regarding the effects of DE on physiological (HR, BP), biological or other echocardiographic parameters. As recently reported, some genetic patterns may influence vascular reactions to DE and explain the variability in the BP response to DE observed among studies (50). Further research is needed to specifically address the mechanistic hypotheses behind the lack of changes in ET-1 and FeNO reported in our study.
Conclusions

We observed, for the first time, an acute increase in pulmonary vasomotor tone following a 2-hour exposure to dilute DE in healthy young male subjects. Impairment in pulmonary vessel distensibility at high cardiac output appears to be the main mechanism involved. Further studies are needed to establish the role of vascular oxidative stress, inflammation and the sympathetic system in DE-related pulmonary vascular dysfunction.

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Disclosure

There is full disclosure of potential conflict of interests.


Figure 1: Study design
R: randomization; AA: ambient air; DE: diesel exhaust; BSL: baseline echographic measures.

Figure 2: Illustrative example of methodology of pulmonary vascular resistance (PVR) measurement and effect of polluted air exposure
Panel A: Echocardiographic measurements of the maximal velocity of tricuspid regurgitation (Vmax TR) and the velocity-time integral in the left ventricular outflow tract (VTI LV outflow) during dobutamine stress echocardiography.
Panel B: PVR calculation based on the linear regression slope of the mPpa/Q relationship during exposure to ambient air (blue line) and polluted air (red line).

Figure 3: Effect of air pollution on pulmonary vascular resistance (PVR)
Panel A: Mean linear regression of the individual Q-mPpa relationships for normalized Q during a cardiac dobutamine stress test after ambient (blue) or polluted (red) air exposure (n=10). Exposure effect p<0.01.
Panel B: Mean linear regression of the individual Q-mPpa relationships for normalized Q during the hypoxemic pulmonary exercise stress test. Normoxia (green), hypoxia after ambient (blue) or polluted (red) air exposure (n=8). NS: not significant.
<table>
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<tr>
<th>Exposure data</th>
<th>Ambient Air</th>
<th>Diesel exhaust</th>
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<td><strong>Pollution parameters</strong></td>
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<td>PM10&lt;sup&gt;a&lt;/sup&gt; (µg/m³)</td>
<td>26±1</td>
<td>311±1</td>
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<td>PM2.5&lt;sup&gt;b&lt;/sup&gt; (µg/m³)</td>
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<td>PM1&lt;sup&gt;c&lt;/sup&gt; (µg/m³)</td>
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<td>SpO₂&lt;sup&gt;h&lt;/sup&gt; (%)</td>
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<td>VE&lt;sup&gt;i&lt;/sup&gt; (l/min)</td>
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<td>FeNO&lt;sup&gt;j&lt;/sup&gt; (ppb)</td>
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<td>ET-1&lt;sup&gt;k&lt;/sup&gt; (pg/ml)</td>
<td>11.7±0.8</td>
<td>12.6±0.8</td>
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<sup>a</sup> PM10: particulate matter < 10 µm
<sup>b</sup> PM2.5: particulate matter < 2.5 µm
<sup>c</sup> PM1: particulate matter < 1 µm
<sup>d</sup> sBP: systolic blood pressure
<sup>e</sup> NS: not significant
<sup>f</sup> dBP: diastolic blood pressure
<sup>g</sup> HR: heart rate
<sup>h</sup> SpO₂: oxygen saturation
<sup>i</sup> VE: minute ventilation
<sup>j</sup> FeNO: fractional exhaled nitric oxide
<sup>k</sup> ET-1: endothelin-1
### TABLE 2

