ORIGINAL RESEARCH

Carnosine Supplementation Mitigates the Deleterious Effects of Particulate Matter Exposure in Mice

Wesley Abplanalp, PhD; Petra Haberzettl, PhD; Aruni Bhatnagar, PhD; Daniel J. Conklin, PhD; Timothy E. O'Toole, PhD

***Background*—**Exposure to fine airborne particulate matter ($PM_{2.5}$) induces quantitative and qualitative defects in bone marrow–derived endothelial progenitor cells of mice, and similar outcomes in humans may contribute to vascular dysfunction and the cardiovascular morbidity and mortality associated with $PM_{2.5}$ exposure. Nevertheless, mechanisms underlying the pervasive effects of $PM_{2.5}$ are unclear and effective interventional strategies to mitigate against $PM_{2.5}$ toxicity are lacking. Furthermore, whether $PM_{2.5}$ exposure affects other types of bone marrow stem cells leading to additional hematological or immunological dysfunction is not clear.

***Methods and Results*—**Mice given normal drinking water or that supplemented with carnosine, a naturally occurring, nucleophilic di-peptide that binds reactive aldehydes, were exposed to filtered air or concentrated ambient particles. Mice drinking normal water and exposed to concentrated ambient particles demonstrated a depletion of bone marrow hematopoietic stem cells but no change in mesenchymal stem cells. However, HSC depletion was significantly attenuated when the mice were placed on drinking water containing carnosine. Carnosine supplementation also increased the levels of carnosine-propanal conjugates in the urine of CAPs–exposed mice and prevented the concentrated ambient particles–induced dysfunction of endothelial progenitor cells as assessed by in vitro and in vivo assays.

***Conclusions*—**These results suggest that exposure to $PM_{2.5}$ has pervasive effects on different bone marrow stem cell populations and that $PM_{2.5}$-induced hematopoietic stem cells depletion, endothelial progenitor cell dysfunction, and defects in vascular repair can be mitigated by excess carnosine. Carnosine supplementation may be a viable approach for preventing $PM_{2.5}$-induced immune dysfunction and cardiovascular injury in humans. **(*J Am Heart Assoc*. 2019;8:e013041. DOI: 10.1161/JAHA.119.013041.)**

Key Words: air pollution • endothelial progenitor cells • hematopoietic stem cells • ischemia

Extensive epidemiological and laboratory studies show that exposure to air pollution is associated with adverse health outcomes.[1] The World Health Organization estimates that air pollution is linked to 6.5 million deaths per year worldwide and that 92% of the world's population lives in areas where the levels of fine particulate matter ($PM_{2.5}$) exceed the World Health Organization recommended annual mean concentration limit of 10 $\mu g/m^3$. While inhalation exposures are proximally associated with pulmonary disorders, increasing evidence suggests that exposure to $PM_{2.5}$ induces systemic effects and contributes to a wide range of cardiovascular,[2–5] immune,[6–9] and neurological[10–12] disorders as well. One of the unexpected, distal targets of $PM_{2.5}$-induced toxicity is the bone marrow. We and others have shown that exposure to $PM_{2.5}$ impacts bone marrow resident, endothelial progenitor cells (EPCs) in both humans and mice, limiting their inherent functionality[13] and mobilization into peripheral circulation.[14–16] These impairments may contribute to the establishment or maintenance of a dysfunctional endothelium and lend mechanistic insight into the robust association between $PM_{2.5}$ exposure and cardiovascular disease. Similar quantitative or qualitative impairments of bone marrow hematopoietic cells (HSCs) or mesenchymal stem cells (MSCs) could likewise form the underlying basis of $PM_{2.5}$-induced immune response defects or defects in tissue regeneration and repair. While some results using tracheal installation approaches do support the idea that $PM_{2.5}$ can broadly impact stem cell populations,[17,18] a complete understanding of the extent and mechanisms whereby $PM_{2.5}$

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Clinical Perspective

What Is New?

- Exposure to fine particulate matter air pollution ($PM_{2.5}$) has differential effects on discrete populations of bone marrow stem cells in mice.
- The effects of particulate matter air pollution exposure on hematopoietic stem cells and endothelial progenitor cells can be mitigated by supplementation with carnosine.

What Are the Clinical Implications?

- Dietary carnosine supplementation may be a viable approach for preventing particulate matter air pollution–induced immune dysfunction and cardiovascular injury in humans.

inhalation impacts bone marrow stem cell subsets is lacking. Consequently, effective strategies for mitigating the deleterious effects of $PM_{2.5}$ exposure are also lacking.

Current ideas suggest that the systemic effects of $PM_{2.5}$ are secondary to $PM_{2.5}$-induced pulmonary inflammation and oxidative stress, and involve the generation and distribution of oxidatively modified biomolecules. Although the exact chemical identity of these mediators remains elusive, there is evidence to suggest that they are derived from the oxidation of unsaturated lipids,[19] leading to the generation of a wide range of stable, but highly reactive end products such as 4-hydroxy-*trans*-2-nonenal and acrolein. Indeed, exposure to these unsaturated aldehydes is associated with an increase in the risk of cardiovascular disease in humans,[20] by promoting hypertension,[21] arrhythmia,[22] atherogenesis,[23] dyslipidemia,[24] and thrombosis.[25] Thus, lipid peroxidation–derived aldehydes could amplify and prolong the injurious potential of pulmonary reactive oxygen species (ROS), which paradoxically, because of their high reactivity and short half-lives, have low biological toxicity. Therefore, detoxification of highly reactive aldehydes could be an effective strategy in mitigating tissue injury caused by the exposure to $PM_{2.5}$ and other inhaled pollutants.

Reactive aldehydes derived from diet, lipid peroxidation, and other metabolic processes are catalytically detoxified by aldehyde reductases and dehydrogenases as well as glutathione-*S*-transferases.[26] In addition, we have recently described a nonenzymatic pathway for the detoxification of these aldehydes by carnosine.[27] Carnosine is an endogenous dipeptide (β-alanyl-L-histidine) that, because of its high nucleophilicity, binds to and neutralizes aldehydes.[28,29] Carnosine also has metal binding, anti-glycating, and anti-inflammatory activities.[30] Supplementation with carnosine in animals has been previously shown to prevent inflammation[31,32] and promote the removal of lipid peroxidation products and decrease atherosclerotic lesion formation in mice.[33]

In this study, to gain further insight into the systemic outcomes of $PM_{2.5}$ exposure, we characterized effects on discrete bone marrow stem cell subtypes. Furthermore, given that the effects of $PM_{2.5}$ are likely to be mediated in part by a sequence of metal-catalyzed oxidative and pro-inflammatory events, we tested whether supplementation with carnosine would attenuate these effects. We found that while mice exposed to concentrated ambient particles (CAPs) demonstrated no quantitative changes in MSCs, there was a decrease in HSC colony-forming units (CFUs) that was abrogated when CAPs-exposed mice had access to water containing carnosine. Carnosine supplementation also mitigated CAPs-induced defects in EPC function, including those on tube formation and proliferation ex vivo as well as vascular repair in vivo. These results suggest that $PM_{2.5}$ exposure has variable effects on different subgroups of bone marrow stem cells and that a practical intervention (ie, carnosine supplementation) to limit intermediates of oxidative stress could mitigate some of these effects.

Materials and Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Animals, Exposures, and Cell Culture

Male C57BL/6 mice (Jackson Laboratories) at 12 weeks of age were used for exposures. Animals in groups of 5 of identical age and similar weight were exposed to filtered air or CAPs generated from downtown Louisville, KY air by a Versatile Aerosol Concentration and Enrichment System (VACES) operated by the Inhalation Facility at the University of Louisville.[14,16] The VACES enriched ambient levels of air pollution in real time by 7.4±2.4-fold with no changes in elemental composition, and exposures to CAPs were for 6 hours per day continuously for 9 days. For carnosine supplementation, mice were provided with water ad libitum containing carnosine (1 mg/mL) beginning 1 week before the onset of exposure and were maintained on this water throughout the exposure time course. Urine was collected from individual animals for 12 hours before the beginning of exposure and for 12 hours after a single-day, 6-hour exposure. Exposed animals were euthanized immediately after a 6-hour exposure on the final day of a 9-day exposure regimen. Bone marrow cells were flushed from the femurs and tibiae with media and washed with PBS. EPCs were cultured from these aspirates by plating on fibronectin-coated dishes (Corning) in the presence of EGM-2 media (Lonza) with a

change of this media every 48 hours for up to 12 days. All animal procedures were approved by the University of Louisville Institutional Animal Care and Use Committee.

