Free Radical Biology and Medicine 110 (2017) 345–367

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Review article

Role of oxidative stress in cardiovascular disease outcomes following exposure to ambient air pollution

Frank J. Kelly*, Julia C. Fussell

NIHR Health Protection Research Unit in Health Impact of Environmental Hazards, Facility of Life Sciences and Medicine, King's College London, 150 Stamford Street, London SE1 9NH, UK

ARTICLE INFO

Keywords:
Ambient air pollution
Cardiovascular disease
Oxidative stress
Metabolic disease
Particulate matter
Gaseous pollutants

ABSTRACT

Exposure to ambient air pollution is associated with adverse cardiovascular outcomes. These are manifested through several, likely overlapping, pathways including at the functional level, endothelial dysfunction, atherosclerosis, pro-coagulation and alterations in autonomic nervous system balance and blood pressure. At numerous points within each of these pathways, there is potential for cellular oxidative imbalances to occur. The current review examines epidemiological, occupational and controlled exposure studies and research employing healthy and diseased animal models, isolated organs and cell cultures in assessing the importance of the pro-oxidant potential of air pollution in the development of cardiovascular disease outcomes. The collective body of data provides evidence that oxidative stress (OS) is not only central to eliciting specific cardiac endpoints, but is also implicated in modulating the risk of succumbing to cardiovascular disease, sensitivity to ischemia/reperfusion injury and the onset and progression of metabolic disease following ambient pollution exposure. To add to this large research effort conducted to date, further work is required to provide greater insight into areas such as (a) whether an oxidative imbalance triggers and/or worsens the effect and/or is representative of the consequence of disease progression, (b) OS pathways and cardiac outcomes caused by individual pollutants within air pollution mixtures, or as a consequence of inter-pollutant interactions and (c) potential protection provided by nutritional supplements and/or pharmacological agents with antioxidant properties, in susceptible populations residing in polluted urban cities.

1. Introduction

Exposure to ambient air pollution is associated with adverse cardiovascular outcomes [1–4]. These are manifested through several, likely overlapping, pathways including at the functional level, endothelial dysfunction, atherosclerosis, pro-coagulant changes, alterations in autonomic nervous system (ANS) balance and changes in blood pressure (BP) [1]. At the molecular level, principal pathways involved in eliciting such effects will depend upon the nature of the air pollutants (i.e. particles plus their components versus gases). These include (a) the

Abbreviations: 1-OHP, 1-hydroxypyrene; 3-NT, 3-nitrotyrosine; 8-oxodGuo, 8-oxo-7,8-dihydro-2'-deoxyguanosine; 15-F2t-IsoP, 15-F2t-isoprostane; +dP/ dt, rate of change of pressure development; -dP/dt, rate of change of pressure decay; Ach, acetylcholine; p-AKT, phosphorylated protein kinase B; ANG II, angiotensin II; ApoE$^{-/-}$, apolipoprotein E$^{-/-}$; ANS, autonomic nervous system; BALF, bronchoalveolar lavage fluid; BC, black carbon; BMSC, bone marrow stem cell; BP, blood pressure; BSO, buthionine sulphoximine; CAT, catalase; CB, carbon black; CO, carbon monoxide; CAP, concentrated ambient particle; DCFH-DA, dichlorodihydrofluorescein diacetate; DE, diesel exhaust; DEP, diesel exhaust particle; DHT, dihydroethidium; DM, diabetes mellitus; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cell; EPR, electron paramagnetic resonance; EPFRs, environmentally persistent free radicals; ERK1/2, extracellular signal-regulated kinase; ET-1, endothelin-1; ET_A, ET-1-endothelial receptor A; evmiRNAs, extracellular vesicle-encapsulated microRNAs; GEE, gasoline engine exhaust; GPx, glutathione peroxidase; GST, glutathione transferase; GSTM1, glutathione S-transferase-M1; H_2O_2, hydrogen peroxide; HAEC, human aortic endothelial cell; Hcy, homocysteine; HDL, high density lipoprotein; HO-1, hemeoxygenase-1; HRV, heart rate variability; HUVEC, human umbilical vein endothelial cell; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; iNOS, inducible nitric oxide synthase; IR, insulin resistance; IT, intratracheal; I/R, ischemia/reperfusion; JNK, c-Jun NH2-terminal kinases; LDP, low density lipoprotein; LOX-1, lectin-like oxidized low-density lipoprotein; LVDP, left Ventricular Developed Pressure; LVEDP, left ventricular end diastolic pressure; LVM, left ventricular mass; MACE, major adverse cardiovascular event; MAPK, mitogen activated kinase; MeS, metabolic syndrome; MDA, malondialdehyde; mDNA, mitochondrial DNA; MMP, matrix metalloprotease; MPMVEC, microvascular endothelial cell; miRNA, microRNA; NAC, N-acetylcysteine; NADPH, nicotinamide adenine dinucleotide phosphate, reduced; Ni, nickel; NO, nitric oxide; NO_2, nitrogen dioxide; OP, oxidative potential; OS, oxidative stress; oxLDL, oxidized low-density lipoprotein; $O_2^{\bullet-}$, superoxide anion radical; O_3, ozone; PAH, polycyclic aromatic hydrocarbon; PAI-1, plasminogen activator inhibitor-1; p-AKT, phosphorylated protein kinase B; PCO, protein carbonyl; PM, particulate matter; $PM_{2.5}$, particulate matter less than 2.5 μm in diameter; PM_{10}, particulate matter less than 10 μm in diameter; QTc, QT interval; SH, spontaneously hypertensive; SOD, superoxide dismutase; sICAM-1, soluble ICAM-1; sP-selectin, soluble platelet selectin; sVCAM-1, soluble VCAM-1; TAC, total antioxidant capacity; TLR, Toll-like receptor; TF, tissue factor; TNF, tumor necrosis factor; tPA, tissue plasminogen activator; UCAPs, concentrated ambient ultrafine particles; VCAM-1, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor

* Corresponding author.
E-mail address: frank.kelly@kcl.ac.uk (F.J. Kelly).

http://dx.doi.org/10.1016/j.freeradbiomed.2017.06.019
Received 31 January 2017; Received in revised form 2 June 2017; Accepted 28 June 2017
Available online 29 June 2017

instigation of an inflammatory response within the alveoli, causing secondary systemic inflammation via pulmonary and systemic oxidative injury which, via pro-oxidative and/or proinflammatory mediators, brings about or exacerbates cardiovascular responses [5]; (b) the translocation of ultrafine and nanosize particles and/or particle constituents (organic compounds, metals) across the alveolar membrane into the systemic circulation possibly giving rise to direct interaction and localized toxicity within the vascular endothelium and/or cardiac tissue [6,7]; (c) the activation of airway sensitive receptors or nerves and subsequent ANS imbalance [8]. At numerous points within each of these functional and molecular pathways, there is potential for cellular oxidative imbalances to occur [9]. Human studies encompassing controlled, occupational, panel and cross-sectional analyses have detected associations between exposure and biomarker molecules that are formed by the action of oxidants on lipids (eg malondialdehyde [MDA], 8-isoprostane) or nucleic acids (eg 8-oxo-7,8-dihydro-2′-deoxyguanosine [8-oxodGuo]) [10]. In addition, epidemiological studies have examined the degree to which polymorphisms in antioxidant related genes modify cardiovascular responses to air pollution [11]. These studies are in turn helping to translate research investigating potential mechanistic pathways in healthy and diseased animal models, isolated organs and cell cultures where evidence of a contributory role of OS in the target tissue has been documented [12–14]. The use of diseased or susceptible animal models (atherosclerosis, hypertension, diabetes) is particularly useful in that in such circumstances, the cellular machinery to elicit OS is already prepared if not operative.

In 2010 Brook and colleagues [1] presented persuasive evidence that oxidative stress is a critically important cause and consequence of PM-mediated cardiovascular effects. The objective of this review is to examine subsequent evidence that has assessed the importance of the pro-oxidant potential of particulate and gaseous air pollution in the development of cardiovascular disease outcomes. Apart from some key earlier findings that are included for contextual background, the studies selected for inclusion were collected through a PubMed search for articles published between January 2009 to October 2016 using the following keywords: oxidative stress OR oxidative potential AND air pollution OR PM OR ozone (O_3) OR carbon monoxide (CO) OR nitrogen dioxide (NO_2) AND cardiovascular OR cardiovascular disease OR myocardial OR heart OR cardiac OR stroke OR heart rate OR arrhythmia OR heart rate variability OR autonomic OR sympathetic OR atherosclerosis OR vascular OR blood pressure OR hypertension OR diabetes OR metabolic OR thrombosis OR coagulation. Studies investigating the effects of particulate air pollution encompass PM in urban air, concentrated ambient particles (CAPs), diesel exhaust (DE) and PM from DE, i.e. diesel exhaust particles (DEPs). Manufactured nanoparticles are not included in this review. The titles and abstracts of over 1300 original research and review articles were examined (including studies that were identified from the reference lists of relevant review articles stemming from the primary search results). Those that were not relevant to the focus of this review were discarded, leaving 267 articles that were then appraised in detail. Of these, the studies that have been included in this review are limited to those deemed to be have produced results of special interest, thereby adding to our understanding of how OS contributes towards cardiovascular disease induced by exposure to ambient air pollution.

2. Endothelial dysfunction

The endothelium forms a single layer of cells lining the inner surface of blood vessels, thereby forming a natural interface between the vascular wall and systemic circulation. By ensuring a quiescent vascular blockade to inflammation, cellular proliferation and thrombosis, and through the synthesis and release of active mediators, the endothelium is a key player in regulating vasomotor function i.e. the ability of blood vessels to dilate and contract. This is a complex process, involving a fine balance between biological mediators, of which one of the most important is nitric oxide (NO), owing to its many protective functions that include relaxation of underlying vascular smooth muscle to control blood flow through arteries and blood pressure, inhibition of smooth muscle proliferation and remodeling, regulation of blood clotting and inhibition of circulating inflammatory cells [15–17]. It is not surprising therefore that abnormal endothelial function is one of the major pathways leading to pathological changes in the cardiovascular system as a consequence of an array of perturbations including fibrinolytic imbalance, aggregation of platelets and subsequent thrombogenesis and atheroma formation. In fact endothelial dysfunction is one of earliest events in the formation of an atheroma and the magnitude of endothelial dysfunction correlates with the extent of atherosclerosis [18]. Endothelial activation, characterized by increased inflammation, is an early event in endothelial dysfunction and involves increased expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1). These adhesion molecules promote monocyte adhesion onto the endothelium, their differentiation into macrophages, migration to the intima and subsequent transformation to lipid-laden foam cells [19–21]. The expression of ICAM-1 and VCAM-1 on endothelial cells is thus an early event in the development of atherosclerosis.

Robust evidence demonstrates that vascular OS following ambient pollution (particularly particulate) exposure is central in the pathogenesis of endothelial-dependent vasomotor dysfunction (Table 1). Endothelial dysfunction in association with OS can stem from the production of the superoxide anion radical ($O_2^{\bullet-}$) via activation of nicotinamide adenine dinucleotide phosphate, reduced (NADPH) oxidases that are widely distributed within the heart. The superoxide anion radical promotes oxidative degradation of the essential NO synthase cofactor, tetrahydrobiopterin (BH4), leading to uncoupling of endothelial nitric oxide synthase (eNOS) and $O_2^{\bullet-}$ (rather than NO) generation [5,22]. Scavenging of NO by $O_2^{\bullet-}$ therefore not only generates harmful peroxynitrite as a product of the reaction [23], but importantly also leads to a compromise in the diverse actions of NO.

2.1. Human studies

To gain insight into relevant biological pathways for the association between PM and adverse cardiovascular outcomes, Madrigano et al. [24] examined the potential impact of antioxidant gene polymorphisms on the relationship between black carbon (BC) and $PM_{2.5}$ (particulate matter less than 2.5 μm in diameter) exposure and serum concentrations of soluble ICAM-1 (sICAM-1) and soluble VCAM-1 (sVCAM-1) in 809 participants of the Normative Aging Study. Whilst ambient $PM_{2.5}$ concentrations were not associated with changes in sICAM-1 or sVCAM-1, associations between BC and sVCAM were magnified in subjects with a glutathione S-transferase M1 (GSTM1*)* deletion. A genetic score approach, investigating interactions between relevant pathways and the environment, also suggests that OS plays a role in the association of ambient particles derived from oil combustion and endothelial dysfunction [25,26]. Research examining the association between PM oxidative potential (OP; the capacity of particles to cause damaging oxidative reactions) and adverse health outcomes is in its infancy, but a cohort panel study in 93 elderly non-smoking adults suggests that short-term exposures to traffic-related air pollutants with a high OP contribute to microvascular endothelial dysfunction, represented by reactive hyperemia index (RHI) [27] (Fig. 1). Finally in 12 healthy subjects, acute (2 h) experimental exposure to DE (300 $\mu g/m^3$ $PM_{2.5}$), a major component of near-road PM, impaired NO–mediated endothelial vasomotor function and promoted $O_2^{\bullet-}$ production (lucigenin chemiluminescence) in human umbilical vein endothelial cells (HUVEC) pre-incubated with serum from 5 of the subjects [28].

2.2. Animal studies

An OS pathway has also been implicated in vasomotor dysfunction

Table 1
Studies linking air pollution exposure to endothelial dysfunction mediated by oxidative stress.

Human

Study [reference]	Population/design	Air pollutant	Main findings
Madrigano et al. [24]	809 participants of NAS cohort	Ambient $PM_{2.5}$ and BC	Association between ambient BC and sVCAM−1 modified by GSTM1 deletion
Bind et al. [25]	922 participants of NAS cohort	Ambient $PM_{2.5}$ and BC	Significant associations between particle number and fibrinogen among participants with higher genetic scores within OS pathway
Dai et al. [26]	712–739 participants of NAS cohort	Ambient $PM_{2.5}$ species & sources	Associations between particles derived from oil combustion & ICAM−1 and VCAM−1 might be stronger in individuals with higher allelic risk profiles related to OS
Zhang et al. [27]	93 adults of an elderly cohort	Ambient traffic-related pollutants	Traffic-related air pollutants with high OP are major components contributing to microvascular dysfunction
Wauters et al. [28]	Randomized, controlled crossover study in 12 healthy male adults	Ambient air or DE (300 μg/m^3 $PM_{2.5}$); 120 min	DE impaired NO–mediated endothelial vasomotor function (hyperemic provocative tests) & promoted $O_2^{\bullet-}$ generation (lucigenin chemiluminescence) in endothelial cells

Animal

Study [reference]	Animal model	Air pollutant exposure	Main findings (exposed versus control)
Cherng et al. [22]	Sprague-Dawley rats	DE (300 μg/m^3 PM); 5 h	Impaired Ach-mediated relaxation and increased $O_2^{\bullet-}$ (DHT) in coronary arteries of rats; signals blocked with $O_2^{\bullet-}$ scavenging, NOS inhibition or BH4 supplementation
Ying et al. [29]	ApoE$^{-/-}$ mice	Concentrated ambient $PM_{2.5}$ plus nickel (66.5 ± 44.6 μg/m^3); 6 h/d, 5 d/ week for 3mo	Increased aortic contractile response and systemic OS (increased plasma 8-isoprostane) and decreased eNOS dimers in the aorta. Effects on NOS monomer formation in cultured endothelial cells attenuated by Tiron
Kampfrath et al. [30]	Balb/c (TLR4^{wt}) and Tlr4$^{Lps\text{-}d}$ (TLR4^{d}, background strain BALB/cAnPt)	CAP exposure (92.4 μg/m^3); 6 h/d, 5 d/wk for 20wk	Impaired aortic tonal responses and induction of NADPH oxidase-derived $O_2^{\bullet-}$ (lucigenin chemiluminescence) in monocytes, aortic tissue and perivascular fat mediated by TLR 4
Lund et al. [31]	ApoE$^{-/-}$ mice	GEE (60 μg/m^3); 6 h/d for 7d	Increased production of aortic lipid peroxidation (TBARS) and $O_2^{\bullet-}$ (DHT staining); aortic upregulation of MMP−2, MMP−9, TIMP−2, ET−1 mediated in part through activation of ET_A
Lund et al. [32]	ApoE$^{-/-}$ mice	Mixed vehicle exhaust (250 μg/m^3 PM diesel plus 50 μg/m^3 PM GE); 6 h/d for 7d	Increased production of aortic lipid peroxidation (TBARS) and $O_2^{\bullet-}$ (DHT staining); aortic upregulation of MM−9 and ET−1 mRNA expression, mediated in part through activation of LOX−1
Labranche et al. [33]	Normotensive Wistar or SH rats	IT DEP (0.8 mg) 3x/wk for 4 wk	Impaired Ach-induced relaxations and upregulation of p22phox in aortas in the SHRs only
Li et al. [34]	Wistar rats	NO_2 (5–20 mg/m^3) 6 h/d for 7 d	Decreased Cu/Zn-SOD activity, increased Mn-SOD and GPx activity, increased MDA and PCO and upregulation of ET−1
Chuang et al. [35]	C57Bl/6 mice	O_3 (0.5 ppm) 8 h/d for 5 or 5d	Decreased aortic SOD activity, eNOS protein and indices of NO production
Kodavanti et al. [36]	Wistar rats	O_3 (0.4 ppm) or DEP (2.1 mg/m^3); 5 h/day, 1 day/ week for 16 weeks	Increased aortic (but not heart) HO−1, ET−1, ET_A, ET_B, eNOS, TF, PAI−1, tPA, vWF, MMP−2, MMP−3, and TIMP−2
Paffett et al. [37]	Sprague-Dawley rats	O_3 (1 ppm) 4 h	Diminished dilatory response of coronary vascular bed to Ach, restored to different degrees by SOD, CAT and NADPH oxidase inhibition
Cui et al. [40]	C57BL/6 mice	Intranasal PM (10 μg) 3x/wk for 1 m	Reduced BMSC population, increased DCFH-DA oxidation, decreased p-AKT
Cui et al. [41]	C57BL/6 mice	Intranasal PM (10 μg) 3x/wk for 1 m	Decreased circulating EPC population, increased DCFH-DA oxidation; treatment with NAC or use of a transgenic model with overexpression of the antioxidant enzyme network effectively prevented effects

In vitro

Study [reference]	Cells/tissue	Air pollutant exposure	Main findings (exposed versus control)
Li et al. [48]	HAEC	UF DEPs (50 μg/ml)	Dose-dependent induction of OS (increased cytosolic and mitochondrial $O_2^{\bullet-}$ production and expression of HO−1) via JNK activation
Mo et al. [50]	MPMVEC	UFP (10–100 μg/ml)	NADPH is major source of oxidants generated, involving the translocation of p47phox, p67phox and rac 1 to plasma membrane
Forchhammer et al. [43]	HUVEC	Wood smoke particles (1–100 μg/ml)	Increased VCAM- 1 expression (1 μg/ml); THP−1 monocyte adhesion (50 or 100 μg/ml); unchanged intracellular production of DCFH-DA oxidation
Cao et al. [46]	HUVEC	CB (100 μg/ml)	DCFH-DA oxidation; increased VCAM−1 and ICAM−1

(continued on next page)

Table 1 *(continued)*

In vitro			
Study [reference]	**Cells/tissue**	**Air pollutant exposure**	**Main findings (exposed versus control)**
			expression unaffected by BSO pre-treatment
Frikke-Schmidt et al. [51]	HUVEC	CB (50–100 μg/ml) and DEP (10–100 μg/ml)	DCFH-DA oxidation and oxidatively damaged DNA; increased VCAM−1 and ICAM−1 expression attenuated by desferrioxamine but not vitamin C
Rui et al. [47]	HUVEC	$PM_{2.5}$ (25–200 μg/ml)	NAC prevented increased oxidant generation, attenuated phosphorylation of JNK, ERK1/2, p38 MAPK and AKT, decreased NF-κB activation and expression of ICAM−1 and VCAM−1
Du et al. [49]	HAEC	UFP (12.5–50 μg/ml)	Reduced NO production; increased eNOS S-glutathionylation; UFP-mediated reduction in NO production restored in the presence of JNK and NADPH oxidase inhibitors NAC and SOD mimetics
Miller et al. [5]	Isolated rat aortic rings	DEP (10–100 μg/ml)	Increased $O_2^{\bullet-}$ production, inhibition of endothelium-dependent NO-mediated relaxation; effects reversed in the presence of SOD
Labranche et al. [33]	isolated rat aortic rings	DEP (100 μg/ml)	Inhibited relaxations to Ach reversed in the presence of SOD
Tseng et al. [53]	HUVEC tube cells	DEP (10 and 100 μg/ml)	H_2O_2 production and release of TNF-α & IL−6 suppressed by NAC through increased endogenous glutathione

Ach: acetylcholine; BMSC: bone marrow stem cell; BSO: buthionine sulphoximine; CAT: catalase; CB: carbon black; CAP: concentrated ambient particle; DCFH-DA: dichlorodihydrofluorescein diacetate; DEP: diesel exhaust particle; DHT: dihydroethidium; eNOS: endothelial nitric oxide synthase; EPC: endothelial progenitor cell; ERK1/2: extracellular signal-regulated kinase; ET-1: endothelin-1; ET_A: ET-1-endothelial receptor A; GEE: gasoline engine exhaust; GPx: glutathione peroxidase; GSTM1: glutathione S-transferase-M1; H_2O_2: hydrogen peroxide; HAEC: human aortic endothelial cell; HO-1: hemeoxygenase-1; HUVEC: human umbilical vein endothelial cell; ICAM-1: intercellular adhesion molecule 1; IL-6: interleukin-6; IT: intratracheal; JNK: c-Jun NH2-terminal kinases; LOX-1: lectin-like oxidized low-density lipoprotein; MAPK: mitogen activated kinase; MMP: matrix metalloprotease; MPMVEC: microvascular endothelial cell; NAC: N-acetylcysteine; NADPH: nicotinamide adenine dinucleotide phosphate, reduced; NAS: Normative Ageing Study; NO: nitric oxide; NO_2: nitrogen dioxide; NOS: nitric oxide synthase; O_3: ozone; OP: oxidative potential; OS: oxidative stress; $O_2^{\bullet-}$:superoxide anion radical; PAI-1: plasminogen activator inhibitor-1; p-AKT: phosphorylated protein kinase B; PM: particulate matter; $PM_{2.5}$: particulate matter less than 2.5 μm in diameter; SH: spontaneously hypertensive; SOD: superoxide dismutase; sICAM-1: soluble ICAM-1; sVCAM-1: soluble VCAM-1; TLR: Toll-like receptor; TF: tissue factor; TIMP-2: tissue inhibitor of matrix metalloprotease-2; TNF-α: tumor necrosis factor-α; tPA: tissue plasminogen activator; UF: ultrafine; UFP: ultrafine particle; UCAP: concentrated ambient ultrafine particle; VCAM-1: vascular cell adhesion molecule; vWF: von Willebrand factor.

observed in animals following exposure to particulate air pollution. Acute DE (300 μg/m^3 PM, 5 h) inhalation impairs acetylcholine (Ach)-mediated relaxation and increases $O_2^{\bullet-}$ production (dihydroethidium [DHT] staining) in coronary arteries of rats, signals that were blocked with $O_2^{\bullet-}$ scavenging, NOS inhibition or BH4 supplementation [22]. Further evidence of NOS uncoupling comes from a murine 3-month nickel (Ni) plus CAP exposure by Ying et al. [29], which induced endothelial dysfunction, systemic OS (increased plasma 8-isoprostane) and a redox-dependent decrease in eNOS dimers in the aorta, as evidenced by reversal of the effect by the antioxidant Tiron in cultured endothelial cells. The same researchers demonstrated that impaired aortic tonal responses and induction of NADPH oxidase-derived $O_2^{\bullet-}$ (lucigenin chemiluminescence) in monocytes, aortic tissue and perivascular fat of mice following chronic CAP exposure (92.4 μg/m^3, 6 h/d, 5 d/wk for 20 weeks) is mediated by Toll like receptor 4 [30]. Oxidative stress following exposure to vehicular source air pollutants has been implicated in the imbalance of vasoactive factors produced by the endothelium. A 7-day exposure to gasoline engine exhaust (GEE; 60 μg/m^3, 6 h/d) or mixed vehicle exhaust (250 μg/m^3 PM diesel plus 50 μg/m^3 PM gasoline exhaust) was associated with increased production of lipid peroxides (TBARS) and $O_2^{\bullet-}$ (DHT staining) in the aorta of $ApoE^{-/-}$ mice along with an upregulation of circulating and vascular factors (matrix metalloproteinase [MMP]−2, MMP-9, tissue inhibitor of metalloproteinases [TIMP]−2, ET-1) mediated in part through activation of ET-1-endothelial receptor A (ET_A) and lectin-like oxidized low-density lipoprotein (LOX-1) receptor pathways [31,32]. LOX-1 is the major endothelial cell-surface receptor responsible for macrophage binding and internalization of oxidized low-density lipoprotein (oxLDL). Of interest, when normotensive and spontaneously hypertensive (SH) rats were exposed via intratracheal (IT) instillation to DEP for 4 weeks (0.8 mg, 3 times a week for 4 weeks), impaired Ach-induced relaxations, accompanied with a significant upregulation of the p22phox NADPH oxidative component in aortas, were only observed in the SH rat group, suggesting a possible synergism between DEP-induced oxidative stress and classical risk factors [33].

