Exposure to combustion-derived fine particulate air pollution is a recognized risk factor for cardiorespiratory mortality and morbidity. There is a strong relationship between acute exposure to traffic-derived particulate matter and the incidence of acute myocardial infarction and hospital readmission in survivors of myocardial infarction. The World Health Organization estimates that annually ∼3 million deaths worldwide can be attributable to air pollution.

Recent controlled exposure studies have demonstrated that inhalation of concentrated ambient particles and ozone causes acute arterial vasoconstriction 2 hours after the exposure. Inhalation of diesel exhaust, a major component of fine particulate air pollution in urban environments, impairs vasomotor function and endogenous fibrinolysis. The fundamental mechanisms underlying these detrimental vascular endothelial effects remain poorly understood. Furthermore, the exact components of air pollution responsible for these effects have not been defined, although it is proposed that airborne particulate matter is likely to be the major arbiter.

Endothelin (ET) 1, an endogenous vasoconstrictor 100-fold more potent than norepinephrine, is a 21-amino acid peptide produced by the vascular endothelium in response to stress. It is produced initially as preproendothelin-1, which is processed to form big-ET-1, before being cleaved by the endothelin-converting enzyme into ET-1. The actions of ET-1 are mediated by 2 G protein–coupled receptors, the ETA and ETB receptors. Stimulation of either the ETA or ETB receptor causes vasoconstriction, although the ETB receptor is also involved in the maintenance of basal vascular tone and blood pressure in humans.

Recent work has suggested that plasma ET-1 concentrations are increased by exposure to air pollution. Rats raised with daily exposure to diesel exhaust particles and urban particulate matter have increased blood pressure, plasma ET-1 concentrations, and ET-1 expression in cardiac tissue. In children from Mexico City, Mexico, plasma ET-1 concentrations correlated with the degree of air pollution exposure. Peretz et al recently demonstrated elevated
plasma ET-1 concentrations in a heterogeneous population of healthy volunteers and patients with the metabolic syndrome 3 hours after a controlled 2-hour resting exposure to diesel exhaust.

The aims of this study were to assess the effect of diesel exhaust inhalation on plasma ET-1 and big-ET-1 concentrations, ET-1–mediated vasoconstriction, and the contribution of ET-1 to basal vascular tone.

Methods

Subjects

Fifteen healthy male volunteers were recruited between February and March 2008 at Umeå University Hospital. All of the subjects had normal lung function, were nonsmokers, and took no regular medication. Those with a significant occupational exposure to air pollution and those with an intercurrent illness were excluded. The trial was performed with the approval of the local research ethics committee, in accordance with the Declaration of Helsinki, and with the written informed consent of each participant.

Study Design

Subjects attended for 2 consecutive days on 4 occasions ≥1 week apart. In a double-blind, randomized crossover study, subjects were exposed to either filtered air or dilute diesel exhaust for 1 hour in a specially built diesel exposure chamber, as described previously. During the exposure, subjects performed 15-minute periods of exercise on a bicycle ergometer (minute ventilation: 25 L/min per meter squared) alternated with 15-minute periods of rest. Temperature and humidity in the chamber were controlled at 22°C and 50%, respectively. On the basis of previous studies,7 vascular assessments and intra-arterial infusions were commenced 2 hours after the exposure. All of the subjects abstained from alcohol for 24 hours and remained indoors at rest between the exposure and the vascular study. All of the subjects underwent brachial artery cannulation in the nondominant arm using a 27-gauge steel needle under controlled conditions. After a 30-minute baseline infusion of 0.9% saline at 1.4 ml/min and 60 minutes, respectively.

Vascular Studies

All of the subjects underwent brachial artery cannulation in the nondominant arm using a 27-gauge steel needle under controlled conditions. After a 30-minute baseline infusion of 0.9% saline, subjects received either a 60-minute infusion of ET-1 (American Peptide) at 5 pmol/min17 or infusion of BQ-123 (an ET₁ receptor antagonist, American Peptide) at 10 nmol/min for 60 minutes, followed by coinfusion of BQ-123 (10 nmol/min) and BQ-788 (an ET₆ receptor antagonist, American Peptide; 1 nmol/min)19,20 for a further 60 minutes.