HSC and MSC Assays

To quantify HSC CFUs, flushed bone marrow cells (5×10^3) were plated in Colony Gel (BioReach) and plates were incubated at 37°C. Enumeration of CFUs was performed as per manufacturer's recommendation. To quantify MSCs, 5×10^5 cells were plated out in Mesencult (Stem Cell Technologies) and cultured for 2 weeks under hypoxic conditions (5% O_2, 10% CO_2, 85% N_2). At the end of this time period, cells were harvested, stained with a fluorescently tagged, anti-CD45 antibody (Becton Dickinson), and $CD45^-$ cells were quantified by flow cytometry.

Measurement of Urinary Carnosine-Aldehyde Conjugates

Mouse urine samples were diluted in a solution of 75% acetonitrile:25% water containing 30 nmol/L $^{13}C_9$ carnosine as an internal standard. Samples were separated and aldehyde conjugates were identified using a Waters ACQUITY UPLC H-Class System (BEH HILIC column equipped with an in-line frit filter unit) coupled with a Xevo TQ-S micro triple quadrupole mass spectrometer. The analytes were eluted using a binary solvent system consisting of 10 mmol/L ammonium formate, 0.125% formic acid in 50% acetonitrile:50% water for mobile phase A and 10 mmol/L ammonium formate 0.125% formic acid in 95% acetonitrile:5% water for mobile phase B at a flow rate of 0.55 mL/min. Initial conditions were 0.1:99.9 A:B ramping to 99.9:0.1 A:B over 5 minutes then quickly ramping to 0.1:99.9 A:B over 0.5 minutes. Aldehyde conjugates were quantified using the peak ratio of histidyl-dipeptide and $^{13}C_9$ carnosine internal standard, interpolated using a standard curve and expressed as nmol/mg creatinine.

Tube Formation and Proliferation

After 10 to 12 days of culture, EPCs were harvested with Cell Dissociation Buffer (Thermo Fisher), washed, and aliquots of 1.5×10^4 were layered on top of 50 μL of Matrigel (Corning) in a 96-well plate. Tubes were allowed to form for 4 hours and microscopic images were acquired with an EVOS Cell Imager (Thermo Fisher). The average number of tubes for each condition was determined over 3 individual exposures. To determine proliferative capacity, cultured cells were plated in triplicates of 0.5×10^5 in 24-well tissue culture plates. Cell number was assessed daily over the following 72 hours using the CellQuant dye (Life Technologies) as per the manufacturer's directions. A doubling time was determined using online software (http://www.doubling-time.com/compute_more.php).

Hind Limb Ischemia and Laser Doppler Perfusion Imaging

Revascularization potential was assessed by Laser Doppler Perfusion Imaging (LDPI) in ischemic hind limbs as previously described.[34] To induce hind limb ischemia, mice were anesthetized by isoflurane inhalation (3% isoflurane mixed with 100% O_2) and anesthesia was maintained by continued isoflurane inhalation (1–2% isoflurane). The left groin area was then shaved, a small incision was made through the skin, and the femoral artery and vein were exposed. Sterile 7.0 sutures were threaded gently under and around the femoral artery and vein, and 2 ligatures were knotted (3 mm apart) for permanent ligation and then blood vessels were cut transversely between ligatures. Skin was closed using sterile nylon 6.0 sutures and tissue adhesive sealant. Sham hind limb ischemia surgery was conducted in the same manner, except that the blood vessels were neither ligated nor cut.

To assess the capacity of bone marrow–derived EPCs to promote vascular repair, we injected these cells (5×10^5 total cells isolated and cultured from CAPs- and air-exposed mice) at 4 upper leg sites (10 μL/site) distal to the wound. Two and 3 weeks following surgery, mice were anesthetized as above and LDPI (MoorLDI2; Moor Instruments) was performed to assess blood flow in both ischemic and nonischemic feet and hind limbs. LDPI values scale linearly with the product of red blood cell velocity and the number of blood cells within the tissue, and are listed in arbitrary units. Two images were acquired from every mouse, and analyzed for return of blood flow (recovery) to ischemic leg as a percentage of blood flow in nonischemic leg.

Histology

Mice were euthanized after the final LDPI scan and lower leg skeletal muscle (gastrocnemius) from ischemic limbs was collected, formalin-fixed, and paraffin-embedded. Sections (5 μm) were stained with Sirius Red (0.1%; RA Lamb LLC; Apex, NC) and Fast Green FCF (0.1%; Sigma-Aldrich) to assess fibrosis. Up to 3 of these stained sections were viewed on an Olympus IOM microscope and imaged with a SPOT camera using SPOT advanced image-capture software. Each image was embedded with a SPOT software-generated calibration line (eg, 1000 μm) stamp for subsequent image analysis. Analysis of staining was performed using the National Institutes of Health free software ImageJ, version 1.45s. Each digital photomicrograph (up to 3 different sections per slide) was analyzed as reported.[35]

Statistical Analysis

Data are reported as mean±SE. Repeated-measures ANOVA with interactions was performed to test whether CAPS exposure had an effect on carnosine-propanal levels in mice fed with normal drinking water versus mice supplemented on carnosine. Two-way ANOVA with interactions were performed to test for differences in normal- versus carnosine-fed animals that were exposed to air and CAPS. The least-square means procedure with Tukey adjustment was used to obtain the *P* values for multiple comparisons. All statistical analyses were performed using SAS, version 9.4, software (SAS Institute, Inc, Cary, NC) and GraphPad Prism, version 7 (GraphPad Software, La Jolla, CA). Summary statistics for all figures are presented in Table S1.

Results

CAPs Exposure Differentially Affects Bone Marrow Stem Cell Populations

The bone marrow serves as a reservoir of discrete stem cell populations. While exposure to CAPs induces quantitative changes and qualitative defects of bone marrow EPCs,[13,14] its effects on other stem cell populations is not clear. MSCs are multipotent stromal cells that can terminally differentiate into multiple tissues, including bone, cartilage, muscle, and adipose. To determine whether CAPs exposure affects MSC levels, we exposed mice to filtered air or CAPs and, at termination of exposure, bone marrow cells were isolated and cultured under hypoxic conditions in Mesencult for 14 days. At this time the cells were harvested and $CD45^-$ cells were enumerated by flow cytometry (Figure 1A). Over 3 unique exposures (Figure 1B and 1C), we observed that there were no statistically significant (*P*: 0.185) quantitative differences in the number of MSCs isolated from control, filtered air–exposed mice (1.54±0.08; n=22) and from CAPs-exposed mice (1.42±0.04; n=22).

In addition to MSCs, the bone marrow also contains HSCs, which are the bone marrow precursors of all mature lymphoid and myeloid blood cell lineages. To measure HSC levels in exposed mice, we collected the flushed bone marrow cells and used them in a colony-forming assay (Figure 1D and 1E). When we plated out equal numbers of cells, we observed fewer CFUs from CAPs-exposed mice (13.8±1.0; n=16) than from the filtered air controls (19.3±0.82; n=16) (Figure 1F; *P*: 0.0002). Overall, we observed that CFUs decreased with increasing CAPs levels (Figure 1G), suggesting a dose effect. Thus, while CAPs-exposed mice demonstrated defects in HSC growth and/or differentiation, there were no observable effects on MSCs.

Carnosine Supplementation Neutralizes Aldehydes and Protects From HSC Depletion in CAPs-Exposed Mice

The generation and systemic delivery of oxidatively modified biomolecules (eg, aldehydes) in response to $PM_{2.5}$ inhalation is a proposed mechanism whereby $PM_{2.5}$ impacts cell and tissue function. Carnosine is a naturally occurring, nucleophilic, di-peptide (β-alanyl-L-histidine) that scavenges free radicals and aldehydes.[30] To determine whether excess carnosine could detoxify aldehydes in our experimental protocol, we measured urinary levels of carnosine-propanal adducts (Figure 2A). In mice drinking normal water, we detected low levels of these adducts in urine collected either pre-exposure or postexposure (Figure 2B through 2D). However, we observed that levels of these adducts increased ≈35-fold in pre-exposure mice drinking carnosine-containing water (Figure 2D), indicating that carnosine supplementation facilitates the removal of endogenous aldehydes. After CAPs exposure, adduct levels increased ≈43% in mice drinking carnosine-containing water, consistent with the idea that particle inhalation promotes oxidative stress and aldehyde generation (Figure 2D). In the same exposure protocol, mice drinking normal water had only a 4% increase in carnosine-propanal adducts after CAPs exposure (Figure 2D), indicating that even though lipid-derived aldehyde generation is increased upon CAPs exposure, the extrusion of these aldehydes in the urine is limited by carnosine availability.