Endothelial dysfunction accompanied by OS in animals exposed to gaseous pollutants has also been reported [34–37]. High concentrations of NO_2 (5–20 mg/m^3, 6 h/d for 7 days) produced mild pathology in the heart of rats, and marked OS (reduction/induction of Cu/Zn-superoxide dismutase [SOD], Mn-SOD and glutathione peroxidase [GPx] activity and increased MDA and protein carbonyl [PCO]) along with an upregulation of the vasoconstrictor ET-1 [34]. Subchronic (0.5 ppm for 1 or 5 days, 8 h/day) O_3 exposure has also been shown to decrease SOD activity, eNOS protein and indices of NO production in the mouse aorta [35]. Following weekly episodic (5 h/day, 1 day/week for 16 weeks) exposure of rats to either O_3 (0.4 ppm) or DEP (2.1 mg/m^3), mRNA biomarkers of OS (hemeoxygenase-1 [HO-1], vasoconstriction (ET-1, ET_A, ET_B, eNOS) and proteolysis (MMP-2, MMP-3, and TIMP-2) were increased in the aorta, but not in the heart [36]. Twenty-four hours following a single inhalation of 1 ppm O_3, the coronary vascular bed of rats exhibited a markedly diminished dilatory response to Ach, which was restored to different degrees by SOD, catalase (CAT) and NADPH oxidase inhibition [37].

PM exposure also suppresses the number and function of bone marrow-derived endothelial progenitor cells (EPCs) [38,39]. Moreover, early research in this area suggests a mechanism related to an increased level of oxidants [40,41]. PM treated (as low as 10 μg 3 times per week for 1 month via intranasal instillation) mice exhibit a reduced bone marrow stem cell (BMSC) population in association with increased dichlorodihydrofluorescein diacetate (DCFH-DA) oxidation, decreased phosphorylated protein kinase B (p-AKT) and inhibited proliferation of BMSCs without induction of apoptosis [40]. The exposure also decreased the circulating EPC population and promoted apoptosis of EPCs in association with increased oxidant production, again detected using the DCFH-DA probe [41]. Treatment with the thiol antioxidant *N*-acetylcysteine (NAC) or use of a transgenic model with overexpression of the antioxidant enzyme network effectively prevented these effects.

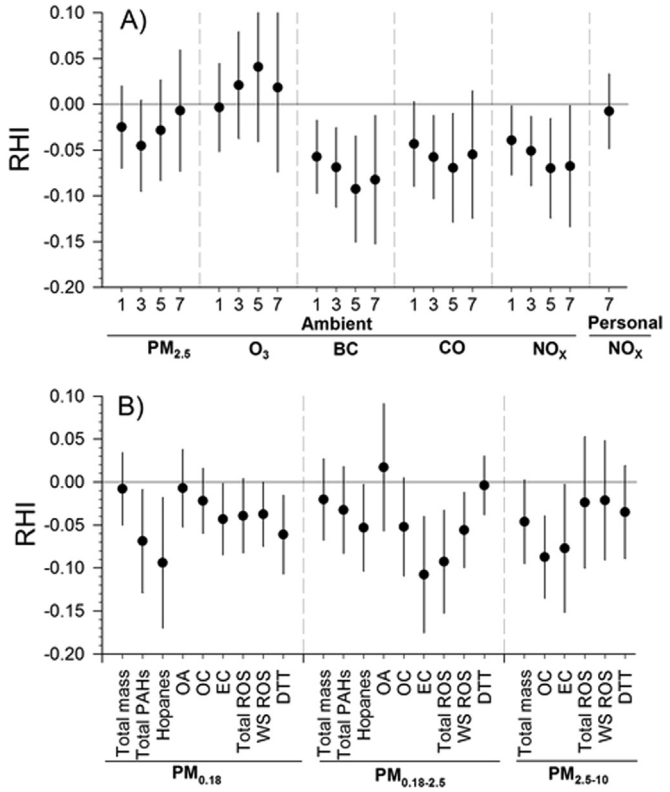

Fig. 1. Association of microvascular function with a one interquartile range increase of ambient and personal pollutants.Exposures were averaged across 1 day, 3 days, 5 days, and 7 days preceding each subject's reactive hyperemia index (RHI) measurement (A) and the PM components in three different size-fractions for exposures averaged across 5 days preceding each subject's RHI measurement (B). BC: black carbon; DTT: dithiothreitol; EC: elemental carbon; OA: Organic acids; OC: organic carbon; PAHs: polycyclic aromatic hydrocarbons; PM particulate matter; ROS: reactive oxygen species; WS: water-soluble.Reproduced from Environmental Health (Zhang et al. [27]) (http://creativecommons.org/licenses/by/4.0/).

2.3. In vitro studies

Cell culture work with end points encompassing cell activation, altered expression and production of messenger molecules and NO are shedding further light on the mechanism by which various sources of particulate air pollution induce OS and subsequent dysfunction in vascular endothelial cells [42–50]. Ultrafine particles emitted from diesel engines have been shown to dose-dependently induce OS (increased cytosolic and mitochondrial $O_2^{\bullet-}$ production and expression of HO-1) via c-Jun NH2-terminal kinases (JNK) activation in HAEC [48], whilst in mouse pulmonary microvascular endothelial cells (MPMVEC), NADPH has been demonstrated to be the major source of oxidants generated (DCFH-DA oxidation) following ambient UFP exposure, involving the translocation of the cytosolic proteins p47phox, p67phox and rac 1 to the plasma membrane [50]. On investigating the subsequent activation of endothelial cells by UFP-induced oxidants, these investigators demonstrated that this was mediated through phosphorylation of p38 mitogen activated kinase (MAPK) and extracellular signal-regulated kinase (ERK1/2). The role of particulate-generated-oxidants in the increased expression of ICAM-1 and VCAM-1 as well as promoted adhesion of monocytes onto endothelial cells has also been investigated [43,46,47,51,52]. Wood smoke particles (1 μg/ml) increased VCAM- 1 expression on HUVECs and at 50 or 100 μg/ml caused adhesion of THP-1 monocytes without having any effect on DCFH-DA oxidation [43]. Although increased oxidants were observed in HUVECs after exposure to DEP and CB nanoparticles, results from inhibitor studies indicate that this is not a prerequisite for increased adhesion factor expression and foam cell formation, [46,51]. However using NAC and selective kinases inhibitors, Rui et al. [47] demonstrated that $PM_{2.5}$-induced increase in intracellular oxidants generation triggers the cell surface expression of ICAM-1 and VCAM-1 and adhesion of THP-1 cells to HUVECs through JNK, ERK1/2, p38 MAPK and AKT phosphorylation, and nuclear translocation of NF-κB in human EA.hy926 cells in a dose- and time- dependent manner. Oxidative stress has also been implicated in an alternative mechanism underlying the regulation of eNOS activity by ambient UFP, namely particulate pollutant-induced increase in glutathione oxidation, protein S-glutathionylation, and eNOS S-glutathionylation, leading to a decrease in NO production [49]. In that the UFP-mediated reduction in NO production was restored in the presence of JNK and NADPH oxidase inhibitors, NAC and SOD mimetics, supports the previous reports that UFP induced vascular OS via NADPH oxidase and JNK activation [48,50]. Investigations into the mechanisms behind vascular permeability exerted by DEP, using an endothelial tube model, has also identified the importance of OS. This is accompanied by the release of pro-inflammatory tumor necrosis factor (TNF)-α and interleukin (IL)−6 from tube cells, subsequently stimulating the secretion of vascular endothelial growth factor (VEGF)-A – a cascade of events that could result in the disruption cell-cell borders and increase vasculature permeability [53]. Exposure of isolated rat aortic rings (thereby ruling out prior interaction with the lung or vascular tissue) to DEP (10–100 μg/ml) generates $O_2^{\bullet-}$ and inhibits endothelium-dependent NO-mediated relaxation, whilst these effects are reversed in the presence of SOD [5,33].

3. Atherosclerosis

Atherosclerosis is a progressive disease of the vasculature, characterized by accumulation of lipids and fibrous material (atherosclerotic plaques) within arteries over a prolonged period of time [54]. An early event in atherogenesis is endothelial cell injury described in the previous section and with that, increased expression of adhesion molecules and secretion of chemoattractant cytokines that induce monocyte attachment and migration into the intima and sub-endothelial space respectively [20,21]. Monocyte differentiation into macrophages ensues and with that, engulfment of normal and oxo-LDL, primarily mediated by CD36 (also known as oxoLDL receptor), a macrophage scavenger receptor [55]. The oxidative modification of LDL accelerates uptake by macrophages and in doing so promotes the formation of lipid-laden foam cells – the landmark in the development of atherosclerosis. These progenitor lesions eventually develop into atherosclerotic plaques as a consequence of further uptake of LDL, smooth muscle cell proliferation and the development of a collagenous fibrous cap [20,21,56]. The presence of advanced lesions leads to a narrowing of the artery, resulting in reduced blood flow to tissues (ischemia). Moreover, the plaques can become unstable and rupture, leading to onset of myocardial infarction or stroke.

The biological pathways involved in atherosclerosis following exposure to air pollution are undoubtedly complex, dependent upon various cell types and an array of molecular mediators related to coagulation, blood lipids, inflammation and OS. Indeed, the observation of increased oxidants in many types of endothelial cells makes OS a key candidate for initiating atheroma development following cellular dysfunction. In the sections that follow, evidence of contributory effects, such as enhancing, through oxidation, the pro-atherogenic properties of LDLs and plaque vulnerability and diminishing the anti-atherogenic function of protective high-density lipoproteins HDL(s), are described (Table 2). Measures of oxidation in circulating blood are highly relevant since they imply the involvement of oxidatively modified plasma LDL and/or HDL, key players in the promotion or protection from atherosclerosis, respectively [57].

3.1. Human studies

A panel of 40 healthy university students relocating between two

campuses with different air pollution scenarios in Beijing, China provides evidence that certain $PM_{2.5}$ chemical constituents/pollution sources are more closely associated with changes in biomarkers of OS associated with atherosclerosis [58]. Whilst $PM_{2.5}$ iron and Ni and $PM_{2.5}$ from traffic emissions and coal combustions were positively associated with ox-LDL, calcium was associated with an increase in soluble CD36. Other studies examining the relationship between air pollution exposure and OS, as indicated by higher levels of plasma ox-LDL, have also found positive associations in occupationally exposed individuals, patients with diabetes and children [48,59–61]. The study by Brucker et al [61] reported increased urinary 1-hydroxypyrene (1-OHP; a biomarker of exposure to polycyclic aromatic hydrocarbons [PAHs] from traffic emissions) levels in taxi drivers that were positively correlated with ox-LDL and homocysteine (Hcy) but negatively correlated with antioxidants (CAT and GST). In 2348 participants living in London, the strength of association between intima-media thickness, a measure of subclinical atherosclerosis, and PM_{10} mass concentration was stronger than that for the OP of PM_{10}, possibly however owing to OP assay underestimating the total oxidative burden of PM [62]. In 22 randomly selected participants in the Normative Aging Study cohort, a significant association was found between long-term ambient $PM_{2.5}$ exposures and levels of multiple extracellular vesicle-encapsulated microRNAs (evmiRNAs) circulating in serum [63]. Extracellular vesicles are double-lipid membrane vesicles that play an important role in the cell-to-cell communication process by transporting biologically active molecules such as non-coding micro RNA (miRNA) molecules that repress target gene expression by translational inhibition or mRNA degradation. In silico pathway analysis on $PM_{2.5}$-associated evmiRNAs identified several key related pathways, including OS, inflammation and atherosclerosis.

3.2. Animal studies

The use of transgenic mouse models (deficient in ApoE [$ApoE^{-/-}$] or LDL [$LDL^{-/-}$] receptors) in demonstrating the role of a localized oxidative insult in the development and/or enhancement of atherosclerosis following PM exposure is well established [64,65]. More recently, evidence stemming from earlier studies has been strengthened by research investigating the molecular pathways through which OS operates following experimental exposure to different types of PM. For DE, Bai et al [66] showed that exposure (200 $\mu g/m^3$; 6 h/day, 5 days/week for 7 weeks) to $ApoE^{-/-}$ mice augmented plaque lipid content, cellularity, foam cell formation and smooth muscle as well as increasing expression of plaque OS markers, iNOS, CD36, and 3-nitrotyrosine (3-NT; the well documented biomarker of peroxynitrite [23]), and enhancing systemic lipid and DNA oxidation detected by urinary 15-F2t-isoprostane (15-F2t-IsoP) and 8-oxodGuo respectively. Miller et al [67] employed a DEP instillation study at a dose (35 μg twice a week for 4 weeks) representing the upper range a person may be exposed to in a heavily polluted city over 24 h. This regimen increased plaque size, number, lipid rich area and frequency of buried fibrous caps in ApoE-/- mice, which despite the lack of systemic inflammation, correlated with lung inflammation and antioxidant gene expression in livers (HO-1, NADPH-quinone oxidoreductase 1 and NF-E2-related factor-2), indicative of a counter-regulatory response to systemic pro-oxidative effects. Lipid peroxidation in plasma and liver following inhalational exposure of $ApoE^{-/-}$ mice to DE (250 $\mu g/m^3$, 2 weeks) was associated with impaired HDL anti-oxidant capacity [68]. UFP exposure has also been shown to trigger reduced HDL antioxidant capacity, pro-atherogenic lipid metabolism and a greater atherosclerotic lesion in $LDLR^{-/-}$ mice, whilst D-4F (an apolipoprotein A-1 mimetic peptide) attenuated these effects, suggesting a role for lipid oxidation in UFP-mediated atherosclerosis [69]. Inhalational exposure of $ApoE^{-/-}$ mice to environmental air pollutants from vehicular sources (250 $\mu g/m^3$ diesel PM and 50 μg PM/m^3 gasoline exhausts; 6 h/d for 7 days) resulted in LOX-1-mediated vascular OS (TBARS; DHT staining), expression of MMP-9 and ET-1 and monocyte/macrophage infiltration, all of which, as discussed above, are associated with progression of atherosclerosis and atherosclerotic plaque rupture [32]. In a Manhattan CAP exposure (6 h/day, 5 days/week, 4 months) study using $ApoE^{-/-}$ mice, Ying et al [70] demonstrated that ambient PM enhances atherosclerosis through the NADPH oxidase–dependent induction of $O_2^{\bullet-}$ and reactive nitrogen species in the aorta, causing decreased guanine cyclase–dependent arterial constriction in response to phenylephrine.

3.3. In vitro studies

The pro-oxidative effects of PM has been observed in a number of cell types that are key players in the development of atherosclerotic lesions including endothelial cells [46,48,71], macrophages/monocytes [46] and possibly smooth muscle cells [72]. Increased OS (DCFH-DA oxidation) may not however completely explain particle-induced lipid accumulation [46,73]. Whilst exposure of THP-1 derived human macrophages to CB nanoparticles (25 μg/ml 24 h) increased cellular lipid load suggesting that the monocytes were transforming into foam cells, this occurred at concentrations lower than those required to trigger increased intracellular oxidant production [46]. Furthermore, whilst the presence of the antioxidant buthionine sulphoximine (BSO) increased the CB-induced DCFH-DA oxidation, it showed no effect on particle-induced lipid accumulation. Automobile DEP (10 μg/ml, 24 h) has also been demonstrated to induce lipid droplet formation in macrophages but again, at concentrations that were not associated with increased generation of oxidants [73].

4. Pro-coagulant changes

If an atherosclerotic plaque becomes physically disrupted, the procoagulant material within its core is exposed to coagulation proteins in the circulating blood and this triggers thrombosis (blood clot formation) that can block an artery. Thrombus formation thus underlies the acute complications of atherosclerosis, increasing the risk of potentially fatal events such as myocardial infarction, stroke or limb ischemia. Studies evaluating the involvement of OS in effects that air pollution exposure on triggering thrombotic cardiovascular events are discussed below (Table 3).

4.1. Human studies

In a panel of 60 elderly subjects with coronary artery disease, traffic-related air pollutants were associated with increased systemic inflammation, soluble platelet selectin (sP-selectin; a biomarker of thrombosis) and decreased erythrocyte antioxidant enzyme activity (GPx1 and Cu,ZnSOD) [74]. Within subject inverse associations of sP-selectin with Cu,ZnSOD were observed in mixed models. Changes in gene expression connected with key OS and coagulation pathways have also been identified in a group of 14 young healthy subjects exposed to DE (300 $\mu g/m^3$, 60 min on 2 separate days) in a controlled setting [75]. A decrease in proteins involved in the fibrinolytic pathway (plasminogen and thrombomodulin) was detected following a 2-h controlled exposure of 34 subjects with metabolic syndrome (MeS) to concentrated ambient ultrafine particles (UCAPs), however GSTM1-individuals were not more responsive than the entire study population [76]. Furthermore on investigating coagulation pathways in a panel of 31 healthy, young volunteers semi-experimentally exposed for 5 h to ambient air pollution at 5 locations with contrasting air pollution characteristics, PM_{10} OP did not display a strong or consistent association with the examined endpoints [77]. As discussed by the authors, this could potentially reflect the fact that the OP assay employed only examined a fraction of PM in vivo biological activity.

Table 2
Studies linking air pollution exposure to atherosclerosis mediated by oxidative stress.

