Endothelial Dysfunction in Normal and Prediabetic Rats With Metabolic Syndrome Exposed by Oral Gavage to Carbon Black Nanoparticles

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Exposure to nanosized particles may increase the risk of cardiovascular diseases by endothelial dysfunction, particularly in susceptible subjects with metabolic syndrome. We investigated vasomotor dysfunction in aorta from obese and lean Zucker rats after oral exposure to nanosized carbon black (CB). Rats were exposed to 1 or 10 weekly doses of 0, 0.064, 0.64 or 6.4 mg/kg body-weight and sacrificed 24 h or 13 weeks later. The exposure to 10 doses of 0.064 or 0.64 mg/kg reduced the acetylcholine-induced vasorelaxation in the lean and obese rats. The half maximal effect concentration values increased by twofold (95% CI: 1.1–3.5-fold) and fourfold (95% CI: 2.3–6.9-fold) in the rats exposed to 0.064 and 0.64 mg/kg compared with the controls, respectively. The rats exposed to 10 doses of 0.64 mg/kg had also 20% (95% CI: 10–29%) lower maximal effect value compared with the controls. However, the nitroglycerin-induced vasorelaxation and phenylephrine-induced vasoconstriction was not affected in rats exposed to CB. The endothelial dysfunction was not observed in rats sacrificed 13 weeks after the last CB exposure. There was unaltered expression of Chrm3, Nos3, Nos2, Ccl2, and Hmox1 in aorta tissue of CB-exposed rats. In conclusion, repeated oral exposure to CB was associated with endothelial dysfunction in rats, further aggravating the effect of metabolic syndrome.

Key Words: cardiovascular disease; cholesterol; gastrointestinal tract; glucose; insulin; nanoparticles; particulate matter; triglycerides; Zucker rats.

The gastrointestinal tract is an important route of exposure to nanoparticles, although it has been considerably less investigated than pulmonary exposure (Oberdörster et al., 2005). Based on consultation from 35 interested parties, including academia, industry, and national and international agencies, it has been recognized that the risk assessment concerning nanoparticles in food is difficult because of a lack in current knowledge of detection and characterization of nanoparticles in complex matrices such as food (European Food Safety Authority, 2011). We have previously shown that oral exposure to carbon-based particles was associated with oxidative stress and inflammation-related DNA damage and gene expression responses in liver and lung (Danielsen et al., 2008, 2010; Folkmann et al., 2009). Oral exposure to nanoparticles may also lead to risk of cardiovascular dysfunction and possible disease in susceptible populations. Experimental studies in humans and animal models indicate that development of vasomotor dysfunction is an important mechanistic event by which inhalation of particulate matter is associated with cardiovascular disease (Mills et al., 2009; Møller et al., 2011).

Obesity and diabetes appear to convey susceptibility to cardiovascular disease in relation to particulate air pollution as shown in a large cohort study on atherosclerosis progression in women and a panel study of endothelial function (Miller et al., 2007; O’Neill et al., 2005). Mice fed with high-fat diet had increased visceral adiposity, insulin resistance, systemic inflammation, and endothelial dysfunction after whole-body exposure to ambient air PM1,2 for 24 weeks, and results in p47phox−/− mice indicate a role of NADPH oxidase for these effects (Sun et al., 2009; Xu et al., 2010). There is also experimental evidence showing that ApoE−/− mice on high-fat diet had vasomotor dysfunction and accelerated progression of atherosclerosis after inhalation of concentrated air pollution particles, whereas there was less effect in mice of the same strain fed with a normal diet (Sun et al., 2005). However, the ApoE−/− mouse is not considered an experimental model of metabolic syndrome although it is excellent for studies of atherosclerosis. Obese Zucker rats, which suffer from hyperphagia because of lacking leptin receptors, develop a number of abnormalities similar to those seen in humans with metabolic syndrome, including obesity, hyperlipidemia, mild glucose intolerance, and hyperinsulinemia (Alexandre de Artinano and Castro, 2009). The metabolic syndrome is associated with increased risk of cardiovascular diseases and type II diabetes (Eckel et al., 2005).

