REVIEW ARTICLE

Atherosclerosis and vasomotor dysfunction in arteries of animals after exposure to combustion-derived particulate matter or nanomaterials

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\textbf{ABSTRACT}

Exposure to particulate matter (PM) from traffic vehicles is hazardous to the vascular system, leading to clinical manifestations and mortality due to ischemic heart disease. By analogy, nanomaterials may also be associated with the same outcomes. Here, the effects of exposure to PM from ambient air, diesel exhaust and certain nanomaterials on atherosclerosis and vasomotor dysfunction in animals have been assessed. The majority of studies have used pulmonary exposure by inhalation or instillation, although there are some studies on non-pulmonary routes such as the gastrointestinal tract. Airway exposure to air pollution particles and nanomaterials is associated with similar effects on atherosclerosis progression, augmented vasoconstriction and blunted vasorelaxation responses in arteries, whereas exposure to diesel exhaust is associated with lower responses. At present, there is no convincing evidence of dose-dependent effects across studies. Oxidative stress and inflammation have been observed in the arterial wall of PM-exposed animals, leading to clinical manifestations and mortality due to ischemic heart disease. By analogy, exposure to diesel exhaust has been associated with enhanced systemic inflammation and oxidative stress, and exposure to diesel exhaust particles has been associated with enhanced systemic inflammation. Furthermore, there is inconsistent evidence with regard to altered plasma lipid profile and systemic inflammation as a key step in vasomotor dysfunction and progression of atherosclerosis in PM-exposed animals. In summary, the results show that certain nanomaterials, including TiO\textsubscript{2}, carbon black and carbon nanotubes, have similar hazards to the vascular system as combustion-derived PM.

Table of contents

Introduction .................................................. ... 438
Cardiovascular risk factors ........................................ 440
Animal models used in studies on cardiovascular effects after PM exposure ........................................ 441
Progression of atherosclerosis ................................... 443
Inflammation and oxidative stress ................................. 444
Qualitative description of studies on vasomotor function and progression of atherosclerosis ................. 445
Vascular effects after exposure to authentic air pollution .......................................................... 445
Beijing, China ....................................... 445
Sao Paulo, Brazil ....................................... 445
Vascular effects after exposure to concentrated ambient air particles (CAPs) ........................................ 445
Tuxedo, NY ........................................... 446
New York City, NY ...................................... 447
Five-city study ........................................... 447
Los Angeles, CA ....................................... 447
Columbus, OH .......................................... 448
Sao Paulo, Brazil ....................................... 448
Utrecht, The Netherlands ................................... 449
Vascular effects after exposure to particulate matter in urban air ........................................ 449
SRM1648 .............................................. 449
SRM1649b ............................................. 449
EHC-93 ............................................... 449
Chapel Hill, NC ........................................ 450
Jinchang and Zhangye, China ................................ 450
Summary of studies on exposure to ambient air pollution particles ........................................ 450
Vascular effects after exposure to diesel exhaust particles .................................................. 451
Single cylinder Yanmar ................................... 451
Cummings .............................................. 451
Ingersoll Rand ......................................... 452
Bredenoord (35 KVA diesel generator under idling condition) ........................................ 452

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Vascular effects after exposure to particulate matter from diesel exhaust
SRM1650b
SRM2975
Summary of studies on exposure to DE and DEP
Vascular effects after exposure to nanomaterials
Multi-walled carbon nanotubes (MWCNTs)
Single-walled carbon nanotubes (SWCNTs)
Carbon black
Fullerene C60
Nickel hydroxide NMs
Cerium dioxide (CeO2)
Silicon-based NMs
Quantum dots
Summary of studies on exposure to NMs

Quantitative analysis of association between exposure to particulate matter and vascular effects
Effect of exposure to particulate matter on vasomotor function
Summary
Effect of exposure to particulate matter on progression of atherosclerosis
Summary
Dose–response relationships for CVD outcomes
Summary
Particle characteristics and effect on vascular outcomes
Summary
Mechanistic link between pulmonary exposure to particulate matter and vascular effects
PM-induced pulmonary inflammation and CVD outcomes
Systemic inflammation as link between PM exposure and CVD outcomes
Systemic levels of lipids and CVD outcomes
PM exposure, inflammation and oxidative stress in vessel walls
Summary
Limitations
Summary
Conclusions
Acknowledgements
Declaration of interest
References

Introduction

There is a well-established link between long-term air pollution exposure and increased mortality due to cardiovascular disease (CVD), particularly ischemic heart disease (IHD) and myocardial infarction (Brook et al. 2010). The excess risk in mortality per 10 μg/m³ increase in fine particles in urban air is 11% (95% confidence interval: 5–16%) for CVDs, particularly IHD, in a large meta-analysis of studies from USA, Asia and Europe (Hoek et al. 2013). This translates into particulate matter (PM) in ambient air contributing to 3.1% of the worldwide global burden of disease, which is dominated by IHD (Lim et al. 2012). Recent meta-analysis of cross-sectional studies from urban areas in USA and Europe provide support by findings of associations between long-term exposure to fine particles near residences and carotid intima-media thickness, which is a measure of preclinical atherosclerosis (Adar et al. 2013, Perez et al. 2015, Provost et al. 2015). However, the role of specific sources of ambient air PM, including diesel exhaust (DE), in relation to CVD outcomes has not been sufficiently elucidated in such epidemiological studies. Moreover, despite concern over increasing exposure to nanomaterials (NMs), which due to the particulate nature could have similar effects, has this yet to be established in epidemiological or human exposure studies related to CVD outcomes. Thus, a feasible way to bridge the knowledge gap between exposure to different types of particles and a comparison of CVD outcomes in a quantitative fashion is via the utilization of animal experimental models. As links between exposure to PM and CVD outcomes were first described in relation to inhalation of ambient air pollution, there has been a tendency to view this association as a secondary effect in respiratory toxicology. However, gastrointestinal exposure to NMs may also occur or even directly systemic application by intravenous (i.v.) injection of nanomedicine (De Jong and Borm 2008; Card et al. 2011).

It is generally acknowledged that the underlying cause of PM-induced CVD is atherogenesis and promotion of atherosclerosis, although pro-thrombotic tendency (or imbalance of fibrinolysis) and dysrhythmia are important steps for myocardial infarction and heart failure (Brook et al. 2010). The purpose of the present review is to summarize and integrate studies on ambient air pollution particles and NMs in the same quantitative analysis. We have included results on atherosclerosis and vasomotor function from experiments in animal models.

Vasomotor function refers to the ability of blood vessels to dilate and contract when stimulated with certain vasoactive compounds. This measurement is directly linked to the integrity of the blood vessels, whereas pro-thrombosis tendency occurs in the blood and dysrhythmia is a relatively complex measure of the cardiac electrical conductivity. Endothelium-dependent vasomotor dysfunction is observed in plaque-laden vessel segments, but it is also an early event in atherogenesis (Ross 1999). In general, bioassays for
progression of atherosclerosis are often prolonged due to the time required for plaque development. Hence, the long-term exposure period and requirement of special atherosclerosis-prone animals make the assessment of PM-generated plaque progression a costly investigation. The measurement of vasomotor dysfunction is an attractive alternative to detection of progression of atherosclerosis because it is applicable in traditional wild-type animals, although laboratory procedures require considerable training and skill. We have included data from experiments on vasoconstriction and vasorelaxation in terms of endothelium-dependent and independent responses to vasoactive agents. These responses are recorded by treating vessels either in situ by infusion with a vasoactive agent or ex vivo by the isolation of a vessel segment and exposure to the agent in a wire (i.e. without pressure) or pressure myograph. Phenylephrine (PE) is the most commonly used vasoconstrictor in studies on PM-generated vasomotor dysfunction. Endothelium-dependent and endothelium-independent vasorelaxations are most commonly assessed by the use of acetylcholine (ACH) and sodium nitroprusside (SNP), respectively. The selection of an exact vasoactive agent may depend on the research of the altered function of a specific receptor that promotes a vasomotor response. In addition, the response to a vasoactive agent typically differs between animal species and between blood vessels in the same animal. Thus, there are currently no recommendations regarding the choice or relevance of specific vasoactive agents for assessment of PM-generated vasomotor dysfunction. Here within we have reported observations of myogenic responses in studies of pressurized vessel segments that in the context of the present paper may be interpreted as a “pre-vasoactive drug treatment condition”. This is typically observed as a reduced ability to sustain normal luminal diameter when the vessel is exposed to increased pressure. In the context of this review, this can be interpreted as a vulnerability of the vessel, but may not affect measurements of drug-induced vasorelaxation or vasoconstriction as these parameters are usually normalized to standardized maximal vasoconstriction responses.

This review encompasses results from 78 publications on exposures to ambient air pollution particles, diluted whole DE, diesel exhaust particles (DEP) and NMs (a description of the literature search is provided in the Supplement) (Figure 1). The figure illustrates that CVD effects following ambient air pollution exposures were assessed first chronologically, whereas there has been a relatively fast accumulation of studies on NMs in recent years. A steady accumulation of studies on DE (or DEP) is also seen, which clearly highlights that the assessment of CVD outcomes related to exposure to PM is an on-going process. Some studies have investigated ambient air pollution particles and DEP that has been obtained on samples that represent “historic exposures” in the sense that traffic-related air pollution changes over time due to different fuel and engine technology (Hesterberg et al. 2012, McClellan et al. 2012). However, the studies on DE and ambient air pollution particles have been carried out within the last decade and these exposures can be considered to have been caused by “modern” fuel combustion technology. Still, it should be recognized that new diesel engines, coming on roads today, emit little PM because of improved combustion technology and use of efficient particle filters.

The introductory section of this review will place PM-generated CVD outcomes into a broader perspective with regard to classical risk factors of CVD. We have briefly summarized histopathological features of atherosclerosis and the association with oxidative stress and inflammation. The individual studies on PM-generated vascular effects are summarized in sections that have been segregated into ambient air pollution particles, DE (and DEP) and NMs. The studies on air pollution particles have been segregated according to the location of exposure because the particle characteristics (size distribution and chemical composition) differ considerably by region and by proximity to roadways. The subsequent sections ignore the compositional variation in ambient air PM chemical characteristics and integrate the studies in a collective analysis with focus on dose–response relationships and association among pulmonary inflammation, oxidative stress and CVD outcomes. This strategy means that, within each category, the comparison of exposures is inherently
different. However, we do believe that an attempt to bridge observations from different types of PM is meaningful for better understanding of effect of size and physicochemical characteristics that promote the development and exacerbation of CVD.

**Cardiovascular risk factors**

CVD constitutes an assortment of conditions that affect the heart and the blood vessels. The two main causes of mortality of CVD are IHD and ischemic stroke (i.e. occlusion of blood vessel in the brain) (GBD 2013 Mortality and Causes of Death Collaborators 2015). Interestingly, ischemic stroke and air pollution have received relatively little attention, although epidemiological studies indicate an association between acute exposure to air pollution particles and stroke (Wang et al. 2014, Shah et al. 2015). To the best of our knowledge, there are no studies on vasomotor dysfunction or atherosclerosis in brain arteries of animals that have been exposed to combustion-derived PM or NMs.

Coronary heart disease (also called coronary artery disease) is caused by a build-up of plaques in arteries to the heart, which results in the narrowing of the lumen and reduced blood supply. This typically includes IHD (including myocardial infarction and angina pectoris) and hypertensive heart diseases (International Classification of Diseases 9 codes 402 and 410–414). There are numerous risk factors for coronary heart disease, which are interlinked in a highly complex causal pathway. These factors include obesity, physical inactivity, family history of disease, ethnicity, psychosocial factors, elevated blood lipids, hypertension, prothrombotic factors and inflammatory markers (Grundy et al. 1999). In calculation of the absolute risk of coronary heart disease from the Framingham risk factors, advanced age (score = 7–8) is a stronger risk factor than total cholesterol, hypertension, smoking and plasma glucose (individual scores up to 4) (Grundy et al. 1999). Similar assessment of risk scores has been conducted in studies on fatal CVDs in European populations (Conroy et al. 2003). Obesity, hypertension, elevated levels of triglycerides, cholesterol and fasting glucose in plasma are also components in the definition of the metabolic syndrome, which is associated with increased risk for developing type 2 diabetes and CVD (Eckel et al. 2005). Table 1 outlines a stratification of risk factors for coronary heart disease in humans, which is driven by atherosclerosis, from observations in epidemiological studies. Certain risk factors have been explored in animal experimental models as intermediate steps in a presumed causal pathway of PM-generated CVD outcomes (e.g. plasma lipids, glucose, systemic inflammation and oxidative stress), whereas others have used animals with predisposition to CVD (e.g. hyperlipidemic or spontaneous hypertensive strains). In this review, we have abstracted results from these well-known risk factors for coronary artery disease in humans, namely Framingham risk factors, including European equivalents, and risk factors for metabolic disease (outlined in Supplementary Tables 1–4 and discussed below).

**Table 1.** Classification of risk factors for coronary heart disease in humans.

| Risk factor   | Description                                                                                  | Examples                                                                                     |
|---------------|---------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| Causal        | Strong evidence of a causal relationship                                                   | Cigarette smoking                                                                          |
|               | Acts independently of other factors                                                        | Hypertension                                                                                 |
|               |                                                                                             | Elevated serum cholesterol                                                                  |
|               |                                                                                             | Low HDL cholesterol                                                                          |
|               |                                                                                             | High plasma glucose                                                                          |
| Conditional   | Associated with increased risk of coronary heart disease                                     | Serum triglycerides                                                                          |
|               | Lack of broad acceptance that the factor is a causal agent because (1) the effect is small | Small LDL particles                                                                          |
|               | compared to causal agents or (2) there is low frequency in the population (difficult to | Homocysteine                                                                                 |
|               | assess in prospective cohort studies)                                                       | Fibrinogen                                                                                   |
|               |                                                                                             | Plasminogen activator inhibitor-I                                                              |
|               |                                                                                             | C-reactive protein                                                                           |
| Predisposing  | Complex association                                                                         | Obesity                                                                                      |
|               | Intensify causal (or conditional) risk factors                                              | Physical inactivity                                                                          |
|               | Acts through unidentified risk factors                                                      | Family history                                                                               |
|               |                                                                                             | Male sex                                                                                     |
|               |                                                                                             | Behavioral, socioeconomic and ethnic factors                                                 |
|               |                                                                                             | Insulin resistance                                                                           |
| Plaque burden | Promotes progression to more advanced plaques (plaque instability and risk of rupture)   | Age                                                                                         |
|               |                                                                                             | Air pollution exposure                                                                       |

*The classification is based on observations in humans as reviewed by Grundy 1999.

*Based on observations of associations between exposure to traffic-related air pollution and sudden death of myocardial infarction (Peters et al. 2004).
Animal models used in studies on cardiovascular effects after PM exposure

Table 2 lists general information about the most commonly used animal models in studies of PM-associated CVD outcomes. It is important to state that, within each species, susceptibility to CVD outcomes varies, therefore it is not logical to regard certain species as particularly susceptible to CVD. In addition, none of the non-human animal models are fully representative of human CVD. The information has been abstracted from webpages from suppliers and selected articles (Paigen et al. 1990, Doggrell and Brown 1998, Pinto et al. 1998, Yanni 2004, Aleixandre de Artinano and Castro 2009, Shiomi and Ito 2009, Kennedy et al. 2010, Getz and Reardon 2012). Body weights are reported for female (F) and male (M) animals at 10 weeks of age.

The development of high-fat diet induced obesity and atherosclerosis is less pronounced in BALB/c mice as compared to C57BL/6 mice. BALB/c mice also seem to be resistant to high-fat diet induced increases in plasma levels of glucose, insulin and triglycerides (Montgomery et al. 2013).

Plaque progression is typically assessed in the aortic arch or brachiocephalic artery, whereas the strain is resistant to coronary atherosclerosis.

| Type (strains) | Developed | Characteristic |
|---------------|-----------|----------------|
| Resistant     |           |                |
| ICR mice      | 1948      | Outbred (albino) Weight: 27–35 g (F), 37–42 g (M) |
| FVB mice      | Early 1970s | Inbred (albino) Weight: 18–22 g (F), 25–29 g (M) Susceptible to asthma-like airway responsiveness |
| Fischer F344 rats | 1920 | Inbred (albino) Weight: 140–150 g (F), 210–240 g (M) |
| Wistar rats   | 1905      | Outbred (albino) Weight: 220–250 g (F), 350–400 g (M) |
| Susceptible   |           |                |
| C57BL/6 mice  | 1921      | Inbred (black) Weight: 19–21 g (F), 23–27 g (M) Develops obesity on high-fat diet (hyperglycemia and hyperinsulinemia) Develops atherosclerosis on high-fat diet |
| BALB/c mice   | 1913      | Inbred (albino) Weight: 18–21 g (F), 23–25 g (M) Moderate development of obesity on high-fat diet Develops atherosclerosis on high-fat diet |
| Sprague-Dawley rats | 1925 | Outbred (albino) Weight: 200–220 g (F), 280–320 g (M) Sensitive to diet-induced obesity |
| New Zealand White rabbits | 1908 | Albino Weight 2200–2500 g (MF) |
| Disease model | | |
| Spontaneous hypertensive rats | 1963 | Inbred albino strain (selectively breed from Wistar Kyoto rats) Weight: 150–160 g (F), 230–250 g (M) Pre-hypertensive by 6–8 weeks (progressive development thereafter) Insulin resistance Impaired endothelium-dependent vasorelaxation Develop cardiovascular complications (mainly ventricular fibrillation from micro infarctions and heart failure). Males more sensitive than females |
| Zucker rats (obese) | 1961 | Leptin receptor deficient (developed by crossing Merck M-strain and Sherman rats) Outbred (colored) Weight: 340 g (F), 420 g (M) Onset of obesity at 4–5 weeks Hyperlipidemia and, hyperinsulinemia Impaired vasomotor function Develop renal dysfunction by 30–40 weeks |
| Watanabe heritable hyperlipidemic rabbits | 1973 | LDL receptor deficient (selectively breed) Dyslipidemic (very high LDL levels) Weight: same as wild-type Develop coronary atherosclerosis (progress to myocardial infarction) Normotensive |
| ApoE<sup>−/−</sup> mice | 1992 | ApoE deficient (constituent in lipoproteins) Developed from C57BL/6 (black) Weight: normal (slightly lower than wild-type) Dyslipidemic (moderately high LDL levels) Develop spontaneously atherosclerosis in various vessels<sup>1</sup> Normotensive |
| LDLr<sup>−/−</sup> mice | 1993 | Developed from C57BL/6 (black) Weight: normal (slightly lower than wild-type) Dyslipidemic (moderately high LDL levels) Hyperglycemia and impaired glucose tolerance (on Western-type diet) Develop only atherosclerosis in various vessels on high-fat diet<sup>2</sup> Normotensive |
species are perfect models for CVD in humans. However, these animal models can be regarded as ideal, to some extent even “clean”, models of specific mechanisms of human CVD. For instance, people at risk of CVD typically display several Framingham risk factors or descriptors of the metabolic syndrome, which makes it difficult to pinpoint the contribution of a single factor. The use of animals allows for many of these risk factors to be investigated separately as exemplified by studies on obesity (Alexandr de Artinano and Castro 2009) and diabetes (King 2012).

The animal models can be segregated into strains that are “resistant” with regard to development of CVD, strains that are “susceptible” to development of CVD and “disease models” that have been bred or transgenically altered to develop risk factors of CVD. From these, transgenic mice and rabbits that spontaneously develop atherosclerotic plaques have been mainly used in particle toxicology. Atherosclerosis can be accelerated by utilization of an atherogenic diet containing a high content of cholesterol, lipids or a Western-type diet, although dyslipidemic animal strains also develop atherosclerosis over time at a slower rate on a normal diet (Getz and Reardon 2012). The most widely used experimental model has been the apolipoprotein E knockout mouse (ApoE−/−) that develops atherosclerosis spontaneously, although this process is accelerated if the mice are fed a high-fat diet. ApoE is a component of lipoproteins, with the exception of low-density lipoprotein (LDL). It functions principally to clear chylomicrons and very low density lipoproteins from the circulation. ApoE also plays a role in macrophage biology, immune function and regulation of adipose tissue. In ApoE−/− mice, atherosclerosis develops in the aortic root as well as the brachiocephalic, carotid and pulmonary arteries. The morphology of these lesions tend to be more “foamy” when mice are fed a high-fat diet, whereas ApoE−/− mice on normal chow develop plaques with a more complex cellular morphology (Getz and Reardon 2012). LDL receptor (Ldlr−/−) knockout mice are the most widely used murine alternative to the ApoE−/− mouse model. This mouse model has impaired lipoprotein clearance from the circulation, but it only develops atherosclerosis on a high-fat diet. In general, mouse models do not span the entire pathophysiology of atherosclerosis as they do not develop vulnerable plaques with risk of rupture, thrombosis and arterial occlusion. Thus, they may not be reliable models for predicting myocardial infarction in humans. A few studies have used rabbits in studies of PM-generated atherosclerosis. As a species, rabbits are sensitive to cholesterol overload (Yanni 2004). However, selective breeding of a mutant rabbit showing hypercholesterolemia has yielded the Watanabe heritable hyperlipidemic (WHHL) rabbit model that has a defect in LDLr, which is associated with very high LDL levels in plasma (Shiomi and Ito 2009). These rabbits develop atherosclerosis in the aorta as well as coronary, cerebral, carotid and pulmonary arteries. In addition, the occurrence of myocardial infarction in WHHL rabbits makes this model more representative of IHD in humans as compared with the murine knockout models. The majority of studies in the present review have investigated plaque progression in ApoE−/− mice (23 studies; Chen and Nadziejko 2005, Sun et al. 2005, Li et al. 2007, Araujo et al. 2008, Sun et al. 2008a, Ying et al. 2009a, Campen et al. 2010, Quan et al. 2010, Vesterdal et al. 2010, Bai et al. 2011, Kang et al. 2011, Mikkelsen et al. 2011, Cassee et al. 2012, Chen et al. 2013a,b, Lippmann et al. 2013, Miller et al. 2013, Pöss et al. 2013, Vedel et al. 2013, Cao et al. 2014, Rao et al. 2014, Han et al. 2015, Keebaugh et al. 2015); while, three studies have used Ldlr−/− mice on high-fat diet (Niwa et al. 2007, Soares et al. 2009, Li et al. 2013b) and one study has utilized a double knockout for both ApoE and Ldlr (Chen and Nadziejko 2005). The rabbit models encompass one study on New Zealand white rabbits on high-fat diet (Miyata et al. 2013) and three studies on WHHL rabbits (Suwa et al. 2002, Goto et al. 2004, Yatera et al. 2008).