Forearm blood flow was measured in the infused and noninfused arms by venous occlusion plethysmography with mercury-in-silicone elastomer strain gauges, as described previously.21 Supine heart rate and blood pressure were determined in the noninfused arm at intervals throughout the study using the ambulatory blood pressure monitor.

Venous cannulae (17-gauge) were inserted into large subcutaneous veins in the antecubital fossae of both arms. Blood (10 mL) was drawn simultaneously from each arm at the end of the baseline saline infusion and at the end of each 60-minute infusion period. Blood samples were also obtained by separate venipuncture at baseline, immediately, and 6 and 24 hours after the exposure.

Biochemical Analyses

Blood samples taken at baseline and 6 and 24 hours after the exposure were analyzed for total and differential cell counts by an autoanalyzer. Plasma samples were collected into ethylene diamine tetra-acetic acid and kept on ice until centrifuged at 3000 rpm for 30 minutes. Plasma samples were immediately frozen and stored at −80°C. Plasma ET-1 and big-ET-1 concentrations were measured according to an acetic acid extraction technique using a commercial radioimmunoassay with rabbit antihuman ET-1 or big-ET-1 (Peninsula Laboratories Europe Ltd), as described previously.

Data and Statistical Analysis

Nursing staff at the clinical research facility at the Umeå University Hospital randomized exposure type and the associated vascular study protocol. The investigators were blinded to the exposure received. Plethysmography data were analyzed as described previously.23 Data are expressed as mean±SEM unless otherwise stated. Statistical analyses were performed using paired Student t tests and 2-way ANOVA with repeated measures, including time and exposure as variables, where appropriate. Statistical significance was taken at the 5% level. All of the analyses were performed using GraphPad Prism (version 4 for Macintosh, GraphPad Software) on a Macintosh personal computer.

Results

Thirteen subjects, with a median age of 23 years (Table), completed the study (Figure 1). The diesel exposure generated a mean particle mass concentration of 331±13 µg/m³, and this was associated with concentrations of NO₂ of 1.0±0.04 ppm, NO of 3.3±0.13 ppm, and total hydrocarbons of 1.4±0.04 ppm. There were no differences in 24-hour mean systolic or diastolic blood pressures or mean heart rate after exposure to air or diesel exhaust (P>0.05 for all), although there was a slightly lower nocturnal diastolic blood pressure after diesel exhaust exposure (62±1 versus 65±1 mm Hg; P<0.01). There was a rise in blood pressure after exercise on both study visits, although there was no difference between the 2 exposures (data not shown; P>0.05).

Table. Baseline Characteristics of the 13 Subjects Who Completed the Study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Data (N=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range), y</td>
<td>23 (21 to 28)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>181±2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>79±3</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24±1</td>
</tr>
<tr>
<td>Hemoglobin concentration, g/L</td>
<td>153±3</td>
</tr>
<tr>
<td>White blood cell count, ×10⁹/L</td>
<td>5.1±0.4</td>
</tr>
<tr>
<td>Neutrophil count, ×10⁹/L</td>
<td>2.5±0.3</td>
</tr>
<tr>
<td>Lymphocyte count, ×10⁹/L</td>
<td>2.0±0.1</td>
</tr>
<tr>
<td>Monocyte count, ×10⁹/L</td>
<td>0.40±0.03</td>
</tr>
<tr>
<td>Platelet count, ×10⁹/L</td>
<td>209±11</td>
</tr>
</tbody>
</table>

Data show the mean±SEM unless otherwise stated.
Infusion of ET-1 caused a slow-onset vasoconstriction after diesel exhaust inhalation (17±10% peak reduction in blood flow), although there was little effect after filtered air (Figure 2; ANOVA, \( P<0.001 \) for exposure effect). Infusion of the ET receptor antagonists, BQ-123 and BQ-788, caused a slow-onset vasodilatation (77±14% peak increase in blood flow after filtered air; Figure 3). This vasodilatation was greater after filtered air compared with the diesel exhaust exposure (Figure 3; ANOVA, \( P<0.001 \)). The difference was greatest at 60 minutes, but, after infusion of the BQ-788, there was little difference in blood flow by 120 minutes (\( P>0.05 \)).