Human studies

Study (reference)	Population/design	Air pollutant	Main findings
Wu et al. [58]	Panel of 40 healthy university students	Ambient $PM_{2.5}$ chemical constituents/pollution sources	$PM_{2.5}$ Fe and Ni and $PM_{2.5}$ from traffic emissions and coal combustions positively associated with ox-LDL; calcium associated with an increase in soluble CD36
Brucker et al. [61]	39 taxi drivers and 21 non-occupationally exposed persons	Traffic related	Increased urinary 1-OHP levels positively correlated with ox-LDL and Hcy but negatively correlated with CAT and GST
Tonne et al. [62]	2348 participants of the Whitehall II cohort	Ambient PM_{10}	Stronger association between CIMT and PM_{10} mass concentration than that for PM_{10} OP
Rodosthenous et al. [63]	22 participants of NAS cohort	Ambient $PM_{2.5}$	Significant association between long-term ambient $PM_{2.5}$ exposures and levels of evmiRNAs circulating in serum several of which were enriched in OS inflammation and atherosclerosis pathways

Animal studies

Study (reference)	Animal model	Air pollutant exposure	Main findings (exposed versus control)
Bai et al. [66]	ApoE^{-}/$^{-}$ mice	DE (200 μg/m^3); 6 h/d, 5 d/wk for 7 wk	Augmented plaque lipid content, cellularity, foam cell formation and smooth muscle; increased expression of plaque iNOS, CD36, and 3-NT, and enhanced systemic lipid and DNA oxidation (15-F2t-IsoP and 8-oxodGuo)
Miller et al. [67]	ApoE^{-}/$^{-}$ mice	DEP (35 μg) 2 x/wk for 4 wk	Increased plaque size, number, lipid rich area and frequency of buried fibrous caps; correlated with liver HO−1, NADPH-quinone oxidoreductase 1 and NF-E2-related factor−2 gene expression
Yin et al. [68]	ApoE^{-}/$^{-}$ mice	DE (250 μg/m^3) 2 wk	Lipid peroxidation in plasma (8-isoprostanes, 12-hydroxyeicosatetraenoic acid, 13-hydroxyoctadecadienoic acid) and liver (MDA) associated with impaired HDL anti-oxidant capacity
Li et al. [69]	LDLR^{-}/$^{-}$ mice	UFP (360 μg/m^3) 5 h/d, 3 d/wk, for 10 wks	Reduced HDL anti-oxidant capacity, pro-atherogenic lipid metabolism, greater atherosclerotic lesion; effects attenuated by D-4F
Lund et al. [32]	ApoE/$^{-}$ mice	DEP (250 μg/m^3) and gasoline exhaust particles (50 μg/m^3) 6 h/d for 7d	LOX−1-mediated vascular OS (TBARS; DHT staining), expression of MMP−9 and ET−1 and monocyte/macrophage infiltration
Ying et al. [70]	ApoE^{-}/$^{-}$ mice	$PM_{2.5}$ CAP exposure (138 μg/m^3) 6 h/d, 5 d/wk, for 4 mo	Increased aortic expression of p47phox, rac1 and iNOS, increased $O_2^{\bullet-}$ and protein nitration in the aorta, increased plaque area of thoracic aorta, macrophage infiltration and lipid deposition

In vitro studies

Study (reference)	Cells/tissue	Air pollutant exposure	Main findings (exposed versus control)
Cao et al. [46]	THP−1 derived human macrophages	CB (25 μg/ml)	Increased cellular lipid load at concentrations lower than those required to trigger intracellular DCFH-DA oxidation; BSO increased CB-induced oxidants but showed no effect on particle-induced lipid accumulation.
Cao et al. [73]	THP−1 derived human macrophages	DEP (10 μg/ml)	Induce lipid droplet formation at concentrations not associated with increased DCFH-DA oxidation

1-OHP: 1-hydroxypyrene; 3-NT: 3-nitrotyrosine; 8-oxodGuo: 8-oxo-7,8-dihydro-2'-deoxyguanosine; 15-F2t-IsoP: 15-F2t-isoprostane; BSO: buthionine sulphoximine; CAP: concentrated ambient particle; CAT: catalase; CB: carbon black; CD36: oxoLDL receptor; CIMT: carotid intima-media thickness; DCFH-DA: dichlorodihydrofluorescein diacetate; DE: diesel exhaust; DEP: diesel exhaust particle; DHT: dihydroethidium; ET-1: endothelin-1; Fe: iron; GST: glutathione transferase; Hcy: homocysteine; HDL: high density lipoprotein; HO-1: hemeoxygenase-1; iNOS: inducible nitric oxide synthase; LOX-1: lectin-like oxidized low-density lipoprotein; MDA: malondialdehyde; MMP: matrix metalloprotease; NADPH: nicotinamide adenine dinucleotide phosphate, reduced; Ni: nickel; NAS: Normative Ageing Study; OP: oxidative potential; OS: oxidative stress; $O_2^{\bullet-}$: superoxide anion radical; oxLDL: oxidized low-density lipoprotein; $PM_{2.5}$: particulate matter less than 2.5 μm in diameter; PM_{10}: particulate matter less than 10 μm in diameter; UFP: ultrafine particle.

4.2. Animal studies

Gaseous and particulate air pollution exposures in animals have been shown to be associated with both OS and disturbed coagulatory hemostasis. These include an effect of an ultrafine carbon particle (180 μg/m^3, 24 h) inhalation on pulmonary and systemic inflammation in aged SH rats that was associated with increased pulmonary expression of HO-1 as well as systemic changes in biomarkers of thrombosis (fibrinogen, TF) [78]. In rats exposed to O_3 (0.4 ppm) or DEP (2.1 mg/m^3) for 16 weeks, biomarkers of OS (HO-1) and thrombosis (tissue factor [TF], plasminogen activator inhibitor-1 [PAI-1], tissue plasminogen activator [tPA], and von Willebrand factor [vWF]) were increased in the aorta, but not in the heart, possibly triggered by oxidatively modified lipids and proteins through LOX-1 and/or RAGE signaling [36]. Pre-treatment of mice with the cysteine (the amino acid that is limiting for GSH synthesis) prodrug l-2-oxothiazolidine-4-carboxylic acid, before IT instillation of DEP (30 μg), suggests that OS is at least partly responsible for the pulmonary and systemic inflammation and thrombotic events in the pial cerebral microvessels (intracranial vessels on the surface of the brain within the pia–arachnoid) [79]. Similarily, emodin (1,3,8-trihydroxy-6-methylanthraquinone; an antraquinone that is extracted from the root of the rhubarb plant), which has strong antioxidant and anti-inflammatory effects, ameliorated the following effects of DEP (1 mg/kg): increased heart tissue levels of IL-1β and TNF, decreased SOD and glutathione reductase activities and the prothrombotic effect of DEP in pial arterioles and venules [80]. Furthermore, emodin prevented platelet aggregation in vitro in whole blood, and the shortening of activated partial thromboplastin time and prothrombin time caused by DEP.

4.3. In vitro studies

A causal link between the oxidative effects of PM exposure and procoagulant responses has also been suggested in human endothelial cells following exposure to soluble UFPs [81]. The exposure induced thrombin generation and fibrin clot formation via TF upregulation,

Table 3
Studies linking air pollution exposure to pro-coagulant changes mediated by oxidative stress.

Human

Study [reference]	Population/design	Air pollutant	Main findings
Delfino et al. [74]	Panel study of 60 elderly subjects with coronary artery disease	Traffic-related air pollutants	Association with increased systemic inflammation and sP-selectin and decreased erythrocyte GPx1 and Cu,ZnSOD activity
Pettit et al. [75]	Controlled exposure; 14 young healthy subjects	DE (300 μg/m^3) 60 min on 2 separate days	Changes in gene expression connected with key OS and coagulation pathways
Devlin et al. [76]	Controlled exposure; 34 individuals with MeS	UCAP (98 μg/m^3) 2 h	Changes in plasminogen and thrombomodulin in GSTM1 null individuals not more responsive than the entire study population
Strak et al. [77]	Semi-experimental exposure; 31 healthy young volunteers	Ambient air pollution at 5 locations with contrasting air pollution characteristics for 5 h	No strong or consistent association between coagulation and PM_{10} OP

Animal

Study [reference]	Animal model	Air pollutant exposure	Main findings (exposed versus control)
Upadhyay et al. [78]	Aged SH rats	Ultrafine carbon particle (180 μg/m^3) 24 h	Pulmonary and systemic inflammation associated with increased pulmonary expression of HO−1 and systemic changes in fibrinogen and TF
Kodavanti et al. [36]	Wistar rats	O_3 (0.4 ppm) or DEP (2.1 mg/m^3) for 16 weeks	Increased aortic HO−1, PAI−1, tPA and vWF, possibly triggered by oxidatively modified lipids and proteins through LOX−1 and/or RAGE signaling
Nemmar et al. [79]	Tuck Ordinary mice pre-treated with cysteine prodrug OTC	DEP (IT, (15 or 30 μg)	Macrophage and neutrophil influx in BALF, elevated TEAC, decreased plasma TEAC, shortened bleeding time, platelet proaggregatory effect in pial cerebral venules; effects reversed by OTC
Nemmar et al. [80]	Tuck Ordinary mice pre-treated with emodin	DEP (IT, 1 mg/kg)	Cardiac tissue: increased IL−1β and TNF, decreased SOD and glutathione reductase activities; pial arterioles and venules: prothrombotic effect; whole blood: platelet aggregation and shortening of activated partial thromboplastin time and prothrombin time. All effects prevented by emodin

In vitro

Study [reference]	Cells/tissue	Air pollutant exposure	Main findings (exposed versus control)
Snow et al. [81]	HAEC	Soluble UFP (10, 50 and 100 μg/ml)	Earlier thrombin generation and fibrin clot formation abolished by an anti-TF antibody, increased intracellular H_2O_2 production involving NOX−4 enzyme, antioxidants attenuated UFP-induced upregulation of TF
Chiarella et al. [82]	Murine alveolar macrophages	CAP (10 μg/m^2)	PM induced mitochondrial oxidant generation primes adenylyl cyclase, enhancing β_2AR-mediated generation of cAMP and phosphorylation CREB to augment *IL6* transcription NB in mice, β_2AR signaling promotes prothrombotic state sufficient to accelerate arterial thrombosis

β_2-AR: β_2-adrenergic receptor; BALF: bronchoalveolar lavage fluid; cAMP: Cyclic adenosine monophosphate DE: diesel exhaust; CREB: cAMP response element-binding protein; GPx: glutathione peroxidase; GSTM1: glutathione S-transferase-M1; H_2O_2: hydrogen peroxide; HAEC: human aortic endothelial cell; HO-1: hemeoxygenase-1; IL-1β: interleukin-1β; IT: intratracheal; LOX-1: lectin-like oxidized low-density lipoprotein; MeS: metabolic syndrome; O_3:ozone; OP: oxidative potential; OS: oxidative stress; OTC: l-2-oxothiazolidine-4-carboxylic acid; PAI-1: plasminogen activator inhibitor-1; RAGE: receptor for advanced glycation end products; SH: spontaneously hypertensive; SOD: superoxide dismutase; TEAC: Trolox equivalent antioxidant capacity; TF: tissue factor; TNF: tumor necrosis factor; tPA: tissue plasminogen activator; UCAP: concentrated ambient ultrafine particle; vWF: von Willebrand factor.

involving intracellular hydrogen peroxide (H_2O_2) production and the NOX-4 isoform of NADPH oxidase. Exemplifying the likely overlapping effects of cellular oxidative imbalances on cardiovascular events, mitochondrial OS is believed to play a role in a recently uncovered mechanism by which activation of the sympathetic nervous system following particulate air pollution exposure increases the risk of thrombotic cardiovascular events [82]. In an elegant and extensive series of animal and in vitro experiments utilizing non-selective and mitochonrial antioxidants as well as electron transport chain inhibitors, Chiarella et al [82] describes a systemic increase of catecholamine induced by CAPs ($PM_{2.5}$) that augments the release of IL-6 from lung macrophages via a pathway that requires mitochondrial oxidants and adenylyl cyclase, and cAMP response element-binding protein, which in turn contributes to a hypercoagulable state (Fig. 2).

5. Autonomic nervous system dysfunction

One way in which the body responds to environmental stressors is by triggering autonomic reflexes in pulmonary receptors, baroreceptors and chemoreceptors. Indeed, modulation of autonomic neural input to the heart and vasculature following direct activation of sensory nerves in the respiratory system, is thought to be a key mechanistic pathway by which exposure to air pollution affects cardiovascular health indicators - especially acute outcomes [8]. Moreover, there is evidence that these effects may be mediated through OS related mechanisms (Table 4). Much of the evidence linking changes in cardiac autonomic tone with exposure to air pollution comes from studies of heart rate variability (HRV). Heart rate variability is the measurement of variation in the time interval between heartbeats, and is a quantitative and non-invasive indicator of the balance between the sympathetic and parasympathetic branches of the ANS and hence cardiovascular disease. High HRV is traditionally considered positive because the heart has the ability to respond to rapidly changing environments. Low HRV, reflecting increased sympathetic tone, is associated with a heightened risk of cardiac arrhythmia and mortality in people with heart disease.

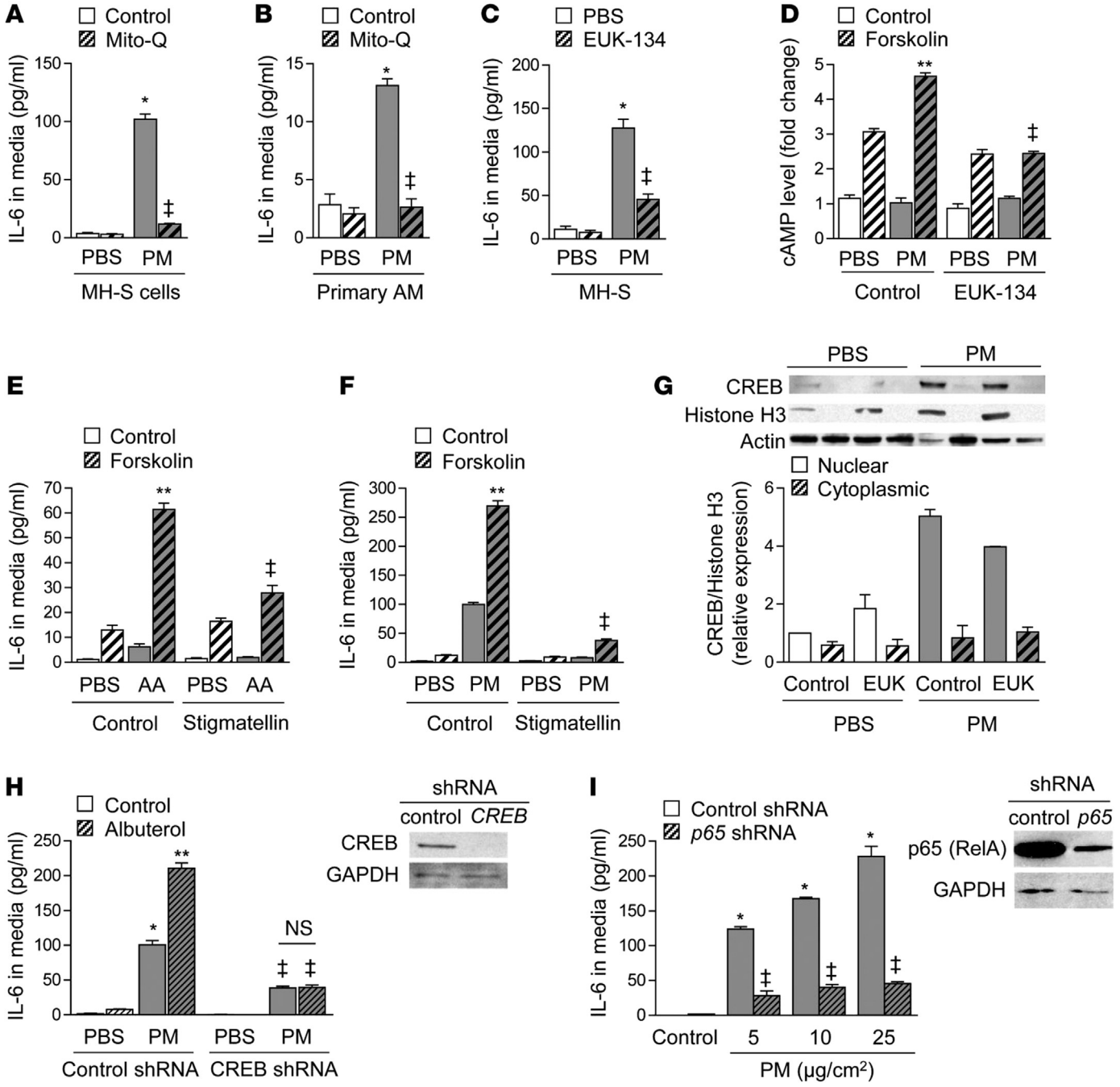

Fig. 2. PM-induced oxidant generation and priming of adenylyl cyclase are required for β_2 agonist–mediated worsening of IL-6 release. (**A**) MH-S cells and (**B**) primary alveolar macrophages (AM) were treated with PM (10 μg/cm^2) or control (medium) and IL-6 levels were measured in the absence or presence of a mitochondrially targeted antioxidant (Mito-Q or control [TPP]) or (**C**) a nontargeted antioxidant (EUK-134) (20 μM). (**D**) MH-S cells were treated with PM or control and with forskolin (50 μM) or control and cAMP levels were measured 1 min after forskolin treatment. (**E**) MH-S cells were treated with antimycin A (AA) (1 μM) or vehicle and with forskolin (50 μM) and IL-6 was measured in the medium 24 h later in the absence or presence of stigmatellin (1 μM). (**F**) MH-S cells were treated with PM or control and with forskolin (50 μM) or control and IL-6 levels were measured 24 h later in the absence or presence of stigmatellin. (**G**) MH-S cells were treated with PM or control and with EUK-134 or control and immunoblotting was performed against cAMP CREB in the nuclear and cytoplasmic fractions 4 h later. (**H**) IL-6 levels were measured in control and CREB shRNA–transfected MH-S cells after treatment with PM or PBS. (**I**) Control and p65-shRNA–transfected cells were treated with PM or control and with albuterol or control and IL-6 levels were measured. *P < 0.05, PM vs. PBS; **P < 0.05, albuterol or forskolin vs. control; ‡P < 0.05, Adrb1$^{+/+}$Adrb2$^{-/-}$ vs. Adrb1$^{+/+}$Adrb2$^{+/+}$, AC inhibitor, mito-Q, EUK-134, or stigmatellin vs. control, CREB or p65 vs. control shRNA. Reprinted with permission from Chiarella et al. [82].

5.1. Human studies

In the initial epidemiological studies using elderly male participants of the Normative Aging Study, functional genetic variations in GSTM1, HMOX-1 and HFE, all of which are involved in defense against OS, modified susceptibility to $PM_{2.5}$ induced changes in HRV [83–85]. More recently, Baja et al [86] reported a stronger association between the heart-rate–corrected QT interval (QTc; a marker of ventricular repolarization and risk factor for ventricular arrhythmias and sudden cardiac death) and elevated short-term exposure to BC among 580 Normative Aging Study participants who had a high number of unfavorable genotypes related to OS, as well those who were obese or diabetic. Another study conducted among a panel of elderly Asian residents using genetic risk scores again suggests that the association of

Table 4
Studies linking air pollution exposure to autonomic nervous system dysfunction mediated by oxidative stress.

Human

Study [reference]	Population/design	Air pollutant	Main findings
Baja et al. [86]	580 NAS participants of NAS cohort	Traffic-related pollution	Stronger association between QT and elevated short-term exposure to BC among subjects with high number of unfavorable genotypes related to OS, as well as in non-smokers, obese individuals or diabetics
Kim et al. [87]	Panel of 547 elderly Asian residents	Ambient PM_{10}, SO_2, NO_2	Genetic risk scores suggests association of air pollution with HRV are mediated by OS pathways
Wang et al. [88]	76 patients with a recent coronary event	Ambient $PM_{2.5}$	TAC does not appear to modify the association between short-term ambient $PM_{2.5}$ concentrations and HRV/ repolarization biomarkers
Zhang et al. [90]	125 healthy young subjects	Ambient air pollutants pre, during and post Beijing Olympics	Improved air quality brought about by the 2008 Olympic Games in Beijing was not mirrored by a change in HRV measurements despite decreases observed in urinary 8-oxodGuo
Lee et al. [91]	44 adults	Personal $PM_{2.5}$	OS modified the association between personal $PM_{2.5}$ exposure and HRV
Sarnat et al. [92]	21 asthmatic and 21 non-asthmatic subjects	2-h highway commute during rush hour	Slight and insignificant elevation of exhaled MDA levels in subjects who experienced decreases in HRV following
Hemmingson et al. [93]	Controlled exposure; 60 overweight middle-aged, elderly adults	real-life levels of $PM_{2.5}$ from an urban street (24 μg/m^3) for 5 h	Reduced HRV and vasomotor dysfunction; effects not associated with OS biomarkers (dihydrobiopterin, biopterin, uric acid, ascorbic acid, dehydroascorbate)
Tong et al. [96]	Controlled exposure; 29 healthy middle-aged adults supplemented with fish oil or olive oil (3 g/day for 4 weeks)	CAP (278 ± 19 μg/m^3) for 2 h	Fish oil supplementation attenuated CAP-induced changes in HRV and cardiac repolarization in
Byun et al. [94]	Occupational exposure; 48 workers in a boilermaker unit	Metal-rich $PM_{2.5}$ from welding	Blood mtDNA methylation levels negatively associated with $PM_{2.5}$ exposure; greater susceptibility of workers with higher levels of mtDNA methylation to effect of $PM_{2.5}$ exposure on HRV measures

Animal

Study [reference]	Animal model	Air pollutant exposure	Major findings (exposed versus control)
Kim et al. [98]	Sprague–Dawley rats	DEP (ET, 200 μg/ml) for 30 min and perfused rat hearts (12.5 μg/ml for 20 min)	Action potential duration prolongation, early afterdepolarization and ventricular arrhythmia - all prevented by pretreatment with NAC as well as active Ca^{2+}/calmodulin-dependent protein kinase II blockade
Robertson et al. [99]	Wistar ats	DEP (IT, 0.5 mg)	Oxidant stress (EPR) of heart perfusate before IR, increased vulnerability to ischemia-associated arrhythmia
Wang et al. [100]	Wistar rats	$PM_{2.5}$ (0.2, 0.8, 3.2 mg) and/or O_3 (0.81 ppm)	O_3 potentiated $PM_{2.5}$-induced increase in heart MDA and decreased HRV
Andre et al. [101]	Wistar rats	CO (30 ppm) 12 h, including five 1 h peaks at 100 ppm for 4 wks	Increased ventricular MDA, altered Ca^{2+} homeostasis and ventricular arrhythmia

8-oxodGuo: 8-oxo-7,8-dihydro-2'-deoxyguanosine; BC: black carbon; CAP: concentrated ambient particle; CO: carbon monoxide; DEP: diesel exhaust particle; EPR: electron paramagnetic resonance; HRV: heart rate variability; IR: insulin resistance; MDA: malondialdehyde; NAC: N-acetylcysteine; NAS: Normative Ageing Study; NO_2: nitrogen dioxide; O_3:ozone; OS: oxidative stress; $PM_{2.5}$: particulate matter less than 2.5 μm in diameter; PM_{10}: particulate matter less than 10 μm in diameter; SO_2: sulphur dioxide; TAC: total antioxidant capacity.

air pollution with HRV, as well BP, are mediated by OS pathways [87]. In a post-infarction population, total antioxidant capacity (TAC) however did not appear to modify the association between short-term ambient $PM_{2.5}$ concentrations and biomarkers of HRV and repolarization [88]. That oxidative pathways exacerbate the adverse effects of ambient levels of air pollution on the cardiac autonomic function has also been investigated by looking at associations between air pollution exposure, biomarkers of OS and HRV [89–93]. Whilst Lee et al [91] reported that OS modified the association between continuous personal exposure to $PM_{2.5}$ and HRV, the markedly improved air quality brought about by the 2008 Olympic Games in Beijing was not mirrored by a change in HRV measurements despite decreases observed in urinary 8-oxodGuo [90]. Exhaled MDA levels were slightly, but insignificantly, elevated in a group of asthmatic and non-asthmatic subjects who experienced decreases in HRV following a 2-h highway commute during rush hour [92]. Another study observed that a 5-h exposure to real-life levels of $PM_{2.5}$ from an urban street (24 μg/m^3) reduced HRV as well as causing vasomotor dysfunction in overweight middle-aged and elderly adults. However these effects were not associated with the altered biomarkers chosen to measure OS (dihydrobiopterin, biopterin, uric acid, ascorbic acid and dehydroascorbate) [93]. The mitochondrion is the primary target of OS in response to exogenous environments, leading to mitochondrial DNA (mtDNA) damage through methylation of nucleotides. An occupational exposure study thus specifically examined the association between metal-rich $PM_{2.5}$ from welding and blood mtDNA methylation in relation to HRV [94]. Blood mtDNA methylation levels were significantly lower in participants who were highly exposed to PM, whilst workers with higher levels of mtDNA methylation were more susceptible to the effect of $PM_{2.5}$ exposure on HRV measures. Studies examining the impact of antioxidant supplementation on the cardiovascular effects of pollution exposure include that of Romieu et al [95] who reported omega-3 polyunsaturated fatty acid supplementation attenuated the effect of same-day indoor $PM_{2.5}$ on HRV among elderly residents of a nursing home in Mexico City. More recently, in a controlled human exposure study (CAP mean mass concentration 278 ± 19 μg/m^3 for 2 h) omega-3 fatty acid supplementation (3 g/day for 4 weeks), attenuated the CAP-induced changes in HRV and cardiac repolarization in healthy, middle-aged healthy adults [96] (Fig. 3).