The purpose of our study was to investigate whether oral exposure to nanosized carbon black (CB) particles cause vasomotor dysfunction particularly in relation to the metabolic...
syndrome. We investigated CB because it is used as a food coloring agent and a remedy with substantial human exposure. In addition, it has been estimated with computer models that 20% and 60% of the inhaled mass of particles are cleared to the stomach after 5 and 200 h, respectively (Sturm, 2007). Recent SPECT/CT imaging studies showed that 8.3–18.7% and 23.3–50.6% of the deposited dose of particles after short-term inhalation was cleared to the stomach in rats and mice, respectively (Kuehl et al., 2012). Similar images of particles in the oropharynx and gut were obtained in humans after inhalation of 0.7-μm size particles (Leach et al., 2012). CB is widely used as reference particle in studies of pulmonary exposure, whereas oral exposure to CB causes hepatic oxidative stress with DNA damage (Danielsen et al., 2010). Moreover, we have previously shown that metabolic exposure to CB had larger effect on vasomotor dysfunction than titanium dioxide, which may be more prevalent in food (Mikkelsen et al., 2011; Vesterdal et al., 2010). A previous study found that exposure of aortic segments from rats with metabolic syndrome had increased vasoconstriction after inhalation of diesel exhaust (Proctor et al., 2008). We have previously demonstrated that diesel exhaust particles or fullerene C60 nanoparticles, injected into the peritoneum of ApoE−/− mice, caused mainly dysfunction of the vasorelaxation response in aorta segments, whereas the vasocostriction response was unaltered (Hansen et al., 2007; Vesterdal et al., 2009). We have, therefore, focused on alterations in the relaxation response of the vessels. The gene expression of cholinergic receptor muscarinic 3 (Chrm3), nitric oxide synthase 3 endothelial cell (NOS3), nitric oxide synthase 2 inducible (NOS2), chemokine (C-C motif) ligand 2 (Ccl2), and heme oxygenase (decycling) 1 (Hmox1) was assessed in aorta tissue as markers of endothelial nitric oxide (NO) production ability, inflammation, and oxidative stress. Hmox1 expression was also measured in the liver.

MATERIALS AND METHODS

Particle exposure of animals. We obtained 72 female obese Zucker rats and 72 age-matched female lean Zucker rats from Charles River (Sulzfeld, Germany). The rats were acclimatized for at least 1 week before entering the experiment. The rats were housed in a temperature-controlled (22°C–24°C) and moisture-controlled (40–70%) room with a 12-h light and 12-h dark cycle. They were housed in pairs receiving the same dose to avoid contamination. All rats had free access to tap water and Standard Altromin no. 1314 rat chow (Altromin, Lage, Germany). The intake of water and food was not monitored in the study. We administered CB to the rats by oral gavage of 500 μl distilled water with or without particles. After the last exposure, the rats were housed in metabolic cages 24 h prior to sacrificing and urine and feces were collected. The rats were anesthetized with Hypnorm/Dormicum (0.3 ml/100 g bodyweight) and blood was drawn from the tip of the tail prior to sacrifice by cervical dislocation.

The rats were allocated randomly to three treatment experiments with the same number of lean and obese Zucker rats. Each group had eight rats at the start of the experiment. We have previously shown that a single oral dose of 0.64 mg/kg bodyweight of CB in Sprague Dawley rats was associated with increased hepatic oxidative stress causing oxidative DNA damage (Danielsen et al., 2010). We used 10-fold dose differences and 10 repeated oral gavages in the present study because it was possible to compare the accumulated doses across different experiments. The largest dose (6.4 mg/kg bodyweight) corresponds to a daily dose of 7.1 mg/kg bodyweight for a human being (70 kg) who ingests one tablet of CB (500 mg per tablet). There appears to be a market for tablets that contain CB—typically labeled as “activated carbon” or “vegetable carbon”—as remedies for weight loss as advertised on the Internet. These tablets can contain about 500 mg CB, although it should be stressed that the labeling on such products is sometimes lacking or insufficient. The Printex 90 is generated by the combustion of fossil fuel that might generate different effects in biological systems than vegetable-based CB, which is a registered food coloring agent in Europe (E153) widely used in black licorices and with no upper limit of acceptable daily intake (http://ec.europa.eu/food/food/FAEF/adjitives/lists_authorised_1A_en.htm).

Experiment 1 consisted of 32 lean Zucker rats and 32 obese Zucker rats that were exposed to a single dose of CB (0.064, 0.64 or 6.4 mg/kg bodyweight) or distilled water for 24 h. The rats were sacrificed at 14 weeks of age.