The disease models develop certain phenotypes that potentiate intermediate steps in PM-generated CVD. As an example, it has been shown that young ApoE−/− on normal chow developed more pulmonary inflammation than wild-type C57BL/6 mice after intratracheal (i.t.) instillation of nanosized carbon black (Jacobsen et al. 2009). It has also been demonstrated that ApoE−/− on regular chow have higher rate of age-dependent oxidatively damaged DNA in the liver, indicative of a higher rate of oxidative stress as compared with wild-type mice (Folkmann et al. 2007). ApoE−/− mice had elevated number of cells, predominantly macrophages, tumor necrosis factor-α (TNF-α), macrophage inflammatory protein 1α (MIP-1α) and interferon-γ (INF-γ) in bronchoalveolar lavage fluid (BALF) after high-fat feeding as compared with normal chow (Naura et al. 2009). ApoE−/− mice also develop emphysema after only 10 weeks of Western-type diet feeding, whereas Ldlr−/− mice do not, which has been attributed to altered efflux of cholesterol in macrophages in the lung (Goldklang et al. 2012).

Spontaneous hypertensive rats have been the species of choice in studies of hypertension in animal experiments (Pinto et al. 1998). Male spontaneous hypertensive rats have been shown to produce a higher influx of neutrophils in BALF following i.t. instillation on 3 consecutive days to 1.6–40 mg/kg of fine particles from
Shanghai, China (Cao et al. 2007). A more realistic mode of exposure by inhalation of residual oil fly ash (15 mg/m³, 6 h/d) for 3 consecutive days elicited the same influx of neutrophils in BALF in spontaneous hypertensive rats and wild-type Wistar–Kyoto counterparts (Kodavanti et al. 2000). Likewise, inhalation of DE (DEP concentration in the mixture: 500 or 2000 µg/m³, 4 h/d for 4 weeks) was associated with the same extent of concentration-dependent increase in neutrophilic influx in BALF in spontaneous hypertensive and wild-type Wistar–Kyoto rats (Gottipolu et al. 2009). Thus spontaneous hypertensive rats may not be specifically sensitive to PM-induced airway inflammation as compared with wild-type rats.

In summary, several animal models have been developed to investigate CVD. The leading causes of CVD mortality in humans are IHD and ischemic stroke. Diminished oxygen supply to the myocardium in patients with IHD gives rise to clinical manifestations such as angina pectoris. In humans, CVD manifests and progresses as a pre-clinical and/or clinical period with a gradual increase of atherosclerosis, endothelial dysfunction, arterial stiffness and hypertension before deterioration and eventual death. Importantly, in transgenic animals, only a number of these manifestations can be mimicked. Thus, these animal models are in all probability not entirely reliable for predicting risk of myocardial infarction in humans. This being said, these animals are still useful for hazard identification of CVD and for comparison of the effect caused by different types of particles. The latter may pertain to vasomotor dysfunction, progression of atherosclerosis or histological composition of plaques. However, once again it should be stated that animals are not perfect models for the pathophysiology or histopathology of IHD or ischemic stroke in humans.

Progression of atherosclerosis

The development and progression of atherosclerosis is the hallmark of coronary heart disease in humans. In animals, it is possible to assess the extent of atherosclerosis with ultrasound imaging, although direct detection in isolated blood vessels is currently the most common method. Atherosclerosis is typically assessed and visualized en face as the area of a blood vessel (visible as white areas). Staining with Sudan IV or Oil Red O is typically used for visualization of lipids in the plaques. The extent of atherosclerosis can also be assessed by histology in cross-section of a blood vessel as the percentage of the lumen that is occupied by the plaque.

The process of atherosclerosis is typically divided into stages based on the morphology of the plaques. Table 3 outlines the American Heart Association’s (AHA) classification of disease progression in human coronary arteries and aorta and the corresponding characteristics that are observed in animal models. The AHA lesion types I through to III are regarded as “early” precursors to more advanced lesions and most typically present in children’s arteries. Lesions I–III mostly affect the intima by the accumulation of extracellular lipids, whereas the adjacent media and adventitia are unaffected. Nevertheless, lesions I–III are regarded as clinically silent, although in these cases there are still increasing levels of cytokines produced, vasoconstriction and decreased endothelium-dependent vasodilation (Stary et al. 1994). Moreover, extracellular deposition of calcium in the arterial wall is intimately related to hypertension, while vascular calcification in the intima is associated with atherosclerosis, whereas medial calcification is typically related to patients with diabetes and renal failure (Kalra and Shanahan 2012). In more advanced lesions, plaques are classified as atheroma (type IV), fibroatheroma (type V) and complicated (type VI) and produce an increasing narrowing of lumen, obstruction and clinical manifestations.

It is possible to mimic a range of manifestations of atherosclerosis found in humans in transgenically altered murine models, if not necessarily a perfect histopathological match of plaques in humans. WHHL rabbits develop unstable plaques and may, therefore, be a more relevant model for myocardial infarction in humans. However, rabbits are less desirable as experimental model as they are more expensive with regard to housing costs and maintenance. Typically, it takes 11–15 months to develop atheromas in WHHL rabbits, whereas it only takes 14–16 weeks in ApoE⁻/⁻ mice on high-fat diet. In addition, the studies in rabbits require much more PM material due to the larger body mass (100-times higher than mice). This may restrict rabbits as an experimental model in studies of NMs due to limited available quantities of well-characterized material.

In summary, several animal models have been developed to investigate the histopathological condition related to CVD in humans. Atherosclerosis in coronary arteries is the most important cause of IHD and myocardial infarction in humans. Transgenically altered murine models do not develop atherosclerosis in coronary arteries and atheromas do not progress to complicated lesions that are more vulnerable to rupture. However, WHHL rabbits do develop coronary atherosclerosis and complicated plaques, but due to financial constraints, long periods of time required for development of disease and large quantities of PM material required for experiments make these animals a less attractive experimental model as compared with transgenically altered murine.
### Table 3. Types of plaques in human and animal arteries*

| Type       | Characteristic in humans                              | Animal models                                      |
|------------|-------------------------------------------------------|---------------------------------------------------|
| I (initial)| Small isolated groups of macrophages with lipid droplets (foam cells) in intima | ApoE<sup>Ⅰ</sup> (normal): 1–2 months              |
|            |                                                      | Ldlr<sup>Ⅰ</sup> (normal): 4–6 weeks               |
|            |                                                      | Ldlr<sup>Ⅰ</sup> (normal): 6–7 months              |
|            |                                                      | WHHL (normal): 3 months                            |
|            |                                                      | Human: <10 years                                   |
| II (fatty streak)| Yellow-colored streaks, patches or spots in intima (stains with Sudan-type of dyes) | ApoE<sup>Ⅰ</sup> (normal): 4–5 months               |
|            | Macrophages in adjacent layers                        | Ldlr<sup>Ⅰ</sup> (normal): 9–10 months              |
|            | Lipid droplets in smooth muscle cells                 | Ldlr<sup>Ⅰ</sup> (normal): >12 months              |
|            | Macrophages without lipid droplets                    | Ldlr<sup>Ⅰ</sup> (HFD): 16–20 weeks                |
|            | Lipids consists of cholesterol esters (majority), cholesterol and phospholipids | WHHL (normal): 11–15 months                        |
|            | Onset at puberty (coronary arteries, common in aorta of young people (up to 30 year) | Human: >30 years                                   |
| III (intermediate)| Adaptive intimal thickening in atherosclerosis-prone locations | ApoE<sup>Ⅲ</sup> (normal): 7–9 months               |
|            | Extracellular lipid droplets                          | ApoE<sup>Ⅲ</sup> (HFD): 12–14 weeks                |
|            | Occur in young adults                                 | Ldlr<sup>Ⅲ</sup> (normal): >12 months              |
|            |                                                      | Ldlr<sup>Ⅲ</sup> (HFD): >24 weeks                  |
|            |                                                      | WHHL (normal): 11–15 months                        |
|            |                                                      | Human: >30 years                                   |
| IV (atheroma) | Lipid core (with calcium particles)                  | ApoE<sup>Ⅳ</sup> (normal): 8–11 months              |
|            | Increased external boundary of arteries (lumen size normal) | ApoE<sup>Ⅳ</sup> (HFD): 14–16 weeks                |
|            |                                                      | Ldlr<sup>Ⅳ</sup> (normal): Not observed             |
|            |                                                      | Ldlr<sup>Ⅳ</sup> (HFD): >24 weeks                  |
|            |                                                      | WHHL (normal): 11–15 months                        |
|            |                                                      | Human: >30 years                                   |
| V (fibroatheoroma) | Fibrous connective sites (mainly collagen)      | ApoE<sup>V</sup> (normal): >10 months               |
|            | Calcified lipid core                                 | ApoE<sup>V</sup> (HFD): 18–20 weeks                |
|            | Narrowing of lumen                                   | Ldlr<sup>V</sup> (normal): Not observed             |
|            | Typically above 40 years of age                      | Ldlr<sup>V</sup> (HFD): Not observed                |
|            |                                                      | WHHL (normal): 20–24 months                        |
|            |                                                      | Human: >40 years                                   |
| VI (complicated) | Surface disruption, hematoma and thrombosis    | ApoE<sup>Ⅵ</sup> (normal): Not observed             |
|            | Associated with morbidity and mortality              | ApoE<sup>Ⅵ</sup> (HFD): Not observed                |
|            | Above 40 years of age                                | Ldlr<sup>Ⅵ</sup> (normal): Not observed             |
|            |                                                      | Ldlr<sup>Ⅵ</sup> (HFD): Not observed                |
|            |                                                      | WHHL (normal): 20–24 months                        |
|            |                                                      | Human: >40 years                                   |

HFD: high-fat diet.

*The description is based on observations in Stary et al. (1994, 1995), Stary (2000) and Whitman (2004). Classification of plaque type in WHHL rabbits has been based on observations from Buja et al. (1983), Shiomi et al. (1994, 1995), Stary (2000) and Shiomi and Fan (2008).*

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### Inflammation and oxidative stress

In order to explain the association between CVD mortality and air pollution exposure, hypotheses from the early 2000s suggested that pulmonary oxidative stress and inflammation would instigate the release of cytokines (and oxidation products such as oxidized LDL), promote atherosclerosis, autonomic dysregulation and increase blood coagulability (Pope III 2000, Donaldson et al. 2001). The hypothesis hinges on the dogma that inhalation of particles induces pulmonary inflammation that “spillover” to the circulation or increase blood levels of signaling factors. In support of this hypothesis, an early study in rats showed that pulmonary exposure to residual oil fly ash was associated with increased plasma levels of fibrinogen (Gardner et al. 2000). However, it is interesting to note that a commentary to this paper highlighted that CVD outcomes have been demonstrated without pulmonary inflammation or injury in healthy individuals (Gordon and Reibman 2000). The systemic inflammation hypothesis, including oxidative stress, has subsequently been scrutinized in animal experimental models as well as studies employing molecular epidemiology in humans. There appears to be different opinions on the importance of systemic inflammation and oxidative stress in development and progression of CVD following exposure of PM. Previously published reviews have discussed systemic inflammation as a link between exposure and CVD outcomes (Campen et al. 2012, Mann et al. 2012, Stapleton et al. 2012b). One review specifically focused on immune responses by macrophages as an important mechanism for PM-induced CVD outcome (Miyata and van Eeden 2011). In another review, progression of atherosclerosis was correlated with pro-oxidant and pro-inflammatory responses as well as the importance of the small size of ultrafine particles (UFPs) and their increasing toxicity in comparison to larger particles (Araujo and Nel 2009). However, Lippmann et al. (2005) argued that long-term
inhalation exposure to concentrated ambient air particles (CAPs) is not associated with persistent pulmonary inflammation due to tolerance to the exposure. Later, Lippmann and Chen (2009) concluded that acute exposure to CAPs was associated with pulmonary inflammation in humans, dogs and rats. The review did not, however, mention pulmonary inflammation in CAPs-exposed mice, which is somewhat puzzling because the authors had conducted several studies on inhalation exposure to CAPs in this species, including atherosclerosis-prone knockout mice. This may be explained by the absence of a positive effect (or conflicting data) or pulmonary inflammation not being investigated as an outcome. In an updated review by the one of the authors, a number of studies on systemic inflammation and oxidative stress were summarized, but there was no definitive conclusion concerning whether the accumulated data provide a convincing association between pulmonary inflammation and CVD outcomes (Lippmann 2014). It should also be emphasized that there are more skeptical views concerning the role of systemic inflammation as a mechanistic link between inhalation of PM and CVD outcomes (Miller 2014). It is our belief that systemic inflammation, i.e. elevated levels of pro-inflammatory cytokines, cannot explain the CVD outcomes that are observed at low-dose exposures to PM. However, there seems to be some evidence linking local inflammation and oxidative stress in the vessel or plaques/atheromas with vasomotor dysfunction and progression of atherosclerosis.

**Qualitative description of studies on vasomotor function and progression of atherosclerosis**

Within the context of this review, the studies are described based on the mass dose or concentration because it is the only descriptor that can be compared across all publications. Other characteristics of particles include the particle size distribution, shape, agglomeration and chemical components. The latter may be used for source appointment in air pollution studies, descriptor of impurities in NMs or identification of active constituents in particles. The particle size is an important predictor of toxicity of particles, but the size distribution may only be reported for the dry form of the sample (e.g. from electron microscopy). The most important descriptors of particle size, including the size distribution, are count median aerodynamic diameter (CMAD) and mass median aerodynamic diameter (MMAD) for inhalation studies. The hydrodynamic diameter in the exposure vehicle is the most important descriptor of particles that have been administered in a suspension. Information on the particle size range is typically reported as the standard deviation (SD) or geometric SD (GSD), assuming a mono-modal distribution. Certain studies have reported the particle size of the peak number concentrations in multi-modal distributions of PM (Supplementary Tables 1–4 contains information on particle characteristics in all studies highlighted in the review).

**Vascular effects after exposure to authentic air pollution**

To date, very few studies have investigated the direct effect of authentic air pollution exposure without focus on particulate fractions. This exposure is only applicable in locations with high emissions of PM, i.e. sourced from major roads in urban areas.

**Beijing, China**

Male ApoE−/− on Western-type diet were exposed for 2 months to air from a major road (average PM2.5 concentration: 61 μg/m3 (exposure group) and 18 μg/m3 (filtered air group), exposure period 18 January–18 March 2010). This exposure was associated with elevated levels of interleukin-6 (IL-6) and TNF-α in the BALF, systemic inflammation (C-reactive protein (CRP) and IL-6) and oxidative stress (decreased superoxide dismutase activity and increased oxidized LDL), whereas plasma triglycerides and high-density lipoprotein (HDL) levels were unaltered. There was also increased aortic plaque area in mice that were exposed to air pollution as compared to filtered air (Chen et al. 2013b).

**Sao Paulo, Brazil**

Male LDLr−/− mice on either normal or high-fat diet were exposed to air pollution from a busy traffic intersection for 4 months (average PM2.5 concentration: 20.4 μg/m3 (exposure group) and 1.6 μg/m3 (filtered air group)). This exposure did not affect the levels of cholesterol and triglycerides in plasma and there was no increased plaque progression in the aorta, whereas high-fat feeding and PM exposure were associated with increased arterial wall thickness (Soares et al. 2009). This indicates that the exposure was associated with vascular remodeling rather than increased plaque size.

**Vascular effects after exposure to concentrated ambient air particles (CAPs)**

The exposure to CAPs in animal models has been used mainly in studies from USA. These exposures mainly
focus on particles in the 0.1–2.5 μm range without affecting the composition (Sioutas et al. 1995, 1997). The health effects of inhalation exposure to CAPs in both humans and experimental animals have been summarized in previous reviews, showing consistency in effects across species (Lippmann and Chen 2009) and a coherence between effects in animal experimental models and risks of CVD from the same locations in epidemiological studies (Lippmann 2014).

It seems that pulmonary inflammation has not been routinely assessed or even discussed as a possible mediator of CVD outcomes in the majority of studies on CAPs exposure. There appears to be an inconsistency in observations of pulmonary inflammation following inhalation of CAPs (Ghio and Huang 2004). This may be contributed to the differences between studies in terms of the concentration of CAPs in the exposure chamber and duration of the exposure. In these experiments, a range of concentrations between 100 and 500 μg/m³ for several hours per day during several months were utilized. It has been argued that low-cytotoxicity particles should not evoke pulmonary inflammation at concentrations below 1000 μg/m³ (Oberdörster 1995). On a similar note, it has been shown that the surface area of urban air particles ranged from 150 μm²/cm³ of air next to a freeway to 50–70 μm²/cm³ at urban background sites in Los Angeles, CA (Ntziachristos et al. 2007). Assuming that rats inhale 50 × 10³ cm³ air during a single 6-h session, the total deposited surface area of particles will be 0.005–0.015 cm² (20% deposition). It has been argued that infiltration of polymorphonuclear neutrophils (PMNs) in BALF occurs at a lung burden particle surface of 200–300 cm² (Tran et al. 2000). As the surface area in CAPs is dominated by small particles, it can be speculated that the inhalation exposure per se has insufficient particle surface area to elicit pulmonary inflammation. In general, it is very difficult to state with any certainty if in CAPs inhalation studies sufficiently large doses have been administered to promote pulmonary inflammation (level of UFPs, mass concentration (i.e. dominated by the non-UFP fraction), exposure frequency and duration). Nevertheless, it is our impression that in the majority of CAPs exposure studies highlighted in this review doses utilized were not high enough to evoke pulmonary inflammation. This is extremely important as a substantial number of CAPs inhalation studies have shown CVD outcomes, which largely can be ascribed to mechanisms that are not secondary events to pulmonary inflammation. Below, the text has been organized according to the location of CAPs exposure in a similar way as that used by Lippmann and Chen (2009).

**Tuxedo, NY**

The publications from this exposure location contain little information on the characterization of CAPs, although an earlier publication described the experimental setup and showed that the particle number size distribution was practically identical between the CAPs aerosol (135 ± 2 nm) and ambient air (123 ± 12) (Maciejczyk et al. 2005). The first study showed that 6 months exposure (85 μg/m³, 6 h/d, 5 d/week) was associated with increased plaque progression in aorta and reduced responsiveness to ACH-induced vasorelaxation and increased response to PE or serotonin vasoconstriction in ApoE⁻/⁻ mice on high-fat diet, whereas there was no effect in mice on regular chow (Sun et al. 2005). These effects were accompanied by signs of inflammation and oxidative stress in the aorta. Plasma lipid profiles showed unaltered cholesterol and triglyceride levels in filtered air versus CAPs exposed mice (Sun et al. 2005). In a concordant study with similar exposure size and duration, increased plaque size in the aortic arch was noted by ultrasound imaging in CAPs exposed ApoE⁻/⁻ mice on high-fat diet, whereas there was no statistically significant in mice on normal chow (Sun et al. 2008a). Inhalation of CAPs (110 μg/m³, 6 h/d, 5 d/week for 5 months) was associated with increased plaque progression in aorta of ApoE⁻/⁻ mice on high-fat diet, whereas there was no difference in plaque area, assessed by en face staining of aorta with Sudan IV, in ApoE and LDLr double knockout mice (Chen and Nadziejko 2005). The CAPs exposure was not associated with pulmonary inflammation (Lippmann et al. 2005).

A more recent study assessed plaque progression at either 3 or 5 months exposure (105 μg/m³, 5 h/d, 4 d/week) in ApoE⁻/⁻ mice on normal chow (Sun et al. 2010). The characterization of the aerosol showed a CMAD of 80 nm (GSD = 1.9 nm), whereas the MMAD was 223 nm (GSD = 1.6 nm). First, en face measurement of aorta with Sudan IV staining showed increased plaque progression at 5 months of exposure. The assessment of plaque progression in brachiocephalic artery (BCA) by ultrasound imaging of lumen size and hematoxylin and eosin (H&E) stain of cross-sections also indicated increased plaque progression, especially after 5 months of exposure. The determination of vasomotor function in aorta showed increased PE-induced vasoconstriction, whereas there was an unaltered response to serotonin, ACH and SNP. In addition, the exposure did not cause pulmonary inflammation (i.e. unchanged levels of PMNs in BALF) and IL-6 and IL-10 levels were unaltered in serum (Quan et al. 2010).

Inhalation of CAPs (73 μg/m³, 6 h/d, 5 d/week for 128 d) in C57BL/6 mice on high-fat diet was associated with
accompanied by an increased expression of NADPH oxidase subunits, collagen deposition and adhesion molecules was assessed. Initially, ApoE−/− mice on high-fat diet were exposed to CAPs for 4 months in a laboratory setting located on the fourth floor above a busy street (138 μg/m³, 6 h/d, 5 d/week for 10 weeks, with angiotensin II infusion at the last week) displayed increased PE-induced vasoconstriction in aorta rings, which was prevented by addition of a Rho-associated protein kinase to PE-precontracted aorta rings ex vivo (Sun et al. 2008b). There was also decreased ACHe response in aorta rings and vasomotor dysfunction was accompanied by an increased expression of NADPH oxidase subunits (Sun et al. 2008b).

New York City, NY

ApoE−/− mice on high-fat diet were exposed to CAPs for 4 months in a laboratory setting located on the fourth floor above a busy street (138 μg/m³, 6 h/d, 5 d/week). The exposure was associated with reduced PE-induced vasoconstriction in aorta rings, decreased responsiveness to SNP-induced vasodilatation and abolished vasorelaxation response to a Ca²⁺ ionophore (A23187), whereas there was no effect on ACHe responsiveness (Ying et al. 2009a). The same study showed a strong plaque burden increase in the aorta (35.4% and 14.8% plaque progression in cross-section of vessels from exposed and controls, respectively), which was accompanied by increased macrophage infiltration, expression of NADPH oxidase subunits, collagen deposition and triglycerides build up in the plaques (Ying et al. 2009a).