Plasma ET-1 and big-ET-1 concentrations were unchanged at all of the time points after diesel exhaust or filtered air exposure (\( P>0.05 \) for both). Comparison of the infused and noninfused arm plasma ET-1 concentrations confirmed that the ET-1 infusion increased local plasma ET-1 concentrations by 58±9% (Figure 2; \( P<0.01 \) for infused arms and \( P>0.05 \) for noninfused arms for both exposures).

**Discussion**

Although diesel exhaust inhalation had no effect on plasma ET-1 or big-ET-1 concentrations, there was an increase in vascular sensitivity to ET-1 associated with a reduced \( \text{ET}_A \) induced vasodilatation. These apparently contradictory findings can be explained by impaired ET-1–induced NO release and are consistent with preclinical evidence of NO-mediated alterations in vascular reactivity to ET-1.24 We conclude that diesel exhaust inhalation, at levels commonly encountered in the urban environment, does not affect plasma ET-1 concentrations but alters vascular reactivity to ET-1 probably through effects on NO release and bioavailability.

**Endothelin 1**

We did not demonstrate any change in plasma concentrations of ET-1 or its immediate precursor, big-ET-1, after exposure to filtered air or diesel exhaust. Although this is consistent with our own previous work,7 it is at odds with other reports.

![Figures](image1.png)

**Figure 1.** Consort flowchart of study participants.

![Figures](image2.png)

**Figure 2.** A, Forearm blood flow (FBF) during infusion of ET-1 (5 pmol/min) after exposure to air (\( \square \)) and diesel exhaust (\( \bullet \); ANOVA, \( P<0.001 \)). B, Maximal effect at 60 minutes and (C) comparison of plasma ET-1 concentrations in infused and noninfused arms before and at the end of the forearm vascular study after air (\( \square \); \( P<0.001 \) for infused and \( P>0.05 \) for noninfused) and diesel exhaust inhalation (\( \bullet \); \( P<0.0001 \) for infused and \( P>0.05 \) for noninfused). Data are from a paired Student t test of air vs diesel.
In rodent studies, plasma ET-1 (and ET-3) concentrations were upregulated after exposure to diesel exhaust and concentrated urban particles.\textsuperscript{12,13} It is possible that there are species differences in the response to diesel exhaust inhalation, and upregulation of ET-1 in rats may not translate into humans. However, Peretz et al\textsuperscript{15} studied a heterogeneous group of individuals composed of patients with metabolic syndrome and healthy volunteers and showed an increase in plasma ET-1 concentrations 3 hours after diesel exhaust exposure. Their study was not designed specifically to look at ET-1 and was limited by missing data and small numbers in a heterogeneous population in which vascular endothelial function may not be equivalent.\textsuperscript{25} In contrast, our study was specifically designed to address the ET hypothesis and used a robust crossover study design, in a homogenous group of healthy volunteers, with samples optimally collected to assess plasma ET-1 and big-ET-1 concentrations.\textsuperscript{22} Taken together with our previous study, our experience represents the largest sample size to date (n=11005). Therefore, we think it is unlikely that diesel exhaust inhalation causes major changes in plasma ET concentrations.

We recognize that plasma ET-1 concentrations may not reflect the activity of the ET system because 90% of ET-1 synthesized by the vascular endothelium is secreted abluminally and acts locally on vascular smooth muscle in a paracrine manner.\textsuperscript{8} Therefore, in addition to measuring plasma ET-1 concentrations, we assessed the effects of ET agonism and antagonism on peripheral vascular tone.

