Final Technical Report

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Center Name: University of Rochester–EPA PM Center
Center Director: Gunter Oberdorster
Title: Clinical Studies of Ultrafine Particle Exposure In Susceptible Human Subjects
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Period Covered by the Report: June 1, 1999–May 31, 2006
RFA: Airborne Particulate Matter (PM) Centers (1999)
Research Category: Particulate Matter

Objective(s) of the Research Project: The project has consisted of an extensive series of controlled inhalation studies examining the effects of ultrafine carbon particles in healthy subjects, asthmatics, and diabetics. We developed a clinical inhalation facility for ultrafine carbon particles and studied particle deposition and retention, respiratory symptoms, pulmonary function, immunologic responses, endothelial function, and electrophysiologic responses to these particles using a dose-response paradigm with rest and exercise exposures. The details of the studies, findings, and implications are detailed in the following sections.

We developed a facility for experimental exposure of humans to ultrafine particles, which permits the quantitative determination of the exposure levels, respiratory intakes, and depositions of the aerosol (Chalupa, et al., 2002). Our overall objectives are to utilize controlled human exposures to examine, in healthy and potentially susceptible subjects, the role of ultrafine particles (UFP) in inducing respiratory and cardiovascular health effects. Our hypothesis was that inhalation of UFP alters pulmonary vascular function, circulating leukocyte activation, and cardiac repolarization. We speculated that these alterations reflect mechanisms involved in the observed increase in cardiovascular morbidity and mortality associated with particulate air pollution.

Summary of Findings:

(a) UFP Exposure in Healthy Subjects

For our initial studies, exposures were conducted at rest with a relatively low concentration of elemental carbon UFP (~10 $\mu g/m^3$, ~2 x 10^6 particles/cm^3, count median diameter 26.4 nm, GSD 2.3). The overall deposition fraction (DF) was 0.66 ± 0.12 (mean ± SD) by number, and 0.58 ± 0.14 by mass (Daigle, et al., 2003). We found no significant differences in respiratory symptoms, blood pressure, pulse-oximetry, spirometry, exhaled NO, blood markers of coagulation and endothelial activation, leukocyte activation, or sputum cell differential counts (Pietropaoli, et al., 2004a,b). There was no convincing evidence for significant effects on heart rate variability, repolarization, or arrhythmias. We concluded that exposure to 10 $\mu g/m^3$

elemental carbon UFP for 2 hours at rest does not cause significant respiratory or cardiac effects in healthy nonsmokers.

We then initiated studies to examine concentration-response effects, and to incorporate exercise. Subjects received each of three exposures (air, 10, and 25 $\mu g/m^3$ UFP). Analyses indicated that exercise further increased the relatively high resting deposition of UFP (number deposition fraction at rest: 0.63±0.04; exercise: 0.76±0.06; means ±SD) (Chalupa, et al., 2004). There was evidence for a concentration-related effect of UFP exposure on the percentage of blood monocytes. In addition, monocyte expression of CD54 (ICAM-1) decreased after exposure in a concentration-response pattern ($p=0.001$), with the greatest effect occurring at 0 and 3.5 hours after exposure, and the differences resolved at 21 hours after exposure (Frampton, et al., 2006). Overall, the findings provided evidence for effects of UFP exposure, with exercise, on blood monocyte number and leukocyte expression of surface markers. In general, surface marker expression decreased in association with UFP exposure, consistent with retention of higher expressing cells within the capillary bed. ECG recording analyses showed that the response of the parasympathetic system (measured by normalized units of high-frequency [HF] components) was blunted during recovery from exercise immediately after exposure to UFP in comparison to air exposure. The analysis of QT interval duration and T wave amplitude also showed a blunted response after UFP exposure in comparison to pure air exposure. The QT and QTc shortened during exercise more substantially during UFP particle exposure than during pure air exposure, and that the QT and QTc interval remained shortened for several hours after UFP exposure but not after pure air exposure. These findings suggested that inhalation of UFP at both concentrations altered myocardial repolarization in healthy subjects.

We next initiated a study to confirm and extend these observations in a larger group of healthy men and women, using a higher, yet still environmentally relevant, concentration of UFP. Our hypothesis was that inhalation of UFP alters pulmonary vascular function, circulating leukocyte activation, and cardiac repolarization. We speculated that these alterations reflect mechanisms involved in the observed increase in cardiovascular morbidity and mortality associated with particulate air pollution.