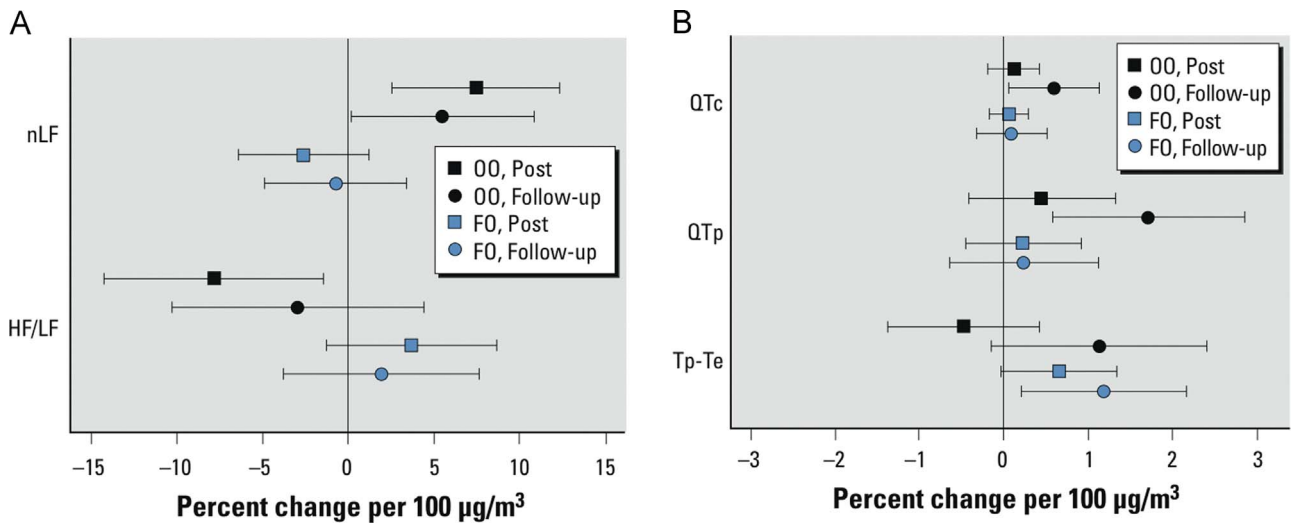

Fig. 3. A Effect of CAP exposure on frequency domain indices of HRV. nLF HRV and HF/LF ratio were analyzed in the ECG recordings of participants at rest before, immediately after exposure to filtered air and CAP (Post), and again the next morning (Follow-up) as described in "Methods." Data shown are average changes per 100-µg/m^3 increase in CAP relative to the filtered air and 95% confidence intervals. Reproduced from Environ Health Perspect (Tong et al., 2012). **B** Effect of CAP exposure on indices of cardiac repolarization. QTc, QTp, and Tp-Te were analyzed in ECG recordings of participants at rest before, immediately after exposure to filtered air and CAP (Post), and again the next morning (Follow-up) as described in "Methods." Data shown are average changes per 100-µg/m^3 increase in CAP relative to the filtered air and 95% confidence intervals. Reproduced from Tong et al. [96].

5.2. Animal studies

Findings from animal studies investigating the arrhythmogenic mechanism of particulate pollution are also consistent with OS mediated pathways of injury [97–99]. Following endotracheal DEP exposure to rats (200 µg/ml for 30 min) and perfused rat hearts (12.5 µg/ml for 20 min), Kim et al [98] observed action potential duration prolongation, early after depolarization and ventricular arrhythmia that were prevented by pretreatment with NAC as well as active Ca^{2+}/calmodulin-dependent protein kinase II blockade. Oxidant stress, determined by electron paramagnetic resonance of heart perfusate before induction of ischemia and reperfusion, in the absence of recruited inflammatory cells, along with increased vulnerability to ischemia-associated arrhythmia has also been observed in rats following a large IT DEP installation (0.5 mg) [99]. Whilst Wang et al [100] reported that O_3 alone (0.81 ppm) exposures did not significantly alter indicators (increased heart MDA, decreased HRV) adversely affected by $PM_{2.5}$ (0.2, 0.8, 3.2 mg/rat), the gaseous pollutant potentiated the effects induced by $PM_{2.5}$. The only other gaseous pollutant that has been investigated in this regard is CO in a chronic exposure (30 ppm for 12 h, including five 1 h peaks at 100 ppm for 4 weeks) to rats, which promoted OS (ventricular MDA), altered Ca^{2+} homeostasis and ventricular arrhythmia [101].

6. Hypertension

It is believed that elevations in BP brought about by constituents of ambient air pollution occur in a biphasic fashion [12]. Owing to the rapidity of observed effects and associations with parameters of HRV, the activation of pulmonary reflexes and subsequent imbalanced activation of the ANS has been postulated to play a role in the initial response (minutes to hours). Controlled exposure studies in animals suggest the later-onset effects following longer-term exposures however maybe explained by heightened arterial vasoconstrictor responsiveness mediated via a variety of mechanisms including OS and inflammation, activation of vasoactive mediators [72] and vascular remodeling [72,78,99,102]. Studies that allow an evaluation of a role of OS in the pro-hypertensive effects of air pollution are discussed below (Table 5).

6.1. Human studies

Relatively few studies have investigated interactions between genetic polymorphisms and air pollution exposure for hypertension and moreover, results have been inconsistent. Among 461 elderly men in the Normative Aging Study, traffic-related BC particles were associated with increased in systolic (SBP) and diastolic (DBP) blood pressure, an effect that was not however modified by gene variants related to OS defense (GSTM1, GSTP1, GSTT1, GSTCD, NQO1, CAT, HMOX-1) [103]. Similarly, in a larger Swedish study (n = 1429), associations between long-term exposure to vehicle NO_2 and hypertension were not stronger among individuals with potentially impaired antioxidant defenses (gene variants in GSTP1, GSTT1, GSTCD) [104]. In a panel study of post-infarction patients (n = 76), no evidence of effect modification by TAC was found on the associations between ambient $PM_{2.5}$, accumulation mode particle and UFP concentrations in the previous 6–120 h and SBP [88]. A more recent study conducted among elderly Asian participants and employing genetic risk scores did however suggest that associations of air pollution with BP were mediated, at least partly by OS pathways [87]. Again using the Normative Aging Study, Zhong et al. [105] evaluated the role of mitochondrial abundance, an adaptive mechanism to compensate for cellular-redox-imbalance following environmental challenges, in a BC-BP relationship. Exposure to short-to moderate-term ambient BC concentrations was associated with increased BP and blood mitochondrial abundance (as reflected in elevated mitochondrial DNA to nuclear DNA copy number). Furthermore, individuals with a higher blood mitochondrial abundance appeared less susceptible to the impact of ambient BC on BP, prompting speculation that this may be a compensatory response that attenuates the cardiac effects of traffic-related pollution (Fig. 4). In a panel of 50 healthy people, short-term PM_{10} exposure was associated with retinal arteriolar narrowing and venular widening, both being independent risk factors for cardiovascular disease. Furthermore, analysis of miRNA expression hinted to a possible role for OS and inflammatory pathways [106].

6.2. Animal studies

Exposure to various types of particulate pollution has been reported to increase BP in rodents along with increased oxygen free radicals (EPR) in coronary perfusate (0.5 mg DEP by IT in adult rats) and in association with an induction of HO-1 within the lung (180 µg/m^3

Table 5
Studies linking air pollution exposure to hypertension mediated by oxidative stress.

Human

Study [reference]	Population/design	Air pollutant	Main findings
Mordukhovich et al. [103]	461 participants of NAS cohort	Traffic-related BC particles	Associations with increased SBP and DBP not modified by gene variants related to OS defense (GSTM1, GSTP1, GSTT1, GSTCD, NQO1, CAT, HMOX − 1)
Levinsson et al. [104]	119 AMI cases and 1310 controls	Traffic-related NO_2	Associations between long-term vehicle NO_2 and hypertension not stronger among individuals with potentially impaired antioxidant defenses (gene variants in GSTP1, GSTT1, GSTCD)
Wang et al. [88]	Panel study of 76 post-infarction patients	Ambient $PM_{2.5}$ and UFP	No evidence of effect modification by TAC on associations between ambient $PM_{2.5}$, accumulation mode particle and UFP concentrations and SBP
Kim et al. [87]	Panel of 547 elderly Asian residents	Ambient PM_{10}, SO_2, NO_2	Genetic risk scores suggest associations of air pollution with BP were mediated by OS pathways
Zhong et al. [105]	675 participants of NAS cohort	Ambient BC	Short-to moderate-term ambient BC concentrations associated with increased BP and blood mitochondrial abundance; individuals with a higher blood mitochondrial abundance appeared less susceptible to the impact of ambient BC on BP
Louwies et al. [106]	50 healthy people	Ambient PM_{10}	Short-term PM_{10} exposure associated with retinal arteriolar narrowing and venular widening; analysis of miRNA expression hinted to a possible role for OS and inflammatory pathways

Animal

Study [reference]	Animal model	Air pollutant exposure	Major findings (exposed versus control)
Upadhyay et al. [78]	Aged SH rats	Ultrafine carbon particles (180 μg/m^3) for 24 h	Increased BP in association with an induction of pulmonary HO − 1
Robertson et al. [99]	Wistar rats	DEP (IT, 0.5 mg DEP)	Increased BP and oxygen free radicals (EPR) in coronary perfusate before induction of IR
Wang et al [156]	Wistar rats	$PM_{2.5}$ (IT, 3.2 mg) alone or in combination with O_3 (0.81 ppm) for 4 h	$PM_{2.5}$ alone or in combination with O_3 (but not O_3 alone), increased SBP and heart MDA
Gottipolu et al. [107]	SH rats	DE (0.5 or 2 mg/m^3) 4 h/d, 5 d/wk for 4 wks	Hypertensive-like cardiac gene expression pattern associated with mitochondrial oxidative stress in healthy rats
Aztatzi-Aguilar et al. [108]	Sprague–Dawley rats	UFP (107 μg/m^3) 5 h/day, 4 d/wk for 8 wk	Increased expression of the angiotensin II receptor type 1 in heart accompanied by decreased HO − 1, induction of Acta1 and Col3a1 and increased coronary wall thickness

AMI: acute myocardial infarction; BC: black carbon; BP: blood pressure; CAT: catalase; DBP: diastolic blood pressure; DE: diesel exhaust; DEP: diesel exhaust particle; EPR: electron paramagnetic resonance; GST: glutathione S-transferase; HO-1: hemeoxygenase-1; IT: intratracheal; MDA: malondialdehyde; NAS: Normative Ageing Study; NO_2: nitrogen dioxide; O_3:ozone; OS: oxidative stress; $PM_{2.5}$: particulate matter less than 2.5 μm in diameter; PM_{10}: particulate matter less than 10 μm in diameter; SBP: systolic blood pressure; SH: spontaneously hypertensive; SO_2: sulphur dioxide; TAC: total antioxidant capacity; UFP: ultrafine particle.

ultrafine carbon particle by inhalation in aged SH rats) [78,99]. High dose IT $PM_{2.5}$ (3.2 mg per rat) alone or in combination with O_3 (0.81 ppm), but not O_3 alone, increases SBP and heart MDA [100]. In healthy and SH rats, a 4-week DE inhalation (0.5 or 2 mg/m^3, 4 h/day, 5 days/week for 4 weeks) enhanced cardiac mitochondrial OS (aconitase activity) in both species and produced a hypertensive-like gene expression pattern in the ventricles of healthy rats, characterized by a generalized suppression of genes (including those related to mitochondrial function and compensatory response to OS) that are already suppressed in SH rats at baseline without DE [107]. Of interest, hearts of already hypertensive rats were spared from DE-induced further impairment in gene expression. Earlier work by Sun et al [72] suggests that at least some of the pro-hypertensive effects of short-term (10 weeks) $PM_{2.5}$ exposure (79.1 ± 7.4 μg/m^3) in rats can be explained by O_2^{*-}, generated through NADPH oxidase, which activates RhoA/Rho-kinase (ROCK). Moreover since angiotensin II (ANG II) is a well-known regulator of NADPH oxidases, a pathway has been hypothesized by which $PM_{2.5}$ exerts additive effects on ANG II-mediated BP. On investigating the effect of different size PM fractions on renin-angiotensin-aldosterone and kallikrein-kinin elements in rats, Aztatzi-Aguilar et al [108], observed an increased expression of the ANG II receptor type 1 in the heart that was accompanied by a decrease in HO-1 together with the induction of Acta1 and Col3a1 (markers of myocardial adaptive response to damage) and increased coronary wall thickness following an UFP subchronic exposure (107 μg/m^3, 8 weeks (5 h/day, 4 days/week)).

7. CV risk

In addition to being central to eliciting specific cardiac responses, evidence also exists that an OS pathway plays a role in modulating the risk of succumbing to cardiovascular disease following air pollution exposure (Table 6).

7.1. Human studies

Hyperhomocysteinemia is an important independent predictor for several CV diseases including atherosclerosis, thrombosis, myocardial infarction and stroke. Elevations in plasma Hcy is also associated with traffic-related pollutant exposures, especially $PM_{2.5}$ and BC and moreover, gene polymorphisms related to OS modify these effects [109]. Specifically, individuals carrying the deletion of GSTT1 or those with polymorphisms in the hemochromatosis genes (HFE C282Y) had higher plasma Hcy when they exposed to BC whilst HFE C282Y and CAT (rs2300181) modified effects of $PM_{2.5}$. In another study, whilst increased urinary 1-OHP levels in taxi drivers were negatively correlated with CAT & GST, the occupationally exposed individuals also displayed increased biomarkers of oxidatively generated damage (MDA and PCO) and serum Hcy levels [61]. Another prominent predictor of negative cardiac outcomes (such as heart failure, arrhythmia, stroke and sudden cardiac death) is an elevated left ventricular mass (LVM). Work within the MultiEthnic Study of Atherosclerosis initially demonstrated a link between close (< 50 m) residential major roadway proximity and

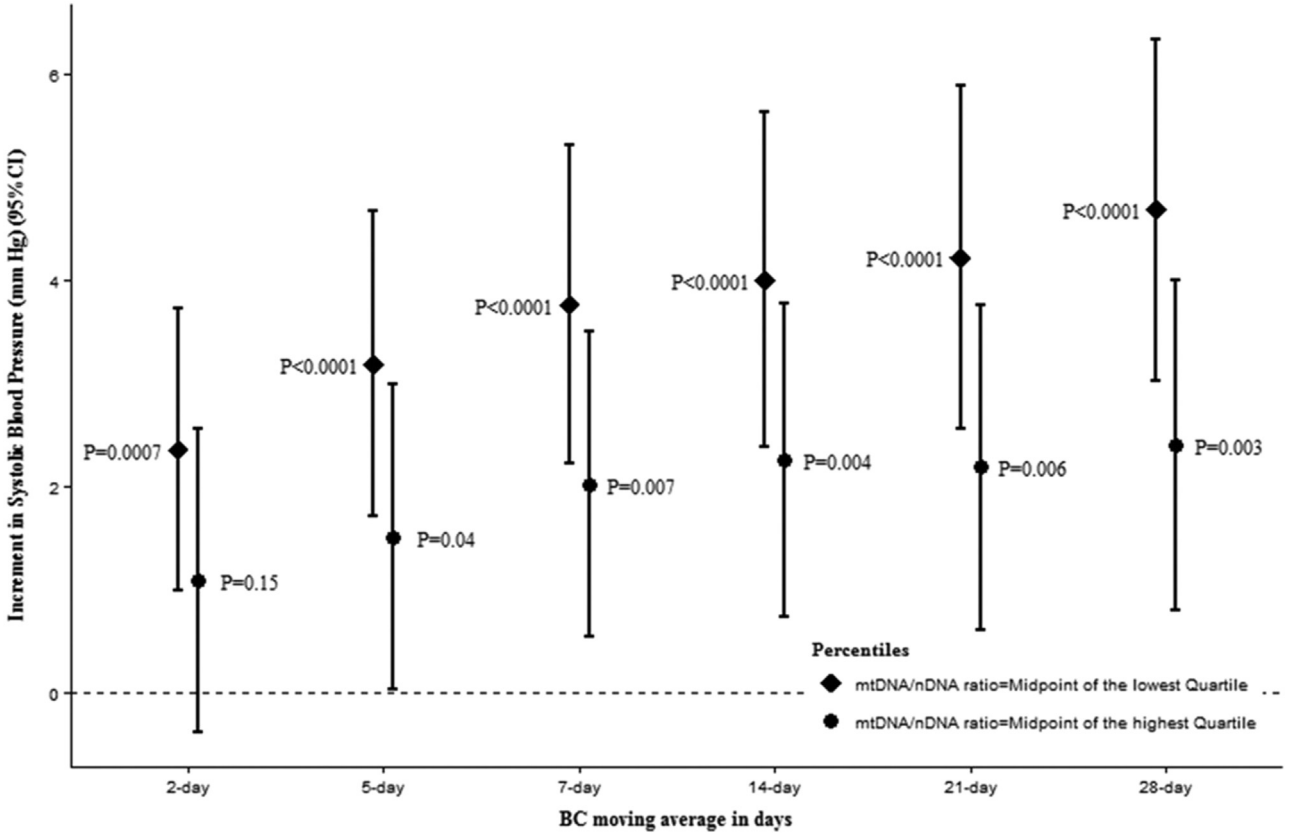

Fig. 4. Association of ambient black carbon (BC) levels with systolic blood pressure (SBP) at different blood mitochondrial DNA to nuclear DNA copy number ratio (mtDNA/nDNA) levels, Normative Aging Study, 1999–2012. Results were adjusted for: apparent temperature and absolute humidity at the matching time window; race; percentage of major cell types (lymphocytes, neutrophils, and platelets); age; weekday; date of visit; body mass index; C- reactive protein > 10 mg/L; smoking; alcohol use; season; physical activity; education level; use of calcium channel blockers, -blockers, and angiotensin-converting enzyme inhibitor. CI: confidence interval. Reprinted with permission from Zhong et al. [105]. Promotional and commercial use of the material in print, digital or mobile device format is prohibited without the permission from the publisher Wolters Kluwer Health. Please contact healthpermissions@wolterskluwer.com for further information.

Table 6
Studies linking air pollution exposure to cardiovascular risk mediated by oxidative stress.

Human

Study [reference]	Population/design	Air pollutant	Main findings
Ren et al. [109]	1000 participants of NAS	Traffic-related	Individuals carrying the deletion of GSTT1 or those with polymorphisms in the hemochromatosis genes (HFE C282Y) had higher plasma Hcy when they exposed to BC whilst HFE C282Y and CAT (rs2300181) modified effects of $PM_{2.5}$.
Brucker et al. [61]	39 taxi drivers and 21 non-occupationally exposed persons	Traffic-related	Increased urinary 1-OHP levels negatively correlated with CAT & GST, increased biomarkers of oxidatively generated damage (MDA and PCO) and serum Hcy levels
Van Hee et al. [111]	1376 participants of MultiEthnic Study of Atherosclerosis	Proximity to major road	Link between close (< 50 m) residential major roadway proximity and higher LVM modified by polymorphisms in genes important in vascular function and inflammation/OS (AGTR1 and ALOX15)
Levinsson et al. [104]	119 AMI cases and 1310 controls	Traffic-related NO_2	AMI risk modified (not significantly) by genetic variants in the GST genes
Dominguez-Rodriguez et al. [112]	307 patients admitted to hospital due to acute coronary syndrome	Ambient BC	High BC exposure and OS marker (MDA levels) independently associated with MACE at 30 days
Weichenthal et al. [113]	Case-crossover study including 30101 cases of myocardial infarction	Ambient $PM_{2.5}$	Regional differences in glutathione-related OP modifies relationship between $PM_{2.5}$ mass concentrations and risk of myocardial infarction; combined oxidant capacity of NO_2 and O_3 may further amplify the effect

Animal

Study [reference]	Animal model	Air pollutant exposure	Major findings (exposed versus control)
Weldy et al. [114]	Pregnant female C57Bl/6 J mice	In utero DE (~300 μg/m^3 $PM_{2.5}$) 6 h/d, 5 d/wk	Placental injury manifested by OS (3-NT), hemorrhage, focal necrosis, embryo resorption and inflammatory cell infiltration; surviving embryos develop weight gain, altered BP, increased susceptibility to heart failure as adults

1-OHP: 1-hydroxypyrene; 3-NT: 3-nitrotyrosine; AMI: acute myocardial infarction; BC: black carbon; BP: blood pressure; CAT: catalase; DE: diesel exhaust; GST: glutathione S-transferase; LVM: left ventricular mass; MACE: major adverse cardiovascular event; MDA: malondialdehyde; NAS: Normative Ageing Study; NO_2: nitrogen dioxide; O_3: ozone; OS: oxidative stress; OP: oxidative potential; PCO: protein carbonyl; $PM_{2.5}$: particulate matter less than 2.5 μm in diameter.

higher LVM and subsequently found that polymorphisms in genes important in vascular function and inflammation/OS (AGTR1 and ALOX15) were associated with substantial alterations in the associations [110,111]. Another study reported that in patients admitted to hospital due to acute coronary syndrome, BC concentration averaged the 7 days preceding and MDA levels on admission were associated with a major adverse cardiovascular event (MACE; defined as the combined result of cardiovascular death, non-fatal myocardial infarction or re-admission for unstable angina) at 30 days follow-up [112]. Again focusing on coronary vulnerability, Levinsson et al. [104] reported that the effect of long-term traffic-related air pollution exposure (NO_2 annual means) on risk of acute myocardial infarction was modified by genetic variants in the GST genes, although the variations were not statistically significant. That regional differences in the oxidative properties of $PM_{2.5}$ may modify its impact on emergency room visits for myocardial infarction has also been investigated [113]. Findings suggested that not only regional differences in glutathione-related OP modify the relationship between $PM_{2.5}$ mass concentrations and risk of myocardial infarction, but also that the combined oxidant capacity of NO_2 and O_3 may further amplify such an effect.

