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Supplemental Material

Exposure to Fine Particulate Air Pollution Causes Vascular Insulin Resistance by Inducing Pulmonary Oxidative Stress

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Methods

Figure S1: Effects of CAP exposure on adiposity and adipose inflammation. **(A)** Changes in body weight during the 30-day exposure to air or CAP in mice fed control (13% kcal fat) or high fat diet (HFD, 60% kcal fat, Study I). Data are mean \pm SE (* $p < 0.05$ HFD vs. matching controls; # $p < 0.05$ air vs. CAP; $n = 8$). **(B)** Epididymal adipose tissue mass and quantification of **(C)** adipocyte size and **(D)** cells positive for F4/80 (F4/80⁺-cells) and crown-like structures (CLS) in epididymal adipose tissue of control (10% kcal fat) or HFD-fed mice exposed for 30 days to air or CAP (Study II). Epididymal adipose tissue sections were labeled with F4/80 and stained with H&E or fluorescence-labeled with Texas red and DAPI. **(E)** Adipose tissue mRNA levels of tumor necrosis factor- α (TNF- α), macrophage inflammatory protein-1 α (MIP-1 α), monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), interleukin-1 β (IL-1 β), leptin, adiponectin and peroxisome proliferators activated receptor γ (PPAR γ) in control diet or HFD-fed mice exposed for 30 days to air or CAP (Study II). Data are mean \pm SE (* $p < 0.05$ HFD vs. matching controls; # $p < 0.05$ air vs. CAP; $n = 4$).

Figure S2: Cardiovascular effects of CAP exposure. **(A)** Western blot analysis of insulin stimulated Akt phosphorylation in hearts ($n = 5-7$) isolated from control (13% kcal fat) or high fat diet (HFD, 60% kcal fat) fed mice exposed for 9 days to air or CAP (Study III). Insulin-stimulated Akt phosphorylation was examined *ex vivo* in hearts perfused in the Langendorff mode (Wetzelsberger et al. 2010) with saline or insulin (150 mU) for 10min. **(B)** Western blot analysis of insulin-stimulated ERK (p44/p42) phosphorylation ($n = 10$) in aortas isolated from control diet fed mice exposed for 9 days to air or CAP. Data are mean \pm SE (* $p < 0.05$ control vs. insulin; # $p < 0.05$ vs. insulin stimulated air-exposed control group). **(C)** Aortic contractility in response to phenylephrine (PE) and high potassium (HI K^+) or thromboxane A_2 analog (U46,619) in control or HFD-fed mice exposed for 9 (i, $n = 4$) or 30 (ii, $n = 6-8$) days to air or CAP (Study I and III). Data are mean \pm SE (* $p < 0.05$, + $0.10 > p < 0.05$ vs. air-exposed control group).

Figure S3: CAP-induced oxidative stress in lymphocytes and aorta is prevented in lung-specific ecSOD transgene (ecSOD-Tg) mice. **(A)** Flow cytometry analysis of the monochlorobimane (MCB) fluorescence in blood lymphocytes of (i) WT and (ii) ecSOD-Tg mice exposed for 9 days to air or CAP (Study V). Representative flow cytometry plots of side (SSC) and forward (FSC) scatter (left) used to define the lymphocyte cell population for the analysis of MCB-fluorescence. Representative histograms and quantification (right) of the median MCB fluorescence in the gated lymphocytes demonstrate a CAP-induced decrease in cellular GSH in WT but not in ecSOD-Tg mice. Data are mean \pm SE (# $p < 0.05$, air vs. CAP; $n = 8-12$). **(B)** Representative Western blots and densitometric analysis of the abundance protein-acrolein adducts in the aorta isolated from WT (i) and ecSOD-Tg (ii) mice exposed for 9 days to air or CAP. Data are mean \pm SE (# $p < 0.05$, + $p < 0.1$ air vs. CAP; $n = 8$).

Figure S4: Full width I κ B α Western blots shown in Figures 3Aiv and 4B and protein-acrolein adduct Western blots including loading controls (protein, amido black protein stain) shown in Fig. 3C, Fig. 5C and Supplemental Material, Fig. S3B.

Figure S5: Proposed mechanism by which the exposure to PM_{2.5} increases the risk for the development of both CVD and T2D. Inhaled PM_{2.5} particles deposited in the lungs generate reactive oxygen species (ROS, e.g. the formation of superoxide, O₂⁻) and lipid peroxidation products. The induction of pulmonary oxidative stress leads to the generation of a diffusible mediator(s) that transfers the oxidative stress response into peripheral tissues (e.g. blood

lymphocytes and aorta). This triggers the development of inflammation and insulin resistance in blood vessels. The induction of vascular insulin resistance in turn affects cardiovascular processes such as endothelium dysfunction, thrombosis, blood pressure regulation, tissue perfusion and atherogenesis that promote the development of both CVD and diabetes. Treatment with the antioxidant TEMPOL and lung-specific overexpression of ecSOD leads to catalyzed disproportionation of superoxide and prevents PM_{2.5}-induced vascular insulin resistance and inflammation, suggesting that vascular insulin resistance and inflammation are secondary to oxidative stress particularly in the lung.

Table S1: RT-PCR primer.

Table S2: CAP exposure concentrations, enrichment factors, sizes and elemental compositions of the exposures in Study I-V.

Table S3: Effects of CAP exposure for 30 or 9 days on lung inflammation (Study I, III).

Table S4: Mean CAP concentrations (determined by filter weight) of exposures performed from July 2010 to August 2013 (see Fig. 3B).

Table S5: Vascular effects of 9 or 30 days CAP exposure (Study I and III).

Table S6: Effects of CAP exposure for 9 days on lung antioxidant defense.

Table S7: Systemic effects of CAP exposure for 9 days in WT and ecSOD-Tg mice (Study V).

References