Published in final edited form as:

Cardiovasc Toxicol. 2016 July ; 16(3): 260–275. doi:10.1007/s12012-015-9334-y.

Air Pollution-Induced Vascular Dysfunction: Potential Role of Endothelin-1 (ET-1) System

Jordan Finch¹ and Daniel J. Conklin²

¹ Department of Pharmacology and Toxicology, School of Medicine, University of Louisville, 580 S. Preston Street, Louisville, KY 40202, USA

² Division of Cardiovascular Medicine, Department of Medicine, Diabetes and Obesity Center, University of Louisville, 580 S. Preston Street, Delia Baxter Building, Rm 404E, Louisville, KY 40202, USA

Abstract

Exposure to air pollution negatively impacts cardiovascular health. Studies show that increased exposure to a number of airborne pollutants increases the risk for cardiovascular disease progression, myocardial events, and cardiovascular mortality. A hypothesized mechanism linking air pollution and cardiovascular disease is the development of systemic inflammation and endothelium dysfunction, the latter of which can result from an imbalance of vasoactive factors within the vasculature. Endothelin-1 (ET-1) is a potent peptide vasoconstrictor that plays a significant role in regulating vascular homeostasis. It has been reported that the production and function of ET-1 and its receptors are upregulated in a number of disease states associated with endothelium dysfunction including hypertension and atherosclerosis. This mini-review surveys epidemiological and experimental air pollution studies focused on ET-1 dysregulation as a plausible mechanism underlying the development of cardiovascular disease. Although alterations in ET-1 system components are observed in some studies, there remains a need for future research to clarify whether these specific changes are compensatory or causally related to vascular injury and dysfunction. Moreover, further research may test the efficacy of selective ET-1 pharmacological interventions (e.g., ET_A receptor inhibitors) to determine whether these treatments could impede the deleterious impact of air pollution exposure on cardiovascular health.

Keywords

Air pollution; Cardiovascular disease; Endothelin-1; Endothelium dysfunction; ET-1; Vascular dysfunction; Vasoconstriction

Introduction

Air pollution is a complex mixture of gases, particulates, and other molecules that permeate the atmosphere, making exposure largely unavoidable in most indoor and outdoor settings. Outdoor, or ambient, pollution can be produced by exhaust from numerous sources,

including motor vehicles, coal-fired power plants, industrial processes, agricultural dust, and smoke from biomass, structural, and wild fires. Incomplete fuel combustion from open fires (wood, coal, organic matter) and cooking stoves is the major source of indoor or household air pollution [1]. Sources of particulate matter (PM), such as cigarette smoke, can affect individuals in both indoor and outdoor locations. All sources of PM have the potential to impact human health, but while household pollution impacts individuals on a personal level, ambient pollution has the potential to influence global health. To address this issue, several countries have passed air quality laws designed to reduce air pollution through the regulation of a variety of specific air toxics. For example, in the USA, 187 air toxics, including acrolein, lead, and ozone, are regulated by the Clean Air Act passed in 1963 [2]. Numerous other policies have been introduced to decrease annual pollution levels, and new technologies have been introduced that work to remove contaminants from smoke and other aerosols before these reach the atmosphere [3]. These efforts led to a 49 % decrease in aggregate emissions between 1980 and 2006 [4] and to an average 72 % drop in emissions of the six most common pollutants: particles, ozone, lead, carbon monoxide, nitrogen dioxide, and sulfur dioxide [2].

Despite the concerted efforts, decreases in PM are not sufficiently effective enough to prevent pollution-induced impacts on human health. The World Health Organization (WHO) attributed 7 million premature fatalities in 2012 to exposure from ambient and indoor air pollution, which roughly equates to 1 in 8 global deaths [4]. WHO attributed the majority of these deaths to cardiovascular diseases (CVD), with 37 and 33 % due to stroke and ischemic heart disease, respectively. Minor causes of death included respiratory conditions (24 %) and lung cancer (6 %) [5]. In the USA, the Centers for Disease Control and Prevention attributes an annual 600,000 American deaths to heart disease, emphasizing the significant burden of heart disease on a national level [6]. Although air pollution has a low odds ratio for the increased risk of myocardial infarction (MI) (1.02; CI 1.01–1.02), the 100 % exposure rate significantly increases the percentage of MIs attributable to air pollution, with levels similar to physical exertion and alcohol [7]. Although epidemiological studies well support an association between air pollution and CVD, this research is insufficient to elucidate the mechanisms responsible, and thus, more research is required in order to ultimately improve pollution-related health outcomes. Based on the health and financial burden of CVD, it is necessary from medical, legislative, and economic standpoints to continue working toward decreased air pollution emissions in an effort to collectively reduce morbidity and mortality and to reveal the biological mechanism(s) by which air pollution causes and/or exacerbates CVD.

Air Pollution and Cardiovascular Disease

Over the past 20 years, a number of clinical and epidemiological studies have established the damaging effects of air pollution on human health. The majority of this damage is found in the pulmonary and cardiovascular systems, leading to an increased risk of heart disease or stroke. Many of these health effects are linked to different forms of air pollution, including ambient air pollution and diesel and gasoline engine exhausts (GEEs). Ambient air pollution is composed of a gaseous suspension of aerosolized particles that typically have an aerodynamic diameter <10 μm [8, 9]; this range is classified into specific particle sizes:

ultrafine ($<0.1 \mu\text{m}$), fine ($\text{PM}_{2.5}$, $0.1\text{--}2.5 \mu\text{m}$), and coarse (PM_{10} , $>2.5\text{--}10 \mu\text{m}$). There is strong evidence linking increased levels of $\text{PM}_{2.5}$ with increased cardiovascular effects [8, 10, 11]. A $10 \mu\text{g}/\text{m}^3$ increase in $\text{PM}_{2.5}$ levels is associated with an increased risk of heart failure hospitalization [12] and with long-term all-cause mortality [13–17].

Additional studies of specific types of air pollutants further strengthen the link between exposure and cardiovascular events. $\text{PM}_{2.5}$ exposure significantly increases left ventricular mass [18] and systolic [19–21] and mean ambulatory arterial [22] blood pressures. Changes in $\text{PM}_{2.5}$ are associated with the onset of MIs [23] and increased intima media thickness progression [24, 25], with accelerated carotid intima media thickness (CIMT) progression of up to $5.5 \mu\text{m}$ (per $10 \mu\text{g}/\text{m}^3$) per year [26]. Similarly, exposure to diesel exhaust (DE) and traffic-related pollution increases arterial stiffness [27] and systolic blood pressure [28] and is associated with both increased mortality hazard ratio and increased risk of dying after hospital discharge [29]. Collectively, these pathogenic changes (e.g., increased CIMT, increased blood pressure) suggest that an underlying vascular alteration or dysfunction is responsible, and thus, there is a need to explore mechanisms consistent with these vascular manifestations.

Brook et al. [11] propose three likely mechanisms by which air pollution may contribute to the development of CVD: (1) an imbalance in the autonomic nervous system; (2) translocation of particles, specifically ultrafine PM, into the circulation; and (3) the development of systemic oxidative stress and inflammation within the vasculature. These hypothetical pathways are generally accepted [10, 22, 27, 30–35]. A primary outcome of these mechanisms is the initiation of endothelium dysfunction, which appears central (perhaps as a result of or in combination with oxidative stress and inflammation) to further development of CVD. Evidence suggests a strong association between endothelium dysfunction and cardiovascular events, leading to high morbidity and mortality from CVD [36] as well as other disease states [37–42]. Endothelium dysfunction is characterized by an imbalance between the dilator and constrictor forces in the vasculature. For example, this is best exemplified by concurrent decreased formation and bioactivity of nitric oxide (NO, derived from endothelial nitric oxide synthase, eNOS) and increased levels or activity of the endothelium-derived vasoconstrictor peptide endothelin-1 (ET-1) [36]. Thus, the latter is the focus of this mini-review.

The Endothelin System

Endothelin-1 (ET-1)—Research in the early 1980s led to the chemical identification of the endothelium-derived relaxant factor NO that is recognized as one of the most important factors in regulating vascular smooth muscle tone [43, 44]. Soon after, it became obvious that the endothelium also produces a vasoconstrictor to counteract NO's effect and maintain vascular homeostasis. Yanagisawa et al. [45] isolated and sequenced the gene for a vasoconstrictive endothelin peptide; two more isoforms were later discovered, and the peptide sequenced by Yanagisawa was termed ET-1, with the subsequent peptides named endothelin-2 (ET-2) and endothelin-3 (ET-3) [46–51]. A fourth isoform has since been discovered, but evidence suggests that this peptide serves solely as an intestinal smooth muscle constrictor [49, 52, 53]. Of these four, ET-1 is the predominant isoform, and it exerts

the greatest vasoconstrictive effect [49, 51, 54]. ET-1 is a 21-amino acid peptide formed by the cleavage of pro-ET-1 (aka big ET-1) by the endothelial cell-specific endothelin-converting enzyme, ECE-1, into the active form [45, 49, 51, 54, 55] (Fig. 1). As expected, the endothelium is the most common source of ET-1 production, but the peptide may also be produced by vascular smooth muscle cells, cardiomyocytes, macrophages, leukocytes, and fibroblasts [47, 54, 56]. A number of physical and chemical factors upregulate the expression of the ET-1 gene: reactive oxygen species (ROS) [57], angiotensin II [48, 54, 56–58], cytokines [56, 58], tumor necrosis factor- α (TNF α) [54, 57], interleukins [54, 57], insulin [48, 54], norepinephrine [54], transforming growth factor- β (TGF β) [48, 54, 57–59], thrombin [48, 54, 56, 57, 60], and exposure to cold temperature [61]. Despite the number of factors that have the potential to increase ET-1 production, the physiological range of plasma ET-1 levels in a healthy individual is both relatively low (pg/ml) and narrow, emphasizing the tight homeostatic regulation of this potent peptide [47, 57, 58, 62].