Five-city study

In a very interesting study by the National Particle Inhalation Laboratory located 300 m from a freeway (Araujo et al. 2008). Inhalation of UF-CAPs (113 μg/m³, 5 h/d, 3 d/week for 40 d) was associated with increased plaque progression in the aortic root of male ApoE−/− mice on regular diet, whereas the same exposure to fine CAPs (438 μg/m³) had no effect (Araujo et al. 2008). The particle number concentration (6.6 × 10⁵ versus 4.6 × 10⁵ particles/cm³) and organic carbon content (52% versus 25%) was higher in the UF-CAPs exposure as compared to fine CAPs. Although no results were presented, the authors noted that the level of inflammatory cells in BALF were unaltered in these mice, while increased plasma levels of total cholesterol were observed in the group of mice that had been exposed to CAPs. Thus, a proatherogenic plasma lipid response rather than pulmonary inflammation seems to explain the accelerated plaque progression.

In a later study, UFPs were collected from an area with close proximity to major freeways and subsequently aerosolized for inhalation experiments (CMAD ≈ 50 nm). LDLr−/− mice on high-fat diet were exposed to 360 μg/m³, 5 h/d, 3 d/week of UFPs for 10 weeks. This
exposure resulted in increased plaque area in the aorta, which was ameliorated by inhibition of nuclear factor-κB activity (NF-κB). The same study also showed systemic inflammation (increased serum amyloid A and TNF levels) and elevated levels of triglycerides in plasma and lower levels of HDL, whereas there were unaltered levels of LDL and cholesterol (Li et al. 2013b). In a parallel study using the same type of re-aerosolized UFPs, concentration and exposure duration increased calcification of atherosclerotic plaques in aortic root sections, concurrently with increased NF-κB staining in LDLr−/− mice was noted (Li et al. 2013a). The data suggest that the exposure to UFPs was associated with both increased plaque area and progression of plaques to advanced stages, although it should be emphasized that there is uncertainty about whether the findings originate from the same study as cross references are missing.

The inhalation of UF-CAPs sourced from a location close to heavy traffic (58 µg/m³, 5 h/d, 4 d/week for 8 weeks) was associated with increased plaque area in BCA in male ApoE−/− mice (Keebaugh et al. 2015). In this study, one group of mice was exposed to UF-CAPs after removal of organic compounds using a thermal denuder. The removal of the organic components reduced the mass of organic carbon from 25 to 9 µg/cm³, whereas the overall mass of metals and other trace elements was not affected. The removal of organic components rendered the UF-CAPs non-atherogenic after inhalation exposure in ApoE−/− mice (Keebaugh et al. 2015).

**Columbus, OH**

The publications from this exposure location have little information about particle characteristics, except for some data on the chemical composition of PM. There seems to be some variation in the chemical composition of PM used in different studies. For instance, the concentration of iron and copper display a three-fold difference between publications and contain little discussion about the role of the chemical composition of PM for the observed effects on vascular endpoints. The inhalation exposure to CAPs (74 µg/m³, 6 h/d, 5 d/week for 12 weeks) in wild-type C57BL/6, which were treated with angiotensin II for the last 2 weeks of exposure, resulted in increased PE-induced vasoconstriction in aortic rings (Ying et al. 2009b). This effect was blunt when PE-precontracted aortic rings were treated ex vivo with a RhoA/Rho-kinase inhibitor, indicating that the augmented vasoconstriction was mediated by Rho-associated protein kinase signaling (Ying et al. 2009b). In the same location, CAPs inhalation exposure (111 µg/m³, 6 h/d, 5 d/week for 10 weeks) in wild-type C57BL/6 on either normal or high-fat diet showed increased PE-induced vasoconstriction and decreased ACH-induced vasorelaxation response in aortic rings, whereas there was no effect in NAD(P)H p47phox knockout mice (Xu et al. 2010, Liu et al. 2014). Insulin-induced vasorelaxation was blunted by CAPs exposure in both strains of mice. Nevertheless, systemic inflammation in terms of TNF-α plasma levels was only observed in wild-type animals and this strain also displayed insulin resistance after exposure to CAPs (Xu et al. 2010, Liu et al. 2014). The exposure to CAPs from the same location, (101.6 µg/m³, 6 h/d, 5 d/week for 6 months) was also associated with increased plaque progression in aorta of ApoE−/− mice on regular diet (Rao et al. 2014). In another study, 6 months exposure (107 µg/m³, 6 h/d, 5 d/week) increased the vasoconstriction response to PE or U-46619 (synthetic analog of prostaglandin H₂), and decreased ACH-induced vasoconstriction in mesenteric arteries of C57BL/6 mice on regular diet (Ying et al. 2014). CAPs exposure to BALB/c mice (92.4 µg/m³, 6 h/d, 5 d/week) for 20 weeks increased PE-induced vasoconstriction and reduced ACH-induced vasorelaxation in aorta rings (Kampfrath et al. 2011). There were increased levels of TNF-α and MCP-1 in lung tissue and serum of CAPs exposed mice. Toll-like receptor 4-deficient mice did not develop pulmonary or systemic inflammation or vasomotor dysfunction following inhalation of CAPs. In the same study, exposure to CAPs increased PE-induced vasoconstriction in the aorta of C57BL/6. However, NOS−/− mice with the same genetic background as C57BL/6 mice did not develop PE-induced vasoconstriction. There was unaltered ACH-induced vasorelaxation in aorta of wild-type C57BL/6 and NOS−/− mice. The systemic inflammation response was deemed to be mediated by oxidized derivatives of 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine in BALF, which triggered the systemic inflammation via Toll-like receptor 4 and NADPH oxidase activation (Kampfrath et al. 2011).

In a final study from Columbus, 15 weeks of exposure to CAPs (128 µg/m³, 6 h/d and 5 d/week) increased vasoconstriction in aortic rings by PE and U-46199 and reduced ACH-induced vasorelaxation in spontaneous hypertensive rats (Ying et al. 2015). These rats had markedly increased expression levels of TNF-α and IL-6 in lung tissue (approximately 40-fold increased), which was persistent (50-fold increased, TNF-α or reduced (8-fold, IL-6) after a 5 week recovery period that was associated with a normalization of vasomotor function (Ying et al. 2015).

**Sao Paulo, Brazil**

The exposure of male Wistar rats to CAPs for 14 consecutive days (593 µg/m³, 3.3 h daily exposure on
average) was associated with a blunted ACH-induced vasorelaxation response and unaltered SNP-response in pulmonary arteries. The exposure also increased TNF-α and oxidative stress (dihydroethidium staining) levels in vessel segments, whereas there were unchanged plasma levels of TNF-α, IL-1β or IL-6 (Davel et al. 2012).

**Utrecht, The Netherlands**

In the set of trials male Fischer rats were exposed at a roadside location (485 μg/m³, 5 d/week, 6 h/d) for 4 weeks to CAPs with a MMAD of 104 nm (GSD = 30 nm). Prior to the CAPs exposure, the rats were exposed to ozone (0.4 ppm for 12 h), to induce mild pulmonary inflammation (Gerlofs-Nijland et al. 2010). It was stated that the CAPs exposure did not generate pulmonary inflammation; while no significant alteration in PE-induced vasoconstriction or vasorelaxation responses to ACH, SNP and isoprenaline in aortic rings was noted (Gerlofs-Nijland et al. 2010).

**Vascular effects after exposure to particulate matter in urban air**

A number of studies have investigated CVD outcomes after bolus administration of PM into the airways by either i.t. instillation or oropharyngeal aspiration. This mode of administration is non-physiological, but the PM samples are typically better characterized than real-time urban air or CAPs exposure. In addition, some of these samples can be purchased from the National Institute of Standards and Technology as a standard reference material (SRM) or they have been sourced in “large” quantities and available to independent researchers.

**SRM1648**

This sample, also referred to as “urban particulate matter”, was collected in St. Louis, MO, in a baghouse dust collector over a 12 month period between 1976 and 1977. I.t. instillation of 5 mg of SRM1648 to male Wistar rats was associated with reduced ACH response in intrapulmonary arteries at 12 h post-exposure, whereas there was no effect at 6, 24 or 72 h (Courtois et al. 2008). Likewise, ex vivo exposure of rat pulmonary arteries to the material was associated with vasomotor dysfunction at concentrations between 1 and 100 μg/ml (Li et al. 2005).

**SRM1649b**

This sample, also referred to as “urban dust”, was collected in Washington, DC, in a baghouse over a 12-month period between 1976 and 1977. In an experiment, ApoE−/− mice were exposed to SRM1649b by i.t. instillation (0.5 mg/kg twice during 24 h, total dose = 1 mg/kg – hydrodynamic particle size in saline was 224 nm (SD = 44 nm)) with no effect on ACH-induced vasorelaxation in aortic rings or inflammatory response in terms of Ccl2 expression in lung tissue (Vesterdal et al. 2014).

**EHC-93**

This was recovered from baghouse videlon filters at the Environmental Health Center in Ottawa, Canada, in 1993. EHC-93 is described as PM_{10} and is similar to SRM1649 and PM_{2.5} samples from the Great Lakes in 1992 (Vincent et al. 1997). The publications on vascular effects describe EHC-93 as having a mean diameter of 0.8 μm (SD = 0.4 μm) with reference to previously published work which described the CMAD (0.35 μm, GSD = 1.7 μm) and MMAD (4.6 μm, GSD = 2.3 μm) (Vincent et al. 1997). Subsequent studies on vascular effects have administered EHC-93 by bolus instillation in animals and the hydrodynamic diameter has not been reported in the suspension vehicle.

Next, the exposure of female WHHL rabbits by intrapulmonary instillation (5 mg twice weekly for 4 weeks) was associated with increased plaque progression in the aorta and coronary arteries, whereas there was no difference in total cholesterol, HDL and LDL in plasma (Suwa et al. 2002). The scoring of the plaque morphology according to the AHA guidelines indicated a progression from fatty streak lesions (type II) to plaques with extracellular lipids and fibrotic layers (type III) (Suwa et al. 2002). A subsequent study also showed increased plaque progression in the aorta of WHHL rabbits (29% versus 20.9%), although this did not reach statistical significance as compared with unexposed rabbits (Goto et al. 2004). Nevertheless, in another study of EHC-93 exposure in WHHL rabbits, plaque progression in the aorta increased and associated with higher expression of cell adhesion proteins in plaques and higher attachment propensity of monocytes to the endothelium overlaying the atherosclerotic plaques (Yatera et al. 2008). This indicates that the EHC-93 exposure was associated with accelerated recruitment of monocytes into plaques.

The administration of an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (i.e. Lovastatin) reduced the complexity of atherosclerotic lesions in EHC-93 exposed (1 mg/kg, 3 times per week for 4 weeks) New Zealand White rabbits on high-fat diet that were subjected to balloon injury in the abdominal aorta (Miyata et al. 2013). The EHC-93 exposed rabbits
displayed increased PE-induced vasoconstriction and reduced ACH-induced vasorelaxation in carotid arteries, whereas the SNP response was unaltered. In the same animals, EHC-93 exposure was associated with increased plasma endothelin 1 (ET-1) levels, whereas cholesterol, triglyceride, HDL and LDL levels were unchanged (Miyata et al. 2013). Lovastatin administration abolished the observed vasomotor dysfunction and reduced ET-1 levels as well as blunted inflammation responses in atherosclerotic lesions. Similarly, utilization of female New Zealand White rabbits showed that exposure by intraparyngeal instillation (2.6 mg/kg every second day for 5 d or 2.0 mg/kg twice weekly for 4 weeks) was associated with a reduced vasorelaxation response to ACH in carotid arteries, whereas the SNP and PE responses were unaltered (Tamagawa et al. 2008). Additionally, increased pulmonary inflammation, predominantly characterized by the infiltration of macrophages and elevated plasma levels of IL-6, but not ET-1, was also noted (Tamagawa et al. 2008). The role of IL-6 in vasomotor dysfunction was further documented in IL-6 knockout mice, which were not affected by i.t. instillation of EHC-93 (10 or 200 μg), whereas wild-type C57BL/6 mice had reduced responsiveness to ACH-induced vasorelaxation in abdominal aorta rings (Kido et al. 2011b).

Furthermore, studies of vasomotor function in spontaneous hypertensive rats showed that exposure via a single i.t. instillation of EHC-93 (10 mg/kg) was associated with increased vasorelaxation response to ACH and SNP in aorta rings at 4 h post-exposure, whereas there was no effect at 24 h and the PE-induced vasoconstriction response was not affected at both post-exposure times (Bagate et al. 2004b). The same authors also showed that ex vivo exposure to EHC-93 (10–100 μg/ml) in the aorta and mesenteric artery segments from wild-type Wistar Kyoto rats, and aorta segments from spontaneous hypertensive rats was associated with vasomotor dysfunction (Bagate et al. 2004a, 2006).

Chaple Hill, NC

Particles less than 150 nm in diameter were collected and described as UFPs. UFPs were collected on filters over 7-d periods in October 2002 (source of PM was not specified). The particles were extracted from the filters by sonication in aqueous solution and lyophilized before suspension in sterile water and administered to ICR mice by i.t. instillation (100 μg/mouse). This exposure resulted in pulmonary inflammation and impaired ACH-induced vasorelaxation in the aorta, whereas the PE-induced vasoconstriction response was unaltered (Cozzi et al. 2006).

Jinchang and Zhangye, China

PM$_{2.5}$ was collected simultaneously in a period from 6 March 2009 to 26 March 2010 from a city with a nickel refinery (Jinchang) and a “control” city (Zhangye). In these sets of trials, vasomotor function was assessed after repeated exposure (50 μg/mouse, twice/week for 3 weeks by oropharyngeal aspiration) in male FVB/N mice. The exposure to PM$_{2.5}$ from Jinchang decreased the responsiveness to ACH in mesenteric arteries, whereas there was no difference in SNP-induced vasorelaxation and PE-induced vasoconstriction. The ACH response was restored by ex vivo addition of a NO synthase inhibitor (L-NAME) or NADPH oxidase inhibitors (apocynin or VAS2870) to vessel rings. Additionally, gene expression analysis of mesenteric arteries showed a larger response to PM$_{2.5}$ from Jinchang (increased TNF-α, IL-6, superoxide dismutase and NADPH oxidase 4), whereas serum levels of cytokines remained unaltered with no significant difference between PM$_{2.5}$ exposure from the two cities. The exposure to PM$_{2.5}$ from both cities also evoked pulmonary inflammation and toxicity (increased protein content in BALF). The general impression from these results is that pulmonary and systemic inflammation could not explain the difference in vasomotor dysfunction between PM$_{2.5}$ from these two cities (Cuevas et al. 2015).

Summary of studies on exposure to ambient air pollution particles

A review of the literature demonstrated that 17 out of 19 studies on CAPs originate from cities located in USA. All studies on CAPs inhalation exposure from USA have shown vascular effects in terms of vasomotor dysfunction and progression of atherosclerosis. However, the majority of the CAPs exposure studies have only used a single dose, with the exception comparing CAPs exposure at different sites within multiple concentrations (Lippmann et al. 2013). There are only two studies on vascular effects in animals after real-life exposure to air pollution and these have shown mixed results. Importantly, 10 out of 12 studies have reported vascular effects after instillation of urban air PM in the airways. The studies have demonstrated both vasomotor dysfunction and progression of atherosclerosis in animals after exposure to ambient air pollution particles. There is consistent evidence showing CVD outcomes in mice, rats (only vasomotor dysfunction) and rabbits following exposure to ambient air pollution particles.
Vascular effects after exposure to diesel exhaust

A number of studies have investigated CVD outcomes after inhalation exposure of DE to mice or rats. These studies are segregated into the type of diesel engine used, whereas the impact of engine type or fuel has not been assessed.

Single cylinder Yanmar

No significant alteration in responsiveness to PE, ACH and SNP in aorta rings of male C57BL/6 mice was noted following material exposure at doses of 300 μg/m³ for 6 h (Weldy et al. 2013). The short-term exposure to DE (300 μg/m³ for 5 h) in Sprague-Dawley rats was associated with increased ET-1 induced vasoconstriction response in coronary arteries, whereas plasma levels of ET-1 and cytokines were unaffected (Cherng et al. 2009). The same exposure also increased ROS levels and reduced ACH-induced vasorelaxation in coronary arteries with effects abrogated by supplementation with sepiapterin (precursor of tetrahydrobiopterin) and inhibition of nitric oxide synthase (Cherng et al. 2011). The short-term exposure to DE (350 μg/m³ for 4 h) augmented vasoconstriction response to ET-1 and reduced vasorelaxation response to NONOate (a nitric oxide donor) in pressurized mesenteric arteries from male C57BL/6 mice (Knuckles et al. 2008). The same group also showed that ex vivo exposure of mice coronary arteries to DE was associated with an increased vasoconstriction response to ET-1 and decreased responsiveness to SNP-induced vasorelaxation (Campen et al. 2005). Moreover, ex vivo exposures resulted in ACH-induced vasorelaxation which was blunted when aorta segments from naïve mice were incubated with plasma from ApoE−/− mice, exposed to combined gasoline and diesel engine exhaust (300 μg/m³) for 50 d (Campen et al. 2014). Interestingly, this exposure did not affect protein and lactate dehydrogenase (LDH) levels in BALF, whereas there were augmented responses in terms of macrophage infiltration, superoxide radical generation and matrix metalloproteinase expression in the aorta (Campen et al. 2014). These observations indicate that pulmonary inflammation was not the principal driver of vascular effects.

A study on DE inhalation (436 μg/m³, 5 h/d, 4 d/week) assessed plaque progression at either 3 or 5 months exposure in ApoE−/− mice on normal chow (Quan et al. 2010). First, en face measurement of atherosclerosis in aorta with SudanIV staining showed increased plaque progression. Ultrasound imaging demonstrated unaltered lumen size in BCA, whereas there was a marginal decreased lumen size in BCA when assessed by H&E stain of cross-sections at 5 months of exposure. The evaluation of vasomotor function in aorta showed increased PE-induced vasoconstriction, whereas there were unaltered responses to serotonin, ACH and SNP. In addition, the exposure did not cause pulmonary inflammation (i.e. PMNs in BALF) and IL-6 and IL-10 levels were unaltered in serum (Quan et al. 2010). The study also showed that inhalation of DE gases had similar effect as whole DE on pulmonary/systemic inflammation and vasomotor function, whereas whole DE had a more pronounced effect on plaque progression as compared with DE gases.

Cummings

The exposure to DE (109, 305 or 1012 μg/m³) with a MMAD of 100–150 nm for 50 consecutive days in male ApoE−/− mice on high-fat diet did not increase the plaque area in the aortic leaflet region, whereas there was increased macrophage and collagen staining, suggesting that the morphology of plaques was driven toward an advanced and fragile stage (Campen et al. 2010). The same exposure was also used in a study on mixed vehicle exhaust from gasoline and diesel combustion (83.3% of the emission was DE). ApoE−/− mice on a high-fat diet were exposed for 50 consecutive days (6 h/d) to 100 or 300 μg/m³, with plaque progression in the aortic leaflet region reported to be statistically nonsignificant, although an increased plaque areas in the DE-exposed mice was noted. The assessment of vasomotor function in aorta rings showed increased PE-induced vasoconstriction and unaltered ACH-induced vasorelaxation. However, in the exposed animals, there was an increased monocyte/macrophage infiltration into atherosclerotic plaques, which was not observed in mice that were only exposed to the gases. In addition, exposure to gases yielded the same level of atherosclerosis and PE-induced vasoconstriction, indicative that particulate fraction of the DE might not be driving the vascular outcomes (Vedal et al. 2013).

Inhalation of DE (200 or 400 μg/m³, 6 h/day) for 3 consecutive days or 7 weeks (5 d/week) in 30 week ApoE−/− mice on normal chow showed no effect on ACH-induced vasorelaxation, whereas there was decreased PE-induced vasoconstriction in aorta (Kido et al. 2011a). The authors also showed increased expression of heat shock protein 70 in lung tissue and plasma as a possible link between pulmonary and systemic responses. In addition, it was shown that the level of atherosclerosis was unaltered in the aortic root, whereas assessment of morphology indicated that plaques had a more advanced stage of atherosclerosis following inhalation to 200 μg/m³ of DE with a MMAD of 104 nm (Bai et al. 2011).
**Ingersoll Rand**

*ApoE−/−* mice on Western-type diet were exposed by nose-only inhalation to DE (1700 µg/m³, 3 h/d, 5 d/week for 28 d) from regular diesel fuel with or without addition of CeO₂. The addition of CeO₂ did not affect the MMAD of exhaust particles (≈ 83 nm), whereas the particle number concentration was lower in the exhaust from diesel with CeO₂ (3.6 × 10⁶ particles/cm³) as compared with regular diesel (5.3 × 10⁶ particles/cm³). The exposure to DE from regular fuel accelerated plaque progression in BCA, whereas fuel supplemented with CeO₂ had no proatherogenic effect. There were increased number of pigmented macrophages in lung tissue and the results indicated a statistically non-significant increased plaque progression in BCA, whereas the plaques displayed signs of progression to a more advanced state with a buried fibrous cap (Cassee *et al.* 2012).

**Bredenoord (35 KVA diesel generator under idling condition)**

In an inhalation study, male Fischer rats were exposed for 4 weeks (174 µg/m³, 5 d/week, 6 h/d) to DE with a MMAD of 76 nm (GSD = 2 nm). Prior to the DE exposure, the rats were exposed to ozone (0.4 ppm for 12 h) to induce mild pulmonary inflammation (Gerlofs-Nijland *et al.* 2010). The DE exposure did not generate pulmonary inflammation and there were unaltered responses to PE-induced vasoconstriction and vasorelaxation responses to ACH, SNP and verapamil in aortic rings (Gerlofs-Nijland *et al.* 2010).

**Vascular effects after exposure to particulate matter from diesel exhaust**

It is important to state that studies on CVD outcomes after exposure to DEP have used standardized material from the National Institute of Standards and Technology. SRM1650 (or later re-bottled samples) and SRM2975 have been used extensively to investigate pulmonary endpoints and oxidative stress. The two samples have similar oxidative stress potential, although they have been obtained from different combustion conditions (Møller *et al.* 2010a). Another type of material, referred to as A-DEP, was collected from a light-duty diesel engine. To the best of our knowledge, this material has not been used for *in vivo* studies of CVD endpoints, although it has been shown that it can reduce vasorelaxation responses to ACH and SNP, as well as reducing PE-induced vasoconstriction in thoracic aorta rings from male Sprague–Dawley rats after *ex vivo* exposure (Ikeda *et al.* 1995). Additionally, A-DEP can also affect the ACH-induced vasorelaxation response in rabbit aorta segments (Muto *et al.* 1996).