Experiment 2 consisted of 24 lean Zucker rats and 24 obese Zucker rats that were exposed to 10 doses (one dose/week at the age of 15–24 weeks) of CB (0.064, 0.64 mg/kg bodyweight) or distilled water. The rats were sacrificed at 24 weeks of age.

Experiment 3 consisted of 16 lean Zucker rats and 16 obese Zucker rats that were exposed to 10 doses (one dose/week at the age of 15–24 weeks) of CB (0.64 mg/kg bodyweight) or distilled water. The rats were sacrificed at 37 weeks of age. We sacrificed the rats at 37 weeks of age because we had no prior knowledge about the required recovery period and, therefore, aimed at the longest possible recovery time. This requirement had to be balanced with observations that obese Zucker rats develop renal disease at about 40 weeks of age. The obese Zucker rats in our experiment were beginning to show signs of deprivation, such as reduced fur grooming behavior and their obesity precluded movement.

All animal procedures followed the guidelines for care and handling of laboratory animals established by the Danish government, and the Animal Experiment Inspectorate, Ministry of Justice, approved the study (no. 2006/561-1161). The Printex 90 CB powder (gift from Evonik Degussa, GmbH, Frankfurt, Germany) was declared to have a primary particle size of 14 nm and organic content of approximately 1% according to the manufacturer. We suspended the CB powder in distilled water. It was sonicated on ice using a Branson Sonifier S-450D (Branson Ultrasonics Corp., Danbury, CT) equipped with a disruption horn (model number 101-147-037) for 5 min (55 s pulse on and 5 s pulse off) 1 h before it was administered to the rats. We analyzed the particle size and distribution by nanoparticle tracking analysis, which is based on laser-illuminated microscopy (laser output of 30 MW at 650 nm) where particles are visualized directly in real-time as light-scattering centers moving under Brownian motion (NanoSight Ltd, Amesbury, U.K.). The mean particle size was 104 nm in the solution, whereas the peak (mode) was 86 nm (Supplementary figure S1). The endotoxin level in Printex 90 has been determined by the Pyrogen Gel Clot LAL assay with a sensitivity of 0.06 EU/ml; gel clots were observed at 25 μg/ml corresponding to 4.8 EU/ml (Danielsen et al., 2011). We think it is unlikely that the endotoxin content can affect the vasomotor function because we did not observe pulmonary inflammation in rats after exposure to 0.64 mg/kg of CB (or the same dose of ambient air particles with fivefold more endotoxin content) by intratracheal instillation (Danielsen et al. 2010, 2011). In addition, endotoxin is already highly present in the gastrointestinal tract because of the gut flora and possibly microbial presence in bedding because of nonsterile housing of the animals.

Vasomotor function. We analyzed the vasomotor function immediately after sacrifice of the rats. The heart and the aorta were dissected out and placed in ice-cold oxygenated physiological saline solution (PSS: 119mM NaCl, 25mM NaHCO3, 4.7mM KCl, 1.18mM KH2PO4, 1.17mM MgSO4, 7H2O, 1.5mM CaCl2·2H2O, 0.027mM EDTA, 5.5mM glucose; pH = 7.4). Surrounding connective tissue was carefully removed and the aorta was cut in four segments
of approximately 1.5 mm in length starting immediately after the three large side branches of the aortic arch. The aorta segments were mounted on steel pins with a diameter of 150 µm in the organ baths of the myograph (Multi Channel Myograph 610M; Danish Myo Technology, Aarhus, Denmark) containing 5 ml cold oxygenated PSS continuously perfused with a 95% O₂ and 5% CO₂ gas mixture and pH was kept at 7.4. The myograph was connected to a computer and the data were processed by Myodaq (Danish Myo Technology). The temperature in the organ baths was slowly raised to 37°C and the segments were allowed to equilibrate for 30 min.

A normalization procedure was performed in which the segments were stretched to their optimal lumen diameter (lₒ) in order to ensure optimal development of active tension in the aorta segments, as described previously (Vest- erdal et al., 2009). The optimal lumen diameter was calculated by the Myo- daq program from a standard curve (lₒ = 0.991lₒ) estimated by recording the tonus in the aorta segments although increasing the mechanical stretching in a stepwise manner. The value lₒ was the diameter of the vessel at the physiologic transmural pressure of 13.3 kPa. The vessels were again allowed to equilibrate for 30 min after the normalization procedure. Then, the aorta segments were contracted three times with the buffer solution (KPSS) containing 125mM K⁺ (119mM KCl, 25mM NaHCO₃, 4.7mM KCL, 1.18mM KH₂PO₄, 1.17mM MgSO₄·7H₂O, 1.5mM CaCl₂, 2H₂O, 0.027mM EDTA, 5.5mM glucose; pH = 7.4) separated by four PSS-washouts. This was done in order to verify the viability of aorta segments and the reproducibility of contractions and to deplete the sympathetic nerve endings of neurotransmitters, noradrenaline in particular.