ET-1 Receptors—Once secreted, ET-1 binds to one of its two G-protein-coupled receptor types to mediate its downstream effects [56, 63, 64]. The ET_A receptor, found on vascular smooth muscle cells and cardiomyocytes, mediates a contractile response, while the ET_B receptor, found on vascular endothelial, epithelial, endocrine, and nerve cells, generally causes the release of vasodilators and is also responsible for the rapid clearance of ET-1 from the vasculature [56, 63–66]. Binding to the ET_B receptor also stimulates the Akt pathway, leading to phosphorylation of eNOS and production of NO [56, 63]. As expected, these receptors are distributed in high levels throughout the vasculature, and although the ET_A receptor is found in higher levels in the heart, the ET_B receptor has a greater distribution in the lungs and kidneys [54, 62, 67, 68]. A subtype of the ET_B receptor, ET_{B2}, also causes vasoconstriction, but it is not found widely throughout the vasculature, and its activity is negligible in healthy individuals [54, 58, 69]. Upon binding to the ET_A receptor, ET-1 produces a vasoconstrictive response that is 100-times more potent than norepinephrine, thus making it the most potent vasoconstrictor known [51, 70]. ET-1-mediated vasoconstriction decreases forearm blood flow and causes a significant increase in blood pressure [71–75]; prolonged ET-1-induced constriction facilitates the development of hypertension [20, 76, 77] and promotes atherosclerosis [47, 48, 51, 54, 78–80]. ET-1, however, under normal conditions, maintains vascular tone [64, 81] in balance with NO. Moreover, ET-1 is necessary for proper skeletal development of the head and neck [82–84] and the formation of the vasculature in and around the heart [85], as well as control of embryonic heart rate [86].

Physiology and Pathology of the Endothelin System

Like all homeostatic mechanisms in the body, the interaction between ET-1 and NO must be in balance in order for the vascular system to function properly, and it is when these two factors become unbalanced that pathological states such as endothelium dysfunction can begin to develop. Although endothelium dysfunction is commonly assessed as a decrease in NO production, it can be caused by an increase in ET-1 production [49, 51, 78]. Nonetheless, it is important to consider that increased ET-1 activity could also be caused by decreased ET-1 clearance and/or by lower levels of NO production or stability [78]. Decreased NO in the vasculature allows a greater effect of ET-1 independent of any change

in ET-1 levels. Regardless of the cause, many studies, including a number of pharmacological trials, show ET-1-dependent activity initiates pleiotropic effects beyond only vasoconstriction. ET-1, working through a variety of pathways, generates ROS [87–89] such as superoxide [47, 78, 80, 88–90]; activates macrophages, leading to increased production of pro-inflammatory mediators [64, 78–80, 89, 91–93]; induces extracellular matrix formation [94]; and stimulates vascular smooth muscle cell proliferation [47, 58, 80, 87, 94, 95]. Furthermore, increased ET-1 inhibits eNOS and blocks the production of NO [78, 95]. These actions can disrupt the equilibrium within the vascular wall and initiate endothelium dysfunction [11, 31, 80, 89, 96–99] and, thereby, generally promote the development of CVD. Therefore, it is plausible and likely that altered ET-1 production and/or activity is one mechanism by which air pollution enhances the development of vascular dysfunction [39, 40, 75–78, 99].

The endothelin system is spread along 60,000 miles of vasculature in the body, so it is within reason that dysregulation of ET-1 could have effects in multiple organ systems and potentially augment the development of other diseases. As with CVD, the vasoconstrictive effect of ET-1 is the proposed major cause of these conditions. In the extremities, cold-temperature-induced increases in ET-1 contribute to the pathogenesis of Raynaud's phenomenon [37, 100], in which exaggerated vasoconstriction causes discomfort (ischemic pain) and discoloration of the affected areas. Likewise, ET-1-related vascular dysfunction in the eye leads to intraocular inflammation; this plays an important part in the development of primary open-angle glaucoma [41]. In the kidneys, ET-1 controls renal blood flow and the reabsorption of water and salt, as well as maintains acid–base balance [54, 57, 78, 101]. If any of these factors are disrupted, it can result in the development of hypertensive neuropathy, glomerulonephritis, or proteinuria [57, 79]. Within the pulmonary system, ET-1 is produced both by endothelial cells and by airway epithelial cells and cleared by the high levels of the ET_B receptor found in the pulmonary tissue [54, 62, 67, 68]. ET-1 influences the maintenance of airway tone, and so increased production or decreased clearance may lead to bronchial constriction [54, 102]. Chronic bronchial constriction can promote asthma and chronic obstructive pulmonary disease (COPD) [38, 103–105]. ET-1 also affects the arteries and veins in the lungs, and thus, elevated ET-1 can lead to the development of pulmonary hypertension, just as it can stimulate the development of systemic hypertension [97, 106, 107]. Thus, changes in ET-1 or its activity can be a link between exposure to air pollution and the development of a variety of pathogenic states through the induction of vasoconstriction and endothelium dysfunction as prevalently seen in CVD.

Air Pollution and Endothelin-1

Human Studies—Despite the wealth of evidence described above, few studies have examined the relationship between air pollution exposure and circulating levels of ET-1 in humans, and those studies that have been performed have produced conflicting results (Tables 1, 2). In epidemiological studies, Calderon-Garciduenas and colleagues have repeatedly examined the effects of elevated levels of air pollution on children in Mexico City, which has been shown to significantly exceed the WHO's annual mean PM_{2.5} standard [108, 109]. In a 2007 study, the group measured plasma ET-1 levels in healthy children ($n = 81$) from both Mexico City and Polotitlán, a control city. They observed that the cumulative

PM_{2.5} level of the previous 7-days was associated with higher plasma ET-1 levels in the children of Mexico City [108]. Similarly, there was a positive correlation between the number of hours a child spent outdoors and plasma ET-1 levels [108]. The authors suggest that the time spent outside increased each child's exposure to air pollution that, in turn, increased plasma ET-1. In a related study, clinically healthy children ($n = 28$) from Mexico City and Polotitlán were used to investigate the effect of PM_{2.5} on markers of systemic inflammation and endothelial cell injury. Again, Mexico City children were shown to have significantly higher levels of ET-1 and various inflammatory markers positively associated with elevated levels of PM_{2.5} exposure [110]. Controlled human exposures have been performed to substantiate the findings of these few epidemiological studies and to provide a better understanding as to whether air pollution alters ET-1. A small study exposed healthy adults ($n = 10$) to DE (100 µg/m³) and showed that DE significantly increases plasma ET-1 and matrix metalloproteinase-9 (MMP-9) compared with filtered air-exposed controls [111]. Dysregulation of MMP-9 contributes to pathological changes in the vasculature, and MMP-9 levels are increased via ET_A receptor activation [111].

Other studies, however, have found no change in the ET-1 system related to air pollution exposure or no alteration in the functional outcomes related to ET-1-induced vasoconstriction. Peretz et al. [112] exposed a mixed group of both healthy subjects and people with metabolic syndrome to 2 h of DE. They reported an average 0.11-mm greater vasoconstriction in the brachial artery in the DE exposure group regardless of health status. Likewise, plasma levels of ET-1 increased in both groups following DE exposure and to a greater degree within the healthy adults. The authors attributed this latter finding to the arterial stiffness associated with metabolic syndrome; the vessels in the diseased individuals are less responsive to the effects of DE due to decreased plasticity, whereas the vessels of the healthy individuals are capable of producing a normal vascular response and, thus, could mount a greater range of vasoconstriction. DE exposure, however, did not affect blood pressure or flow-mediated dilatation (FMD), the latter being a common indicator of endothelium function. Similarly, after a 2-h exposure to concentrated ambient air particulate matter (CAP), healthy non-smokers had no change in either FMD or in blood pressure, although they did have a significant reduction in brachial artery diameter [113]. Both Mills et al. [114] and Langrish et al. [115] exposed participants to higher levels of DE-derived PM_{2.5}, which was expected to have a greater effect on the endothelium, but neither investigation demonstrated any change in the plasma levels of ET-1 or big ET-1. The varied results of these human exposure studies indicate that the effect of air pollution exposure on the ET-1 system is difficult to measure and may differ based on factors such as pollution type, the length and concentration of exposure, and each measurement protocol.

Other research has focused on the targets of ET-1—the ET-1 receptors. For example, administration of the selective ET_A receptor antagonist, BQ-123, is used to explore the possibility of an increased secretion of ET-1 abluminally, which would not necessarily change ET-1 levels in the plasma [48, 115]. Exposure to DE decreased the amount of vasodilation seen as a result of the BQ-123 infusion, yet previous research indicated that BQ-123-induced vasodilation was in part due to endothelial cell-derived NO release mediated by the ET_B receptor [116]. Thus, Langrish et al. [115] propose that DE-elicited vasoconstriction results from decreased NO bioavailability and not increased ET-1. Because

NO and ET-1 must be delicately balanced in order to properly maintain the tone of the vasculature, a decrease in NO likely produces a similar effect as an increase in ET-1, so this remains a difficult relationship to disentangle. Further use of ET receptor blockers and observation of the effects of direct ET-1 administration should allow for elucidation of which of these two factors more significantly influences alterations within the vasculature.

Occupational Exposure—Occupational exposures often result in unique forms of personal air pollution exposure. A few studies have examined the cardiovascular effects of occupational traffic pollutant exposure [103, 117]. A large study of professional drivers ($n = 1922$) revealed that bus drivers who had more than 10 years' experience, theoretically with more engine exhaust exposure, had a twofold increased odds ratio of MI, even after adjustment for unfavorable lifestyle factors; taxi drivers also had an increased odds ratio, while there was no evidence of increased risk for truck drivers [117]. In another study, air quality monitors were used to measure personal and ambient exposure of North Carolina State Highway Patrol troopers during four successive 9-h shifts [103]. Although the report indicates lower in-vehicle PM_{2.5} levels compared with ambient PM_{2.5}, higher levels of other pollutants, including carbon monoxide, nitrogen dioxide, and hydrocarbons, were present in the vehicle. Furthermore, they showed that for every 10 µg/m³ increase in PM_{2.5}, the officers exhibited increased C-reactive protein (CRP), which serves as an indicator of inflammation, and increased next-morning heart rate variability (HRV). HRV, like ET-1, has been shown to be altered by increased PM_{2.5}, which indicates a relationship, either direct or indirect, between these two factors [19].

Workers in other occupations are also prone to high-level exposures of pollution. In a study of sugarcane harvesters, higher levels of PM_{2.5} are measured during the harvest season [118]. Sugarcane fields are cleared by burning, and workers are exposed to high amounts of smoke and pollutants during burning and subsequently in the harvesting of the burnt sugarcane. Both systolic and diastolic blood pressures are significantly increased in the workers during the harvest season compared with the non-harvest period [118]. Welding is another profession wherein workers are exposed to higher amounts of air pollution [119]. However, Jarvela et al. [120] determined that an 8-h dust and welding fume exposure (inhalable dust; 1.5–35 mg/m³) did not significantly change the workers' levels of plasma ET-1, yet exposure to welding fumes (alveolar particle fraction; 1.0–25.3 mg/m³) significantly decreased ET-1 [121]. The investigators hypothesized that this contradictory response could be due to the heterogeneous mix of the welding fumes, which included high levels of nitrogen oxides that could disrupt the homeostasis in the vasculature. Regardless, the study had insufficient evidence to accurately describe the mechanism of the response [121], and so further research is needed to determine the cause of this somewhat paradoxical result.