**ET Agonism**

We demonstrated increased vasoconstriction after exposure to diesel exhaust, but little effect after exposure to filtered air, suggesting an increased vascular sensitivity to ET-1. We were surprised to see little vasoconstriction with ET-1 after exposure to filtered air, having previously reported \textasciitilde50% to 40% reductions in forearm blood flow during infusions of 5 pmol/min.\textsuperscript{10,26} In the present study, we used an alternative preparation of ET-1 and suggest that the disparity in vascular effects is attributable to differing potencies of the preparations. Because of this, we measured plasma ET-1 concentrations in both forearms and demonstrated a selective 60% increase in plasma ET-1 concentrations in the infused arm. Assuming a forearm blood flow of 25 mL/min, we achieved an end-organ concentration approximately one tenth of that anticipated. However, this simple calculation does assume that there is no clearance or extraction of ET-1 across the forearm, but we do not believe that the modest increase in venous ET-1 concentrations can be solely accounted for by clearance, and conclude that it reflects a reduced activity of the infused peptide preparation. Although the reduced activity of ET-1 limits comparisons with other studies, this was perhaps fortuitous, because it enabled us to assess the vasoreactivity to ET-1 at the threshold for vasoconstriction and to observe an alteration in ET-1 sensitivity.

**ETA Receptor Antagonism**

The reduction in vasodilatation to BQ-123 infusion after diesel exhaust inhalation has several potential explanations. First, this may relate to reduced production or increased clearance of active ET-1 from the vasculature. However, this seems unlikely given that plasma ET-1 and big-ET-1 concentrations were unchanged, although we acknowledge that an effect on the abluminal release of ET-1 cannot be excluded. Second, a reduced sensitivity of the vascular smooth muscle ETA receptor could have occurred, but this is at odds with the increased ET-1 vasoconstriction and is, therefore, unlikely. We believe that there is a third, more likely, explanation.

Looking closely at ETA receptor antagonism, it is clear that the mechanism of vasodilatation is complex. This reflects the distribution and basal activity of both the ETA and ETB receptors. Both receptors contribute to the maintenance of basal vascular tone but have differing actions and vascular distributions: ETA receptors are present on vascular smooth muscle cells only and mediate vasoconstriction, whereas ETB receptors are present on both vascular smooth muscle and endothelial cells, where they mediate vasoconstriction and vasodilatation, respectively. Moreover, selective ETA receptor antagonism leads not only to inhibition of the ETA
receptor but potentially to hyperstimulation of the ET\textsubscript{B} receptor. Indeed, we have demonstrated that BQ-123-induced vasodilatation can be markedly attenuated by concomitant blockade of NO release,\textsuperscript{18} suggesting that selective ET\textsubscript{A} receptor antagonism does indeed lead to significant ET\textsubscript{B} receptor–mediated vasodilatation through endothelial NO release. Given the central role of NO in modulating and balancing the effects of the ET system, we propose that changes in the L-arginine-NO pathway offer the most plausible hypothesis for the impaired vasodilatation to BQ-123. This would also explain the enhanced vasoconstriction to ET-1 with a reduction in the opposing vasodilatory actions of NO. Moreover, we have shown previously that diesel exhaust impairs NO bioavailability\textsuperscript{7,27} and suggest that the observed effects on ET-1 vasoreactivity can be explained by the reduction in ET-induced NO release and bioavailability.

The finding of reduced vasodilatation in response to ET\textsubscript{A} receptor blockade is at odds with previous studies demonstrating enhanced response in patients with conditions such as hypertension and hypercholesterolemia who have preexisting endothelial dysfunction mediated by reduced NO bioavailability.\textsuperscript{28,29} The reason for this discrepancy is unclear, but here we have induced an acute and brief episode of endothelial dysfunction in an otherwise healthy population of volunteers. Chronic dysfunctional states are likely to invoke compensatory mechanisms that may result in important differences in these vascular responses.