The endothelium-dependent vasorelaxation was tested by first precontracting the segments with prostaglandin F₂α (PGF₂α; Dinolitic vet. 5 mg/ml; Pharmacia NV/SA, Puurs, Belgium) and after reaching a stable plateau adding acetylcholine (Sigma Aldrich Chemie GmbH, Schelldorf, Germany) to the organ bath in a cumulative manner (0.1nM–0.01mM). To examine the endothelium-independent vasorelaxation, the NO donor, nitroglycerine (Glyceryl nitrate 5 mg/ml; Dinolytic vet. 5 mg/ml; Pharmacia NV/SA, Puurs, Belgium) and after reaching a stable plateau adding acetylcholine (Sigma Aldrich Chemie GmbH, Schelldorf, Germany) to the organ bath in a cumulative manner (0.1nM–0.01mM). To examine the endothelium-independent vasorelaxation, the NO donor, nitroglycerine (Glyceryl nitrate 5 mg/ml; Region Hovedstadsen Apoteka, Herlev, Denmark), was added to the organ bath in a cumulative manner (0.1nM–0.03mM) after inducing a stable precontraction in the aorta segments with PGF₂α. In other segments, the effect of blocker of voltage-dependent (L-type) calcium channels in the smooth muscle cells, felodipine was completely blocked from the organ bath.

Tension (Nm⁻¹) curves were recorded using “Myodaq” and specific values of tension were extracted from these curves to a datasheet using “Myodata” (Danish Myo Technology). The relaxation caused by acetylcholine, nitroglycerine, and felodipine was calculated as the percent relaxation of the preconstriction tension produced by PGF₂α or KPSS. The contraction caused by PE was calculated as the percentage of the maximal contraction obtained by the final cocktail solution. The basal tone of the aorta segments was recorded at different time points during the experiments. Data from the cumulative concentration-response curve with acetylcholine, nitroglycerine, and phenylephrine were fitted to sigmoid curves using nonlinear regression analysis for each experiment, and the half maximal effect concentration (EC₅₀) and maximal effect (E₉₀) values were calculated using the GraphPad Prism 5 (GraphPad Software, La Jolla, CA). The estimated pharmacodynamic param-

### RESULTS

#### Vasomotor Function

The treatment with acetylcholine induced a concentration-dependent relaxation in isolated aorta segments (Fig. 1, Supplementary table S1). The young obese Zucker rats had similar
acetylcholine-dependent vasorelaxation as the lean counterparts, whereas the rats in the middle and old age groups had 26% (95% CI: 19–34%) and 36% (95% CI: 26–34%) lower $E_{\text{max}}$ values than the lean rats, respectively. Rats exposed to 10 doses of 0.064 and 0.64 mg/kg had statistically significant right-shift in $EC_{50}$ values, although there was no statistically...
significant difference in the estimated effect between the two types of strain. The dose of 0.064 and 0.64 mg/kg increased the EC50 values by twofold (95% CI: 1.1–3.5-fold) and fourfold (95% CI: 2.3–6.9-fold) compared with the controls, respectively. The dose of 0.64 mg/kg was associated with a twofold (95% CI: 1.1–3.6) higher EC50 value than the dose of 0.064 mg/kg. Furthermore, the rats exposed to 10 doses of 0.64 mg/kg had 20% (95% CI: 10–29%) lower Emax value than

FIG. 2. Nitroglycerine-mediated vasorelaxation in aorta of lean and obese Zucker rats after exposure to CB. The rats were sacrificed 24 h after either a single dose (14 weeks) or 10 doses administered once a week for 10 weeks (24 weeks). The oldest group of rats (37 weeks) was sacrificed 13 weeks after cessation of CB exposure once a week for 10 weeks. Data are mean and SE (n = 5–8). *p < 0.05 compared with EC50 in the lean rats (control group). p < 0.05 compared with Ecmax in the lean rats (control group). EC50 and Emax values are reported in Supplementary table S2.
the controls. There was no statistically significant effect of CB exposure in the groups of rats that were exposed to only one dose or sacrificed 13 weeks after the last of 10 oral gavage doses.