Diffusing Capacity: In order to test our hypothesis, and to determine concentration-response relationships, we initiated exposures of healthy subjects to a higher concentration, 50 $\mu g/m^3$ UFP, using the same protocol. In these studies, we also measured the pulmonary diffusing capacity for carbon monoxide, which is affected by changes in pulmonary capillary blood volume, in 16 subjects. We also observed a significant reduction in the pulmonary diffusing capacity for carbon monoxide (DLCO), 21 hours after exposure to 50 $\mu g/m^3$ UFP when compared with air (Figure 1) (Pietropaoli, et al., 2004b). There was also a significant reduction in blood NO products throughout the post-exposure period (Figure 2). The DLCO is a function of the diffusing capacity of the pulmonary "membrane," Dm, and the pulmonary capillary blood volume, Vc. The reduction in DLCO in these studies may be caused by mild pulmonary vasoconstriction, as a consequence of reduced NO availability, leading to a reduction in the pulmonary capillary blood volume. In addition, the results of this study confirmed our previous observations of UFP effects on leukocyte expression of adhesion molecules. Monocyte expression was significantly decreased for CD18, CD11b, and CD54.

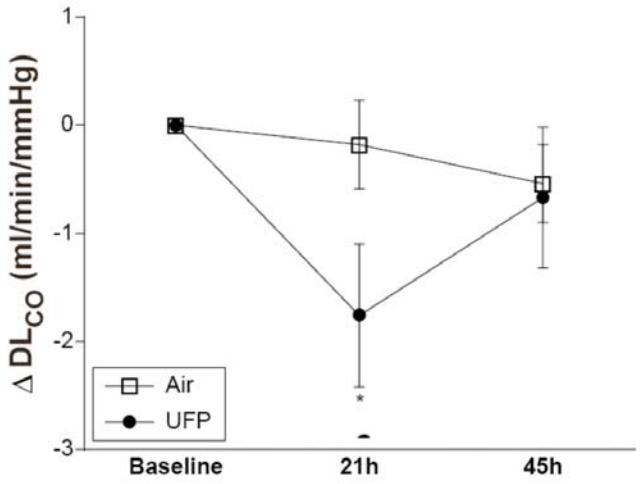

Figure 1. Change in DLCO After Exposure to Air or 50 mcg/m^3 UFP

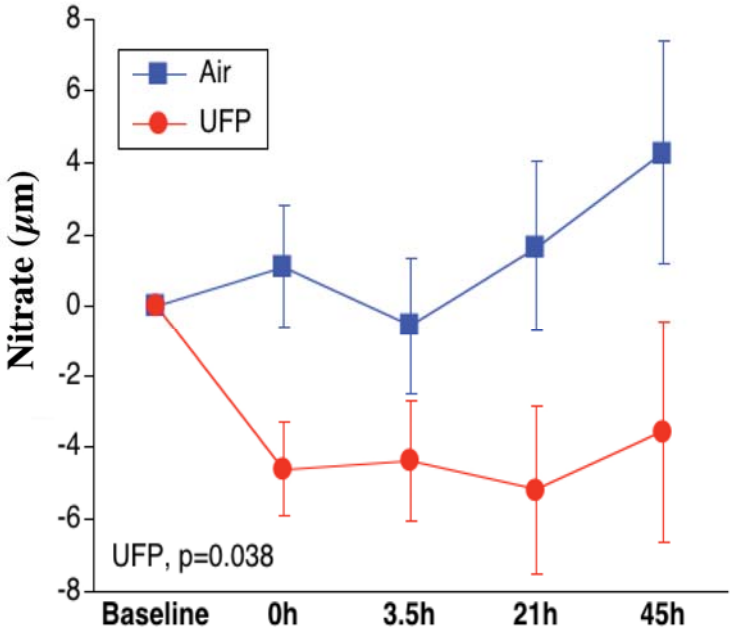

Figure 2. Change in Venous Nitrate

We subsequently tested the hypothesis that the effects on pulmonary diffusing capacity are a consequence of the high surface area of ultrafine particles, with their potential to deliver reactive oxygen species to the endothelium. In this study, 12 healthy never-smoking adults underwent three separate exposures, separated by at least 2 weeks: 1) air; 2) 50 μg/m^3 UFP (count median diameter ~30 nm, particle number ~1 x 10^7/cm^3, surface area ~750 m^2/g); and 3) 100 μg/m^3 fine particles (FP) (count median diameter ~300 nm, particle number ~1 x 10^3/cm^3, surface area ~7 m^2/g). The higher mass concentration used for FP relative to UFP was designed to provide an equivalent mass deposition in the lung, in view of the lower predicted deposition efficiency for

FP. Exposures were by mouthpiece for 2 hours with intermittent exercise, randomized and double-blinded. Effects on oxygen saturation, DLCO, and diffusing capacity for nitric oxide (DLNO) were assessed before and at intervals after exposure. Blood plasma was analyzed for nitric oxide metabolites and nitration products.