Given this observation that carnosine supplementation increases the extrusion of lipid-derived aldehydes, we next determined whether carnosine supplementation had protective effects on HSC depletion (Figure 3A). Consistent with our earlier observation, mice drinking normal water and exposed to CAPs demonstrated a reduction of HSC colonies (0.65±0.04; n=7) compared with control mice breathing filtered air (1.0±0.07; n=9) (Figure 3B and 3C). However, when mice were placed on carnosine-containing water, there was no statistically significant loss of HSCs in the CAPs-exposed group (1.10±0.17; n=9) versus the filtered air–exposed group (1.0±0.09; n=9) (Figure 3B and 3C). Thus, carnosine supplementation limits the quantitative effects of CAPs exposure on bone marrow HSCs.

Carnosine Supplementation Mitigates the Effects of CAPs on EPC Function Ex Vivo

We have previously reported that CAPs inhalation induces EPC dysfunction.[13] Hence, we next determined whether carnosine supplementation could protect against these adverse outcomes (Figure 4A). As previously reported, we found that EPCs isolated and cultured from the bone marrow of CAPs-

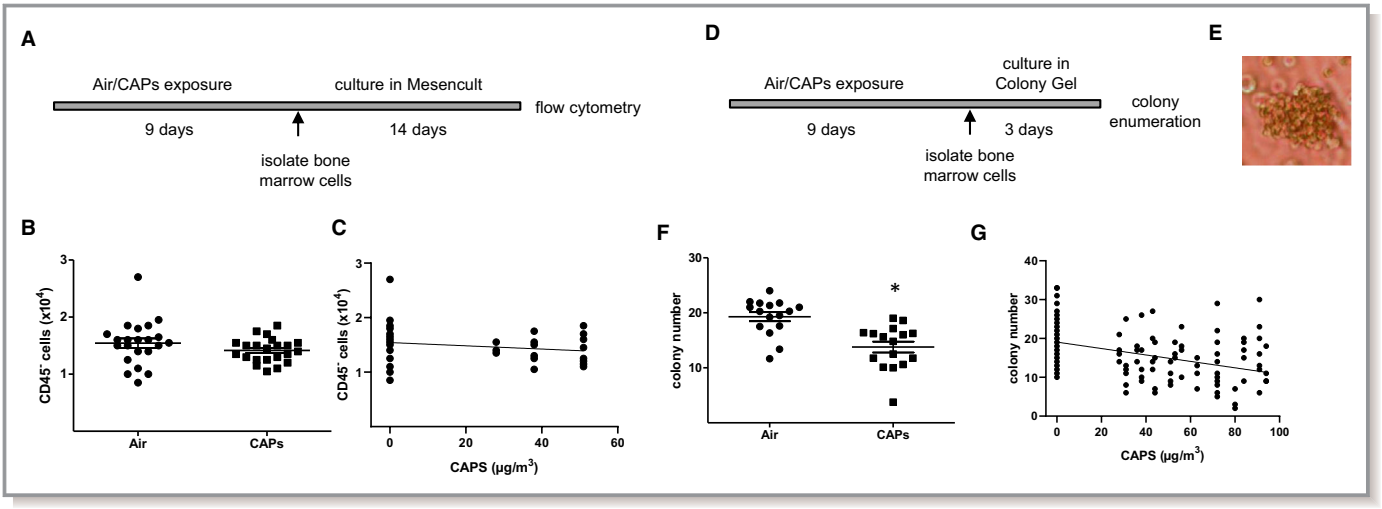

Figure 1. Quantitative impact of CAPs exposure on mesenchymal stem cells and hematopoietic stem cell colony formation. Mice exposed to filtered air or CAPs for 9 days were euthanized and bone marrow cells were collected. MSCs in these isolates were quantified after culture in Mesencult and flow cytometry (**A**). Illustrated are the cumulative results (**B**: n=22 mice) and actual MSC numbers in 3 separate exposures of different CAPs levels (**C**; β: −0.0030; *R*2: 0.041; *P*: 0.180). From 16 exposures of different CAPs levels, HSCs were quantified in a colony-forming assay (**D**). Illustrated is a representative colony (**E**) and the cumulative results (**F**; **P*<0.05). Also illustrated (**G**; β: −0.0827; *R*2: 0.177; *P*<0.001) are actual colony counts in the filtered air (n=70 mice over 16 exposures) and CAPS-exposed groups (n=4–7 mice per exposure). Levels of MSCs (**C**) and HSC colonies (**G**) in control mice inhaling filtered air are depicted at the 0 $\mu g/m^3$ concentration. CAPs indicates concentrated ambient particles; HSCs, hematopoietic cells; MSCs, mesenchymal stem cells.

exposed mice had a slower rate of proliferation (longer doubling time) (2.16±0.50; n=5) compared with those cells isolated from mice breathing filtered air (1.0±0.0; n=5). However, when we performed these experiments using cells isolated from CAPs-exposed mice drinking carnosine-containing water, we observed no defect in proliferation versus the filtered air controls (0.90±0.22; n=5 versus 1.10±0.17; n=4) (Figure 4B).

We also analyzed EPC function using a tube-forming assay, which is indicative of angiogenic capacity. We found that even though EPCs isolated from CAPs-exposed mice drinking normal water were impaired in forming tubes versus mice breathing filtered air (0.43±0.14 versus 1.01±0.14: n=5, 7, respectively), EPCs from CAPs-exposed mice drinking carnosine-containing water had a tube-forming capacity similar to those mice breathing filtered air (1.12±0.16 versus 1.00±0.06: n=7) (Figure 4C and 4D). Taken together, these observations suggest that carnosine supplementation protects from CAPs-induced EPC dysfunction ex vivo.

Carnosine Supplementation Mitigates the Effects of CAPs on EPC Function In Vivo

We have previously shown that EPCs isolated from CAPs-exposed mice demonstrated an impaired capacity for vascular repair in vivo.[13] To determine whether carnosine supplementation could mitigate these effects, we isolated and cultured EPCs from CAPs-exposed animals and injected them into naïve mice subjected to hind limb ischemia. The recovery of vascular perfusion in these treated mice was assessed after 3 weeks by LDPI (Figure 5A). We observed that mice receiving cells isolated from CAPs-exposed animals drinking carnosine-containing water exhibited a 66.93±1.43% recovery (n=20), whereas those mice receiving cells isolated from CAPs-exposed animals drinking normal water exhibited only a 48.11±2.40% recovery (n=24) (Figure 5B and 5C). Furthermore, those mice receiving cells from CAPs-exposed animals placed on carnosine-containing water exhibited enhanced tissue repair, as evidenced by a greater deposition of collagen fibers, compared with mice receiving cells from CAPs-exposed animals drinking normal water (7.32±0.70 versus 1.00±0.22, respectively) (Figure 5D and 5E) These observations suggest that carnosine protects against CAPs-induced EPC dysfunction as these cells retain their competence in promoting tissue repair in vivo.

Discussion

In this work we show that acute exposure to CAPs has differential effects on bone marrow stem cell populations in mice. While exposure had no effect on MSC levels, there was a depletion of HSC CFUs. Together with our previous work showing that CAPs induces an accumulation of EPCs in the bone marrow,[14,16] these results provide evidence that exposure to $PM_{2.5}$, initially and primarily a lung insult, can