The treatment with nitroglycerine induced a concentration-dependent relaxation in isolated aorta segments (Fig. 2, Supplementary table S2). The obese Zucker rats had decreased nitroglycerin-induced vasorelaxation, which persisted throughout the experiment; this was observed as both rightward shifts in EC50 values and decline in Emax values. However, there was no significant alteration in nitroglycerine-induced effect related to CB exposure.

The assessment of felodipine-mediated vasorelaxation and phenylephrine-mediated vasocontraction responses was unaltered in CB-exposed rats as compared with the unexposed counterparts (Supplementary figures S3 and S4).

**mRNA Expression**

Figure 3 shows the mRNA expressions in aorta tissue of the rats that had been exposed to 10 doses of CB. There was low or no detectable expression level of Nos2, whereas the expression of Ccl2 was not changed significantly by CB, indicating no sign of an inflammatory response in aorta from exposed rats. The expression of Hmox1 was unaltered in the exposed rats, whereas the obese rats had less expression compared with the lean counterparts (p < 0.001). The expressions of Chrm3 and Nos3 were also unaltered in regard to the CB exposure and strain of rats.

Figure 4 shows the mRNA expression level of Hmox1 in the liver of the lean and obese Zucker rats. In the single dose exposure group, there was increased Hmox1 expression in the obese Zucker rats at all doses (p < 0.05), whereas the lean counterparts had unaltered expression of Hmox1. The expression of Hmox1 was unaltered in relation to the CB exposure in the 24-week-old rats, whereas the obese Zucker rats had increased Hmox1 expression level compared with the lean counterparts (p < 0.05).

There was no statistically significant dose-response relationship in the gene expression of Tjp1 and Ocln in colon epithelial cells (Supplementary table S5). However, there was a tendency towards a decrease in the expression in CB-exposed lean rats, whereas the expression tended to increase in the obese rats; the difference in Ocln expression level was statistically significant in the rats exposed to 0.064 (p < 0.05) and 0.64 mg/kg (p < 0.05), whereas it was of borderline statistical significance for the Tjp1 expression level (p = 0.051).

**Metabolic Variables**

The obese and lean Zucker rats gained weight throughout the study; when compared with the lean Zucker rats, obese Zucker

![Graph](image-url)
rats were heavier throughout the study (Supplementary table S6). The exposure to CB was not associated with statistically significant differences in serum concentrations of nonfasting cholesterol and triglycerides (Supplementary table S7), although there could be a modest increase in the triglyceride concentration of the CB-exposed obese Zucker rats that were sacrificed at week 24 (0.64 mg/kg; 1.70-fold, 95% CI: 0.94–3.08, \( p = 0.08 \)). However, the concentration of triglycerides were 7.0 (95% CI: 5.2–9.6), 23.1 (95% CI: 16.5–32.3), and 15.9 (95% CI: 9.7–26.2) fold higher in the obese Zucker rats at the age of 14, 24, and 37 weeks as compared with their lean counterparts, respectively. The concentration of cholesterol in serum was increased by 1.3 (95% CI: 1.2–1.8), 2.3 (95% CI: 1.7–3.1), and 3.4 (95% CI: 2.7–4.3) in the same age groups of rats (Supplementary table S7). In contrast, there was no difference in the serum concentration of nonfasting glucose between the obese and lean Zucker rats (Supplementary table S7). We have considered nonfasting glucose concentrations in serum as normal when below 11mM. Samples from a few rats were higher than this threshold, whereas the means were below. In addition, all urine samples were tested negative for glucose. The serum concentration of insulin had an age-dependent decline in the obese Zucker rats (Supplementary table S8). Young obese Zucker rats displayed 8.2 (95% CI: 6.8–9.8)-fold increase in serum insulin concentration compared with the lean strain, whereas there was 2.8 (95% CI: 2.3–3.5)-fold difference when comparing to the 24-week-old rats, and no statistical difference was observed in comparison with the oldest group of rats.