Environmental and Secondhand Tobacco Smoke—When examining how air pollution contributes to the development of chronic diseases, it is important to consider not just general air pollution, but also situations where individuals may be exposed to different forms or relatively higher levels of PM_{2.5} as well as other pollutants (e.g., acrolein). Cigarette smoke, for example, is strongly associated with cardiovascular and respiratory

diseases, including those linked to endothelium dysfunction [122], and again the involvement of ET-1 in the mechanistic pathway is a reasonable hypothesis. Working through the mitogen-activated protein kinase (MAPK) pathway, specifically the extracellular signal-regulated kinase (ERK) pathway, cigarette smoke upregulates the expression of mRNA for both the ET_A and ET_B receptors [123], as well as ET-1 in a variety of tissues [122, 124]. These effects are readily seen in smokers, but also in individuals who are exposed to environmental or secondhand smoke (SHS), also known as passive smoking. Environmental exposure to cigarette smoke at one-hundredth the level of active smoking increases an individual's risk of coronary artery disease by 30 % [125]. Likewise, both animal and human models have shown that passive smoking is associated with increased CIMT [125] and platelet activation [125, 126], as well as decreased vasodilatory function at levels comparable to those seen in active smokers [125]. Similarly, Cao et al. [123] demonstrate a direct effect of SHS exposure in rats on the ET-1 system as SHS activates the MAPK pathway and upregulates both ET_A and ET_B receptor mRNA and protein in coronary arteries. Additionally, Pinnamaneni et al. [127] show that SHS exposure as brief as 1 min causes a reversible impairment of endothelium-dependent vasodilatory function as measured by FMD in rats. Together, these studies show that the impact of SHS exposure on endothelium function and overall CVD health via dysregulation of the ET-1 system is just as relevant as exposure from active smoking and may in fact have a greater disproportional impact on CVD due to the unintentional exposure of the general public.

Animal Studies—Both environmental and controlled exposures in animals show a consistent trend between air pollution and alterations in ET-1 levels (Tables 3, 4). Mice exposed to GEE or DE have significant increases in plasma ET-1 [128], ET-1 mRNA expression in the aorta [104, 128], and ET-1-induced vasoconstriction [129]. Some studies also noted elevated plasma levels of MMPs [104, 111, 128], which, as noted, are upregulated via activation of the ET_A receptor [111]. Similarly, air pollution exposures in rats induced effects consistent with those observed in exposed mice. For example, exposure of rats to DE enhances both the arterial vasoconstriction response to ET-1 [130] and the expression of ET-1 and ET_A receptor mRNAs [131]. A combination exposure of DE particles and ozone also increases the expression of the ET_B receptor in the aorta [131]. Similarly, CAP exposure in rats upregulates the mRNA expression of the ET_A receptor in the heart [132], and inhalation of urban dust particles increases plasma ET-1 levels and decreases the production of pulmonary NO [133]. In rabbits, PM₁₀ exposure significantly increases plasma ET-1 levels [134]. Thus, in a number of different animal models, well-designed exposure experiments provide evidence that a variety of air pollutants (e.g., PM, PM + O₃, DE, GEE) are capable of increasing the level of plasma ET-1 and/or enhancing its receptors in the vasculature while decreasing NO status. These changes likely prime blood vessels for endothelium and vascular dysfunction.

ET-1, Air Pollution, and Diabetes: Is There a Connection?

A 2013 study stated that there are an estimated 381.8 million people with diabetes; that number is expected to climb another 55 % by 2035 [135]. Although this estimate includes both type 1 and type 2 diabetes, the more important form of the disease in regard to ET-1 is type 2 (T2D). T2D is caused by cellular insulin resistance (IR) [136, 137], which in turn

causes high levels of glucose to remain in the circulation, leading to hyperglycemia. A number of studies have shown that endothelium dysfunction often occurs in conjunction with IR; because dysregulated levels of ET-1 are known to contribute to endothelium dysfunction, many have suggested that ET-1 can thus cause IR [80, 136–140]. A study of genetically obese rats supports this hypothesis: basal plasma ET-1 levels are increased in the first 7 weeks during the progression of IR [138]. Alternatively, insulin itself can stimulate the production of ET-1 [54, 136, 137] via ERK signaling [80], which can then act to reduce the insulin sensitivity of target tissues and exacerbate IR [137]. Yet other studies find no significant increase in plasma ET-1 levels in diabetic or IR individuals [141, 142]. At least one of these studies, however, measured ET-1 in venous samples 2 h after inducing physiological hyperinsulinemia [141]. The authors concede that they could not rule out a transient increase in ET-1, and because the half-life of ET-1 is only approximately 1 min [47, 65, 66], it is possible that the blood collection protocol used was unlikely to detect rapid changes in plasma ET-1. Furthermore and importantly, both Dhaun et al. [57] and Langrish et al. [115] point out that most of the ET-1 is released abluminally with only small amounts making it into the plasma, and so plasma concentrations of ET-1 may not accurately reflect physiological alterations within the vascular wall. Also, there is an inability to measure endothelial cell ET-1 in vivo to verify whether or not more ET-1 is being made in the endothelium. Collectively, these factors could account for the general lack of significant findings that link plasma ET-1 levels with cardiovascular outcomes [113].

Recently, research has begun to look into possible associations between air and water pollution and chronic diseases such as T2D. Reviews of studies relating air pollution and T2D show support for an association between the two factors [143, 144], particularly air pollution from traffic exposure and human T2D [144]. Exposures to PM_{2.5} are associated with elevated fasting blood glucose and insulin in mice, leading to the development or exaggeration of whole-body IR [145, 146], and PM₁₀ exposure has been associated with elevated glycated hemoglobin (HbA_{1c}) levels in newly diagnosed diabetics [147]. These studies have shown that exposure to air pollution downregulates insulin receptor substrate 1 signaling and inhibits the Akt pathway, resulting in decreased Akt signaling while leaving the ERK pathway unaffected [144, 146]. Based on this evidence, it is logical to propose that, as with CVD, air pollution can lead to the development of T2D in part via increased signaling of the ERK pathway, leading to increased production of ET-1 and endothelium dysfunction. Thus, aside from CVD, diabetes mellitus would likely be the most prevalent condition resulting from dysregulation of the ET-1 system.

Endothelial Progenitor Cells (EPCs) and ET-1

It would furthermore be beneficial to examine the relationship between ET-1 and endothelial progenitor cells (EPCs). EPCs are stem cells produced by the bone marrow in response to vascular injury. Once activated, these cells differentiate into new endothelial cells to replace damaged cells in the vasculature [148]. Exposure to PM_{2.5} has been shown to deplete circulating levels of EPCs in humans [149] and mice [150], the latter study indicating a mechanism related to VEGF resistance; both studies suggest lower EPCs reduce vascular repair. Similar EPC and VEGF resistance effects were found in mice exposed acutely to acrolein [151], although ET-1 was not measured. Studies with tyrosine kinase inhibitors have

shown that inhibition of the VEGF receptor stimulates the ERK pathway, increasing the expression of prepro-ET-1 while also inhibiting the Akt pathway and decreasing NO production [152–155]. These results strengthen the hypothesis that VEGF resistance is a key component in the mechanism through which air pollution influences ET-1 and, downstream, EPCs. A small number of studies have looked at ET-1 and EPCs in relation to ischemic stroke [148], diabetes [156], and cancer [157], but none of these studies have been specifically designed to relate ET-1 and EPCs with air pollution. A study with this focus could shed light on a new role of ET-1, if any, in the release of EPCs from the bone marrow and how the two factors also may interact within the vasculature.

Concluding Remarks

Our understanding of the role of air pollution in the development of CVD has been furthered by examining the role of ET-1 in the vasculature. Epidemiological observations and controlled exposures in both humans and animals show an association between air pollution exposure and cardiovascular effects; many have identified dysregulation of ET-1 as a possible mechanism underlying this association, likely via inhibition of the Akt pathway and increased stimulation of the ERK pathway, thus leading to greater ET-1 production and decreased production of NO. There seems to be a more consistent relationship in animal studies, however, compared with human studies, although this is likely more reflective of our ability to control all aspects of the exposure and a more complete set of measurements made in animal studies. Additional human studies specifically designed to measure ET-1 levels in relation to functional vascular parameters (e.g., blood pressure, forearm blood flow, and brachial artery diameter) are needed to improve our understanding of the peptide's role in homeostatic maintenance after exposure to pollution. As ET-1 is more likely a paracrine signaling molecule, measuring local ET-1 levels would more accurately reflect the levels produced in response to exposures. For example, more invasive and direct evaluations of released ET-1 by using endothelial cell biopsy or abluminal wick measurements would allow for assessment of ET-1 levels in specific vascular locations.

Additional studies are also needed to determine what role pollution exposure plays in the levels of ET-1 in healthy versus diseased individuals. It is known that older or obese individuals or those with preexisting heart or respiratory conditions are more susceptible to the effects of air pollution [11]; it is therefore necessary to study the effects of air pollution in a number of different population groups. It would also be beneficial to examine ET-1 levels in more individuals with higher levels of occupational exposure. Firefighters in particular have high incidence of CVD and are exposed to high levels of pollutants on a frequent yet irregular basis, making them an ideal population in which to study the relationship between exposure and ET-1 [158]. Only one study has currently examined ET-1 levels in a population of firefighters, examining firefighters who participated in a computerized fire strategies and tactics drill, a program meant to simulate the physical and mental response to fighting an actual fire. This study showed no difference in plasma ET-1 compared with an exercise only group [159]. Levels did show a significant rise in the simulation group during a recovery period, which may indicate increased levels of ET-1 in response to mental stress. However, since neither group was exposed to true air pollution, and especially not at the levels to which they would be subjected during a response to an

actual fire, it is unclear what acute exposure to high levels of smoke may have on ET-1 levels. Furthermore, the study reports levels of ET-1 in ng/mL, which is three orders of magnitude higher than what is reported in other studies of ET-1 and air pollution exposure, making a true comparison difficult. Thus, more studies are needed to gain a clearer understanding of this relationship in individuals who are occupationally exposed to high levels of air pollution.

Further research could make more effective use of endothelin receptor blockers. A number of ET receptor blockers have been developed and tested [49, 76, 98, 106, 160, 161], but only a small number have been approved for medical use. Bosentan, one of the approved drugs, is a dual endothelin receptor (ET_A, ET_B) antagonist approved for the treatment of pulmonary arterial hypertension that has proven to be quite effective in blocking the effects of ET-1 in the lung vasculature [107, 162–164]. The development and approval of other endothelin receptor blockers may help reduce the number of new cases of hypertension, atherosclerosis, and IR by decreasing the influence of ET-1 in the vasculature and maintaining the balance between ET-1 and NO. Balancing these two factors pharmacologically could help improve overall cardiovascular health in spite of general or specific pollution exposures. Nonetheless, thorough investigation using explicitly designed experimental exposures and collection protocols should better define ET-1's role as a link between air pollution exposure and CVD. This information could then be used to combat the detrimental impact of air pollution on the cardiovascular health of the human public.