Our findings confirm that exposure to carbon UFP decreases the DLCO relative to air exposure. When the changes in DLCO following UFP exposure in this study were combined with data from our previous study in healthy subjects, the DLCO decreased from 30.15 ± 1.28 to 28.23 ± 1.16 ml/min/mmHg 24 hours after UFP exposure ($p=0.002$ vs. air exposure). The DLCO also decreased after exposure to fine carbon particles, but the difference was not significant. We also found an increase in the DLNO/DLCO ratio, suggesting a decrease in pulmonary capillary blood volume, with both UFP and FP exposure. However, this ratio was not significantly changed when the two studies were combined. There were no significant exposure effects on NO products, including nitrate, nitrite, S-nitrosohemoglobin, and iron-nitrosyl hemoglobin. Additional analyses are in progress. These findings provide important confirmation of effects of inhaled UFP on the pulmonary diffusing capacity for carbon monoxide. To our knowledge, no previous human clinical study has demonstrated pulmonary effects following exposures to such low mass concentrations of particulate matter. The data support the hypothesis that UFP inhalation alters endothelial function in the pulmonary vasculature of healthy nonsmokers.

Endothelial Function: In addition, we measured flow-mediated vascular dilatation of the forearm (FMD), before and at intervals up to 48 hours after exposure, using forearm plethysmography before and after ischemia, which measures the response in resistance vessels to the post ischemic increase in flow. FMD is mediated in part by endothelial NO action on vascular smooth muscle, and we hypothesized that UFP-induced reductions in vascular responsiveness would be accompanied by reduction in plasma NO reaction products. We therefore measured changes in the products of NO metabolism, nitrite and nitrate. We did not see any significant effect of UFP exposure on total forearm blood flow, either before or after ischemia. However, UFP exposure appeared to cause a blunting of the increase in peak flow in response to exercise. Figure 3 shows forearm blood flow following ischemia, measured 3.5 hours after exposure. The values represent change from the pre-exposure measurement. Peak flow (0 minutes) after air exposure increased, representing increased flow-mediated dilatation in response to exercise, which is an expected change. However, peak flow did not increase with UFP exposure. Figure 4 shows the change in peak flow at each of the time points measured. The difference in peak flows at 3.5 hours after exposure was significant by paired t-test, but not by analysis of variance. Minimal vascular resistance, which is the mean arterial pressure divided by the peak flow, was significantly increased compared with air exposure at this time point. Mean arterial pressure did not change significantly. These findings suggest that exposure to UFP reduced or delayed the exercise-induced increase in flow-mediated dilatation. Thus, inhalation of ultrafine carbon particles may have subtle vasoconstrictive effects in both the pulmonary and systemic vasculature.

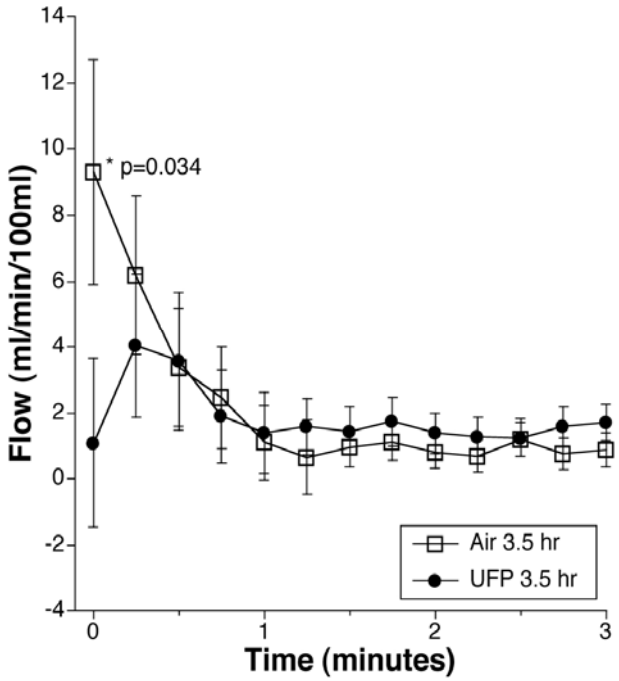

Figure 3. Forearm Blood Flow Post-Ischemia 3.5 Hours After Exposure, Difference From Pre-exposure