**DISCUSSION**

This study demonstrates that gastrointestinal exposure to CB impaired the endothelium-dependent vasorelaxation to the same absolute extent in lean and obese Zucker rats, suggesting that particle-elicted vasomotor dysfunction is independent of the metabolic syndrome. Importantly, it required repeated exposures to develop endothelial dysfunction and the effect vanished upon cessation of the exposure. The endothelial dysfunction was not directly linked to alteration in the metabolic parameters in terms of blood lipids, insulin, or nonfasting glucose. We measured the metabolic parameters in nonfasting rats because fasting might affect the vasomotor function response, and the oral glucose fasting test, for assessment of insulin sensitivity, might be affected by CB-mediated damage to glucose carriers in the gastrointestinal epithelium. Obese Zucker rats have unaltered nonfasting glucose concentration in serum, and the age-dependent decreased insulin concentration in the obese Zucker rats suggests insulin resistance, which might be due to exhaustion of pancreatic beta cells and inflammation effects mediated by, for instance, IL-1\( \beta \) (Dinarello et al., 2010). We observed unaltered expression level of Chrm3 and Nos3, indicating that particle-mediated, endothelium-dependent vasorelaxation is not related to a transcriptional downregulation of key components in the acetylcholine-induced NO production. A direct measurement of decreased eNOS-mediated NO production in the endothelial cells would have been optimal, although this is difficult because other sources of NO may affect the measurement. It is possible that the gastrointestinal exposure to CB caused vasomotor dysfunction by mechanisms that involve oxidative stress in the endothelial cells and low-grade inflammation, although we did not detect changes in the Nos2, Ccl2, or Hmox1 expression in
aorta tissue or TNF in serum samples. The CB exposure may not generate oxidative stress in the smooth muscle cells, as suggested by the unaltered vasocontraction and nitroglycerine-mediated vasorelaxation. It has been shown that 16 weeks of inhalation exposure to diesel exhaust particles was associated with increased expression of Hmox1 in aorta of rats, whereas the levels of inflammatory genes were unaltered (Kodavanti et al., 2011). We have found that ApoE−/− mice exposed to a single dose of diesel exhaust particles by intraperitoneal injection had increased level of oxidatively damaged DNA and inflammation indicated by increased expression of Nos2 in the liver as well as endothelial dysfunction in the aorta (Folkmann et al., 2007; Hansen et al., 2007). Moreover, a single oral dose of diesel exhaust particles, single wall carbon nanotubes, or C60 fullerene causes oxidative stress-related DNA damage and gene expression responses in liver and lung (Danielsen et al., 2008; Folkmann et al., 2009). Recently, we have shown that a single oral exposure to CB at a dose of 0.64 mg/kg in wild-type rats also generated oxidatively damaged DNA and inflammation indicated by increased expression of Ccl2 in the liver, whereas the Hmox1 expression was statistically nonsignificantly increased by twofold (Danielsen et al., 2010). There was a similar tendency of increased hepatic Hmox1 expression in the single dose experiment, which reached statistical significance in the obese Zucker rats. The same preparation of CB as used here (Printex 90) generated high levels of reactive oxygen species and oxidatively damaged DNA in cultured cells (Jacobsen et al., 2008) as well as inflammation and DNA damage in the lung of dyslipidemic ApoE−/− mice after pulmonary exposure (Jacobsen et al., 2009). In the same experimental setup, a single intratracheal instillation of CB in the dose range of 0.05–2.7 mg/kg was not associated with vasomotor dysfunction, whereas intratracheal instillations of 0.5 mg/kg on two consecutive days was associated with endothelial dysfunction (Vesterdal et al., 2010). A particle-induced oxidative stress effect on vasomotor function is in keeping with observations that exposure to diesel exhaust was associated with increased basal levels of superoxide anion radicals in coronary arteries and diminished acetylcholine-mediated vasorelaxation (Cheng et al., 2011). The same level of exposure did not alter the endothelium-independent vasorelaxation (Cheng et al., 2009), suggesting that oxidative stress may have stronger effect on NO production in endothelial cells than vascular smooth muscle sensitivity to basal NO.