**SRM1650b**

This material is representative of heavy-duty diesel engine PM since it has been collected from different four-cycle diesel engines, operating under different combustion conditions. The original batch (SRM1650) has been rebottled and thus superseded to first SRM1650a and subsequently to SRM1650b. The material was produced in 1983 and issued in 1985; hence, it represents a relatively old diesel combustion technology (Risom *et al.* 2005). In a study, utilizing intranasal instillation (2 µg/mouse, 5 instillations/week for 6 weeks) of SRM1650b in *ApoE−/−* mice on a Western-type diet there was increased plaque progression in the aorta (Pöss *et al.* 2013).

**SRM2975**

This material was collected from an industrial diesel-powered forklift and issued in 2000. A short-term exposure (hydrodynamic particle size in saline was 280 nm (SD = 9 nm)) in *ApoE−/−* mice (0.5 mg/kg at 26 and 2 h before sacrifice, total dose = 1 mg/kg) by i.t. instillation did not alter ACH-induced vasorelaxation in aorta rings and there was no inflammatory response in terms of *Ccl2* expression in lung tissue (Vesterdal *et al.* 2014). The same group also exposed *ApoE−/−* and wild-type C57BL/6 mice by intraperitoneal injection (0.5 or 5 mg/kg) and showed that only the low dose was associated with reduced ACH-induced vasorelaxation in *ApoE−/−* mice at 1 h post-exposure, whereas there was no difference in the SNP or PE response in aorta rings (Hansen *et al.* 2007). In a subsequent experiment, there was no effect on vasomotor function responses by ACH, SNP or PE in aorta rings at 1 h post-exposure to i.v. injection of 0.25 or 0.50 mg/kg (Bai and van Eeden 2013).

Using male Wistar rats, it was shown that a single i.t. instillation of SRM2975 (500 µg/rat) was associated with pulmonary inflammation and systemic inflammation in terms of IL-6 and TFN-α (Robertson *et al.* 2012). While, intra-arterial injection of these materials in the hind-limb vascular bed did not affect ACH-induced vasodilation in femoral arteries at 6 and 24 h post-exposure. However, the authors stated a reduced vasodilation in response to SNP in exposed rats. In the i.t. exposed rats, assessment of vasomotor function of aorta rings only indicated an augmented vasoconstriction response to PE at 6 h post-exposure. In the same vessels, there was no alteration in response to ACH and SNP at 2, 6 and 24 h post-exposure. Likewise, the vasomotor function response to PE, ACH...
and SNP in femoral and mesenteric arteries were largely unaffected with the exception of an increased vasorelaxation response to SNP in mesenteric arteries at 6 h post-exposure (Robertson et al. 2012). The authors concluded that there was no association between pulmonary/systemic inflammation and vasomotor dysfunction. The modest responses in vasomotor function outcome by SRM2975 was in sharp contrast to effects observed by ex vivo treatment of isolated rat aortic rings (10–100 μg/ml) that showed attenuation of especially the vasorelaxation function (Miller et al. 2009).

Next, repeated exposure to SRM2975 (hydrodynamic size of particles in saline buffer was 257 nm (SD = 46 nm)) by oropharyngeal aspiration (35 μg/mouse, twice per week for 4 weeks) was associated with increased plaque area in both the aorta and the BCA of ApoE−/− mice on a Western-type diet, whereas there was no effect in wild-type animals (Miller et al. 2013). The histological examination of atherosclerotic plaques in exposed ApoE−/− mice indicated a transition toward a phenotype associated with increased vulnerability to rupture. The same study showed no effect on vasorelaxation response to ACH or SNP in the distal portion of the thoracic aorta or any systemic effects in terms of plasma levels of cholesterol, triglycerides, CRP or fibrinogen (Miller et al. 2013).

In another study, a dysfunction in ACH-induced vasorelaxation was observed in spontaneous hypertensive rats after repeated exposure of SRM2975 by i.t. instillation (0.8 mg/rat, three times per week for 4 weeks), whereas there was no response on SNP-induced vasorelaxation response (Labranche et al. 2012). The hydrodynamic particle size in phosphate buffer saline with Tween 80 was 189 nm (SD = 16 nm). In addition, ex vivo treatment of aorta rings with the particular matter (100 μg/ml for 30 min) attenuated vasorelaxation responses to ACH and SNP (Labranche et al. 2012). Interestingly, this sign of endothelial dysfunction was not observed in wild-type Wistar rats and it correlated with increased expression of p22phox in the aorta tissue of spontaneous hypertensive rats (Labranche et al. 2012).

**Summary of studies on exposure to DE and DEP**

The studies on pulmonary exposure to DE or DEP have shown mixed outcomes on CVD progression with approximately equal number of studies demonstrating either an altered or no effect on progression of atherosclerosis and vasomotor function. There does not appear to be a relationship between specific types of diesel engines and effect on CVD outcomes. Direct exposure of blood vessels to DEP either via i.v. injection in animals or by ex vivo exposure of vessels has been associated with vasomotor dysfunction, but the relevance of these observations for prediction of in vivo effects is questionable due the higher concentrations utilized.

**Vascular effects after exposure to nanomaterials**

NMs have various applications either as components in industrial products, additives and drugs. The prominent body of research in the nanoparticle field is primarily focused on the development and exploitation of these materials while a smaller body of work has concentrated on the putative hazardous properties of these NMs. In the first category of studies, NMs have been highlighted for their special properties in drug delivery or diagnostics. This may introduce publication bias because potentially hazardous NMs might have been screened out in projects on development of nanomedicines i.e. development of nanocarriers in drugs or therapeutics. Naturally nanotoxicology research is dominated by studies on NMs with the priority in establishing the potential hazardous nature of the materials. In the present review, we have included publications that have strived to assess toxicological effects to the cardiovascular system after exposure to NMs. Consequently, it is mainly “classic” types of particles from inhalation toxicology, e.g. TiO2 and carbon black, carbon nanotubes (CNTs) and fullerenes C60. A few studies also have investigated types of specific NMs that can be found in air pollution PM (i.e. nickel hydroxide) and DE (i.e. CeO2). Hereafter, the term “nanomaterial” is used to distinguish these materials from anthropogenic particles in ambient air. These NMs have a primary size in nanometer range, although the actual particle size in air or suspension may be above 100 nm as a consequence of agglomeration. The majority of NMs can be classified as “spherical” in comparison with CNTs that are long and thin fibers. Thus, the toxic potential of CNTs is typically compared with other types of fibers such as asbestos (Donaldson et al. 2013).

**TiO2**

Exposure to nanosized TiO2 (primary particle size was reported to be 15 nm, whereas the size distribution in exposure vehicle was not reported) altered ACH-induced vasodilation in second-order branch intralobar pulmonary arteries of male Wistar rats at 21 d post-exposure following i.t. instillation of 100 μg/rat (Courtois et al. 2010). These results were supported by observations that 24 h incubation of pulmonary arteries (200 μg/ml) was not associated with differences in ACH-induced vasorelaxation (Courtois et al. 2008, 2010).
Nurkiewicz et al. have conducted a number of studies on whole-body inhalation exposure to nanosized TiO$_2$ in male Sprague–Dawley rats that document impaired vasodilatation response in arterioles of the microcirculation. The primary particle size of these materials was 21 nm – 80% anatase and 20% rutile. The aerosol displayed a bimodal size distribution with peaks at 100 and 400 nm (CMAD = 138 nm). In these investigations, different concentrations (1.5–12 mg/m$^3$) and exposure times (2–8 h) were used to produce a range of well-defined deposited doses (4, 6, 10, 19 and 38 ng/g/rat) for the assessment of vasodilation in situ in pressurized spinotrapezius muscle arterioles at 24 h post-exposure. The data revealed a dose-dependent impairment of A23187-induced vasodilatation exemplified by a dose of ≥ 10 ng/g/rat (≈ 0.05 mg/kg) associated with reduced vasodilatation (Nurkiewicz et al. 2008). A subsequent study showed impaired endothelium-dependent A23187-induced vasodilatation in spinotrapezius muscle arterioles, whereas the endothelium-independent SNP-induced vasodilatation was unaltered at a deposited dose of 10 ng/g/rat (Nurkiewicz et al. 2009). This suggests the development of endothelium dysfunction, which occurred concurrently with oxidative stress (i.e. elevated ROS levels) and nitrosative stress in vessels of the microcirculation (Nurkiewicz et al. 2009). The addition of antioxidants (i.e. tempol and catalase) and inhibitors of ROS producing enzymes (i.e. apocynin (inhibitor of NADPH oxidase) and 4-aminobenzoic hydrazide (inhibitor of myeloperoxidase) restored the A23187-induced vasodilatation, indicating that the TiO$_2$-induced dysfunction in vasodilatation might be caused by oxidative stress (Nurkiewicz et al. 2009). Additionally, pretreatment of the animals with cyclophosphamide to deplete neutrophils had no effect on the blunted vasodilatation response in the pressurized spinotrapezius muscle arterioles in Fisher 344 rats; while the levels of 26 different circulating cytokines measured were not significantly different from the controls (Nurkiewicz et al. 2011). In another study, reduced vasodilation in spinotrapezius muscle arterioles in response to active hyperemia in Sprague–Dawley rats following inhalation of TiO$_2$ (corresponding to a deposited dose of 10 ng/g) was attributed to action via cyclooxygenases, microvascular nitric oxide bioavailability and augmented sympathetic responsiveness (Knuckles et al. 2012). The same authors further documented a blunted vasodilation response to ACH and A23187 in subepicardial arterioles of rats after whole-body inhalation exposure to 6 mg/m$^3$ for 4 h, corresponding to a deposited dose of 10 ng/g/rat, whereas endothelial-independent vasodilation response to SNP was unaltered (LeBlanc et al. 2009). In keeping with observations in the spinotrapezius muscle arterioles, coronary resistance arterioles had increased ROS production and impaired ACH-induced vasodilation which was restored by pretreatment with tempol (LeBlanc et al. 2010). The same coronary resistance arterioles displayed only modest vasoconstriction response to arachidonic acid, whereas there was augmented vasoconstriction response to a thromboxane analog (U46619) (LeBlanc et al. 2010). Moreover, in utero exposure to TiO$_2$ (10 mg/m$^3$, 5 h/d for an average of 6.8 d) from gestation day 6 in Sprague–Dawley rats showed a blunted ACH-induced vasorelaxation response in coronary and uterine arterioles (Stapleton et al. 2015). Meanwhile, a similar response was observed for endothelium-independent vasorelaxation by spermine NONOate treatment, whereas the vasoconstriction response was unchanged. These observations are interesting as they suggest a transcended priming of vaso-motor function.

The inhalation of a commercial spray product with nanosized TiO$_2$ (CMAD: 110 nm, 2.62 mg/m$^3$ for 2 h, 1.72 mg/m$^3$ for 4 h or 3.79 mg/m$^3$ for 4 h for 4 d) was associated with pulmonary inflammation, whereas it did not alter the vasomotor function response to ACH or PE in the ventral tail arteries of Sprague–Dawley rats (McKinney et al. 2012).

In another study, administration of nanosized rutile or photocatalytic TiO$_2$ to ApoE$^{-/-}$ mice (0.5 mg/kg at 26 and 2 h before sacrifice, total dose = 1 mg/kg) by i.t. instillation showed unaltered vasorelaxation response in aorta rings to endothelium-dependent (ACH or calcitonin-gene related peptide) and endothelium-independent vasodilators (nitroglycerin or filodipine) in the presence or absence of the superoxide dismutase mimic tempol (Mikkelsen et al. 2011). The primary particle sizes of the samples were 21 and 12 nm, whereas the hydrodynamic diameter in suspension was well above the nanosize range (518 and 2321 nm) for the rutile and photocatalytic TiO$_2$, respectively. However, the same study showed that i.t. instillation of rutile TiO$_2$ (0.5 mg/kg once a week for 4 weeks, total dose = 2 mg/kg) was associated with a minor increase in plaque area in the aorta, which was not accompanied by pulmonary inflammation as assessed by gene expression levels of monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein 2 (MIP-2) (Mikkelsen et al. 2011).

Finally, exposure of 5–10 nm anatase TiO$_2$ by i.t. instillation (5, 25 or 50 µg twice a week for 6 weeks) in ApoE$^{-/-}$ mice on regular diet resulted in a bell-shaped dose–response relationship where a statistically significant increase in plaque progression by en face staining of the whole aorta was observed in the group of mice that had received 25 µg (≈ 2.5 mg/kg per week). On the contrary, assessment of plaque progression by H&E stain of cross-sections of the aortic arch indicated only
statistical significance at 50 μg (≈ 5 mg/kg per week). Hence, both methods indicated unaltered plaque progression at the lowest administered dose (≈ 0.50 mg/kg per week). These observations may indicate a threshold for plaque progression following repeated i.t. instillations at about 0.5 mg/kg per week in ApoE−/− mice on regular diet (Chen et al. 2013a). These findings are in line with other observations that a single i.t. instillation of nanosized TiO2 (primary particle size = 20 nm), count median diameter = 16 nm, GSD = 1.4 nm) was associated with PMN infiltration in BALF at doses of 25 and 100 μg/mouse (≈ 1.25 and 5 mg/kg), whereas there was no effect after exposure to 6 μg/mouse (≈ 0.3 mg/kg) (Oberdörster et al. 2000).

**Multi-walled carbon nanotubes (MWCNTs)**

MWCNTs consist of multiple sheets of graphite rolled into a hollow tube. It has been suggested that due to their physicochemical characteristics (long, thin and biopersistent), these materials behave more like fibers than spherical particles in the airways.

In one investigation of cardiovascular complications associated with MWCNT (commercially available – 5% iron impurity with a fiber length of several microns and a hydrodynamic size in suspension with bimodal distribution of 200 and 1000 nm) in male Sprague–Dawley rats were exposed to 100 μg of materials (≈ 0.27 mg/kg) by i.t. instillation and sacrificed at 24 h post-exposure. The data showed unaltered vasorelaxation response to ACH and SNP in the left anterior descending coronary arteries. There was a statistically non-significant increase in vasoconstriction response to ET-1, whereas the response to serotonin was unaltered (Thompson et al. 2014a). The general impression from the wire myograph data was that the exposure to MWCNTs did not alter the vasomotor function. In contrast, there were signs of augmented vasoconstriction and increased cardiac infarct size following ex vivo ischemia/reperfusion in Langendorf experiments. The exposure did not generate pulmonary inflammation, assessed by total cell counts in BALF, whereas there was increased protein concentration (Thompson et al. 2014a).

MWCNT-7 was the material of choice in one study on inhalation exposure of male Sprague–Dawley rats (5 mg/m³ for 5 h). The MWCNT was characterized via electron microscopy image analysis, which demonstrated that the fibers were bended with a mean length of 3.9 μm. The rats were exposed to a deposited dose of 13.5 μg (≈ 0.04 mg/kg) and sacrificed at 24–168 h post-exposure.

This exposure was associated with pulmonary inflammation and cytotoxicity (increased PMNs and LDH in BALF). The study further indicated endothelial dysfunction, based on a blunted vasodilation response to ACH and A23187 in pressurized subepicardial arterioles and unaltered SNP-induced endothelium-independent dilation (Stapleton et al. 2012a). Furthermore, there was a statistically statistical significant (p = 0.08, ANOVA) reduced PE-induced vasoconstriction (statistically significant in a post-hoc test and considered to be “additional exploration” by the authors) (Stapleton et al. 2012a). Nevertheless, the authors suggested that the reduced vasoconstriction response could be a protective mechanism to endothelium-dependent vasomotor dysfunction. Indeed, this somewhat surprising finding is interesting in light of the complex regulation of the vascular tone, which may not be easily and readily manipulated by functional tests with the use of vasoactive compounds.

In another study, two different types of MWCNTs (NM400 and NM402) sourced from the European Commission Joint Research Center Nanomaterials Repository was used to investigate the impact of differences in fiber dimensions on progression of atherosclerosis. These MWCNTs were originally selected to represent a short and a long fiber type, based on the supplier’s description of the material. However, a thorough characterization of the samples by the authors showed that the materials had similar dimensions (a fiber length of 0.7–3 μm and 0.4–4 μm) and hydrodynamic diameter in saline (i.e. NM400 and NM402 had a median particle size 116 and 147 nm, respectively). The study showed increased plaque progression in aorta of ApoE−/− mice on a Western-type diet at 24 h after 5 i.t. instillations (once a week for 5 weeks, total dose = 128 μg/mouse), whereas there was only a statistically non-significant increase for one type of MWCNT after a 4-week recovery period (Cao et al. 2014). The exposure was associated with pulmonary inflammation in terms of neutrophilic infiltration in the BALF and elevated cytokine levels, whereas there were unaltered cytokine levels in serum (Cao et al. 2014).

The sub-chronic exposure of MWCNTs in female ApoE−/− mice (40 μg MWCNT by pharyngeal aspiration once a week for 16 weeks and sacrificed at day 1 or 7 post-exposure) did not accelerate atherosclerotic lesions in the aorta or affect plasma cholesterol levels, whereas the exposure increased the numbers of PMNs in BALF, total protein and LDH (Han et al. 2015). The hydrodynamic size of MWCNTs in suspension was 98 ± 10 nm with agglomerates of 30–300 nm. The primary dimension of MWCNTs was 20–30 nm with a mean fiber length of 20–50 μm.

**Single-walled carbon nanotubes (SWCNTs)**

SWCNTs consist of a singular cylindrical graphite sheet that is rolled into a hollow tube. In a study, ApoE−/− mice
were exposed to one such SWCNT (less than 1 μm in length – 0.5 mg/kg twice during 24 h, total dose = 1 mg/kg). The data showed no effect on ACH-induced vasorelaxation in aorta rings, whereas there was an increased gene expression of CCL-2 (approximately seven-fold) in lung tissue, indicating a potential inflammatory response (Vesterdal et al. 2014).

Next, the i.t. instillation of SWCNT (20 μg/mouse every other week for 8 weeks, total dose ≈3.2 mg/kg) increased plaque area in the aorta and BCA of ApoE−/− mice on a high-fat diet, whereas results from animals on regular diet were not quantitated due to changes being too small (Li et al. 2007). There was unaltered plasma level of inflammation markers (MCP-1, IL-12, IL-6, TNF-α and INF-γ), yet other signs of inflammation (increased macrophage-3 antigen and vascular cell adhesion molecule-1 protein in BCA) and oxidative stress (increased protein carbonyls and decreased GSH/GSSG ratio in aorta) were noted in SWCNT exposed mice (Li et al. 2007). In this study, the fiber length was not reported for the SWCNTs; however, it was stated that the material had low content of impurities (0.23% Fe).

The i.v. injection of SWCNTs (1.8 μm in length) in Wistar rats (5 mg/rat and sacrifice at 3 h post-exposure) was not associated with vasomotor dysfunction in terms of response to ACH, SNP and PE in aorta rings (Vlasova et al. 2014). Using the same material, it was shown that a concentration of 200 μg/ml increased vasorelaxation response in pressurized mesenteric arteries and blockage of nitric oxide synthesis by N-nitro-L-arginine methyl ester (L-NAME) pretreatment abolished the effect (Vlasova et al. 2014). Other observations from ex vivo exposure studies indicated that SWCNT exposure in aorta rings in a concentration-dependent manner (0.1–10 μg/ml) produced relaxation of PE pre-contracted aorta rings independent of endothelium involvement (Gutierrez-Hernandez et al. 2015).

In a recent study, the injection of Fe-SWCNT (length: 1.5–3.0 μm) or Gd-SWCNT (length: 0.6–0.8 μm) near an arteriole in the left cheek pouch of hamsters or the right cremaster muscle of mice was carried out and vasomotor function by intravital microscopy measured (Frame et al. 2014). In general, there was little difference in response among the SWCNTs, animal models and location of measurement of vasomotor function (arcade or terminal location). It was noted that the suspensions of aggregated SWCNTs caused an immediate vasodilation, whereas non-aggregated suspensions caused vasoconstriction. These effects subsided within 1 min after injection and a blunted ACH-induced vasodilation response was observed at 15 min post-exposure.

**Carbon black**

An early study on in vivo exposure of a nanosized carbon black with a primary size of 13–14 nm showed unaltered ACH-induced vasorelaxation in second-order branch intralobar pulmonary arteries of male Wistar rats at day 21 after i.t. instillation of 100 μg/rat (Courtois et al. 2010). These results were supported by observations that 24 h incubation of pulmonary arteries (200 μg/ml) did not affect the ACH-induced vasorelaxation response (Courtois et al. 2010). The particle size in exposure vehicle was not reported. However, earlier observations from the same group also showed unaltered response to ACH-induced vasorelaxation in rat pulmonary arteries after exposure to a high-concentration (200 μg/ml) for 24 h to two different samples of nanosized carbon black with a primary particle size of 13 and 21 nm (Courtois et al. 2008).

A 4 weeks exposure to carbon black with a mean aerodynamic diameter of 85 nm (1.3 × 10⁵–42 × 10⁵ particles/cm³, 4 h/d and 5 d/week) did not cause pulmonary inflammation and unaltered vasomotor response to ACH-induced vasorelaxation and vasoconstriction response to PE and 5-HT in the aorta of Sprague–Dawley (Kim et al. 2011). Still, opposite observations have been obtained in studies using a different sample of carbon black (Printex 90, primary particle size = 14 nm), in which concentration-dependent ACH-induced vasorelaxation ex vivo was decreased in aorta rings after a 30 min exposure period to 100 μg/ml, whereas SNP-induced endothelium-independent vasorelaxation was only marginally altered and PE-induced vasoconstriction was increased (Vesterdal et al. 2012). The same study also showed an increased pressure–diameter relationship in mesenteric arteries from rats following a 30 min exposure to 10 μg/ml of Printex 90 (Vesterdal et al. 2012). There was no difference in vasomotor function, assessed by ACH, SNP or PE responses in aorta rings, following a single i.t. instillation in ApoE−/− mice (0.05–2.7 mg/kg), whereas a dosing regimen of two i.t. instillations (26 and 2 h before sacrifice) reduced the responsiveness to ACH-induced vasorelaxation (Vesterdal et al. 2010). Analysis of the particle size in exposure vehicle indicated a bimodal distribution with peaks at 1.4 and 5.5 μm (Vesterdal et al. 2010, 2012). The highest dose increased neutrophilic influx in BALF (Jacobsen et al. 2009) and MCP-1 gene expression in lung tissue (Vesterdal et al. 2010, 2012), without concurrent vasomotor dysfunction, whereas there was no indication of pulmonary inflammation following two i.t. instillations of 0.5 mg/kg (total dose = 1 mg/kg). This suggests that pulmonary inflammation was not necessary for vasomotor dysfunction in aorta following airway...
exposure to Printex 90. The same study also investigated plaque progression in 48–49 weeks old ApoE \(^{-/-}\) mice as a model for advanced CVD. It was planned that the mice should receive repeated i.t. instillations over several weeks, but the exposure was stopped after two i.t. instillations due to death of a couple of mice in each group following exposure. Thus the total received dose (1 mg/kg) was relatively low, which could be the reason for the unaltered plaque progression in aorta and BCA (Vesterdal et al. 2010).