**Cardiac stress tests measurements**

<table>
<thead>
<tr>
<th></th>
<th>Dobutamine stress test (n=10)</th>
<th>Hypoxic exercise stress test (n=8)</th>
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<td></td>
<td>AA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>DE&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>BSL&lt;sup&gt;c&lt;/sup&gt; 8 μg/kg/min</td>
<td>BSL 8 μg/kg/min</td>
</tr>
<tr>
<td><strong>Echocardiographic data</strong></td>
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</tr>
<tr>
<td>mPpa (mmHg)</td>
<td>13.7±0.4</td>
<td>24.3±1</td>
</tr>
<tr>
<td>Q&lt;sup&gt;d&lt;/sup&gt; (l/min)</td>
<td>4.9±0.3</td>
<td>8.9±0.6</td>
</tr>
<tr>
<td>α (%/mmHg)</td>
<td>0.96±0.15</td>
<td>0.64±0.12</td>
</tr>
<tr>
<td>Mitral E/A</td>
<td>1.8±0.1</td>
<td>2.5±0.8</td>
</tr>
<tr>
<td>Lateral mitral E/E'</td>
<td>4.3±0.3</td>
<td>5.4±0.6</td>
</tr>
<tr>
<td>Tr S wave&lt;sup&gt;e&lt;/sup&gt; (cm/s)</td>
<td>14.3±0.8</td>
<td>25.1±1.6</td>
</tr>
<tr>
<td>TAPSE&lt;sup&gt;f&lt;/sup&gt; (mm)</td>
<td>25.7±0.8</td>
<td>35.1±1.7</td>
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<tr>
<td><strong>Physiological parameters</strong></td>
<td></td>
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<tr>
<td>sBP&lt;sup&gt;g&lt;/sup&gt; (mmHg)</td>
<td>118±2</td>
<td>161±6</td>
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<tr>
<td>dBP&lt;sup&gt;h&lt;/sup&gt; (mmHg)</td>
<td>67±2</td>
<td>73±2</td>
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<tr>
<td>HR&lt;sup&gt;i&lt;/sup&gt; (/min)</td>
<td>63±1</td>
<td>80±1</td>
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<tr>
<td>SpO₂&lt;sup&gt;j&lt;/sup&gt; (%)</td>
<td>98±1</td>
<td>98±1</td>
</tr>
</tbody>
</table>

<sup>a</sup> AA: ambient air, <sup>b</sup> DE: diesel exhaust, <sup>c</sup> BSL: baseline measurements, <sup>d</sup> Q: cardiac output, <sup>e</sup> Tr S wave: tricuspid S wave, <sup>f</sup> TAPSE: tricuspid annular plane systolic excursion, <sup>g</sup> sBP: systolic blood pressure, <sup>h</sup> dBP: diastolic blood pressure, <sup>i</sup> HR: heart rate, <sup>j</sup> SpO₂: oxygen saturation, * p<0.01
<table>
<thead>
<tr>
<th></th>
<th>AMBIENT AIR</th>
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<th>POLLUTED AIR</th>
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<tbody>
<tr>
<td><strong>Vmax TR</strong></td>
<td></td>
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<tr>
<td><strong>VTI LV outflow</strong></td>
<td></td>
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<tr>
<td><strong>HR (/min)</strong></td>
<td>61</td>
<td>62</td>
<td>75</td>
<td>61</td>
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<tr>
<td><strong>PVR (mmHg.min/l)</strong></td>
<td>3.17</td>
<td>4.38</td>
<td>3.5</td>
<td>3.8</td>
<td>3.27</td>
<td>4.06</td>
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<tr>
<td><strong>Vmax TR</strong></td>
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<tr>
<td><strong>VTI LV outflow</strong></td>
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</tr>
<tr>
<td><strong>HR (/min)</strong></td>
<td>61</td>
<td>62</td>
<td>55</td>
<td>63</td>
<td>61</td>
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<tr>
<td><strong>PVR (mmHg.min/l)</strong></td>
<td>3.27</td>
<td>4.06</td>
<td>5.13</td>
<td>4.71</td>
<td>3.3</td>
<td>7.2</td>
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<tr>
<td><strong>Dobutamine</strong></td>
<td><strong>/kg/min</strong></td>
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<td>0</td>
<td>4</td>
<td>6</td>
<td>8</td>
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</tbody>
</table>

**B**

![Graph showing the relationship between cardiac output (l/min) and mPpa (mmHg).](image)

Slope AA: 3.3 mmHg.min/l
Slope AP: 7.2 mmHg.min/l