7.2. Animal studies

Evidence from experimental animal work of a role played by OS in cardiovascular disease susceptibility mediated by particulate air pollution comes from an in utero DE (300 μg/m^3 $PM_{2.5}$, 6 h/day, 5 days/week; equating to a 53 μg/m3/h $PM_{2.5}$ time weighted average) exposure study in mice [114]. This regimen promotes placental injury manifested by OS (3-NT) as well as hemorrhage, focal necrosis, embryo resorption and inflammatory cell infiltration, whilst surviving embryos develop weight gain, altered blood pressure and increased susceptibility to heart failure as adults. Of note, the observed increase in placental vascular OS did not overlap with areas of inflammatory cell infiltration, suggesting an alternative source of radical generation.

8. Cardiac dysfunction

Evidence exists that cardiac dysfunction subsequent to air pollutant exposure is linked with increased OS and/or decrease in antioxidant reserve (Table 7). Following a chronic O_3 exposure (0.8 ppm 8 h/day for 28 and 56 days) in rats, left ventricular developed pressure (LVDP), rate of change of pressure development (+dP/ dt) and rate of change of pressure decay (-dP/dt) values significantly decreased and left ventricular end diastolic pressure (LVEDP) was significantly increased [115,116]. The attenuation of cardiac function was associated with decreased myocardial activities of SOD and increased myocardial lipid peroxidation. Short-term inhalational exposure to environmentally persistent free radicals (EPFRs, 20 min/day for 7 days), specifically 1,2-dichlorobenzene chemisorbed to 0.2-μm-diameter silica/CuO particles at 230 °C (DCB230), significantly reduce baseline cardiac function in otherwise healthy rats as a consequence of EPFR-mediated increases in pulmonary arterial pressure [117]. Moreover, levels of HO-1 and manganese-dependent SOD in the left ventricle were significantly increased. Chronic O_3 exposure (0.8 ppm, 8 h/day for 28 and 56 days) of rats also enhances the sensitivity to ischemia/reperfusion (I/R) injury in isolated perfused hearts, and again evidence points to a role for OS [118]. Cardiac function (LVDP, +dP/dt, -dP/dt, and LVEDP) after I/R was significantly compromised and this was associated with increased myocardial lipid peroxidation and decreased myocardial activities of SOD. Other work has shown that regular exposure to a simulated urban CO pollution (air enriched with 30–100 ppm CO, 4 weeks) of rats worsens myocardial I/R injuries, resulting in increased severity of post ischemic ventricular arrthymia, impaired recovery of myocardial function, increased infarct size and a reduced myocardial enzymatic antioxidant status (SOD, GPx) [119]. More recent work, using a specific blocker to inhibit iNOS, suggests that up-regulation of this enzyme mediates the higher sensitivity of the myocardium to ischemic events following daily low non-toxic CO exposure, via NO/pro-oxidant dependent pathways, having deleterious effects on diastolic Ca^{2+} overload and myofilaments Ca^{2+} sensitivity [120] (Fig. 5). In rats, treatment with the antioxidant vanillic acid prior to IT exposure to PM_{10} (0.5–5.0 mg/kg), improved the observed myocardial dysfunction after I/R as well as rectifying the observed perturbations in cardiac oxidant imbalance (xanthine oxidase, lactate dehydrogenase), antioxidant enzyme activities (SOD, GPx, CAT) and expression of iNOS and eNOS mRNA [121].

9. Metabolic disease

Air pollution poses a greater risk of adverse cardiovascular events for people with metabolic conditions such as insulin resistance (IR), diabetes mellitus (DM) and obesity. Individuals with DM appear to be particularly sensitive to the effects that ambient PM exposure has on heart disease hospital admissions [122–124]. Nanoparticle and CO air pollution has been reported to elicit ANS dysfunction, manifesting in significant heart rate variations among individuals with MeS but not in normal subjects [125], whilst obese people may also be at increased risk [126,127]. Potential mechanisms linking PM to impaired cardiovascular function in such susceptible populations have attracted increased interest and are undoubtedly the result of a complex interaction of individual risk factors that are still under investigation. A major underlying factor behind increased susceptibility in DM appears to be the higher propensity for cardiovascular complications within this population, for example, preexisting vascular endothelial dysfunction, reduced HRV and accelerated atherosclerosis [49,122,123,128–130]. Indeed studying IR and the development of type 2 DM in relation to air pollution exposure may well aid in clarifying causative associations between environmental factors and cardiovascular risk [131,132]. Increased OS has emerged as playing a central role in MeS and as such, may constitute a unifying mechanism underlying the increased propensity for cardiovascular disease in this complex phenotype. (Table 8).

9.1. Human studies

In evaluating associations between ambient air pollutants and markers of IR and effect modification by genes involved in OS among 560 elderly participants in the Korean Elderly Environmental Panel study, Kim & Hong [133] reported that PM_{10}, NO_2, O_3 may increase IR in the elderly, and that *GSTM1*-null, *GSTT1*-null, and *GSTP1* AG or GG genotypes may increase susceptibility. Two-hour controlled exposures to UFP (50 μg/m^3) produced mild changes in gene expression in circulating mononuclear cells of healthy subjects, and pathway analysis identified two insulin-related pathways (IGF-1 and insulin receptor signaling) and pathways related to host defense against environmental insults (including NRF2-mediated OS signaling) [134].

9.2. Animal studies

Experimental data from studies in rodents investigating mechanisms linking air pollution and increased risk of cardio-metabolic status have demonstrated the ability of various $PM_{2.5}$ exposure regimens to decrease insulin sensitivity in various adipose tissues including brown, white epicardial and perirenal, and induce an inflammatory response plus OS in these tissues [135,136]. The findings in epicardial adipose tissue are noteworthy in respect to the possible role of this tissue in maintaining myocardial form and function. Using wild type versus genetically modified mice (p47phox homozygous knockout) which are unable to release $O_2^{\bullet-}$ from NADPH oxidase, Xu et al. [137] found that early life (at 3 weeks of age) exposure to $PM_{2.5}$ exposure, increases obesity and systemic IR later in life, likely by NADPH oxidase-derived oxidants. Recently, a novel link between pulmonary OS and vascular insulin signaling has been reported, and one that may contribute to the

Table 7
Studies linking air pollution exposure to cardiac dysfunction mediated by oxidative stress.

Study [reference]	Animal model	Air pollutant	Main findings (exposed versus control)
Perepu et al. [115]	Sprague–Dawley rats	O_3 exposure (0.8 ppm) 8 h/d for 28 and 56 d	Cardiac dysfunction (decreased LVDP, + dP/ dt and -dP/dt and increased LVEDP) associated with decreased myocardial activities of SOD and increased myocardial lipid peroxidation
Sethi et al. [116]	Sprague–Dawley rats	O_3 exposure (0.8 ppm) 8 h/d for 28 and 56 d	Cardiac dysfunction (decreased LVDP) associated with decreased myocardial activities of SOD
Mahne et al. [117]	Sprague–Dawley rats	EPFRs (DCB230), 20 min/d for 7 d	Reduced baseline cardiac function accompanied by increased HO − 1 and manganese-dependent SOD in the left ventricle
Perepu et al. [118]	Sprague–Dawley rats	O_3 exposure (0.8 ppm) 8 h/d for 28 and 56 d	Enhanced sensitivity to I/R injury in isolated perfused hearts associated with increased myocardial lipid peroxidation and decreased myocardial SOD
Meyer et al. [119]	Wistar rats	Simulated urban CO pollution (air enriched with 30–100 ppm CO) 4 wk	Increased severity of post ischemic ventricular arrthymia, impaired recovery of myocardial function, increased infarct size and reduced myocardial enzymatic antioxidant status (SOD, GPx)
Meyer et al. [120]	Wistar rats	Simulated urban CO pollution (air enriched with 30–100 ppm CO) 4 wk	Specific blocker to inhibit iNOS, suggests that up-regulation of iNOS mediates the higher sensitivity of the myocardium to I/R, via a NO/pro-oxidant dependent pathway, having deleterious effects on diastolic Ca^{2+} overload and myofilaments Ca^{2+} sensitivity
Dianat et al. [121]	Wistar ats pretreated with vanillic acid	PM_{10} (IT, 0.5–5.0 mg/kg)	Pretreatment with vanillic acid improved observed myocardial dysfunction after I/R and rectified perturbations in cardiac oxidative imbalance (xanthine oxidase, lactate dehydrogenase), antioxidant enzyme activities (SOD, GPx, CAT) and expression of iNOS and eNOS mRNA

+dP/ dt : rate of change of pressure development; -dP/dt : rate of change of pressure decay; CAT: catalase; CO: carbon monoxide; eNOS : endothelial nitric oxide synthase; GPx: glutathione peroxidase; HO hemeoxygenase-1; iNOS: inducible nitric oxide synthase; I/R: ischemia/reperfusion; LVDP: left Ventricular Developed Pressure; LVEDP: left ventricular end diastolic pressure; NO: nitric oxide; O_3: ozone; PM_{10}: particulate matter less than 10 μm in diameter; SOD: superoxide dismutase.

mechanism by which air pollution increases the risk for cardiovascular and metabolic disease. Specifically, short-term exposure of $PM_{2.5}$ CAPs induces IR and inflammation in blood vessels of mice, mediated at least in part by OS in the lungs reflected by an increase in the expression SOD2, SOD3 and GST-α [138]. Pulmonary inflammation and increased tissue and systemic OS (decreased GSH and increased MDA) has also been postulated to contribute to increased weight gain and metabolic dysfunction in rat dams and their offspring following pre and postnatal exposure to unfiltered Beijing air [139]. Another study has demonstrated increased cardiovascular vulnerability in a mouse model of type 1 diabetes exposed to IT DEP (0.4 mg/kg) comprising hypoxemia, hepatotoxicity and acceleration of coagulation comprising thrombosis in vivo, platelet aggregation in vitro, and the increase in plasma concentrations of PAI-1 and vWF [140]. These exacerbations were attributed to observations of increases in systemic OS (increased plasma 8-isoprostane) and inflammation.

Studies, albeit a limited number, have also investigated whether OS is involved in O_3-induced insulin resistance [136,141,142]. Repeated O_3 inhalation (0.5 ppm, 4 h/day for 13 weeks) induces IR, inflammation and increased expression of OS-related genes (Cox4, Cox5a, Scd1, Nrf1, Nrf2) in visceral adipose tissue [142]. Another study reported that overnight exposure of rats to environmentally realistic concentrations of O_3 (0.8 ppm) triggers peripheral IR and systemic OS, in association with endoplasmic reticulum stress and JNK activation [141]. In that increases in many OS biomarkers in blood were observed as well as a decreased GSH-to-GSSG ratio in erythrocytes and an increase in protein carbonyls in gastrocnemius muscle, suggests OS can spread into peripheral tissues and may mediate remote effects of O_3. Muscle cells treated with bronchoalveolar lavage fluid (BALF) from O_3-exposed rats exhibited an inhibition of insulin-stimulated glucose uptake, whilst pretreatment of rats with a JNK inhibitor or NAC prior to O_3 exposure alleviated IR. Moreover, BALF from NAC treated O_3-exposed animals failed to impair insulin-signaling pathways in skeletal muscle cells (Fig. 6). Put together, these findings suggest that OS, stemming from toxic lung mediators is instrumental in eliciting the peripheral effects of O_3 on muscle insulin sensitivity.

9.3. *In vitro studies*

In vitro studies investigating the effects of PM exposure in the setting of DM or high glucose have focused on macrophages/monocytes, as they are one of the first lines of defense against inhaled particles and respond to PM by secreting various mediators of a vicious cycle that contribute to cardiovascular disease [143–145]. Mo et al. [146] demonstrated that in vitro urban PM (SRM 1648, 600 μg/ml) exposure led to greater DCFH-DA oxidation in alveolar macrophages from rabbits with DM (as opposed to rabbits without DM) as well as heightened up-regulation of cytokine expression and pro-MMP-9 activity. Findings from the same group reported that co-exposure of human monocytes to the same urban PM plus high glucose caused a significant increase in DCF fluorescence, phosphorylation of p38, MMP-2 and MMP-9 mRNA expression and pro-MMP-2/pro-MMP-9 activity compared with that of urban PM alone [147]. Furthermore, use of NAC and CAT and a specific p38 inhibitor suggest that activation of MMPs by UFP within a high glucose setting is partly mediated via p38 phosphorylation induced by OS.

10. Concluding remarks

The large and diverse evidence base, stemming from epidemiological research, experimental human and animal studies as well as isolated organ and cellular data indicates that cellular oxidative imbalances can occur at each of the potential junctures (endothelial dysfunction, atherosclerosis, pro-coagulant changes, autonomic nervous system dysfunction, hypertension, cardiac dysfunction and increased susceptibility) at which inhaled air pollutants can exert adverse effects on the cardiovascular system. The studies reviewed have uncovered numerous mechanistic pathways, acting on several different organ systems, through which pro-oxidative effects may operate (Fig. 7). Even in the absence of a polluted ambient atmosphere, the heart is inherently susceptible to oxidative injury [148]. It is an extremely active organ and as a consequence, has a high metabolic rate to satisfy the high energy demand. This in turn leads to an increased rate of production of oxidizing species but at the same time, compared with other tissues, it is characterized by lower concentrations of SOD, CAT and GPx [149,150]. It is therefore not surprising that an increased oxidative burden elicted by air pollution, in addition to being central to eliciting specific cardiac endpoints, is also implicated in modulating the risk of succumbing to cardiovascular disease, sensitivity to I/R injury and the onset and progression of metabolic conditions and their

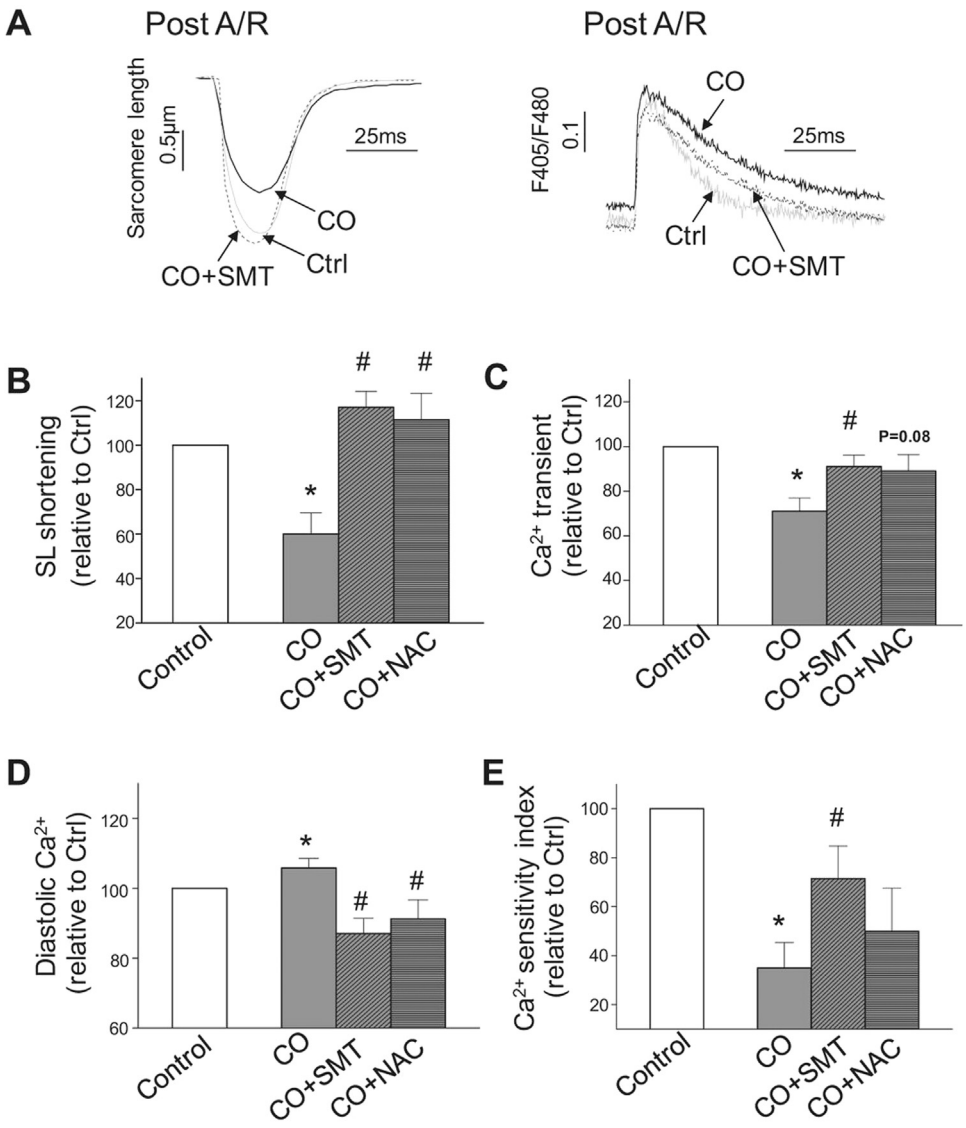

Fig. 5. Effect of iNOS inhibition and antioxidant treatment on the sensitivity to anoxia-reoxygenation (A/R) of cardiomyocytes from Ctrl and CO rats. **A**: representative contraction (*left*) and intracellular Ca^{2+} signal (*right*) of intact cardiomyocytes following a protocol of A/R. **B–E**: contraction of intact myocytes in presence or absence SMT or *N*-acetylcystein was evaluated by measuring SL shortening (**B**), Ca^{2+} transient amplitude (**C**), diastolic intracellular Ca^{2+} (**D**), and myofilament Ca^{2+} sensitivity index (**E**). Data are expressed relative to Ctrl group and presented as means ± SE; $n = 4$ rats/$n = 20$ cells per group; two-way ANOVA. $^{*}P < 0.05$ vs. Ctrl rats; $\#P < 0.05$ SMT/*N*-acetylcystein-treated vs. without treatment. Reprinted with permission from Meyer et al. [120].

associated complications.

Although both gasses and particles have been linked to detrimental outcomes, more evidence implicates the PM components for a large portion of the outcomes investigated. Much of the experimental data supporting an oxidative pathway originates from studies investigating the toxic effects of DEPs that contribute significantly to the air shed in many of the world's largest cities. The toxicological capacity of DEPs is facilitated by the size (80% of DEPs have an aerodynamic diameter of < 1 µm) as well as their surface chemistry characteristics. For instance, DEPs have a highly adsorptive carbon core that act as a vector for the delivery, deep into the lung, of redox active metals, polyaromatic hydrocarbons and quinones. Air pollution in urban areas is however a complex heterogeneous mixture of reactive gases combined with primary and secondary PM. These particles differ not only in chemical composition, mass, size, number, shape and surface area, but also source, solubility and reactivity. Particulate matter can also vary in space and time as a consequence of atmospheric chemistry and weather conditions, as well as interactions with O_3 and NO_2. However, little is known about OS pathways and cardiac effects caused by individual pollutants within air pollution mixtures, or as a consequence of inter-pollutant interactions. In characterizing free radical pathways and the endothelinergic system in rats after inhalation of urban PM, O_3 and a PM/O_3 combination, Kumarathasan et al [151] reported that such pollutant-specific changes can be amplified or abrogated following multi-pollutant exposures and called upon further studies, adopting a systems biology approach to validate and give greater insight into these air pollutant exposure-specific mechanistic pathways.

The presence of an oxidative imbalance in association with air pollution induced cardiovascular disease is inferred in many ways including increased oxidant production, attenuation or reversal of effect in the presence of antioxidants or use of transgenic models, markers of oxidative damage in biological systems, effect modification by polymorphisms in antioxidant genes and genetic pathway analysis. Technical difficulties associated with analytical approaches, together with uncertainties in interpretation, mean that despite the considerable evidence base discussed herein, any limitations must be understood and when necessary, results should be interpreted with caution [152,153]. Before definitely attributing a given event to an increased generation of a specific reactive oxygen species, accurate detection and characterization is paramount. For example, whilst DCFH-DA oxidation to fluorescent DCF is widely employed for detecting intracellular H_2O_2, the complexity of the system's intracellular redox chemistry means that there are several limitations and artifacts associated with this technique for an accurate assessment of intracellular OS. Whilst oxidative damage biomarkers are routinely employed to indicate production of an oxidant such as the hydroxyl radical, the link may well be tenuous in that biomarker accumulation can reflect change in clearance rather than in the production of a given ROS. It is also essential to understand the

Table 8
Studies linking air pollution exposure to metabolic disease mediated by oxidative stress.