Acknowledgments

The authors thank the University of Louisville School of Medicine Integrated Programs in Biomedical Sciences for academic and financial support. We thank Natasha DeJarnett, PhD, MPH, for her editorial input. This work was supported by NIH Grants (ES019217, GM0103492, HL089083).

Abbreviations

Akt	Protein kinase B
Ca²⁺	Calcium
CAP	Concentrated ambient air particulate matter
CIMT	Carotid intima media thickness
cGMP	Cyclic guanosine monophosphate
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DE	Diesel exhaust
EC	Endothelial cell

ECE	Endothelin-converting enzyme
ECM	Extra cellular matrix
eNOS	Endothelial nitric oxide synthase
EPCs	Endothelial progenitor cells
ERK	Extracellular signal-regulated kinases
ET-1	Endothelin-1
ET_A	Endothelin receptor A
ET_B	Endothelin receptor B
ET_{B1}	Endothelin receptor B1
FBF	Forearm blood flow
FMD	Flow-mediated dilation
GEE	Gasoline engine exhaust
HbA_{1c}	Glycated hemoglobin
HO-1	Heme oxygenase 1
HRV	Heart rate variability
HTN	Hypertension
IL	Interleukin
InR	Insulin receptor
IR	Insulin resistance
MAPK	Mitogen-activated protein kinase
MI	Myocardial infarction
MMP	Matrix metalloproteinase
NMD	Endothelial-independent nitroglycerin-mediated dilatation
NO	Nitric oxide
O₃	Ozone
PGE2	Prostaglandin E2
PI3K	Phosphoinositide 3-kinase
PKC	Protein kinase C

PKG	Protein kinase G
PM	Particulate matter
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SGC	Soluble guanylyl cyclase
SHS	Secondhand smoke
SMC	Smooth muscle cell
T2D	Type 2 diabetes
TIMP-2	TIMP metalloproteinase inhibitor 2
TNFα	Tumor necrosis factor- α
VEGF	Vascular endothelial growth factor
VR	VEGF receptor
VSMC	Vascular smooth muscle cell
WHO	World Health Organization

References

1. World Health Organization. [June 17, 2014] Health topics: Air pollution. 2014. Available from: www.who.int
2. US Environmental Protection Agency. [June 17, 2014] Latest findings of national air quality: Status and trends through 2006. 2008. Available from: www.epa.gov
3. Rubin ES. Climate change, technology innovation, and the future of coal. *Cornerstone*. 2013; 1:37–43.
4. US Environmental Protection Agency. [June 17, 2014] Progress cleaning the air and improving people's health. 2014. Available from: www.epa.gov
5. World Health Organization. [June 17, 2014] 7 million premature deaths annually linked to air pollution. 2014. Available from: www.who.int
6. Centers for Disease Control and Prevention. [July 16, 2014] Heart disease facts. 2014. Available from: www.cdc.gov
7. Nawrot TS, Perez L, Künzli N, Munters E, Nemery B. Public health importance of triggers of myocardial infarction: A comparative risk assessment. *The Lancet*. 2011; 377:732–740.
8. Brook RD, Franklin B, Cascio W, Hong Y, Howard G, Lipsett M, et al. Air pollution and cardiovascular disease: A statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. *Circulation*. 2004; 109:2655–2671. [PubMed: 15173049]
9. Bhatnagar A. Environmental cardiology: Studying mechanistic links between pollution and heart disease. *Circulation Research*. 2006; 99:692–705. [PubMed: 17008598]
10. Pope CA, I. I. I. Dockery DW. Health effects of fine particulate air pollution: Lines that connect. *Journal of the Air & Waste Management Association*. 2006; 56:709–742. [PubMed: 16805397]
11. Brook RD, Rajagopalan S, Pope CA, I. I. I. Brook JR, Bhatnagar A, Diez-Roux AV, et al. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. *Circulation*. 2010; 121:2331–2378. [PubMed: 20458016]

12. Shah AS, Langrish JP, Nair H, McAllister DA, Hunter AL, Donaldson K, et al. Global association of air pollution and heart failure: A systematic review and meta-analysis. *Lancet*. 2013; 382:1039–1048. [PubMed: 23849322]
13. Dockery DW, Pope CA, Xu X, Spengler JD, Ware JH, Fay ME, et al. An association between air pollution and mortality in six US cities. *New England Journal of Medicine*. 1993; 329:1753–1759. [PubMed: 8179653]
14. Pope CA, I. I. I. Burnett RT, Thurston GD, Thun MJ, Calle EE, Krewski D, et al. Cardiovascular mortality and long-term exposure to particulate air pollution: Epidemio-logical evidence of general pathophysiological pathways of disease. *Circulation*. 2004; 109:71–77. [PubMed: 14676145]
15. Laden F, Schwartz J, Speizer FE, Dockery DW. Reduction in fine particulate air pollution and mortality: Extended follow-up of the Harvard Six Cities study. *American Journal of Respiratory and Critical Care Medicine*. 2006; 173:667–672. [PubMed: 16424447]
16. Puett RC, Hart JE, Yanosky JD, Paciorek C, Schwartz J, Suh H, et al. Chronic fine and coarse particulate exposure, mortality, and coronary heart disease in the Nurses' Health Study. *Environmental Health Perspectives*. 2009; 117:1697–1701. [PubMed: 20049120]
17. Lepeule J, Laden F, Dockery D, Schwartz J. Chronic exposure to fine particles and mortality: An extended follow-up of the Harvard Six Cities study from 1974 to 2009. *Environmental Health Perspectives*. 2012; 120:965–970. [PubMed: 22456598]
18. Van Hee VC, Adar SD, Szpiro AA, Barr RG, Blue-mke DA, Diez Roux AV, et al. Exposure to traffic and left ventricular mass and function: The Multi-Ethnic Study of Atherosclerosis. *American Journal of Respiratory and Critical Care Medicine*. 2009; 179:827–834. [PubMed: 19164703]
19. Ibal-d-Mulli A, Stieber J, Wichmann H, Koenig W, Peters A. Effects of air pollution on blood pressure: A population-based approach. *American Journal of Public Health*. 2001; 91:571–577. [PubMed: 11291368]
20. Auchincloss AH, Diez Roux AV, Dvornch JT, Brown PL, Barr RG, Daviglius ML, et al. Associations between recent exposure to ambient fine particulate matter and blood pressure in the Multi-ethnic Study of Atherosclerosis (MESA). *Environmental Health Perspectives*. 2008; 116:486–491. [PubMed: 18414631]
21. Dvornch JT, Kannan S, Schulz AJ, Keeler GJ, Mentz G, House J, et al. Acute effects of ambient particulate matter on blood pressure: Differential effects across urban communities. *Hypertension*. 2009; 53:853–859. [PubMed: 19273743]
22. Langrish JP, Li X, Wang S, Lee MMY, Barnes GD, Miller MR, et al. Reducing personal exposure to particulate air pollution improves cardiovascular health in patients with coronary heart disease. *Environmental Health Perspectives*. 2012; 120:367–372. [PubMed: 22389220]
23. Peters A, Dockery DW, Muller JE, Mittleman MA. Increased particulate air pollution and the triggering of myocardial infarction. *Circulation*. 2001; 103:2810–2815. [PubMed: 11401937]
24. Adar SD, Sheppard L, Vedal S, Polak JF, Sampson PD, Diez Roux AV, et al. Fine particulate air pollution and the progression of carotid intima-medial thickness: A prospective cohort study from the multi-ethnic study of atherosclerosis and air pollution. *PLoS Medicine*. 2013; 10:e1001430. [PubMed: 23637576]
25. Wilker EH, Mittleman MA, Coull BA, Gryparis A, Bots ML, Schwartz J, et al. Long-term exposure to black carbon and carotid intima-media thickness: The normative aging study. *Environmental Health Perspectives*. 2013; 121:1061–1067. [PubMed: 23820848]
26. Künzli N, Jerrett M, Garcia-Esteban R, Basagaña X, Beckermann B, Gilliland F, et al. Ambient air pollution and the progression of atherosclerosis in adults. *PLoS One*. 2010; 5:1–10.
27. Lundback M, Mills NL, Lucking A, Barath S, Donaldson K, Newby DE, et al. Experimental exposure to diesel exhaust increases arterial stiffness in man. *Part Fibre Toxicology*. 2009; 6:7.
28. Cosselman KE, Krishnan RM, Oron AP, Jansen K, Peretz A, Sullivan JH, et al. Blood pressure response to controlled diesel exhaust exposure in human subjects. *Hypertension*. 2012; 59:943–948. [PubMed: 22431582]
29. Medina-Ramon M, Goldberg R, Melly S, Mittleman MA, Schwartz J. Residential exposure to traffic-related air pollution and survival after heart failure. *Environmental Health Perspectives*. 2008; 116:481–485. [PubMed: 18414630]