The utilization of carbon black at high doses by i.t. dispersion (1 mg/week for 10 weeks, total dose = 10 mg/mouse, MMAD = 121 nm) in Ldlr \(^{-/-}\) mice on a cholesterol-rich diet was associated with increased plaque progression in aorta, whereas there was no statistically significant effect in mice on a regular diet (Niwa et al. 2007). However, it should be emphasized that the doses in this study were very high and possibly beyond any relevance for human exposures.

The exposure to Printex 90 once a week for 10 weeks via oral gavage (total dose = 0.64 or 6.4 mg/kg, hydrodynamic mean size: 104 ± 53 nm) was associated with a dose-dependent decrease in ACH-induced vasoconstriction of aorta rings from lean and obese Zucker rats, whereas the same total dose administered as a bolus exposure had no effect (Folkmann et al. 2012). The vasoconstriction responses to nitroglycerin and felodipine were unaltered in exposed rats, as was the vasoconstriction response to PE (Folkmann et al. 2012). These results indicated that repeated oral exposure to nanosized carbon black was associated with endothelial dysfunction, which may further depend on sustained exposure (vasomotor dysfunction had subsided after a 13-week recovery period) (Folkmann et al. 2012).

**Fullerenes C\(^{60}\)**

This material has a bucky-ball structure and is commonly regarded as the classic NM. In a recent study, i.t. instillation or i.v. exposure to fullerenes C\(^{60}\) (formulated in polyvinylpyrrolidone with a hydrodynamic diameter of 371 ± 1.2 nm) in male and female Sprague-Dawley rats was proceeded by vasomotor function tests carried out in coronary arteries 24 h post-exposure to 28 \(\mu\)g (±0.93 mg/kg) of the materials. The exposure resulted in minimal pulmonary inflammation and slightly increased the protein content in BALF (Thompson et al. 2014b). In general, there was slightly increased vasoconstriction response to ET-1 in coronary arteries after i.t. exposure, which was ameliorated by pretreatment with indomethacin (a non-steroidal anti-inflammatory agent) in male rats. This was supported by a slightly increased vasoconstriction response to serotonin, although it did not reach statistical significance (\(p = 0.06, \) ANOVA). There were unaltered vasorelaxation responses to ACH or SNP. The putative augmented vasoconstriction response after i.t. exposure was not accompanied by increases in serum levels of IL-6 or MCP-1, whereas these cytokines were elevated following i.v. exposure without any effect on vasomotor response. It should be noted that only five animals per group were utilized, which could affect the statistical power (Thompson et al. 2014b). Another study using the same formulation and dose of fullerenes C\(^{60}\) assessed vasomotor function in pregnant (gestational day 17–19) and non-pregnant Sprague-Dawley rats at 24 h after an i.v. injection (Vidanapathirana et al. 2014). Based on the measurements of vasomotor function in aorta, main uterine artery and mesenteric artery, it was concluded that the fullerene C\(^{60}\) exposure increased vasoconstriction in pregnant rats mediated by Rho-kinase activity. It should be stated that the conclusions were based on a thorough assessment of the vasoconstriction response using 2–3 different vasoconstrictors in each blood vessel. The results indicate that the fullerenes C\(^{60}\) in polyvinylpyrrolidone was not different from the vehicle control, whereas there was a different vasoconstriction response compared to the group of rats that was only exposed to saline. Thus the data indicated an effect induced by the polyvinylpyrrolidone rather than fullerenes C\(^{60}\) on vasomotor function. Finally, the authors noted a smaller response on ACH-induced vasorelaxation; while the no alteration in cytokine levels in serum were determined after i.v. exposure of fullerenes C\(^{60}\).

The effect on vasomotor function following intraperitoneal injection (0.05 or 0.5 mg/kg) was investigated in ApoE \(^{-/-}\) mice at different ages (age being important in levels of plaque progression in the aorta) (Vesterdal et al. 2009). The fullerenes C\(^{60}\) had a small primary diameter of 0.7 nm, but suspension in saline a solution supplemented with BALF resulted in high agglomeration with diameters of above 1 \(\mu\)m. There was reduced ACH-induced vasorelaxation in aortic arch rings of 11–13 weeks old female ApoE \(^{-/-}\) mice at both doses of fullerenes C\(^{60}\). The same dysfunction in ACH-response was observed in rings from the descending part of the aorta in 40–42 weeks old female mice, which had a similar plaque level as the aortic arch of the young mice. On the contrary, it was not possible to obtain reliable information about fullerenes C\(^{60}\) generated vasomotor dysfunction in rings from the aortic arch of 40–42 weeks old mice due to low ACH-induced vasorelaxation in the control group. A similar dose-dependent relationship in SNP-induced
vasoconstriction response was also observed, although it only reached statistical significance in the group of young mice in the high-dose group (0.5 mg/kg). Additionally, there was no effect on PE-induced vasoconstriction response. The aggregated data thus indicate that intraperitoneal injection of fullerenes C60 was associated with dysfunction of the vasorelaxation response, whereas this cannot be only attributed to endothelial dysfunction.

**Nickel hydroxide NMs**

This NM has been investigated due to its use in power and energy industries. One research group has investigated vascular effects after inhalation of nickel hydroxide particles with a primary size of 5 nm (CMAD = 40 nm). In a series of studies, it has been demonstrated that whole-body inhalation exposure of nickel hydroxide (100–900 µg/m³ for 5 h/d on 1, 3 or 5 d) was associated with a blunted ACH-induced vasorelaxation response in carotid arteries of C57BL/6 mice (Cuevas et al. 2010). Earlier studies from the same group of authors showed that the exposure was associated with pulmonary inflammation (Gillespie et al. 2010). It was also shown that a 5 months exposure (79 µg/m³ for 5 h/d, 5 d/week) resulted in pulmonary inflammation and increased plaque progression in the aorta of ApoE-/- mice (Kang et al. 2011).

**Cerium dioxide (CeO2)**

This material is used as diesel fuel additive to increase the combustion efficiency, although the material may also have applications in food, nanomedical and industrial products (Cassee et al. 2011). Two studies conducted by the same group investigated vascular effects of CeO2 exposure in animals after i.t. instillation, i.v. injection or gastrointestinal administration. The primary particle size of the material was 4–6 nm, whereas the hydrodynamic diameter in suspension displayed a distribution with peaks at 191, 900 and 5081 nm. The effect of pulmonary exposure on vasomotor function in pressurized coronary and mesenteric arterioles was investigated in male Sprague–Dawley rats following a single i.t. instillation (10, 50, 100, 200 or 400 µg/rat). First, reduced endothelium-dependent vasorelaxation was observed in both coronary and mesenteric arterioles by use of ACH and A23187 at all doses. A similarly blunted response was observed in spermine NONOate-induced endothelium-independent vasorelaxation at all doses; whereas, an augmented serotonin-induced vasoconstriction response was only observed in coronary arterioles at the highest dose (400 µg/rat) and the myogenic response was not affected. In addition, the influx of PMNs and LDH activity in BALF were unaltered at 10 µg/rat, however, these levels were increased at 100 µg/rat (Minarchick et al. 2013). This suggests that the dysfunction in vasorelaxation response occurred at doses that did not generate pulmonary inflammation and cytotoxicity. The same group has also investigated CeO2 induced effects following i.v. injection and gastrointestinal tract exposures in rats. There was reduced ACH-induced vasorelaxation in mesenteric arteries in rats exposed by i.v. injection (50, 100 and 900 µg/rat), gastrointestinal (600 µg/rat) and i.t. instillation (65 µg/rat). Moreover, impaired endothelium-independent vasorelaxation was also observed following i.v. injection, whereas gastrointestinal tract exposure was associated with either an augmented (100 µg/rat) or reduced (600 µg/rat) response. There was no difference in myogenic response and assessment of PE-induced vasoconstriction did not provide conclusive results with regard to sign of vascular dysfunction. A further investigation into mechanisms of the endothelial dysfunction by co-incubation of vessel segments with inhibitors of nitric oxide synthase (i.e. N G-mono-methyl-L-arginine) or cyclooxygenase (i.e. indomethacin) indicated a dependency of the exposure route of CeO2 on the vasorelaxation response to ACH (Minarchick et al. 2015).

**Silicon-based NMs**

These NMs are typically developed for a variety of diagnostic and therapeutic applications in medicine (Napieraska et al. 2010). It has been shown that exposure to mesoporous silicon NMs (hydrodynamic diameter = 90 nm) by i.v. injection (5 mg and sacrifice at 3 h post-exposure) was associated with reduced response to ACH in aorta rings of male Wistar rats and unaltered SNP response. There was also an augmented PE-induced vasoconstriction response in aorta rings. On the contrary, there was no effect on vasorelaxation after ex vivo exposure to 200 µg/ml in pressurized PE-precontracted mesenteric arteries (Vlasova et al. 2014). Similarly, in another study ex vivo exposure to amorphous silica NMs (50 nm, 2–50 µg/ml) resulted in a concentration-dependent reduction of ACH-induced vasorelaxation in PE-precontracted mesenteric arterioles from Wistar rats (Nemmar et al. 2014).

**Quantum dots**

As a group of NMs, quantum dots represent a relatively large group of semiconductor nanocrystals that are applied in biomedical imaging and electronics. The
absorption, distribution, metabolism, excretion and toxicity of quantum dots depend on both inherent physicochemical properties and exposure conditions (Hardman 2006). These materials have received much attention with regard to their potential toxicity due to their high-scale manufacture. In one such study, male Wistar rats were exposed to mercaptoendecanoic acid-coated quantum dots by i.v. injection (100 μg or ≈333 mg/kg) for 2 h. The NMs were described as having a diameter of 5.0 ± 0.9 nm, although transmission electron microscopy images indicated at agglomerated particles with larger diameters. There was unaltered PE-induced vasoconstriction and ACH-induced endothelium-dependent vasorelaxation in pressurized fourth-order mesenteric arteries, whereas there was a blunted endothelium-independent (SNP-induced) vasorelaxation (Shukur et al. 2013). Next, ex vivo exposure of mesenteric arteries to quantum dots (15 μg/ml) showed unaltered level of ACH-induced vasorelaxation, whereas there was a slightly decreased response to SNP-induced endothelium-independent vasorelaxation (statistical significance not reported).

**Summary of studies on exposure to NMs**

CVD outcomes have been assessed after pulmonary or non-pulmonary exposure to a number of different types of NMs. On one hand, pulmonary exposure to TiO₂, MWCNTs and SWCNTs have been associated with adverse CVD outcomes, with some consistency across laboratories. On the other hand, pulmonary exposure to carbon black has yielded mixed results which might be related to the animal strain and type of carbon black utilized. Interestingly, both TiO₂ and carbon black have been shown to cause vasomotor dysfunction after oral exposure. Moreover, adverse CVD outcomes have been observed after exposure to nickel hydroxide NMs, CeO₂, silicon-based NMs and quantum dots. However, these observations need to be confirmed in future experiments. The data from the literature collectively indicate that exposure to a range of NMs is associated with vasomotor dysfunction and progression of atherosclerosis.

### Quantitative analysis of association between exposure to particulate matter and vascular effects

#### Effect of exposure to particulate matter on vasomotor function

Currently, it is not possible to establish the differences in effect size in a systematic analysis of vasomotor function because some studies have not reported actual values in null effect findings. For the analysis of vasomotor dysfunction, we have dichotomized the response to either showing a statistically significant or null effect (Table 4). Furthermore, the percentage of studies with altered vasomotor function in the full dataset as well as for the studies that have investigated effects after pulmonary exposure to PM have been reported. In this analysis, a “study” refers to the measurement of one PM sample in an experimental system. Thus, a single reference can refer to more than one study if it incorporates more than one material (e.g. DE and CAPs), strain (e.g. assessment of the same sample in wild-type and spontaneous hypertensive rats) or experimental system (e.g. ApoE⁻/⁻ mice fed normal chow or high-fat diet). Overall, approximately 40% of the studies have reported altered responses in terms of vasoconstriction, whereas 60% have shown altered endothelium-dependent vasorelaxation (Table 4). It should be noted that there is an uneven distribution of studies that have reported altered vasoconstriction response after

| Response                          | Vasoconstriction | Endothelium-dependent vasorelaxation | Endothelium-independent vasorelaxation |
|----------------------------------|------------------|---------------------------------------|---------------------------------------|
| **Pulmonary exposure**           |                  |                                       |                                       |
| Altered (number)                 | 18 (12/4/2)      | 32 (19/2/11)                          | 3 (2/0/1)                            |
| Unaltered (number)               | 23 (7/5/11)      | 23 (5/9/9)                            | 22 (7/7/8)                           |
| Percentage of study showing altered response (95% CI) | 44 (29–59)c     | 58 (45–71)c                           | 12 (0–25)                            |
| **All studies**                  |                  |                                       |                                       |
| Altered (number)                 | 20 (12/4/4)      | 39 (19/3/17)                          | 6 (2/0/4)                            |
| Unaltered (number)               | 31 (7/7/14)      | 26 (5/10/11)                          | 27 (7/7/13)                          |
| Percentage of study showing altered response (95% CI) | 39 (26–53)c     | 60 (48–72)c                           | 18 (5–31)c                           |

Numbers in bracket are type of particulate matter (ambient air pollution particles/diesel exhaust/nanomaterials). Ambient air pollution particles encompass studies on authentic air pollution, concentrated ambient air particles and particulate matter from ambient air.

*Different distribution of the type of particulate matter between studies showing altered and unaltered vasorelaxation response (pulmonary exposure: χ² = 11.7, p < 0.01, all studies: χ² = 11.1, p < 0.05).

*Different distribution of the type of particulate matter between studies showing altered and unaltered response on vasorelaxation response (χ² = 7.2, p < 0.05).

*p < 0.05 (there is a non-random distribution in case the 95% lower confidence (95% CI) interval does not include 5%. The 95% confidence interval has been calculated for the binomial distribution).
pulmonary exposure to particles ($\chi^2 = 7.2, p < 0.05$), which is mainly driven by an over-representation of studies showing vasoconstriction after exposure to air pollution particles and an over-representation of studies showing unaltered vasoconstriction after exposure to NMs. There were also differences in the endothelium-dependent vasorelaxation response in studies of ambient air pollution particles, DE and NMs (pulmonary exposure: $\chi^2 = 11.7, p < 0.01$, all studies: $\chi^2 = 11.1, p < 0.05$). This heterogeneity is mainly driven by studies that have shown unaltered response after exposure to DE or DEP and a high number of studies on ambient air pollution particles that have shown vasomotor dysfunction. There appears to be relatively few studies on endothelium-independent vasorelaxation response and the majority of these studies show unaltered effect after PM exposure. It has not been possible to ascertain whether PM-exposure causes endothelium dysfunction because there are relatively few studies that have assessed both endothelium-dependent and endothelium-independent vasorelaxation.

**Summary**

It appears that exposure to PM is associated with increased vasoconstriction (approximately 40% of the studies) and reduced endothelium-dependent vasorelaxation responses (approximately 60% of the studies). The majority of studies have investigated pulmonary exposure to PM. Vasoconstriction responses have consistently been observed after exposure to ambient air pollution particles, whereas exposures to DE (or DEP) and NMs have been less consistent with regard to the generated vasoconstriction. There is a similar pattern of endothelium-dependent vasorelaxation after exposure to ambient air pollution particles and NMs, whereas there are fewer studies than anticipated that have shown endothelium-dependent vasomotor response following exposure to DE and DEP.

**Effect of exposure to particulate matter on progression of atherosclerosis**

The results on plaque progression have been reported with different scales. A positive value of SMD indicates plaque progression. This effect is statistically significant if the 95% CI does not include zero. The SMD is depicted in a Forest plot, which displays means and 95% CIs for subgroups and the overall analysis with the heterogeneity between studies assessed in a Funnel plot. This depicts the standard error and as function of the SMD. An asymmetrical funnel shape indicates heterogeneity between studies.

The effect of exposure to PM and subsequent plaque progression is shown in a Forest plot (Figure 2). The analysis indicates that exposure to DE (and DEP) has the least effect (SMD = 0.36, 95% CI: 0.05 to 0.77), followed by NMs (SMD = 0.67, 95% CI: 0.26–1.09) and ambient air pollution particles (SMD = 1.26, 95% CI: 0.80–1.72). However, it has to be noticed that the group of ambient air pollution particles displays heterogeneity, which is attributed to 4 studies with high effect sizes (Ying et al. 2009a, Chen et al. 2013b, Li et al. 2013b, Rao et al. 2014). The heterogeneity is lost by omission of these publications from the meta-analysis, with the effect size decreased (SMD = 0.70, 95% CI: 0.46–0.94), while there is no difference in plaque progression between exposure to ambient air particles, DE (and DEP) and NMs (Supplementary Figures 2 and 3).

The quantitation of plaque progression with the AHA classification (Table 3) has been used in a few studies on EHC-93 exposure to rabbits (referred to as “atherosclerosis score”). The first study showed increased atherosclerosis score in the left main coronary artery (score 2.7 versus 1.9 in exposed and controls) and aorta (score 3.1 versus 2.4) (Suwa et al. 2002). In a later study of New Zealand White rabbits on high-cholesterol diet also showed increased atherosclerosis score in the abdominal aorta after exposure to EHC-93 (score 1.7 and 1.0 in exposed and unexposed, respectively) (Miyata et al. 2013). It has been reported that inhalation of DE was associated with increased collagen content and macrophage infiltration in the aorta leaflet region of ApoE−/− mice on high-fat diet [photographic documentation and description of plaque morphology suggest that DE exposure was associated with a transition from AHA types 4–5 to 5] (Campen et al. 2010). Vedal et al. (2013) showed increased monocyte infiltration in aorta plaques from ApoE−/− mice after exposure to mixed vehicle exhaust [photographic documentation indicates a plaque development in AHA types 3–4, whereas it is not possible to score differences between exposed and unexposed animals]. Furthermore, exposure to DE increased the complexity of atherosclerotic plaques as occurrence of buried fibrous layers in plaques in BCA, whereas there was unaltered lipid content and
macrophages/foam cells [this is probably equivalent to an increase in AHA score from 5 to score <6] (Cassee et al. 2012). The development of atherosclerosis following exposure to ambient air pollution in LDLr⁻/⁻ mice on high-fat diet has also been documented [photographic documentation indicates a uniform lipid deposition with numerous cellular structures (Oil Red with hematoxylin counterstain), which differs somewhat from a regular cross-section of plaques]. The plaque morphology was described as a thickening of the arterial wall, which appears to be seen in the intima-media [photographic documentation provides insufficient detail to suggest AHA types] (Soares et al. 2009). It should be emphasized that the quantitative assessment of plaque progression throughout this review is speculative as the assessment is based on a relatively few cross-sections of arteries in different animal models. However, the assessment does suggest that PM exposure is associated with less than one unit AHA score over an exposure period of 1–2 months. This is based on observations from studies on DE and ambient air pollution particles, whereas there are no studies on NMs. As a recommendation for the future it is imperative that investigations into plaque morphology are carried out, although it should be kept in mind that the atherosclerotic histology differs between humans and animal models.

**Summary**

Exposure to PM is associated with accelerated plaque progression in atherosclerosis-prone mice and rabbits. The studies on ApoE⁻/⁻ mice have used either normal...
chow or high-fat diet. The latter accelerates progression of atherosclerosis and may also produce a different histopathological composition of the atheroma. To date, only a few studies have assessed histopathological changes in atheromas after PM exposure in groups of high-fat or regular chow fed animals to provide conclusions about differences in plaque morphology. However, there appears to be no difference in plaque progression between animals fed a normal chow and high-fat diet which is not entirely surprising as the high-fat diet merely accelerates the progression of atherosclerosis.

Dose–response relationships for CVD outcomes

PM-induced vascular effects have mainly been studied at a singular dose in each study. Hence, there is a lack of dose–response relationships on progression of atherosclerosis. For ambient air pollution particles, there is one study on vasomotor dysfunction after exposure to EHC-93 that has used more than one dose (Kido et al. 2011b). It has been shown that inhalation of DE was not associated with dose-dependent responses of vasomotor dysfunction in the aorta and plaque progression (Campen et al. 2010, Kido et al. 2011a). Furthermore, pulmonary exposure to carbon black, MWCNT, TiO$_2$, CeO$_2$ and nickel hydroxide NMs has not revealed dose–response relationships on vasomotor dysfunction endpoints or plaque progression (Cuevas et al. 2010, Vesterdal et al. 2010, Chen et al. 2013a, Minarchick et al. 2013, Cao et al. 2014). One study on intraperitoneal injection of fullerenes C$_{60}$ was non-conclusive because the effect on vasomotor function was small at all doses investigated (Vesterdal et al. 2009). However, i.v. injection of CeO$_2$ was associated with a blunted ACH-response at all doses tested, whereas only the highest dose (600 $\mu$g/rat) reduced ACH-induced vasorelaxation in mesenteric arterioles of rats (Minarchick et al. 2015). In another study, oral exposure of nanosized carbon black showed a dose-dependent decrease in ACH-dependent vasorelaxation in the aorta of lean and obese Zucker rats (Folkmann et al. 2012).

Figure 3 shows the analysis of the dose–response relationship for vasomotor function test in studies on instillation or inhalation of PM. The instilled dose (mg/kg bodyweight per week) was higher in studies that showed altered endothelium-dependent vasorelaxation response ($p < 0.05$). There is a positive association between the administered dose and altered vasomotor response (odds ratio $= 6.1$ (95% CI: 1.2–31) for effect in studies that have used a dose above versus below the geometric mean). These results indicate a dose-dependent relationship on endothelium-dependent vasorelaxation in the instillation studies, whereas there was no difference in inhalation studies. Finally, there were no dose-dependent relationships on endothelium-independent vasorelaxation or vasoconstriction.