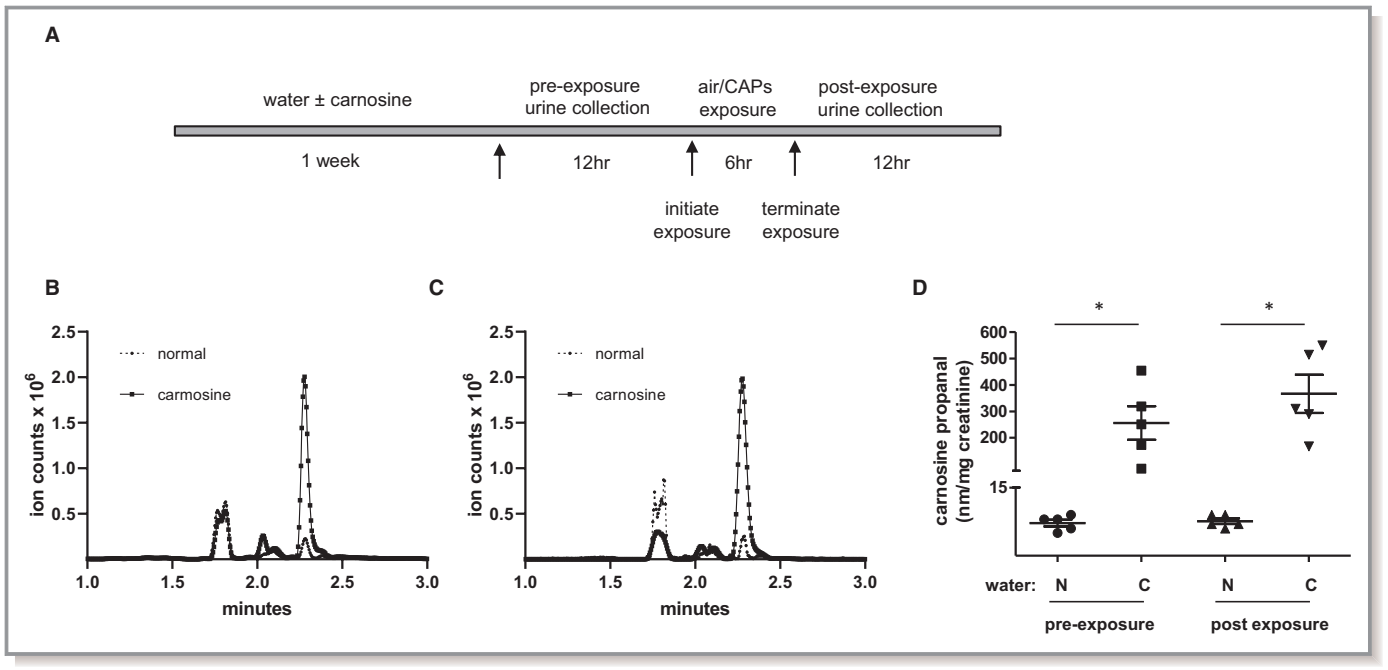

Figure 2. CAPs exposure increases urinary levels of carnosine-propanal. Illustrated is the experimental outline (**A**). Mice were placed on normal drinking water or water supplemented with 1 mg/mL carnosine for 1 week before exposure to filtered air or CAPs. Urine was then collected for 12 hours before exposure and for 12 hours after a single-day, 6-hour exposure. Levels of carnosine-propanal in the urine were measured as described in Methods and normalized to urinary creatinine. Illustrated are representative LC-MS/MS ionograms obtained from pre-exposure urine (**B**) and postexposure urine (**C**), where carnosine propanal elutes at ≈2.3 minutes. Also illustrated are the cumulative results (**D**). n=5; **P*<0.05. C indicates carnosine-containing water; N, normal water; LC-MS/MS, liquid chromatography-massspectrometry.

impact distal tissues, including the bone marrow. Moreover, the pervasive effects of $PM_{2.5}$ exposure on discrete stem cell populations are indicative of the complex and sometimes cell-type-specific physiological responses to $PM_{2.5}$ exposure. These findings add a new facet to the pathophysiology of $PM_{2.5}$ exposures and provide new insights on how such exposures could affect tissue capacity for repair and regeneration.

The mechanistic basis whereby $PM_{2.5}$ inhalation impacts bone marrow cells is uncertain. Some proposed mechanisms for the pathophysiological outcomes resulting from inhalation exposures ascribe this to an altered redox balance with excessive generation of ROS.[36,37] Thus, oxidized biomolecules generated in the lungs are distributed through peripheral circulation and induce systemic effects. The view that ROS mediate $PM_{2.5}$-induced toxicity is supported by our recent studies showing that limiting ROS production at its source, through pulmonary overexpression of extracellular superoxide dismutase, not only alleviates oxidative stress in distal cells and tissues, but also mitigates the $PM_{2.5}$-induced dysfunction of bone marrow–derived EPCs.[13] Although the identity of these putative oxidized biomolecules remains unclear, increased levels of discrete, oxidized phospholipids have been found in bronchoalveolar lavage fluid of $PM_{2.5}$-exposed animals.[19] The concept that inhaled toxins generate systemic signaling intermediates is also consistent with the finding that serum or plasma isolated from exposed animals or humans influences naïve, unexposed cells and tissues.[38,39] Even though ROS themselves seem to support HSC growth and differentiation,[40] certain oxidized phospholipids (eg, 4-hydroxynonenal)[41,42] or aldehydes generated by oxidative stress or impaired metabolism[43] may be important mediators of $PM_{2.5}$ toxicity. This is consistent with our finding that carnosine both formed adducts with, and neutralized aldehydes, and reversed the impact of $PM_{2.5}$ on HSC levels. Related studies using a model of particle tracheal installation also show that limiting oxidative stress reverses a quantitative deficit of bone marrow Lin^-/Sca^+ and $Lin^-/CD133^+$ cells induced by exposure.[17] While our results suggested that CAPs inhalation did not affect MSC levels, the direct application of particles to MSCs in vitro has been reported to induce membrane damage, as well as cell shrinkage and death.[44] The lack of in vivo effects in our study could be because of the inaccessibility of $PM_{2.5}$ particles to bone marrow cell populations and further supports the idea that $PM_{2.5}$-mediated effects are caused by oxidative or inflammatory events initiated in the lung.

The $PM_{2.5}$-induced loss of HSCs provides possible mechanistic insight into the correlation between exposure and

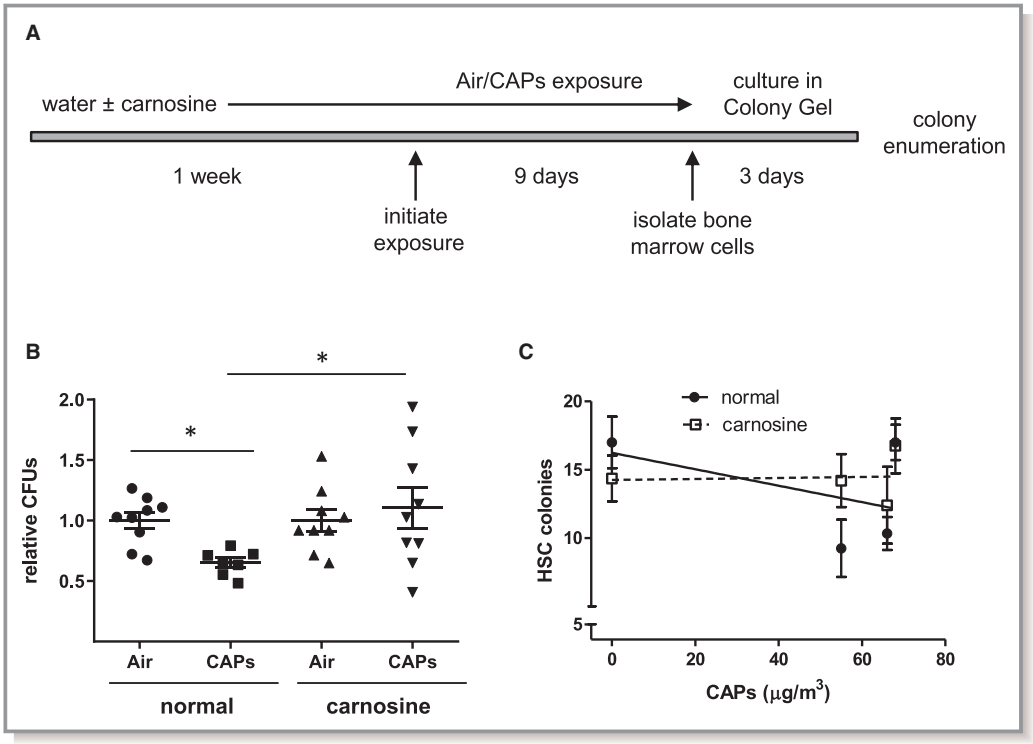

Figure 3. Carnosine supplementation limits HSC depletion. Illustrated is the experimental outline (**A**). Mice were placed on normal drinking water or that supplemented with 1 mg/mL carnosine for 1 week before exposure and then exposed to filtered air or CAPs for 9 days. Bone marrow cells were then isolated and HSC CFUs were quantified after culture in Colony Gel. Illustrated are the cumulative results (**B**; n=7–9 mice) and the average CFUs in 3 independent exposures (**C**). The average number of HSC colonies in control mice inhaling filtered air is depicted at the 0 μg/mL concentration (**C**). CAPs indicates concentrated ambient particles; CFUs, colony-forming units; HSCs, hematopoietic cells.

immune response disorders. There is a strong association between exposure to air toxins and the development of autoimmune disorders,[6,45] asthma,[46,47] and respiratory[48] or systemic infections.[48,49] Furthermore, several studies have shown that prior exposure to $PM_{2.5}$ impairs subsequent antibacterial and antiviral responses in exposed animals[7,9,50,51] and that this may be attributable to defective granulocyte or natural killer cell function.[9,51] Cell-based immunity is dependent upon the generation and maturation of functional myeloid and lymphoid cell populations that derive from bone marrow HSCs. Thus, individuals chronically exposed to $PM_{2.5}$ may have a persistent loss of HSCs and defects in the production of immune cell populations. As with exposures to $PM_{2.5}$, cigarette smoking has also been associated with impaired immune responses and an increased incidence of infection.[52] These outcomes may likewise reflect the effects of cigarette toxins (eg, aldehydes) on HSC viability.[53,54] Limitations on HSC growth and defects in hematopoiesis may thus be a general mechanism underlying impaired immune responses upon exposure to several inhaled toxins.