Human

Study [reference]	Population/design	Air pollutant	Main findings
Kim et al. [133]	560 elderly participants of Korean Elderly Environmental Panel	Ambient PM_{10}, NO_2, O_3	*GSTM1*-null, *GSTT1*-null, and *GSTP1* AG or GG genotypes may increase susceptibility of potential effects of ambient air pollutants on IR
Huang et al. [134]	Controlled exposure	UFP (50 μg/m^3) 2 h	Gene expression changes in circulating mononuclear cells; pathway analysis identified IGF − 1 and insulin receptor signaling and including NRF2-mediated OS signaling

Animal

Study [reference]	Animal model	Air pollutant exposure	Main findings (exposed versus control)
Xu et al. [135]	C57BL/6 mice	$PM_{2.5}$ (~12.7 μg/m^3) 6 h/d, 5 d/wk for 10 wk	Systemic and local IR, decreased glucose tolerance, inflammatory response, oxidative stress (elevated 3-NT and increased Nrf2-regulated antioxidant genes), mitochondrial dysfunction in brown and white adipose tissue
Sun et al. [136]	Sprague–Dawley rats fed high fructose diet prior to exposure	CAPs (441 μg/m^3) + O_3 (0.497 ppm) 8 h/d, 5 d/wk, for 9 d over 2 wk	Inflammation and OS (increase iNOS protein expression), decreased mitochondrial area in epicardial and perirenal adipose tissue
Xu et al. [137]	C57BL/6 mice (wild type) versus genetically modified mice (p47phox homozygous knockout)	Early life (3 wk) exposure to $PM_{2.5}$ (~111 μg/m^3) 6 h/d, 5 d/wk for 10 wk	Increased obesity and systemic IR later in life, likely by NADPH oxidase-derived oxidants
Haberzetti et al. [138]	C57BL/6 mice fed a high fat diet	$PM_{2.5}$ CAPs (30–120 μg/m^3) 6 h/d for 9 or 30 d	Vascular insulin resistance and inflammation triggered in part by pulmonary oxidative stress (increased expression of SOD2, SOD3 and GST-α)
Wei et al. [139]	Pregnant Sprague Dawley and offspring	Pre (14d) and postnatal (3 or 8 wk) exposure to unfiltered Beijing $PM_{2.5}$ (~73.5 μg/m^3)	Pulmonary inflammation and increased tissue and systemic OS (decreased GSH and increased MDA) contributes to increased weight gain and metabolic dysfunction
Nemmar et al. [140]	Tuck Ordinary mouse model of type 1 diabetes	IT DEP (0.4 mg/kg)	Hypoxemia, hepatotoxicity, acceleration of coagulation, attributed to increased systemic OS (increased plasma 8-isoprostane) and inflammation
Zhong et al. [142]	Diabetes-prone KK mice	O_3 (0.5 ppm) 4 h/d for 13 wk	IR, inflammation, increased expression of OS-related genes (Cox4, Cox5a, Scd1, Nrf1, Nrf2) in visceral adipose tissue
Vella et al. [141]	Wistar rats	O_3 (0.8 ppm) overnight	Whole-body insulin resistance and OS, associated endoplasmic reticulum stress, JNK) activation, disruption of insulin signaling in skeletal muscle. Inhibition of insulin-stimulated glucose uptake in muscle cells treated with BALF from O_3-exposed rats; but not from BALF from NAC treated O_3-exposed rats

In vitro

Study [reference]	Cells/tissue	Air pollutant exposure	Main findings (exposed versus control)
Mo et al. [146]	Alveolar macrophages from rabbits with or without DM	Urban PM (SRM 1648, 600 μg/ml)	OS (DCFH-DA oxidation) in macrophages from DM rabbits and up-regulation of cytokine expression and MMP − 9 activity
Zhang et al. [147]	Human monocytes	Urban PM (SRM 1648, 600 μg/ml) plus high glucose	Increased OS (DCFH-DA oxidation) phosphorylation of p38, MMP − 2 and MMP − 9 mRNA expression and pro-MMP − 2/pro-MMP − 9 activity compared with that of urban PM alone; use of NAC, CAT and specific p38 inhibitor suggest activation of MMPs by UFP within a high glucose setting is partly mediated via p38 phosphorylation induced by OS

3-NT: 3-nitrotyrosine; BALF: bronchoalveolar lavage fluid; CAP: concentrated ambient particle; DCFH-DA: dichlorodihydrofluorescein diacetate; DEP: diesel exhaust particle; GSH: reduced glutathione; GST: glutathione S-transferase; iNOS: inducible nitric oxide synthase; IR: insulin resistance; IT: intratracheal; JNK: c-Jun NH2-terminal kinases; MDA: malondialdehyde; NAC: N-acetylcysteine; NADPH: nicotinamide adenine dinucleotide phosphate, reduced; NO_2: nitrogen dioxide; O_3: ozone; OS: oxidative stress; PM: particulate matter; $PM_{2.5}$: particulate matter less than 2.5 μm in diameter; SOD: superoxide dismutase; UFP: ultrafine particle.

precise biological origins of a given biomarker prior to an accurate interpretation. For example whilst urinary 8-oxodGuo is widely assumed to be a product of DNA repair, and as such reflective of DNA oxidation, the precise source (ie 2′-deoxyribonucleotide pool or otherwise) is not understood [154]. Another potentially problematic area is interpreting the effects of generic antioxidants such as NAC, many of the observed effects of which may be related to increasing thiol levels within a cell or culture medium or changes in cell surface proteins rather than the direct scavenging of oxidants such as H_2O_2. Away from the analytical approach to the experimental system applied, since cell culture systems not only impose an OS per se, but also represent an environment in which antioxidants are unstable, the likelihood that further misleading data can easily be generated should be borne in mind.

Much of the epidemiological support for a role of OS in influencing cardiovascular outcomes stems from studies examining how antioxidant defence pathways (polymorphisms in antioxidant genes, antioxidant supplementation, PM oxidative potential) may modify the impact of ambient pollutant exposure on health effects. Studies, primarily limited to the Normative Aging Study cohort of elderly Caucasian men, have revealed an effect modification of the association between traffic pollutants and markers of endothelial function, changes in HRV and risk of acute cardiovascular disease by gene variants related to antioxidant defenses [24,83–86,104]. Results from a smaller number of investigations into the interactions between such genetic polymorphisms and urban air pollution for hypertension and markers of thrombosis and

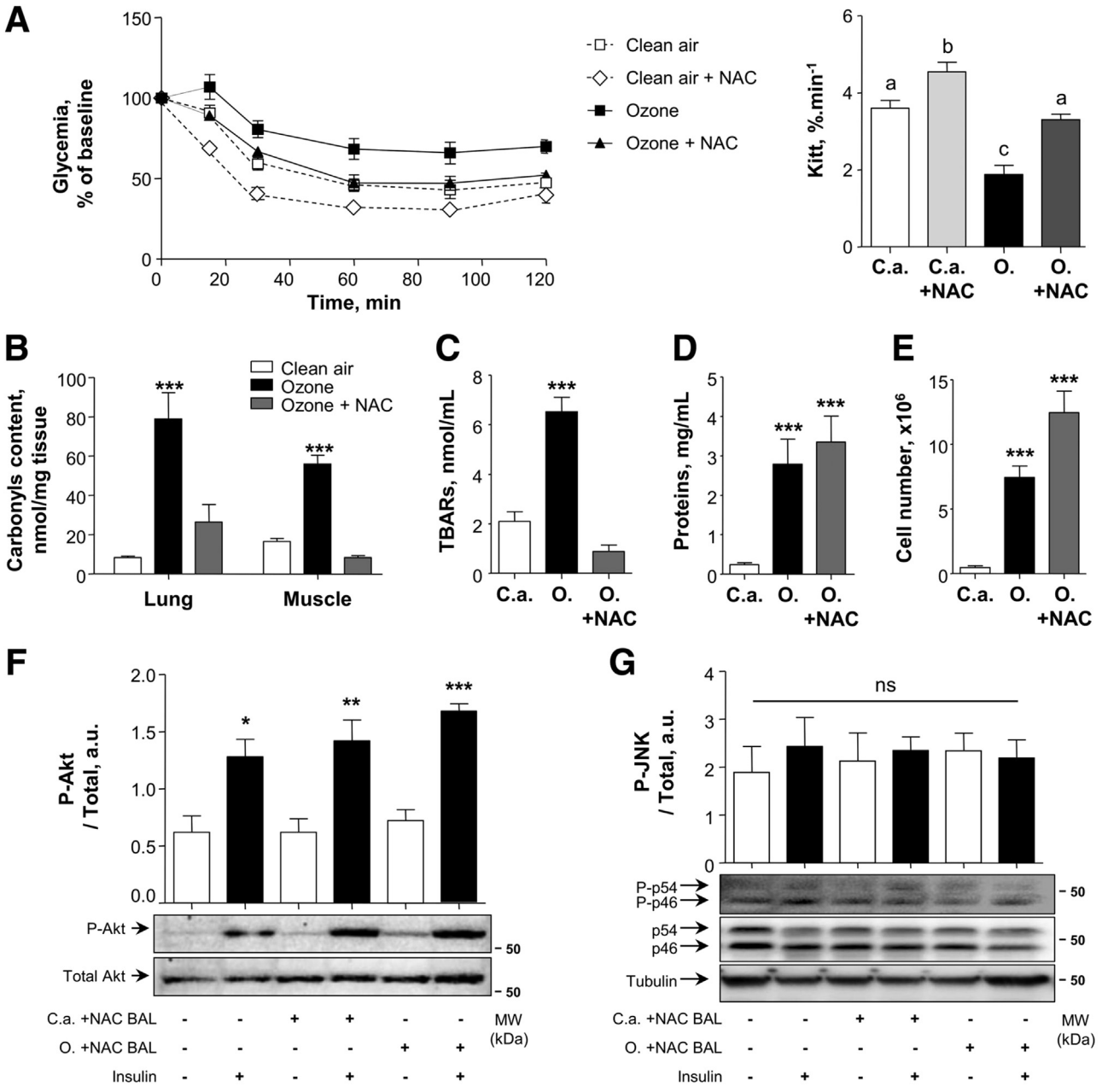

Fig. 6. The antioxidant NAC prevented O_3-induced disruption of insulin signaling pathways. Rats were given NAC (225 mg kg^{-1}) for 10 days prior to O_3 exposure. Insulin sensitivity was explored using ITT. *A*: Blood glucose concentration was measured after intraperitoneal injection of insulin (0.5 IU/kg) in fasting rats, and plasma K_{ITT} was calculated. Results are expressed as the mean ± SEM for $n = 5$ rats. $^{**}P < 0.01$, $^{***}P < 0.005$. Different letters (a, b, and c) indicate a significant difference at $P < 0.05$. Carbonyl content in muscle and lung (*B*), TBARS concentration (*C*), total protein concentration (*D*), and total cell count (*E*) in BALF ($n = 5$). $^{**}P < 0.01$, $^{***}P < 0.005$. C2C12 myotubes were incubated for 30 min with BALF (10%, v/v) from rats pretreated with NAC and exposed to O_3, and stimulated by 100 nmol/L insulin for 20 min. Insulin-induced phosphorylation of PKB/Akt (*F*) and JNK (*G*) was measured by Western blotting. Results are reported as the mean ± SEM for $n = 4$. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$. C.a., clean air; MW, molecular weight; ns, nonsignificant; O., O_3; P, phosphorylated. Reprinted with permission from Vella et al. [141].

coagulation have been more inconsistent [103,104]. Further evaluation is therefore warranted, particularly for chronic cardiovascular outcomes and in more generalized populations to broaden our understanding of susceptibilities to pollutant-induced health effects. The few, small scale studies that have explored potential effect modification by either fish oil supplementation [95,96] or statins [83] again support the role of OS in an ambient PM-induced decline in HRV. These studies are supported by animal data, showing that pretreatment with antioxidants or functional foods represents a valuable approach for the prevention of DEP-induced thrombotic complications [79,80] – a caveat here however is that laboratory animals appear to be more responsive to dietary antioxidants compared with humans [155]. More epidemiological studies, ideally incorporating mechanistic investigations, into such beneficial effects are therefore needed since if nutritional supplements and/or pharmacological agents can blunt the adverse health cardiac effects of air pollution exposure, populations residing in polluted urban cities might be offered at least some protection. This may be particularly effective for vulnerable individuals – for instance those genetically susceptible to oxidative stress or people with underlying cardiovascular conditions on the basis that there may in fact be some synergism between classical risk factors and DEP-induced OS [33].

Further work is also required to ascertain, for each of the cardiovascular endpoints discussed, whether enhanced OS triggers and/or worsens the effect and/or is representative of the consequence of disease progression. This may well depend upon the nature of the ambient pollutant as well as the specific adverse cardiac event or susceptibility

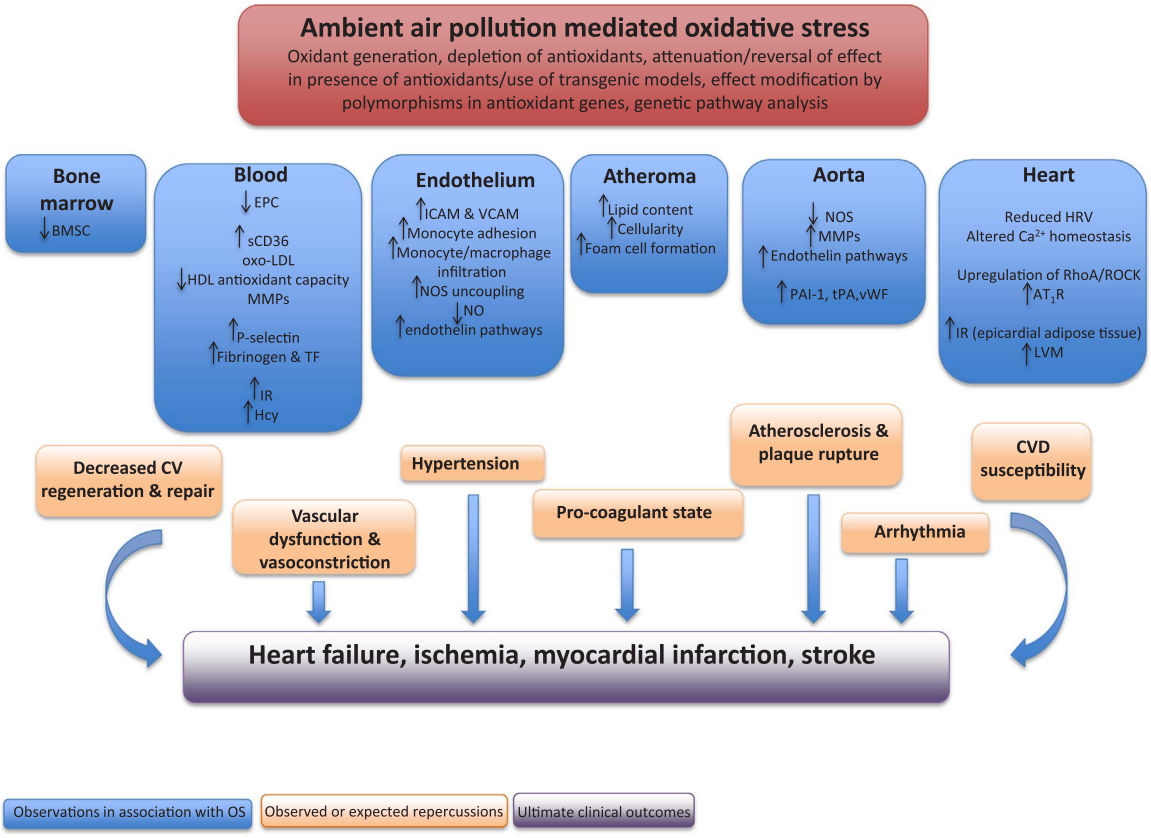

Fig. 7. Illustration of potential oxidative stress-mediated mechanisms for effect of ambient air pollution on cardiovascular diseases. Observations in association with oxidative stress (eg increased oxidant production; attenuation/reversal of effect in the presence of antioxidants/use of transgenic models; effect modification by polymorphisms in antioxidant genes; genetic pathway analysis) originate from epidemiological and experimental findings described in this review. AT_1R: angiotensin II receptor type 1; BMSC: bone marrow stem cells; CVD: cardiovascular disease susceptibility; EPC: endothelial progenitor cell; HDL: high density lipoprotein; HRV: heart rate variability; IR: insulin resistance; MMP: matrix metalloproteinases; NO: nitric oxide; NOS: nitric oxide synthase; oxo-LDL: oxidized low density lipoprotein; PAI-1: plasminogen activator inhibitor-1; sCD36: soluble CD36 receptor; TF: tissue factor; tPA: tissue plasminogen factor; vWF: von Willebrand factor.

to associated conditions under question. For example if OS is a trigger for the acceleration or exacerbation of pollution-induced atherosclerosis, signs of cellular oxidative imbalance should coincide with or precede the atherosclerotic effects. However, animal research employing weeks to months of inhalation exposures, very often undertake OS and lesion development measures at the end of the study, making it difficult to determine the degree to which OS may initiate versus represent a consequence of the process. The contribution of OS pathways to acute versus chronic cardiovascular disease following exposure to air pollutants is also currently unclear. However it is unlikely to be an either/or scenario. An acute oxidative insult could escalate into a cumulative effect following repeated environmental insults and/or provoke end stage adverse outcomes in already chronic cardiac states. It is highly likely that such questions are related to the substantial degree of interconnectivity between the pro-oxidant and pro-inflammatory effects that occur following exposure and inhalation of ambient pollutants and as such the difficulty in determining whether the induction of vascular OS precedes or follows that of inflammation.

We can undoubtedly look forward to many more years of research in this area, leading to a clearer and more precise definition of the role of OS versus indeed other mechanisms. In the meantime, the collective body of evidence emerging from multi-disciplinary work, continues to support a role for OS in the fundamental machinery behind the development of cardiovascular disease outcomes associated with ambient air pollution.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This work was funded by the National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in Health Impacts of Environmental Hazards at King's College London in partnership with Public Health England (PHE) (HPRU-2012-10030). The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, the Department of Health or Public Health England

References

[1] R.D. Brook, S. Rajagopalan, C.A. Pope 3rd, J.R. Brook, A. Bhatnagar, A.V. Diez-Roux, F. Holguin, Y. Hong, R.V. Luepker, M.A. Mittleman, A. Peters, D. Siscovick, S.C. Smith Jr., L. Whitsel, J.D. Kaufman, American Heart Association Advocacy Coordinating Committee, Council on Epidemiology and Prevention, Council on the Kidney in Cardiovascular Disease, Council on Nutrition, Physical Activity and Metabolism, Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association, Circulation 121 (21) (2010) 2331–2378.

[2] M. Franchini, P.M. Mannucci, Short-term effects of air pollution on cardiovascular diseases: outcomes and mechanisms, J. Thromb. Haemost. 5 (2007) 2169–2174.

[3] H. Mustafic, P. Jabre, C. Caussin, M.H. Murad, S. Escolano, M. Tafflet, M.-C. Perier, E. Marijon, D. Vernerey, J.-P. Empana, X. Jouven, Main air pollutants and myocardial infarction: a systematic review and meta-analysis, JAMA 307 (7) (2012) 713–721.

[4] C.A. Pope, D.W. Dockery, Health effects of fine particulate air pollution: lines that connect, J. Air Waste Manag. Assoc. 56 (6) (2006) 709–742.

[5] M.R. Miller, S.J. Borthwick, C.A. Shaw, S.G. McLean, D. McClure, N.L. Mills, R. Duffin, K. Donaldson, I.L. Megson, P.W. Hadoke, D.E. Newby, Direct impairment of vascular function by diesel exhaust particulate through reduced bioavailability of endothelium-derived nitric oxide induced by superoxide free radicals, Environ. Health Perspect. 117 (4) (2009) 611–616.

[6] P. Moller, L. Mikkelsen, L.K. Vesterdal, J.K. Folkmann, L. Forchhammer, M. Roursgaard, P.H. Danielsen, S. Loft, Hazard identification of particulate matter