30. McDonald JD, Reed MD, Campen MJ, Barrett EG, Seagrave J, Mauderly JL. Health effects of inhaled gasoline engine emissions. *Inhalation Toxicology*. 2007; 19(Suppl. 1):107–116. [PubMed: 17886058]
31. Brook RD, Rajagopalan S. Particulate matter, air pollution, and blood pressure. *Journal of the American Society of Hypertension*. 2009; 3:332–350. [PubMed: 20409976]
32. Peters A. Air quality and cardiovascular health: Smoke and pollution matter. *Circulation*. 2009; 120:924–927. [PubMed: 19720930]
33. Sun Q, Hong X, Wold LE. Cardiovascular effects of ambient particulate air pollution exposure. *Circulation*. 2010; 121:2755–2765. [PubMed: 20585020]
34. Meng Q, Richmond-Bryant J, Lu S-E, Buckley B, Welsh WJ, Whitsel EA, et al. Cardiovascular outcomes and the physical and chemical properties of metal ions found in particulate matter air pollution: A QICAR Study. *Environmental Health Perspectives*. 2013; 121:558–564. [PubMed: 23462649]
35. Uzoigwe JC, Prum T, Bresnahan E, Garelnabi M. The emerging role of outdoor and indoor air pollution in cardiovascular disease. *North American Journal of Medical Sciences*. 2013; 5:445–453. [PubMed: 24083218]
36. Lerman A, Zeiher AM. Endothelial function: Cardiac events. *Circulation*. 2005; 111:363–368. [PubMed: 15668353]
37. Leppert J, Ringqvist A, Karlberg BE, Ringqvist I. Whole-body cooling increases plasma endothelin-1 levels in women with primary Raynaud's phenomenon. *Clinical Physiology*. 1998; 18:420–425. [PubMed: 9784937]
38. Spiropoulos K, Trakada G, Nikolaou E, Prodromakis E, Efremidis G, Pouli A, et al. Endothelin-1 levels in the pathophysiology of chronic obstructive pulmonary disease and bronchial asthma. *Respiratory Medicine*. 2003; 97:983–989. [PubMed: 12924528]
39. O'Neill MS, Veves A, Zanobetti A, Sarnat JA, Gold DR, Economides PA, et al. Diabetes enhances vulnerability to particulate air pollution-associated impairment in vascular reactivity and endothelial function. *Circulation*. 2005; 111:2913–2920. [PubMed: 15927967]
40. Calderon-Garciduenas L, Franco-Lira M, Torres-Jardon R, Henriquez-Roldan C, Barragan-Mejia G, Valencia-Salazar G, et al. Pediatric respiratory and systemic effects of chronic air pollution exposure: Nose, lung, heart, and brain pathology. *Toxicologic Pathology*. 2007; 35:154–162. [PubMed: 17325984]
41. Cellini M, Strobbe E, Gizzi C, Balducci N, Toschi PG, Campos EC. Endothelin-1 plasma levels and vascular endothelial dysfunction in primary open angle glaucoma. *Life Sciences*. 2012; 91:699–702. [PubMed: 22406080]
42. Toda N, Okamura T. Obesity impairs vasodilatation and blood flow increase mediated by endothelial nitric oxide: An overview. *Journal of Clinical Pharmacology*. 2013; 53:1228–1239. [PubMed: 24030923]
43. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*. 1980; 288:373–376. [PubMed: 6253831]
44. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*. 1987; 327:524–526. [PubMed: 3495737]
45. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*. 1988; 332:411–415. [PubMed: 2451132]
46. Battistini B, D'Orleans-Juste P, Sirois P. Endothelins: Circulating plasma levels and presence in other biologic fluids. *Laboratory Investigation*. 1993; 68:600–628. [PubMed: 8515652]
47. Rubanyi GM, Polokoff MA. Endothelins: Molecular biology, biochemistry, pharmacology, physiology, and pathophysiology. *Pharmacological Reviews*. 1994; 46:325–415. [PubMed: 7831383]
48. Levin ER. Endothelins. *New England Journal of Medicine*. 1995; 333:356–363. [PubMed: 7609754]
49. Luscher TF, Barton M. Endothelins and endothelin receptor antagonists: Therapeutic considerations for a novel class of cardiovascular drugs. *Circulation*. 2000; 102:2434–2440. [PubMed: 11067800]

50. Fagan KA, McMurtry IF, Rodman DM. Role of endothelin-1 in lung disease. *Respiratory Research*. 2001; 2:90–101. [PubMed: 11686871]
51. Barton M, Yanagisawa M. Endothelin: 20 years from discovery to therapy. *Canadian Journal of Physiology and Pharmacology*. 2008; 86:485–498. [PubMed: 18758495]
52. Saida K, Mitsui Y, Ishida N. A novel peptide, vasoactive intestinal contractor, of a new (endothelin) peptide family. Molecular cloning, expression, and biological activity. *Journal of Biological Chemistry*. 1989; 264:14613–14616. [PubMed: 2768235]
53. Cunningham ME, Huribal M, Bala RJ, McMillen MA. Endothelin-1 and endothelin-4 stimulate monocyte production of cytokines. *Critical Care Medicine*. 1997; 25:958–964. [PubMed: 9201047]
54. Kedzierski RM, Yanagisawa M. Endothelin system: The double-edged sword in health and disease. *Annual Review of Pharmacology and Toxicology*. 2001; 41:851–876.
55. Howard PG, Plumpton C, Davenport AP. Anatomical localization and pharmacological activity of mature endothelins and their precursors in human vascular tissue. *Journal of Hypertension*. 1992; 10:1379–1386. [PubMed: 1336523]
56. Ohkita M, Tawa M, Kitada K, Matsumura Y. Pathophysiological roles of endothelin receptors in cardiovascular diseases. *Journal of Pharmacological Sciences*. 2012; 119:302–313. [PubMed: 22863667]
57. Dhaun N, Webb DJ, Kluth DC. Endothelin-1 and the kidney—beyond BP. *British Journal of Pharmacology*. 2012; 167:720–731. [PubMed: 22670597]
58. Brunner F, Bras-Silva C, Cerdeira AS, Leite-Moreira AF. Cardiovascular endothelins: Essential regulators of cardiovascular homeostasis. *Pharmacology & Therapeutics*. 2006; 111:508–531. [PubMed: 16457892]
59. Kurihara H, Yoshizumi M, Sugiyama T, Takaku F, Yanagisawa M, Masaki T, et al. Transforming growth factor-beta stimulates the expression of endothelin mRNA by vascular endothelial cells. *Biochemical and Biophysical Research Communications*. 1989; 159:1435–1440. [PubMed: 2649101]
60. Emori T, Hirata Y, Imai T, Ohta K, Kanno K, Equchi S, et al. Cellular mechanism of thrombin on endothelin-1 biosynthesis and release in bovine endothelial cell. *Biochemical Pharmacology*. 1992; 44:2409–2411. [PubMed: 1472106]
61. Chen GF, Sun Z. Effects of chronic cold exposure on the endothelin system. *Journal of Applied Physiology*. 2006; 100(5):1719–1726. [PubMed: 16384835]
62. Mazzuca MQ, Khalil RA. Vascular endothelin receptor type B: Structure, function and dysregulation in vascular disease. *Biochemical Pharmacology*. 2012; 84:147–162. [PubMed: 22484314]
63. Bremnes T, Paasche JD, Mehllum A, Sandberg C, Bremnes B, Attramadal H. Regulation and intracellular trafficking pathways of the endothelin receptors. *Journal of Biological Chemistry*. 2000; 275:17596–17604. [PubMed: 10747877]
64. Ihling C, Szombathy T, Bohrmann B, Brockhaus M, Schaefer HE, Loeffler BM. Coexpression of endothelin-converting enzyme-1 and endothelin-1 in different stages of human atherosclerosis. *Circulation*. 2001; 104:864–869. [PubMed: 11514370]
65. Vierhapper H, Wagner O, Nowotny P, Waldhausl W. Effect of endothelin-1 in man. *Circulation*. 1990; 81:1415–1418. [PubMed: 2180594]
66. Thorin E, Clozel M. The cardiovascular physiology and pharmacology of endothelin-1. *Advances in Pharmacology*. 2010; 60:1–26. [PubMed: 21081213]
67. Battistini B, Dussault P. Biosynthesis, distribution and metabolism of endothelins in the pulmonary system. *Pulmonary Pharmacology & Therapeutics*. 1998; 11:79–88. [PubMed: 9918740]
68. Kastin, A. *Handbook of biologically active peptides*. Elsevier; Amsterdam: 2013.
69. Moreland S, McMullen DM, Delaney CL, Lee VG, Hunt JT. Venous smooth muscle contains vasoconstrictor ETB-like receptors. *Biochemical and Biophysical Research Communications*. 1992; 184:100–106. [PubMed: 1314581]
70. Miyauchi T, Tomobe Y, Shiba R, Ishikawa T, Yanagisawa M, Kimura S, et al. Involvement of endothelin in the regulation of human vascular tonus. Potent vasoconstrictor effect and existence in endothelial cells. *Circulation*. 1990; 81:1874–1880. [PubMed: 2188755]

71. Haynes WG, Clarke JG, Cockcroft JR, Webb DJ. Pharmacology of endothelin-1 in vivo in humans. *Journal of Cardiovascular Pharmacology*. 1991; 17(Suppl. 7):S284–S286. [PubMed: 1725356]
72. Gasic S, Wagner OF, Vierhapper H, Nowotny P, Waldhausl W. Regional hemodynamic effects and clearance of endothelin-1 in humans: Renal and peripheral tissues may contribute to the overall disposal of the peptide. *Journal of Cardiovascular Pharmacology*. 1992; 19:176–180. [PubMed: 1376785]
73. Kiowski W, Sutsch G, Hunziker P, Muller P, Kim J, Oechslin E, et al. Evidence for endothelin-1-mediated vasoconstriction in severe chronic heart failure. *Lancet*. 1995; 346:732–736. [PubMed: 7658874]
74. Bohm F, Johansson BL, Hedin U, Alving K, Pernow J. Enhanced vasoconstrictor effect of big endothelin-1 in patients with atherosclerosis: Relation to conversion to endothelin-1. *Atherosclerosis*. 2002; 160:215–222. [PubMed: 11755940]
75. Knuckles TL, Lund AK, Lucas SN, Campen MJ. Diesel exhaust exposure enhances venoconstriction via uncoupling of eNOS. *Toxicology and Applied Pharmacology*. 2008; 230:346–351. [PubMed: 18455212]
76. Kodavanti UP, Thomas RF, Ledbetter AD, Schladweiler MC, Bass V, Krantz QT, et al. Diesel exhaust induced pulmonary and cardiovascular impairment: The role of hypertension intervention. *Toxicology and Applied Pharmacology*. 2013; 268:232–240. [PubMed: 23415681]
77. Nar G, Soyulu K, Akcay M, Gulel O, Yuksel S, Meric M, et al. Evaluation of the relationship between arterial blood pressure, aortic stiffness and serum endothelin-1 levels in patients with essential hypertension. *Clinical and Experimental Hypertension*. 2013; 35:589–594. [PubMed: 23530911]
78. Bohm F, Pernow J. The importance of endothelin-1 for vascular dysfunction in cardiovascular disease. *Cardiovascular Research*. 2007; 76:8–18. [PubMed: 17617392]
79. Barton M. Reversal of proteinuric renal disease and the emerging role of endothelin. *Nature Clinical Practice Nephrology*. 2008; 4:490–501.
80. Pernow J, Shemyakin A, Bohm F. New perspectives on endothelin-1 in atherosclerosis and diabetes mellitus. *Life Sciences*. 2012; 91:507–516. [PubMed: 22483688]
81. Haynes WG, Webb DJ. Contribution of endogenous generation of endothelin-1 to basal vascular tone. *Lancet*. 1994; 344:852–854. [PubMed: 7916401]
82. Kurihara Y, Kurihara H, Suzuki H, Kodama T, Maemura K, Nagai R, et al. Elevated blood pressure and craniofacial abnormalities in mice deficient in endothelin-1. *Nature*. 1994; 368:703–710. [PubMed: 8152482]
83. Clouthier DE, Hosoda K, Richardson JA, Williams SC, Yanagisawa H, Kuwaki T, et al. Cranial and cardiac neural crest defects in endothelin-A receptor-deficient mice. *Development*. 1998; 125:813–824. [PubMed: 9449664]
84. Clouthier DE, Williams SC, Yanagisawa H, Wieduwilt M, Richardson JA, Yanagisawa M. Signaling pathways crucial for craniofacial development revealed by endothelin-A receptor-deficient mice. *Development Biology*. 2000; 217:10–24.
85. Yanagisawa H, Hammer RE, Richardson JA, Williams SC, Clouthier DE, Yanagisawa M. Role of endothelin-1/endothelin-A receptor-mediated signaling pathway in the aortic arch patterning in mice. *The Journal of Clinical Investigation*. 1998; 102:22–33. [PubMed: 9649553]
86. Karpainen S, Rapila R, Maäkikallio K, Haänninen SL, Rysä J, Vuolteenaho O, et al. Endothelin-1 signalling controls early embryonic heart rate in vitro and in vivo. *Acta Physiologica*. 2014; 210:369–380. [PubMed: 24325624]
87. Dong F, Zhang X, Wold LE, Ren Q, Zhang Z, Ren J. Endothelin-1 enhances oxidative stress, cell proliferation and reduces apoptosis in human umbilical vein endothelial cells: Role of ETB receptor, NADPH oxidase and caveolin-1. *British Journal of Pharmacology*. 2005; 145:323–333. [PubMed: 15765100]
88. Loomis ED, Sullivan JC, Osmond DA, Pollock DM, Pollock JS. Endothelin mediates superoxide production and vasoconstriction through activation of NADPH oxidase and uncoupled nitric-oxide synthase in the rat aorta. *Journal of Pharmacology and Experimental Therapeutics*. 2005; 315:1058–1064. [PubMed: 16144972]