Figure 3 outlines a comparison of dose–response relationships between inhalation of PM and plaque progression across publications on atherosclerosis-prone mice with the outcome being the daily plaque progression (SMD divided by the number of exposure days) and the exposure calculated as time-integrated weekly dose (mg* h/m$^3$). Interestingly, by using this exposure metric, it has been shown that exposure to 100 mg* h/m$^3$ of carbon black was associated with pulmonary overload in rats (Hesterberg et al. 2012). All studies incorporated in this review have utilized time-integrated weekly doses less than 100 mg* h/m$^3$. In general, there is a large spread in the effect sizes between the studies, with three studies showing relative high effect sizes (SMD/exposure day > 0.03); while one has utilized a high-exposure concentration of UFPs (360 $\mu$g/m$^3$), which may explain the high effect size (Li et al. 2013b). The other studies, originating from Beijing (China) and New York (NY), do not have any striking features that readily explain the high effects (Ying et al. 2009a, Chen et al. 2013b). In general, there is little evidence for dose–response relationships across studies and there does not appear to be an overt difference between PM originating from DE and ambient air pollution.
Figure 4 depicts the dose–response relationship for studies on i.t. instillation (or oropharyngeal aspiration) in atherosclerosis-prone mice and rabbits. The exposure is reported as weekly dose per bodyweight (mg/kg per week) due to the difference in species. To allow for comparison between Figures 3 and 4, a weekly dose of 1 mg/kg bodyweight corresponds to inhalation of 2 mg/m³ of PM for 6 h/d and 5 d/week (i.e. 60 mg*h/m³) in mice. The data show a spread in effect sizes between studies, which is not readily explained by differences in types of PM (Figure 4). The results might indicate that a weekly dose of 1 mg/kg bodyweight produces little effect in terms of plaque progression, whereas the effect at higher doses may depend on the type of PM.

Summary

There is little evidence indicating that pulmonary exposure to increasing PM dose leads to vasomotor dysfunction and plaque progression, whereas one study on oral exposure to nanosized carbon black indicates a dose–response relationship. The few studies that have used multiple doses within the same study have not revealed clear dose–response relationships. From the analysis of the data, it is clear that comparison of findings and conclusions are difficult across studies due to the fact that the physicochemical characteristics of PM utilized are too different. The use of disparate methodology and experimental setups further complicates the above mentioned issue.

Particle characteristics and effect on vascular outcomes

The quantitative analysis of the data indicates a scatter pattern in exposure–effect relationships on vasomotor function and atherosclerosis (Figures 3 and 4). This is not surprising since the analysis is based on the mass concentration, which is a relatively inaccurate descriptor of the exposure to nanosized particles. It can be suggested that particle size, shape and chemical composition might be better parameters for prediction of the toxicological potential of particles. However, there is not sufficient information available in the literature on these factors to allow for the assessment of the differences in CVD outcomes within specific types of PM exposure.

It is apparent that the studies on air pollution particles have rather poor description of particle characteristics.
For instance, the publications on CAPs exposure from Columbus, OH, at best only contain information on the chemical composition of the PM (Ying et al. 2009b, Xu et al. 2010, Kampfrath et al. 2011, Rao et al. 2014, Ying et al. 2014, 2015). A number of studies of CAPs inhalation from Tuxedo, NY, have not provided any data about particle characteristics (Sun et al. 2005, 2008a, 2008b, 2009), whereas basic description on the elemental composition and particle size is available in other publications (Chen and Nadziejko 2005, Ying et al. 2009a, Quan et al. 2010). The assessment of elemental composition in the studies of air pollution particles seems to be related to source appointment rather than assessment of toxic components in the exposure. The studies on DE and NMs appear to have better description of particle characteristics than the investigations into air pollution particles. Nevertheless, the analysis of the chemical components in the NMs seems mainly to have been used to describe the purity of the sample rather than describing constituents that can explain the exposure-effect relationship. There are some studies on NMs with information on shape and agglomeration of the particles, but this is very limited and cannot be used for comparison of studies on this particle characteristic. Additionally, a number of publications have cited other publications when describing the characteristics of the exposure. For instance, the studies on instillation of EHC-93 in the airways have cited Vincent et al. (1997) as the source of particle size characteristics (Suwa et al. 2002, Bagate et al. 2004a,b, Goto et al. 2004, Tamagawa et al. 2008, Yatera et al. 2008, Kido et al. 2011b, Miyata et al. 2013). However, this publication only contains data on primary size and aerodynamic diameter, whereas the most important and interesting information must be the hydrodynamic particle size and agglomeration due to the fact that the ECH-93 has been administered in a suspension vehicle. A similar example of citation to previously published work is seen in studies on DE, in which the description of exposure is vague with little detail on the equipment used and particle characteristics measured and reported (Knuckles et al. 2008, Campen et al. 2010, Cherng et al. 2011). It is important to note that citation of earlier publications for characterization of particles may not provide reliable information of the particle characteristic in the reported study unless the same vehicle of administration is utilized.

The studies on SRM2975 suggest some variation in the reported particles size, although the studies have used a similar vehicle. The hydrodynamic diameter of SRM2975 in saline has been reported as 189 ± 16 nm (Labranche et al. 2012), 257 ± 46 nm (Miller et al. 2013) and 280 ± 9 nm (Vesterdal et al. 2014). The latter also measured the hydrodynamic particles size in water (179 ± 11 nm) and cell medium (197 ± 6 nm) (Vesterdal et al. 2014). There are subtle differences with regard to the addition of compounds that improves the dispersion, but these inter-study differences in particle size may also be due to different equipment and handling. For instance, it has been shown in an intra-laboratory trial that the variation in hydrodynamic particles size for the same NM was dependent on the investigator and equipment (Roursgaard et al. 2014). The magnitude of variation in particle size determination related to differences in vehicle, investigator’s handling procedures and equipment has not been assessed in a thorough manner using the same material.

Table 6 outlines the segregation of the studies into groups based on thoroughness of the particle characterization into four categories: “none” is defined as studies that have only reported the mass concentration/dose of PM; “some” defines publications with additional information on the chemical composition, shape or size in dry form; “well-characterized samples” are exposures where the PM has been characterized in dry form either by the supplier (e.g. EHC-93 or the standard reference materials) or investigators. Finally, the highest level of characterization is obtained in the studies that have assessed the particle size distribution in the vehicle, which are either air or a liquid suspension. Furthermore, Table 6 also contains information on the mode of exposure (inhalation or instillation), dose–response relationship, CVD outcome and its significance (vasomotor dysfunction or plaque progression). The data indicate that the studies on air pollution particles have not assessed the dose–response relationship and in general there is poor characterization of the particle size distributions ($\chi^2 = 32.0, p < 0.001$). The studies on DE (or DEP) and NMs have mainly used well-characterized PM exposures. There is, however, an uneven distribution of studies that have used inhalation (mainly air pollution particles) and instillation (mainly DEP and NMs) ($\chi^2 = 11.5, p < 0.01$). Interestingly, the studies on ambient air pollution particles mainly show statistically significant effect on vascular outcomes as compared with the studies on DE (or DEP) and NMs (crude odds ratio: 7.1, 95% CI: 1.9–26.3) (Table 6). Adjustment for the type of exposure (inhalation or instillation), assessment of dose–response relationship and CVD endpoint (vasomotor dysfunction or plaque progression) did not affect the odds ratio (7.9, 95% CI: 1.8–35.3, logistic regression with particle characterization dichotomized into low level (i.e. “none” and “some”) and high level (i.e. “well-characterized”)). Consequently, the quality of the exposure characterization is not a strong independent predictor of the statistical result of the CVD outcome, whereas the locations of ambient air pollution particle exposures are
Table 6. Segregation of studies with regard to the level of characterization of particles in studies of air pollution, diesel exhaust (DE) or nanomaterials (NMs).

| Level of particle characterization | Types (air pollution particles/DE/NMs) | Mode of exposure (inhalation/instillation) | Dose–response assessment | Endpoint (vasomotor/atherosclerosis) | Effect (positive/null effect) |
|-----------------------------------|---------------------------------------|-------------------------------------------|--------------------------|-------------------------------------|-----------------------------|
| None                              | 9/2/2                                 | 10/3                                      | No dose–response          | 9/4                                 | 8/5                         |
| Some                              | 14/0/0                                | 12/1                                      | No dose–response          | 8/6                                 | 14/0                        |
| Well-characterized sample         | 11/6/10                               | 13/14                                    | Two studies with dose–response | 17/10                              | 25/2                        |
| Well-characterized sample in vehicle | 3/9/14                               | 12/14                                    | Eight studies with dose–response | 17/9                               | 13/13                       |

The levels are “None”: no information except the mass concentration or dose of PM, “Some”: information on chemical composition, shape or primary particle size in dry form (e.g. elemental composition in CAPs exposure studies), “Well-characterized sample”: information on both particle size (or shape) and chemical composition in dry sample (e.g. EHC-93), “Well-characterized sample in vehicle”: information on aerodynamic or hydrodynamic diameter in the suspension vehicle (air or suspension).

There is an uneven distribution of studies with regard to particle characterization ($\chi^2 = 32.0, p < 0.001$, DE and NM studies have been pooled for the analysis).

There is an uneven distribution of studies with regard to inhalation and instillation exposure ($\chi^2 = 11.5, p < 0.01$). Supplementary Table 5 contains additional information on the segregation of the studies.

Summary

There is substantial difference in the quality of particle characterization across studies. Archetypally, studies on ambient air pollution particles have little information on particle size distribution, although some studies have reported data on the chemical composition of the PM. However, investigation on DE (or DEP) and NMs typically have more information on particle characteristics in either dry form or in the exposure vehicle. Nevertheless, currently, it is not possible to determine whether other metrics than the mass dose/concentration are useful predictors for the dose–response relationship between PM and CVD outcomes in terms of vasomotor dysfunction and progression of atherosclerosis.

Mechanistic link between pulmonary exposure to particulate matter and vascular effects

PM-induced pulmonary inflammation and CVD outcomes

It is a prevailing hypothesis that vascular effects emerge as a consequence of an inflammatory responses after pulmonary exposure to PM. The doses or dose-rates utilized in some studies on CVD outcomes have led to pulmonary inflammation. The most convincing evidence of spillover cytokine-mediated effect from the lung was shown in a study on i.t. instillation of EHC-93 in which increased levels of IL-6 in the lungs and serum were noted concurrently with a blunted vasorelaxation response to Ach in wild-type mice, whereas there were no particle-generated vasomotor dysfunction in IL-6 knockout mice (Kido et al. 2011b). However, there are numerous studies that have exposed animals to PM at doses that are not associated with pulmonary inflammation and still have shown vasomotor dysfunction.

In order to assess the association between pulmonary inflammation and CVD outcomes, the studies on pulmonary exposure of PM have been segregated into groups using doses that have yielded inflammation and doses that have not (Supplementary Table 6). In addition, the studies are further divided into short-term (i.e. less than a week) or long-term exposures (i.e. weeks to months). This analysis demonstrated an uneven distribution of studies that have shown altered vasconstriction response after repeated low-dose exposures (Table 7); while studies that have used high doses and short duration of exposure have not observed PM-induced vasconstriction. The distribution of studies that have shown altered endothelium-dependent or endothelium-independent vasorelaxation response was not different between the groups. Next, Table 8 shows a segregation of the studies into three groups based on the susceptibility of the animal model to develop CVD showing an altered vasconstriction response in susceptible and high-risk models for CVD ($\chi^2 = 7.9, p < 0.05$). Furthermore, endothelium-dependent vasorelaxation has predominantly been observed in studies using CVD-susceptible models ($\chi^2 = 12.3, p < 0.01$). The data in Tables 7 and 8 do not support the hypothesis of pulmonary inflammation exacerbation of vasomotor dysfunction responses in animals that have been exposed to PM in the airways.
The studies investigating atherosclerosis have used either regular or high-fat diet and repeated exposures. Therefore, we have separated the studies according to the diet utilized. The low-dose exposures in animals fed a normal chow was associated with the lowest effect (SMD = 0.47, 95% CI: 0.24–0.69), whereas high-dose exposures had higher effect size (SMD = 1.59, 95% CI: 0.62–2.56; high-dose: SMD = 0.86, 95% CI: 0.50–1.09) (Supplementary Figures 5 and 6). However, it should be emphasized that the analysis displayed heterogeneity, which was attributed to few studies with very high effect sizes (Ying et al. 2009a, Chen et al. 2013b, Li et al. 2013b). The omission of these studies from the analysis eliminated the intra-group heterogeneity and reduced the SMD in both the low-dose (SMD = 0.79, 95% CI: 0.21–1.37) and high-dose (SMD = 0.64, 95% CI: 0.28–1.00) groups of animals that were fed on a high-fat diet (Supplementary Figures 7 and 8). There is also a statistically significant subgroup difference in studies using high doses which were associated with higher effect size than low-dose exposure in animals on normal chow (p < 0.05). The observations indicate that high-dose PM exposures, associated with pulmonary inflammation, produced a larger effect in animals on normal chow, whereas the exposure displays no dose–response relationship in animals on high-fat diet. Thus, the observations do not indicate that a high-fat diet is a predisposing factor for accelerated plaque progression in PM-exposed animals. The high-fat feeding may facilitate a faster development of atherosclerosis and, therefore, reduce the required period with PM exposure. In addition, the high-fat diet may also increase the lipid content in atherosclerotic plaques, which could make it easier to measure the plaque size and composition.

Collectively, this assessment of the effect of dose and duration of exposure does not indicate a coherent pattern of CVD outcomes. The vasorelaxation response is clearly affected by pulmonary PM exposure, but this appears to occur independently of lung inflammation. In addition, it is striking that high-dose exposure studies, associated with pulmonary inflammation, have not been associated with altered vasorelaxation, whereas low-dose exposure studies predominantly show vasoconstriction. Importantly, pulmonary inflammation is not
required for vasomotor dysfunction responses and plaque progression, further highlighted by observations on fine size TiO₂ and residual oil fly ash that showed no association between pulmonary inflammation and vasomotor dysfunction, yet notable local oxidative stress and vasomotor responses in blood vessels (Nurkiewicz et al. 2004, 2006). The studies on plaque progression showed a dose-dependent effect in animals fed a regular chow.

**Systemic inflammation as link between PM exposure and CVD outcomes**

Chronic low-grade systemic inflammation has been explored extensively as causal risk factor for particle-induced atherosclerosis. The definition of chronic low-grade systemic inflammation is somewhat elusive, although it is typically a minimum of a two-fold increase in plasma cytokines or acute phase proteins. It has been shown that a 2.7-fold elevated level of CRP (2.4 versus 0.9 mg/l as cutoff values from upper and lower tertile) was associated with an odds ratio of 2.1 (95% CI: 1.4–3.3) of non-fatal myocardial infarction and death from coronary heart disease in humans (Danesh et al. 2000).

In another study, increased hazard ratio for coronary heart disease of 1.26 (95% CI: 1.08–1.46) for IL-6 and 1.14 (95% CI: 1.00–1.31) for TNF-α per 1-SD of log-transformed baseline values, which roughly corresponds to two-fold increases from baseline log-values, was noted (Kaptoge et al. 2014). A further re-analysis of data from 52 prospective cohort studies showed that inclusion of CRP levels to the statistical models of age, systolic blood pressure, smoking status, diabetes and cholesterol for prediction of 10-years CVD risk categories (low, medium and high) led to a re-classification of 1.5% of the subjects to new categories (Kaptoge et al. 2012). These findings indicate that changes in plasma cytokines and acute phase proteins (i.e. two-fold increases) is associated with increased risk of CVD outcomes, although the significance in terms of prediction of risk is small compared with other more established risk factors. In comparison, a review of studies on ambient air pollution particles showed a 1.07-fold (95% CI: 1.06–1.51) level of systemic inflammation markers in exposed humans (Møller et al. 2014). The same study showed that animals had a 1.28 (95% CI: 1.10–1.71) higher fold systemic inflammation after exposure to ambient air pollution particles (Møller et al. 2014). In the present review, nine studies showed the same effect on systemic inflammation biomarkers and vasomotor function outcomes, whereas no association was noted in five studies. Likewise, six studies reported a direct link between systemic inflammation biomarkers and plaque progression, whereas four studies did not (Supplementary Table 7). Thus, it appears that only about half of the studies have shown associations between systemic inflammation markers and CVD outcomes. This may further represent an overestimation of systemic inflammatory, due to non-altered plasma levels in some studies being omitted as uninteresting results. This notion is supported by the fact that all studies that have reported dissimilar responses in terms of systemic inflammation and CVD outcomes found unaltered levels of cytokines and acute phase proteins in plasma/serum.

It has shown that weekly administration of IL-6 was associated with exacerbated atherosclerosis in ApoE⁻/⁻ mice on high-fat (SMD = 2.11, 95% CI: 0.31–3.91, 6 weeks exposure) and normal chow (SMD = 1.72, 95% CI: 0.15–3.30, 21 weeks exposure), with a 7.8-fold and 4.9-fold increased plasma concentration of IL-6, and 3.3-fold and 2.0-fold increased TNF-α levels in high-fat diet and normal chow, respectively (Huber et al. 1999). The SMDs are similar to the effects that have been obtained in other studies on PM-induced atherosclerosis, but the fold-increases in plasma concentrations are substantially larger in this particular study. Likewise, ApoE⁻/⁻ mice displayed accelerated atherosclerosis as compared with ApoE and TNF-α double knockout mice (SMD = 1.87, 95% CI: 0.81–2.93) (Ohta et al. 2005). This effect size was similar to that observed in PM-induced atherosclerosis, but it was accompanied by a marked difference in systemic levels of TNF-α.

It has been speculated that particles, which have passed from the airway space to the circulation, can cause systemic inflammation and oxidative stress. However, translocation of particles is very modest as documented from experiments on gold nanoparticles with a diameter of 80 nm that had less than 0.1% translocation in rats following i.t. instillation (Kreyling et al. 2014). This observation is identical to earlier studies showing that 15 and 80 nm iridium particles has less than 0.2% and 0.1% translocation in rats, respectively (Kreyling et al. 2002). Likewise, the translocation of MWCNT-7 to the liver, kidney, heart and brain was less than 0.01% and 0.04% of the deposited dose at 1 or 336 d post-exposure (Mercer et al. 2013). On the contrary, NMs with higher solubility (e.g. CeO₂ or quantum dots) have slightly higher systemic translocation, which can be attributed to passage of dissolved of constituents from the particles (Kermanizadeh et al. 2015). Observations from ex vivo exposure of vessel segments to PM have typically shown that concentrations within 10–100 μg/ml of PM cause vasomotor dysfunction (Bagate et al. 2004a,b, 2006, Hansen et al. 2007, Miller et al. 2009, Vesterdal et al. 2012). It is difficult to envisage that plasma PM concentrations, which are only a small fraction of the deposited pulmonary doses, will ever be
sufficiently high enough to cause an effect to endothelial cells in the arterial circulation. As an example, a plasma concentration of 10 μg/ml can be reached from a deposited dose of 250 mg/kg in mice, assuming for 0.1% translocation in a 20 g animal with 1 ml of plasma. This dose is even higher than the doses that have been used in the studies of pulmonary instillation of PM. Collectively, there is no consistent evidence to support a notion that low-grade systemic inflammation is an important link between airway exposure to PM and CVD outcomes in terms of vasomotor dysfunction and accelerated plaque progression. Thus, the best explanation remains as a yet unidentified component in serum acting as a signaling factor between the local effect at the site of external exposure (e.g. lung epithelial cells or gastrointestinal mucosa) and arterial wall. This has been investigated in an ex vivo study where aorta rings were incubated with serum from PM-exposed mice. The serum from mice, which had been exposed to mixed vehicle exhaust or wood smoke, impaired the vasorelaxation response to ACH, whereas dust from roadway surfaces on residential streets and urban thoroughfare roads in Phenix and Tucson had no effect (Aragon et al. 2015).

**Systemic levels of lipids and CVD outcomes**

Cholesterol levels and to a lesser extent triglycerides in the blood are traditional risk factors for IHD in humans. Lipotoxicity is related to elevated levels of free fatty acids and associated with insulin resistance and endothelial dysfunction (Imrie et al. 2010). However, studies that have measured vasomotor function in long-term PM-exposed animals while simultaneously measuring triglycerides or cholesterol levels in plasma/serum have reported unaltered levels of the lipids (Sun et al. 2005, 2009, Folkmann et al. 2012, Miller et al. 2013, Miyata et al. 2013). The same analysis of studies on plaque progression also indicated inconsistent association to responses in terms of cholesterol (four out of 12 studies showing consistent results) and triglyceride (three out of nine studies) levels in blood (summarized in Supplementary Table 8).

Overall, there seems to be no consistent association between blood lipid levels and CVD outcomes. However, it can be speculated that total cholesterol and triglyceride levels lack sufficient specificity as proxy measures for atherogenic and toxic lipids. In addition, it has been described that mixed whole engine emission exposure to ApoE−/− mice (300 μg/m³, 6 h/d for 7 or 50 d) increased expression of lectin-like oxidized LDL receptors (LOX-1) in the aorta (Lund et al. 2011). Similarly, another study showed increased LOX-1 expression in the aorta tissue in male Kyoto rats after exposure to mixed ozone (0.4 ppm) and DE (2200 μg/m³) for 16 weeks (5 h/d and 1 d/week), whereas there was no alteration in the levels of total cholesterol, triglycerides, HDL and LDL (Kodavanti et al. 2011).