In addition to the protective effects of carnosine on HSCs, we also observed that bone marrow EPCs derived from CAPs-exposed, carnosine-supplemented mice demonstrated normal tube-forming capacity and growth ex vivo and were effective in restoring vascular perfusion in vivo. Thus, a second major finding of this work is that a practical intervention (ie, dietary carnosine supplementation) can limit $PM_{2.5}$-induced pathology. As an endogenous nucleophile, carnosine acts as an anti-oxidant by sequestering hydroxyl radicals. It can also directly scavenge reactive, unsaturated aldehydes,[30] and in this mode of action, may directly protect EPCs from aldehyde-induced dysfunction. Thus, by removing or neutralizing aldehydes generated from oxidized lipids, carnosine may directly protect bone marrow cells from $PM_{2.5}$-induced dysfunction. In addition to aldehyde quenching, carnosine also has buffering and antiglycating capacity, chelates metals, and, stimulates NO synthesis.[30] In this latter mode of action, carnosine supplementation could potentially protect from HSC loss,[55] and promote NO-dependent EPC function.[56,57] We have previously shown similar protective effects when using ecSOD-Tg mice in exposure experiments,[13] further supporting the idea that pulmonary-initiated oxidative stress and downstream reactive intermediates (eg, aldehydes) play a causative role in $PM_{2.5}$-induced pathogenesis.

Previous attempts to mitigate $PM_{2.5}$-induced pathologies have focused on reducing oxidative stress. In addition to our

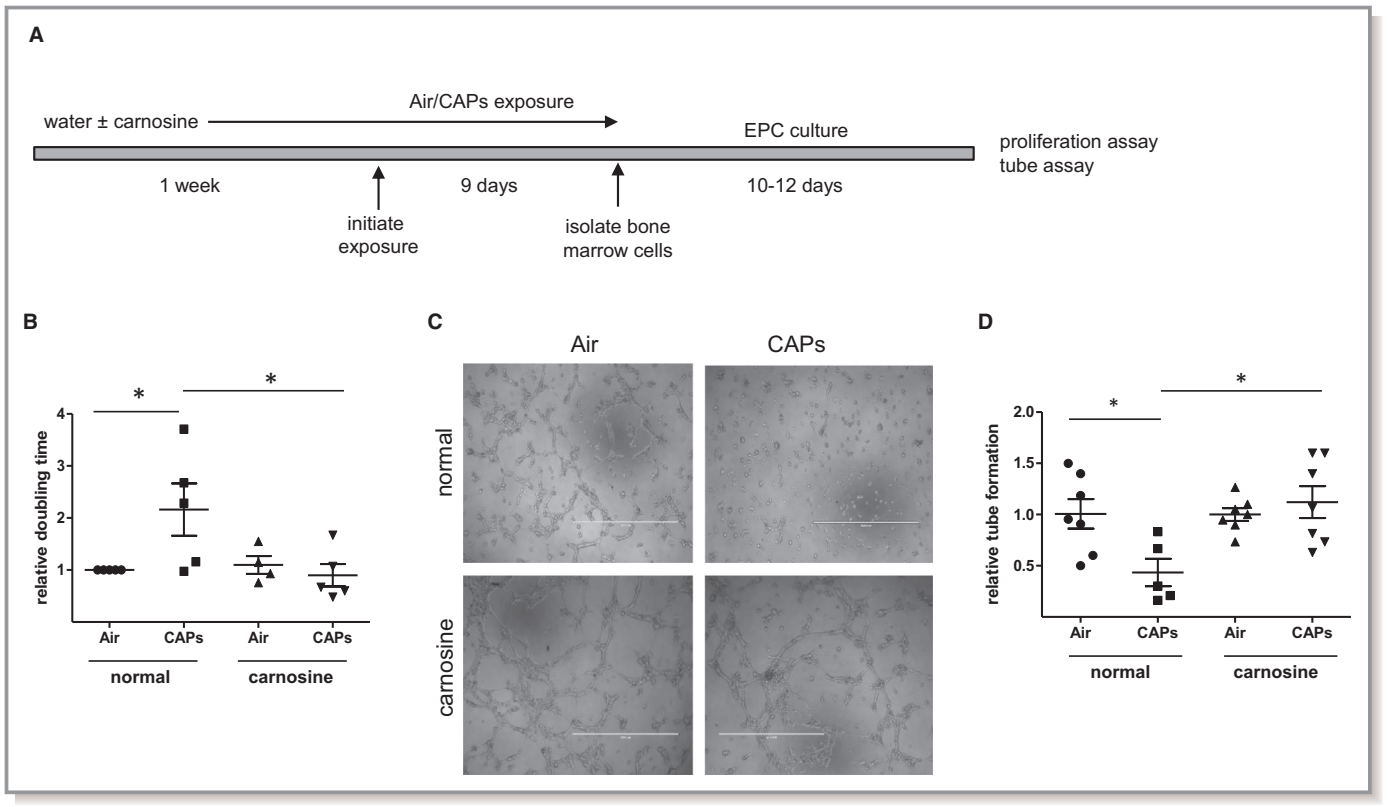

Figure 4. Carnosine supplementation attenuates CAPs-induced EPC dysfunction ex vivo. Illustrated is the experimental outline (**A**). Mice were placed on normal drinking water or that supplemented with 1 mg/mL carnosine for 1 week before exposure and then exposed to filtered air or CAPs for 9 days. Bone marrow cells were isolated and EPCs were cultured for 10 to 12 days and then used in functional assays. **B,** Illustrated are the relative doubling times of these cells normalized to filtered air controls. n=4 to 5; **P*≤0.05. The cultured EPCs were also used in a tube formation assay. Illustrated are representative images (**C**) and quantitative data (**D**). n=5 to 7; **P*<0.05. CAPs indicates concentrated ambient particles; EPCs, endothelial progenitor cells.

exposure studies with ecSOD-Tg mice,[13] treatment with nonspecific antioxidants[58,59] or inhibition of NADPH oxidase or Rho kinase[60] prevent some of the adverse outcomes associated with exposure to $PM_{2.5}$ or diesel exhaust particles. However, other anti-oxidant interventional studies in humans have yielded uncertain and somewhat limited results.[61–63] Reasons for these discrepant results in humans are unclear, but may relate to the fact that low-dose ROS can actually be protective and, therefore, systemically active antioxidants not only alleviate ROS toxicity, but also inhibit the protective, adaptive, and growth-promoting effects of ROS as well. Moreover, there are multiple limitations precluding the practical use of anti-oxidant interventions in humans. Some strategies such as the use of NADPH oxidase and Rho kinase inhibitors, or ecSOD overexpression are experimental animal approaches that cannot be effectively or directly translated to use in humans. Therefore, the ability of carnosine to prevent vascular deficits caused by $PM_{2.5}$ exposure in a preclinical model suggests its potential utility as a practical, therapeutic intervention for humans who are routinely exposed to high levels of $PM_{2.5}$. Given that carnosine is well tolerated by humans and has been used extensively as a dietary supplement, these findings strengthen the rationale for conducting large-scale interventional studies to prophylactically decrease the risk of immune dysfunction and cardiovascular injury imparted by exposure to atmospheres containing high levels of $PM_{2.5}$.