on vasomotor dysfunction and progression of atherosclerosis, Crit. Rev. Toxicol. 41 (4) (2011) 339–368.
[7] M.R. Miller, The role of oxidative stress in the cardiovascular actions of particulate air pollution, Biochem Soc. Trans. 42 (4) (2014) 1006–1011.
[8] C.M. Perez, M.S. Hazari, A.K. Farraj, Role of autonomic reflex arcs in cardiovascular responses to air pollution exposure, Cardiovasc Toxicol. 15 (1) (2015) 69–78.
[9] M.R. Miller, C.A. Shaw, J.P. Langrish, From particles to patients: oxidative stress and the cardiovascular effects of air pollution, Future Cardiol. 8 (4) (2012) 577–602.
[10] R.J. Delfino, N. Staimer, N.D. Vaziri, Air pollution and circulating biomarkers of oxidative stress, Air Qual. Atmos. Health 4 (1) (2011) 37–52.
[11] S.A. Weichenthal, K. Godri-Pollitt, P.J. Villeneuve, PM2.5, oxidant defence and cardiorespiratory health: a review, Environ. Health 12 (2013) 40.
[12] R.D. Brook, S. Rajagopalan, Particulate matter, air pollution, and blood pressure, J. Am. Soc. Hypertens. 3 (5) (2009) 332–350.
[13] H.C. Chuang, T.W. Hsueh, C.C. Chang, J.S. Hwang, K.J. Chuang, Y.H. Yan, T.J. Cheng, Nickel-regulated heart rate variability: the roles of oxidative stress and inflammation, Toxicol. Appl. Pharmacol. 266 (2) (2013) 298–306.
[14] P. Moller, D.V. Christophersen, N.R. Jacobsen, A. Skovmand, A.C. Gouveia, M.H. Andersen, A. Kermanizadeh, D.M. Jensen, P.H. Danielsen, M. Roursgaard, K. Jantzen, S. Loft, Atherosclerosis and vasomotor dysfunction in arteries of animals after exposure to combustion-derived particulate matter or nanomaterials, Crit. Rev. Toxicol. (2016) 1–40.
[15] U. Alheid, J.C. Frolich, U. Forstermann, Endothelium derived relaxing factor from cultured human endothelial cells inhibits aggregation of human platelets, Thromb. Res. 47 (1987) 561–571.
[16] P. Kubes, M. Suzuki, D.N. Granger, Nitric oxide: an endogenous modulator of leukocyte adhesion, Proc. Natl. Acad. Sci. 88 (1991) 4651–4655.
[17] Y. Nunokawa, S. Tanaka, Interferon-γ inhibits proligeration of rat smooth muscle cells by nitric oxide generation, Biochem Biophys. Res Commun. 188 (1) (1992) 409–415.
[18] T.J. Anderson, M.D. Gerhard, I.T. Meredith, F. Charbonneau, D. Delagrange, M.A. Creager, A.P. Selwyn, P. Ganz, Systemic nature of endothelial dysfunction in atherosclerosis, Am. J. Cardiol. 75 (1995) 71B–74B.
[19] E.O. Apostolov, S.V. Shah, E. Ok, A.G. Basnakian, Carbamylated low-density lipoprotein induces monocyte adhesion to endothelial cells through intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, Arterioscler. Thromb. Vasc. Biol. 27 (4) (2007) 826–832.
[20] P. Libby, P.M. Ridker, G.K. Hansson, Progress and challenges in translating the biology of atherosclerosis, Nature 473 (7347) (2011) 317–325.
[21] K.J. Moore, F.J. Sheedy, E.A. Fisher, Macrophages in atherosclerosis: a dynamic balance, Nat. Rev. Immunol. 13 (10) (2013) 709–721.
[22] T. Cherng, M. Paffett, O. Jackson-Weaver, M. Campen, B. Walker, K. NL, Mechanisms of diesel-induced endothelial nitric oxide synthase dysfunction in coronary arterioles, Environ. Health Perspect. 119 (2011) 98–103.
[23] R. Radi, Peroxynitrite, a stealthy biological oxidant, J. Biol. Chem. 288 (37) (2013) 26464–26472.
[24] J. Madrigano, A. Baccarelli, R.O. Wright, H. Suh, D. Sparrow, P.S. Vokonas, J. Schwartz, Air pollution, obesity, genes and cellular adhesion molecules, Occup. Environ. Med 67 (5) (2010) 312–317.
[25] M.A. Bind, B. Coull, H. Suh, R. Wright, A. Baccarelli, P. Vokonas, J. Schwartz, A novel genetic score approach using instruments to investigate interactions between pathways and environment: application to air pollution, PLoS One 9 (4) (2014) e96000.
[26] L. Dai, M.A. Bind, P. Koutrakis, B.A. Coull, D. Sparrow, P.S. Vokonas, J.D. Schwartz, Fine particles, genetic pathways, and markers of inflammation and endothelial dysfunction: analysis on particulate species and sources, J. Expo. Sci. Environ. Epidemiol. 26 (4) (2016) 415–421.
[27] X. Zhang, N. Staimer, T. Tjoa, D.L. Gillen, J.J. Schauer, M.M. Shafer, S. Hasheminassab, P. Pakbin, J. Longhurst, C. Sioutas, R.J. Delfino, Associations between microvascular function and short-term exposure to traffic-related air pollution and particulate matter oxidative potential, Environ. Health 15 (1) (2016) 81.
[28] A. Wauters, C. Dreyfuss, S. Pochet, P. Hendrick, G. Berkenboom, P. van de Borne, J.F. Argacha, Acute exposure to diesel exhaust impairs nitric oxide-mediated endothelial vasomotor function by increasing endothelial oxidative stress, Hypertension 62 (2) (2013) 352–358.
[29] Z. Ying, X. Xu, M. Chen, D. Liu, M. Zhong, L.C. Chen, Q. Sun, S. Rajagopalan, A synergistic vascular effect of airborne particulate matter and nickel in a mouse model, Toxicol. Sci. 135 (1) (2013) 72–80.
[30] T. Kampfrath, A. Maiseyeu, Z. Ying, Z. Shah, J.A. Deiuliis, X. Xu, N. Kherada, R.D. Brook, K.M. Reddy, N.P. Padture, S. Parthasarathy, L.C. Chen, S. Moffatt-Bruce, Q. Sun, H. Morawietz, S. Rajagopalan, Chronic fine particulate matter exposure induces systemic vascular dysfunction via NADPH oxidase and TLR4 pathways, Circ. Res 108 (6) (2011) 716–726.
[31] A.K. Lund, J. Lucero, S. Lucas, M.C. Madden, J.D. McDonald, J.C. Seagrave, T.L. Knuckles, M.J. Campen, Vehicular emissions induce vascular MMP-9 expression and activity associated with endothelin-1-mediated pathways, Arterioscler. Thromb. Vasc. Biol. 29 (4) (2009) 511–517.
[32] A.K. Lund, J. Lucero, M. Harman, M.C. Madden, J.D. McDonald, J.C. Seagrave, M.J. Campen, The oxidized low-density lipoprotein receptor mediates vascular effects of inhaled vehicle emissions, Am. J. Respir. Crit. Care Med. 184 (1) (2011) 82–91.
[33] N. Labranche, C. El Khattabi, L. Dewachter, C. Dreyfuss, J. Fontaine, P. van de Borne, G. Berkenboom, S. Pochet, Vascular oxidative stress induced by diesel exhaust microparticles: synergism with hypertension, J. Cardiovasc. Pharmacol. 60 (6) (2012) 530–537.
[34] H. Li, M. Han, L. Guo, G. Li, N. Sang, Oxidative stress, endothelial dysfunction and inflammatory response in rat heart to NO(2) inhalation exposure, Chemosphere 82 (11) (2011) 1589–1596.
[35] G.C. Chuang, Z. Yang, D.G. Westbrook, M. Pompilius, C.A. Ballinger, C.R. White, D.M. Krzywanski, E.M. Postlethwait, S.W. Ballinger, Pulmonary ozone exposure induces vascular dysfunction, mitochondrial damage, and atherogenesis, Am. J. Physiol. Lung Cell Mol. Physiol. 297 (2) (2009) L209–L216.
[36] U.P. Kodavanti, R. Thomas, A.D. Ledbetter, M.C. Schladweiler, J.H. Shannahan, J.G. Wallenborn, A.K. Lund, M.J. Campen, E.O. Butler, R.R. Gottipolu, A. Nyska, J.E. Richards, D. Andrews, R.H. Jaskot, J. McKee, S.R. Kotha, R.B. Patel, N.L. Parinandi, Vascular and cardiac impairments in rats inhaling ozone and diesel exhaust particles, Environ. Health Perspect. 119 (3) (2011) 312–318.
[37] M.L. Paffett, K.E. Zychowski, L. Sheppard, S. Robertson, J.M. Weaver, S.N. Lucas, M.J. Campen, Ozone inhalation impairs coronary artery dilation via intracellular oxidative stress: evidence for serum-borne factors as drivers of systemic toxicity, Toxicol. Sci. 146 (2) (2015) 244–253.
[38] P. Haberzettl, J. Lee, D. Duggineni, J. McCracken, D. Bolanowski, T.E. O'Toole, A. Bhatnagar, D.J. Conklin, Exposure to ambient air fine particulate matter prevents VEGF-induced mobilization of endothelial progenitor cells from the bone marrow, Environ. Health Perspect. 120 (6) (2012) 848–856.
[39] T.E. O'Toole, J. Hellmann, L. Wheat, P. Haberzettl, J. Lee, D.J. Conklin, A. Bhatnagar, C.A. Pope 3rd, Episodic exposure to fine particulate air pollution decreases circulating levels of endothelial progenitor cells, Circ. Res. 107 (2) (2010) 200–203.
[40] Y. Cui, F. Jia, J. He, X. Xie, Z. Li, M. Fu, H. Hao, Y. Liu, D.Z. Liu, P.J. Cowan, H. Zhu, Q. Sun, Z. Liu, Ambient fine particulate matter suppresses in vivo proliferation of bone marrow stem cells through reactive oxygen species formation, PLoS One 10 (6) (2015) e0127309.
[41] Y. Cui, X. Xie, F. Jia, J. He, Z. Li, M. Fu, H. Hao, Y. Liu, J.Z. Liu, P.J. Cowan, H. Zhu, Q. Sun, Z. Liu, Ambient fine particulate matter induces apoptosis of endothelial progenitor cells through reactive oxygen species formation, Cell Physiol. Biochem. 35 (1) (2015) 353–363.
[42] H. Wei, W. Dan, Y. Shuo, Z. Fang, D. Wenjun, Oxidative stress induced by urban fine particles in cultured EA.hy926 cells, Hum. Exp. Toxicol. 30 (7) (2011) 579–590.
[43] L. Forchhammer, S. Loft, M. Roursgaard, Y. Cao, I.S. Riddervold, T. Sigsgaard, P. Moller, Expression of adhesion molecules, monocyte interactions and oxidative stress in human endothelial cells exposed to wood smoke and diesel exhaust particulate matter, Toxicol. Lett. 209 (2) (2012) 121–128.
[44] A. Montiel-Davalos, A. Gonzalez-Villava, V. Rodriguez-Lara, L.F. Montano, T.I. Fortoul, R. Lopez-Marure, Vanadium pentoxide induces activation and death of endothelial cells, J. Appl. Toxicol. 32 (1) (2012) 26–33.
[45] N. Buchner, N. Ale-Agha, S. Jakob, U. Sydlik, K. Kunze, K. Unfried, J. Altschmied, J. Haendeler, Unhealthy diet and ultrafine carbon black particles induce senescence and disease associated phenotypic changes, Exp. Gerontol. 48 (1) (2013) 8–16.
[46] Y. Cao, M. Roursgaard, P.H. Danielsen, P. Moller, S. Loft, Carbon black nanoparticles promote endothelial activation and lipid accumulation in macrophages independently of intracellular ROS production, PLoS One 9 (9) (2014) e106711.
[47] W. Rui, L. Guan, F. Zhang, W. Zhang, W. Ding, PM2.5 -induced oxidative stress increases adhesion molecules expression in human endothelial cells through the ERK/AKT/NF-kappaB-dependent pathway, J. Appl. Toxicol. 36 (1) (2016) 48–59.
[48] R. Li, Z. Ning, J. Cui, B. Khalsa, L. Ai, W. Takabe, T. Beebe, R. Majumdar, C. Sioutas, T. Hsiai, Ultrafine particles from diesel engines induce vascular oxidative stress via JNK activation, Free Radic. Biol. Med. 46 (6) (2009) 775–782.
[49] Y. Du, M. Navab, M. Shen, J. Hill, P. Pakbin, C. Sioutas, T.K. Hsiai, R. Li, Ambient ultrafine particles reduce endothelial nitric oxide production via S-glutathionylation of eNOS, Biochem Biophys. Res. Commun. 436 (3) (2013) 462–466.
[50] Y. Mo, R. Wan, S. Chien, D.J. Tollerud, Q. Zhang, Activation of endothelial cells after exposure to ambient ultrafine particles: the role of NADPH oxidase, Toxicol. Appl. Pharmacol. 236 (2) (2009) 183–193.
[51] H. Frikke-Schmidt, M. Roursgaard, J. Lykkesfeldt, S. Loft, J.K. Nojgaard, P. Moller, Effect of vitamin C and iron chelation on diesel exhaust particle and carbon black induced oxidative damage and cell adhesion molecule expression in human endothelial cells, Toxicol. Lett. 203 (3) (2011) 181–189.
[52] C.C. Lee, S.H. Huang, Y.T. Yang, Y.W. Cheng, C.H. Li, J.J. Kang, Motorcycle exhaust particles up-regulate expression of vascular adhesion molecule-1 and intercellular adhesion molecule-1 in human umbilical vein endothelial cells, Toxicol. Vitr. 26 (4) (2012) 552–560.
[53] C.Y. Tseng, J.F. Chang, J.S. Wang, Y.J. Chang, M.K. Gordon, M.W. Chao, Protective Effects of N-acetyl cysteine against diesel exhaust particles-induced intracellular ROS generates pro-inflammatory cytokines to mediate the vascular permeability of capillary-like endothelial tubes, PLoS One 10 (7) (2015) e0131911.
[54] J.F. Viles-Gonzalez, V. Fuster, J.J. Badimon, Atherothrombosis: a widespread disease with unpredictable and life-threatening consequences, Eur. Heart J. 25 (14) (2004) 1197–1207.
[55] E. Podrez, M. Febbraio, N. Sheibani, D. Schmitt, R. Silverstein, D. Hajjar, P. Cohen, W. Frazier, H. Hoff, S. Hazen, Macrophage scavenger receptor CD36 is the major receptor for LDL modified by monocyte-generated reactive nitrogen species, J. Clin. Investig. 105 (2000) 1095–1108.
[56] E. Zakynthinos, N. Pappa, Inflammatory biomarkers in coronary artery disease, J. Cardiol. 53 (3) (2009) 317–333.
[57] J.A. Araujo, A.E. Nel, Particulate matter and atherosclerosis: role of particle size,

composition and oxidative stress, Part Fibre Toxicol. 6 (2009) 24.
[58] S. Wu, D. Yang, H. Wei, B. Wang, J. Huang, H. Li, M. Shima, F. Deng, X. Guo, Association of chemical constituents and pollution sources of ambient fine particulate air pollution and biomarkers of oxidative stress associated with atherosclerosis: a panel study among young adults in Beijing, China, Chemosphere 135 (2015) 347–353.
[59] R. Kelishadi, N. Mirghaffari, P. Poursafa, S.S. Gidding, Lifestyle and environmental factors associated with inflammation, oxidative stress and insulin resistance in children, Atherosclerosis 203 (1) (2009) 311–319.
[60] L. Jacobs, J. Emmerechts, C. Mathieu, M.F. Hoylaerts, F. Fierens, P.H. Hoet, B. Nemery, T.S. Nawrot, Air pollution related prothrombotic changes in persons with diabetes, Environ. Health Perspect. 118 (2) (2010) 191–196.
[61] N. Brucker, A.M. Moro, M.F. Charao, J. Durgante, F. Freitas, M. Baierle, S. Nascimento, B. Gauer, R.P. Bulcao, G.B. Bubols, P.D. Ferrari, F.V. Thiesen, A. Gioda, M.M. Duarte, I. de Castro, P.H. Saldiva, S.C. Garcia, Biomarkers of occupational exposure to air pollution, inflammation and oxidative damage in taxi drivers, Sci. Total Environ. 463–464 (2013) 884–893.
[62] C. Tonne, J.D. Yanosky, S. Beevers, P. Wilkinson, F.J. Kelly, PM mass concentration and PM oxidative potential in relation to carotid intima-media thickness, Epidemiology 23 (3) (2012) 486–494.
[63] R.S. Rodosthenous, B.A. Coull, Q. Lu, P.S. Vokonas, J.D. Schwartz, A.A. Baccarelli, Ambient particulate matter and microRNAs in extracellular vesicles: a pilot study of older individuals, Part Fibre Toxicol. 13 (1) (2016) 13.
[64] Q. Sun, A. Wang, X. Jin, A. Natanzon, D. Duquaine, R.D. Brook, J.-G.S. Aguinaldo, Z.A. Fayad, V. Fuster, M. Lippmann, L.C. Chen, S. Rajagopalan, Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model, JAMA 294 (23) (2005) 3003–3010.
[65] A.K. Lund, T.L. Knuckles, C. Obot Akata, R. Shohet, J.D. McDonald, A. Gigliotti, J.C. Seagrave, M.J. Campen, Gasoline exhaust emissions induce vascular remodeling pathways involved in atherosclerosis, Toxicol. Sci. 95 (2) (2007) 485–494.
[66] N. Bai, T. Kido, H. Suzuki, G. Yang, T.J. Kavanagh, J.D. Kaufman, M.E. Rosenfeld, C. van Breemen, S.F. Eeden, Changes in atherosclerotic plaques induced by inhalation of diesel exhaust, Atherosclerosis 216 (2) (2011) 299–306.
[67] M.R. Miller, S.G. McLean, R. Duffin, A.O. Lawal, J.A. Araujo, C.A. Shaw, N.L. Mills, K. Donaldson, D.E. Newby, P.W. Hadoke, Diesel exhaust particulate increases the size and complexity of lesions in atherosclerotic mice, Part Fibre Toxicol. 10 (2013) 61.
[68] F. Yin, A. Lawal, J. Ricks, J.R. Fox, T. Larson, M. Navab, A.M. Fogelman, M.E. Rosenfeld, J.A. Araujo, Diesel exhaust induces systemic lipid peroxidation and development of dysfunctional pro-oxidant and pro-inflammatory high-density lipoprotein, Arterioscler. Thromb. Vasc. Biol. 33 (6) (2013) 1153–1161.
[69] R. Li, M. Navab, P. Pakbin, Z. Ning, K. Navab, G. Hough, T.E. Morgan, C.E. Finch, J.A. Araujo, A.M. Fogelman, C. Sioutas, T. Hsiai, Ambient ultrafine particles alter lipid metabolism and HDL anti-oxidant capacity in LDLR-null mice, J. Lipid Res 54 (6) (2013) 1608–1615.
[70] Z. Ying, T. Kampfrath, G. Thurston, B. Farrar, M. Lippmann, A. Wang, Q. Sun, L.C. Chen, S. Rajagopalan, Ambient particulates alter vascular function through induction of reactive oxygen and nitrogen species, Toxicol. Sci. 111 (1) (2009) 80–88.
[71] A. Montiel-Davalos, J. Ibarra-Sanchez Mde, J.L. Ventura-Gallegos, E. Alfaro-Moreno, R. Lopez-Marure, Oxidative stress and apoptosis are induced in human endothelial cells exposed to urban particulate matter, Toxicol. Vitr. 24 (1) (2010) 135–141.
[72] Q. Sun, P. Yue, Z. Ying, A.J. Cardounel, R.D. Brook, R. Devlin, J.S. Hwang, J.L. Zweier, L.C. Chen, S. Rajagopalan, Air pollution exposure potentiates hypertension through reactive oxygen species-mediated activation of Rho/ROCK, Arterioscler. Thromb. Vasc. Biol. 28 (10) (2008) 1760–1766.
[73] Y. Cao, K. Jantzen, A.C. Gouveia, A. Skovmand, M. Roursgaard, S. Loft, P. Moller, Automobile diesel exhaust particles induce lipid droplet formation in macrophages in vitro, Environ. Toxicol. Pharmacol. 40 (1) (2015) 164–171.
[74] R.J. Delfino, N. Staimer, T. Tjoa, D.L. Gillen, A. Polidori, M. Arhami, M.T. Kleinman, N.D. Vaziri, J. Longhurst, C. Sioutas, Air pollution exposures and circulating biomarkers of effect in a susceptible population: clues to potential causal component mixtures and mechanisms, Environ. Health Perspect. 117 (8) (2009) 1232–1238.
[75] A.P. Pettit, A. Brooks, R. Laumbach, N. Fiedler, Q. Wang, P.O. Strickland, K. Madura, J. Zhang, H.M. Kipen, Alteration of peripheral blood monocyte gene expression in humans following diesel exhaust inhalation, Inhal. Toxicol. 24 (3) (2012) 172–181.
[76] R.B. Devlin, C.B. Smith, M.T. Schmitt, A.G. Rappold, A. Hinderliter, D. Graff, M.S. Carraway, Controlled exposure of humans with metabolic syndrome to concentrated ultrafine ambient particulate matter causes cardiovascular effects, Toxicol. Sci. 140 (1) (2014) 61–72.
[77] M. Strak, G. Hoek, K.J. Godri, I. Gosens, I.S. Mudway, R. van Oerle, H.M. Spronk, F.R. Cassee, E. Lebret, F.J. Kelly, R.M. Harrison, B. Brunekreef, M. Steenhof, N.A. Janssen, Composition of PM affects acute vascular inflammatory and coagulative markers – the RAPTES project, PLoS One 8 (3) (2013) e58944.
[78] S. Upadhyay, T. Stoeger, L. George, M.C. Schladweiler, U. Kodavanti, K. Ganguly, H. Schulz, Ultrafine carbon particle mediated cardiovascular impairment of aged spontaneously hypertensive rats, Part Fibre Toxicol. 11 (2014) 36.
[79] A. Nemmar, S. Al-Salam, S. Dhanasekaran, M. Sudhadevi, B.H. Ali, Pulmonary exposure to diesel exhaust particles promotes cerebral microvessel thrombosis: protective effect of a cysteine prodrug l-2-oxothiazolidine-4-carboxylic acid, Toxicology 263 (2–3) (2009) 84–92.
[80] A. Nemmar, R. Al Dhaheri, J. Alamiri, S. Al Hefeiti, H. Al Saedi, S. Beegam, P. Yuvaraju, J. Yasin, B.H. Ali, Diesel exhaust particles induce impairment of vascular and cardiac homeostasis in mice: ameliorative effect of emodin, Cell Physiol. Biochem. 36 (4) (2015) 1517–1526.
[81] S.J. Snow, W. Cheng, A.S. Wolberg, M.S. Carraway, Air pollution upregulates endothelial cell procoagulant activity via ultrafine particle-induced oxidant signaling and tissue factor expression, Toxicol. Sci. 140 (1) (2014) 83–93.
[82] S.E. Chiarella, S. Soberanes, D. Urich, L. Morales-Nebreda, R. Nigdelioglu, D. Green, J.B. Young, A. Gonzalez, C. Rosario, A.V. Misharin, A.J. Ghio, R.G. Wunderink, H.K. Donnelly, K.A. Radigan, H. Perlman, N.S. Chandel, G.R. Budinger, G.M. Mutlu, beta(2)-Adrenergic agonists augment air pollution-induced IL-6 release and thrombosis, J. Clin. Invest. 124 (7) (2014) 2935–2946.
[83] J. Schwartz, S.K. Park, M.S. O'Neill, P.S. Vokonas, D. Sparrow, S. Weiss, K. Kelsey, Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction, Am. J. Respir. Crit. Care Med. 172 (12) (2005) 1529–1533.
[84] S.K. Park, M.S. O'Neill, R.O. Wright, H. Hu, P.S. Vokonas, D. Sparrow, H. Suh, J. Schwartz, HFE genotype, particulate air pollution, and heart rate variability: a gene-environment interaction, Circulation 114 (25) (2006) 2798–2805.
[85] T. Chahine, A. Baccarelli, A. Litonjua, R.O. Wright, H. Suh, D.R. Gold, D. Sparrow, P. Vokonas, J. Schwartz, Particulate air pollution, oxidative stress genes, and heart rate variability in an elderly cohort, Environ. Health Perspect. 115 (11) (2007) 1617–1622.
[86] E.S. Baja, J.D. Schwartz, G.A. Wellenius, B.A. Coull, A. Zanobetti, P.S. Vokonas, H.H. Suh, Traffic-related air pollution and QT interval: modification by diabetes, obesity, and oxidative stress gene polymorphisms in the normative aging study, Environ. Health Perspect. 118 (6) (2010) 840–846.
[87] K.N. Kim, J.H. Kim, K. Jung, Y.C. Hong, Associations of air pollution exposure with blood pressure and heart rate variability are modified by oxidative stress genes: a repeated-measures panel among elderly urban residents, Environ. Health 15 (1) (2016) 47.
[88] M. Wang, M.J. Utell, A. Schneider, W. Zareba, M.W. Frampton, D. Oakes, P.K. Hopke, J. Wiltshire, C. Kane, A. Peters, S. Breitner, D. Chalupa, D.Q. Rich, Does total antioxidant capacity modify adverse cardiac responses associated with ambient ultrafine, accumulation mode, and fine particles in patients undergoing cardiac rehabilitation? Environ. Res 149 (2016) 15–22.
[89] L.Y. Lin, H.C. Chuang, I.J. Liu, H.W. Chen, K.J. Chuang, Reducing indoor air pollution by air conditioning is associated with improvements in cardiovascular health among the general population, Sci. Total Environ. 463–464 (2013) 176–181.
[90] J. Zhang, T. Zhu, H. Kipen, G. Wang, W. Huang, D. Rich, P. Zhu, Y. Wang, S.E. Lu, P. Ohman-Strickland, S. Diehl, M. Hu, J. Tong, J. Gong, D. Thomas, Cardiorespiratory biomarker responses in healthy young adults to drastic air quality changes surrounding the 2008 Beijing Olympics, Res Rep. Health Eff. Inst. 174 (2013) 5–174.
[91] M.S. Lee, K.D. Eum, S.C. Fang, E.G. Rodrigues, G.A. Modest, D.C. Christiani, Oxidative stress and systemic inflammation as modifiers of cardiac autonomic responses to particulate air pollution, Int J. Cardiol. 176 (1) (2014) 166–170.
[92] J.A. Sarnat, R. Golan, R. Greenwald, A.U. Raysoni, P. Kewada, A. Winquist, S.E. Sarnat, W. Dana Flanders, M.C. Mirabelli, J.E. Zora, M.H. Bergin, F. Yip, Exposure to traffic pollution, acute inflammation and autonomic response in a panel of car commuters, Environ. Res 133 (2014) 66–76.
[93] J.G. Hemmingsen, J. Rissler, J. Lykkesfeldt, G. Sallsten, J. Kristiansen, P.P. Moller, S. Loft, Controlled exposure to particulate matter from urban street air is associated with decreased vasodilation and heart rate variability in overweight and older adults, Part Fibre Toxicol. 12 (2015) 6.
[94] H.M. Byun, E. Colicino, L. Trevisi, T. Fan, D.C. Christiani, A.A. Baccarelli, Effects of air pollution and blood mitochondrial DNA methylation on markers of heart rate variability, J. Am. Heart Assoc. 5 (4) (2016).
[95] I. Romieu, M.M. Tellez-Rojo, M. Lazo, A. Manzano-Patino, M. Cortez-Lugo, P. Julien, M.C. Belanger, M. Hernandez-Avila, F. Holguin, Omega-3 fatty acid prevents heart rate variability reductions associated with particulate matter, Am. J. Respir. Crit. Care Med. 172 (12) (2005) 1534–1540.
[96] H. Tong, A.G. Rappold, D. Diaz-Sanchez, S.E. Steck, J. Berntsen, W.E. Cascio, R.B. Devlin, J.M. Samet, Omega-3 fatty acid supplementation appears to attenuate particulate air pollution-induced cardiac effects and lipid changes in healthy middle-aged adults, Environ. Health Perspect. 120 (7) (2012) 952–957.
[97] E. Ghelfi, G.A. Wellenius, J. Lawrence, E. Millet, B. Gonzalez-Flecha, Cardiac oxidative stress and dysfunction by fine concentrated ambient particles (CAPs) are mediated by angiotensin-II, Inhal. Toxicol. 22 (11) (2010) 963–972.
[98] J.B. Kim, C. Kim, E. Choi, S. Park, H. Park, H.N. Pak, M.H. Lee, D.C. Shin, K.C. Hwang, B. Joung, Particulate air pollution induces arrhythmia via oxidative stress and calcium calmodulin kinase II activation, Toxicol. Appl. Pharmacol. 259 (1) (2012) 66–73.
[99] S. Robertson, A.L. Thomson, R. Carter, H.R. Stott, C.A. Shaw, P.W. Hadoke, D.E. Newby, M.R. Miller, G.A. Gray, Pulmonary diesel particulate increases susceptibility to myocardial ischemia/reperfusion injury via activation of sensory TRPV1 and beta1 adrenoreceptors, Part Fibre Toxicol. 11 (2014) 12.
[100] G. Wang, R. Jiang, Z. Zhao, W. Song, Effects of ozone and fine particulate matter (PM(2.5)) on rat system inflammation and cardiac function, Toxicol. Lett. 217 (1) (2013) 23–33.
[101] L. Andre, F. Gouzi, J. Thireau, G. Meyer, J. Boissiere, M. Delage, A. Abdellaoui, C. Feillet-Coudray, G. Fouret, J.P. Cristol, A. Lacampagne, P. Obert, C. Reboul, J. Fauconnier, M. Hayot, S. Richard, O. Cazorla, Carbon monoxide exposure enhances arrhythmia after cardiac stress: involvement of oxidative stress, Basic Res. Cardiol. 106 (6) (2011) 1235–1246.
[102] Z. Ying, P. Yue, X. Xu, M. Zhong, Q. Sun, M. Mikolaj, A. Wang, R.D. Brook,