89. Feletou M, Vanhoutte PM. Endothelial dysfunction: A multifaceted disorder (the Wiggers Award lecture). *American Journal of Physiology Heart and Circulatory Physiology*. 2006; 291:H985–H1002. [PubMed: 16632549]
90. Duerschmidt N, Wippich N, Goettsch W, Broemme HJ, Morawietz H. Endothelin-1 induces NAD(P)H oxidase in human endothelial cells. *Biochemical and Biophysical Research Communications*. 2000; 269:713–717. [PubMed: 10720482]
91. Ehrenreich H, Anderson RW, Fox CH, Rieckmann P, Hoffman GS, Travis WD, et al. Endothelins, peptides with potent vasoactive properties, are produced by human macrophages. *Journal of Experimental Medicine*. 1990; 172:1741–1748. [PubMed: 1701822]
92. Fan J, Unoki H, Iwasa S, Watanabe T. Role of endothelin-1 in atherosclerosis. *Annals of the New York Academy of Sciences*. 2000; 902:84–93. discussion 84–93. [PubMed: 10865828]
93. Gray GA, Battistini B, Webb DJ. Endothelins are potent vasoconstrictors, and much more besides. *Trends in Pharmacological Sciences*. 2000; 21:38–40. [PubMed: 10733432]
94. Gossel M, Lerman A. Endothelin: Beyond a vasoconstrictor. *Circulation*. 2006; 113:1156–1158. [PubMed: 16520422]
95. Ramzy D, Rao V, Tumiati LC, Xu N, Sheshgiri R, Miriuka S, et al. Elevated endothelin-1 levels impair nitric oxide homeostasis through a PKC-dependent pathway. *Circulation*. 2006; 114:I319–I326. [PubMed: 16820593]
96. Barton M. Endothelial dysfunction and atherosclerosis: Endothelin receptor antagonists as novel therapeutics. *Current Hypertension Reports*. 2000; 2:84–91. [PubMed: 10981133]
97. Attinà T, Camidge R, Newby DE, Webb DJ. Endothelin antagonism in pulmonary hypertension, heart failure, and beyond. *Heart*. 2005; 91:825–831. [PubMed: 15894792]
98. Battistini B, Berthiaume N, Kelland NF, Webb DJ, Kohan DE. Profile of past and current clinical trials involving endothelin receptor antagonists: The novel “-sentan” class of drug. *Experimental Biology and Medicine (Maywood)*. 2006; 231:653–695.
99. Vita JA. Endothelial function. *Circulation*. 2011; 124:e906–e912. [PubMed: 22184047]
100. Sulli A, Soldano S, Pizzorni C, Montagna P, Secchi ME, Villaggio B, et al. Raynaud's phenomenon and plasma endothelin: Correlations with capillaroscopic patterns in systemic sclerosis. *Journal of Rheumatology*. 2009; 36:1235–1239. [PubMed: 19369451]
101. Evans RG, Bergstrom G, Cotterill E, Anderson WP. Renal haemodynamic effects of endothelin-1 and the ETA/ETB antagonist TAK-044 in anaesthetized rabbits. *Journal of Hypertension*. 1998; 16:1897–1905. [PubMed: 9886875]
102. Migneault A, Sauvageau S, Villeneuve L, Thorin E, Fournier A, Leblanc N, et al. Chronically elevated endothelin levels reduce pulmonary vascular reactivity to nitric oxide. *American Journal of Respiratory and Critical Care Medicine*. 2005; 171:506–513. [PubMed: 15579730]
103. Riediker M, Cascio WE, Griggs TR, Herbst MC, Bromberg PA, Neas L, et al. Particulate matter exposure in cars is associated with cardiovascular effects in healthy young men. *American Journal of Respiratory and Critical Care Medicine*. 2004; 169:934–940. [PubMed: 14962820]
104. Lund AK, Knuckles TL, Obot Akata C, Shohet R, McDonald JD, Gigliotti A, et al. Gasoline exhaust emissions induce vascular remodeling pathways involved in atherosclerosis. *Toxicological Sciences*. 2007; 95:485–494. [PubMed: 17065432]
105. Hocher B, Schwarz A, Fagan KA, Thone-Reineke C, El-Hag K, Kusserow H, et al. Pulmonary fibrosis and chronic lung inflammation in ET-1 transgenic mice. *American Journal of Respiratory Cell and Molecular Biology*. 2000; 23:19–26. [PubMed: 10873149]
106. Channick RN, Simonneau G, Sitbon O, Robbins IM, Frost A, Tapson VF, et al. Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: A randomised placebocontrolled study. *The Lancet*. 2001; 358:1119–1123.
107. Leuchte HH, Meis T, El-Nounou M, Michalek J, Behr J. Inhalation of endothelin receptor blockers in pulmonary hypertension. *American Journal of Physiology—Lung Cellular and Molecular Physiology*. 2008; 294:L772–L777. [PubMed: 18296497]
108. Calderon-Garciduenas L, Vincent R, Mora-Tiscareno A, Franco-Lira M, Henriquez-Roldan C, Barragan-Mejia G, et al. Elevated plasma endothelin-1 and pulmonary arterial pressure in children exposed to air pollution. *Environmental Health Perspectives*. 2007; 115:1248–1253. [PubMed: 17687455]

109. World Health Organization. [June 15, 2015] Ambient (outdoor) air pollution in cities database 2014. 2014. Available from: www.who.int
110. Calderon-Garciduenas L, Villarreal-Calderon R, Valencia-Salazar G, Henriquez-Roldan C, Gutierrez-Castrellon P, Torres-Jardon R, et al. Systemic inflammation, endothelial dys-function, and activation in clinically healthy children exposed to air pollutants. *Inhalation Toxicology*. 2008; 20:499–506. [PubMed: 18368620]
111. Lund AK, Lucero J, Lucas S, Madden MC, McDonald JD, Seagrave JC, et al. Vehicular emissions induce vascular MMP-9 expression and activity associated with endothelin-1-mediated pathways. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2009; 29:511–517.
112. Peretz A, Sullivan JH, Leotta DF, Trenga CA, Sands FN, Allen J, et al. Diesel exhaust inhalation elicits acute vasoconstriction in vivo. *Environmental Health Perspectives*. 2008; 116:937–942. [PubMed: 18629317]
113. Brook RD. Inhalation of fine particulate air pollution and ozone causes acute arterial vasoconstriction in healthy adults. *Circulation*. 2002; 105:1534–1536. [PubMed: 11927516]
114. Mills NL, Tornqvist H, Robinson SD, Gonzalez M, Darnley K, MacNee W, et al. Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. *Circulation*. 2005; 112:3930–3936. [PubMed: 16365212]
115. Langrish JP, Lundback M, Mills NL, Johnston NR, Webb DJ, Sandstrom T, et al. Contribution of endothelin 1 to the vascular effects of diesel exhaust inhalation in humans. *Hypertension*. 2009; 54:910–915. [PubMed: 19687345]
116. Verhaar MC, Strachan FE, Newby DE, Cruden NL, Koomans HA, Rabelink TJ, et al. Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade. *Circulation*. 1998; 97:752–756. [PubMed: 9498538]
117. Bigert C, Gustavsson P, Hallqvist J, Hogstedt C, Lewné M, Plato N, et al. Myocardial infarction among professional drivers. *Epidemiology*. 2003; 14:333–339. [PubMed: 12859035]
118. Barbosa CM, Terra-Filho M, de Albuquerque AL, Di Giorgi D, Grupi C, Negrao CE, et al. Burnt sugarcane harvesting—Cardiovascular effects on a group of healthy workers, Brazil. *PLoS One*. 2012; 7:e46142. [PubMed: 23029413]
119. Fang SC, Cavallari JM, Eisen EA, Chen JC, Mittleman MA, Christiani DC. Vascular function, inflammation, and variations in cardiac autonomic responses to particulate matter among welders. *American Journal of Epidemiology*. 2009; 169:848–856. [PubMed: 19153215]
120. Jarvela M, Kauppi P, Tuomi T, Luukkonen R, Lindholm H, Nieminen R, et al. Inflammatory response to acute exposure to welding fumes during the working day. *International Journal of Occupational Medicine and Environmental Health*. 2013; 26:220–229. [PubMed: 23690265]
121. Scharrer E, Hessel H, Kronseder A, Guth W, Rolinski B, Jorres RA, et al. Heart rate variability, hemostatic and acute inflammatory blood parameters in healthy adults after short-term exposure to welding fume. *International Archives of Occupational and Environmental Health*. 2007; 80:265–272. [PubMed: 16791613]
122. Zhang Y, Edvinsson L, Xu CB. Up-regulation of endothelin receptors induced by cigarette smoke —involvement of MAPK in vascular and airway hyper-reactivity. *Scientific World Journal*. 2010; 10:2157–2166. [PubMed: 21057729]
123. Cao L, Zhang Y, Cao YX, Edvinsson L, Xu CB. Cigarette smoke upregulates rat coronary artery endothelin receptors in vivo. *PLoS One*. 2012; 7:e33008. [PubMed: 22412974]
124. Adachi C, Naruse M, Ishihara Y, Tanabe A, Takagi S, Yoshimoto T, et al. Effects of acute and chronic cigarette smoking on the expression of endothelin-1 mRNA of the cardiovascular tissues in rats. *Journal of Cardiovascular Pharmacology*. 2000; 36:S198–S200. [PubMed: 11078376]
125. Ambrose JA, Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: An update. *Journal of the American College of Cardiology*. 2004; 43:1731–1737. [PubMed: 15145091]
126. Law MR, Morris JK, Wald NJ. Environmental tobacco smoke exposure and ischaemic heart disease: An evaluation of the evidence. *British Medical Journal*. 1997; 315:973–980. [PubMed: 9365294]