In summary, the findings of this study suggest that inhalation of $PM_{2.5}$ decreases the levels of HSCs in the bone marrow and that this is associated with increased oxidative stress, as measured by an increase in aldehyde–carnosine conjugates. Pretreatment with carnosine, an endogenous nucleophile, expressed abundantly in skeletal muscle, heart, and brain, prevents not only the depletion of HSCs, but also ameliorates $PM_{2.5}$-induced EPC dysfunction as well. Significantly, we found that oral supplementation with carnosine attenuates $PM_{2.5}$-induced defects in EPC-mediated vascular reperfusion and tissue repair. Although we cannot account for the amount of carnosine-containing water any individual mouse consumes, the carnosine supplementation group as a whole demonstrated protection from the effects of CAPs exposure. Taken together, these observations provide new evidence suggesting a key role

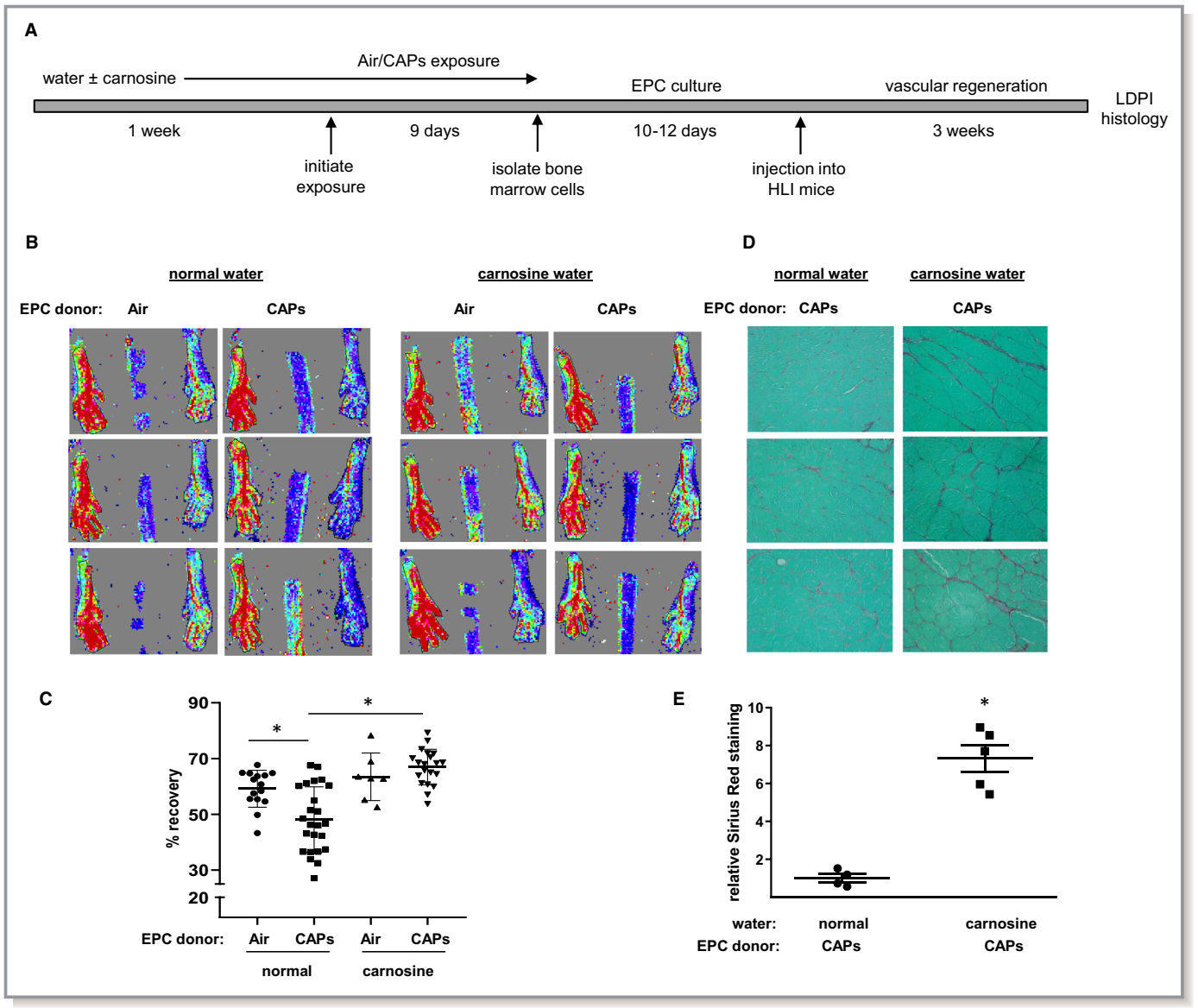

Figure 5. Carnosine supplementation attenuates CAPs-induced defects in EPC-mediated vascular reperfusion and tissue repair. **A**, Illustrated is the experimental outline. Mice were placed on normal drinking water or that supplemented with 1 mg/mL carnosine for 1 week before exposure and then exposed to filtered air or CAPs for 9 days. Bone marrow cells were isolated and EPCs cultured for 10 to 12 days and then injected into naïve mice that were subjected to HLI. Recovery of vascular perfusion in the injured limb was examined after 3 weeks by LDPI. Illustrated are representative LDPI images (**B**). The percent recoveries of vascular perfusion in HLI mice receiving cells from the various donors is also illustrated (**C**). n=7 to 24; **P*<0.05. Isolated muscle sections from HLI mice injected with cells from the CAPs-exposed donors were also stained with Sirius Red. Illustrated are representative images (**D**) and the relative staining intensity (**E**); n=4 to 5; **P*<0.05. CAPs indicates concentrated ambient particles; EPCs, endothelial progenitor cells; HLI, hind limb ischemia; LDPI, Laser Doppler Perfusion Imaging.

of alterations in bone marrow cell populations in the pathophysiological changes induced by inhalation of $PM_{2.5}$, and provide a simple, safe, and effective intervention to mitigate against these potentially toxic effects of $PM_{2.5}$.

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Disclosures

None.