**PM exposure, inflammation and oxidative stress in vessel walls**

A number of studies have investigated inflammation and oxidative stress as mechanism of vasomotor dysfunction in PM-exposed animals. In general, production of ROS has mainly been assessed ex vivo by incubation of tissue cross-sections on microscope slides with dihydroethidium, which is considered to represent superoxide anion radicals (mainly derived from enzymes in inflammatory cells (e.g. NADPH oxidase) or uncoupling of endothelial nitric oxide synthase). The ex vivo incubation means that there is no direct proof of production of ROS in the arterial wall, although it can be considered as an indicator of the potential prooxidant milieu in tissue. These radicals can react with nitric oxide to produce peroxynitrite, which is a highly reactive compound that is typically measured as 3-nitro adducts on tyrosine residues on proteins (3-NT). It has been shown that short-term DE exposure was associated with both vasoconstriction and increased superoxide anion radical production in coronary arteries of Sprague–Dawley rats (Cherng et al. 2011). Similarly, short-term airway exposure to nanosized TiO₂ was associated with increased superoxide anion radical production, increased 3-NT levels and vasomotor dysfunction in spinotrapezius muscle and subepicardial arterioles of Sprague–Dawley rats (Nurkiewicz et al. 2009, LeBlanc et al. 2010). On the contrary, i.t. instillation of nanosized carbon black was associated with vasomotor dysfunction, yet no alteration in 3-NT levels in the aorta of ApoE−/− mice was visible (Vesterdal et al. 2010). Additionally, long-term CAPs inhalation has also been associated with increased activity of NADPH oxidase, 3-NT formation, superoxide anion radical generation and vasomotor dysfunction in the aorta of Sprague–Dawley rats and ApoE−/− mice (Sun et al. 2005, Ying et al. 2009a). Moreover, increased 3-NT staining in the aorta was observed after long-term inhalation of gasoline engine emission and particle-filtered exhaust (60 μg/m³, 6 h/d for 7 weeks), which did not generate pulmonary inflammation (Lund et al. 2007). The same authors also showed that 7 d exposure to gasoline engine exhaust (60 μg/m³, 6 h/d) or mixed vehicle exhaust (300 μg/m³) was associated with increased production of superoxide anion radicals in the aorta of ApoE−/− mice (Lund et al. 2009, 2011).
Collectively, there is evidence indicating that exposure to PM is associated with oxidative stress in blood vessel walls. Several studies have measured biomarkers of oxidative stress in terms of oxidized lipids, DNA and proteins. Unfortunately, a number of assays for assessment of these biomarkers have poor quality. This pertains especially to certain methods for detection of lipid peroxidation products and nucleobase oxidation products in tissues, blood or urine (Møller and Loft 2010). Therefore, studies that have use these methodologies have not been included in review as they provide little concrete evidence of oxidative stress in the target tissue.

Summary

There is little evidence to support the hypothesis of pulmonary/systemic inflammation as the main causal link between pulmonary exposure to PM and CVD outcomes. The concurrent increases in cytokine levels in lung tissue and vascular dysfunction may only reflect parallel, yet unrelated, processes after exposure to PM. In general, there could be a bias towards reporting a positive association between pulmonary/systemic inflammation and CVD outcomes; with this mechanism of action potentially only discussed in publications with a positive association between inflammation and CVD outcomes. It is, therefore, difficult to identify publications with selective omission of null results on biomarkers of inflammation. Furthermore, selective citation of publications showing the same association, or support the prevailing hypothesis by focusing only on the positive data, is another obstacle that may bias research (Møller et al. 2010b). The same limitation pertains to conclusions about other systemic biomarkers such as levels of lipids. Nevertheless, there is compelling evidence of oxidative stress and inflammation in the vessel wall and their importance in the development of CVD outcomes. It is very possible that inflammation and oxidative stress are transient effects after a short-term exposure to PM. Still, atherosclerosis is a pathological process characterized by a pro-inflammatory and pro-oxidant milieu. Therefore, a sudden increase of inflammation and oxidative stress after PM exposure may be associated or at least contribute to the rupture of unstable plaques, which in humans could be the final step in elicitation of a clinical manifestation such as myocardial infarction and ischemic stroke.

Limitations

In the present review, we have only assessed exposure to PM. At least, some studies on DE exposure have indicated that the gaseous components play an important role for vasomotor dysfunction and plaque progression (Quan et al. 2010, Vedal et al. 2013). It is currently uncertain whether the gaseous constituents play a role in the adverse effects of CVD following air pollution exposures, e.g. inhalation of CAPs. It should be stated that oxidizing gases such as ozone and nitrogen dioxide appear to have little direct effect on vascular endpoints or biomarkers in the circulation in controlled exposure studies (Hesterberg et al. 2009, Goodman et al. 2015). However, the inhalation of carbon monoxide (30–100 ppm to mimic fluctuation in urban air) for 4 weeks was associated with reduced vasorelaxation response in coronary arteries of rats, whereas there was no difference in the SNP response (Meyer et al. 2011). Nevertheless, it has been shown that serum from ozone-exposed mice contains vasoactive factors that can directly impair ACH-induced vasorelaxation (Robertson et al. 2013).

Here, the assessment of differences related to vasomotor function is relatively crude as we have used statistical significance rather than effect size as the outcome variable. The concentration–response relationship of vasoactive agents is typically fitted to a sigmoid curve. It is, therefore, possible to obtain the maximal response and the concentration that induces 50% response as outcome measures. Using this information, one can further investigate the mechanisms of vasomotor dysfunction, although re-analysis of original data is most likely necessary to standardize the data.

In this review, the analysis of the characterization of the active components in PM that causes CVD outcomes is imprecise. As previously discussed, many of the publications highlighted contain insufficient information about the particle size, shape and composition in relevant exposure vehicle to compare these metrics across studies. On the contrary, the NPACT studies deserve recognition for their efforts to establish which factors in ambient air pollution contribute to CVD (Vedal et al. 2013, Lippmann 2014). Despite best intentions, the NPACT study did not succeed in identifying the role of PM composition for vascular effects, which emphasizes the challenges in the research of PM-induced adverse effects. Hence, we have heavily focused on the generalizability of PM exposure rather than the cause–effect relationship of specific exposures. As such, there are only few studies that have actually investigated different types of exposures in the same experimental setting. This has been tested in studies on inhalation of DE and CAPs (Gerlofs-Nijland et al. 2010, Quan et al. 2010), the i.t. instillation of carbon black and TiO₂ (Courtois et al. 2010) and SRM2975, SRM1648 and SWCNT (Vesterdal et al. 2014). Interestingly, none of these studies have documented substantial differences in the CVD outcomes by
different types of PM. Nevertheless, it may not be possible to extrapolate the findings from the present analysis to other combustion-derived PM such as biomass or environmental exposures. At present, there seems to be relatively few studies on PM exposure from industrial or environmental sources. An example of such investigation is an interesting study on vasomotor dysfunction following exposure to PM from an Appalachian mountaintop mining site (Knuckles et al. 2013).

As a limitation, the analysis has only included vasomotor dysfunction and atherosclerosis as outcomes. Atherosclerosis is the underlying condition for myocardial infarction and ischemic stroke, but it does not necessarily lead to clinical manifestations. In fact, the progression of atherosclerosis can decelerate, stop or even reverse in humans if cardiovascular risk factors are avoided. Vasomotor dysfunction can occur in both healthy individuals and CVD patients after exposure to PM. CVD patients may already have vasomotor dysfunction and, therefore, diminished capacity to regulate blood flow. The additional impairment of vasomotor function by PM exposure may be the final trigger for clinical manifestations such as myocardial infarction or stroke. However, increased thrombosis tendency and dysrhythmia are also important descriptors of the acute risk of myocardial infarction, although they may not contribute to the chronic plaque development and deteriorated vasomotor function.

Finally, the quantitative analysis has been based on the mass concentration as dose metric. It should be stated that particle number concentration or surface area is more accurate dose metrics for UFPs and NMs. However, many publications do not provide sufficient information to assess the dose–response relationships on metrics other than the mass concentration.

**Summary**

The limitations of this review do not detract from the main conclusion formed based on the securitization of available literature stating that exposure to PM from urban air, DE (or DEP) and certain types of NMs affect vascular system by accelerating atherosclerosis and impair vasomotor function. The animal experiments are not perfect models for atherosclerosis and vasomotor dysfunction in humans. Nevertheless, animals and humans would be expected to show the same ranking of effect by different types of particles (i.e. if exposure to air pollution particles was more hazardous than NMs in animals, one would expect the same pattern in humans, irrespective of species differences in CVD development and progression). The lack of particle characterization data in studies on air pollution particles makes it impossible to compare studies on other metrics than the mass concentration/dose. The studies on NMs have relatively detailed description of particle characteristics, including size distribution, specific surface area and shape. Hence it is possible that future critical reviews, which specifically focus on CVD effects of NMs with a larger number of publications, may identify particle characteristics that are better predictors of CVD outcomes than the mass concentration/dose.

**Conclusions**

The results from the quantitative analysis in this review show that pulmonary and most likely other exposure routes to PM are associated with vasomotor dysfunction and progression of atherosclerosis. Further conclusions are as follows:

1. Airway exposure to PM is associated with augmented vasoconstriction and reduced endothelium-dependent vasorelaxation responses.
2. Vasomotor dysfunction is observed in multiple vessels in the arterial tree of PM-exposed animals, including the aorta, mesenteric, coronary and carotid arteries.
3. Exposure to PM is associated with accelerated plaque progression in atherosclerosis-prone animals on either normal chow or high-fat diet. Accelerated atherosclerosis encompasses both increased plaque size and progression of plaques to more advanced stages. This effect is not consistently associated with altered lipid levels in plasma/serum that may promote a more pro-atherogenic environment.
4. Systemic or pulmonary inflammation is not a prerequisite for dysfunction in the vasomotor response and accelerated progression of atherosclerosis in PM-exposed animals.
5. Oxidative stress and inflammation have been observed in the arterial wall of PM-exposed animals with vasomotor dysfunction or plaque progression.
6. There has been a similar effect on atherosclerosis progression and vasomotor dysfunction after airway exposure to ambient air pollution particles and certain types of NMs, including TiO₂, carbon black and CNTs. The exposure to DE (and DEP) has been associated with lower responses as compared with ambient air pollution particles and NMs. These observations cannot support the suggestion that all types of NMs per se are hazardous to the vascular system.

The observations from the present review point to several challenges in the future. The major limitation with regard to using the findings presented in risk
assessment is a lack of dose–response relationship between exposures and CVD outcomes. Pooled analysis of the existing data is possible, although there are obvious challenges to such an approach because of the differences in methodology and exposures. There is a knowledge gap in the mechanism of CVD outcomes following PM exposure, which has been most clearly visible in studies of pulmonary exposure. A coherent linkage between local pulmonary effects and toxicity in the arterial wall (i.e. oxidative stress and inflammation) remains to be established. The role of mediators in the blood also requires further investigation. The few studies on gastrointestinal exposure to NMs have shown fairly clear associations with vasomotor dysfunction. Further investigations of the relationship between intake of NMs and CVD outcomes are warranted because oral exposure is a very likely route of exposure. This could be stimulated by the observation from the present review that NMs and combustion-derived PMs have similar hazards to the vascular system.

In summary, the analysis shows that certain NMs, including TiO$_2$, carbon black and CNTs, have similar hazards to the vascular system as combustion-derived PM. In addition, airway exposure by inhalation or instillation to air pollution particles and NMs is associated with similar effect size on atherosclerosis progression, augmented vasoconstriction and blunted vasorelaxation responses in arteries. Exposure to DE (or DEP) is associated with lower vascular responses as compared with air pollution particles and NMs. There is not consistent evidence of pulmonary/systemic inflammation and pro-atherogenic plasma lipid profile as intermediate step in vasomotor dysfunction and progression of atherosclerosis in PM-exposed animals. There is, however, experimental evidence of oxidative stress and inflammation in the arterial wall of PM-exposed animals. From the data, it is clear that exposure to air pollution particles from traffic vehicles is hazardous to the vascular system, leading to clinical manifestations and mortality due to IHD. This implies that NM exposure may also be associated with the same diseases in humans, although this has not yet been investigated in observational studies.

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References

Adar, S.D., et al., 2013. Fine particulate air pollution and the progression of carotid intima-medial thickness: a prospective cohort study from the multi-ethnic study of atherosclerosis and air pollution. *PLoS medicine*, 10, e1001430.

Alexandre de Artinano, A. and Castro, M.M., 2009. Experimental rat models to study the metabolic syndrome. *British journal of nutrition*, 102, 1246–1253.

Aragon, M.J., et al., 2015. Inflammatory and vasoactive effects of serum following inhalation of varied complex mixtures. *Cardiovascular toxicology*, 2015; (doi:10.1007/s12012-015-9325-2).

Araujo, J.A. and Nel, A.E., 2009. Particulate matter and atherosclerosis: role of particle size, composition and oxidative stress. *Particle and fibre toxicology*, 6, 24.

Araujo, J.A., et al., 2008. Ambient particulate pollutants in the ultrafine range promote early atherosclerosis and systemic oxidative stress. *Circulation research*, 102, 589–596.

Bagate, K., et al., 2004a. The effect of particulate matter on resistance and conductance vessels in the rat. *Inhalation toxicology*, 16, 431–436.

Bagate, K., et al., 2004b. Vascular effects of ambient particulate matter instillation in spontaneous hypertensive rats. *Toxicology and applied pharmacology*, 197, 29–39.

Bagate, K., et al., 2006. Signal transduction pathways involved in particulate matter induced relaxation in rat aorta-spontaneous hypertensive versus Wistar Kyoto rats. *Toxicology in vitro*, 20, 52–62.

Bai, N. and van Eeden, S.F., 2013. Systemic and vascular effects of circulating diesel exhaust particulate matter. *Inhalation toxicology*, 25, 725–734.

Bai, N., et al., 2011. Changes in atherosclerotic plaques induced by inhalation of diesel exhaust. *Atherosclerosis*, 216, 299–306.

Brook, R.D., et al., 2010. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. *Circulation*, 121, 2331–2378.

Buja, L.M., et al., 1983. Cellular pathology of progressive atherosclerosis in the WHHL rabbit. An animal model of familial hypercholesterolemia. *Arteriosclerosis*, 3, 87–101.

Campen, M.J., Lund, A. and Rosenfeld, M., 2012. Mechanisms linking traffic-related air pollution and atherosclerosis. *Current opinion in pulmonary medicine*, 18, 155–160.

Campen, M.J., et al., 2005. Nonparticulate components of diesel exhaust promote constriction in coronary arteries from ApoE/−/− mice. *Toxicological sciences*, 88, 95–102.

Campen, M.J., et al., 2010. Inhaled diesel emissions alter atherosclerotic plaque composition in ApoE(−/−) mice. *Toxicology and applied pharmacology*, 242, 310–317.
Campen, M., et al., 2014. Engine exhaust particulate and gas phase contributions to vascular toxicity. *Inhalation toxicology*, 26, 353–360.

Cao, Q., et al., 2007. Pulmonary responses to fine particles: differences between the spontaneously hypertensive rats and Wistar Kyoto rats. *Toxicology letters*, 171, 126–137.

Cao, Y., et al., 2011. Vascular effects of multi-walled carbon nanotubes in dyslipidemic ApoE−/− mice and cultured endothelial cells. *Toxicological sciences*, 138, 104–116.

Card, J.W., et al., 2011. An appraisal of the published literature on the safety and toxicity of food-related nanomaterials. *Critical reviews in toxicology*, 41, 22–49.

Cassee, F.R., et al., 2011. Exposure, health and ecological effects review of engineered nanoscale cerium and cerium oxide associated with its use as a fuel additive. *Critical reviews in toxicology*, 41, 213–229.

Cassee, F.R., et al., 2012. The biological effects of subacute inhalation of diesel exhaust following addition of cerium oxide nanoparticles in atherosclerosis-prone mice. *Environmental research*, 115, 1–10.

Chen, L.C. and Nadziejko, C., 2005. Effects of subchronic exposures to concentrated ambient particles (CAPs) in mice. V. CAPs exacerbatate aortic plaque development in hyperlipidemic mice. *Inhalation toxicology*, 17, 217–224.

Chen, T., et al., 2013a. Cardiovascular effects of pulmonary exposure to titanium dioxide nanoparticles in ApoE knockout mice. *The journal of nanoscience and nanotechnology*, 13, 3214–3222.

Chen, T., et al., 2013b. Beijing ambient particle exposure accelerates atherosclerosis in ApoE knockout mice. *Toxicology letters*, 223, 146–153.

Cheng, T.W., et al., 2009. Impairment of coronary endothelial cell ET(B) receptor function after short-term inhalation exposure to whole diesel emissions. *American journal of physiology, regulatory, integrative and comparative physiology*, 297, R640–R647.

Cheng, T.W., et al., 2011. Mechanisms of diesel-induced endothelial nitric oxide synthase dysfunction in coronary arteries. *Environmental health perspectives*, 119, 98–103.

Conroy, R.M., et al., 2003. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *European heart journal*, 24, 987–1003.

Courtios, A., et al., 2008. Impairment of NO-dependent relaxation in intralobar pulmonary arteries: comparison of urban particulate matter and manufactured nanoparticles. *Environmental health perspectives*, 116, 1294–1299.

Courtios, A., et al., 2010. Effect of engineered nanoparticles on vasomotor responses in rat intrapulmonary artery. *Toxicology and applied pharmacology*, 245, 203–210.

Coozi, E., et al., 2006. Ultrafine particulate matter exposure augments ischemia-reperfusion injury in mice. *American journal of physiology: Heart and circulatory physiology*, 291, H894–H903.

Cuevas, A.K., et al., 2010. Inhaled nickel nanoparticles alter vascular reactivity in C57BL/6 mice. *Inhalation toxicology*, (Suppl 2), 2, 100–106.

Cuevas, A.K., et al., 2015. Metal rich particulate matter impairs acetylcholine-mediated vasorelaxation of microvessels in mice. *Particle and fibre toxicology*, 12, 14.

Danesh, J., et al., 2000. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. *British medical journal*, 321, 199–204.

Davel, A.P., et al., 2012. Endothelial dysfunction in the pulmonary artery induced by concentrated fine particulate matter exposure is associated with local but not systemic inflammation. *Toxicology*, 295, 39–46.

De Jong, W.H. and Borm, P.J., 2008. Drug delivery and nanoparticles: applications and hazards. *International journal of nanomedicine*, 3, 133–149.

Doggrill, S.A. and Brown, L., 1998. Rat models of hypertension, cardiac hypertrophy and failure. *Cardiovascular research*, 39, 89–105.

Donaldson, K., et al., 2001. Ambient particle inhalation and the cardiovascular system: potential mechanisms. *Environmental health perspectives*, 109 (Suppl 4), 523–527.

Donaldson, K., et al., 2013. Pulmonary toxicity of carbon nanotubes and asbestos-similarities and differences. *Advanced drug delivery reviews*, 65, 2078–2086.

Eckel, R.H., Grundy, S.M. and Zimet, P.Z., 2005. The metabolic syndrome. *Lancet*, 365, 1415–1428.

Folkman, J.K., Loft, S. and Møller, P., 2007. Oxidatively damaged DNA in aging dyslipidemic ApoE−/− and wild-type mice. *Mutagenesis*, 22, 105–110.

Folkmann, J.K., et al., 2012. Endothelial dysfunction in normal and prediabetic rats with metabolic syndrome exposed by oral gavage to carbon black nanoparticles. *Toxicological sciences*, 129, 98–107.

Frame, M.D., et al., 2014. Vasoactive effects of stable aqueous suspensions of single walled carbon nanotubes in hamsters and mice. *Nanotoxicology*, 8, 867–875.

Gardner, S.Y., Lehmann, J.R. and Costa, D.L., 2000. Oil fly ash-induced elevation of plasma fibrinogen levels in rats. *Toxicological sciences*, 56, 175–180.

GBD 2013 Mortality and Causes of Death Collaborators, 2015. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*, 385, 117–171.

Gerlofs-Nijland, M.E., et al., 2010. Pulmonary and cardiovascular effects of traffic-related particulate matter: 4-week exposure of rats to roadside and diesel engine exhaust particles. *Inhalation toxicology*, 22, 1162–1173.

Getz, G.S. and Reardon, C.A., 2012. Animal models of atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology*, 32, 1104–1115.

Ghio, A.J. and Huang, Y.C., 2004. Exposure to concentrated ambient particles (CAPs): a review. *Inhalation toxicology*, 16, 53–59.

Gillespie, P.A., et al., 2010. Pulmonary response after exposure to inhaled nickel hydroxide nanoparticles: short and long-term studies in mice. *Nanotoxicology*, 4, 106–119.

Goldklang, M., et al., 2012. Activation of the TLR4 signaling pathway and abnormal cholesterol efflux lead to emphysema in ApoE-deficient mice. *American journal of physiology-lung cellular and molecular physiology*, 302, L1200–L1208.

Goodman, J.E., et al., 2015. Ozone exposure and systemic biomarkers: evaluation of evidence for adverse cardiovascular health impacts. *Critical reviews in toxicology*, 45, 412–452.

Gordon, T. and Reibman, J., 2000. Cardiovascular toxicity of inhaled ambient particulate matter. *Toxicological sciences*, 56, 2–4.

Goto, Y., et al., 2004. Exposure to ambient particles accelerates monocyte release from bone marrow in atherosclerotic
rabbits. *American journal of physiology: Lung cellular and molecular physiology*, 287, L79–L85.

Gottipolu, R.R., *et al.*, 2009. One-month diesel exhaust inhalation produces hypertensive gene expression pattern in healthy rats. *Environmental health perspectives*, 117, 38–46.

Grundy, S.M., 1999. Primary prevention of coronary heart disease: integrating risk assessment with intervention. *Circulation*, 100, 988–998.

Grundy, S.M., *et al.*, 1999. Assessment of cardiovascular risk by use of multiple-risk-factor assessment equations: a statement for healthcare professionals from the American Heart Association and the American College of Cardiology. *Circulation*, 100, 1481–1492.

Gutierrez-Hernandez, J.M., *et al.*, 2015. Single-walled carbon nanotubes (SWCNTs) induce vasodilation in isolated rat aortic rings. *Toxicology in vitro*, 29, 657–662.

Han, S.G., *et al.*, 2015. Pulmonary and atherogenic effects of multi-walled carbon nanotubes (MWCNT) in apolipoprotein-E-deficient mice. *Journal of toxicology and environmental health part A*, 78, 244–253.

Hansen, C.S., *et al.*, 2007. Diesel exhaust particles induce endothelial dysfunction in apoE−/− mice. *Toxicology and applied pharmacology*, 219, 24–32.

Hardman, R., 2006. A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. *Environmental health perspectives*, 114, 165–172.

Hesterberg, T.W., *et al.*, 2009. Critical review of the human data on short-term nitrogen dioxide (NO2) exposures: evidence for NO2 no-effect levels. *Critical reviews in toxicology*, 39, 743–781.

Hesterberg, T.W., *et al.*, 2012. Health effects research and regulation of diesel exhaust: an historical overview focused on lung cancer risk. *Inhalation toxicology*, 24 (Suppl 1), 1–45.

Hoek, G., *et al.*, 2013. Long-term air pollution exposure and cardio-respiratory mortality: a review. *Environmental health*, 12, 43.

Huber, S.A., *et al.*, 1999. Interleukin-6 exacerbates early atherosclerosis in mice. *Arteriosclerosis, thrombosis, and vascular biology*, 19, 2364–2367.

Ikeda, M., *et al.*, 1995. Impairment of endothelium-dependent relaxation by diesel exhaust particles in rat thoracic aorta. *Japanese journal of pharmacology*, 68, 183–189.