127. Pinnamaneni K, Sievers RE, Sharma R, Selchau AM, Gutierrez G, Nordsieck EJ, et al. Brief exposure to secondhand smoke reversibly impairs endothelial vasodilatory function. *Nicotine & Tobacco Research*. 2014; 16:584–590. [PubMed: 24302638]
128. Campen MJ, Lund AK, Doyle-Eisele ML, McDonald JD, Knuckles TL, Rohr AC, et al. A comparison of vascular effects from complex and individual air pollutants indicates a role for monoxide gases and volatile hydrocarbons. *Environmental Health Perspectives*. 2010; 118:921–927. [PubMed: 20197249]
129. Campen MJ, Babu NS, Helms GA, Pett S, Wernly J, Mehran R, et al. Nonparticulate components of diesel exhaust promote constriction in coronary arteries from ApoE^{−/−} mice. *Toxicological Sciences*. 2005; 88:95–102. [PubMed: 16093524]
130. Cherng TW, Campen MJ, Knuckles TL, Gonzalez Bosc L, Kanagy NL. Impairment of coronary endothelial cell ETB receptor function after short-term inhalation exposure to whole diesel emissions. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*. 2009; 297:R640–R647.
131. Kodavanti UP, Thomas R, Ledbetter AD, Schladweiler MC, Shannahan JH, Wallenborn JG, et al. Vascular and cardiac impairments in rats inhaling ozone and diesel exhaust particles. *Environmental Health Perspectives*. 2011; 119:312–318. [PubMed: 20980218]
132. Ito T, Suzuki T, Tamura K, Nezu T, Honda K, Kobayashi T. Examination of mRNA expression in rat hearts and lungs for analysis of effects of exposure to concentrated ambient particles on cardiovascular function. *Toxicology*. 2008; 243:271–283. [PubMed: 18077076]
133. Bouthillier L, Vincent R, Goegan P, Adamson IYR, Bjarnason S, Stewart M, et al. Acute effects of inhaled urban particles and ozone. *The American Journal of Pathology*. 1998; 153:1873–1884. [PubMed: 9846977]
134. Miyata R, Hiraiwa K, Cheng JC, Bai N, Vincent R, Francis GA, et al. Statins attenuate the development of atherosclerosis and endothelial dysfunction induced by exposure to urban particulate matter (PM10). *Toxicology and Applied Pharmacology*. 2013; 272:1–11. [PubMed: 23756175]
135. Beagley J, Guariguata L, Weil C, Motala AA. Global estimates of undiagnosed diabetes in adults. *Diabetes Research and Clinical Practice*. 2014; 103:150–160. [PubMed: 24300018]
136. Ferri C, Carlomagno A, Coassin S, Baldoncini R, Cassone Faldetta MR, Laurenti O, et al. Circulating endothelin-1 levels increase during euglycemic hyperinsulinemic clamp in lean NIDDM men. *Diabetes Care*. 1995; 18:226–233. [PubMed: 7729302]
137. Verma S, Yao L, Stewart DJ, Dumont AS, Anderson TJ, McNeill JH. Endothelin antagonism uncovers insulin-mediated vasorelaxation in vitro and in vivo. *Hypertension*. 2001; 37:328–333. [PubMed: 11230293]
138. Berthiaume N, Mika AK, Zinker BA. Development of insulin resistance and endothelin-1 levels in the Zucker fatty rat. *Metabolism*. 2003; 52:845–849. [PubMed: 12870159]
139. Jansson PA. Endothelial dysfunction in insulin resistance and type 2 diabetes. *Journal of Internal Medicine*. 2007; 262:173–183. [PubMed: 17645585]
140. Kolka CM, Bergman RN. The endothelium in diabetes: Its role in insulin access and diabetic complications. *Reviews in Endocrine and Metabolic Disorders*. 2013; 14:13–19. [PubMed: 23306780]
141. Leyva F, Wingrove C, Felton C, Stevenson JC. Physiological hyperinsulinemia is not associated with alterations in venous plasma levels of endothelin-1 in healthy individuals. *Metabolism*. 1997; 46:1137–1139. [PubMed: 9322795]
142. DeLoach S, Huan Y, Daskalakis C, Falkner B. Endothelin-1 response to glucose and insulin among African Americans. *Journal of the American Society of Hypertension*. 2010; 4:227–235. [PubMed: 20728421]
143. Pearson JF, Bachireddy C, Shyamprasad S, Goldfine AB, Brownstein JS. Association between fine particulate matter and diabetes prevalence in the US. *Diabetes Care*. 2010; 33:2196–2201. [PubMed: 20628090]
144. Rajagopalan S, Brook RD. Air pollution and type 2 diabetes: Mechanistic insights. *Diabetes*. 2012; 61:3037–3045. [PubMed: 23172950]

145. Sun Q, Yue P, Deiluiis JA, Lumeng CN, Kampfrath T, Mikolaj MB, et al. Ambient air pollution exaggerates adipose inflammation and insulin resistance in a mouse model of diet-induced obesity. *Circulation*. 2009; 119:538–546. [PubMed: 19153269]
146. Cuiqing L, Xiaohua X, Yuntao B, Tse-Yao W, Xiaoquan R, Aixia W, et al. Air pollution-mediated susceptibility to inflammation and insulin resistance: Influence of CCR2 pathways in mice. *Environmental Health Perspectives*. 2014; 122:17–26. [PubMed: 24149114]
147. Tamayo T, Rathmann W, Krämer U, Sugiri D, Grabert M, Holl RW. Is particle pollution in outdoor air associated with metabolic control in type 2 diabetes? *PLoS One*. 2014; 9:1–6.
148. Paczkowska E, Goł b-Janowska M, Bajer-Czajkowska A, Machali ska A, Ustianowski P, Rybicka M, et al. Increased circulating endothelial progenitor cells in patients with haemorrhagic and ischaemic stroke: The role of endothelin-1. *Journal of the Neurological Sciences*. 2013; 325:90–99. [PubMed: 23290569]
149. O'Toole TE, Hellmann J, Wheat L, Haberzettl P, Lee J, Conklin DJ, et al. Episodic exposure to fine particulate air pollution decreases circulating levels of endothelial progenitor cells. *Circulation Research*. 2010; 107:200–203. [PubMed: 20595651]
150. Haberzettl P, Lee J, Duggineni D, McCracken J, Bolanowski D, O'Toole TE, et al. Exposure to ambient air fine particulate matter prevents VEGF-induced mobilization of endothelial progenitor cells from the bone marrow. *Environmental Health Perspectives*. 2012; 120:848–856. [PubMed: 22418586]
151. Wheat LA, Haberzettl P, Hellmann J, Baba SP, Bertke M, Lee J, et al. Acrolein inhalation prevents vascular endothelial growth factor-induced mobilization of Flk-1+/Sca-1+ cells in mice. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2011; 31:1598–1606.
152. Matsuura A, Yamochi W, Hirata K-I, Kawashima S, Yokoyama M. Stimulatory interaction between vascular endothelial growth factor and endothelin-1 on each gene expression. *Hypertension*. 1998; 32:89–95. [PubMed: 9674643]
153. Grant K, Loizidou M, Taylor I. Endothelin-1: A multifunctional molecule in cancer. *British Journal of Cancer*. 2003; 88:163–166. [PubMed: 12610497]
154. Lankhorst S, Kappers MHW, van Esch JHM, Danser AHJ, van den Meiracker AH. Hypertension during vascular endothelial growth factor inhibition: Focus on nitric oxide, endothelin-1, and oxidative stress. *Antioxidants & Redox Signaling*. 2014; 20:135–145. [PubMed: 23458507]
155. Star GP, Giovinnazzo M, Lamoureux E, Langleben D. Effects of vascular endothelial growth factor on endothelin-1 production by human lung microvascular endothelial cells in vitro. *Life Sciences*. 2014; 118:191–194. [PubMed: 24607779]
156. Jung C, Rafnsson A, Brismar K, Pernow J. Endothelial progenitor cells in relation to endothelin-1 and endothelin receptor blockade: A randomized, controlled trial. *International Journal of Cardiology*. 2013; 168:1017–1022. [PubMed: 23168014]
157. Wu MH, Huang CY, Lin JA, Wang SW, Peng CY, Cheng HC, et al. Endothelin-1 promotes vascular endothelial growth factor-dependent angiogenesis in human chondrosarcoma cells. *Oncogene*. 2014; 33:1725–1735. [PubMed: 23584483]
158. Soteriades ES, Smith DL, Tsismenakis AJ, Baur DM, Kales SN. Cardiovascular disease in US firefighters: A systematic review. *Cardiology in Review*. 2011; 19:202–215. [PubMed: 21646874]
159. Webb HE, Garten RS, McMinn DR, Beckman JL, Kamimori GH, Acevedo EO. Stress hormones and vascular function in firefighters during concurrent challenges. *Biological Psychology*. 2011; 87:152–160. [PubMed: 21382435]
160. Maguire JJ, Davenport AP. ETA receptor-mediated constrictor responses to endothelin peptides in human blood vessels in vitro. *British Journal of Pharmacology*. 1995; 115:191–197. [PubMed: 7647976]
161. Singh AD, Amit S, Kumar OS, Rajan M, Mukesh N. Cardioprotective effects of bosentan, a mixed endothelin type A and B receptor antagonist, during myocardial ischaemia and reperfusion in rats. *Basic & Clinical Pharmacology & Toxicology*. 2006; 98:604–610. [PubMed: 16700825]
162. Rubin LJ, Badesch DB, Barst RJ, Galie N, Black CM, Keogh A, et al. Bosentan therapy for pulmonary arterial hypertension. *New England Journal of Medicine*. 2002; 346:896–903. [PubMed: 11907289]

163. Davie NJ, Schermuly RT, Weissmann N, Grimminger F, Ghofrani HA. The science of endothelin-1 and endothelin receptor antagonists in the management of pulmonary arterial hypertension: Current understanding and future studies. *European Journal of Clinical Investigation*. 2009; 39:38–49. [PubMed: 19335746]
164. Pham I, Wuerzner G, Richalet J-P, Peyrard S, Azizi M. Bosentan effects in hypoxic pulmonary vasoconstriction: Preliminary study in subjects with or without high altitude pulmonary edema-history. *Pulmonary Circulation*. 2012; 2:28–33. [PubMed: 22558517]

Fig. 1.