Humans with metabolic syndrome may at a late stage of their cardiovascular disease be susceptible to myocardial infarction elicited by endothelial dysfunction and/or rupture of atherosclerotic plaques. Data from time-series studies in humans demonstrate that even a small increase in the concentration of ambient air particles is associated with sudden events of cardiovascular diseases (Brook et al., 2010). It is, therefore, possible that nanoparticle-elicited vasomotor dysfunction, superimposed upon the effects of metabolic syndrome, will abolish the remaining vasomotor function, which could elicit sudden cardiovascular events in susceptible individuals. Dysfunctional lipid metabolism and nonalcoholic fatty liver among individuals with metabolic syndrome may contribute to increased susceptibility by cardiac overload that triggers cardiovascular endpoints by exposure to particles. This is supported by observations in mice exposed to CB by inhalation, which showed exacerbated uncoupling of age-dependent Nos3 levels in cardiac myocytes (Tankersley et al., 2008). Studies in humans also indicate that elderly people could be susceptible to vasomotor dysfunction by exposure to particles (Bräuner et al., 2008a,b). Another study in people at risk or having diabetes showed an association between levels of ambient air particles and decreased nitroglycerine-mediated vasodilation, whereas flow-mediated vasodilation was statistically nonsignificantly decreased in the subjects with or without diabetes (O’Neill et al., 2005). However, unlike, for instance, lung cancer, which has a few strong etiological factors, cardiovascular morbidity and mortality are multifactorial diseases where each factor has a low relative risk, whereas the sum of all risk factors can be high. Our observations indicate that oral exposure to nanoparticles may be an independent risk factor for cardiovascular disease, although it needs to be confirmed in controlled exposure studies in humans and animal models related to other susceptibility factors for cardiovascular disease, such as spontaneous hypertensive rats or atherosclerosis-prone ApoE−/− mice.

It has been debated how much of the applied dose of particles is absorbed from the gut. A study on whole-body inhalation exposure to ultrafine 13C particles, generated in an electric spark discharge generator, showed deposition in the liver, which was speculated to originate from gastrointestinal exposure and uptake from the gut (Oberdörster et al., 2002). However, the authors later obtained different results in rats after inhalation of nanosized 192Ir particles and argued that the 13C dose in the former study was about 10% of the naturally occurring endogenous 13C, and the previous results could have been affected by variability in the total content of carbon in the tissues (Kreyling et al., 2009). There are reports showing that less than 1% of the applied dose of polystyrene latex microspheres and 192Ir nanoparticles in the gastrointestinal tract is detected in blood (Kreyling et al., 2002; Semmler et al., 2004). This is in agreement with observations from experiments of pulmonary exposure to nanoparticles that generally indicate only minute translocation to systemic circulation and secondary organs (Geiser and Kreyling, 2010). It is possible that particles in the gut are associated with irritation or damage of the mucosa leading to alteration of the normal barrier function. This could be associated with local inflammation in the mucosa. Interference with digestive enzymes and/or signaling factors in the gut could also be speculated to give rise to dysfunction of the gastrointestinal tract with subsequent development of vasomotor dysfunction. Different particle characteristics and their effect on/or interaction with components in chyle or mucosa cells are important aspects and are still not fully investigated. However, we did not expect that the obese Zucker rats had vasomotor dysfunction because of reduced gastrointestinal
barrier. By the same token, we hypothesized that obese Zucker rats would be more susceptible to particle-elicited vasomotor dysfunction because of metabolic syndrome and not because they would have higher gastrointestinal uptake of particles. This is somewhat confirmed by same expression level of Tip1 and Ocln in the colon of the unexposed rats, whereas the CB exposure suggested that the expression might depend on the strain. The obese Zucker rats develop metabolic syndrome because of hyperphagia, whereas aggravation of the metabolic syndrome in obese Zucker rats would most likely have been due to systemic effects, such as hepatosteatosis, rather than altered function of the gastrointestinal tract.

There are, to the best of our knowledge, no studies on exposure assessment of the oral intake of CB in humans. CB from fossil sources is widely used as a component in rubber, tires, and as pigment in paints and printing inks, but this is probably of limited importance in regard to oral exposure, whereas other combustion particles from fossil sources contaminate food. Indeed, there is clearly paucity of epidemiological studies on health effects of oral exposure to nanoparticles. However, the uncertainty in the current knowledge about the detection and characterization of nanoparticles in foods suggests a strong possibility of nondifferential exposure misclassification in epidemiological studies, which tends to drive relative risk estimates towards zero.

In conclusion, 10 repeated weekly oral exposures to CB nanoparticles caused endothelial dysfunction in lean Zucker rats and a further deterioration of existing vascular dysfunction in obese Zucker rats with metabolic syndrome, whereas this was reversible 13 weeks after the last dose, and single doses had no effects.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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REFERENCES