Imrie, H., Abbas, A. and Kearney, M., 2010. Insulin resistance, lipotoxicity and endothelial dysfunction. *Biochimica et biophysica Acta*, 1801, 320–326.

Jacobsen, N.R., *et al.*, 2009. Lung inflammation and genotoxicity following pulmonary exposure to nanoparticles in ApoE−/− mice. *Particle and fibre toxicology*, 6, 2.

Kalra, S.S. and Shanahan, C.M., 2012. Vascular calcification and hypertension: cause and effect. *Annals of medicine*, 44 (Suppl 1), S85–S92.

Kamprath, T., *et al.*, 2011. Chronic fine particulate matter exposure induces systemic vascular dysfunction via NADPH oxidase and TLR4 pathways. *Circulation research*, 108, 716–726.

Kang, G.S., *et al.*, 2011. Long-term inhalation exposure to nickel nanoparticles exacerbated atherosclerosis in a susceptible mouse model. *Environmental health perspectives*, 119, 176–181.

Kaptoge, S., *et al.*, 2012. C-reactive protein, fibrinogen, and cardiovascular disease prediction. *The new England journal of medicine*, 367, 1310–1320.

Kaptoge, S., *et al.*, 2014. Inflammatory cytokines and risk of coronary heart disease: new prospective study and updated meta-analysis. *The European heart journal*, 35, 578–589.

Keebaugh, A.J., *et al.*, 2015. Is atherosclerotic disease associated with organic components of ambient fine particles? *Science of the total environment*, 533, 69–75.

Kennedy, A.J., *et al.*, 2010. Mouse models of the metabolic syndrome. *Disease models & mechanisms*, 3, 156–166.

Kermanizadeh, A., *et al.*, 2015. Nanomaterial translocation – the biokinetics, tissue accumulation, toxicity and fate of materials in secondary organs – a review. *Critical reviews in toxicology*, 45, 837–872.

Kido, T., *et al.*, 2011a. Diesel exhaust inhalation induces heat shock protein 70 expression in vivo. *Inhalation toxicology*, 23, 593–601.

Kido, T., *et al.*, 2011b. Particulate matter induces translocation of IL-6 from the lung to the systemic circulation. *American journal of respiratory cell and molecular biology*, 44, 197–204.

Kim, J.K., *et al.*, 2011. Effect of nano-sized carbon black particles on lung and circulatory system by inhalation exposure in rats. *Safety and health at work*, 2, 282–289.

King, A.J., 2012. The use of animal models in diabetes research. *The British journal of pharmacology*, 166, 877–894.

Knuckles, T.L., *et al.*, 2008. Diesel exhaust exposure enhances venoconstriction via uncoupling of eNOS. *Toxicology and applied pharmacology*, 230, 346–351.

Knuckles, T.L., *et al.*, 2012. Nanoparticle inhalation alters systemic arteriolar vasoreactivity through sympathetic and cyclooxygenase-mediated pathways. *Nanotoxicology*, 6, 724–735.

Knuckles, T.L., *et al.*, 2013. Air pollution particulate matter collected from an Appalachian mountaintop mining site induces microvascular dysfunction. *Microcirculation*, 20, 158–169.

Kodavanti, U.P., *et al.*, 2000. The spontaneously hypertensive rat as a model of human cardiovascular disease: evidence of exacerbated cardiopulmonary injury and oxidative stress from inhaled emission particulate matter. *Toxicology and applied pharmacology*, 164, 250–263.

Kodavanti, U.P., *et al.*, 2011. Vascular and cardiac impairments in rats inhaling ozone and diesel exhaust particles. *Environmental health perspectives*, 119, 312–318.

Kreiling, W.G., *et al.*, 2002. Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. *Journal of toxicology and environmental health Part A*, 65, 1513–1530.

Kreiling, W.G., *et al.*, 2014. Air-blood barrier translocation of tracheally instilled gold nanoparticles inversely depends on particle size. *American chemical society Nano*, 8, 222–233.

Labranche, N., *et al.*, 2012. Vascular oxidative stress induced by diesel exhaust microparticles: synergism with hypertension. *Journal of cardiovascular pharmacology*, 60, 530–537.

LeBlanc, A.J., *et al.*, 2009. Nanoparticle inhalation impairs endothelium-dependent vasodilation in subepicardial arteries. *The journal of toxicology and environmental health a*, 72, 1576–1584.

LeBlanc, A.J., *et al.*, 2010. Nanoparticle inhalation impairs coronary microvascular reactivity via a local reactive oxygen species-dependent mechanism. *Cardiovascular toxicology*, 10, 27–36.

Li, Z., *et al.*, 2005. Pollutant particles produce vasoconstriction and enhance MAPK signaling via angiotensin type I receptor. *Environmental health perspectives*, 113, 1009–1014.
Li, R., et al., 2013a. Atmospheric ultrafine particles promote vascular calcification via the NF-kappaB signaling pathway. *American journal of physiology: Cell physiology*, 304, C362–C369.

Li, R., et al., 2013b. Ambient ultrafine particles alter lipid metabolism and HDL anti-oxidant capacity in LDLR-null mice. *The journal of lipid research*, 54, 1608–1615.

Li, Z., et al., 2007. Cardiovascular effects of pulmonary exposure to single-wall carbon nanotubes. *Environmental health perspectives*, 115, 377–382.

Lim, S.S., et al., 2012. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, 380, 2224–2260.

Lippmann, M., 2014. Toxicological and epidemiological studies of cardiovascular effects of ambient air fine particulate matter (PM_{2.5}) and its chemical components: coherence and public health implications. *Critical reviews in toxicology*, 44, 299–347.

Lippmann, M. and Chen, L.C., 2009. Health effects of concentrated ambient air particulate matter (CAPs) and its components. *Critical reviews in toxicology*, 39, 865–913.

Lippmann, M., Gordon, T. and Chen, L.C., 2005. Effects of subchronic exposures to concentrated ambient particles in mice. IX. Integral assessment and human health implications of subchronic exposures of mice to CAPs. *Inhalation toxicology*, 17, 255–261.

Lippmann, M., et al., 2013. National Particle Component Toxicity (NPACT) Initiative: integrated epidemiologic and toxicologic studies of the health effects of particulate matter components. *Research report health effects institute*, 177, 5–13.

Liu, C., et al., 2014. Air pollution-mediated susceptibility to inflammation and insulin resistance: influence of CCR2 pathways in mice. *Environmental health perspectives*, 112, 17–26.

Lund, A.K., et al., 2007. Gasoline exhaust emissions induce vascular remodeling pathways involved in atherosclerosis. *Toxicological sciences*, 95, 485–494.

Lund, A.K., et al., 2009. Vehicular emissions induce vascular MMP-9 expression and activity associated with endothelin-1-mediated pathways. *Arteriosclerosis, thrombosis, and vascular biology*, 29, 511–517.

Lund, A.K., et al., 2011. The oxidized low-density lipoprotein receptor mediates vascular effects of inhaled vehicle emissions. *American journal of respiratory and critical care medicine*, 184, 82–91.

Maciejczyk, P., et al., 2005. Effects of subchronic exposures to concentrated ambient particles (CAPs) in mice. II. The design and of a CAPs exposure system for biometric telemetry monitoring. *Inhalation toxicology*, 17, 189–197.

Mann, E.E., et al., 2012. Changes in cardiopulmonary function induced by nanoparticles. *Nanomedicine and nanobiotechnology*, 4, 691–702.

McClellan, R.O., Hesterberg, T.W. and Wall, J.C., 2012. Evaluation of carcinogenic hazard of diesel engine exhaust needs to consider revolutionary changes in diesel technology. *Regulatory toxicology and pharmacology*, 63, 225–258.

McKinney, W., et al., 2012. Pulmonary and cardiovascular responses of rats to inhalation of a commercial antimicrobial spray containing titanium dioxide nanoparticles. *Inhalation toxicology*, 24, 447–457.

Mercer, R.R., et al., 2013. Extrapulmonary transport of MWCNT following inhalation exposure. *Particle and fibre toxicology*, 10, 38.

Meyer, G., et al., 2011. Carbon monoxide pollution impairs myocardial perfusion reserve: implication of coronary endothelial dysfunction. *Cardiovascular toxicology*, 11, 334–340.

Mikkelsen, L., et al., 2011. Modest effect on plaque progression and vasodilatory function in atherosclerosis-prone mice exposed to nanosized TiO_{2}. *Particle and fibre toxicology*, 8, 32.

Miller, M.R., 2014. The role of oxidative stress in the cardiovascular actions of particulate air pollution. *Biochemical society transactions*, 42, 1006–1011.

Miller, M.R., et al., 2009. Direct impairment of vascular function by diesel exhaust particulate through reduced bioavailability of endothelium-derived nitric oxide induced by superoxide free radicals. *Environmental health perspectives*, 117, 611–616.

Miller, M.R., et al., 2013. Diesel exhaust particulate increases the size and complexity of lesions in atherosclerotic mice. *Particle and fibre toxicology*, 10, 61.

Minarick, V.C., et al., 2013. Pulmonary cerium dioxide nanoparticle exposure differentially impairs coronary and mesenteric arteriolar reactivity. *Cardiovascular toxicology*, 13, 323–337.

Minarick, V.C., et al., 2015. Intravenous and gastric cerium dioxide nanoparticle exposure disrupts microvascular smooth muscle signaling. *Toxicological sciences*, 144, 77–89.

Miyata, R. and van Eeden, S.F., 2011. The innate and adaptive immune response induced by alveolar macrophages exposed to ambient particulate matter. *Toxicology and applied pharmacology*, 257, 209–226.

Miyata, R., et al., 2013. Statins attenuate the development of atherosclerosis and endothelial dysfunction induced by exposure to urban particulate matter (PM10). *Toxicology and applied pharmacology*, 272, 1–11.

Møller, P., and Loft, S., 2010. Oxidative damage to DNA and lipids as biomarkers of exposure to air pollution. *Environmental health perspectives*, 118, 1126–1136.

Møller, P., et al., 2010a. Role of oxidative damage in toxicity of particulates. *Free radical research*, 44, 1–46.

Møller, P., et al., 2010b. Aging and oxidatively damaged nuclear DNA in animal organs. *Free radical biology and medicine*, 48, 1275–1285.

Møller, P., et al., 2014. Oxidative stress and inflammation generated DNA damage by exposure to air pollution particles. *Mutation research: Reviews in mutation research*, 762, 133–166.

Montgomery, M.K., et al., 2013. Mouse strain-dependent variation in obesity and glucose homeostasis in response to high-fat feeding. *Diabetologia*, 56, 1129–1139.

Muto, E., et al., 1996. Endothelial-constitutive nitric oxide synthase exists in airways and diesel exhaust particles inhibit the effect of nitric oxide. *Life Sciences*, 59, 1563–1570.

Napierska, D., et al., 2010. The nanosilica hazard: another variable entity. *Particle and fibre toxicology*, 7, 39.

Naura, A.S., et al., 2009. High-fat diet induces lung remodeling in ApoE-deficient mice: an association with an increase in circulatory and lung inflammatory factors. *Laboratory investigations*, 89, 1243–1251.

Nemmar, A., et al., 2014. Amorphous silica nanoparticles impair vascular homeostasis and induce systemic inflammation. *The international journal of nanomedicine*, 9, 2779–2789.
Nurkiewicz, T.R., et al., 2004. Particulate matter exposure impairs systemic microvascular endothelium-dependent dilation. *Environmental health perspectives*, 112, 1299–1306.

Nurkiewicz, T.R., et al., 2006. Systemic microvascular dysfunction and inflammation after pulmonary particulate matter exposure. *Environmental health perspectives*, 114, 412–419.

Nurkiewicz, T.R., et al., 2008. Nanoparticle inhalation augments particle-dependent systemic microvascular dysfunction. *Particle and fibre toxicology*, 5, 1.

Nurkiewicz, T.R., et al., 2009. Pulmonary nanoparticle exposure disrupts systemic microvascular nitric oxide signaling. *Toxicological sciences*, 110, 191–203.

Nurkiewicz, T.R., et al., 2011. Pulmonary particulate matter and systemic microvascular dysfunction. *Research report health effects institute*, 164, 3–48.

Oberdörster, G., 1995. Lung particle overload: implications for occupational exposures to particles. *Regulatory toxicology and pharmacology*, 21, 123–135.

Oberdörster, G., et al., 2000. Acute pulmonary effects of ultrafine particles in rats and mice. *Research report health effects institute*, 96, 5–74.

Ohta, H., et al., 2005. Disruption of tumor necrosis factor-alpha gene diminishes the development of atherosclerosis in ApoE-deficient mice. *Atherosclerosis*, 180, 11–17.

Paigen, B., et al., 1990. Atherosclerosis susceptibility differences among progenitors of recombinant inbred strains of mice. *Arteriosclerosis*, 10, 316–323.

Perez, L., et al., 2015. Air pollution and atherosclerosis: a cross-sectional analysis of four European cohort studies in the ESCAPE study. *Environmental health perspectives*, 123, 597–605.

Peters, A., et al., 2004. Exposure to traffic and the onset of myocardial infarction. *The new England journal of medicine*, 351, 1721–1730.

Pinto, Y.M., Paul, M. and Ganten, D., 1998. Lessons from rat models of hypertension: from Goldblatt to genetic engineering. *Cardiovascular research*, 39, 77–88.

Pope, C.A., 2000. Epidemiology of fine particulate air pollution and human health: biologic mechanisms and who’s at risk? *Environmental health perspectives*, 108 (Suppl 4), 713–723.

Pöss, J., et al., 2013. Diesel exhaust particles impair endothelial progenitor cells, compromise endothelium integrity, reduce neangiogenesis, and increase atherogenesis in mice. *Cardiovascular toxicology*, 13, 290–300.

Provost, E.B., et al., 2015. Carotid intima-media thickness, a marker of subclinical atherosclerosis, and particulate air pollution exposure: the meta-analytical evidence. *PloS One*, 10, e0127014.

Quan, C., et al., 2010. Comparative effects of inhaled diesel exhaust and ambient fine particles on inflammation, atherosclerosis, and vascular dysfunction. *Inhalation toxicology*, 22, 738–753.

Rao, X., et al., 2014. CD36-dependent 7-ketocholesterol accumulation in macrophages mediates progression of atherosclerosis in response to chronic air pollution exposure. *Circulation research*, 115, 770–780.

Risom, L., Møller, P. and Loft, S., 2005. Oxidative stress-induced DNA damage by particulate air pollution. *Mutation research*, 592, 119–137.

Robertson, S., et al., 2012. Diesel exhaust particulate induces pulmonary and systemic inflammation in rats without impairing endothelial function ex vivo or in vivo. *Particle and fibre toxicology*, 9, 9.

Robertson, S., et al., 2013. CD36 mediates endothelial dysfunction downstream of circulating factors induced by O3 exposure. *Toxicological sciences*, 134, 304–311.

Ross, R., 1999. Atherosclerosis – an inflammatory disease. *The new England journal of medicine*, 340, 115–126.

Roursgaard, M., et al., 2014. Variability in particle size determination by Nanoparticle Tracking Analysis. *Advanced science, engineering and medicine*, 6, 1–11.

Shah, A.S., et al., 2015. Short term exposure to air pollution and stroke: systematic review and meta-analysis. *British medical journal*, 350, h1295.

Shiomi, M. and Fan, J., 2008. Unstable coronary plaques and cardiac events in myocardial infarction-prone Watanabe heritable hyperlipidemic rabbits: questions and quandaries. *Current opinion in lipidology*, 19, 631–636.

Shiomi, M. and Ito, T., 2009. The Watanabe heritable hyperlipidemic (WHHL) rabbit, its characteristics and history of development: a tribute to the late Dr. Yoshio Watanabe. *Atherosclerosis*, 207, 1–7.

Shiomi, M., et al., 1994. Cell compositions of coronary and aortic atherosclerotic lesions in WHHL rabbits differ. An immunohistochemical study. *Arteriosclerosis, thrombosis*, 14, 931–937.

Shukur, A., et al., 2013. Altered sensitivity to nitric oxide donors, induced by intravascular infusion of quantum dots, in murine mesenteric arteries. *Nanomedicine*, 9, 532–539.

Sioutas, C., Koutrakis, P. and Burton, R.M., 1995. A technique to expose animals to concentrated fine ambient aerosols. *Environmental health perspectives*, 103, 172–177.

Sioutas, C., et al., 1997. Fine particle concentrators for inhalation exposures – effect of particle size and composition. *Journal of aerosol science*, 28, 1057–1071.

Soares, S.R., et al., 2009. Air pollution and antibodies against modified lipoproteins are associated with atherosclerosis and vascular remodeling in hyperlipemic mice. *Atherosclerosis*, 207, 368–373.

Stapleton, P.A., et al., 2012a. Impairment of coronary arteriolar endothelium-dependent dilation after multi-walled carbon nanotube inhalation: a time-course study. *The international journal of molecular sciences*, 13, 13781–13803.

Stapleton, P.A., et al., 2012b. Xenobiotic particle exposure and microvascular endpoints: a call to arms. *Microcirculation*, 19, 126–142.

Stapleton, P.A., et al., 2015. Microvascular and mitochondrial dysfunction in the female F1 generation after gestational TiO2 nanoparticle exposure. *Nanotoxicology*, 9, 941–951.

Stary, H.C., 2000. Natural history and histological classification of atherosclerotic lesions and a histological classification of intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation*, 89, 2462–2478.

Stary, H.C., et al., 1995. A definition of advanced types of atherosclerotic lesions and a histological classification of...
atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Arteriosclerosis, thrombosis, and vascular biology, 15, 1512–1531.

Sun, Q., et al., 2005. Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. Journal of American medical association, 294, 3003–3010.

Sun, Q., et al., 2008a. Ambient air particulate matter exposure and tissue factor expression in atherosclerosis. Inhalation toxicology, 20, 127–137.

Sun, Q., et al., 2008b. Air pollution exposure potentiates hypertension through reactive oxygen species-mediated activation of Rho/ROCK. Arteriosclerosis, thrombosis, and vascular biology, 28, 1760–1766.

Sun, Q., et al., 2009. Ambient air pollution exaggerates adipose inflammation and insulin resistance in a mouse model of diet-induced obesity. Circulation, 119, 538–546.

Suwa, T., et al., 2002. Particulate air pollution induces progression of atherosclerosis. The journal of the American college of cardiology, 39, 935–942.

Tamagawa, E., et al., 2008. Particulate matter exposure induces persistent lung inflammation and endothelial dysfunction. American journal of physiology: Lung cellular and molecular physiology, 295, L79–L85.

Thompson, L.C., et al., 2014a. Pulmonary instillation of multi-walled carbon nanotubes promotes coronary vasoconstriction and exacerbates injury in isolated hearts. Nanotoxicology, 8, 38–49.

Thompson, L.C., et al., 2014b. C60 exposure augments cardiac ischemia/reperfusion injury and coronary artery constriction in Sprague Dawley rats. Toxicological sciences, 138, 365–378.

Tran, C.L., et al., 2000. Inhalation of poorly soluble particles. II. Influence Of particle surface area on inflammation and clearance. Inhalation toxicology, 12, 1113–1126.

Vedal, S., et al., 2013. National Particle Component Toxicity (NPACT) initiative report on cardiovascular effects. Research report health effects institute, 178, 5–8.

Vesterdal, L.K., et al., 2009. Modest vasomotor dysfunction induced by low doses of C60 fullerenes in apolipoprotein E knockout mice with different degree of atherosclerosis. Particle and fibre toxicology, 6, 5.

Vesterdal, L.K., et al., 2010. Pulmonary exposure to carbon black nanoparticles and vascular effects. Particle and fibre toxicology, 7, 33.

Vesterdal, L.K., et al., 2012. Carbon black nanoparticles and vascular dysfunction in cultured endothelial cells and artery segments. Toxicology letters, 214, 19–26.

Vesterdal, L.K., et al., 2014. Pulmonary exposure to particles from diesel exhaust, urban dust or single-walled carbon nanotubes and oxidatively damaged DNA and vascular function inapoE(-/-) mice. Nanotoxicology, 8, 61–71.

Vidanapathirana, A.K., et al., 2014. PVP formulated fullerene (C60) increases Rho-kinase dependent vascular tissue contractility in pregnant Sprague Dawley rats. Reproductive toxicology, 49, 86–100.

Vincent, R., et al., 1997. Acute pulmonary toxicity of urban particulate matter and ozone. The American journal of pathology, 151, 1563–1570.

Vlasova, M.A., et al., 2014. Injected nanoparticles: the combination of experimental systems to assess cardiovascular adverse effects. The European Journal of Pharmaceutics and Biopharmaceutics, 87, 64–72.

Wang, Y., Eliot, M.N. and Wellenius, G.A., 2014. Short-term changes in ambient particulate matter and risk of stroke: a systematic review and meta-analysis. Journal of the American heart association, 3, e000983.

Weldy, C.S., et al., 2013. Glutathione (GSH) and the GSH synthesis gene Gclm modulate plasma redox and vascular responses to acute diesel exhaust inhalation in mice. Inhalation toxicology, 25, 444–454.

Whitman, S.C., 2004. A practical approach to using mice in atherosclerosis research. The clinical biochemist reviews, 25, 81–93.

Xu, X., et al., 2010. Effect of early particulate air pollution exposure on obesity in mice: role of p47phox. Arteriosclerosis, thrombosis, and vascular biology, 30, 2518–2527.

Yanni, A.E., 2004. The laboratory rabbit: an animal model of atherosclerosis research. Laboratory Animal, 38, 246–256.

Yatera, K., et al., 2008. Particulate matter air pollution exposure promotes recruitment of monocytes into atherosclerotic plaques. American journal of physiology: Heart and circulatory physiology, 294, H944–H953.

Ying, Z., et al., 2009a. Ambient particulates alter vascular function through induction of reactive oxygen and nitrogen species. Toxicological sciences, 111, 80–88.

Ying, Z., et al., 2009b. Air pollution and cardiac remodeling: a role for RhoA/Rho-kinase. American journal of physiology: Heart and circulatory physiology, 296, H1540–H1550.

Ying, Z., et al., 2014. Long-term exposure to concentrated ambient PM2.5 increases mouse blood pressure through abnormal activation of the sympathetic nervous system: a role for hypothalamic inflammation. Environmental health perspectives, 122, 79–86.

Ying, Z., et al., 2015. Exposure to concentrated ambient particulate matter induces reversible increase of heart weight in spontaneously hypertensive rats. Particle and fibre toxicology, 12, 15.