Schematic of vascular wall cell dependent production and receptor-mediated actions of ET-1. *Akt* protein kinase B, Ca^{2+} calcium, *cGMP* cyclic guanosine monophosphate, *EC* endothelial cell, *ECE-1* endothelin-converting enzyme, *ECM* extracellular matrix, *eNOS* endothelial nitric oxide synthase, *ERK* extracellular signal-regulated kinases, *ET-1* endothelin-1, *ET_A* endothelin receptor A, *ET_{B1}* endothelin receptor B1, *HTN* hypertension, *InR* insulin receptor, *NO* nitric oxide, *PKC* protein kinase C, *PKG* protein kinase G, *SGC* soluble guanylyl cyclase, *VEGF* vascular endothelial growth factor, *VR* VEGF receptor, *VSMC* vascular smooth muscle cell

Table 1

Epidemiological and experimental studies linking air pollution exposures and levels of ET-1 in human adults

Age	Patient type	CAP ($\mu\text{g}/\text{m}^3$)	DE ($\mu\text{g}/\text{m}^3$)	Findings	References
Adult	Healthy	150 (2 h)		Significant brachial artery constriction with CAP + O ₃ exposure versus filtered air ($p = 0.007$). No significant differences in FMD, NMD, or blood pressure between exposures	Brook [113]
			300 (1 h)	No effect of DE exposures on SBP, DBP, or mean heart rate. Plasma big ET-1 and ET-1 levels unchanged; DE increased vascular sensitivity to ET-1. Infusion of BQ-123 and BQ-788 caused vasodilatation, with greater effect after DE exposure ($p < 0.001$)	Langrish et al. [115]
			100 (2 h)	DE caused significant elevation in MMP-9 levels ($p \pm 0.05$) and activity ($p \pm 0.05$). Plasma ET-1 ($p \pm 0.05$) upregulated by DE 24 h post-exposure (female: 0.62 ± 0.09 pg/mol, male: 0.87 ± 0.09 pg/mol)	Lund et al. [111]
			300 (1 h)	Big ET-1, ET-1, TNF α , and IL-6 levels not significantly altered by DE or filtered air exposures. Vasodilators increased FBF after both exposures ($p < 0.0001$); effect blunted 2 h post-exposure	Mills et al. [114]
	Metabolic syndrome		100–200 (range 2h)	Greater constriction in brachial artery with DE ₂₀₀ exposure versus filtered air (11 mm, $p = 0.01$). No effect of DE on SBP or DBP. Increased plasma ET-1 with DE ₂₀₀ after 3 h (overall: 1.62 ± 0.13 pg/mol, $\Delta = 0.43$, $p = 0.01$); greater increase seen in healthy participants ($\Delta = 0.76$ vs. $\Delta = 0.31$)	Peretz et al. [112]

CAP concentrated ambient air particulate matter, DBP diastolic blood pressure, DE diesel exhaust, ET-1 endothelin-1, FMD flow-mediated dilation, FBF forearm blood flow, IL-6 interleukin-6, MMP matrix metalloproteinase, NMD endothelial-independent nitroglycerin-mediated dilatation, O₃ ozone, SBP systolic blood pressure, TNF α tumor necrosis factor- α

Table 2

Epidemiological and experimental studies linking air pollution exposures and levels of ET-1 in human children

Age	Patient type	Mean pollution levels	Findings	References
Children	General population	O ₃ , PM _{2.5} , and PM ₁₀	Plasma ET-1 levels significantly higher in northeast (2.09 ± 0.10 pg/mL, $p \pm 0.001$) and southwest (2.40 ± 0.14 pg/mL, $p \pm 0.001$) Mexico City compared to control (1.23 ± 0.06 pg/mL). MPAP elevated in northeast ($p \pm 0.01$) and southwest ($p \pm 0.05$) children and for Mexico City overall (17.3 ± 0.5 mmHg, $p < 0.01$). Positive correlation between MPAP and plasma ET-1 ($p < 0.0001$). Correlation between number of outdoor hours and ET-1 levels ($p = 0.012$) and MPAP ($p = 0.0008$) for Mexico City children. Positive correlation between ET-1 levels and 7-day cumulative PM _{2.5} exposure ($p = 0.03$)	Calderon-Garciduenas et al. [40]
		O ₃ , PM _{2.5} , and PM ₁₀	ET-1 increased in Mexico City children (69.8 ± 2.6 pg/mL, $p \pm 0.0001$) compared to control (39.8 ± 1.9 pg/mL). TNF α ($p = 0.005$), IL-10 ($p = 0.0009$), IL1 β ($p = 0.02$), PGE2 ($p = 0.003$), and CRP ($p = 0.001$) also higher in Mexico City children. All soluble endothelial adhesion molecules significantly lower in Mexico City children ($p < 0.001$ for all)	Calderon-Garciduenas et al. [108]

CRPC-reactive protein, *ET-1* endothelin-1, *IL-10* interleukin-10, *IL1 β* interleukin-1 beta, *MPAP* mean pulmonary arterial pressure, *PGE2* prostaglandin E2, *O3* ozone, *PM* particulate matter, *TNF α* tumor necrosis factor- α

Table 3

Experimental studies of air pollution exposures and levels of ET-1 in mice and rabbits

Type	Pollutant	Concentration and duration	Findings	References
Mouse	Gasoline engine exhaust	8–60 µg/m ³ (range 6 h/day)	Significant concentration-related increase in aortic expression of MMP-3, MMP-7, and MMP-9 with both PM and PM-filtered exposure. MMP-9 protein increase localized to endothelial cells and plaque regions. ET-1 mRNA expression elevated in exposure concentration-dependent manner (2.3-fold at highest level)	Lund et al. [104]
		60 µg/m ³ (6 h/day)	Seven-day GEE exposure increased aorta protein expression of MMP-9 and MMP-2 and plasma MMP-9 ($p < 0.05$ for all). BQ-123 significantly reduced MMP-9 protein levels in exposed group	Lund et al. [111]
		5–60 µg/m ³ (range 7 weeks)	ET-1 increased over 200 % and MMP-7 over 250 % for both whole and filtered GEE. Increases not reduced by filtration: non-PM components of emissions primarily responsible for effects	McDonald et al. [30]
	Diesel engine exhaust	500–3600 µg/m ³ (range 6 h/day)	DE exposure caused significant T-wave depression and bradycardia in ApoE mice. Vessels exposed to DE had greater ET-1 induced constriction than control vessels (47.5 % reduction from original diameter vs. 34.5 %, $p < 0.001$). DE exposure reduced dilatory response with sodium nitroprusside: vessels only returned to 77.4 % of baseline diameter	Campen et al. [129]
	Mixed	PM: 60–300 µg/m ³ (range 6 h/day)	Significant increase in ET-1, MMP-9, HO-1, and TIMP-2 mRNA ($p < 0.05$ for all) with GEE exposure. Slight increase in ET-1 mRNA with DE exposure ($p = 0.068$). Significant elevation of ET-1 and MMP-9 mRNA at 80 ppm CO; doubled with NO at 17 ppm	Campen et al. [128]
Rabbit	PM ₁₀	1.0 mg/kg (3 times/week)	Atherosclerotic score increased by PM ₁₀ ($p < 0.01$) but reduced by lovastatin ($p < 0.05$). Both ET-1 and ROS inhibition significantly improved baseline acetylcholine response. Plasma ET-1 increased by PM ₁₀ ($p < 0.05$) and PM ₁₀ and lovastatin ($p < 0.05$), ET-2/3 levels unaffected	Miyata et al. [134]

CO carbon monoxide, DE diesel exhaust, ET-1 endothelin-1, GEE gasoline engine exhaust, HO-1 heme oxygenase 1, MMP matrix metalloproteinase, NO nitric oxide, PM particulate matter, ROS reactive oxygen species, TIMP-2 TIMP metalloproteinase inhibitor 2

Table 4

Experimental studies of air pollution exposures and levels of ET-1 in rats

Type	Pollutant	Concentration and length	Findings	References
Rat	Diesel engine exhaust	300 µg/m ³ (5 h)	Enhanced vasoconstrictive response to ET-1 in DE exposed vessels ($p < 0.001$), with significantly greater maximal constriction ($p < 0.001$). Majority of ET-1-mediated vasoconstriction blocked with BQ-123 in both DE and filtered air groups. NOS inhibition via L-NNA augmented constrictor sensitivity to ET-1 in air group ($p < 0.05$). Five hours of DE exposure elevated plasma NO almost twofold ($p < 0.05$). Inhibiting NOS and removing endothelium did not affect ET-1-mediated constriction with DE exposure	Cherng et al. [130]
		2.1 mg/m ³ (5 h/day)	ET-1 and ET _A receptor mRNA expression in the aorta enhanced with exposure to ozone or DEP but not both ($p < 0.05$), as was eNOS, MMP-2, and HO-1 mRNA. ET _B receptor mRNA increased in the aorta with ozone + DEP exposure ($p = 0.05$)	Kodavanti et al. [131]
		1500 µg/m ³ (4 h/day)	Hydralazine reduced blood pressure in hypertensive rats ($p < 0.05$). DE increased eNOS expression in hypertensive rats with and without hydralazine ($p = 0.05$ for both). L-NAME increased ET-1 and decreased eNOS expression in 4 week WKY rats with air and gas exposure	Kodavanti et al. [76]
	Urban dust	40 ± 2 mg/m ³ (4 h)	Inhalation of particles alone and ozone alone decreased NO production. Inhalation of ozone alone stimulated ET-1 secretion by lung lavage cells. Increased plasma ET-1 with particles or particles + ozone ($p = 0.018$)	Bouthillier et al. [133]
	CAP	0.7–2.3 mg/m ³ (range 4 days)	ET-1, HO-1, and ET _A receptor mRNA expression upregulated in heart. Significant correlation between mRNA level and cumulative weight of CAP for all three markers. No significant change in heart rate with CAP exposure. No significant change in ET-1 mRNA expression in the lungs with CAP exposure	Ito et al. [132]

CAP concentrated ambient air particulate matter, DE diesel exhaust, DEP diesel exhaust particles, eNOS endothelial nitric oxide synthase, ET-1 endothelin-1, ET_A endothelin receptor A, ET_B endothelin receptor B, HO-1 heme oxygenase 1, MMP matrix metalloproteinase, NOS nitric oxide synthase, NO nitric oxide