**Combined ET\textsubscript{A} and ET\textsubscript{B} Receptor Antagonism**

Previous data, including our own work,\textsuperscript{18} would suggest that combined ET\textsubscript{A}/ET\textsubscript{B} receptor antagonism should produce less vasodilatation than selective ET\textsubscript{A} receptor antagonism. We were, therefore, surprised to observe the continued further modest vasodilatation when ET\textsubscript{B} receptor antagonism was superimposed on ET\textsubscript{A} receptor antagonism. We believe that the explanation for this observation is 3-fold. First, vasodilatation to ET agonism and antagonism is of slow onset and offset. In our own hands, BQ-123–induced vasodilatation appears to reach a peak effect by 60 minutes\textsuperscript{18} but may take up to 90 minutes.\textsuperscript{15} The continued vasodilatation may, therefore, reflect further and more complete ET\textsubscript{A} receptor antagonism. Second, we chose this study design to minimize the number of visits given the invasive nature of the studies. We attempted to assess both ET\textsubscript{A} receptor antagonism and combined ET\textsubscript{A}/ET\textsubscript{B} receptor antagonism on the same visit. This approach has been used once before by Cardillo et al\textsuperscript{30} in a small subgroup of patients with diabetes mellitus. Here, they demonstrated a brisk vasodilatation to BQ-123 of \textasciitilde 65\% with maximal vasodilatation by 60 minutes. Importantly, the predicted “tailing off” of the response when BQ-788 was added did not occur, and the vasodilatation plateaued rather than fell. This is consistent with the findings in our study. Finally, there may be an interaction when ET\textsubscript{A} receptor antagonism precedes combined ET\textsubscript{A}/ET\textsubscript{B} receptor antagonism. This may reflect alterations in ET\textsubscript{B} receptor expression on both the endothelium and vascular smooth muscle cells in the face of ET\textsubscript{A} receptor antagonism. Indeed, there is considerable cross-talk between the receptors, as we have described previously.\textsuperscript{31} Thus, the differing profile of responses may reflect the dynamic interaction of the 2 receptors over the course of the study.

This altered profile of vasodilatation does not detract from the comparison between the filtered air and diesel exhaust exposure. Combined ET\textsubscript{A} and ET\textsubscript{B} receptor antagonism appears to be unaffected by diesel exhaust exposure, whereas selective ET\textsubscript{A} receptor antagonism is impaired. This is likely to reflect the greater and marked dependence of ET\textsubscript{A} receptor antagonism on NO release in comparison with combined ET\textsubscript{A} and ET\textsubscript{B} receptor antagonism.

**Conclusions**

Our data demonstrate that the previously documented impairment of endothelium-dependent vasodilatation after a 1-hour exposure to combustion-derived air pollutants is not mediated by an upregulation of the ET system. Furthermore, we have shown that diesel exhaust inhalation has no effect on plasma ET-1 concentrations or systemic blood pressure. Our data are consistent with the hypothesis that the diesel exhaust–induced vascular effects are predominantly driven by reduced endothelial NO bioavailability. However, we cannot exclude a role for other vasoactive mediators, such as endothelium-derived hyperpolarizing factor, and further studies are warranted to investigate the L-arginine:NO and other pathways in more detail.

**Perspectives**

Air pollution exposure is associated with increased cardiovascular morbidity and mortality and is thought to lead to \textasciitilde 3 million deaths worldwide each year. Understanding the underlying mechanism for these detrimental effects is crucial in trying to reduce this significant disease burden. In this study, we show that the well-established adverse vascular endothelial effects demonstrated after inhalation of diesel exhaust are not directly mediated through the ET system. We propose instead that these may be driven by changes in NO bioavailability. Additional studies are warranted to investigate this hypothesis in more detail.

**Acknowledgments**

We thank Annika Johansson, Frida Holmström, Margot Johansson, Jamshid Pourazar, Ann-Britt Lundström, Ester Roos-Engstrand, Maj-Cari Ledin, and the Department of Respiratory Medicine and Allergy (Umeå). Thanks also to the staff at Svensk Maskinprovning (Umeå) and the Clinical Pharmacology Unit (Edinburgh) for their assistance with the studies.

**Sources of Funding**

This research was supported by a project grant from Chest, Heart, and Stroke Scotland (08/A116); the Swedish Heart Lung Foundation; the County Council of Västerbottens, Sweden; the Swedish National Air Pollution Programme; a British Heart Foundation Programme grant (RG/03/005); and the University of Umeå. J.P.L. is supported by a British Heart Foundation Clinical PhD Studentship (FS/07/048). A.B. is the holder of the Lars Werko¨ Distinguished Research Fellowship from the Swedish Heart Lung Foundation.

**Disclosures**

None.
References


Contribution of Endothelin 1 to the Vascular Effects of Diesel Exhaust Inhalation in Humans

Hypertension. 2009;54:910-915; originally published online August 17, 2009; doi: 10.1161/HYPERTENSIONAHA.109.135947

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/54/4/910

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/