References

1. Chen H, Goldberg MS, Villeneuve PJ. A systematic review of the relation between long-term exposure to ambient air pollution and chronic diseases. *Rev Environ Health*. 2008;23:243–297.
2. Araujo JA, Barajas B, Kleinman M, Wang X, Bennett BJ, Gong KW, Navab M, Harkema J, Sioutas C, Lusis AJ, Nel AE. Ambient particulate pollutants in the ultrafine range promote early atherosclerosis and systemic oxidative stress. *Circ Res*. 2008;102:589–596.
3. Bhatnagar A. Environmental cardiology: studying mechanistic links between pollution and heart disease. *Circ Res*. 2006;99:692–705.
4. Pope CA III, Burnett RT, Thurston GD, Thun MJ, Calle EE, Krewski D, Godleski JJ. Cardiovascular mortality and long-term exposure to particulate air pollution: epidemiological evidence of general pathophysiological pathways of disease. *Circulation*. 2004;109:71–77.
5. Sun Q, Wang A, Jin X, Natanzon A, Duquaine D, Brook RD, Aguinaldo JG, Fayad ZA, Fuster V, Lippmann M, Chen LC, Rajagopalan S. Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. *JAMA*. 2005;294:3003–3010.
6. Bernatsky S, Fournier M, Pineau CA, Clarke AE, Vinet E, Smargiassi A. Associations between ambient fine particulate levels and disease activity in patients with systemic lupus erythematosus (SLE). *Environ Health Perspect*. 2011;119:45–49.
7. Harrod KS, Jaramillo RJ, Rosenberger CL, Wang SZ, Berger JA, McDonald JD, Reed MD. Increased susceptibility to RSV infection by exposure to inhaled diesel engine emissions. *Am J Respir Cell Mol Biol*. 2003;28:451–463.
8. Psoter KJ, De Roos AJ, Mayer JD, Kaufman JD, Wakefield J, Rosenfeld M. Fine particulate matter exposure and initial *Pseudomonas aeruginosa* acquisition in cystic fibrosis. *Ann Am Thorac Soc*. 2015;12:385–391.
9. Sigaud S, Goldsmith CA, Zhou H, Yang Z, Fedulov A, Imrich A, Kobzik L. Air pollution particles diminish bacterial clearance in the primed lungs of mice. *Toxicol Appl Pharmacol*. 2007;223:1–9.
10. Calderon-Garciduenas L, Calderon-Garciduenas A, Torres-Jardon R, Avila-Ramirez J, Kulesza RJ, Angiulli AD. Air pollution and your brain: what do you need to know right now. *Prim Health Care Res Dev*. 2015;16:329–345.
11. Palacios N. Air pollution and Parkinson's disease—evidence and future directions. *Rev Environ Health*. 2017;32:303–313.
12. Peters R, Ee N, Peters J, Booth A, Mudway I, Anstey KJ. Air pollution and dementia: a systematic review. *J Alzheimers Dis*. 2019;70. Available at: https://content.iospress.com/articles/journal-of-alzheimers-disease/jad180631. Accessed June 18, 2019.
13. Haberzettl P, Conklin DJ, Abplanalp WT, Bhatnagar A, O'Toole TE. Inhalation of fine particulate matter impairs endothelial progenitor cell function via pulmonary oxidative stress. *Arterioscler Thromb Vasc Biol*. 2018;38:131–142.
14. Haberzettl P, Lee J, Duggineni D, McCracken J, Bolanowski D, O'Toole TE, Bhatnagar A, Conklin DJ. Exposure to ambient air fine particulate matter prevents VEGF-induced mobilization of endothelial progenitor cells from the bone marrow. *Environ Health Perspect*. 2012;120:848–856.
15. Niu J, Liberda EN, Qu S, Guo X, Li X, Zhang J, Meng J, Yan B, Li N, Zhong M, Ito K, Wildman R, Liu H, Chen LC, Qu Q. The role of metal components in the cardiovascular effects of PM2.5. *PLoS One*. 2013;8:e83782.
16. O'Toole TE, Hellmann J, Wheat L, Haberzettl P, Lee J, Conklin DJ, Bhatnagar A, Pope CA III. Episodic exposure to fine particulate air pollution decreases circulating levels of endothelial progenitor cells. *Circ Res*. 2010;107:200–203.
17. Cui Y, Jia F, He J, Xie X, Li Z, Fu M, Hao H, Liu Y, Liu DZ, Cowan PJ, Zhu H, Sun Q, Liu Z. Ambient fine particulate matter suppresses in vivo proliferation of bone marrow stem cells through reactive oxygen species formation. *PLoS One*. 2015;10:e0127309.
18. Cui Y, Xie X, Jia F, He J, Li Z, Fu M, Hao H, Liu Y, Liu JZ, Cowan PJ, Zhu H, Sun Q, Liu Z. Ambient fine particulate matter induces apoptosis of endothelial progenitor cells through reactive oxygen species formation. *Cell Physiol Biochem*. 2015;35:353–363.
19. Kampfrath T, Maiseyeu A, Ying Z, Shah Z, Deiuliis JA, Xu X, Kherada N, Brook RD, Reddy KM, Padture NP, Parthasarathy S, Chen LC, Moffatt-Bruce S, Sun Q, Morawietz H, Rajagopalan S. Chronic fine particulate matter exposure induces systemic vascular dysfunction via NADPH oxidase and TLR4 pathways. *Circ Res*. 2011;108:716–726.
20. DeJarnett N, Conklin DJ, Riggs DW, Myers JA, O'Toole TE, Hamzeh I, Wagner S, Chugh A, Ramos KS, Srivastava S, Higdon D, Tollerud DJ, DeFilippis A, Becher C, Wyatt B, McCracken J, Abplanalp W, Rai SN, Ciszewski T, Xie Z, Yeager R, Prabhu SD, Bhatnagar A. Acrolein exposure is associated with increased cardiovascular disease risk. *J Am Heart Assoc*. 2014;3:e000934. DOI: 10.1161/JAHA.114.000934.
21. Feron VJ, Til HP, de Vrijer F, Woutersen RA, Cassee FR, van Bladeren PJ. Aldehydes: occurrence, carcinogenic potential, mechanism of action and risk assessment. *Mutat Res*. 1991;259:363–385.
22. Bhatnagar A. Electrophysiological effects of 4-hydroxynonenal, an aldehydic product of lipid peroxidation, on isolated rat ventricular myocytes. *Circ Res*. 1995;76:293–304.
23. Srivastava S, Sithu SD, Vladykovskaya E, Haberzettl P, Hoetker DJ, Siddiqui MA, Conklin DJ, D'Souza SE, Bhatnagar A. Oral exposure to acrolein exacerbates atherosclerosis in apoE-null mice. *Atherosclerosis*. 2011;215:301–308.
24. Conklin DJ, Barski OA, Lesgards JF, Juvan P, Rezen T, Rozman D, Prough RA, Vladykovskaya E, Liu S, Srivastava S, Bhatnagar A. Acrolein consumption induces systemic dyslipidemia and lipoprotein modification. *Toxicol Appl Pharmacol*. 2010;243:1–12.
25. Sithu SD, Srivastava S, Siddiqui MA, Vladykovskaya E, Riggs DW, Conklin DJ, Haberzettl P, O'Toole TE, Bhatnagar A, D'Souza SE. Exposure to acrolein by inhalation causes platelet activation. *Toxicol Appl Pharmacol*. 2010;248:100–110.
26. Srivastava S, Spite M, Trent JO, West MB, Ahmed Y, Bhatnagar A. Aldose reductase-catalyzed reduction of aldehyde phospholipids. *J Biol Chem*. 2004;279:53395–53406.
27. Zhao J, Posa DK, Kumar V, Hoetker D, Kumar A, Ganesan S, Riggs DW, Bhatnagar A, Wempe MF, Baba SP. Carnosine protects cardiac myocytes against lipid peroxidation products. *Amino Acids*. 2019;51:128–138.
28. Baba SP, Hoetker JD, Merchant M, Klein JB, Cai J, Barski OA, Conklin DJ, Bhatnagar A. Role of aldose reductase in the metabolism and detoxification of carnosine-acrolein conjugates. *J Biol Chem*. 2013;288:28163–28179.
29. Orioli M, Aldini G, Benfatto MC, Facino RM, Carini M. HNE Michael adducts to histidine and histidine-containing peptides as biomarkers of lipid-derived carbonyl stress in urines: LC-MS/MS profiling in Zucker obese rats. *Anal Chem*. 2007;79:9174–9184.
30. Boldyrev AA, Aldini G, Derave W. Physiology and pathophysiology of carnosine. *Physiol Rev*. 2013;93:1803–1845.
31. Rashid I, van Reyk DM, Davies MJ. Carnosine and its constituents inhibit glycation of low-density lipoproteins that promotes foam cell formation in vitro. *FEBS Lett*. 2007;581:1067–1070.
32. Tanaka KI, Sugizaki T, Kanda Y, Tamura F, Niino T, Kawahara M. Preventive effects of carnosine on lipopolysaccharide-induced lung injury. *Sci Rep*. 2017;7:42813.
33. Barski OA, Xie ZZ, Baba SP, Sithu SD, Agarwal A, Cai J, Bhatnagar A, Srivastava S. Dietary carnosine prevents early atherosclerotic lesion formation in apolipoprotein E-null mice. *Arterioscler Thromb Vasc Biol*. 2013;33:1162–1170.
34. Abplanalp WT, Conklin DJ, Cantor JM, Ginsberg MH, Wysoczynski M, Bhatnagar A, O'Toole TE. Enhanced integrin alpha4beta1-mediated adhesion contributes to a mobilization defect of endothelial progenitor cells in diabetes. *Diabetes*. 2016;65:3505–3515.
35. Conklin DJ, Kong M; Committee HEIHR. Part 4. Assessment of plasma markers and cardiovascular responses in rats after chronic exposure to new-technology diesel exhaust in the aces bioassay. *Res Rep Health Eff Inst*. 2015;184:111–139; discussion 141–171.
36. Deng X, Zhang F, Wang L, Rui W, Long F, Zhao Y, Chen D, Ding W. Airborne fine particulate matter induces multiple cell death pathways in human lung epithelial cells. *Apoptosis*. 2014;19:1099–1112.
37. Ying Z, Kampfrath T, Thurston G, Farrar B, Lippmann M, Wang A, Sun Q, Chen LC, Rajagopalan S. Ambient particulates alter vascular function through induction of reactive oxygen and nitrogen species. *Toxicol Sci*. 2009;111:80–88.
38. Aragon MJ, Chrobak I, Brower J, Roldan L, Fredenburgh LE, McDonald JD, Campen MJ. Inflammatory and vasoactive effects of serum following inhalation of varied complex mixtures. *Cardiovasc Toxicol*. 2016;16:163–171.
39. Channell MM, Paffett ML, Devlin RB, Madden MC, Campen MJ. Circulating factors induce coronary endothelial cell activation following exposure to inhaled diesel exhaust and nitrogen dioxide in humans: evidence from a novel translational *in vitro* model. *Toxicol Sci*. 2012;127:179–186.
40. Sardina JL, Lopez-Ruano G, Sanchez-Sanchez B, Llanillo M, Hernandez-Hernandez A. Reactive oxygen species: are they important for haematopoiesis? *Crit Rev Oncol Hematol*. 2012;81:257–274.
41. Moneypenny CG, Gallagher EP. 4-Hydroxynonenal inhibits cell proliferation and alters differentiation pathways in human fetal liver hematopoietic stem cells. *Biochem Pharmacol*. 2005;69:105–112.
42. Skorokhod OA, Caione L, Marrocco T, Migliardi G, Barrera V, Arese P, Piacibello W, Schwarzer E. Inhibition of erythropoiesis in malaria anemia: role of hemozoin and hemozoin-generated 4-hydroxynonenal. *Blood*. 2010;116:4328–4337.