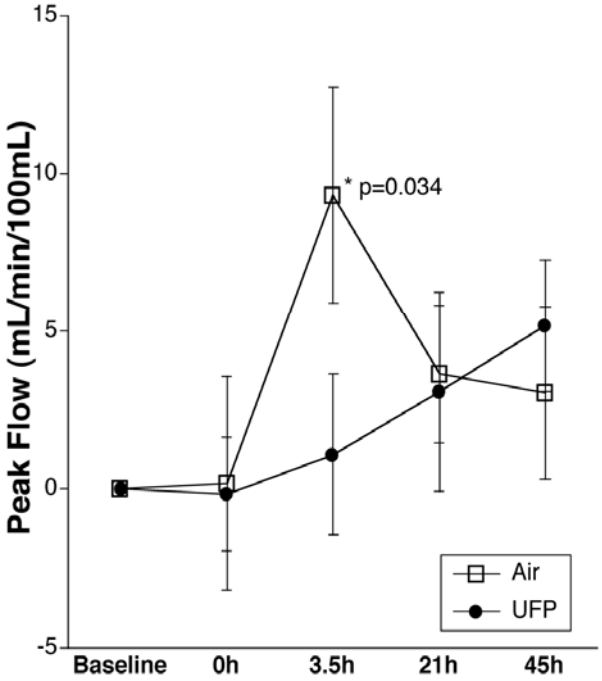

Figure 4. Peak Post-Ischemic Flow, Difference From Pre-exposure Baseline

(b) UFP Exposure in Subjects with Asthma

Subjects with asthma may represent a group with increased susceptibility to the health effects of ultrafine particles, both because of the possibility of increased airways deposition of particles, and because of underlying airway inflammation. We have completed a clinical exposure study (co-funded by the Health Effects Institute) of subjects with mild asthma. Sixteen subjects (8 male, 8 female) were exposed to air and to 10 $\mu g/m^3$ carbon UFP for 2 hours with intermittent exercise. We measured effects on pulmonary function, symptoms, airway inflammation (exhaled NO and induced sputum), blood leukocyte activation, and cardiac electrophysiologic function.

In the asthmatic subjects, we found that total respiratory fractional deposition by particle number was high at rest (0.77±0.05) and increased during exercise (0.86 ± 0.04) Chalupa, et al., 2004). Rest deposition was significantly increased compared with our previous study in healthy subjects at rest (0.63 ± 0.03). We conclude that ultrafine particle deposition is increased in mild asthmatic subjects compared with healthy subjects.

There were no significant changes in respiratory symptoms or pulmonary function in response to these exposures. Blood studies revealed a particle-related decrease in blood eosinophils, basophils, and $CD4^+$ T-lymphocytes. Blood monocytes showed a significant reduction in CD11b expression after exposure (p=0.029) (Frampton, et al., 2006). Expression of CD54 on polymorphonuclear neutrophil (PMN) decreased in a time-related fashion, with the greatest difference from control at 45 hours after exposure, with a significant time-exposure interaction (p=0.031). Expression of CD62L on PMN showed a significant exposure-gender interaction, with an increase in expression of CD62L in males only. The most significant effect on leukocyte surface molecule expression in subjects with asthma appeared to be on eosinophils. There was a small exposure-related reduction in eosinophil percentage from the blood leukocyte differential count. In addition to this early reduction in eosinophil number, there was a delayed reduction in eosinophil expression of CD32 (time-exposure interaction, p=0.015), and CD11b (main effect, p=0.015).

In summary, our study indicates that asthmatics have increased airway deposition of ultrafine particles, and that exposure to even low mass concentrations of ultrafine particles alters circulating leukocyte subsets. These data are most consistent with an alteration in leukocyte retention in the pulmonary circulation. In addition, our preliminary results suggest there are also effects on systemic endothelial function.

Conclusions

The major conclusions from our studies are as follows:

- In healthy individuals, there is a high pulmonary deposition of inhaled ultrafine particles at rest that increases with exercise.

- The reduction in diffusing capacity and changes in forearm flow mediated vascular dilatation suggest that inhalation of low mass concentrations of ultrafine carbon particles cause subtle changes in both pulmonary and systemic endothelial function.

- In addition, the findings provide evidence for effects of UFP exposure, with exercise, on blood monocyte number and leukocyte expression of surface markers. In general, surface marker expression decreased in association with UFP exposure, consistent with retention of higher expressing cells within the capillary bed.

- Asthmatics have increased airway deposition of ultrafine particles, and exposure to even low mass concentrations of ultrafine particles alters circulating leukocyte subsets.

Our findings provide support for the hypothesis that inhalation of ultrafine particles causes subtle alterations in vascular function. Our findings are consistent with the panel studies described in Core 2, which provided evidence for vascular endothelial effects of ambient particles in susceptible humans. In particular, the findings in Core 2 of increased soluble CD40L, and decreases in platelet counts, may indicate that inhalation of particulate matter causes platelet activation, which is a consequence of endothelial injury. This is consistent with our findings of changes in blood leukocyte markers, diffusing capacity, and forearm blood flow.

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Supplemental Keywords: NA

Relevant Web Sites: http://www2.envmed.rochester.edu/envmed/PMC/indexPMC.html