L.C. Chen, S. Rajagopalan, Air pollution and cardiac remodeling: a role for RhoA/Rho-kinase, Am. J. Physiol. Heart Circ. Physiol. 296 (5) (2009) H1540–H1550.
[103] I. Mordukhovich, E. Wilker, H. Suh, R. Wright, D. Sparrow, P.S. Vokonas, J. Schwartz, Black carbon exposure, oxidative stress genes, and blood pressure in a repeated-measures study, Environ. Health Perspect. 117 (11) (2009) 1767–1772.
[104] A. Levinsson, A.C. Olin, L. Modig, S. Dahgam, L. Bjorck, A. Rosengren, F. Nyberg, Interaction effects of long-term air pollution exposure and variants in the GSTP1, GSTT1 and GSTCD genes on risk of acute myocardial infarction and hypertension: a case-control study, PLoS One 9 (6) (2014) e99043.
[105] J. Zhong, A. Cayir, L. Trevisi, M. Sanchez-Guerra, X. Lin, C. Peng, M.A. Bind, D. Prada, H. Laue, K.J. Brennan, A. Dereix, D. Sparrow, P. Vokonas, J. Schwartz, A.A. Baccarelli, Traffic-Related Air Pollution, Blood Pressure, and Adaptive Response of Mitochondrial Abundance, Circulation 133 (4) (2016) 378–387.
[106] V.C. Louwies T, L. Panis, B. Cox, K. Vrijens, T. Nawrot, P. De Boever, MiRNA expression profiles and retinal blood vessel calibers are associated with short-term particulate matter air pollution exposure, Environ. Res. 147 (2016) 24–31.
[107] R.R. Gottipolu, J.G. Wallenborn, E.D. Karoly, M.C. Schladweiler, A.D. Ledbetter, T. Krantz, W.P. Linak, A. Nyska, J.A. Johnson, R. Thomas, J.E. Richards, R.H. Jaskot, U.P. Kodavanti, One-month diesel exhaust inhalation produces hypertensive gene expression pattern in healthy rats, Environ. Health Perspect. 117 (1) (2009) 38–46.
[108] O.G. Aztatzi-Aguilar, M. Uribe-Ramirez, J.A. Arias-Montano, O. Barbier, A. De Vizcaya-Ruiz, Acute and subchronic exposure to air particulate matter induces expression of angiotensin and bradykinin-related genes in the lungs and heart: angiotensin-ii type-I receptor as a molecular target of particulate matter exposure, Part Fibre Toxicol. 12 (2015) 17.
[109] C. Ren, S.K. Park, P.S. Vokonas, D. Sparrow, E. Wilker, A. Baccarelli, H.H. Suh, K.L. Tucker, R.O. Wright, J. Schwartz, Air pollution and homocysteine: more evidence that oxidative stress-related genes modify effects of particulate air pollution, Epidemiology 21 (2) (2010) 198–206.
[110] V.C. Van Hee, S.D. Adar, A.A. Szpiro, R.G. Barr, D.A. Bluemke, A.V. Diez Roux, E.A. Gill, L. Sheppard, J.D. Kaufman, Exposure to traffic and left ventricular mass and function: the Multi-Ethnic Study of Atherosclerosis, Am. J. Respir. Crit. Care Med. 179 (9) (2009) 827–834.
[111] V.C. Van Hee, S.D. Adar, A.A. Szpiro, R.G. Barr, A. Diez Roux, D.A. Bluemke, L. Sheppard, E.A. Gill, H. Bahrami, C. Wassel, M.M. Sale, D.S. Siscovick, J.I. Rotter, S.S. Rich, J.D. Kaufman, Common genetic variation, residential proximity to traffic exposure, and left ventricular mass: the multi-ethnic study of atherosclerosis, Environ. Health Perspect. 118 (7) (2010) 962–969.
[112] A. Dominguez-Rodriguez, S. Rodriguez, P. Abreu-Gonzalez, P. Avanzas, R.A. Juarez-Prera, Black carbon exposure, oxidative stress markers and major adverse cardiovascular events in patients with acute coronary syndromes, Int J. Cardiol. 188 (2015) 47–49.
[113] S. Weichenthal, E. Lavigne, G. Evans, K. Pollitt, R.T. Burnett, Ambient PM2.5 and risk of emergency room visits for myocardial infarction: impact of regional PM2.5 oxidative potential: a case-crossover study, Environ. Health 15 (1) (2016) 46.
[114] C.S. Weldy, Y. Liu, H.D. Liggitt, M.T. Chin, In utero exposure to diesel exhaust air pollution promotes adverse intrauterine conditions, resulting in weight gain, altered blood pressure, and increased susceptibility to heart failure in adult mice, PLoS One 9 (2) (2014) e88582.
[115] R.S. Perepu, D.E. Dostal, C. Garcia, R.H. Kennedy, R. Sethi, Cardiac dysfunction subsequent to chronic ozone exposure in rats, Mol. Cell Biochem. 360 (1–2) (2012) 339–345.
[116] R. Sethi, S. Manchanda, R.S. Perepu, A. Kumar, C. Garcia, R.H. Kennedy, S. Palakurthi, D. Dostal, Differential expression of caveolin-1 and caveolin-3: potential marker for cardiac toxicity subsequent to chronic ozone inhalation, Mol. Cell Biochem. 369 (1–2) (2012) 9–15.
[117] S. Mahne, G.C. Chuang, E. Pankey, L. Kiruri, P.J. Kadowitz, B. Dellinger, K.J. Varner, Environmentally persistent free radicals decrease cardiac function and increase pulmonary artery pressure, Am. J. Physiol. Heart Circ. Physiol. 303 (9) (2012) (H1135-42).
[118] R.S. Perepu, C. Garcia, D. Dostal, R. Sethi, Enhanced death signaling in ozone-exposed ischemic-reperfused hearts, Mol. Cell Biochem. 336 (1–2) (2010) 55–64.
[119] G. Meyer, L. Andre, S. Tanguy, J. Boissiere, C. Farah, F. Lopez-Lauri, S. Gayrard, S. Richard, F. Boucher, O. Cazorla, P. Obert, C. Reboul, Simulated urban carbon monoxide air pollution exacerbates rat heart ischemia-reperfusion injury, Am. J. Physiol. Heart Circ. Physiol. 298 (5) (2010) H1445–H1453.
[120] G. Meyer, L. Andre, A. Kleindienst, F. Singh, S. Tanguy, S. Richard, P. Obert, F. Boucher, B. Jover, O. Cazorla, C. Reboul, Carbon monoxide increases inducible NOS expression that mediates CO-induced myocardial damage during ischemia-reperfusion, Am. J. Physiol. Heart Circ. Physiol. 308 (7) (2015) H759–H767.
[121] M. Dianat, E. Radmanesh, M. Badavi, S.A. Mard, G. Goudarzi, Disturbance effects of PM on iNOS and eNOS mRNA expression levels and antioxidant activity induced by ischemia-reperfusion injury in isolated rat heart: protective role of vanillic acid, Environ. Sci. Pollut. Res. Int. 23 (6) (2015).
[122] F.M.A. Pereira, L.A.A. Pereira, F.F. Arbex, M. Arbex, G.M. Conceição, U.P. Santos, A.C. Lopes, P.H.N. Saldiva, A.L.F. Braga, S. Cendon, Effect of air pollution on diabetes and cardiovascular diseases in São Paulo, Brazil, Brazillian J. Med. Biol. Res. 41 (2008) 526–532.
[123] M.S. O'Neill, A. Veves, A. Zanobetti, J.A. Sarnat, D.R. Gold, P.A. Economides, E.S. Horton, J. Schwartz, Diabetes enhances vulnerability to particulate air pollution-associated impairment in vascular reactivity and endothelial function, Circulation 111 (22) (2005) 2913–2920.
[124] A. Zanobetti, J. Schwartz, Are diabetics more susceptible to the health effects of airborne particles? Am. J. Respir. Crit. Care Med. 164 (2001) 831–833.
[125] S.K. Park, A.H. Auchincloss, M.S. O'Neill, R. Prineas, J.C. Correa, J. Keeler, R.G. Barr, J.D. Kaufman, A.V. Diez Roux, Particulate air pollution, metabolic syndrome, and heart rate variability: the multi-ethnic study of atherosclerosis (MESA), Environ. Health Perspect. 118 (10) (2010) 1406–1411.
[126] K.A. Miller, D.S. Siscovick, L. Sheppard, K. Sheppard, J.H. Sullivan, G.L. Anderson, J.D. Kaufman, Long-term exposure to air pollution and incidence of cardiovascular events in women, N. Engl. J. Med. 356 (2007) 447–458.
[127] R.C. Puett, J. Schwartz, J.E. Hart, J.D. Yanosky, F.E. Speizer, H. Suh, C.J. Paciorek, L.M. Neas, F. Laden, Chronic particulate exposure, mortality, and coronary heart disease in the nurses' health study, Am. J. Epidemiol. 168 (10) (2008) 1161–1168.
[128] B. M, Biochemistry and molecular cell biology of diabetic complications, Nature 414 (2001) 813–820.
[129] U. Hink, H. Li, H. Mollnau, M. Oelze, E. Matheis, M. Hartmann, M. Skatchkov, F. Thaiss, R.A.K. Stahl, A. Warnholtz, T. Meinertz, K. Griendling, D.G. Harrison, U. Forstermann, T. Munzel, Mechanisms underlying endothelial dysfunction in diabetes mellitus, Circ. Res. 88 (2001) e14–e22.
[130] M.S. O'Neill, A. Veves, J.A. Sarnat, A. Zanobetti, D.R. Gold, P.A. Economides, E.S. Horton, J. Schwartz, Air pollution and inflammation in type 2 diabetes: a mechanism for susceptibility, Occup. Environ. Med. 64 (6) (2007) 373–379.
[131] P.W. Franks, R.L. Hanson, W.C. Knowler, M.L. Sievers, P.H. Bennett, H.C. Looker, Childhood obesity, other cardiovascular risk factors, and premature death, N. Engl. J. Med. 362 (6) (2010) 485–493.
[132] N. Mattsson, T. Ronnemaa, M. Juonala, J.S. Viikari, O.T. Raitakari, Childhood predictors of the metabolic syndrome in adulthood. The cardiovascular risk in Young Finns Study, Ann. Med 40 (7) (2008) 542–552.
[133] J.H. Kim, Y.C. Hong, GSTM1, GSTT1, and GSTP1 polymorphisms and associations between air pollutants and markers of insulin resistance in elderly Koreans, Environ. Health Perspect. 120 (10) (2012) 1378–1384.
[134] Y.C. Huang, M. Schmitt, Z. Yang, L.G. Que, J.C. Stewart, M.W. Frampton, R.B. Devlin, Gene expression profile in circulating mononuclear cells after exposure to ultrafine carbon particles, Inhal. Toxicol. 22 (10) (2010) 835–846.
[135] Z. Xu, X. Xu, M. Zhong, I.P. Hotchkiss, R.P. Lewandowski, J.G. Wagner, L.A. Bramble, Y. Yang, A. Wang, J.R. Harkema, M. Lippmann, S. Rajagopalan, L.C. Chen, Q. Sun, Ambient particulate air pollution induces oxidative stress and alterations of mitochondria and gene expression in brown and white adipose tissues, Part Fibre Toxicol. 8 (2011) 20.
[136] L. Sun, C. Liu, X. Xu, Z. Ying, A. Maiseyeu, A. Wang, K. Allen, R.P. Lewandowski, L.A. Bramble, M. Morishita, J.G. Wagner, J. Dvonch, Z. Sun, X. Yan, R.D. Brook, S. Rajagopalan, J.R. Harkema, Q. Sun, Z. Fan, Ambient fine particulate matter and ozone exposures induce inflammation in epicardial and perirenal adipose tissues in rats fed a high fructose diet, Part Fibre Toxicol. 10 (2013) 43.
[137] X. Xu, Z. Yavar, M. Verdin, Z. Ying, G. Mihai, T. Kampfrath, A. Wang, M. Zhong, M. Lippmann, L.C. Chen, S. Rajagopalan, Q. Sun, Effect of early particulate air pollution exposure on obesity in mice: role of p47phox, Arterioscler Thromb. Vasc. Biol. 30 (2010) 2518–2527.
[138] P. Haberzettl, T.E. O'Toole, A. Bhatnagar, D.J. Conklin, Exposure to fine particulate air pollution causes vascular insulin resistance by inducing pulmonary oxidative stress, Environ. Health Perspect. 124 (12) (2016) H1423–H1438.
[139] Y. Wei, J.J. Zhang, Z. Li, A. Gow, K.F. Chung, M. Hu, Z. Sun, L. Zeng, T. Zhu, G. Jia, X. Li, M. Duarte, X. Tang, Chronic exposure to air pollution particles increases the risk of obesity and metabolic syndrome: findings from a natural experiment in Beijing, FASEB J. 30 (6) (2016) 2115–2122.
[140] A. Nemmar, D. Subramaniyan, J. Yasin, B.H. Ali, Impact of experimental type 1 diabetes mellitus on systemic and coagulation vulnerability in mice acutely exposed to diesel exhaust particles, Part Fibre Toxicol. 10 (2013) 14.
[141] R.E. Vella, N.J. Pillon, B. Zarrouki, M.L. Croze, L. Koppe, M. Guichardant, S. Pesenti, M.A. Chauvin, J. Rieusset, A. Geloen, C.O. Soulage, Ozone exposure triggers insulin resistance through muscle c-Jun N-terminal kinase activation, Diabetes 64 (3) (2015) 1011–1024.
[142] J. Zhong, K. Allen, X. Rao, Z. Ying, Z. Braunstein, S.R. Kankanala, C. Xia, X. Wang, L.A. Bramble, J.G. Wagner, R. Lewandowski, Q. Sun, J.R. Harkema, S. Rajagopalan, Repeated ozone exposure exacerbates insulin resistance and activates innate immune response in genetically susceptible mice, Inhal. Toxicol. (2016) 1–10.
[143] A. Seaton, W. MacNee, K. Donaldson, D. Godden, Particle air pollution and acute health effects, Lancet 345 (1995) 176–178.
[144] K. Donaldson, V. Stone, A. Seaton, W. MacNee, Ambient particle inhalation and the cardiovascular system: potential mechanisms, Environ. Health Perspect. 109 (Suppl 4) (2001) 523–527.
[145] A. Bhatnagar, Cardiovascular pathophysiology of environmental pollutants, Am. J. Physiol. Heart Circ. Physiol. 286 (2004) H479–H485.
[146] Y. Mo, R. Wan, J. Wang, S. Chien, D.J. Tollerud, Q. Zhang, Diabetes is associated with increased sensitivity of alveolar macrophages to urban particulate matter exposure, Toxicology 262 (2) (2009) 130–137.
[147] Y. Zhang, Y. Mo, A. Gu, R. Wan, Q. Zhang, D.J. Tollerud, Effects of urban particulate matter with high glucose on human monocytes U937, J. Appl. Toxicol. 36 (4) (2015) 586–595.
[148] A. Zaky, A. Ahmad, L.J. Dell'Italia, L. Jahromi, L.A. Reisenberg, S. Matalon, S. Ahmad, Inhaled matters of the heart, Cardiovasc, Regen. Med. 2 (2015).
[149] R.M. Damiani, M.O. Piva, M.R. Petry, P.H. Saldiva, A. Tavares Duarte de Oliveira, C.R. Rhoden, Is cardiac tissue more susceptible than lung to oxidative effects induced by chronic nasotropic instillation of residual oil fly ash (ROFA)? Toxicol. Mech. Methods 22 (7) (2012) 533–539.
[150] V.M. Costa, F. Carvalho, J.A. Duarte, L. Bastos Mde, F. Remiao, The heart as a target for xenobiotic toxicity: the cardiac susceptibility to oxidative stress, Chem. Res Toxicol. 26 (9) (2013) 1285–1311.
[151] P. Kumarathasan, E. Blais, A. Saravanamuthu, A. Bielecki, B. Mukherjee,

S. Bjarnason, J. Guenette, P. Goegan, R. Vincent, Nitrative stress, oxidative stress and plasma endothelin levels after inhalation of particulate matter and ozone, Part Fibre Toxicol. 12 (2015) 28.
[152] B. Halliwell, Free radicals and antioxidants: updating a personal view, Nutr. Rev. 70 (5) (2012) 257–265.
[153] M.P. Murphy, A. Holmgren, N.G. Larsson, B. Halliwell, C.J. Chang, B. Kalyanaraman, S.G. Rhee, P.J. Thornalley, L. Partridge, D. Gems, T. Nystrom, V. Belousov, P.T. Schumacker, C.C. Winterbourn, Unraveling the biological roles of reactive oxygen species, Cell Metab. 13 (4) (2011) 361–366.
[154] M.D. Evans, V. Mistry, R. Singh, D. Gackowski, R. Rozalski, A. Siomek-Gorecka, D.H. Phillips, J. Zuo, L. Mullenders, A. Pines, Y. Nakabeppu, K. Sakumi, M. Sekiguchi, T. Tsuzuki, M. Bignami, R. Olinski, M.S. Cooke, Nucleotide excision repair of oxidised genomic DNA is not a source of urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine, Free Radic. Biol. Med. 99 (2016) 385–391.
[155] B. Halliwell, Free radicals and antioxidants – quo vadis? Trends Pharmacol. Sci. 32 (3) (2011) 125–130.
[156] G. Wang, L. Zhen, P. Lu, R. Jiang, W. Song, Effects of ozone and fine particulate matter (PM2.5) on rat cardiac autonomic nervous system and systemic inflammation, Wei Sheng Yan Jiu 42 (4) (2013) 554–560.