43. Smith C, Gasparetto M, Jordan C, Pollyea DA, Vasiliou V. The effects of alcohol and aldehyde dehydrogenases on disorders of hematopoiesis. *Adv Exp Med Biol*. 2015;815:349–359.
44. Abu-Elmagd M, Alghamdi MA, Shamy M, Khoder MI, Costa M, Assidi M, Kadam R, Alsehli H, Gari M, Pushparaj PN, Kalamegam G, Al-Qahtani MH. Evaluation of the effects of airborne particulate matter on bone marrow-mesenchymal stem cells (BM-MSCs): cellular, molecular and systems biological approaches. *Int J Environ Res Public Health*. 2017;14:E440.
45. Bernatsky S, Smargiassi A, Johnson M, Kaplan GG, Barnabe C, Svenson L, Brand A, Bertazzon S, Hudson M, Clarke AE, Fortin PR, Edworthy S, Belisle P, Joseph L. Fine particulate air pollution, nitrogen dioxide, and systemic autoimmune rheumatic disease in Calgary, Alberta. *Environ Res*. 2015;140:474–478.
46. Takizawa H. Impacts of particulate air pollution on asthma: current understanding and future perspectives. *Recent Pat Inflamm Allergy Drug Discov*. 2015;9:128–135.
47. Zhang X, Zhong W, Meng Q, Lin Q, Fang C, Huang X, Li C, Huang Y, Tan J. Ambient PM2.5 exposure exacerbates severity of allergic asthma in previously sensitized mice. *J Asthma*. 2015;52:785–794.
48. Ghio AJ. Particle exposures and infections. *Infection*. 2014;42:459–467.
49. Huang WH, Yen TH, Chan MJ, Su YJ. Impact of environmental particulate matter and peritoneal dialysis-related infection in patients undergoing peritoneal dialysis. *Medicine (Baltimore)*. 2014;93:e149.
50. Ciencewicki J, Jaspers I. Air pollution and respiratory viral infection. *Inhal Toxicol*. 2007;19:1135–1146.
51. Zhao H, Li W, Gao Y, Li J, Wang H. Exposure to particular matter increases susceptibility to respiratory *Staphylococcus aureus* infection in rats via reducing pulmonary natural killer cells. *Toxicology*. 2014;325:180–188.
52. Arcavi L, Benowitz NL. Cigarette smoking and infection. *Arch Intern Med*. 2004;164:2206–2216.
53. Serobyan N, Orlovskaya I, Kozlov V, Khaldoyanidi SK. Exposure to nicotine during gestation interferes with the colonization of fetal bone marrow by hematopoietic stem/progenitor cells. *Stem Cells Dev*. 2005;14:81–91.
54. Siggins RW, Hossain F, Rehman T, Melvan JN, Zhang P, Welsh DA. Cigarette smoke alters the hematopoietic stem cell niche. *Med Sci (Basel)*. 2014;2:37–50.
55. Bonafe F, Guarnieri C, Muscari C. Nitric oxide regulates multiple functions and fate of adult progenitor and stem cells. *J Physiol Biochem*. 2015;71:141–153.
56. Lu A, Wang L, Qian L. The role of eNOS in the migration and proliferation of bone-marrow derived endothelial progenitor cells and in vitro angiogenesis. *Cell Biol Int*. 2015;39:484–490.
57. Thum T, Fraccarollo D, Schultheiss M, Froese S, Galuppo P, Widder JD, Tsikas D, Ertl G, Bauersachs J. Endothelial nitric oxide synthase uncoupling impairs endothelial progenitor cell mobilization and function in diabetes. *Diabetes*. 2007;56:666–674.
58. Du X, Jiang S, Bo L, Liu J, Zeng X, Xie Y, He Q, Ye X, Song W, Zhao J. Combined effects of vitamin E and omega-3 fatty acids on protecting ambient PM2.5-induced cardiovascular injury in rats. *Chemosphere*. 2017;173:14–21.
59. Haberzettl P, O'Toole TE, Bhatnagar A, Conklin DJ. Exposure to fine particulate air pollution causes vascular insulin resistance by inducing pulmonary oxidative stress. *Environ Health Perspect*. 2016;124:1830–1839.
60. Sun Q, Yue P, Ying Z, Cardounel AJ, Brook RD, Devlin R, Hwang JS, Zweier JL, Chen LC, Rajagopalan S. Air pollution exposure potentiates hypertension through reactive oxygen species-mediated activation of Rho/ROCK. *Arterioscler Thromb Vasc Biol*. 2008;28:1760–1766.
61. Pope CA III, Bhatnagar A, McCracken JP, Abplanalp W, Conklin DJ, O'Toole T. Exposure to fine particulate air pollution is associated with endothelial injury and systemic inflammation. *Circ Res*. 2016;119:1204–1214.
62. Sack CS, Jansen KL, Cosselman KE, Trenga CA, Stapleton PL, Allen J, Peretz A, Olives C, Kaufman JD. Pretreatment with antioxidants augments the acute arterial vasoconstriction caused by diesel exhaust inhalation. *Am J Respir Crit Care Med*. 2016;193:1000–1007.
63. Tong H, Rappold AG, Diaz-Sanchez D, Steck SE, Berntsen J, Cascio WE, Devlin RB, Samet JM. Omega-3 fatty acid supplementation appears to attenuate particulate air pollution-induced cardiac effects and lipid changes in healthy middle-aged adults. *Environ Health Perspect*. 2012;120:952–957.

SUPPLEMENTAL MATERIAL

Table S1. Summary statistics.

Figure	Group	n	Mean	SE	Test	p-value
1B	Air	22	1.54	0.08	Air vs CAPs	0.185
	CAPs	22	1.42	0.04		
1F	Air	16	19.3	0.82	Air vs CAPs	0.0002
	CAPs	16	13.8	1.00		
2D	Normal Pre	5	7.19	0.58	Carn vs Normal (Pre-exp)	0.004
	Normal Post	5	7.55	0.55		
	Carnosine Pre	5	256.0	63.2	Carn vs. Normal (Post-exp)	0.001
	Carnosine Post	5	367.0	72.1		
3B	Normal/Air	9	1.00	0.07	Air vs CAPs (Normal)	0.038
	Normal/CAPs	7	0.65	0.04	Air vs CAPs (Carnosine)	0.487
	Carnosine/Air	9	1.00	0.09	Normal vs Carn (Air)	1.000
	Carnosine/CAPs	9	1.11	0.17	Normal vs Carn (CAPs)	0.008
4B	Normal/Air	5	1.00	0.00	Air vs CAPs (Normal)	0.013
	Normal/CAPs	5	2.16	0.50	Air vs CAPs (Carnosine)	0.658
	Carnosine/Air	4	1.10	0.17	Normal vs Carn (Air)	0.827
	Carnosine/CAPs	5	0.90	0.22	Normal vs Carn (CAPs)	0.008
4D	Normal/Air	7	1.01	0.14	Air vs CAPs (Normal)	0.007
	Normal/CAPs	5	0.43	0.13	Air vs CAPs (Carnosine)	0.501
	Carnosine/Air	7	1.00	0.06	Normal vs Carn (Air)	0.970
	Carnosine/CAPs	7	1.12	0.16	Normal vs Carn (CAPs)	0.002
5C	Normal/Air	15	59.17	1.72	Air vs CAPs (Normal)	<0.001
	Normal/CAPs	24	48.11	2.40	Air vs CAPs (Carnosine)	0.384
	Carnosine/Air	7	63.47	3.23	Normal vs Carn (Air)	0.299
	Carnosine/CAPs	20	66.93	1.43	Normal vs Carn (CAPs)	<0.001
5E	Normal	4	1.00	0.22	Normal vs Carn	0.0001
	Carnosine	5	7.32	0.70		

p-values were obtained using Tukey adjustment for multiple comparisons.
Carn: carnosine; SE: standard error